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FORM AND DISTRIBUTION OF SENSORY TERMINALS IN CAT HINDLIMB MUSCLE SPINDLES

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The sensory innervation of cat hindlimb muscle spindles was studied by reconstruction, electron microscopy, and examination of teased, silver preparations to ascertain the form of the terminals and their distribution to bag_1 (b_1), bag_2 (b_2), and chain (c) muscle fibres. Reconstructions were made of two primary endings, one S_1 secondary ending, and the branching of four primary and six secondary axons. For the silver analysis spindles were teased from 14 different hindlimb muscles, the largest samples being from tenuissimus, peroneus brevis, p. longus, p. tertius, superficial lumbrical, extensor digitorum longus, and soleus. Among 310 spindles examined, 40 lacked a b_1 fibre. These were all portions of tandem spindles in which the b_2 fibre was continuous from one capsule, where it was accompanied by b_1 and c fibres, to another, in which it was accompanied by c fibres only. These have been designated 'b_2c spindle units' as distinct from 'b_1b_2c spindle units'.

Counts of myonuclei in the primary regions of four b_1b_2c spindle units revealed 52–106 in the nuclear bags, b_1 bags averaging 68, b_2 bags 80. The average number in a myotube region was nine (range 6–12), and in a c fibre 24 (range 11–38). The reconstructions showed a close association between nucleation and innervation, but no constant relation between number of myonuclei and terminal contact area. They also showed that the largest cross-sectional areas of each bag fibre corresponded with the sites of S_1 secondary ending innervation. Approximately 70–90 % of the cross-sectional area of each bag fibre was occupied by myonuclei in the bag region, 30–50 % in the myotube regions, and 10 % in the region of secondary innervation. In b_2c spindle units the equatorial nucleation of the b_2 fibre usually resembled that of a c fibre.

The intramuscular diameter of Ia axons supplying b_1b_2c spindle units (mean 7.5 μm, range 3.4–12.8 μm, n = 213) was generally thicker than that of Ia axons supplying b_2c spindle units (mean 5.1 μm, range 2.2–9.0 μm, n = 37). The distribution of terminals by the first-order branches (usually two) of Ia axons to b_1, b_2, and c fibres was exclusively from heminodes, and was either segregated (b_1 fibres supplied separately from b_2 and c fibres, thereby resulting in separation of dynamic and static inputs) or mixed. Mixing was restricted most frequently to the dynamic input, and usually resulted from b_1 fibres sharing a supply of terminals with a few c fibres. Distribution of terminals was usually segregated in tenuissimus and mixed in superficial lumbrical muscles, but in most muscles neither type of distribution predominated. Among 270 b_1b_2c spindle units, 32 had more than one b_1 fibre, and 12 had primary endings formed by two Ia axons.

Primary terminal systems supplied to bag fibres consisted of a middle portion, in which the terminals were arranged mainly as regular transverse bands, and portions at each end, in which they were disposed irregularly. In the silver analysis, b_1 terminal systems in 151 b_1b_2c primary endings were distinguished from those supplied to b_2 fibres by having more of their total length occupied by irregular portions (on average 57 %, as compared with 33 % in b_2 fibres), and more bands per unit length in the middle. In the two reconstructed primaries the b_1 fibres received 33 and 37 % of the total terminal contact area, the b_2 fibres 25 and 24 %, and the c fibres 5–12 % individually, 42 and 39 % collectively. Primary endings supplied to b_2c spindle units were mostly irregular in appearance.

The polar position of 351 secondary endings was S_1 253, S_2 79, S_3 15, S_4 3, S_5 1; 67.8 % were distributed to b_1b_2c fibres (mostly as S_1 endings), 20.8 % to b_2c fibres, 6.3 % to c fibres, and 5.1 % to b_1c fibres. The intramuscular diameter of II axons terminating as S_1 endings was generally greater (mean 3.9 μm) than that of II axons terminating as S_2–S_5 endings (mean 2.9 μm); 75 % of b_1b_2c II axons had diameters that fell within the lower part of the b_1b_2c Ia diameter range. Most II axons had two first-order branches; the distribution of terminals by the first-order branches of b_1b_2c II axons was usually

mixed. Terminals were derived either exclusively from heminodes (as in most S_1 endings) or from both heminodes and penultimate nodes.

The mean length of 313 secondary endings was 348 μm (range 138–716 μm) as compared with a mean length of 359 μm (range 242–608 μm) for 151 primaries. In 83 b_1b_2c S_1 endings the innervated portions of the bag fibres represented, on average, 42% (b_1) and 51% (b_2) of the total length of the ending. In 64% of the endings the b_2 fibre received more terminals than the b_1. In the reconstructed S_1 ending the b_1 fibre received 8% of the total terminal contact area, the b_2 fibre 17%, and the c fibres 16–22% individually, 75% collectively. Some muscles had fewer secondaries than others; the Ia:II ratio ranged from 1:1.2 (superficial lumbrical) to 1:1.8 (peroneus longus).

The constant features of spindle sensory innervation that emerge from this study (e.g. the dense primary innervation of the b_1 fibre) are discussed in the context of spindle development and in terms of their functional significance. The data on the branching of spindle afferents is related to the work of others on pacemakers and the manner in which nerve impulses are generated from the endings and propagated into the axons. We suggest that transduction may occur by a deformation of the sensory terminal owing to increased tension in the basal lamina. It is supposed that the permeability of the Na^+ channels is affected by an intracellular messenger (probably Ca^{2+}) released from a bound state by increase in cytoskeletal tension. Reasons are given as to why the afferent innervating b_2c spindle units should be regarded as primary. The probable functional significance of these units is discussed, and some correlations are made between the function of certain muscles and the characteristics of their spindle populations.

1. Introduction

It is now accepted that mammalian muscle spindles are composed of nuclear-chain fibres and two types of nuclear-bag fibre, bag_1 and bag_2 (Ovalle & Smith 1972; Banks *et al.* 1975, 1977*b*; Gladden 1976); and it is generally agreed that the dynamic responsiveness of the primary ending is mediated by the bag_1 fibre, and its static responsiveness by bag_2 and chain fibres (Barker *et al.* 1976, 1978; Boyd *et al.* 1977). In arriving at these conclusions attention was necessarily focused on the distribution of the motor innervation to the three types of intrafusal muscle fibre. The main purpose of this investigation has been to make a similar enquiry into the distribution of the intrafusal sensory innervation.

The essential features that emerge from previous descriptions of mammalian spindle sensory innervation (mostly cat) may be summarized under the headings of *primary endings*, *secondary endings* and *diameters of spindle afferents*, as follows.

Primary endings. An annulospiral ending (Ruffini 1898) is supplied by branches of a Ia axon (Hunt 1954), which distributes large spirals to the bag fibres and small spirals to the chain fibres (Boyd 1962). A first-order branch of the Ia axon (usually two are produced) may innervate both bag and chain fibres, or exclusively supply either bag or chain fibres (Barker & Cope 1962). The ending terminates on the densely nucleated equatorial parts of the intrafusal muscle fibres (the nuclear bags, myotube regions, and nuclear chains), and occupies a length of about 300 μm. The terminals consist of spirals of up to four or five turns, many half rings, and a few complete rings. They are set closely together around the middle of each nuclear bag, disposed more loosely to either side, and are usually of an irregular clasping form in the myotube regions (Barker 1948). A terminal may end on a single muscle fibre, or on two or three adjacent ones to form an interlocking 'sensory cross-terminal' (Adal 1969). These usually occur between chain fibres, but may also occur between a bag and a chain fibre (Corvaja *et al.* 1969; Banker &

Girvin 1971; Scalzi & Price 1972). Some primary endings in the cat are irregular in appearance and have only a few rings and spirals. Spindles that lack secondaries usually have primaries of this type, as in 'simple' single spindles (Ruffini 1898) and simple spindle units that form part of tandem spindles (Barker & Ip 1961). A primary ending may occasionally be composed of terminals supplied by two axons (Ruffini 1898), and instances of primary endings restricted to bag fibres have been observed (Barker & Cope 1962; Jones 1966).

Secondary endings. Most cat hindlimb spindles are supplied with one secondary ending next to the primary (Barker & Ip 1961), but up to five may occur, three on one side of the primary and two on the other, each occupying lengths of about 400 μm designated S_1, S_2, S_3 according to their position relative to the primary (Boyd 1962). Secondary terminals are also mainly annulospiral (Barker 1948), though some may be in the form of sprays. The spirals are small and restricted to the chain fibres, whereas the sprays terminate on the bag fibres (Boyd 1962). The ending is more dispersed than the primary, and the terminals, some of which interlock around adjacent muscle fibres, are mostly thin and claw-like (Tello 1922).

Diameters of spindle afferents. Measurements of the total internodal diameters of spindle afferents made in the neighbourhood of spindles show that: (i) the total diameter of primary axons is about twice that of secondary axons (Ia, mean 12.4 μm; II, mean 6.0 μm; for cat tenuissimus (Boyd 1962)); (ii) the thinnest primary axons overlap in diameter with the thickest secondaries (Ia–II diameter overlap 8–11 μm (Boyd 1962)), and are mostly supplied to spindles that do not receive secondary endings (Adal & Barker 1962).

These observations were all made before it was realized that bag fibres were of two types, and that the functional differences between them made it necessary to know about their individual sensory innervation. It was this need that motivated the present enquiry.

We began by making reconstructions from serial sections of the sensory innervation of some of the cat tenuissimus spindles whose motor innervation had already been analysed following direct observation of the effects of fusimotor stimulation (Banks *et al.* 1978; Banks 1981*a*). Reconstructions were made of two primary endings, one S_1 secondary ending, and the branching of four primary and six secondary axons. These showed, among other things, that: (i) there were distinct differences in the form of the primary terminals on bag_1 and bag_2 fibres; (ii) the first-order branches of all the Ia axons supplied bag_1 fibres separately from bag_2 and chain fibres (segregated distribution); and (iii) the S_1 secondary axons innervated all three types of muscle fibre.

These features of the reconstructions were easily recognizable in teased, silver preparations of whole spindles, making it possible to identify bag_1 and bag_2 fibres by distinguishing (as one of several criteria) the differences in their sensory innervation. The findings from the small sample of reconstructed tenuissimus spindles could thus be compared with those from a much larger sample of spindles teased from several different hindlimb muscles. This showed, among other things, that the distribution of terminals by first-order branches of Ia axons is not always segregated, and that irregular primary endings are characteristic of spindle units that lack a bag_1 fibre. Preliminary accounts of some of the results have been published (Banks *et al.* 1977*b*, 1979, 1981).

2. Materials and methods

(*a*) *Reconstructions and electron microscopy*

The sensory endings and axons reconstructed from serial transverse sections belonged to four of the 20 cat tenuissimus spindles studied by Banks *et al.* (1978) in their analysis of intrafusal contractions and motor innervation, namely, spindles 6, 8, 9 and 12 (see their table 1). Additional information was obtained from serial longitudinal sections of spindle 5. Apart from the inclusion of post-fixation with osmium tetroxide, the procedures relating to the fixation of these spindles, their preparation for histological analysis, and the criteria subsequently used for the identification of muscle-fibre type, were as described by Barker *et al.* (1978).

(i) *Microtomy*

Serial transverse sections about 1–2 μm thick, cut for light microscopy, were stained with 1.7 mg/ml toluidine blue in 1.7 mg/ml borax solution, or with a saturated solution of *p*-phenylenediamine in methanol. The sections of spindle 8 were cut on an LKB Ultratome UM1 and collected in batches of about 5–20 on glass slides, the last section being collected separately, since the order of the other sections was not always known. The average thickness of transverse sections relative to the living spindle was estimated by counting the number of sections between a pair of structures, e.g. blood vessels, marked on the photograph of the spindle taken at the conclusion of the experiment (Banks *et al.* 1978) before fixation. No correction for longitudinal shrinkage due to processing was therefore necessary.

Serial transverse sections of spindles 6, 9 and 12 were cut on a Reichert OMU3 ultramicrotome and collected on strips of coverglass in ribbons of ten, five such strips being mounted on each glass slide. The graduated knife-advance control of the microtome provided a nominal section thickness of 1 μm. Longitudinal shrinkage averaged 8% as estimated by comparing distances between pairs of structures in the living preparation with the nominal distances provided by summing section thicknesses. This correction has not been applied to the measurements quoted for these three spindles.

Sections for electron microscopy were cut at selected sites, stained with uranyl acetate and lead citrate, and examined with an A.E.I. EM 801 electron microscope at an accelerating voltage of 80 kV.

(ii) *Photography and reconstructions*

Every section containing part of a sensory axon or terminal was photographed on 35 mm film with a Zeiss Ultraphot with ×40 or ×100 planapo objectives. With ×4.5 enlargements of the photographs, two types of reconstruction were made: schematic diagrams showing the branching and myelination of four primary and six secondary axons supplying the spindles (figure 1); and semi-diagrammatic isometric reconstructions of two primary endings (spindles 6 and 12) and one S_1 secondary ending (spindle 6) (figures 4–6).

The isometric reconstructions were made by tracing the relevant features of each photograph onto acetate film with use of coloured inks to distinguish between the various components. Adjacent tracings were placed along an inclined line to produce the required amount of spatial resolution. Since no external reference was available, the assumption was made that one part of the reconstruction always fell on this line, namely, the centre of the bag_1 fibre. The bag_2

fibre was orientated so that its centre was either vertically above (spindle 6) or horizontally to the right (spindle 12) of this. The bag_1 fibre was thus artificially straightened, while the position of the bag_2 fibre was restricted to a plane that passed through the bag_1 fibre's centre, and the chain fibres were positioned relative to that plane. Examination of longitudinal sections of the equatorial regions of other spindles showed that these constraints produced little distortion. For clarity, the bag fibres were reconstructed separately from the chain fibres. The reconstructions were built up by using the same colours to draw the envelopes of every group of ten tracings, and then by making tracings of the envelopes in indian ink.

(iii) *Measurement of sensory-terminal contact areas*

The photographs used for reconstructing the three sensory endings were also used to estimate the areas of contact between the terminals and the muscle fibres. The length of contact between terminal and fibre, and the length of fibre surface devoid of terminal, were determined in each section of a muscle fibre that included a sensory neuromuscular junction. Measurements were made with a map-measuring wheel, converted into micrometres and summed to give areas in micrometres squared, section thickness being taken as 1 μm. These measurements provided estimates of the total terminal–fibre contact area for each sensory ending, and this could be expressed as a proportion of the total surface area available for contact so as to give an indication of density of innervation. Any error involved in making measurements with the mapping wheel was assessed by using it to determine the lengths of many straight lines of differing lengths, ten measurements being made of each length. A comparison of the means of these measurements with the original terminal–fibre contact lengths showed that the error involved was relatively greater for shorter lengths, and that the probable overall error was an underestimate of about 4%.

(*b*) *Analysis of silver preparations*

(i) *Nature of sample*

The total sample comprised 310 spindle capsules, of which 84 were linked in tandem. They were teased from 72 hindlimb muscles removed from 38 adult cats, as follows.

TABLE 1.

muscle	number of spindle capsules		number of muscles teased	
tenuissimus	70		14	
peroneal muscles				
p. longus	20		4	
p. brevis	61	108	10	23
p. tertius	27		9	
lumbrical muscles				
superficial	34		10	
deep	8	42	1	11
soleus	31		3	
extensor digitorum longus	21		7	
extensor digitorum brevis	7		1	
flexor hallucis longus	11		4	
tibialis anterior	13		5	
tibialis posterior	1		1	
interosseus	5		2	
rectus femoris	1		1	
total . . .	310		72	

The sample of spindles assembled for analysis was selected from the large collection of teased preparations that has accumulated in this laboratory over the years, excellence of staining of sensory innervation being the sole criterion for inclusion. With one exception all the spindles had been stained by de Castro's silver method as modified by Barker & Ip (1963); the exception was a rectus femoris spindle stained with Gairns's (1930) gold chloride method.

Of the 72 muscles, 26 had been the object of experiments involving either de-efferentation (4), de-efferentation and sympathectomy (15), or degeneration of all but one or a few axons in their motor supply (7). Of the total sample of spindle capsules, 83 were teased from these muscles.

(ii) *Diameter and branching of spindle afferents*

The total diameters of 259 Ia and 357 II axons were measured by using a Zeiss micrometer eyepiece and a $\times 40$ objective. The measurements were made as far from spindle entry as possible in order to avoid the length over which diameter increases as the first subdivision before termination is approached. Each measurement recorded was the mean of three internodal readings. Conversion to the equivalent fresh diameter requires multiplication by a factor of 1.5 (Stacey 1969), but this has not been applied to the results.

The branching and distribution of 182 Ia axons was analysed by using a $\times 100$ objective under oil immersion. Each analysis was double-checked; any instance occasioning the slightest doubt resulted in the axon in question being excluded from the final sample.

(iii) *Analysis of sensory endings*

Each primary ending was first examined to distinguish between the terminal systems distributed to the bag_1 and bag_2 fibres on the basis of differences in amount of irregularity. Terminals were regarded as 'regular' when they were set at right angles to the longitudinal axis of the bag fibre, as in the nuclear-bag region, and 'irregular' when they were set diagonally to, or in parallel with, this axis, as in the myotube regions. Confirmation of the identity of the bag fibres was then sought by checking on their length (bag_2 longer); association with (bag_2), or dissociation from (bag_1), chain fibres in the equatorial region; abundance (bag_2) or scarcity (bag_1) of associated elastic fibres in the extracapsular polar region; motor innervation by p_1 and/or p_2 plates (typically supplied to bag_1) and trail endings (typically supplied to bag_2); and sensory innervation by secondary endings (bag_1 usually receives fewer terminals).

Each primary ending was then measured so as to record its total length, the lengths of the regular and irregular portions of the terminal systems on each bag fibre, and the maximum diameter of each terminal system. Finally, the number of terminals encircling each bag fibre in the regular portion of its primary terminal system was counted over a length of 50 μm.

If the spindle under analysis was supplied with one or more secondary endings, the length of each was measured, its position (S_1, S_2, or S_3) was noted, and the supply of terminals to one or both types of bag fibre was observed. In a sample of 83 S_1 secondary endings the number of terminals on each type of bag fibre was counted and the length of the innervated portion of the fibre was measured.

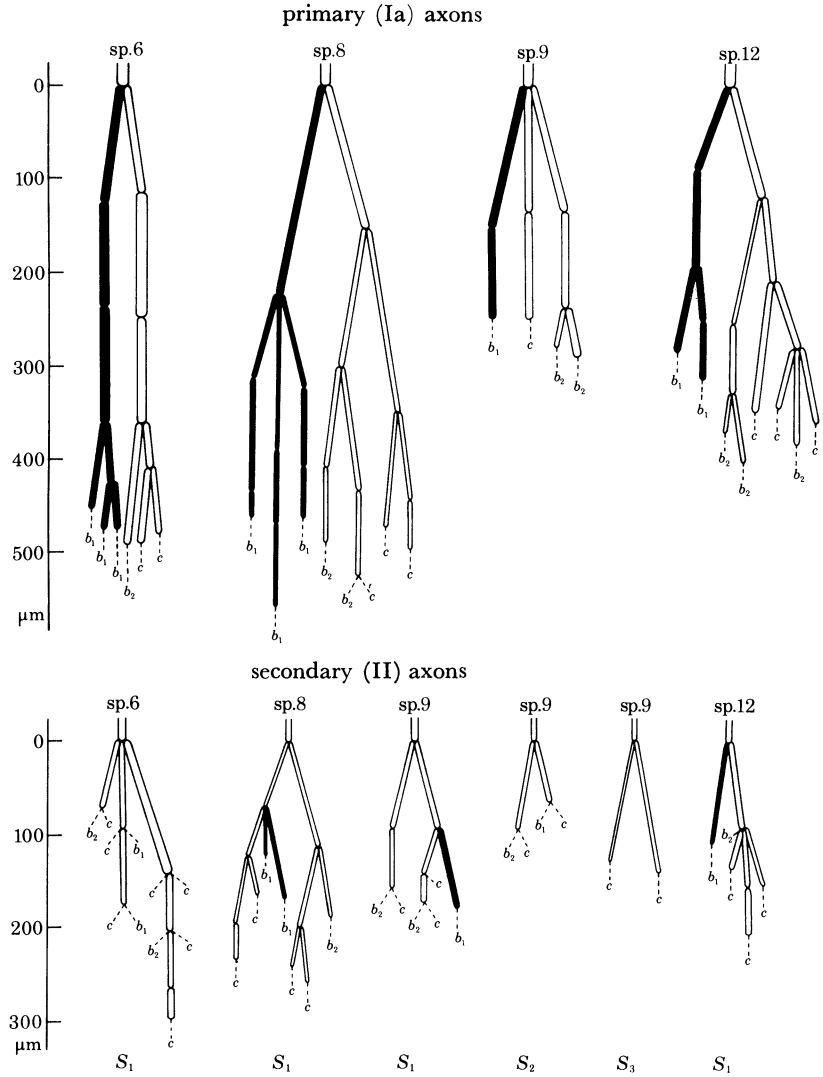


FIGURE 1. Branching of the primary and secondary axons supplying spindles (sp.) 6, 8, 9 and 12, and the distribution of their branches to the three types of intrafusal muscle fibre. Black branches exclusively innervate bag_1 (b_1) fibres; preterminal axons indicated by dotted lines. The primary axon of spindle 12, and the primary and secondary axons of spindle 6, are shown in more detail in the reconstructions reproduced in figures 4, 5 and 6, respectively. Symbols: b_2, bag_2 fibre; c, chain fibre(s); S_{1-3}, secondary endings.

3. RESULTS

(*a*) *Reconstructions and electron microscopy*

(i) *Branching and distribution of spindle afferents*

The sensory innervation of the four spindles studied was: spindle 6, S_1P; 8, $S_1PS_1S_2$; 9, $PS_1S_2S_3$; 12, S_1P (symbols according to Boyd (1962), given in proximal-to-distal order). Figure 1 shows the branching of the four primary axons and six of the secondary axons, and indicates the distribution of the branches to the three types of intrafusal muscle fibre.

The first subdivision of each primary axon produced either two or three first-order branches, one of which was the exclusive distributor of terminals to the bag_1 (b_1) fibre. This branch

usually subdivided further and had two to five internodes. Most of the 18 subdivisions undergone by the four primary axons were dichotomous; only three were trichotomous. One of these was the first subdivision of the axon innervating spindle 9, which resulted in one first-order branch being the exclusive distributor of terminals to each of the three fibre types. In the other three primary axons the bag_2 (b_2) and chain (c) fibres each derived their terminals from the same first-order branch.

The primary axons reached their heminodes after branching over distances of 250–558 μm. The internodes became shorter as the heminodes were approached; the mean lengths of all first to fifth primary internodes in figure 1 are 192, 123, 95, 75 and 58 μm, respectively. When the first subdivision was dichotomous, the first internodes differed in total diameter by about 1.0 μm, the thinner belonging to that which exclusively innervated the b_1 fibre. Total diameter decreased distally by 0.3–6.0 μm (mean 2.6 μm) between first and last internodes, the mean decrease per 100 μm length being 0.4 μm. Myelin thickness was approximately halved as between the last internode of the parent axon and the first internodes of its branches (e.g. from 1.7 to 0.8 μm), and halved again as between first and last internodes (e.g. from 0.6 to 0.3 μm).

The eight secondary axons distributed terminals to the muscle fibres as follows (spindle reference numbers in brackets): S_1 (6), S_1 (8), S_1S_2 (9), S_1 (12) to b_1, b_2 and c fibres; S_1 (8) to b_1 and c fibres; and S_2 (8), S_3 (9) to c fibres only. Reconstructions of the branching of six of the axons (figure 1) showed that the terminals were supplied by preterminal axons that arose, not only from the heminodes, but also from some of the penultimate nodes.

The secondary axons reached their heminodes over distances of 65–300 μm. They did not branch as extensively as primary axons and their internodes were generally shorter; the mean lengths of all first to fourth internodes in figure 1 are 97, 68, 51 and 37 μm, respectively. Trichotomous branching was relatively more frequent (three of 11 subdivisions). Six first-order branches had only one internode; four of these belonged to the dichotomously branching S_2 and S_3 axons innervating spindle 9. There were four instances of first-order branches exclusively supplying one type of muscle fibre, namely, in the S_1 afferents of spindle 6 (to c fibres) and 12 (to the b_1 fibre), and the S_3 afferent of spindle 9 (to c fibres). Total diameter decreased by a mean of 0.4 μm per 100 μm length between first and last internodes, much as in primaries, but there were four instances of increase; these ranged from 0.3 to 1.1 μm. With few exceptions (four in 25) myelin thickness remained the same from the parent axon to the most distal internode, e.g. all 12 internodes in the branching of the S_1 afferent of spindle 8 had a myelin thickness of 0.3 μm.

(ii) *Equatorial nucleation*

The nuclei of intrafusal muscle fibres are located either peripherally underneath the sarcolemma (subsarcolemmal nuclei) or internally among the myofibrils (myonuclei). In teased preparations it is often difficult to distinguish between subsarcolemmal nuclei and those of satellite cells and endomysial fibrocytes. The reconstruction of the distal pole of spindle 6 showed that satellite cells were mostly found in association with the b_2 fibre in the extracapsular region. They were less frequently associated with the b_1 fibre and rarely occurred on c fibres (Banks 1981*b*). Equatorially both satellite cells and subsarcolemmal nuclei were scarce, whereas myonuclei were abundant. The reconstructions produced an opportunity for studying the number and distribution of myonuclei in the area of sensory innervation and the nature of the structural substrate beneath the sensory terminals.

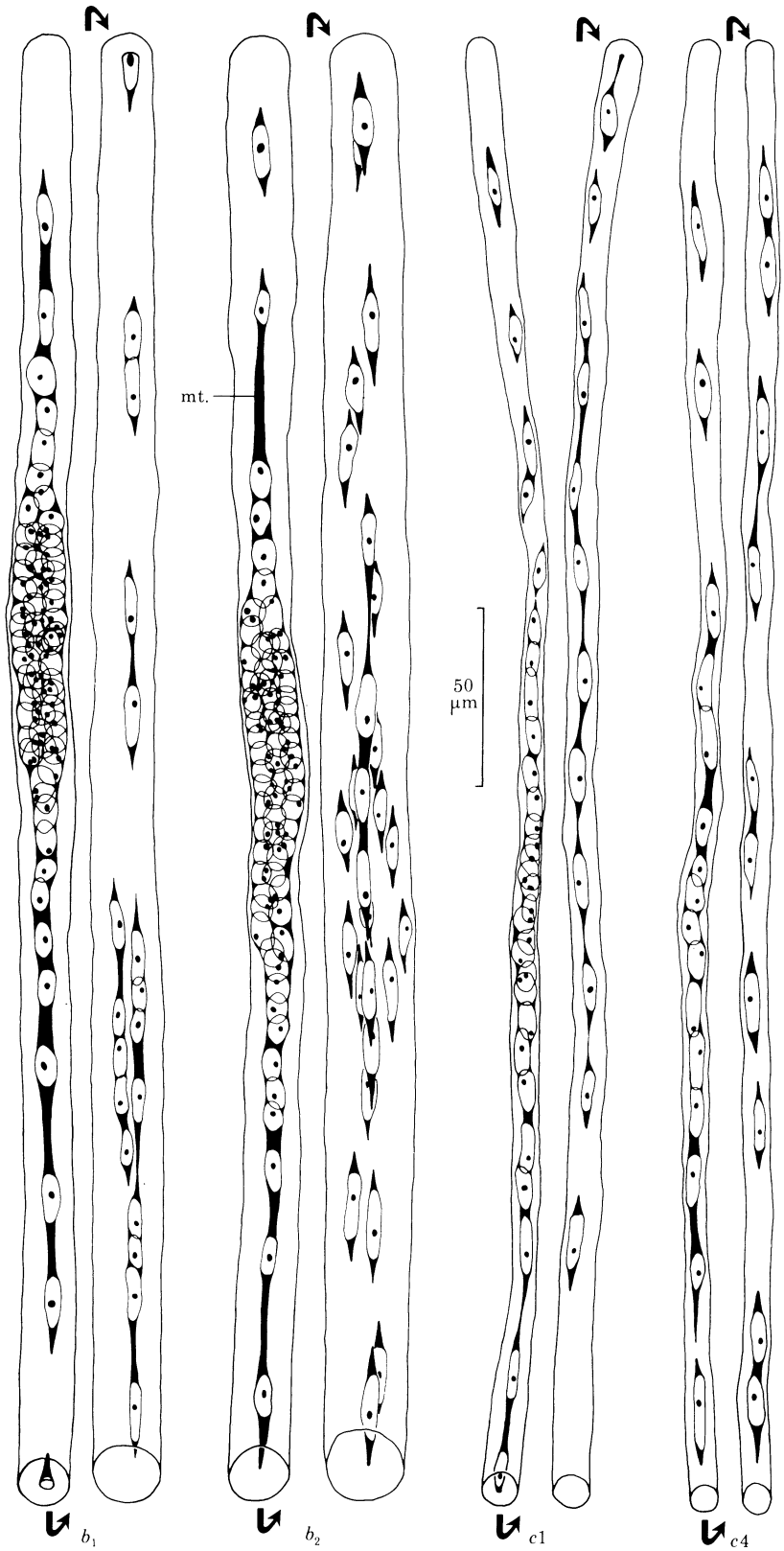


FIGURE 2. For description see opposite.

Figure 2 illustrates the equatorial nucleation of spindle 6 as reconstructed from sections of the primary and S_1 secondary regions of the b_1 and b_2 fibres and the longest and shortest c fibres (see also plates 1 and 2). Each nuclear bag consisted of a sheath of myofibrils that enclosed an aggregation of closely-packed, oval (typically 5 μm × 7 μm) myonuclei. At each end these reduced in number from three to two abreast, and finally to a single row of oblong (typically

TABLE 2. NUMBER AND DISTRIBUTION OF PRIMARY-REGION MYONUCLEI IN FOUR CAT TENUISSIMUS SPINDLES

(Symbols: b_1, bag_1 fibre; b_2, bag_2 fibre. Chain fibres in spindles 6 and 12 numbered in order of decreasing length.†)

spindle no.		bag fibres				chain fibres						combined total
		myotube	bag	myotube	total	1	2	3	4	5	total	
6	b_1	9	52	12	*73*	24	23	22	14		*83*	*228*
	b_2	9	53	10	*72*							
8	b_1	9	52	9	*70*	37	36	23	26	18	*140*	*316*
	b_2	10	88	8	*106*							
10	b_1	6	59	11	*76*	38	30	25			*93*	*256*
	b_2	11	68	8	*87*							
12	b_1	9	34	9	*52*	24	19	18	20	11	*92*	*197*
	b_2	8	35	10	*53*							

† Chain fibres 2 and 3 in spindle 6 are numbered as 3 and 2 by Banks (1981*a*), and he numbers chains 1, 2, 3 and 4 in spindle 12 as 4, 1, 2 and 3.

5 μm × 9 μm) nuclei contained within a centrally placed myotube. It is convenient to define the nuclear bag as being delimited at each end by the level at which there is a consistent presence of three myonuclei abreast in five successive transverse or longitudinal 1 μm sections; and to define a myotube region as occupying a length of fibre from this level to the end of its axial core of sarcoplasm. Thus defined, the nuclear bags measured 70 μm (b_1) and 86 μm (b_2) long and contained 52 and 53 myonuclei, respectively. The lengths of the myotubes were 100 and 172 μm (b_1) and 93 and 148 μm (b_2), the longest being adjacent to the site of the S_1 secondary ending. They contained nine and twelve (b_1) and nine and ten (b_2) myonuclei, respectively. Table 2 includes these counts together with similar counts for spindles 8, 10 and 12. In this small sample it is evident that b_1 and b_2 fibres either have a very similar number of myonuclei in the primary region or have most located in the b_2 fibre.

On the distal side of the primary region myonuclei were scattered at intervals along the length of the bag fibres, becoming fewer as these left the periaxial space for the distal pole. Each nucleus was more or less centrally aligned and enclosed within an envelope of sarcoplasm. On the proximal side, where the S_1 secondary ending was located, there were dense aggregations

FIGURE 2. Equatorial nucleation of spindle 6 as reconstructed from sections of the primary and S_1 secondary regions of the bag_1 (b_1) and bag_2 (b_2) fibres and the longest and shortest chain (c) fibres (numbered 1 and 4, as in table 2). Only myonuclei are shown; the few subsarcolemmal nuclei and satellite cells present have been omitted. The myonuclei are accurately drawn except those filling the nuclear bags, which are drawn as freehand outlines around their nucleoli. The alignment of each muscle fibre relative to the other is that which obtained in the spindle; the distal end of each fibre lies at top left. Sarcoplasm, such as that filling each myotube (mt.), is shown black. See also figures 5 and 6, and plates 1 and 2.

of myonuclei in those areas of the bag fibres that received secondary terminals (see figures 2 and 8). In the b_1 fibre there were two rows of nuclei in this area, each enclosed within a separate myotube, whereas in the b_2 fibre the nuclei were mostly contained within sarcoplasmic envelopes situated at all levels among the myofibrils. The same transverse section might include up to four. Bag-fibre myonuclei in the secondary region were usually longer and narrower than those in the primary region, typically measuring 4 μm × 14 μm.

Counts of the myonuclei present in the primary region of the *c* fibres in spindles 6, 8, 10 and 12 are given in table 2. There was a tendency for the longest fibres to have the most nuclei. In spindle 6 the myonuclei in the longest fibre were enclosed within a myotube that was more or less continuous throughout the primary and secondary regions (see figure 2). The nuclei were closer together in the primary region, and for a short length at the equator they formed a double instead of a single row so as to give the appearance of a miniature nuclear bag. By contrast, the shortest *c* fibre had a single row of myonuclei in a myotube that was confined to the primary region.

Measurements of the total cross-sectional area of the bag fibres in spindle 6, made at intervals during their course through the equatorial region, showed that there was a marked increase in the region of the S_1 secondary ending (see figure 3). The largest cross-sectional areas of each bag fibre corresponded with the sites at which they received secondary terminals. At these levels their cross-sectional areas were approximately twice those at the equivalent levels on the distal side of the primary region, where there was no secondary ending. In each bag fibre the proportion of cross-sectional area occupied by myonuclei was approximately 70–90 % in the region of the nuclear bag, 30–50 % in the myotube regions, and 10 % in the region of the secondary terminals (see figure 3).

The *c* fibres did not show a similar increase in thickness in the secondary region. Indeed they were somewhat thinner here than in the equivalent region on the distal side of the primary ending. From 40 to 60 % of the total cross-sectional area of the longest *c* fibre was occupied by myonuclei in the primary region, and about 15 % in the secondary region.

(iii) *Reconstructed primary endings*

Reconstructions of the primary endings of spindles 6 and 12 are shown in figures 4 and 5. The endings are remarkably alike in several respects. For example, in the proportional distribution of terminal contact area to the three types of intrafusal fibre, it is the b_1 fibre in each ending that is the most densely innervated. Thus, in spindle 6, 37 % of the total terminal contact area was distributed to the b_1 fibre as against 24 % to the b_2 fibre and 39 % to the four *c* fibres. This compares with proportions of 33 % (b_1), 25 % (b_2) and 42 % (*c* fibres) in spindle 12, and in each ending it was the b_1 fibre terminals that covered the largest proportion of muscle-fibre surface (see table 3).

There were also consistent differences in the form and disposition of the terminals on the two bag fibres. In the b_1 fibre, transversely orientated, regularly arranged terminals wrapped closely together around the nuclear bag were flanked on either side by an irregular array of terminals, many of which lay parallel with the fibre's axis. By contrast the terminals on the b_2 fibre were more widely spaced apart and nearly all were transversely orientated, with minimal irregularity at each end. In figure 7 these differences between the primary terminals on the bag fibres of spindle 6 have been emphasized by changing the alignment of the nuclear bags so that, instead

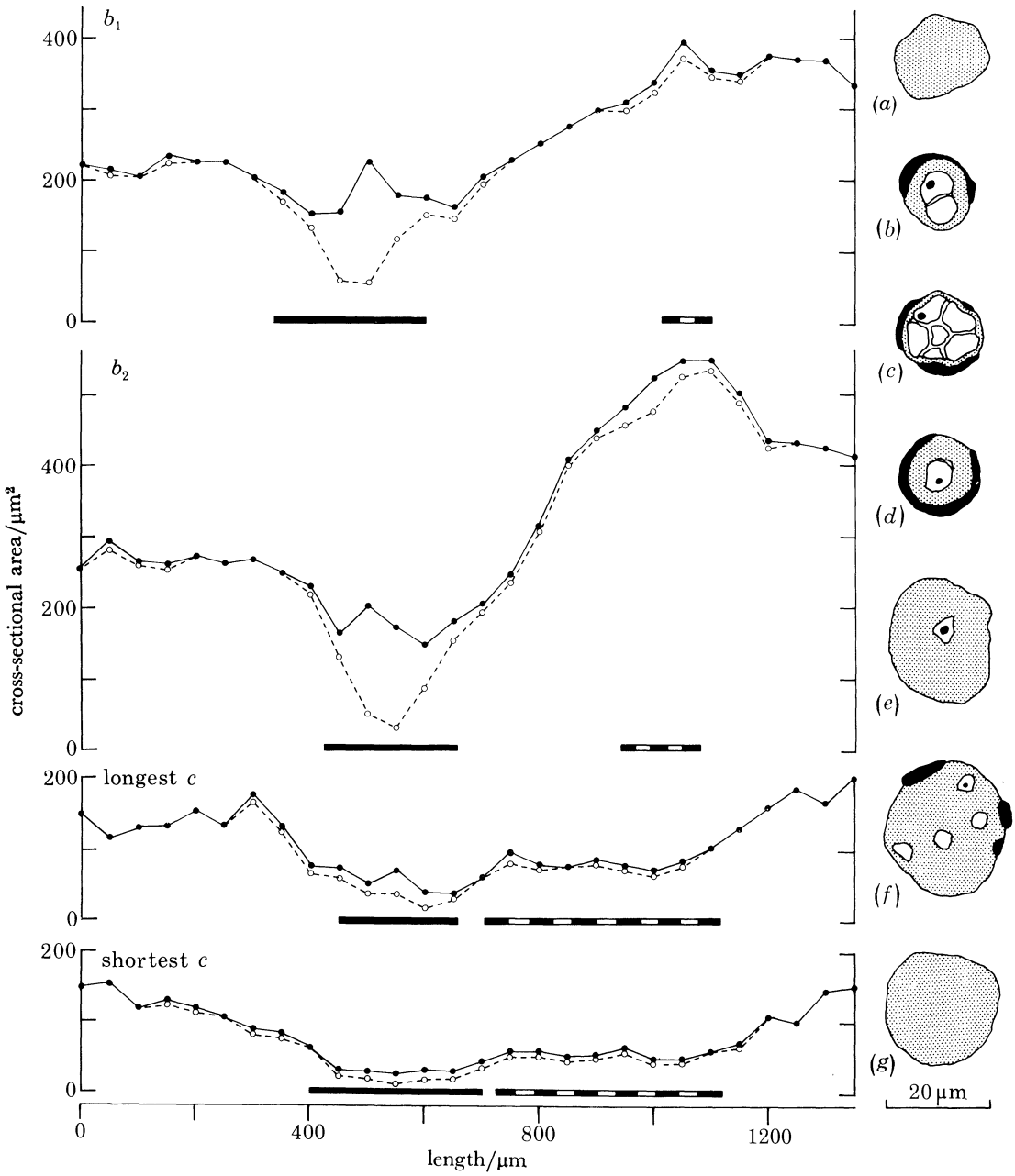


FIGURE 3. Graphs illustrating the cross-sectional areas of the bag fibres (b_1, b_2) and longest and shortest chain (c) fibres of spindle 6 during their course through the equatorial region. Areas plotted at 50 μm intervals; filled circles indicate total area of muscle fibre, unfilled circles the area occupied by myofibrils. Solid bar underneath each graph indicates length of fibre occupied by primary-ending terminals, broken bar indicates the length occupied by secondary-ending terminals.

(a)–(g) Cross-sectional profiles of b_2 fibre based on sections selected to represent condition at various levels during the course of the fibre through the equatorial region, as follows: (a) distal equatorial limit of periaxial space; (b) transition between distal myotube region and nuclear bag; (c) middle of nuclear bag; (d) proximal myotube region; (e) region between primary and secondary terminals; (f) region of secondary innervation; (g) proximal equatorial limit of periaxial space. Myofibrils indicated by dots; myonuclei as hollow profiles, some with nucleoli; sensory terminals as peripheral black shapes.

of their staggered arrangement in the ending, each is centred on the equator. In addition each bag fibre is repeated alongside to show its myonucleation. There was a close association between nucleation and innervation, but no constant relation between number of myonuclei and terminal contact area.

The terminals on each muscle fibre may be regarded as being arranged in an essentially helical fashion, with the pitch progressively increasing towards each end. Various modifications are superimposed upon this basic pattern, such as branches to form a double helix, fusion of adjacent turns, and longitudinal anastomoses, which may themselves bear lateral branches. The irregularities produced by these factors were minimal among the terminals on *c* fibres and maximal among those on the b_1 fibre. Thus regular spirals of up to four complete turns occurred among the *c* fibre terminals in both primary endings, whereas the longest stretch of regular spiral among the b_1 terminals in spindle 6 was one and a half turns around one end of the nuclear bag.

Description of figures 4–6

These figures illustrate the reconstructions of the primary and secondary endings described in the text. They should be examined with reference to figures 1–3, 7 and 8, which illustrate information abstracted from them, e.g. the branching of the I a axon supplying spindle 12, shown as reconstructed in figure 4, is shown schematically in figure 1; the primary terminals supplied to the bag figures of spindle 6, shown as reconstructed in figure 5, are schematically represented in figure 7; and so on.

The main conventions of line thickness and shading used are as follows. The *standard line* is that used to indicate the sarcolemma of the intrafusal muscle fibres. A *thick line* delineates internodal myelin and sensory terminals located on the near side of muscle fibres. A *thin line* indicates the outline of structures seen through other structures, e.g. most myonuclei. Preterminal axons and terminals have *graded shading* when located on the near side of muscle fibres, *even shading* when located on their far side.

Nuclear-bag nuclei are represented by their nucleoli only.

Figure 4. Isometric reconstruction of the primary ending of spindle 12.

(*a*) The terminals on the chain (*c*) fibres are seen to be interconnected by sensory cross-terminals (s.c.t.) and to form two separate systems each supplied by one or two short preterminal axons. One *c* fibre in the lower group is almost completely obscured by two others. Some terminals belonging to the adjacent S_1 secondary ending are shown in outline at the end on the right.

(*b*) The bag_1 (b_1) and bag_2 (b_2) terminal systems are each supplied by two preterminal axons. Note dense innervation of b_1 fibre. The gap between the bag fibres was occupied by the *c* fibres.

(*c*), (*d*) First-order branches of I a axon supplying (*c*) the b_2 and *c* fibres, and (*d*) the b_1 fibre.

Symbols: n.R., node of Ranvier; pt.a., preterminal axon; sat.c., satellite cell; sbs.n., sub-sarcolemmal nucleus; Sch.c.n., Schwann cell nucleus.

Figure 5. Isometric reconstruction of the primary ending of spindle 6. Compare with figure 4.

(*a*) Terminals on the *c* fibres; they are interconnected to form a single system supplied by two preterminal axons.

(*b*) Terminals on the bag fibres. The b_1 terminal system is supplied by three preterminal axons, two of which subdivide close to their heminodes, whereas the b_2 terminal system is supplied by two preterminal axons derived from a single heminode.

(*c*) First-order branches of the Ia axons and their subdivisions that lie in front of the muscle fibres shown in relation to the outline of the intrafusal bundle.

Symbols: b_1 br., b_2c br., first-order branches of I a axon supplying, respectively, the b_1 fibre and the b_2 and *c* fibres.

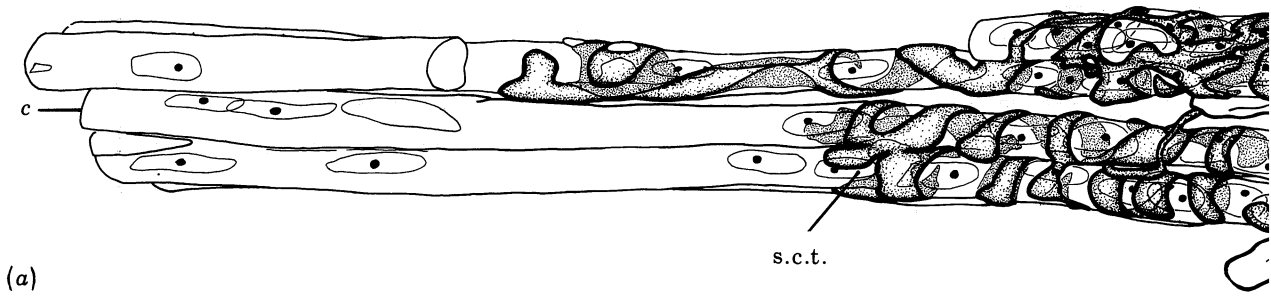
Figure 6. Isometric reconstruction of the S_1 secondary ending of spindle 6. The left end of the intrafusal bundle is continuous with the right end of that shown in figure 5.

(*a*) Terminals on the *c* fibres.

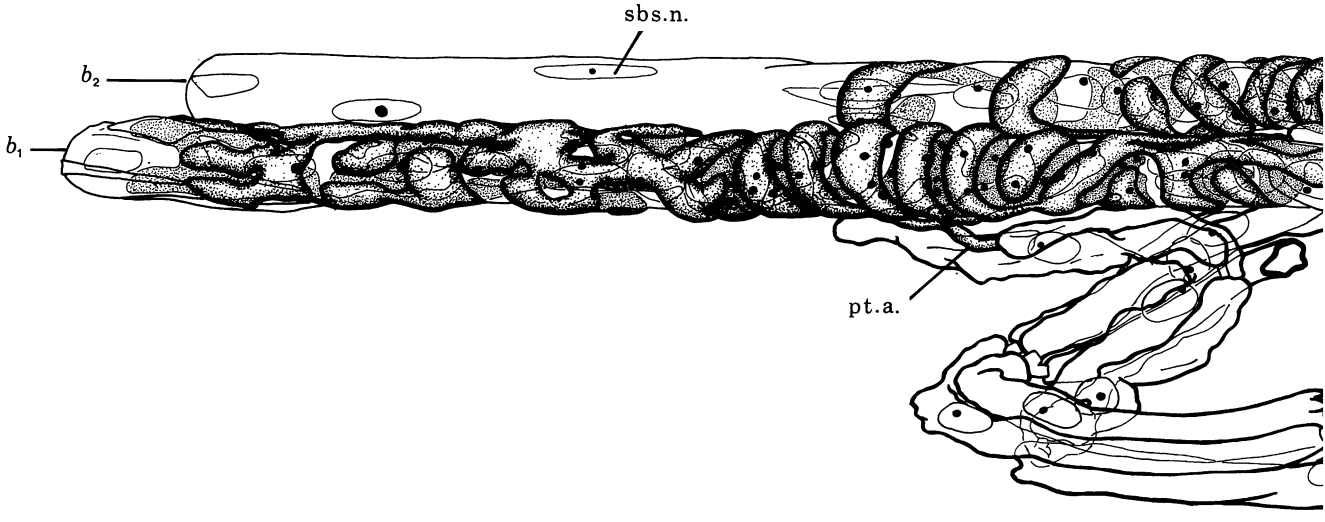
(*b*) Terminals on the bag figures.

(*c*) Part of the II axon and branches that lie in front of the muscle fibres shown in relation to the outline of the intrafusal bundle.

Note that the preterminal axons (pt.a.), as compared with those in primary endings, arise from nodes as well as heminodes and are relatively longer and more abundant.



(*a*)



(*b*)

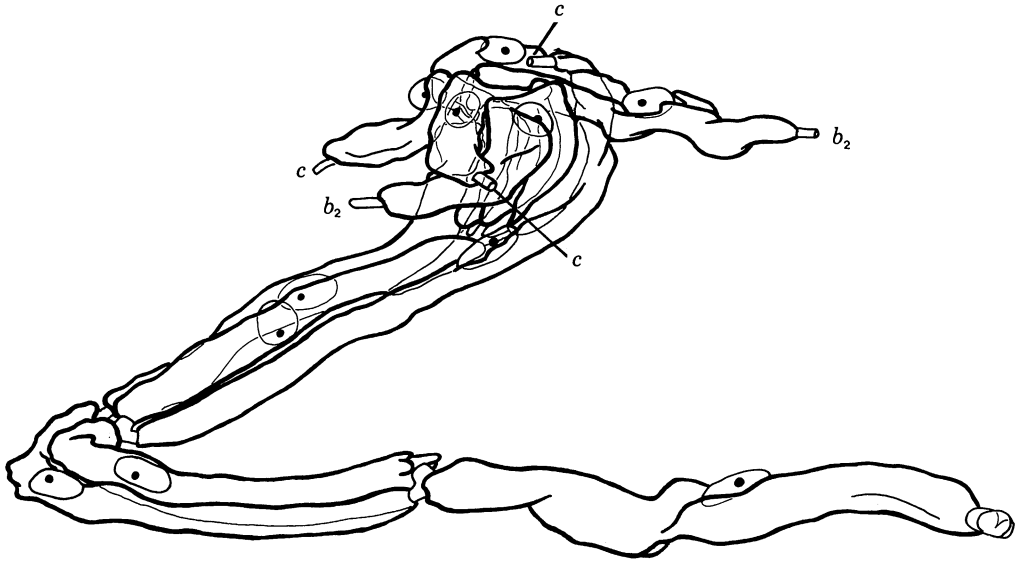


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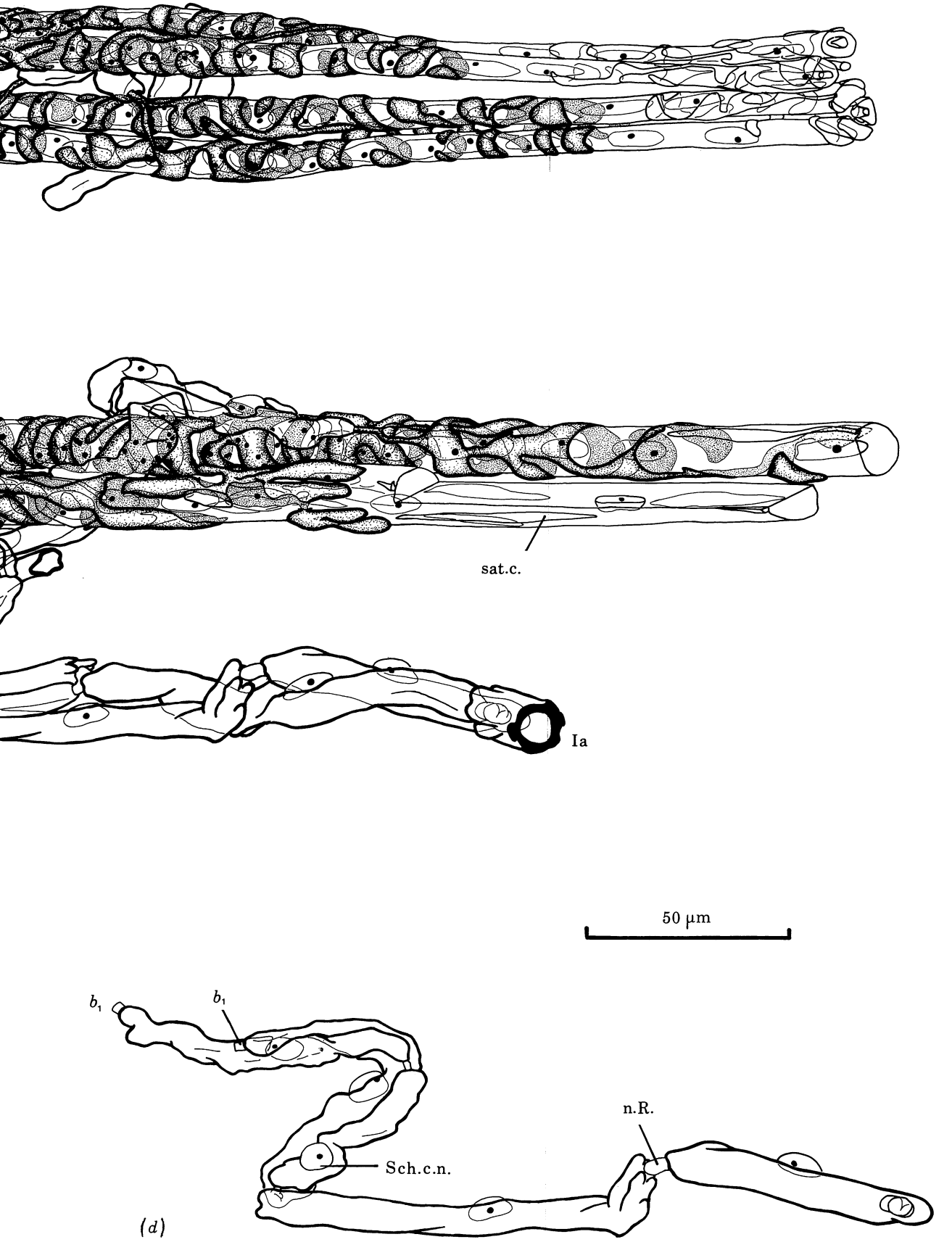
FIGURE 4. Fo



GURE 4. For description see opposite.

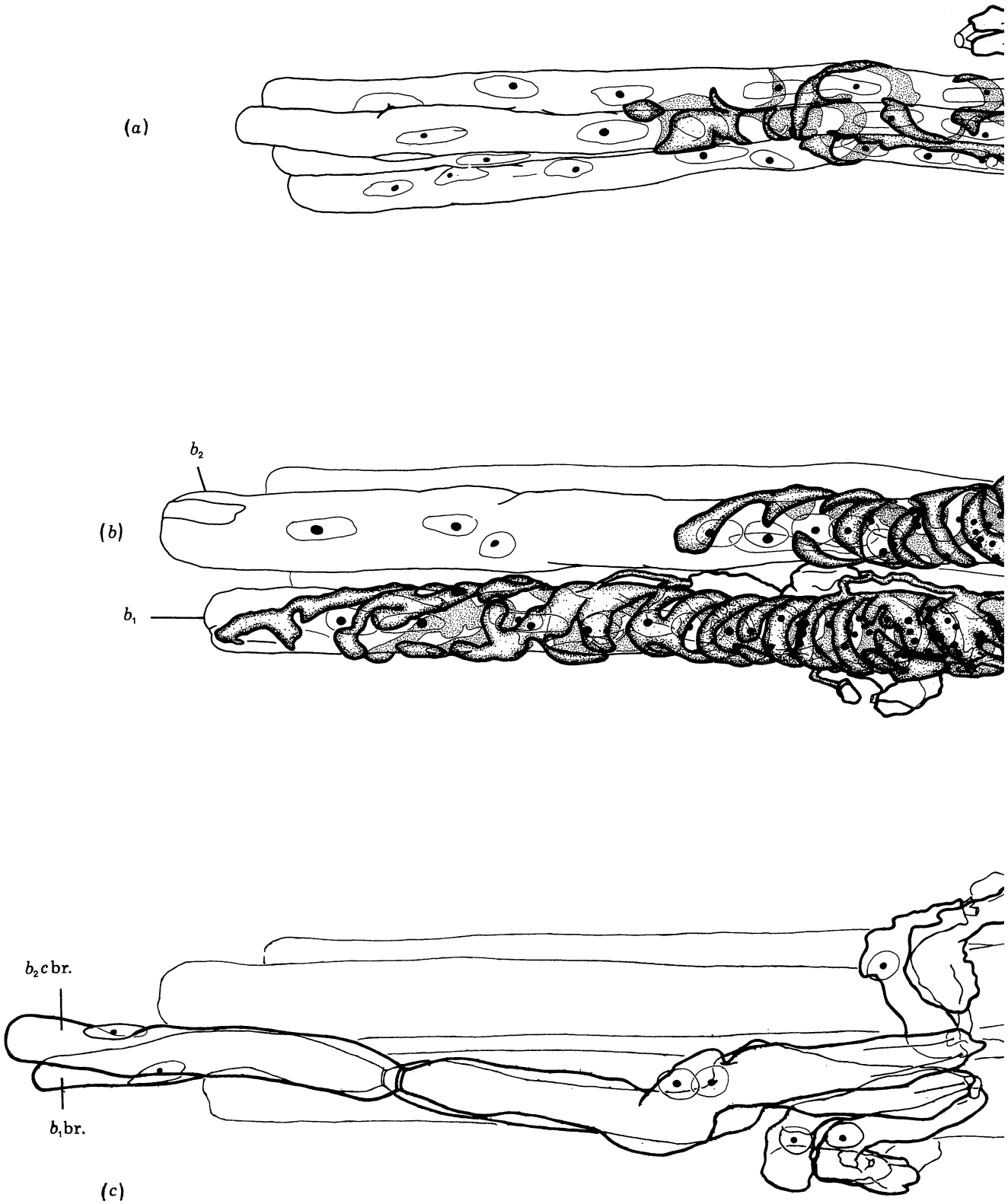
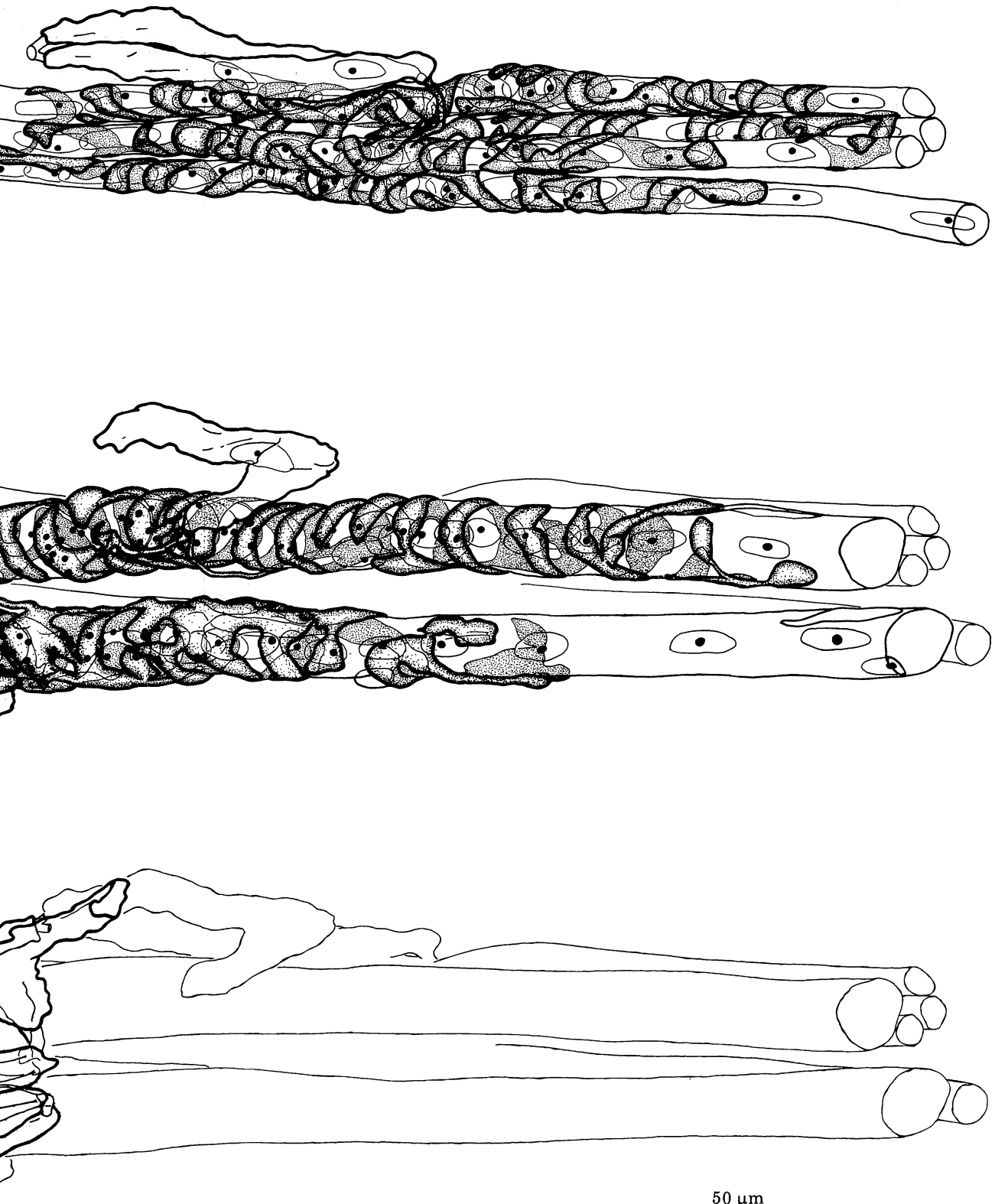
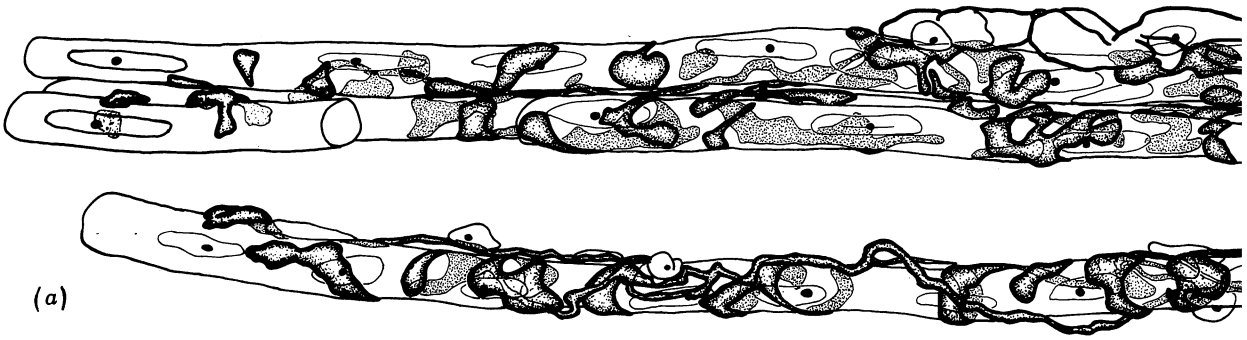


FIGURE 5. For descrip



For description see page 342.



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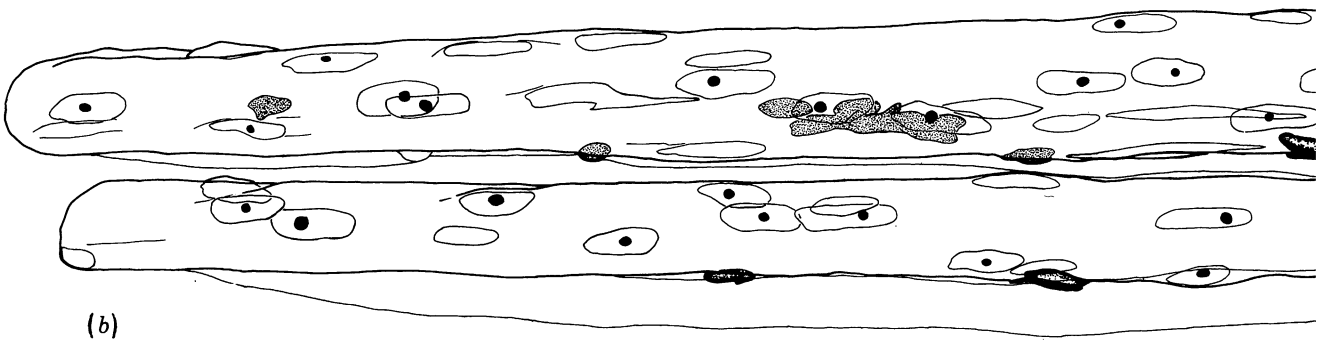

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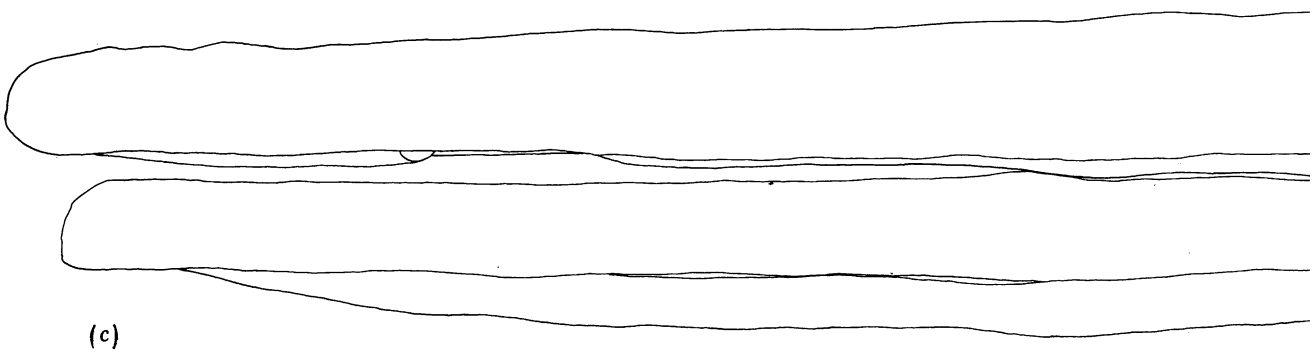

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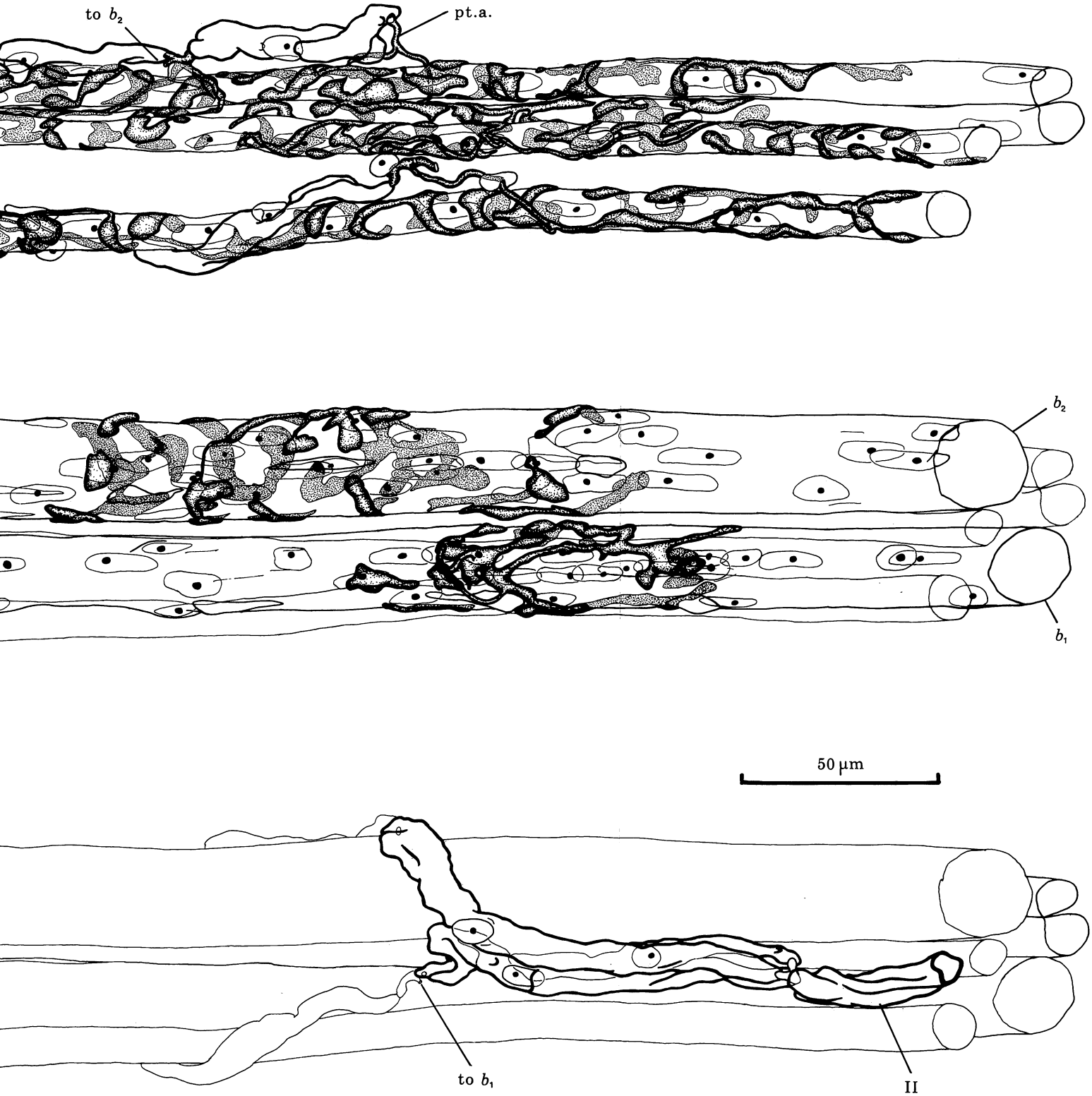

FIGURE 6. For description see page 342.

FIGURE 7. Schematic representation of parts of the reconstructed primary ending of spindle 6 shown in figure 5. The terminals shown are those supplied to the bag_1 (b_1) and bag_2 (b_2) fibres and the longest and shortest chain (c) fibres (numbered 1 and 4, as in table 2). In addition each fibre is repeated alongside to show its myonucleation and thus demonstrate the relation between nucleation and innervation. Adjustments have been made to the original alignment of each muscle fibre relative to the others, mainly in order to position the centre of each nuclear bag on a common midline and thus facilitate comparison between b_1 and b_2 terminal systems. Terminals shown in outline at bottom end of $c4$ belong to adjacent S_1 secondary ending. Asterisks alongside c terminals indicate positions of sensory cross-terminals with other c fibres.

FIGURE 8. For description see opposite.

Cross-terminals occurred frequently in both primary endings and were formed exclusively among *c* fibres. There was a tendency for the shortest *c* fibres to be involved with the greatest number of cross-terminals. The interconnections were usually made between two fibres, occasionally three. They resulted in the terminals on all four *c* fibres being interconnected in spindle 6, and those in spindle 12 being interconnected among one group of two fibres and another of three. This made it possible for the chain-fibre terminals to be distributed by fewer preterminal axons than were bag-fibre terminals. Only two such axons distributed terminals to the *c* fibres in spindle 6, as compared with seven supplying the bag fibres (five b_1, two b_2). The preterminal axons measured 1–2 μm in diameter and were of much smaller cross-sectional area than either the terminals they supplied or the myelinated axons from which they were derived. They connected with the terminals somewhere within the middle third of the ending on each bag fibre and on each group of *c* fibres.

Examination of the 1 μm thick serial transverse sections used in reconstruction revealed the presence of darkly stained granules located mostly towards the ends of the terminal systems supplied to each muscle fibre. To identify the nature of these granules we cut serial longitudinal sections of the equatorial and juxtaequatorial regions of spindle 5, including some ultrathin sections for study with electron microscopy (see plate 2). These confirmed the presence and distribution of the granules, which, under high magnification, proved to be osmiophilic bodies with a membranous organization and a range of electron densities. We concluded that they represented mitochondria in various stages of degeneration (cf. Hudson & Hartmann 1961), since the least dense, most highly organized bodies closely resembled nearby mitochondria (figure 17, plate 2).

(iv) *Reconstructed secondary ending*

The reconstruction of the S_1 secondary ending supplied to spindle 6 is shown in figure 6. As in the adjacent primary ending, the terminals were distributed to all three types of intrafusal muscle fibre, but they covered the fibres more sparsely and were spread over a greater length (445 μm as against 363 μm). The total area of contact made by the terminals was less (12639 μm^2 as against 18492 μm^2), but proportionately much more was distributed to the *c* fibres (75 % as against 39 %) (see table 3). Of the remainder, the b_2 fibre received about twice (17 %) that received by the b_1 fibre (8 %). The close association between myonuclei and innervation was especially marked in the region of the bag-fibre terminals (see figure 8).

The terminals clasped and partially encircled the fibres in an irregular array that lacked spirals. Many terminals appeared to be completely isolated; presumably the strands connecting them to other parts of the ending were so thin as to escape detection with the light microscope. Cross-terminals occurred between *c* fibres, and also between bag (b_1 and b_2) fibres and *c* fibres. The shortest *c* fibre was involved with the greatest number of cross-terminals (see figure 8). The preterminal axons supplying the terminals arose from three heminodes and three penultimate

FIGURE 8. Schematic representation of parts of the reconstructed S_1 secondary ending of spindle 6, shown in figure 6. As in figure 7, the terminals shown are those supplied to the b_1, b_2, *c*1 and *c*4 fibres, and each fibre has been repeated alongside to show its myonucleation and demonstrate the relation between nucleation and innervation. Asterisks indicate positions of 11 sensory cross-terminals; 10 of these linked terminals on the *c*4 fibre to those on the b_1 fibre (1), b_2 fibre (5), and other *c* fibres (4), and there was one cross-terminal between the b_1 and *c*1 fibres.

nodes. As in the primary endings, these axons measured 1–2 μm in diameter, but they differed in occasionally extending for considerable distances (up to 200 μm).

(*b*) *Analysis of silver preparations*

(i) *Composition of the spindle sample*

The nature of the sensory innervation of the 310 spindles studied is shown in table 4. Most of the spindles were composed of three types of muscle fibre, but a small proportion (12.9%) lacked a b_1 fibre. It is convenient to refer to these as 'b_2c spindle units', as distinct from 'b_1b_2c spindle units'. The b_2c spindle units were all portions of tandem spindles in which the b_2 fibre was continuous from one capsule, where it was accompanied by b_1 and c fibres, to another, in which it was accompanied by c fibres only (usually two or three). In these units the equatorial nucleation of the b_2 fibre usually resembled that of a c fibre. After passing through an encapsulated sensory region, each unit formed a very short pole that usually inserted into tendon, leaving the excluded b_1 fibre to insert separately. In extensively sampled muscles the proportion of b_2c spindle units was 23.8% in extensor digitorum longus, 23% in peroneus brevis, and between 6 and 11% in the rest. In muscles less extensively sampled the highest proportions of b_2c units were 28.6% in extensor digitorum brevis and 27.3% in flexor hallucis longus (see table 4).

Two-hundred-and-seventy b_1b_2c spindle units received a total of 282 Ia and 440 II axons, a Ia:II ratio of 1:1.6. Twelve of these spindles (4.4%) had primary endings formed by two Ia axons (double primaries). Secondary endings (total 444) were usually located on both sides of the primary ending when two or more were present. The majority (70.7%) terminated in the S_1 position; the rest terminated in the S_2 (23.4%), S_3 (5.0%), S_4 (0.7%) and S_5 (0.2%) positions. When several secondaries were distributed to a pole their positional arrangement was

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Description of plate 1

Photographs of some of the transverse sections used in reconstructing the sensory region of spindle 6. The distances between the sections selected for illustration in figures 9–14 are, respectively, 123, 6, 0, 39 and 466 μm. The two large muscle fibres in each section are nuclear-bag fibres (bag$_1$ (b_1) left; bag$_2$ (b_2) right), the four smaller ones nuclear-chain fibres (c). Epon sections, 1 μm thick, stained with toluidine blue. Abbreviations: ax.sh.n., axial sheath nucleus; cap., capsule; p.s., periaxial space; pt.a., preterminal axon; s.c.t., sensory cross-terminal; s.t., sensory terminal.

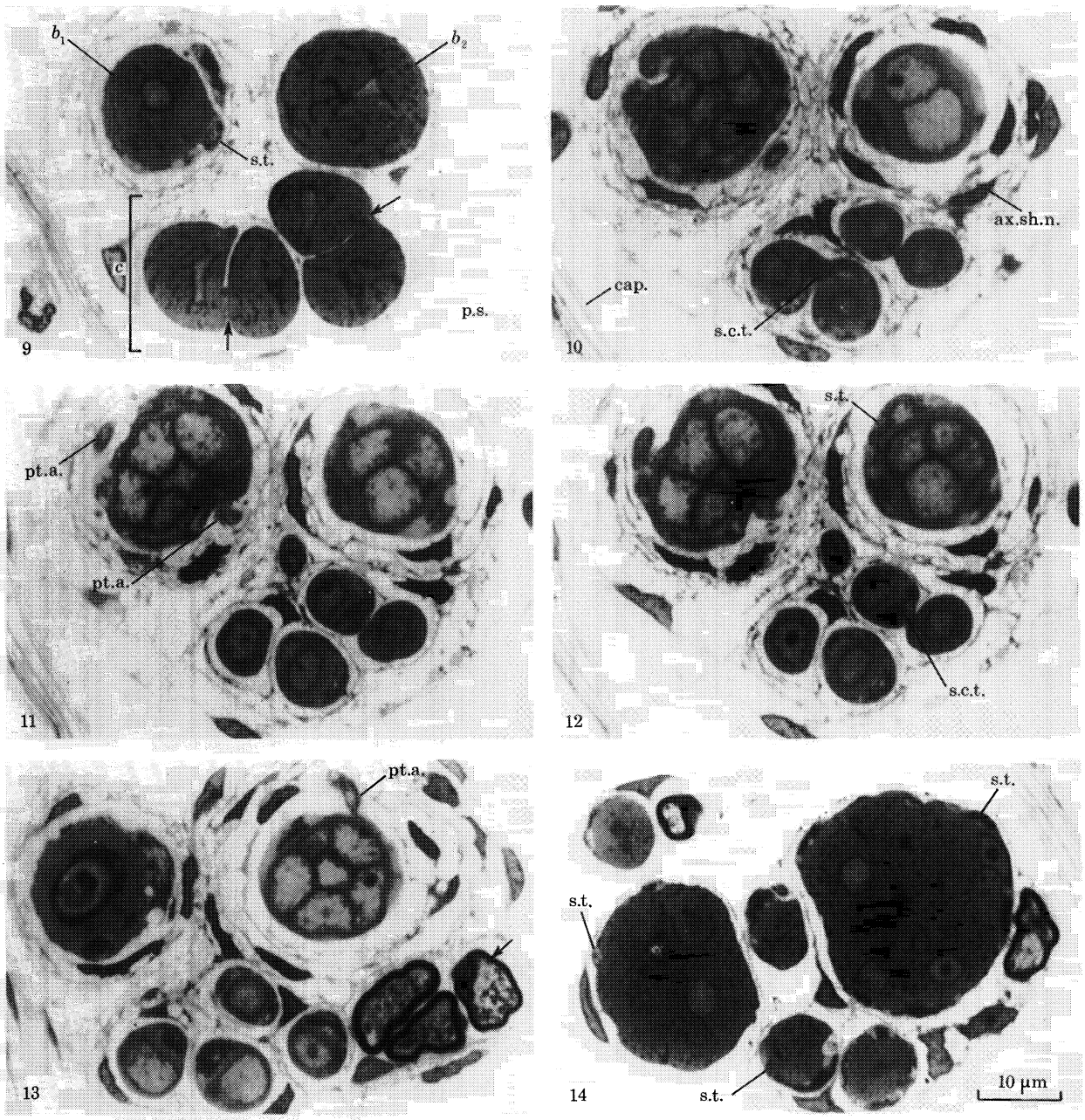
Figure 9. Section through distal myotube of the b_1 fibre, here dissociated from the b_2 and c fibres. The c fibres are joined together as two pairs by regions of close apposition (arrows) such as described by Corvaja *et al.* (1967). Primary sensory terminals present at this level only on b_1 fibre.

Figure 10. Section through nuclear bag of the b_1 fibre, distal myotube of the b_2 fibre, and nuclear chains of the c fibres. The characteristic euchromatic nature of the bag and chain myonuclei is evident in this and the next three figures. Note prominent sensory cross-terminal between two of the c fibres.

Figures 11 and 12. Adjacent sections through the nuclear bags of both bag fibres. Two preterminal axons close to the b_1 fibre in figure 11 can be seen contributing primary sensory terminals to it in figure 12. The two sections also show the start of a sensory cross-terminal between a pair of c fibres.

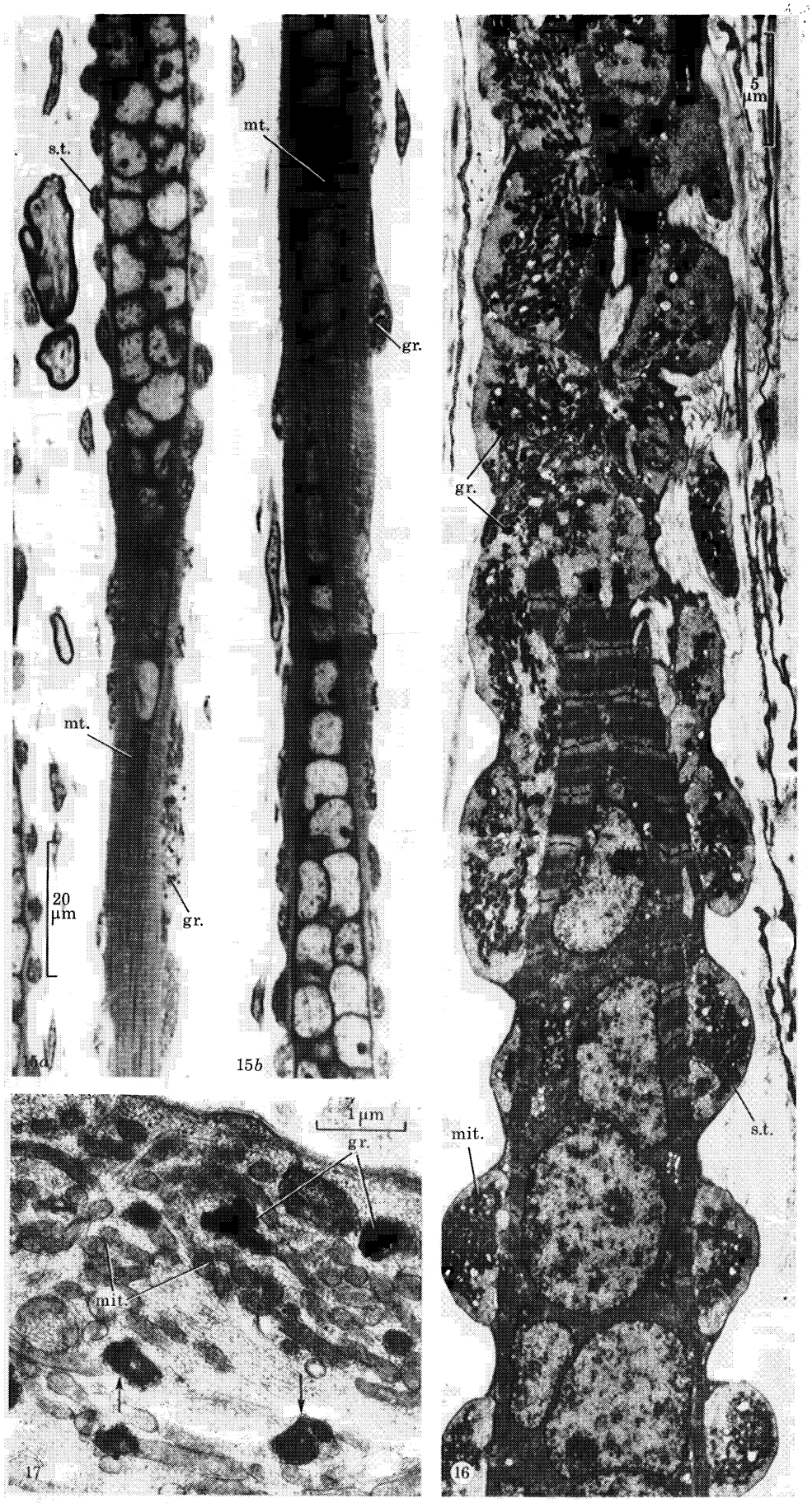
Figure 13. Section through proximal myotube of the b_1 fibre and nuclear bag of the b_2 fibre. The preterminal axon seen contributing a primary sensory terminal to the b_2 fibre was derived from the heminode of the myelinated Ia axon branch arrowed at bottom right. The other two myelinated branches supplied terminals to the c fibres.

Figure 14. Section through S_1 secondary ending in a region where terminals are present on b_1, b_2 and c fibres. Note nucleation and relatively large diameters of the bag fibres.



FIGURES 9–14. For description see opposite.

(*Facing p.* 344)



FIGURES 15–17. For description see opposite.

TABLE 3. AREAS AND DISTRIBUTION OF SENSORY ENDINGS IN TWO CAT TENUISSIMUS SPINDLES

(Symbols: b_1, bag$_1$ fibre; b_2, bag$_2$ fibre; c, chain fibre. Chain fibres numbered in order of decreasing length.)

		terminal area µm²		area of fibre surface covered (%)	proportion of ending on fibre (%)	
primary ending (spindle 12)	b_1	6154		59	33	
	b_2	4738		49	25	
	$c1$	1364	7869	40	7	42
	$c2$	2097		45	11	
	$c3$	1772		45	9	
	$c4$	1617		41	9	
	$c5$	1019		43	5	
primary ending (spindle 6)	b_1	6842		51	37	
	b_2	4391		41	24	
	$c1$	1617	7259	33	9	39
	$c2$	2267		41	12	
	$c3$	1417		26	8	
	$c4$	1959		38	11	
S_1 secondary ending (spindle 6)	b_1	974		16	8	
	b_2	2205		16	17	
	$c1$	2775	9460	28	22	75
	$c2$	2554		30	20	
	$c3$	2082		26	16	
	$c4$	2049		27	16	

normally sequential starting with S_1, but one spindle received a single secondary located in the S_2 position. Spindles were most frequently supplied with one primary and one secondary ending (PS_1, 30.0%) or a primary ending only (P, 25.2%; 42 among 270 b_1b_2c units, 36 among 40 b_2c units).

(ii) *Branching and distribution of primary (Ia) axons*

Examination of the branching undergone by 182 Ia axons before terminating showed that they either remained unbranched (3.3%) or divided to produce two (84.0%), three (11.0%) or four (1.7%) first-order branches. The axons examined included 39 supplied to b_2c spindle

DESCRIPTION OF PLATE 2

Longitudinal sections through the primary sensory region of the b_1 fibre of spindle 5 illustrating nucleation, innervation, and nature of granules located in parts of the terminal system. Abbreviations: gr., granules; mit., mitochondria; mt., myotube; s.t., sensory terminal.

FIGURE 15. Section through the entire length of the primary terminal system on the b_1 fibre. The micrograph has been divided into two parts close to the middle of the nuclear bag. Terminals forming the regular, middle portion of the system are seen as profiles on each side of the bag, whereas those forming the irregular portions at each end are seen as profiles associated with the myotube regions. These terminals contain densely stained granules that do not occur in those forming the middle portion of the system. Epon section 1 µm thick, stained with toluidine blue.

FIGURE 16. Electron micrograph of the b_1 fibre including one end of the nuclear bag and part of one myotube region; the myotube itself lies largely outside the plane of section. Note abundance of mitochondria that occur in the sensory terminals. Osmiophilic granules, presumed to correspond with those shown in figure 15, occur in the irregular portion of the primary terminal system associated with the myotube region.

FIGURE 17. Electron micrograph of part of the irregular portion of the primary terminal system, showing detail of granules, which appear to represent stages (arrows) in the degeneration of mitochondria.

units and eight involved in contributing terminals to two fibre types in double primary endings (see table 5).

The distribution of first-order branches to b_1, b_2, and c fibres was ascertained for 132 Ia axons and gave the results shown in table 6. Among tenuissimus axons, the first-order branches of 73% (24 of 33) had a segregated distribution, i.e. were exclusively supplied either to b_1 or to b_2 and/or c fibres, thereby resulting in separation of dynamic and static inputs. By contrast, the

TABLE 4. SILVER ANALYSIS: SENSORY INNERVATION OF SPINDLE SAMPLE

(Symbols and abbreviations: b_1, b_2, c, bag_1, bag_2, chain fibres; P, primary ending; S, secondary ending; ten., tenuissimus; p.b., p.l., p.t., peroneus brevis, p. longus, p. tertius; s. and d.lum., superficial and deep lumbricals; sol., soleus; e.d.l., e.d.b., extensor digitorum longus and brevis; f.h.l., flexor hallucis longus; t.a., t.p., tibialis anterior and posterior; p. int., pes interosseus; r.f., rectus femoris.)

sensory innervation	muscles sampled														
b_1b_2c spindle units	ten.	p.b.	p.l.	p.t.	s.lum.	d.lum.	sol.	e.d.l.	e.d.b.	f.h.l.	t.a.	t.p.	p.int.	r.f.	total
P	10	4	—	6	10	1	6	1	1	2	1	—	—	—	42
PS_1	15	21	8	5	11	4	9	5	1	2	3	1	4	—	89
PS_1S_2	12	6	2	3	4	—	3	3	1	—	1	—	1	—	36
P–S_2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
$PS_1S_2S_3$	6	—	—	1	1	—	1	1	—	—	1	—	—	—	11
$PS_1S_2S_3S_4$	—	—	1	—	—	—	—	—	—	—	1	—	—	—	2
$PS_1S_2S_3S_4S_5$	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
S_1PS_1	9	6	3	6	3	2	7	2	1	1	—	—	—	—	40
$S_1PS_1S_2$	5	6	2	2	3	—	2	1	1	3	2	—	—	—	27
$S_1PS_1S_2S_3$	1	—	1	—	—	—	—	—	—	—	—	—	—	—	2
$S_2S_1PS_1S_2$	2	—	—	—	—	—	1	1	—	—	1	—	—	—	5
$S_2S_1PS_1S_2S_3$	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
$S_3S_2S_1PS_1S_2S_3$	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1
PPS_1	1	—	—	—	—	—	—	1	—	—	—	—	—	—	2
PPS_1S_2	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
$PPS_1S_2S_3$	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2
S_1PPS_1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
$S_1PPS_1S_2$	1	2	1	—	—	—	—	1	—	—	—	—	—	—	5
$S_2S_1PPS_1S_2S_3$	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
b_2c spindle units															
P	4	13	2	3	2	1	2	4	1	3	1	—	—	—	36
PS_1	1	1	—	—	—	—	—	1	1	—	—	—	—	—	4
total . . .	70	61	20	27	34	8	31	21	7	11	13	1	5	1	310

first-order branches of 83% (19 of 23) of Ia axons supplying superficial lumbrical spindles had a mixed distribution, and such distributions were only slightly less frequent than segregated ones among the samples of Ia axons examined from peroneus brevis, p. tertius and soleus muscles. The most common type of mixed distribution was that in which the b_1 fibre derived its terminals from the same first-order branch as one or two c fibres. Mixing was usually restricted to the dynamic input. This was so in 74.6% (44 of 59) of Ia axons in a sample where all had two first-order branches; mixing was restricted to the static input in 11.9%, and affected both inputs in 13.5%.

The distribution of Ia first-order branches was ascertained in eight double primary endings. Six of these were formed by one Ia axon supplying terminals to b_1, b_2 and c fibres in combination with another whose distribution was restricted to two fibre types, i.e. to b_1 and b_2 fibres

(2), b_2 and c fibres (2), or b_1 and c fibres (2). In one double primary each Ia axon supplied all three fibre types, and in another b_1 and c fibres were supplied by one Ia axon, b_2 and c fibres by the other.

(iii) *Branching and distribution of secondary (II) axons*

Branching was examined among 272 secondary axons, i.e. 201 S_1, 58 S_2, 11 S_3, one S_4, and one S_5. The proportion remaining unbranched was 17.7%; the rest divided to produce two (74.6%), three (7.0%) or four (0.7%) first-order branches. Unbranched axons occurred less

TABLE 5. BRANCHING AND DISTRIBUTION OF PRIMARY AFFERENTS SUPPLYING CAT HINDLIMB MUSCLES

(Abbreviations as in table 4.)

distribution to fibre types	number of first-order branches	afferents innervating ten.	s.lum.	p.b.	p.l.	p.t.	sol.	others†	total
				b_1b_2c spindle units: single primaries					
b_1b_2c	0	—	—	1	—	1	1	—	3
	2	24	16	24	4	10	16	10	104
	3	6	4	2	1	1	1	2	17
	4	—	3	—	—	—	—	—	3
				b_1b_2c spindle units: double primaries					
b_2c	2	3	—	—	—	—	—	—	3
b_1c	2	2	—	—	—	—	—	1	3
b_1b_2	2	—	—	—	1	—	—	1	2
b_1b_2c	2	2	—	—	1	—	—	3	6
	3	1	—	—	—	—	—	1	2
				b_2c spindle units					
b_2c	0	—	—	2	—	—	—	1	3
	2	5	2	12	2	3	1	10	35
	3	—	—	—	—	—	1	—	1
	total ...	43	25	41	9	15	20	29	182

† Ia afferents innervating d.lum. (4), e.d.l. (9), e.d.b. (2), f.h.l. (4), t.a. (6), p.int. (2), r.f. (2).

frequently among S_1 secondary axons (12.2% unbranched) than among those terminating in more polar positions (S_2, 29.3% unbranched; S_3, 36.4%; S_4 and S_5 axons both unbranched). One secondary axon in soleus branched at spindle entry and supplied two secondary endings, one on each side of the primary in the S_1 position.

The preterminal axons that supplied secondary terminals were derived either exclusively from heminodes (as in primary endings) or from both heminodes and penultimate nodes. The latter were usually those distal to the first branching node, but this node itself, as well as that immediately proximal to it, were also occasionally involved. Secondary endings supplied exclusively from heminodes were more frequent in the S_1 position than in more polar positions. For example, among 65 S_1 endings, 42 (64.6%) were supplied exclusively from heminodes, whereas this was so in only six of 15 S_2 endings (40.0%). Generally speaking, the more polar the position of a secondary the more likely it was for the terminals to be partly derived from penultimate nodes, and for the parent axon to be unbranched.

Table 7 correlates the position of 351 secondary endings with their distribution to the three

types of intrafusal muscle fibre. The most common secondary (59.0%) was the S_1 ending distributed to b_1, b_2 and c fibres, and this kind of distribution was the most common (67.8%) in the sample as a whole. Restriction of terminals to one or two fibre types was more prevalent among secondaries terminating in the more polar positions. Thus the terminals of 68.4% of S_2 and 60.0% of S_3 endings were restricted to one or two fibre types as against only 18.2% S_1 endings.

TABLE 6. DISTRIBUTION OF FIRST-ORDER BRANCHES OF PRIMARY AFFERENTS SUPPLYING CAT HINDLIMB MUSCLES

(Symbols: b_1, bag_1 fibre; b_2, bag_2 fibre; c, chain fibre(s). Abbreviations of muscles as in table 4.)

type of distribution	number of first-order branches	distribution to muscle fibres mediating dynamic (*D*) and static (*S*) responses		ten.	s.lum.	p.b.	p.t.	sol.	others†	total
segregated	2	*D*:*S*	b_1:b_2c	19	—	14	5	8	5	51
	3	*D*:*S*:*S*	b_1:b_2 :c	3	—	—	—	1	—	4
			b_1:b_2c:b_2	1	—	—	—	—	—	1
			b_1:b_2c:c	—	—	—	1	—	1	2
			b_1:b_2c:b_2c	—	1	—	—	—	—	1
		D:*D*:*S*	b_1:b_1 :b_2c	1	—	1	—	—	—	2
	4	*D*:*D*:*S*:*S*	b_1:b_1 :b_2c:c	—	1	—	—	—	—	1
			b_1:b_1 :b_2 :c	—	1	—	—	—	—	1
			subtotal . . .	24	4	15	6	9	6	64
mixed	2	*DS*:*S*	b_1b_2 :c	—	—	1	—	1	—	2
			b_1b_2 :b_2c	—	1	1	—	—	—	2
			b_1c :b_2	1	—	1	—	—	—	2
			b_1c :b_2c	4	6	1	1	2	3	17
			b_1b_2c :b_2	—	—	1	1	—	—	2
			b_1b_2c :c	1	3	1	—	2	6	13
			b_1b_2c :b_2c	—	1	2	—	2	1	6
		D:*DS*	b_1 :b_1b_2c	1	2	2	—	—	2	7
		DS:*DS*	b_1b_2 :b_1b_2c	—	1	—	—	—	1	2
			b_1c :b_1b_2	—	—	—	—	1	—	1
			b_1c :b_1b_2c	—	1	—	3	—	—	4
			b_1b_2c :b_1b_2c	—	1	—	—	—	—	1
	3	*D*:*DS*:*S*	b_1:b_1c:b_2	1	—	—	—	—	—	1
			b_1:b_1c:b_2c	—	1	1	—	—	—	2
		DS:*S*:*S*	b_1c :b_2c:c	1	2	—	—	—	—	3
			b_1c :b_2c:b_2c	—	—	—	—	—	1	1
			b_1c :b_2c:b_2	—	—	—	—	—	1	1
			b_1b_2 :c :c	—	—	—	—	—	1	1
			subtotal . . .	9	19	11	5	8	16	68
		total . . .		33	23	26	11	17	22	132
		percentage segregated . . .		73	17	58	55	53	27	48.5
		percentage mixed . . .		27	83	42	45	47	73	51.5

† Ia afferents innervating p.l. (6), d.lum. (4), e.d.l. (3), f.h.l. (1), t.a. (4), p.int. (2), r.f. (2).

A sample of 44 S_1 secondary axons innervating b_1, b_2 and c fibres was examined to ascertain the distribution of first-order branches to the three fibre types. The sample comprised axons from tenuissimus (19), soleus (11), peroneus brevis (6), p. tertius (2) and lumbrical (6) muscles, and all had two first-order branches. A segregated distribution, in which one branch supplied the b_1 fibre while the other supplied b_2 and c fibres, was infrequent in all muscles and occurred

in only 22.7 % of the sample. In mixed distributions the usual condition (61.8 %) was for one first-order branch to supply the b_1 fibre together with c fibres, or b_2 and c fibres, and for the other to supply b_2 and c fibres.

(iv) *Diameters of spindle afferents*

Measurements of the intramuscular diameters of 259 Ia and 357 II axons showed that on average Ia axons were about twice as thick as II axons (mean diameters: Ia, 7.1 μm; II, 3.6 μm) (see table 8). The diameters of Ia axons supplying b_2c spindle units were generally

TABLE 7. SECONDARY ENDINGS: CORRELATION OF POSITION WITH DISTRIBUTION TO FIBRE TYPES

(Abbreviations as in table 4.)

position of ending	distribution to fibre types				total
	b_1b_2c	b_1c	b_2c	c	
S_1	207	7	38	1	253
S_2	25	9	31	14	79
S_3	6	1	3	5	15
S_4	—	1	1	1	3
S_5	—	—	—	1	1
total . . .	238	18	73	22	351
percentage . . .	67.8	5.1	20.8	6.3	

thinner than those supplying b_1b_2c spindle units. Thus the mean diameter of 37 b_2c Ia axons was 5.1 μm in a range of 2.2–9.0 μm (peak 4.0 μm), whereas 213 b_1b_2c Ia axons had a mean diameter of 7.5 μm in a range of 3.4–12.8 μm (peaks at 6.0 and 8.0 μm). There was no correlation between the diameter of b_1b_2c Ia axons and the presence, absence or number of secondary endings.

The diameter of II axons terminating as S_2–S_5 endings was generally less (mean 2.9 μm) than that of those terminating as S_1 endings (mean 3.9 μm). This decrease appeared to be related to polar position rather than to number of muscle-fibre types innervated. Thus among II axons distributed to all three fibre types the mean diameter of 196 terminating in the S_1 position was 3.8 μm as compared with a mean of 3.0 μm for 28 terminating in the S_2–S_5 positions. Similarly, among those II axons distributed to only one or two fibre types the mean diameter of 40 S_1 axons was 3.8 μm as against a mean of 2.8 μm for 65 S_2–S_5 axons.

The histograms in figure 18 compare the diameters of b_1b_2c Ia axons, firstly, with those of other Ia axons in the sample that supplied only two fibre types (figure 18*a*), and, secondly, with those of II axons that also supplied b_1, b_2 and c fibres (figure 18*b*). There is overlap in each case: the lower part of the b_1b_2c Ia diameter range includes all but one of the Ia axons supplied to two fibre types, and 75 % of the II axons supplied to three fibre types. However, in all three of the possible comparisons the differences between the mean values are significant (b_1b_2c Ia > b_2c Ia) or very highly significant (b_1b_2c Ia > b_1b_2c II; b_2c Ia > b_1b_2c II).

(v) *Primary endings*

The form of primary endings supplied to b_1b_2c spindle units was remarkably constant, the only major variation being that due to the presence of bag fibres additional to the normal complement of one of each type. In the total sample of 270 such spindles (see table 4) this

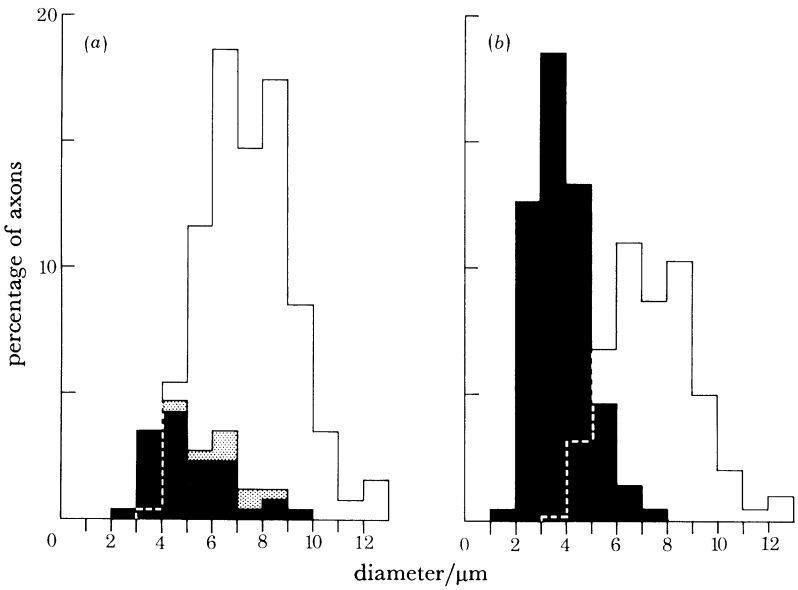


FIGURE 18. Histograms of fibre diameter for cat hindlimb spindle afferents as measured in teased, silver preparations.

(*a*) Comparison between the diameters of Ia axons supplying three types of intrafusal muscle fibre (unfilled columns; $n = 213$) and those supplying two. The latter group consisted of 37 Ia axons supplying b_2c spindle units (filled columns), and eight supplying two fibre types (stippled columns) as follows: seven to double primary endings (three b_2c, two b_1c, two b_1b_2) and one (b_1c) to a compound spindle.

(*b*) Comparison between the diameters of Ia axons supplying three types of intrafusal muscle fibre (unfilled columns; same sample as in (*a*)), and II axons that also supplied three types of intrafusal muscle fibre (filled columns; $n = 224$).

TABLE 8. INTRAMUSCULAR DIAMETERS OF SPINDLE AFFERENTS

(Abbreviations as in table 4.)

type of axon measured	number of axons measured													diameter/μm	
	ten.	p.b.	p.l.	p.t.	s.lum	d.lum	sol.	e.d.l.	e.d.b.	f.h.l.	t.a.	p.int.	total	range	mean
primary (Ia) supplying:															
(*a*) 3 fibre types															
b_1b_2c spindle units	51	37	11	17	28	7	23	12	5	7	10	5	213	3.4–12.8	7.5
(*b*) 2 fibre types															
b_2c spindle units	5	12	1	3	2	1	2	5	2	3	1	—	37	2.2–9.0	5.1
others†	5	—	1	—	—	—	—	1	—	—	2	—	9	4.6–8.1	6.5
total . . .	61	49	13	20	30	8	25	18	7	10	13	5	259	2.2–12.8	7.1
secondary (II) supplying:															
b_1b_2c spindle units															
S_1	69	40	21	23	24	8	27	14	4	4	15	4	253	1.7–7.7	3.9
S_2	28	15	5	4	6	—	7	5	—	2	9	—	81	1.4–4.6	2.9
S_3	6	1	2	1	—	—	1	1	—	—	3	—	15		
S_4	1	—	1	—	—	—	—	—	—	—	1	—	3	1.5–4.5	2.7
S_5	1	—	—	—	—	—	—	—	—	—	—	—	1		
b_2c spindle units															
S_1	1	1	—	—	—	—	—	1	1	—	—	—	4	2.6–4.3	3.1
total . . .	106	57	29	28	30	8	35	21	5	6	28	4	357	1.4–7.7	3.6

† Eight Ia axons contributing to double primary endings; one innervating b_1c fibres in an aberrant primary supplied to a compound spindle.

occurred in 32 (11.9%) and most frequently involved the duplication of the b_1 fibre: 28 spindles had two b_1 fibres, two had three, and two had two bag fibres of each type. In some spindles duplication of the b_1 fibre was accompanied by a reduction in the number of c fibres. In most of the muscles sampled the frequency of spindles with more than one b_1 fibre was below 10%, but in tibialis anterior it was 16.7% (two of 12), extensor digitorum longus 18.8% (three of 16), superficial lumbrical 28.1% (nine of 32) and pes interosseus 80.0% (four of five).

The mean length of 151 single primary endings was 359 μm in a range of 242–608 μm; the lengths of seven double primaries fell mostly in the upper part of this range (mean 466 μm). There was no consistent difference between the two types of bag fibre in the length occupied by primary terminals; the mean length innervated in b_1 fibres was 294 μm, in b_2 fibres 282 μm. There was also no significant difference between the maximum diameters of the b_1 and b_2 terminal systems; the mean of 134 b_1 measurements was 24.6 μm (s.e. 0.56 μm) as compared with a mean of 25.0 μm (s.e. 0.49 μm) for 120 b_2 measurements. According to Homma & Seki (1964) spindles in the fast tibialis anterior muscle have wider nuclear bags than those in the slow soleus, but our measurements did not reveal any significant difference.

The terminal systems supplied to bag fibres in the sample of 151 single primary endings consisted of a middle portion, in which the terminals were arranged mainly as regular transverse bands, and portions at each end, in which the terminals were disposed as irregular forms in mainly diagonal and longitudinal arrangements. The terminal systems supplied to b_1 and b_2 fibres were distinguished from each other by the following features.

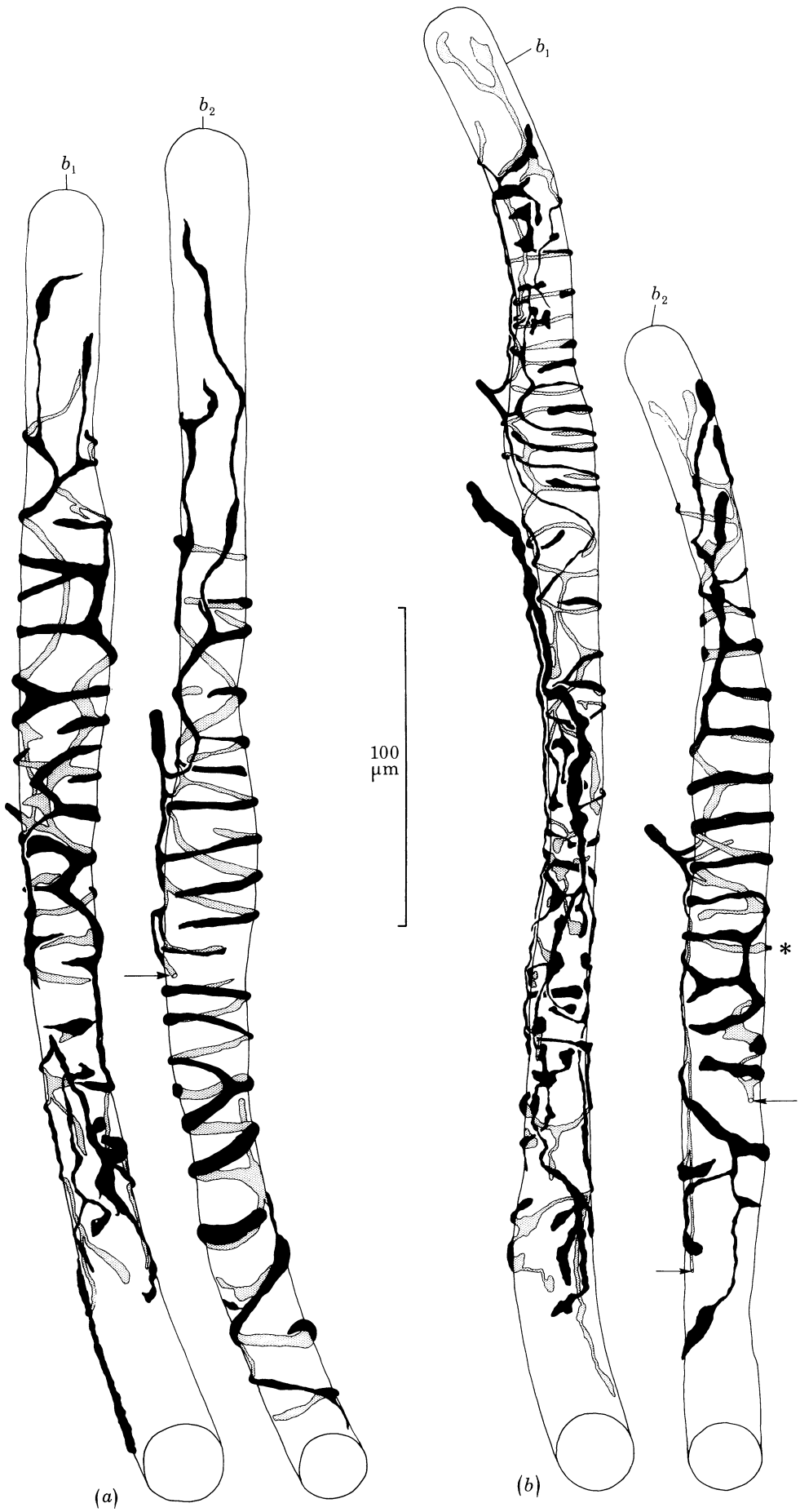
(i) Lack of an irregular portion at one end of the terminal system. This occurred frequently (43.4%) among b_2 systems, seldom (4.4%) among b_1 systems. In four endings the b_2 systems were entirely regular.

(ii) Relative proportions of regularity and irregularity in the two systems. Partly as a consequence of (i), the terminal systems supplied to b_2 fibres were mainly regular; on average their regular portions occupied 67% of their total length. By contrast, the irregular portions predominated in b_1 systems, occupying, on average, 57% of their total length.

(iii) Difference in number of bands encircling each fibre in the regular portion of its terminal system. In most endings the number of bands per unit length was greatest in the b_1 system; this was so in 78% of the sample, there being no difference among the rest. The excess number per 50 μm length ranged from one to four, and was usually one (48%) or two (22%).

Drawings of b_1 and b_2 terminal systems traced from photographs are shown in figure 19. The most notable feature of the terminals supplied to c fibres was the fairly common occurrence of continuous sequences of spirals, sometimes with as many as six or seven complete turns, about twice the maximum number that occurred among spirals supplied to bag fibres. This, and other features of b_1b_2c primary endings, are illustrated in plates 3, 4 and 6.

The primary endings supplied to b_2c spindle units were mostly irregular in appearance and formed a straggling array that generally extended over a greater length than b_1b_2c primaries. The mean length of 32 was 402 μm in a range of 243–833 μm, with most measuring between 400 and 500 μm. Continuous sequences of spirals were sometimes present among the c fibre terminals, and regular transverse bands occurred around the most heavily nucleated part of the b_2 fibre when this constituted a bag rather than the more usual chain of nuclei. Photographs of these endings, and of the equatorial nucleation of the b_2c unit, are illustrated in plate 5.


b_1
b_2
b_1
b_2
100 µm
*
(a)
(b)

(vi) *Secondary endings*

A constant feature of all secondary endings was the distribution of terminals to *c* fibres, largely in the form of widely spaced spirals and incomplete loops, and in most endings this was the dominant feature. The terminals were more dispersed and generally thinner, more delicate and irregular than those supplied to *c* fibres in primary endings, but viewed collectively they presented a similar annulospiral appearance. When bag fibres were included in the innervation, their terminals were usually much more irregular, and those supplied to the b_1 fibre were often in the form of sprays. Occasionally these sprays were a dominant feature so that the appearance of the ending could then more appropriately be described as 'flower-spray'. In such endings the b_1 innervation was extensive and the *c* innervation was reduced, sometimes because the *c* fibres were fewer than usual. There was frequently a strong resemblance between the spray terminals of an S_1 secondary ending supplied to a b_1 fibre and the adjacent irregular portion of the primary terminal system supplied to the same fibre.

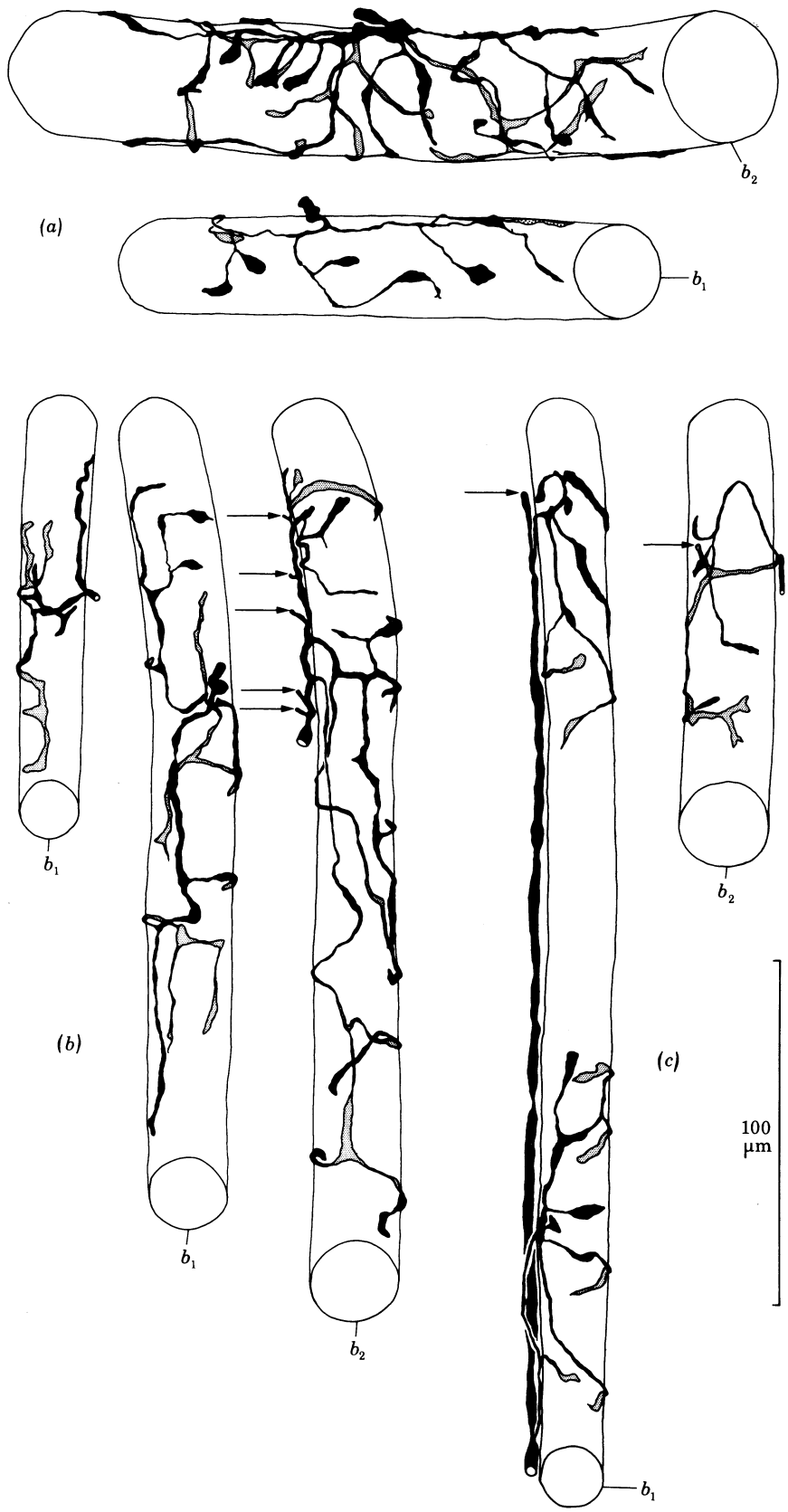
The lengths of the secondary endings studied were about the same as those of the primary endings. The mean length of 313 secondaries (221 S_1, 71 S_2, 17 S_3, 4 S_4) was 348 μm in a range of 138–716 μm; S_2 endings tended to be the longest (mean 385 μm, range 202–716 μm). Instances of overlap between sensory and motor innervation were occasionally observed in the S_1–S_3 positions involving secondary terminals on *c* fibres and trail endings on bag fibres.

A study of the supply of terminals to bag fibres in 83 b_1b_2c S_1 secondary endings was made in order to quantify the amount of innervation received and its proportional distribution between the two types. The sample comprised endings from tenuissimus (26), peroneus brevis (16), p. tertius (15), soleus (13) and superficial lumbrical (13) muscles supplied to a total of 67 spindles. The mean lengths of the b_1 and b_2 fibres innervated in these endings were, respectively, 143 μm (range 18–360 μm) and 173 μm (range 16–434 μm). On average the innervated portions represented 42% (b_1) and 51% (b_2) of the total length of the ending. In the endings sampled from tenuissimus and soleus the longest innervated portion belonged to either type of bag fibre with about equal frequency, but in 63% of all endings sampled it belonged to the b_2 fibre. The b_1 fibres received an average of ten terminals (range 2–31), the b_2 fibres an average of 13 (range 2–46). The presence of an additional b_1 fibre in the spindle did not always result in an excess of b_1 innervation, though this was often the case. The b_2 fibre received the most terminals in 64% of the endings. A supply of eight terminals provided an innervation that was arbitrarily judged to be visually significant. The proportion of endings on b_1 fibres with eight or more terminals was 65%, that on b_2 fibres 76%.

In four of the 40 b_2c spindle units the primary ending was accompanied by an S_1 secondary ending (see table 4). The predominance of terminals supplied to the b_2 fibre gave the endings a markedly irregular appearance.

In some muscles secondary endings were noticeably fewer than in others. In muscles exten-

FIGURE 19. Primary terminals supplied to bag$_1$ (b_1) and bag$_2$ (b_2) fibres in spindles from (*a*) tenuissimus and (*b*) superficial lumbrical muscles. Drawings traced from photographs of teased, silver preparations; a photograph of the tenuissimus preparation used is shown in figure 25, plate 4. Arrows indicate preterminal axons that have been cut, but which in the ending ran on to supply terminals to chain (*c*) fibres. Asterisk indicates position of a sensory cross-terminal with a *c* fibre. The b_1 terminal system in (*a*) and the b_2 system in (*b*) are each derived from a single heminode. The difference in spacing of the bands encircling the regular portions of the b_1 and b_2 systems is especially marked in (*b*). Note the characteristically irregular portions of the b_1 systems.


(a)
b_2
b_1
(b)
b_1
b_1
b_2
(c)
b_2
b_1
100 μm

sively sampled the lowest Ia:II ratio was 1:1.2 in superficial lumbrical; amongst the highest were tenuissimus (1:1.7) and peroneus longus (1:1.8). Drawings, traced from photographs, of secondary terminals supplied to b_1 and b_2 fibres are shown in figure 20, and the main features of secondary endings that have been described are illustrated in plates 3, 5 and 6.

4. Discussion

This study has shown that there are a number of constant features in the form and distribution of sensory terminals in cat hindlimb spindles. Among these are: (i) the close association between sensory terminals and equatorial myonuclei; (ii) the fact that among individual fibres the b_1 fibre always receives the most innervation in primary endings and usually the least in secondary endings; (iii) the almost exclusive restriction of sensory cross-terminals (Adal 1969) in primary endings to interconnections between the terminals supplied to c fibres; and (iv) the occurrence of S_1 secondary endings distributed to b_1, b_2 and c fibres as the most common type of secondary in all muscles sampled. We shall explore the functional significance of these features having first considered them in the context of spindle development.

The association between innervation and nucleation begins when spindle development is initiated by the contact of a Ia axon with a developing myotube that would otherwise have matured into an extrafusal muscle fibre. There follows a period of morphogenesis induced by the Ia axon during which the three types of intrafusal muscle fibre and their equatorial nucleation are differentiated in the sequence b_2, b_1, c (Zelená 1957; Landon 1972; Milburn 1973; Barker & Milburn 1982). Thereafter the Ia axon appears to play a major role in the maintenance of the adult spindle, so that, for example, after deafferentation, the equatorial nuclei disappear and are ultimately replaced by myofibrils, and the periaxial space is reduced (Tower 1932; Boyd 1962; Kucera 1980). An abundant and varied population of vesicles in the terminals of the primary ending, as well as beneath them in the muscle fibres, strongly supports Zelená's (1957, 1964) assumption that the influence of the Ia axon is mediated neurohumorally.

Secondary axons seem to have much less influence. Spindles that lack them, though slightly smaller, appear to be entirely normal. Our data indicate that secondary axons are supplied to developing spindles in a random fashion, since the frequency distribution of spindles that received different numbers of secondary endings is binomial (see figure 41). We suggest that the axons are led to the developing spindles by contact guidance, growing down paths already established by the Ia axons. They reach developing cat spindles after Ia and β axons, at about the same time as γ axons (A. Milburn 1981, personal communication). At this stage the process of separation of interlocked myotubes has started to spread from the poles to the equator and the b_1 myotube is becoming increasingly dissociated from the rest of the intrafusal bundle

Figure 20. Terminals of S_1 secondary endings supplied to bag_1 (b_1) and bag_2 (b_2) fibres. Drawings traced from photographs of teased, silver preparations.

(*a*) Tenuissimus spindle; the b_1 fibre receives nine terminals in the form of a spray, whereas the b_2 fibre is surrounded by a complex configuration of 46 terminals.

(*b*) Superficial lumbrical spindle with two b_1 fibres (a photograph of the preparation is shown in figure 38, plate 6). The b_1 fibres together receive 19 terminals, the b_2 fibre 16.

(*c*) Tenuissimus spindle; in this ending the b_1 fibre, with 19 terminals, is more densely innervated than the b_2 fibre, with six terminals. Note that each configuration of terminals supplied to the b_1 fibre is derived from a penultimate node. Arrows indicate preterminal axons that have been cut, but which in the ending ran on to supply terminals to c fibres.

Description of plate 3

Photographs of teased, silver preparations illustrating features of primary (P) and secondary (S_1) endings innervating spindles in cat hindlimb muscles. Abbreviations: Ia, primary axon; II, secondary axon; b_1, bag$_1$ fibre; b_1 br., b_2 br., b_1b_2 br., b_2c br., first-order branch of Ia axon supplying b_1 fibre, b_2 fibre, b_1b_2 fibres or b_2c fibres; b_1 p.t.s., b_1 primary terminal system; b_2, bag$_2$ fibre; b_2 p.t.s., b_2 primary terminal system; c, chain fibre(s); c sp., chain-fibre spiral terminals.

Figure 21. The primary ending of a soleus spindle is supplied by a Ia axon that has a segregated distribution; S_1 secondary endings lie adjacent to it in the upper and lower parts of the figure. The b_1 fibre is dissociated from the others, and the position of the three types of muscle fibre in the intrafusal bundle alters, left to right, from b_1, b_2, c at the top of the figure, to b_2, c, b_1 at the bottom. Note irregular terminals (i.t.) at each end of the b_1 primary terminal system (otherwise only faintly stained) and the supply of secondary terminals to the b_1 fibre at top left and bottom right of spindle. Asterisk denotes example of preterminal axon being derived from the penultimate node of a II axon.

Figure 22. Two Ia axons supply a double primary ending in a peroneus longus spindle; that at the top of the figure (b_1b_2 Ia) innervates the b_1 and b_2 fibres, whereas that at the bottom (b_1b_2c Ia) innervates all three fibre types.

Figure 23. The primary ending of a superficial lumbrical spindle is supplied by a Ia axon that has a mixed distribution; an S_1 secondary ending lies adjacent to it in the lower part of the figure. The equatorial dissociation of the b_1 fibre provides a clear view of its primary terminal system and secondary innervation. Asterisk denotes point where II axon divides to produce two first-order branches; the branch on the right gives rise to three preterminal axons, two of which travel downwards to supply terminals to the b_1 and b_2 fibres, while the third travels upwards to supply terminals to b_2 and c fibres.

Figure 24. Photographs taken at different focal planes to illustrate a primary ending supplied to a peroneus brevis spindle with two b_1 fibres. The focal plane in (*a*) shows primary terminal systems supplied to the b_2 fibre (left) and one of the b_1 fibres, whereas that in (*b*) shows the chain fibre terminals (left) and the primary terminal system supplied to the other b_1 fibre (right). The bands encircling the b_2 fibre are seen to be spaced wider apart than those around the b_1 fibres. Three first-order branches were produced by the Ia axon supplying the ending, and their distribution was segregated.

Description of plate 4

Photographs of teased, silver preparations illustrating features of primary endings innervating spindles in cat hindlimb muscles. Abbreviations as in plate 3.

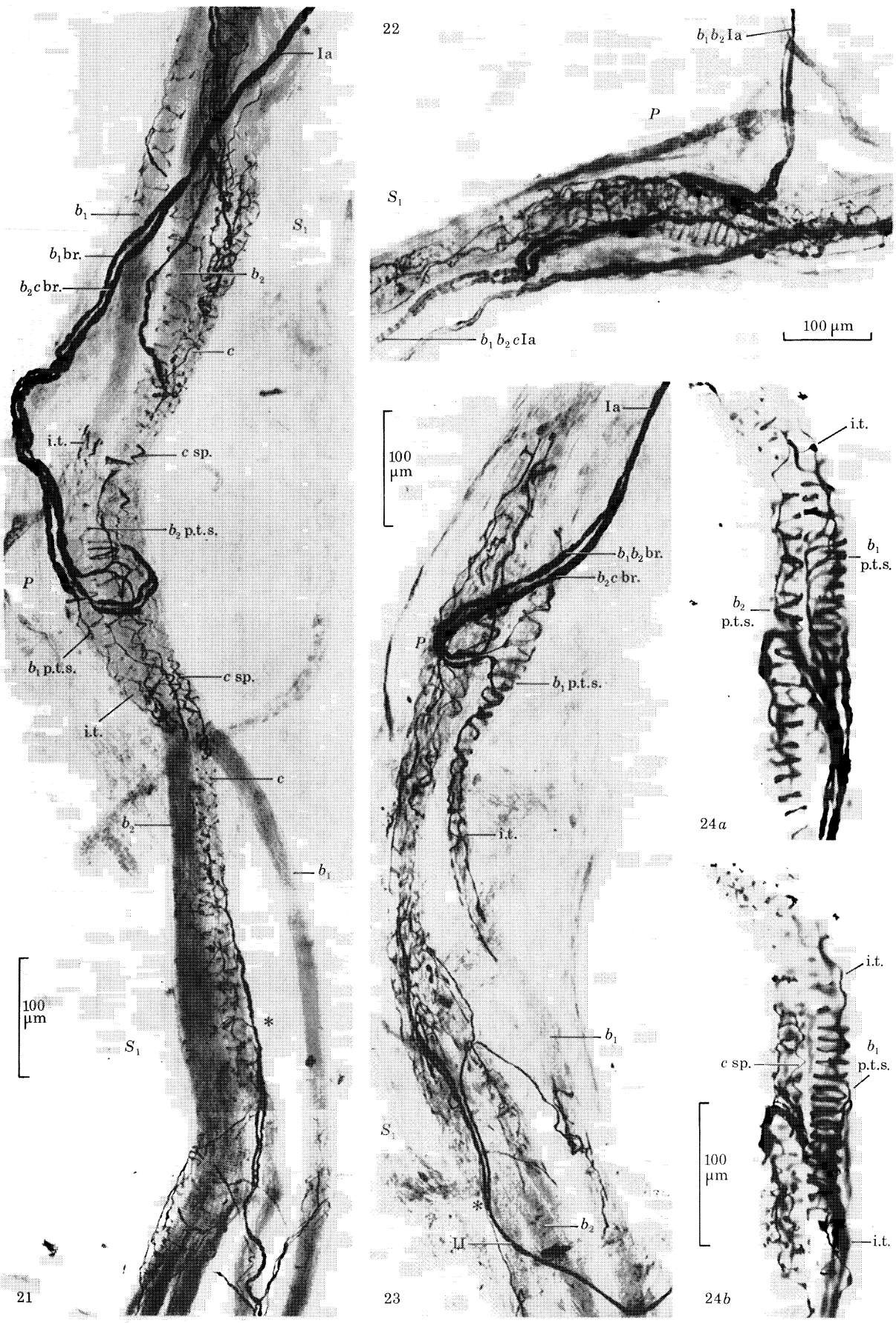
Figure 25. Primary ending of a tenuissimus spindle supplied by a Ia axon whose segregated distribution is clearly evident. The focal plane selected is that which best demonstrates the b_1 terminal system. The terminals supplied to the b_2 fibre lie on the right underneath the chain-fibre terminals. Drawings of the bag-fibre primary terminals supplied to this spindle are shown in figure 19*a*.

Figure 26. Part of a primary ending supplied to a peroneus brevis spindle provides an example of a continuous sequence of spirals around a chain fibre. Irregular terminals belonging to the b_1 terminal system lie immediately above.

Figure 27. Example of an unbranched Ia axon. The preterminal axons distributing the primary terminals are all derived from one heminode. Peroneus brevis spindle.

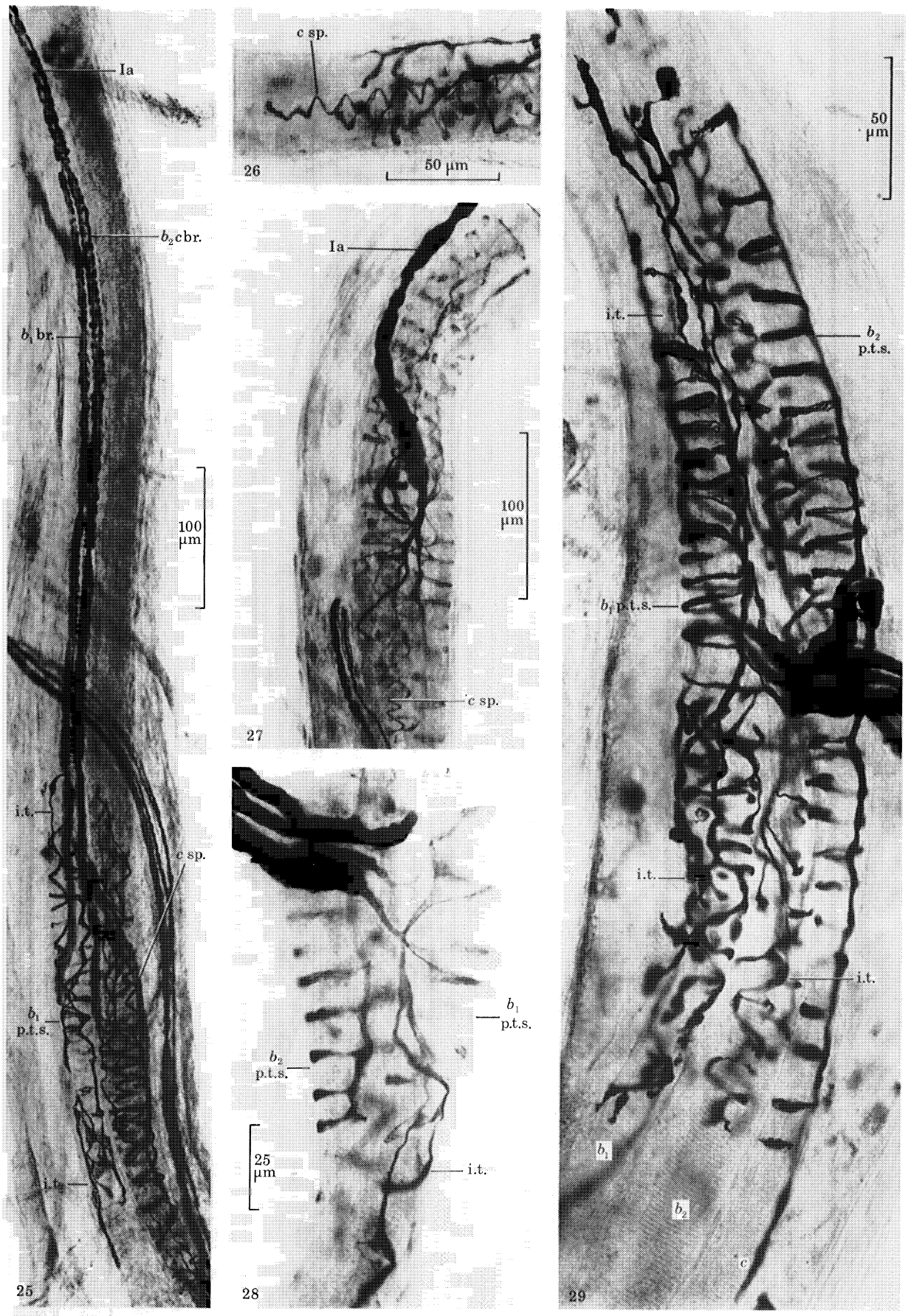
Figure 28. Part of primary ending supplied to a peroneus brevis spindle illustrating typical contrast between the end portions of the terminal systems on the b_1 and b_2 fibres.

Figure 29. Primary ending supplied to a peroneus brevis spindle. The position of the three types of muscle fibre in the intrafusal bundle alters, left to right, from b_1, c, b_2 in the top half of the ending, to b_1, b_2, c in the bottom half. The b_1 terminal system, with its irregular portions at both ends, contrasts with the terminal system supplied to the b_2 fibre, which is regular at one end (top of figure) and irregular at the other (bottom). The terminals supplied to the b_2 fibre in the top half of the ending all originate from one centrally placed preterminal axon and wrap around the fibre in ribcage fashion, a fairly common type of configuration. Terminals are similarly disposed around the chain fibre in the bottom right half of the ending.

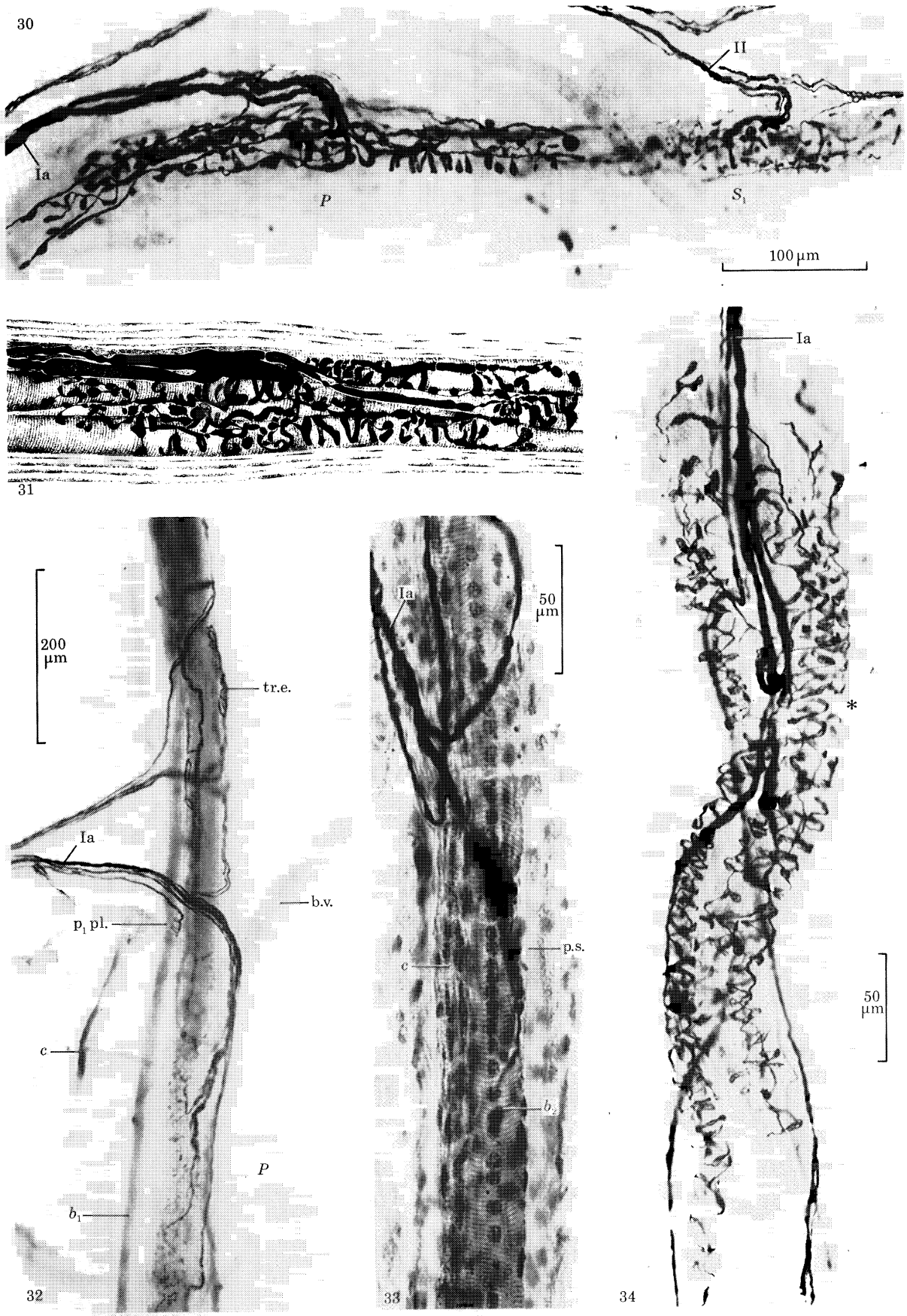


FIGURES 21–24. For description see opposite.

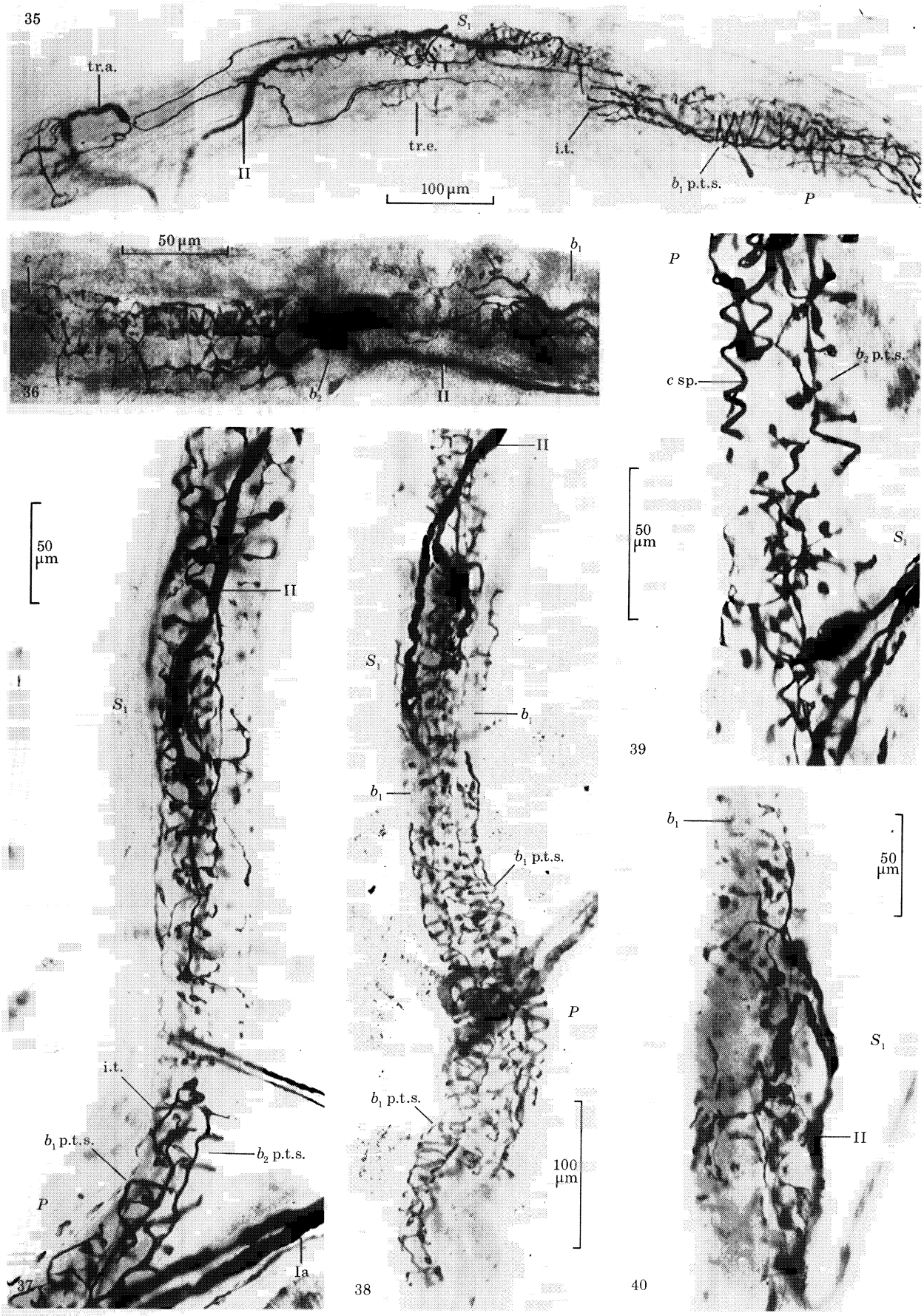
(*Facing p.* 356)



FIGURES 25–29. For description see page 356.



FIGURES 30–34. For description see page 357.



FIGURES 35–40. For description see opposite.

Description of plate 5

Photographs of teased, silver preparations illustrate the sensory innervation and equatorial nucleation of b_2c spindle units, and are compared with a figure from Ruffini (1898). Abbreviations: b.v., blood vessel; p.s., periaxial space; $p_1$pl., p_1 plate; tr.e., trail ending. Other abbreviations as in plate 3.

Figure 30. A b_2c spindle unit from peroneus brevis is supplied with a primary ending and one S_1 secondary ending. The b_2 fibre lacked a nuclear bag and was accompanied by three c fibres.

Figure 31. Ruffini (1898), in his figure 3, plate 2, reproduced here, illustrates a type of primary ending in which 'the pure spiral or ring form occurs only here or there, the greater part showing S or C forms intercalated among forked, hooked or comma-shapes' (p. 199). The figure and description strongly suggest what we recognize as a primary ending supplied to a b_2c spindle unit.

Figure 32. A b_2c spindle unit from peroneus brevis illustrating the exclusion of the b_1 fibre belonging to the tandem-linked b_1b_2c spindle unit. The b_2c unit, consisting of one b_2 and three c fibres, is seen to be innervated by a Ia axon and fusimotor axons terminating as trail endings, while the excluded b_1 fibre receives a p_1 plate. A c fibre belonging to the tandem-linked b_1b_2c unit inserts alongside the b_2c unit.

Figure 33. Equatorial region of a b_2c spindle unit from tenuissimus. Three c fibres accompany the b_2 fibre, which is seen to possess a chain of myonuclei. The terminals of the primary ending are unstained.

Figure 34. Primary ending supplied to a b_2c spindle unit from peroneus brevis. The b_2 fibre was accompanied by four c fibres. The regularly spaced bands that can be seen in the ending at the level of the asterisk encircle the b_2 fibre.

Description of plate 6

Photographs of teased, silver preparations illustrating features of primary and S_1 secondary endings innervating spindles in cat hindlimb muscles. Abbreviations: tr.a., trail axon; tr.e., trail ending. Other abbreviations as in plate 3.

Figure 35. Peroneus brevis spindle illustrates overlap that may occur in the distribution of secondary and fusimotor endings. The b_1 fibre, positioned on the lower side of the intrafusal bundle, receives a typical system of terminals in the primary ending on the right, while in the adjacent S_1 position a secondary ending is distributed to the b_2 and c fibres. The trail axon on the left innervates all three muscle-fibre types; the terminals supplied to the b_1 fibre are seen to overlap in position with the S_1 secondary ending. The innervation of b_1 fibres by trail axons occurs in 17% of spindles in peroneus brevis (Barker & Stacey 1981).

Figure 36. An S_1 secondary ending supplied to a superficial lumbrical spindle clearly demonstrates the distribution of terminals to all three types of intrafusal muscle fibre.

Figure 37. Example of S_1 secondary innervation of a b_2 fibre in a peroneus brevis spindle. The b_2 fibre lies on the right of the intrafusal bundle, the b_1 fibre mostly at top centre with the c fibres beneath it. The regular end portion of the b_2 terminal system in the primary ending contrasts typically with the irregular end portion of the b_1 terminal system. The b_2 fibre received an extensive secondary innervation of 30 terminals distributed over a length of 434 μm, as compared with 11 terminals distributed to the b_1 fibre over a length of 182 μm.

Figure 38. Primary and S_1 secondary innervation of superficial lumbrical spindle that possessed two b_1 fibres. Each b_1 fibre is supplied with a primary terminal system with typically irregular portions at each end, and is also supplied with terminals in the S_1 secondary ending (cf. figure 20*b*).

Figure 39. Example of typical contrast between the regular features of primary-ending terminals (spirals on c fibres, regular end of b_2 terminal system) and the irregularity of S_1 secondary terminals. Peroneus brevis spindle.

Figure 40. The elaborate sprays of terminals supplied to the b_1 fibre in this S_1 secondary ending were its dominant feature, giving a general appearance more aptly described as 'flower-spray' than 'annulospiral'. Peroneus tertius spindle.

(Barker & Milburn 1982). Thus the first secondary axon to reach any given spindle is most likely to terminate in an S_1 position on a bundle of interlocked myotubes and supply all three fibre types, whereas any that arrive later will be obliged to terminate in more polar positions where the myotubes have already become separate and the innervation of only one or two fibre types is more probable. The proportional distribution of secondary endings to the three types

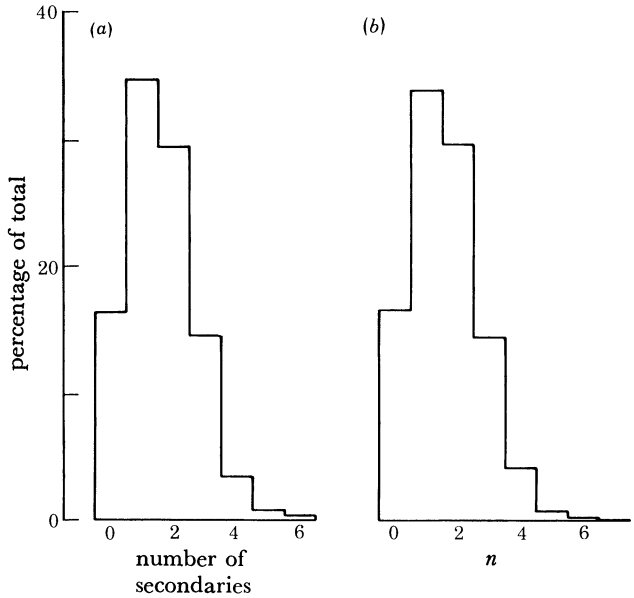


FIGURE 41. (*a*) Histogram showing the frequency distribution of b_1b_2c spindle units receiving different numbers of secondary endings. Data used are those in table 4 that relate to units supplied with a single primary ending. (*b*) Histogram showing the frequencies expected from a binomial distribution with parameters ($p = 0.22$, $n = 7$) calculated from the histogram in (*a*). (χ^2 goodness-of-fit test; $\chi^2 = 0.43$, for 5 degrees of freedom $P < 0.01$.)

of muscle fibre in adult spindles (see table 7) is thus accounted for. Equatorially the process of separation is never completed among *c* fibres, hence the regions of close apposition and sensory cross-terminals that occur among them in the adult.

Chain fibres frequently contract as a group on fusimotor stimulation (Boyd 1966, 1976), and their effect on the primary response is so powerful as to drive it at the frequency of their own contractions, up to about 60 Hz under suitable conditions (Boyd 1981). In the context of other characteristics of *c* fibres it seems likely that their cross-terminals represent a positive adaptation to such behaviour, perhaps to produce as large a generator potential as possible. Nevertheless, if the functional unity of *c* fibres is important, it is not clear why there should be so many. It may be that in view of the relatively poor vascularization of the spindle, a high surface/volume ratio is essential for their metabolism. Also more fibre surface is made available for sensory innervation in a given length of equatorial region, and, as we have shown, *c* fibres collectively receive the greatest amount of contact area with both primary and secondary terminals (see table 3).

The dense innervation of the b_1 fibre in primary endings is presumably adapted to generate a sufficiently large dynamic component in the receptor potential, since considerably more fibres (b_2 and *c*) are involved in the static input. The innervation of the b_1 fibre by secondary endings

appears to be of little functional significance. Although some secondary endings have a high dynamic sensitivity to passive stretch (Jami & Petit 1979), there appears to be no obvious correlation between this and the extent of their b_1 secondary innervation. Moreover, stimulation of dynamic fusimotor axons rarely increases the dynamic sensitivity of secondary endings (Appelberg *et al.* 1966; Boyd 1981), although it does occasionally increase their static sensitivity (L. Jami & J. Petit 1980, personal communication). The static effect may be due to the enhanced 'creep' of the b_1 fibre that occurs during dynamic fusimotor stimulation following the completion of ramp stretch (Boyd 1981). This could lead to a compensatory extension of the secondary terminals while the primary region was shortening so as to produce what Matthews (1964) envisaged as 'an inverse dynamic response, consisting of a static response developing with a lag'.

Our data on the branching of spindle afferents have important implications for the manner in which nerve impulses are generated from the endings and propagated into the axons. Quick *et al.* (1980), using a cytochemical method, have shown that within the final branches of Ia axons the heminodes and some of the penultimate nodes are all potential sites of spike initiation. If the membrane properties of these nodes are similar, the preferred sites for impulse generation would be the heminodes, resulting in a complex pattern of spikes converging on the parent axon. Since abortive spikes have been observed in isolated frog spindle preparations (Katz 1950; Ito 1976; Ito & Komatsu 1976), it may be assumed that not all spikes propagate into the parent axon. This may be attributed to the relatively low safety factor of the branch points (Krnjević & Miledi 1958) resulting in some of the nodes at these points acting as further stages in the encoding of the afferent response.

For Ia axons whose first-order branches have a segregated distribution there would presumably be separate dynamic and static pacemakers. These have been demonstrated by Hulliger & Noth (1979), whose results show that the outputs of the separate pacemakers do not summate linearly; indeed the dynamic pacemaker may be totally occluded when its output falls below that of the static one. Our reconstructions of the branching patterns of Ia axons (figure 1) suggest how this may occur. When an impulse, propagating centrally in a first-order branch, reaches the first branching node it will then propagate orthodromically into the parent axon, and, provided that it is not refractory, antidromically into the other first-order branch. During the following refractory period this branch would then be less likely to transmit another impulse, thus suppressing its pacemaker. However, the antidromic impulse might itself fail to propagate into some of the smaller branches, or be eliminated by collisions with abortive orthodromic spikes within them. Since these effects would be cumulative, a profusely branching first-order system would recover from an antidromic impulse more rapidly than one with fewer branches.

Examination of the Ia branching patterns shown in figure 1 shows that the first-order branches associated with static input (from b_2 and c fibres) tend to be more profusely branched than those associated with dynamic input (from b_1 fibres). There is thus a correlation between the morphological and physiological asymmetry. If this interpretation is correct the branching pattern of spindle 6 shown in figure 1 should produce a more symmetrical interaction between its dynamic and static pacemakers than is typical. We would suppose that the branching patterns of Ia axons exert a major influence on the pattern of the afferent response, and suspect that the dynamic pacemaker is more easily occluded in Ia axons that have a mixed distribution.

Similar considerations apply to secondary axons, though in some instances their branching allows for a terminal input from penultimate nodes as well as heminodes. Presumably these

nodes act as spike generators, or as modulators of impulse transmission. The variable dynamic sensitivity of secondary endings may partly be accounted for by a variable input from b_1 terminals, though, as already mentioned this sensitivity is rarely affected by dynamic fusimotor stimulation.

The spiking activity of the final branches of spindle afferents is presumably generated by receptor potentials produced by their terminals. An electrotonic potential has been recorded in the Ia axon about 1 mm from its terminal by Hunt & Ottoson (1975) using an isolated cat-spindle preparation in which spiking activity was blocked with tetrodotoxin. They refer to this as the receptor potential, but it is perhaps more accurately regarded as the algebraic sum of several receptor potentials, each associated with a single terminal branch system. Thus in primary endings there would be at least three receptor potentials separately produced by terminals on b_1, b_2 and c fibres. By analogy with impulse activity in a group of axons, the potential recorded by Hunt & Ottoson could be referred to as the 'compound receptor potential' (c.r.p.). However, the c.r.p. is probably an abstraction since its appearance would normally be prevented by spiking activity in the final afferent branches. Hunt *et al.* (1978) have shown that the c.r.p. is generated mainly by increased Na^+ conductance, and presumably the individual receptor potentials are generated in the same way.

The actual transduction mechanism is still a matter for speculation. In its simplest form it would involve direct coupling of the ion-permeability changes to stretch deformation of the terminal membrane adjacent to the intrafusal muscle fibre (Katz 1950), and this seems to be the mechanism favoured by Hunt *et al.* (1978) and Ito *et al.* (1980). However, consideration of the fluid mosaic model of membrane structure (Singer & Nicholson 1972) makes it difficult to see how the membrane could support the necessary longitudinal tension changes for this to occur. Moreover, since the terminals are convex structures occupying grooves in the muscle fibres (Adal 1969), it seems more likely that the membranes flatten out rather than stretch during muscle lengthening. Flattening of frog sensory terminals has been described by Karlsson *et al.* (1971). Furthermore there is no evidence that the terminals adhere directly to the muscle fibres; they appear instead to be pressed against them by the basal laminae of the muscle fibres, which pass over the free surfaces of the terminals.

We believe that the deformation leading to transduction may be due to a deformation of the whole sensory terminal brought about by increased tension in the basal lamina, the terminal being squeezed between the lamina and the underlying muscle fibre. We suggest that the Na^+ channels are distributed over the whole terminal membrane, rather than being restricted to that part adjacent to the muscle fibre, and that their permeability is affected by an intracellular messenger released from a bound state by the increase in cytoskeletal tension that may be presumed to accompany the bulk deformation of the terminal. Such a process has the advantage of introducing a possible amplification stage between the initial mechanically linked event and the permeability changes, thereby allowing for very high sensitivity. The intracellular messenger would most probably be Ca^{2+} since it is thought to play a similar role in retinal receptors (Hagins 1979) as well as acting as an intracellular messenger in a number of other subcellular systems (Kretsinger 1979). Removal of Ca^{2+} from the extracellular fluid has profound effects on crustacean muscle receptors (Wiermsa *et al.* 1953), as does the inclusion of Ca^{2+} channel blockers on frog spindles (Ito *et al.* 1980), but so far no effect has been reported for mammalian spindles (Hunt *et al.* 1978).

Soon after it was recognized that intrafusal muscle fibres were of two types, nuclear-bag and

nuclear-chain (Cooper & Daniel 1956; Boyd 1956), it became evident that pairs of spindle capsules were sometimes linked together in tandem by a single bag fibre, the smaller capsule of the two being supplied by a markedly irregular primary ending (Barker & Ip 1961; Barker & Cope 1962). We have established that the link is made by the b_2 fibre, and have recognized the smaller capsule, from which the b_1 fibre is excluded, as a b_2c spindle unit innervated by a Ia axon of relatively small diameter. Despite some features that are intermediate we regard the axon and its ending as primary rather than secondary for the following reasons: (i) the myotubal chains of nuclei, and the occasional nuclear bag that occurs in the b_2 fibre, resemble the chains and bags of myonuclei found at the equator of spindles where primary endings terminate; (ii) the relation of the ending to the surrounding capsule and periaxial space is typical of primary innervation; and (iii) the ending is occasionally accompanied by another axon and ending that have all the appearances of being S_1 secondary. Furthermore, it seems very probable that the ending is the same one that Ruffini (1898) recognized as an irregular type of primary in his original description (compare figures 30 and 31, plate 5).

These b_2c spindle units were present in most of the muscles sampled for the silver analysis. They usually inserted into tendon and were more common in some muscles than in others, e.g. they were about four times more frequent in extensor digitorum longus and peroneus brevis than in superficial lumbrical and soleus. Bakker & Richmond (1981) have found much higher frequencies in some cat neck muscles: 45% of the spindle population were b_2c units in complexus (sample of 40) and biventer cervicis (40), 33% in splenius (33). They have also demonstrated that the ATPase profile of the bag fibre is that of a b_2 fibre.

The functional properties of b_2c Ia axons and their endings remain to be elucidated. The usual criteria for the identification of primaries (axon conduction velocity, dynamic response of the ending to passive stretch) are of little use since the relatively small diameters of the axons will place their conduction velocities somewhere between 60 m/s (obviously secondary) and 80 m/s (obviously primary), and in the absence of a b_1 fibre their endings will have little dynamic sensitivity. Richmond & Abrahams (1979) found that the stretch responses of a considerable number of neck-spindle sensory endings were intermediate between primary and secondary. Many of these were probably primaries innervating b_2c spindle units, and it seems likely that these also account for most of the 'truly intermediate sensory endings' identified by Dutia (1980) in soleus spindles.

Banks *et al.* (1980) have examined the responses of peroneal spindle afferents to ramp-and-hold stretches in an attempt to identify b_2c Ia axons. These did not emerge in plots of dynamic index against static response, or of the dynamic index/static response ratio against conduction velocity. Further progress in this matter must await experiments in which the responses of b_2c spindle units are monitored during motor stimulation. Boyd (1981) has described the effects that separately activating each type of intrafusal muscle fibre has on the dynamic and position (or length) sensitivities of primary and secondary endings. Some response patterns of b_2c units could be predicted from these data, but it is perhaps unwise to ascribe particular functions to subsystems of the spindle and expect them to combine linearly under varying conditions.

The high proportion of b_2c units in muscles that raise the head, such as complexus, is due to the fact that their spindles are mostly arranged in elaborate tandem systems in which there are many capsules that exclude the b_1 fibre (Richmond & Abrahams 1975; Bakker & Richmond 1981). Spindle systems of this kind presumably have a high primary static sensitivity to cater for the exclusive antigravity function of such muscles. By contrast, occipitoscapularis, which

rotates the scapula, has a spindle population that lacks tandem systems and resembles that of a hindlimb muscle (Richmond & Abrahams 1975). In our own sampling of tenuissimus, soleus, extensor digitorum longus, peroneal and lumbrical muscles, the spindle population of the superficial lumbrical muscles stands apart as having the lowest proportion of b_2c units, the lowest supply of secondary endings, and the highest proportion of b_1b_2c units with more than one b_1 fibre. A relatively high dynamic sensitivity may thus be achieved, which presumably relates to the function that these muscles serve in carrying out finely adjusted movements of digits. With the amount of information about spindles now available, attempts such as these to correlate the function of a muscle with the structural and functional properties of its spindle population are becoming more feasible, and perhaps the time has come to pay more attention to these considerations.

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References

Adal, M. N. 1969 The fine structure of the sensory region of cat muscle spindles. *J. Ultrastruct. Res.* **26**, 332–354.

Adal, M. N. & Barker, D. 1962 Intramuscular diameters of afferent nerve fibres in the rectus femoris muscle of the cat. In *Symposium on muscle receptors* (ed. D. Barker), pp. 249–256. Hong Kong University Press.

Appelberg, B., Bessou, P. & Laporte, Y. 1966 Action of static and dynamic fusimotor fibres on secondary endings of cat's spindles. *J. Physiol., Lond.* **185**, 160–171.

Bakker, G. J. & Richmond, F. J. R. 1981 Two types of muscle spindles in cat neck muscles: a histochemical study of intrafusal fibre composition. *J. Neurophysiol.* **45**, 973–986.

Banker, B. Q. & Girvin, J. P. 1971 The ultrastructural features of the mammalian muscle spindle. *J. Neuropath. exp. Neurol.* **30**, 155–195.

Banks, R. W. 1981*a* A histological study of the motor innervation of the cat's muscle spindle. *J. Anat.* **133**, 571–591.

Banks, R. W. 1981*b* The number and distribution of satellite cells of intrafusal muscle fibres in a muscle spindle of the cat. *J. Anat.* **133**, 694.

Banks, R. W., Barker, D., Bessou, P., Pagès, B. & Stacey, M. J. 1978 Histological analysis of cat muscle spindles following direct observation of the effects of stimulating dynamic and static motor axons. *J. Physiol., Lond.* **283**, 605–619.

Banks, R. W., Barker, D., Harker, D. W. & Stacey, M. J. 1975 Correlation between ultrastructure and histochemistry of mammalian intrafusal muscle fibres. *J. Physiol., Lond.* **252**, 16*P*–17*P*.

Banks, R. W., Barker, D. & Stacey, M. J. 1977*a* Intrafusal branching and distribution of primary and secondary afferents. *J. Physiol., Lond.* **272**, 66*P*–67*P*.

Banks, R. W., Barker, D. & Stacey, M. J. 1979 Sensory innervation of cat hindlimb muscle spindles. *J. Physiol., Lond.* **293**, 40*P*–41*P*.

Banks, R. W., Barker, D. & Stacey, M. J. 1981 Structural aspects of fusimotor effects on spindle sensitivity. In *Muscle receptors and movement* (ed. A. Taylor & A. Prochazka), pp. 5–16. London: Macmillan.

Banks, R. W., Ellaway, P. H. & Scott, J. J. 1980 Responses of de-efferented muscle spindles of peroneus brevis and tertius muscles in the cat. *J. Physiol., Lond.* **310**, 53*P*.

Banks, R. W., Harker, D. W. & Stacey, M. J. 1977*b* A study of mammalian intrafusal muscle fibres using a combined histochemical and ultrastructural technique. *J. Anat.* **123**, 783–796.

Barker, D. 1948 The innervation of the muscle-spindle. *Q. Jl microsc. Sci.* **89**, 143–186.

Barker, D., Bessou, P., Jankowska, E., Pagès, B. & Stacey, M. J. 1978 Identification of intrafusal muscle fibres activated by single fusimotor axons and injected with fluorescent dye in cat tenuissimus spindles. *J. Physiol., Lond.* **275**, 149–165.

Barker, D. & Cope, M. 1962 The innervation of individual intrafusal muscle fibres. In *Symposium on muscle receptors* (ed. D. Barker), pp. 263–269. Hong Kong University Press.

Barker, D., Emonet-Dénand, F., Harker, D. W., Jami, L. & Laporte, Y. 1976 Distribution of fusimotor axons to intrafusal muscle fibres in cat tenuissimus muscle spindles as determined by the glycogen-depletion method. *J. Physiol., Lond.* **261**, 49–69.

Barker, D. & Ip, M. C. 1961 A study of single and tandem types of muscle-spindle in the cat. *Proc. R. Soc. Lond.* B **154**, 377–397.

Barker, D. & Ip, M. C. 1963 A silver method for demonstrating the innervation of mammalian muscle in teased preparations. *J. Physiol., Lond.* **69**, 73*P*–74*P*.

Barker, D. & Milburn, A. 1982 Development of cat muscle spindles. *J. Physiol., Lond.* **325**, 85*P*.

Barker, D. & Stacey, M. J. 1981 On the innervation of bag_1 fibres in cat muscle spindles by static γ axons. *J. Physiol., Lond.* **320**, 93*P*.

Boyd, I. A. 1956 The tenuissimus muscle of the cat. *J. Physiol., Lond.* **133**, 35*P*–36*P*.

Boyd, I. A. 1962 The structure and innervation of the nuclear bag muscle fibre system and the nuclear chain muscle fibre system in mammalian muscle spindles. *Phil. Trans. R. Soc. Lond.* B **245**, 81–136.

Boyd, I. A. 1966 The behaviour of isolated mammalian muscle spindles with intact innervation. *J. Physiol., Lond.* **186**, 109*P*–110*P*.

Boyd, I. A. 1976 The response of fast and slow nuclear bag fibres and nuclear chain fibres in isolated cat muscle spindles to fusimotor stimulation, and the effect of intrafusal contraction on the sensory endings. *Q. Jl exp. Physiol.* **61**, 203–254.

Boyd, I. A. 1981 The action of the three types of intrafusal fibre in isolated cat muscle spindles on the dynamic and length sensitivities of primary and secondary sensory endings. In *Muscle receptors and movement* (ed. A. Taylor & A. Prochazka), pp. 17–32. London: Macmillan.

Boyd, I. A., Gladden, M. H., McWilliam, P. N. & Ward, J. 1977 Control of dynamic and static nuclear bag fibres and nuclear chain fibres by γ- and β-axons in isolated cat muscle spindles. *J. Physiol., Lond.* **265**, 133–162.

Cooper, S. & Daniel, P. M. 1956 Human muscle spindles. *J. Physiol., Lond.* **133**, 1*P*–3*P*.

Corvaja, N., Marinozzi, V. & Pompeiano, O. 1967 Close appositions and junctions of plasma membranes of intrafusal muscle fibres in mammalian muscle spindles. *Pflügers Arch. ges. Physiol.* **296**, 337–345.

Corvaja, N., Marinozzi, V. & Pompeiano, O. 1969 Muscle spindles in the lumbrical muscle of the cat. *Archs. ital. Biol.* **107**, 365–543.

Dutia, M. B. 1980 Activation of cat muscle spindle primary, secondary and intermediate sensory endings by suxamethonium. *J. Physiol., Lond.* **304**, 315–330.

Gairns, F. W. 1930 A modified gold chloride method for the demonstration of nerve endings. *Q. Jl microsc. Sci.* **74**, 151–153.

Gladden, M. H. 1976 Structural features relative to the function of intrafusal muscle fibres in the cat. In *Understanding the stretch reflex* (ed. S. Homma), *Prog. Brain Res.* **44**, 51–59.

Hagins, W. A. 1979 Excitation in vertebrate photoreceptors. In *The neurosciences: fourth study program* (ed. F. O. Schmitt & F. G. Worden), pp. 183–191. Cambridge, Massachusetts and London: M.I.T. Press.

Homma, S. & Seki, Y. 1964 Muscle spindles in phasic and tonic muscle. *Tohoku J. exp. Med.* **83**, 391–397.

Hudson, G. & Hartmann, J. F. 1961 The relationship between dense bodies and mitochondria in motor neurones. *Z. Zellforsch. mikrosk. Anat.* **54**, 147–157.

Hulliger, M. & Noth, J. 1979 Static and dynamic fusimotor interaction and the possibility of multiple pacemakers operating in the cat muscle spindles. *Brain Res.* **173**, 21–28.

Hunt, C. C. 1954 Relation of function to diameter in afferent fibres of muscle nerves. *J. gen. Physiol.* **38**, 117–131.

Hunt, C. C. & Ottoson, D. 1975 Impulse activity and receptor potential of primary and secondary endings of isolated mammalian muscle spindles. *J. Physiol., Lond.* **252**, 259–281.

Hunt, C. C., Wilkinson, R. S. & Fukami, Y. 1978 Ionic basis of the receptor potential in primary endings of mammalian muscle spindles. *J. gen. Physiol.* **71**, 683–698.

Ito, F. & Komatsu, Y. 1976 Sensory terminal responses of frog muscle spindle recorded across vaseline gap onto intrafusal muscle fibre. *Pflügers Arch. Eur. J. Physiol.* **366**, 25–30.

Ito, F., Komatsu, Y. & Kaneko, N. 1980 A model of transduction in the frog muscle spindle. *Nagoya J. med. Sci.* **42**, 89–91.

Ito, Y. 1976 Existence of multiple abortive spike generators and their independence in frog spindle terminal. *Proc. Japan Acad.* **52**, 248–251.

Jami, L. & Petit, J. 1979 Dynamic and static responses of primary and secondary spindle endings of the cat peroneus tertius muscle. *J. Physiol., Lond.* **296**, 109*P*.

Jones, E. G. 1966 The innervation of muscle spindles in the Australian oppossum *Trichosurus vulpecula*, with special reference to the motor nerve endings. *J. Anat., Lond.* **100**, 733–759.

Karlsson, U. L., Hooker, W. M. & Bendeich, E. G. 1971 Quantitative changes in the frog muscle spindle with passive stretch. *J. Ultrastruct. Res.* **36**, 743–756.

Katz, B. 1950 Action potentials from a sensory nerve ending. *J. Physiol., Lond.* **111**, 248–260.

Kretsinger, R. H. 1979 Calcium in neurobiology: a general theory of its function and evolution. In *The neurosciences: fourth study program* (ed. F. O. Schmitt & F. G. Worden), pp. 617–622. Cambridge, Massachusetts and London: M.I.T. Press.

Krnjević, K. & Miledi, R. 1958 Failure of neuromuscular propagation in rats. *J. Physiol., Lond.* **140**, 440–461.

Kucera, J. 1980 Myofibrillar ATPase activity of intrafusal fibres in chronically de-afferented rat muscle spindles. *Histochemistry* **66**, 221–228.

Landon, D. M. 1972 The fine structure of the equatorial regions of developing muscle spindles in the rat. *J. Neurocytology* **1**, 189–210.

Matthews, P. B. C. 1964 Muscle spindles and their motor control. *Physiol. Rev.* **44**, 219–288.

Milburn, A. 1973 The early development of muscle spindles in the rat. *J. Cell Sci.* **12**, 175–195.

Ovalle, W. K. & Smith, R. S. 1972 Histochemical identification of three types of intrafusal muscle fibre in the cat and monkey based on the myosin ATPase reaction. *Can. J. Physiol. Pharmac.* **50**, 195–202.

Quick, D. C., Kennedy, W. R. & Poppele, R. E. 1980 Anatomical evidence for multiple sources of action potentials in the afferent fibres of muscle spindles. *Neuroscience* **5**, 109–115.

Richmond, F. J. R. & Abrahams, V. C. 1975 Morphology and distribution of muscle spindles in dorsal muscles of the cat neck. *J. Neurophysiol.* **38**, 1322–1339.

Richmond, F. J. R. & Abrahams, V. C. 1979 Physiological properties of muscle spindles in dorsal muscles of the cat neck. *J. Neurophysiol.* **42**, 604–617.

Ruffini, A. 1898 On the minute anatomy of the neuromuscular spindles of the cat, and on their physiological significance. *J. Physiol., Lond.* **23**, 190–208.

Scalzi, H. A. & Price, H. M. 1972 Electron-microscopic observations of the sensory region of the mammalian muscle spindle. In *Research in muscle development and the muscle spindle* (ed. B. Q. Banker *et al.*), pp. 254–263. Amsterdam: Excerpta Medica.

Singer, S. J. & Nicholson, G. C. 1972 The fluid mosaic model of the structure of cell membranes. *Science, N.Y.* **175**, 720.

Stacey, M. J. 1969 Free endings in skeletal muscle of the cat. *J. Anat.* **105**, 231–254.

Tello, J. F. 1922 Die Entstchung der motorischen und sensiblen Nervenendigungen. *Z. ges. Anat.* **64**, 348–440.

Tower, S. S. 1932 Atrophy and degeneration in the muscle spindle. *Brain* **55**, 77–90.

Wiermsa, C. A. G., Furshpan, E. & Florey, E. 1953 Physiological and pharmacological observations on muscle receptor organs of the crayfish, *Cambarus clarkii* Girard. *J. exp. Biol.* **30**, 136–150.

Zelená, J. 1957 Morphogenetic influence of innervation on the ontogenetic development of muscle spindles. *J. Embryol. exp. Morph.* **5**, 283–292.

Zelená, J. 1964 Development, degeneration and regeneration of receptor organs. In *Mechanisms of neural regeneration of receptor organs* (ed. M. Singer & J. P. Schadé). *Prog. Brain Res.* **13**, 175–211.

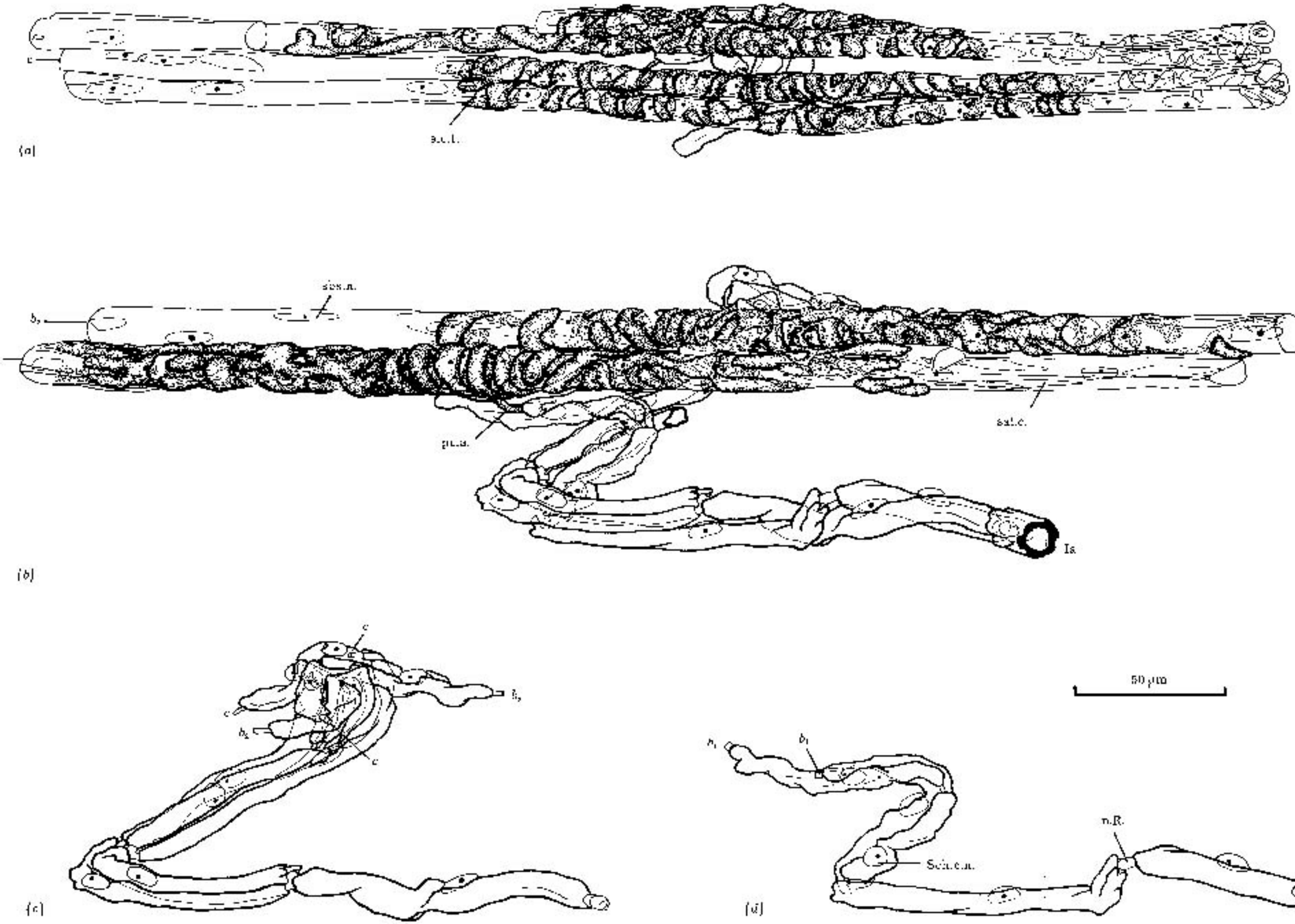


FIGURE 4. For description see opposite.

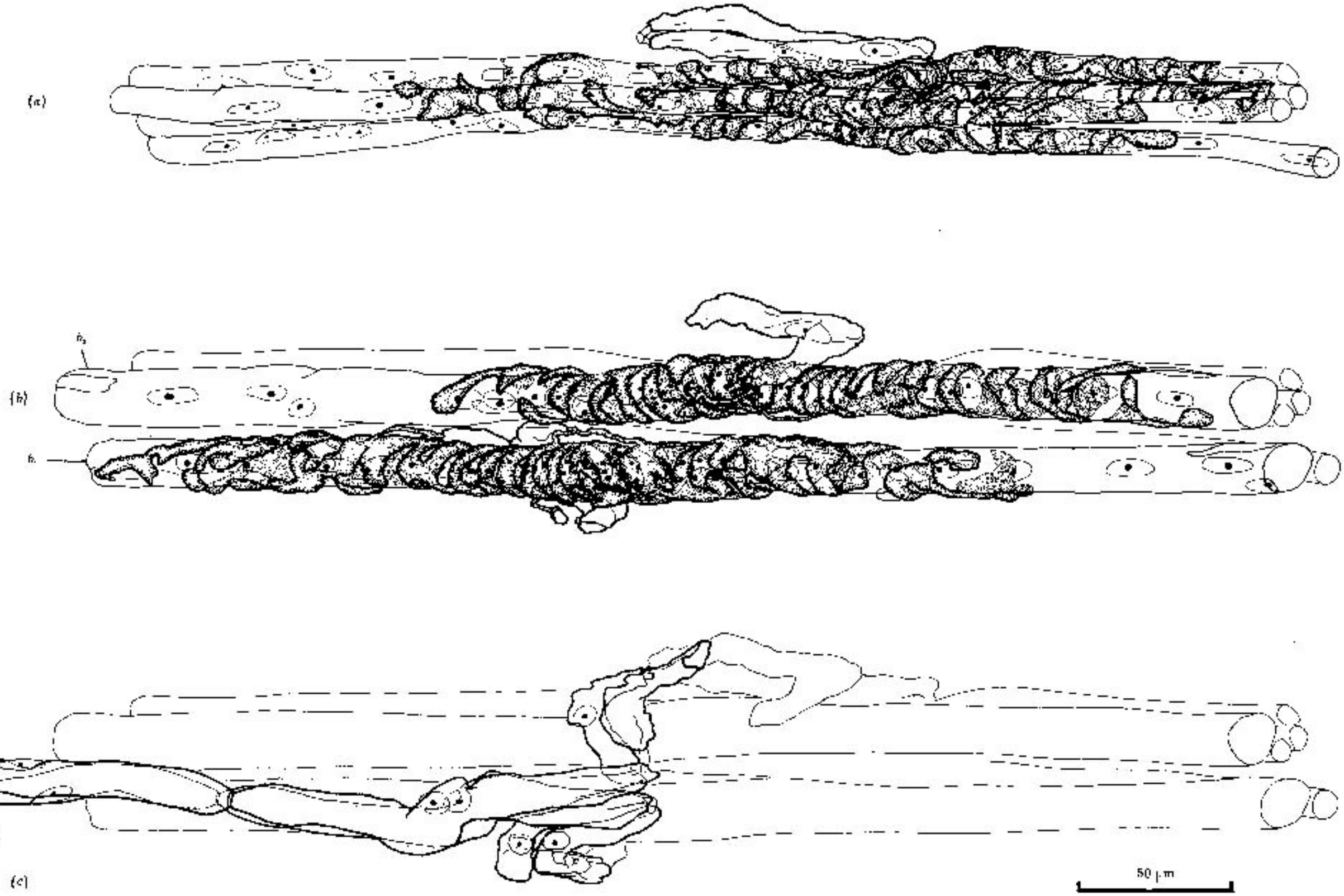
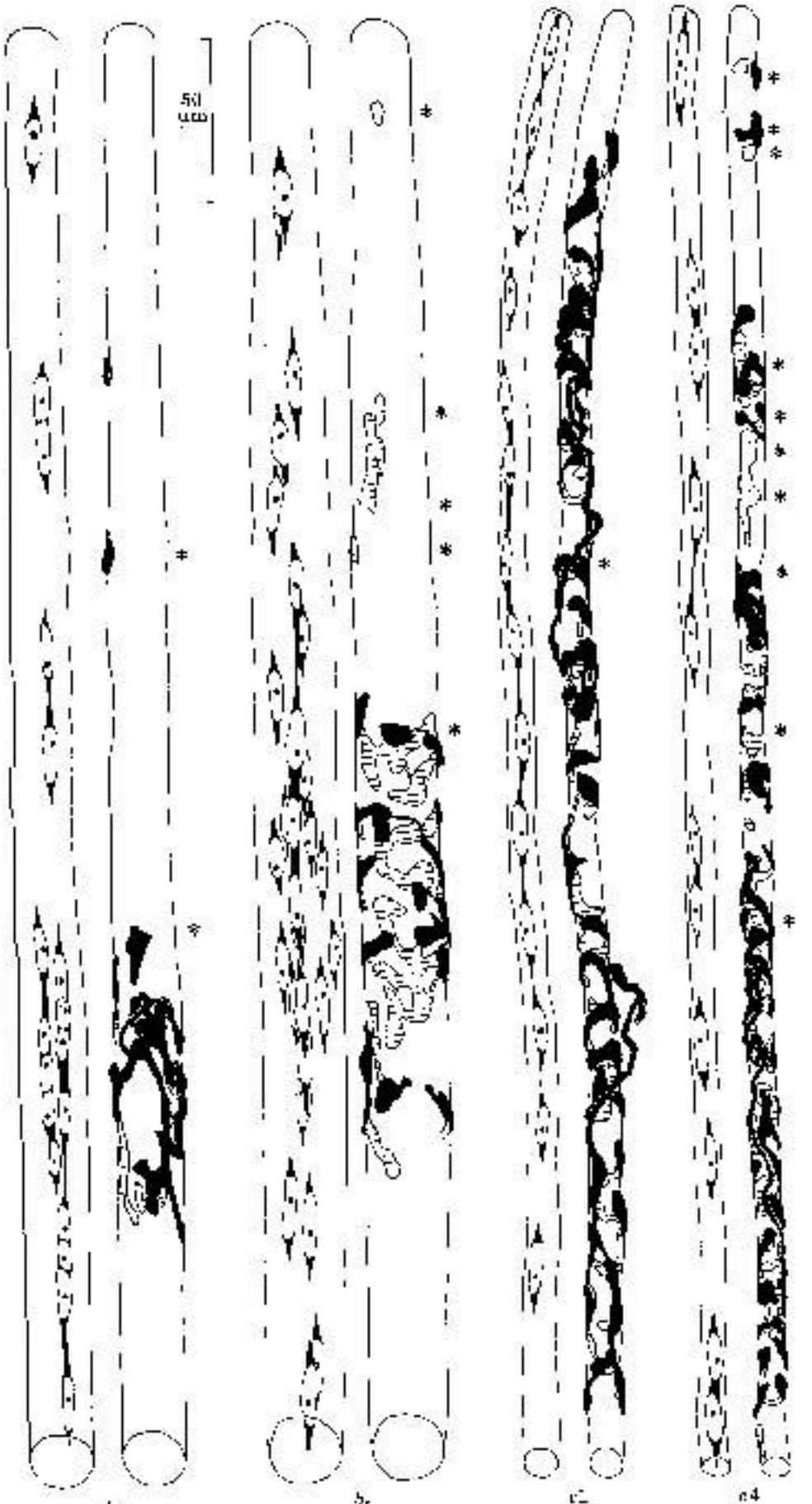


FIGURE 5. For description see page 342.

FIGURE 6. For description see page 342.

FIGURE 7. Schematic representation of parts of the reconstructed primary ending of spindle 6 shown in figure 6. The terminals shown are those supplied to the bag_1 (b_1) and bag_2 (b_2) fibres and the longest and shortest chain (c) fibres (numbered 1 and 4, as in table 2). In addition each fibre is repeated alongside to show its myonucleation and thus demonstrate the relation between nucleation and innervation. Adjustments have been made to the original alignment of each muscle fibre relative to the others, mainly in order to position the centre of each nuclear bag on a common midline and thus facilitate comparison between b_1 and b_2 terminal systems. Terminals shown in outline at bottom end of c1 belong to adjacent S_1 secondary ending. Asterisks alongside γ termina's indicate positions of sensory cross-terminals with other γ fibres.



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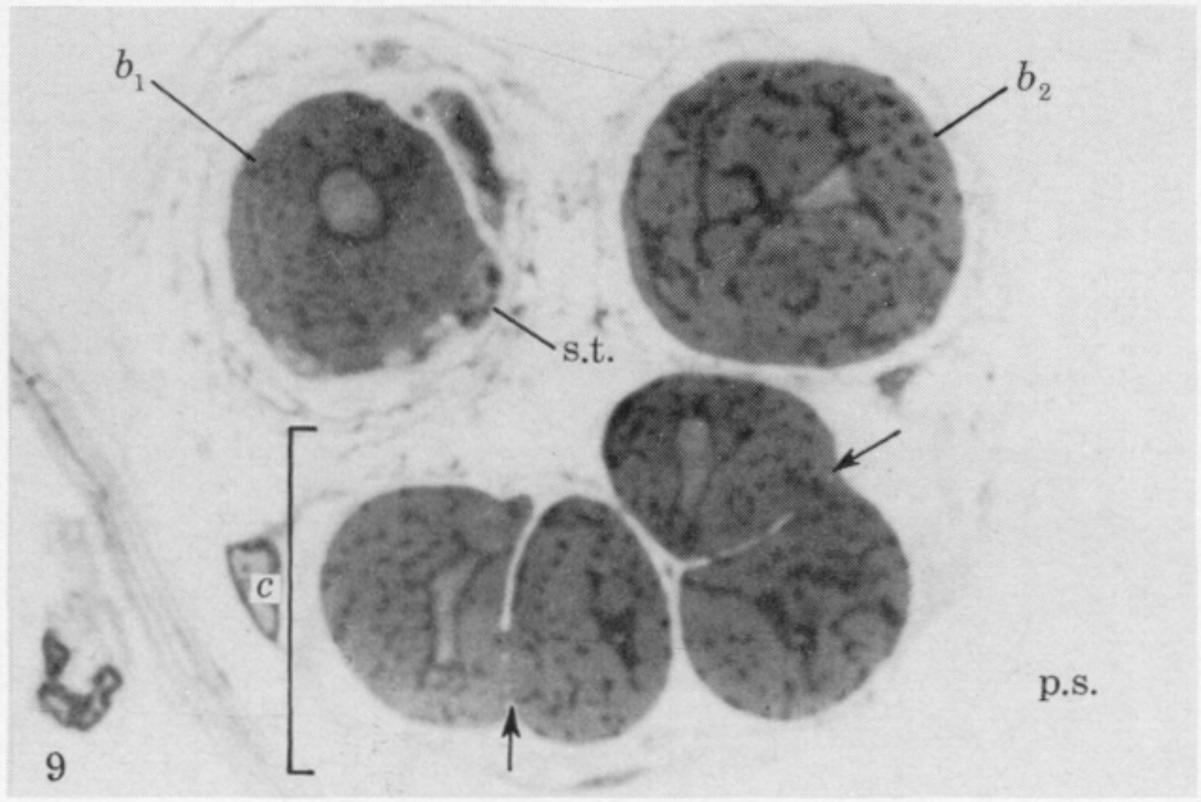
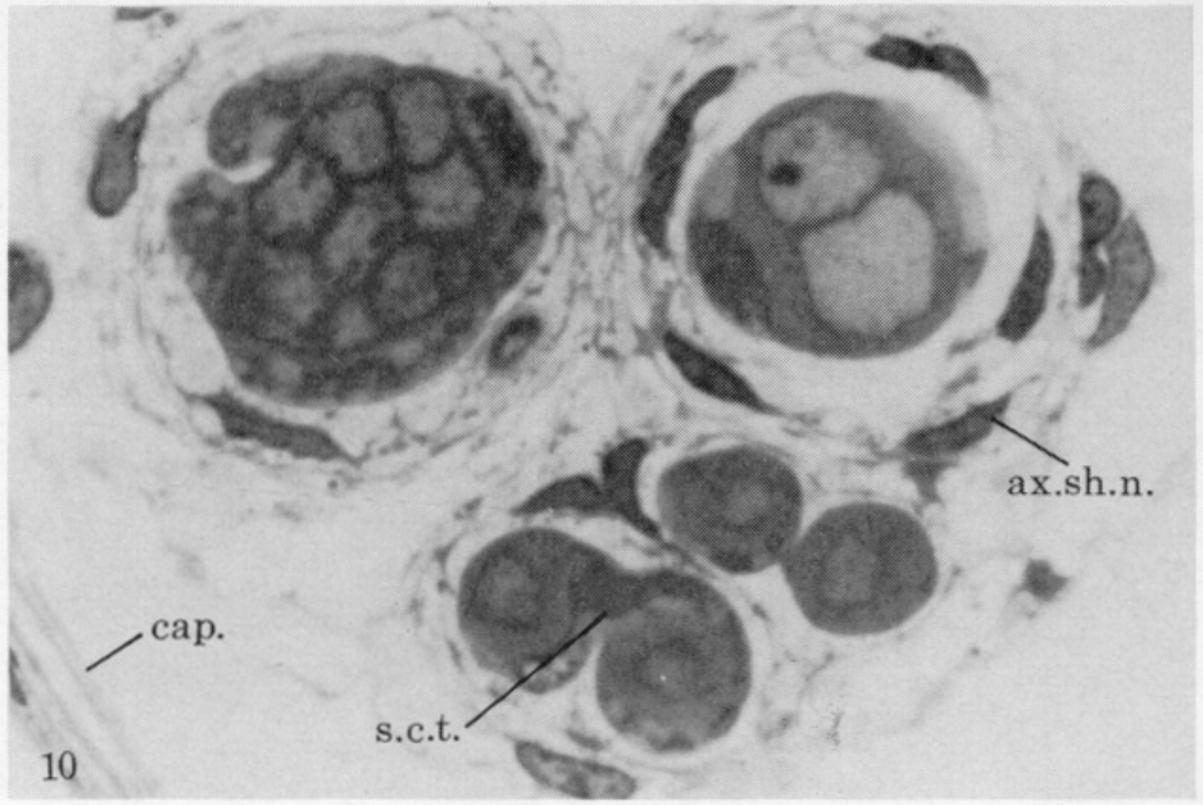
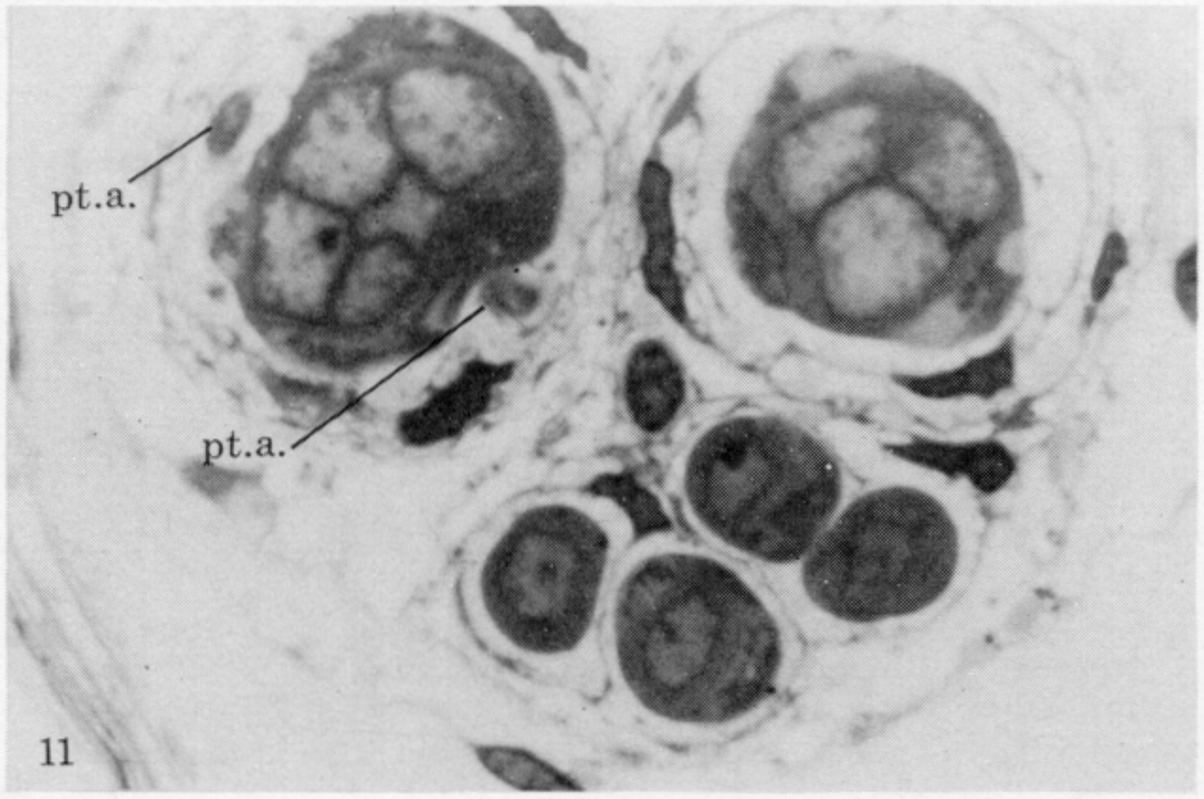
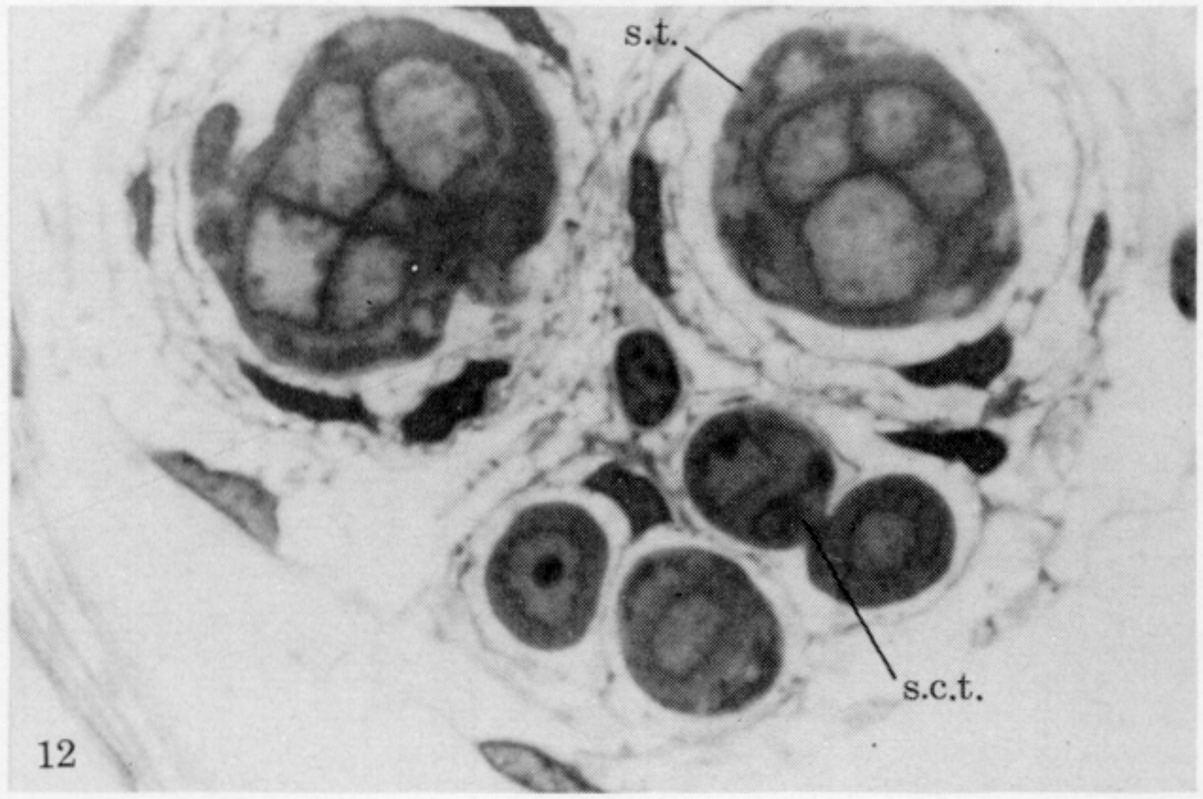
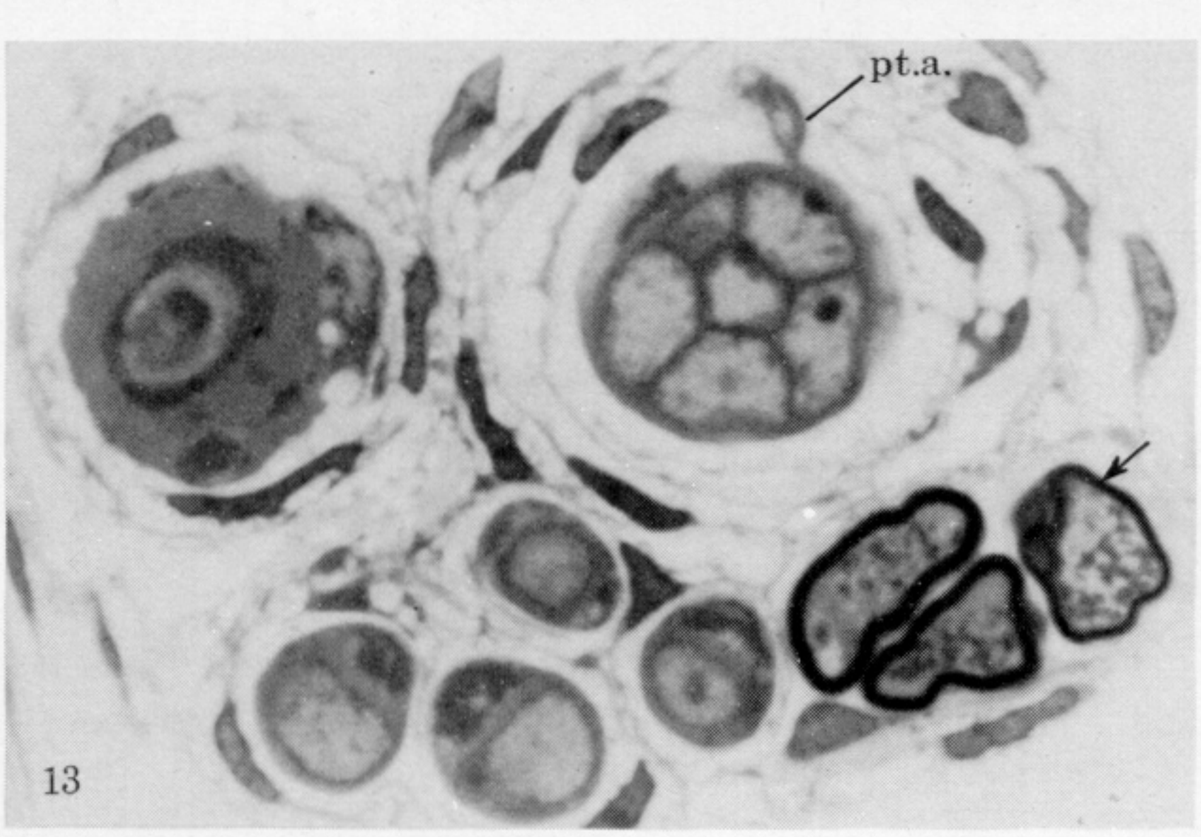
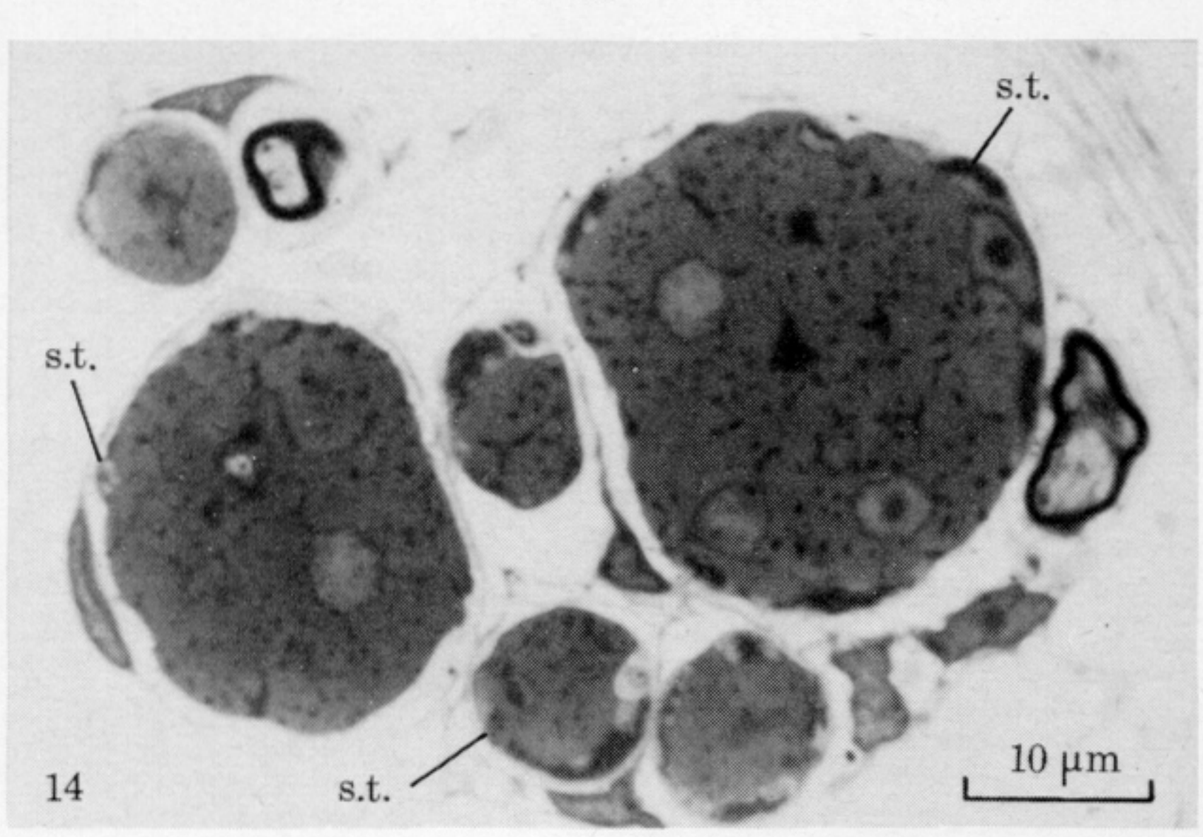






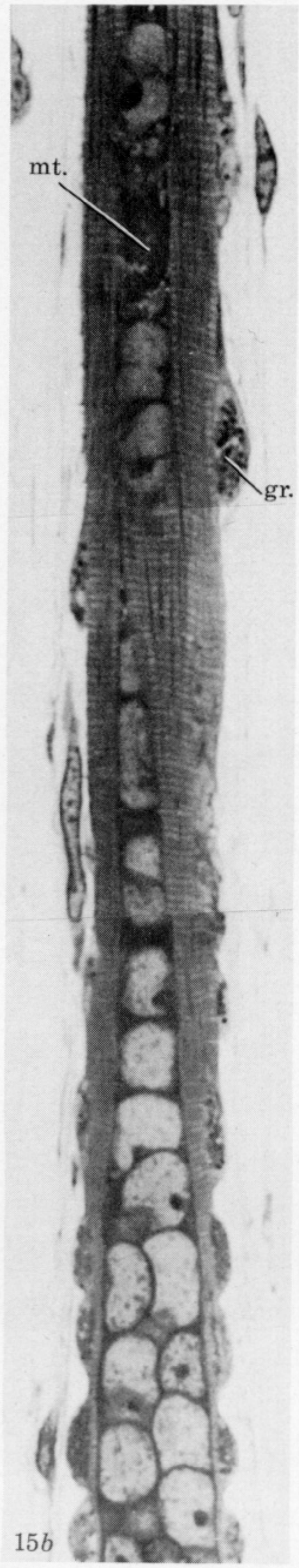






FIGURES 9–14. For description see opposite.

15a



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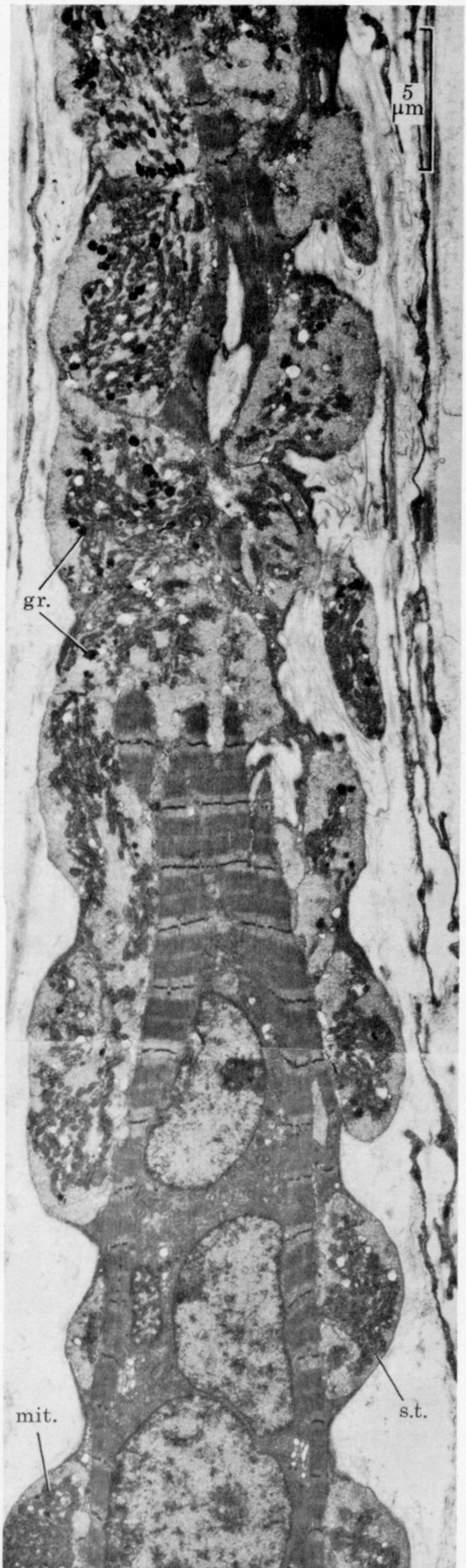

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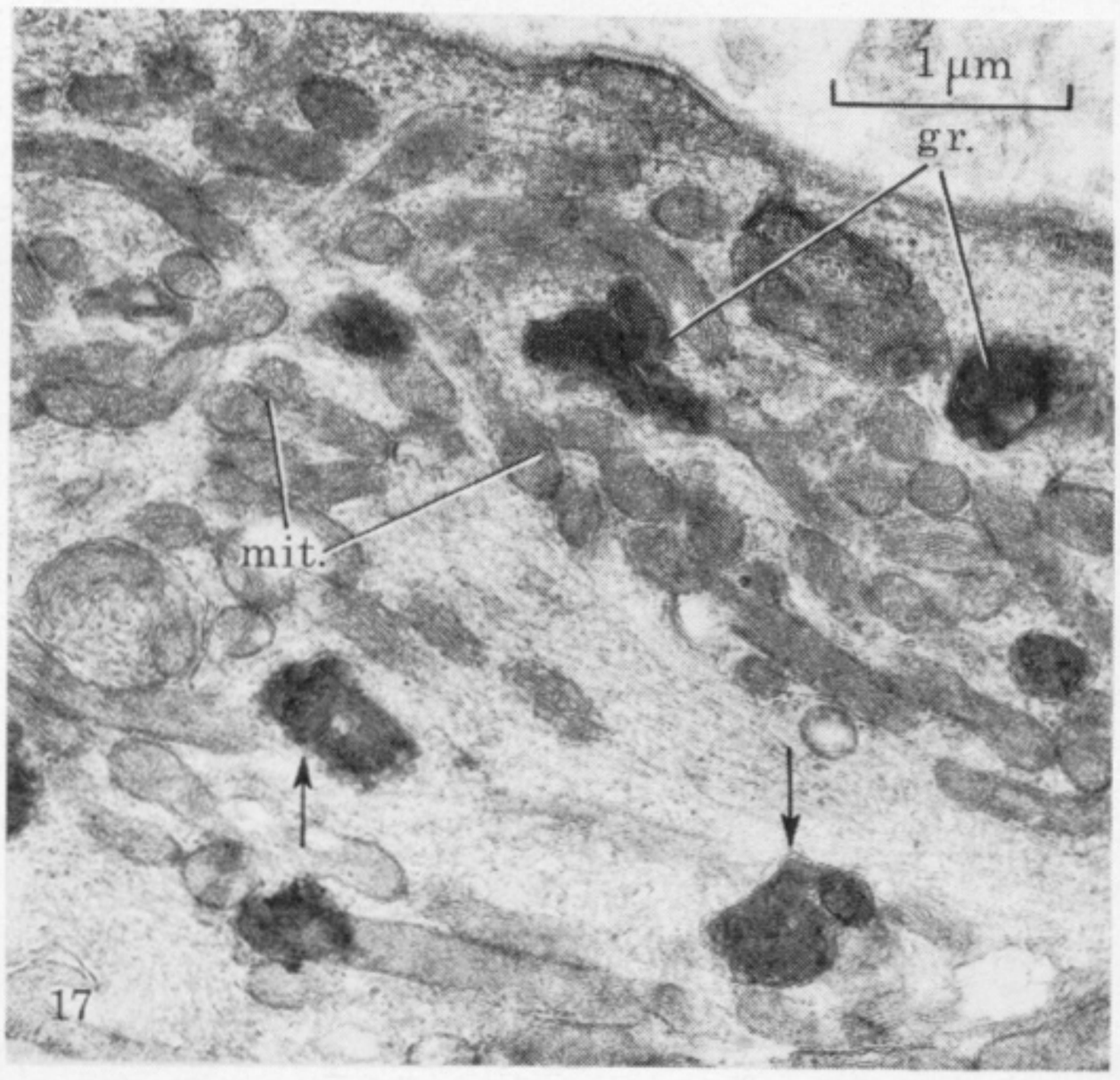

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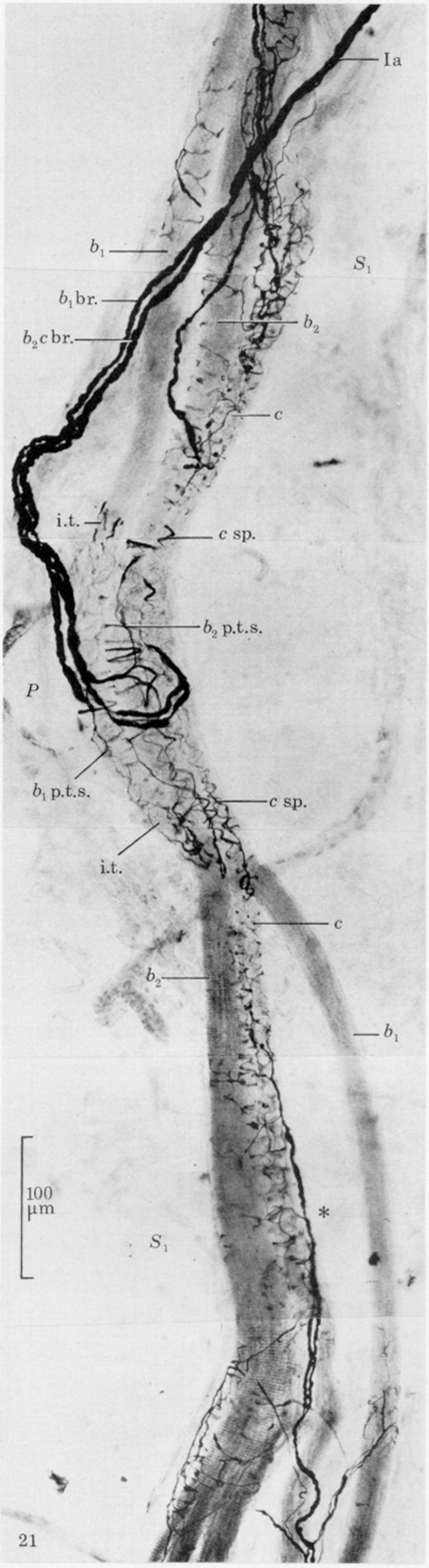
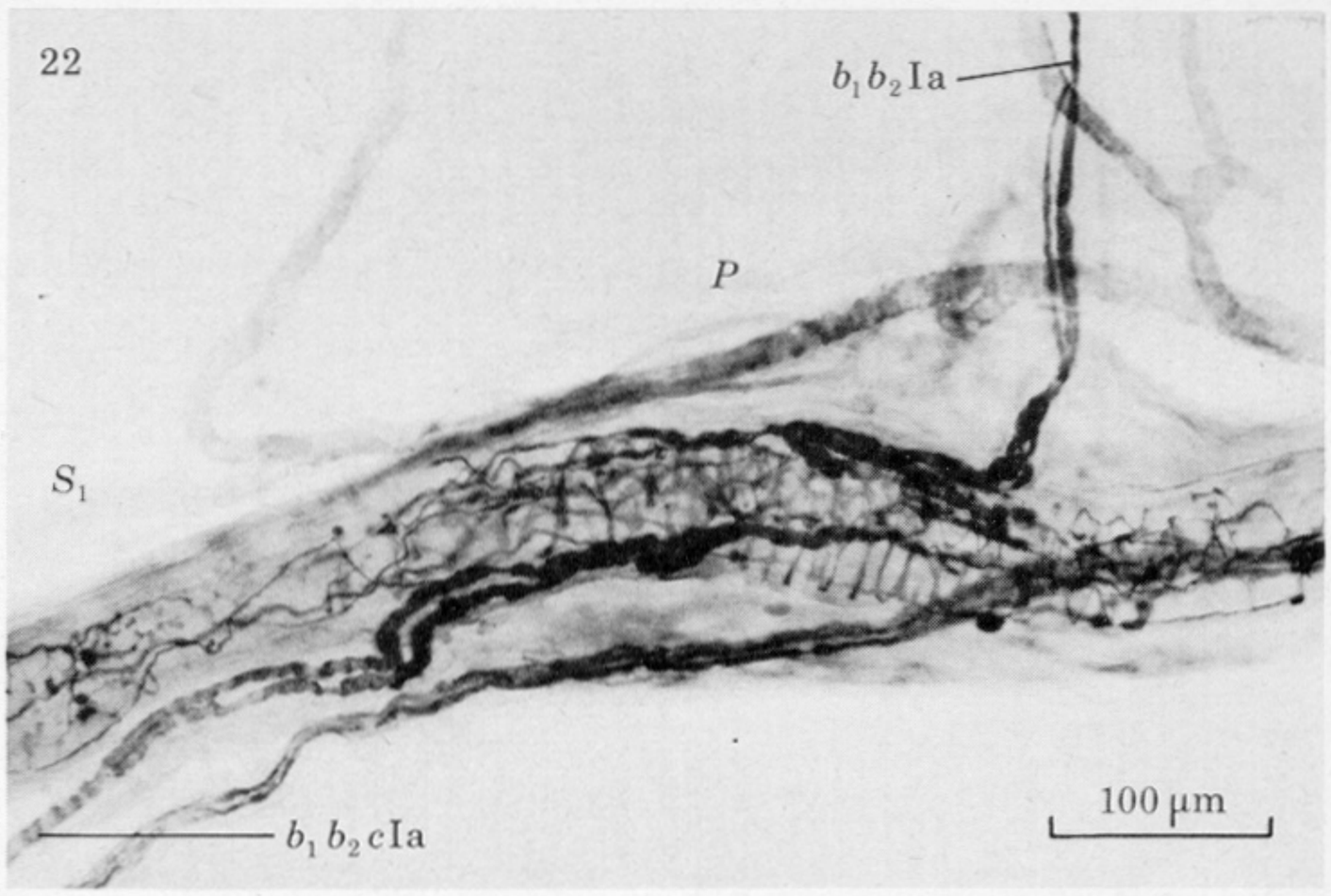
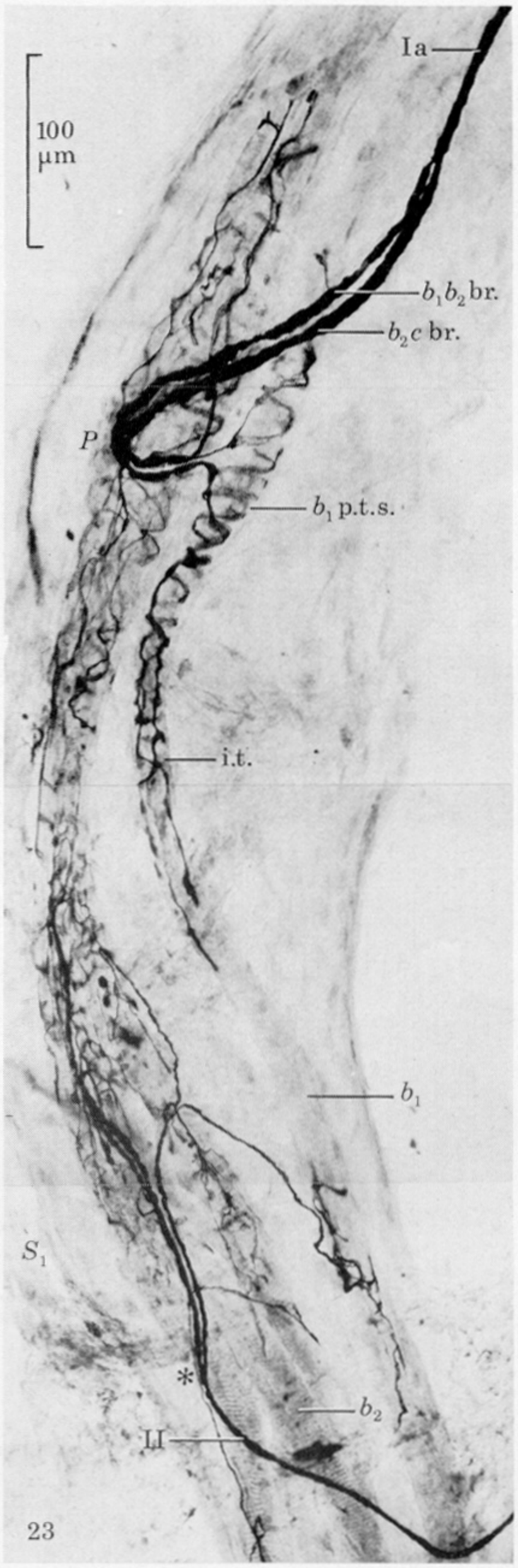
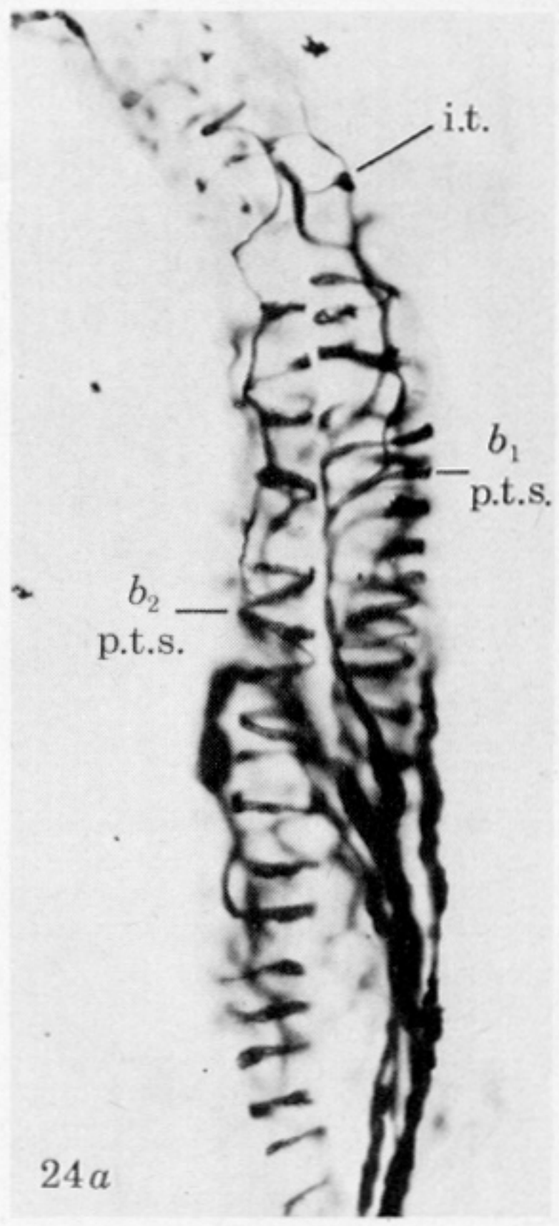
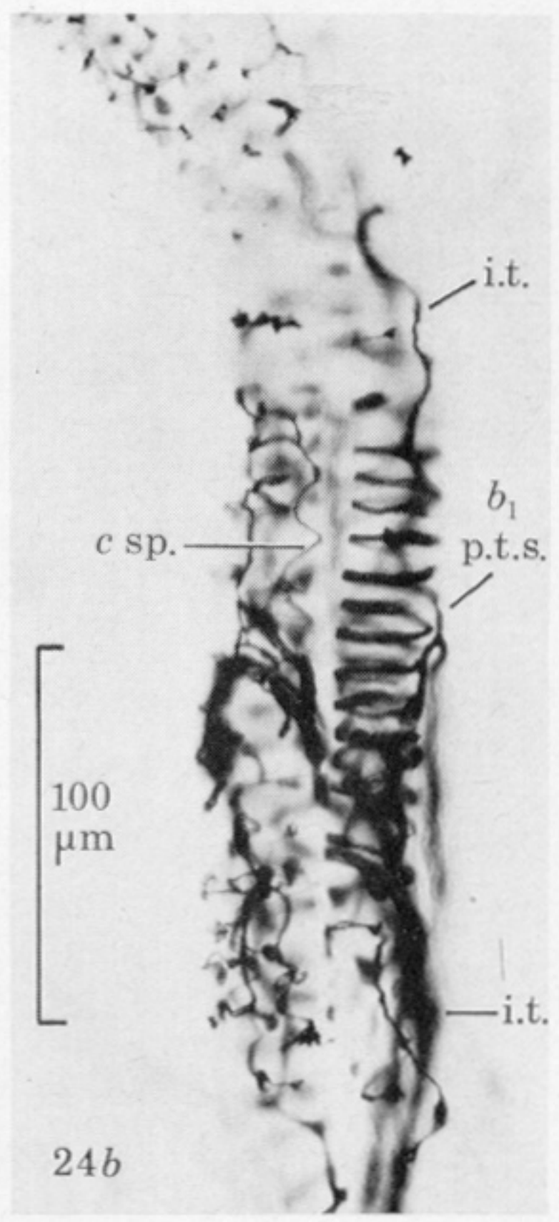
FIGURES 15–17. For description see opposite.



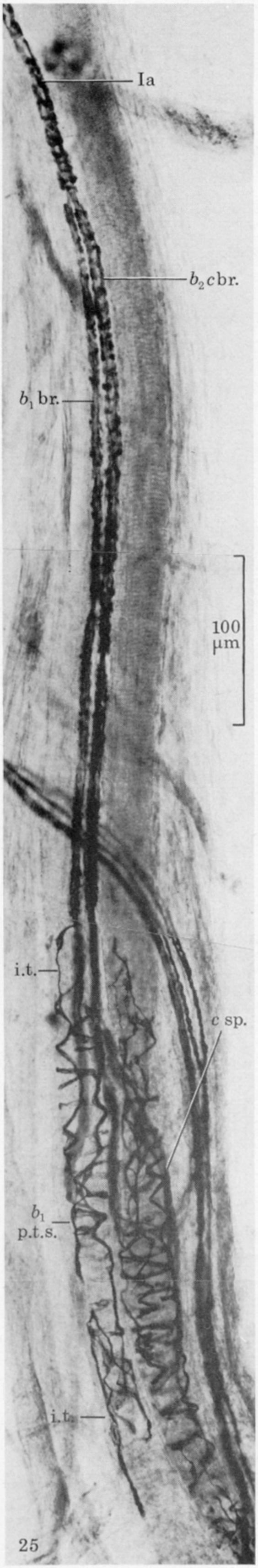








FIGURES 21–24. For description see opposite.



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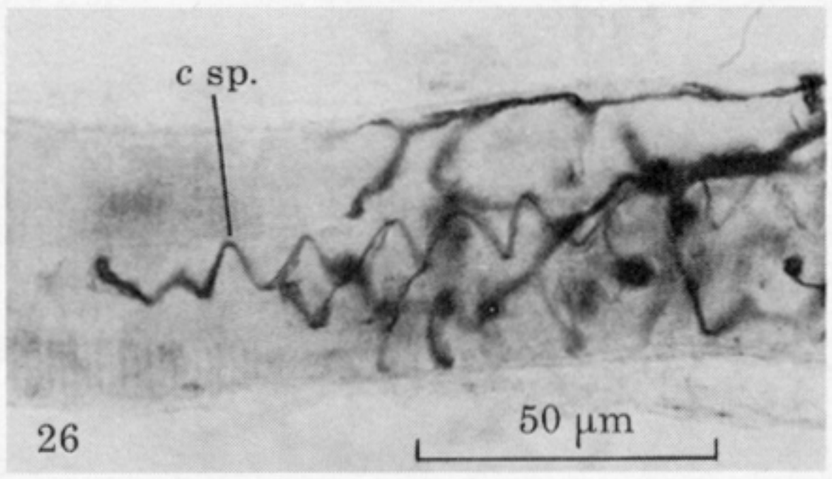

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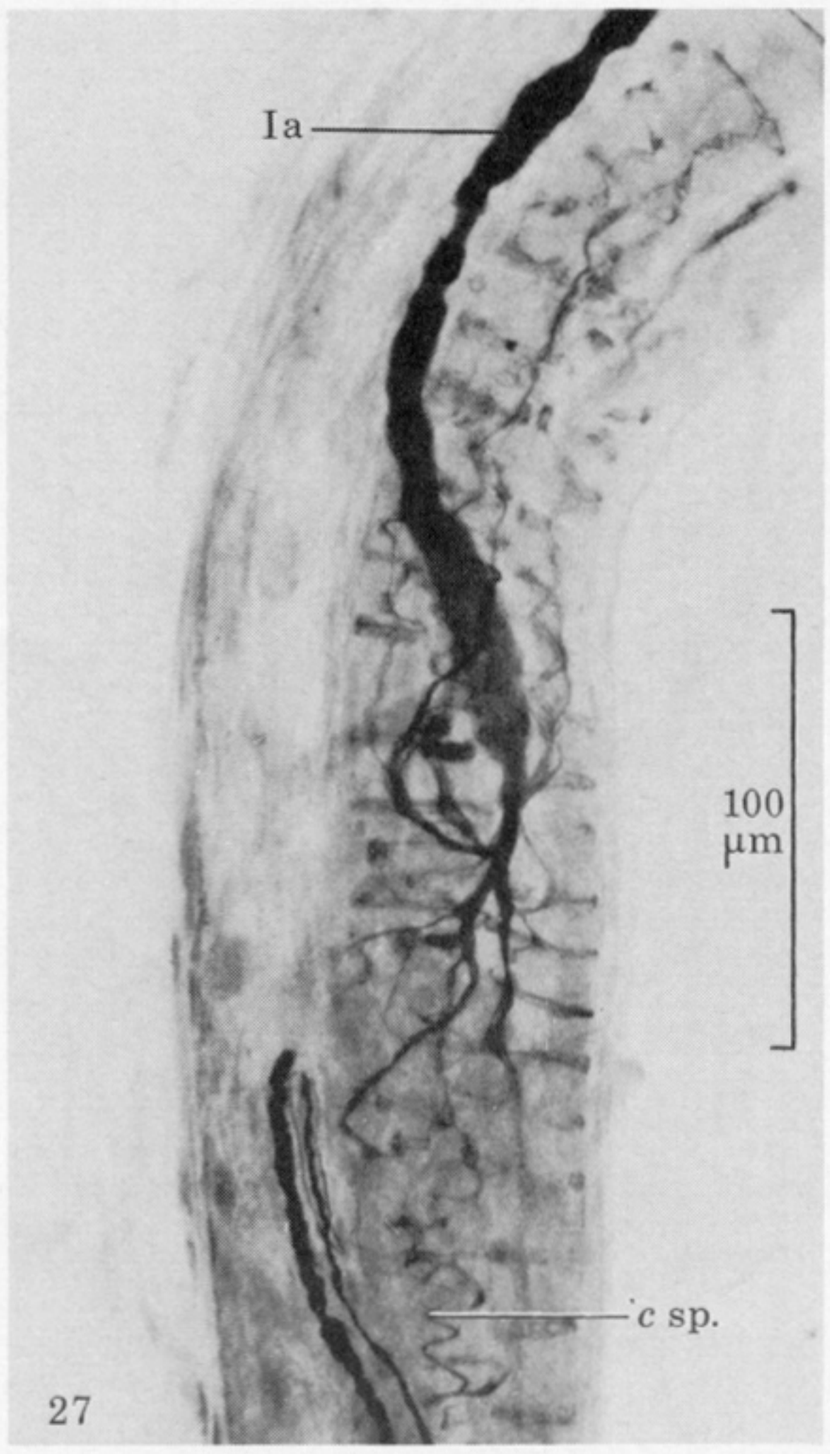

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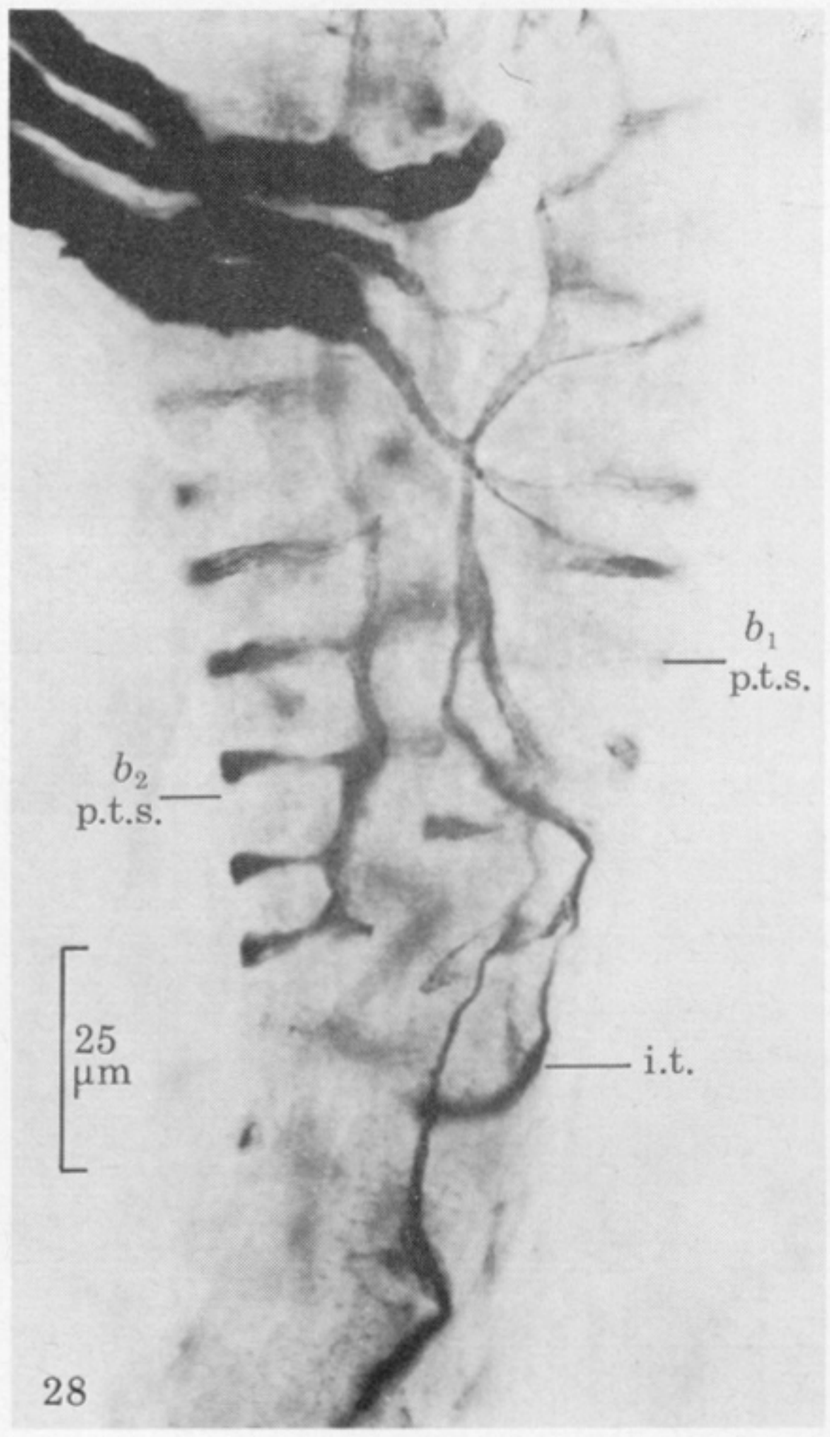

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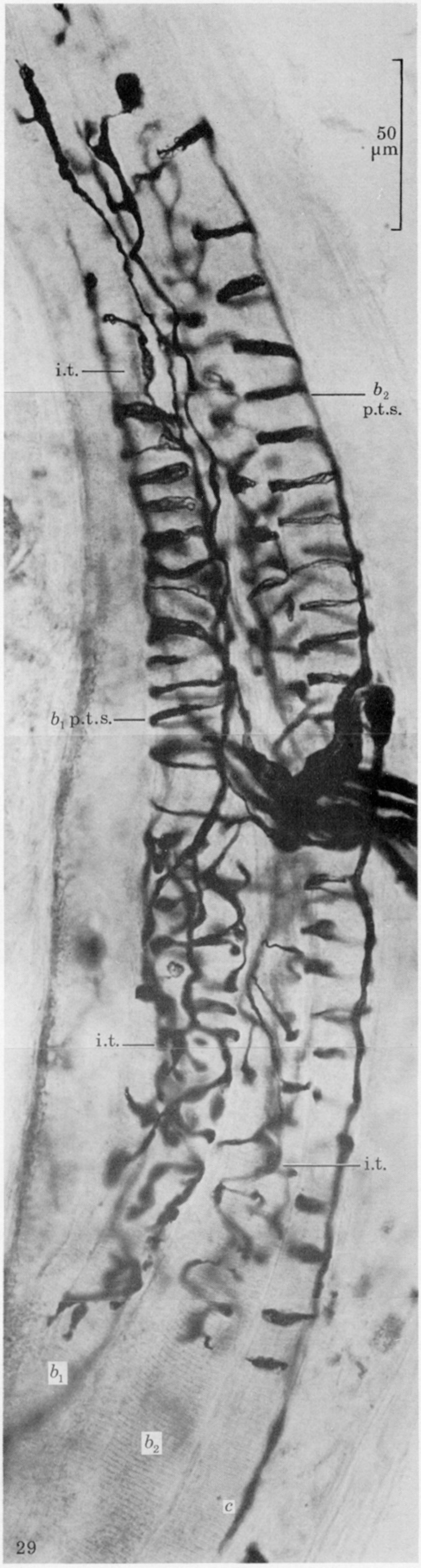

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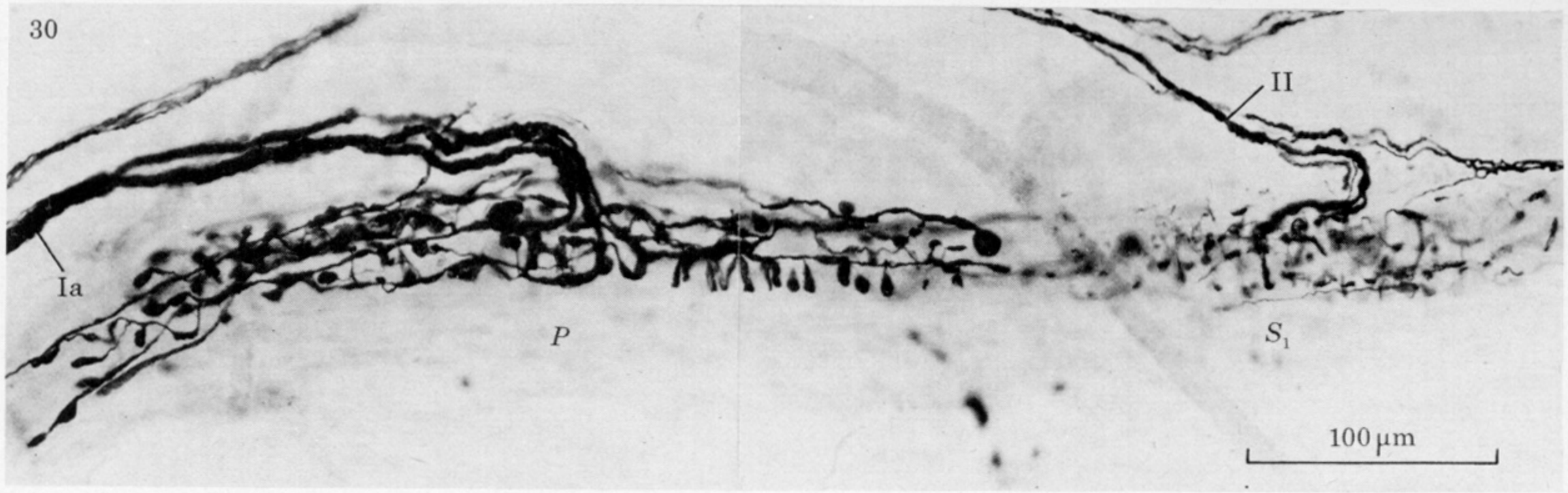
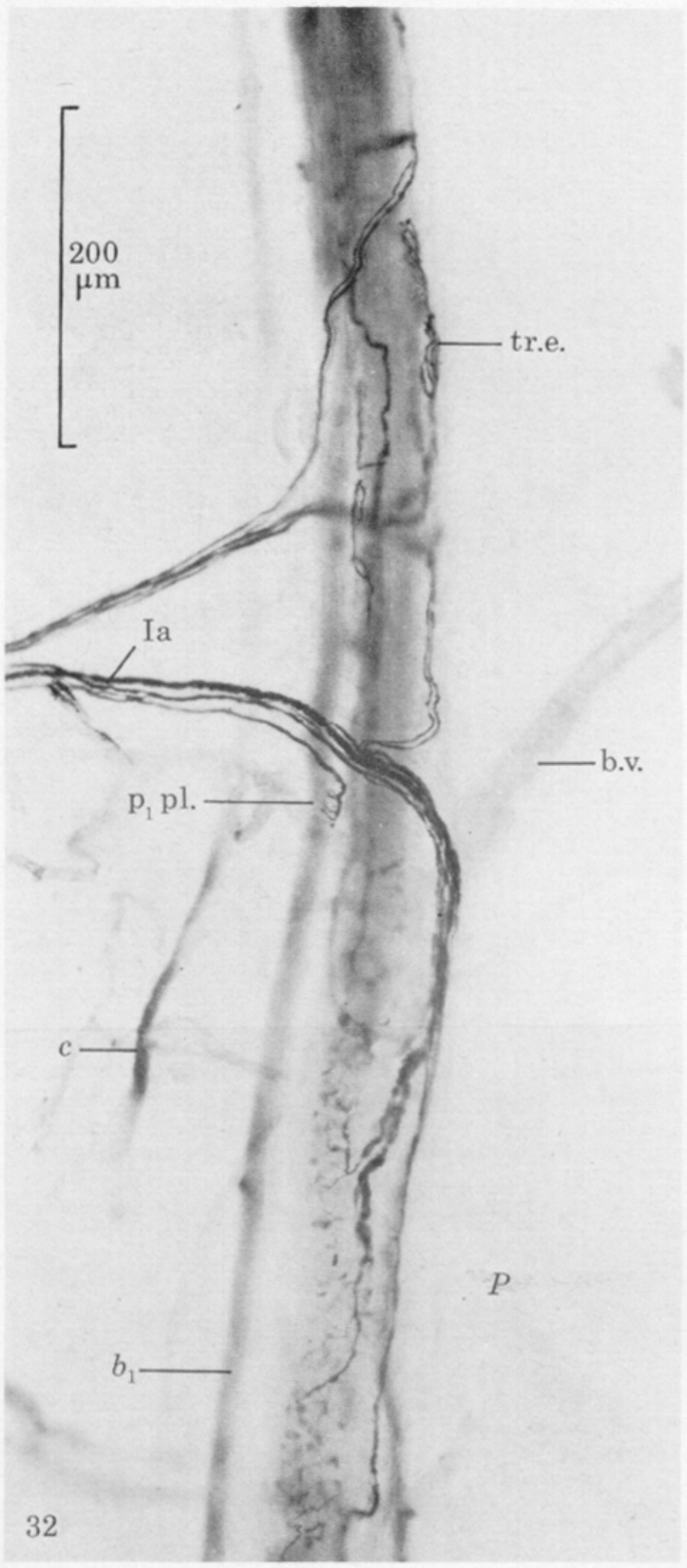
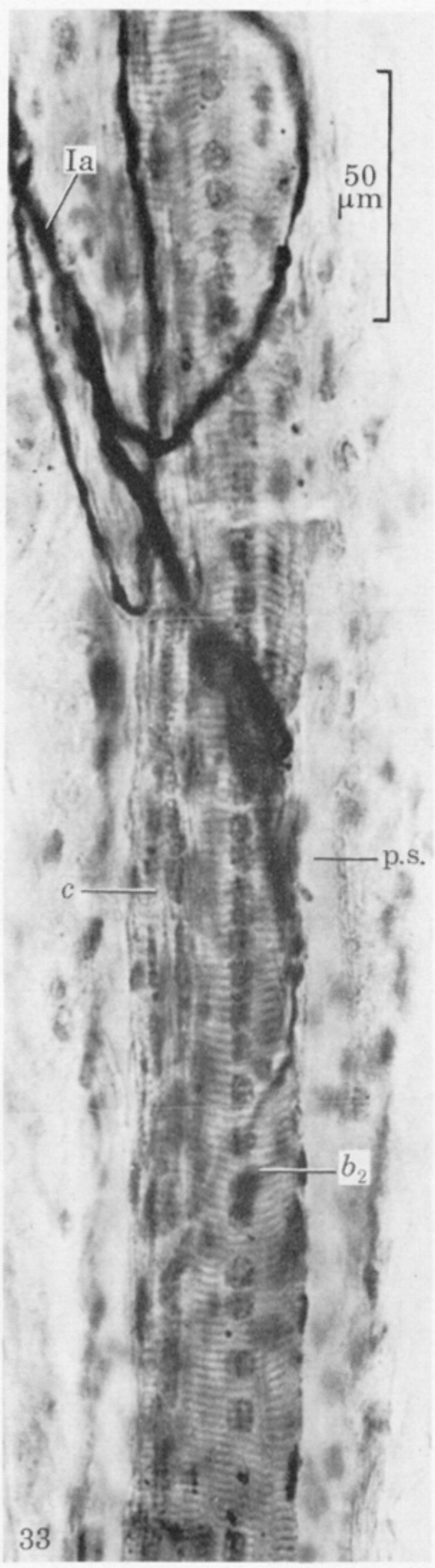
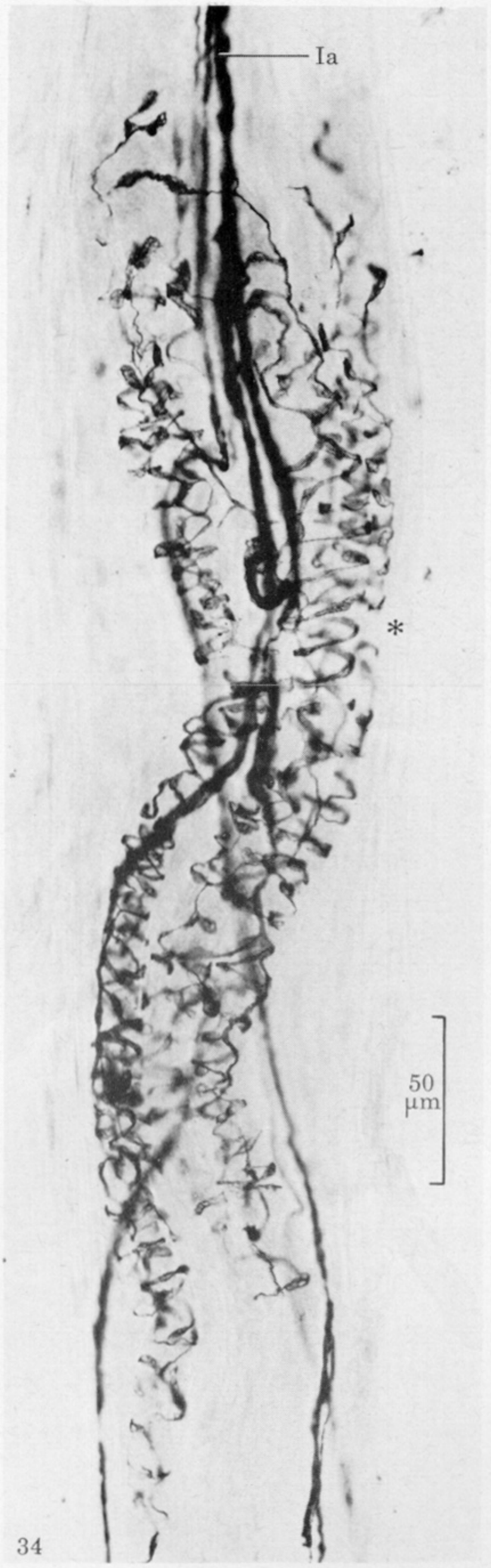
FIGURES 25–29. For description see page 356.

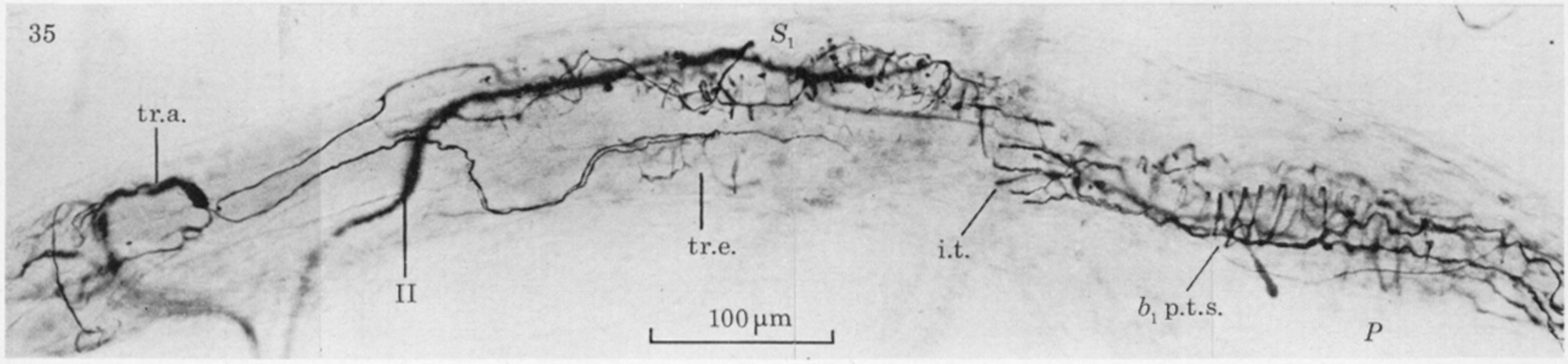


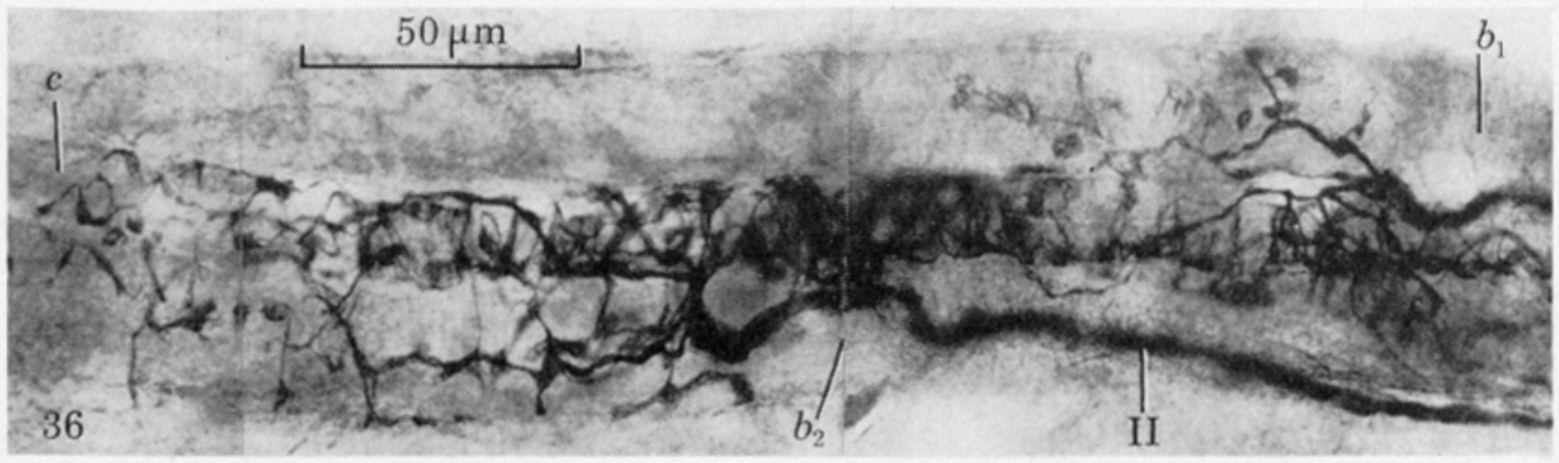


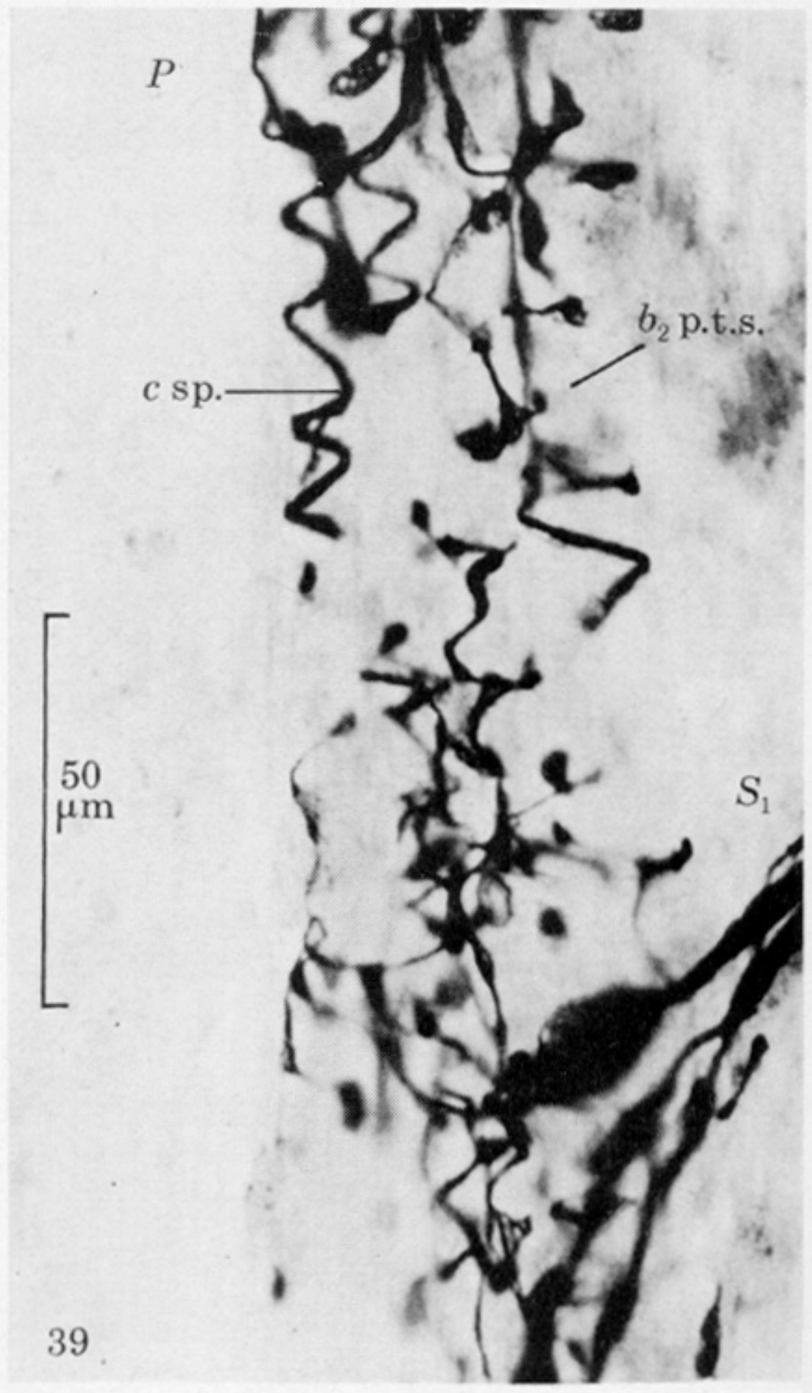


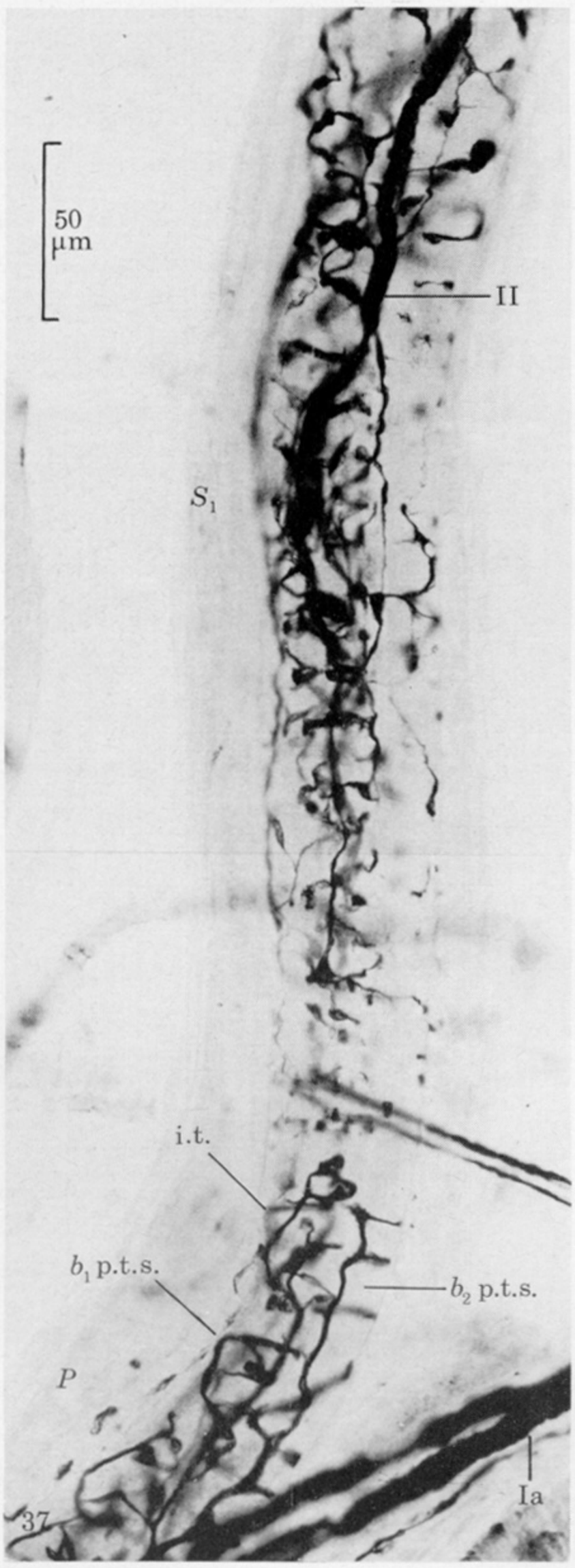


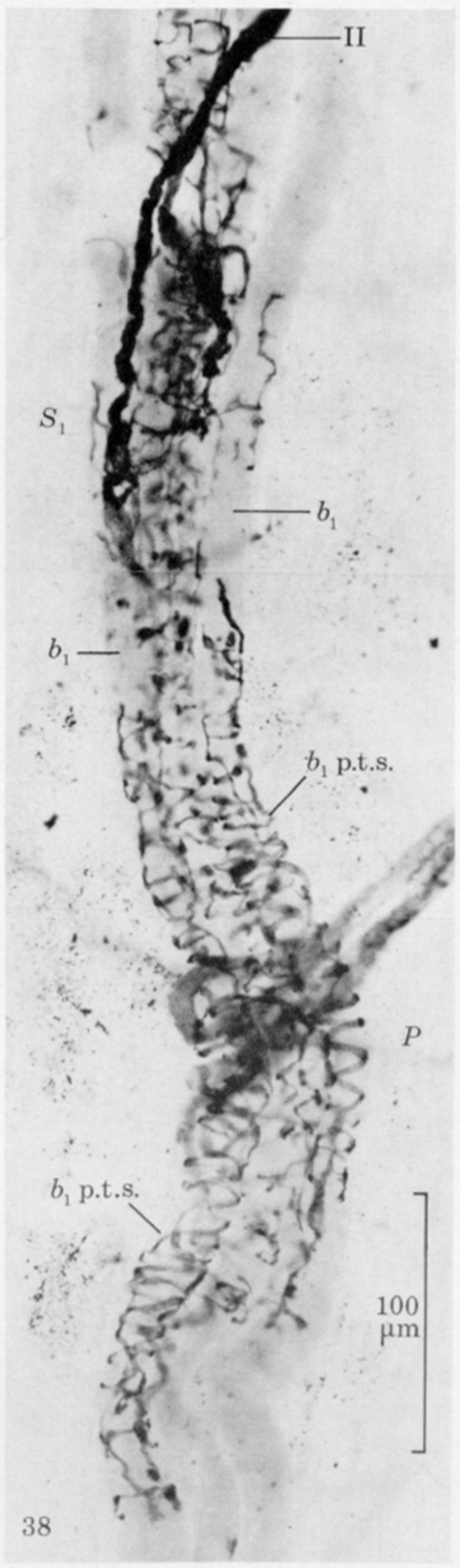
FIGURES 30–34. For description see page 357.

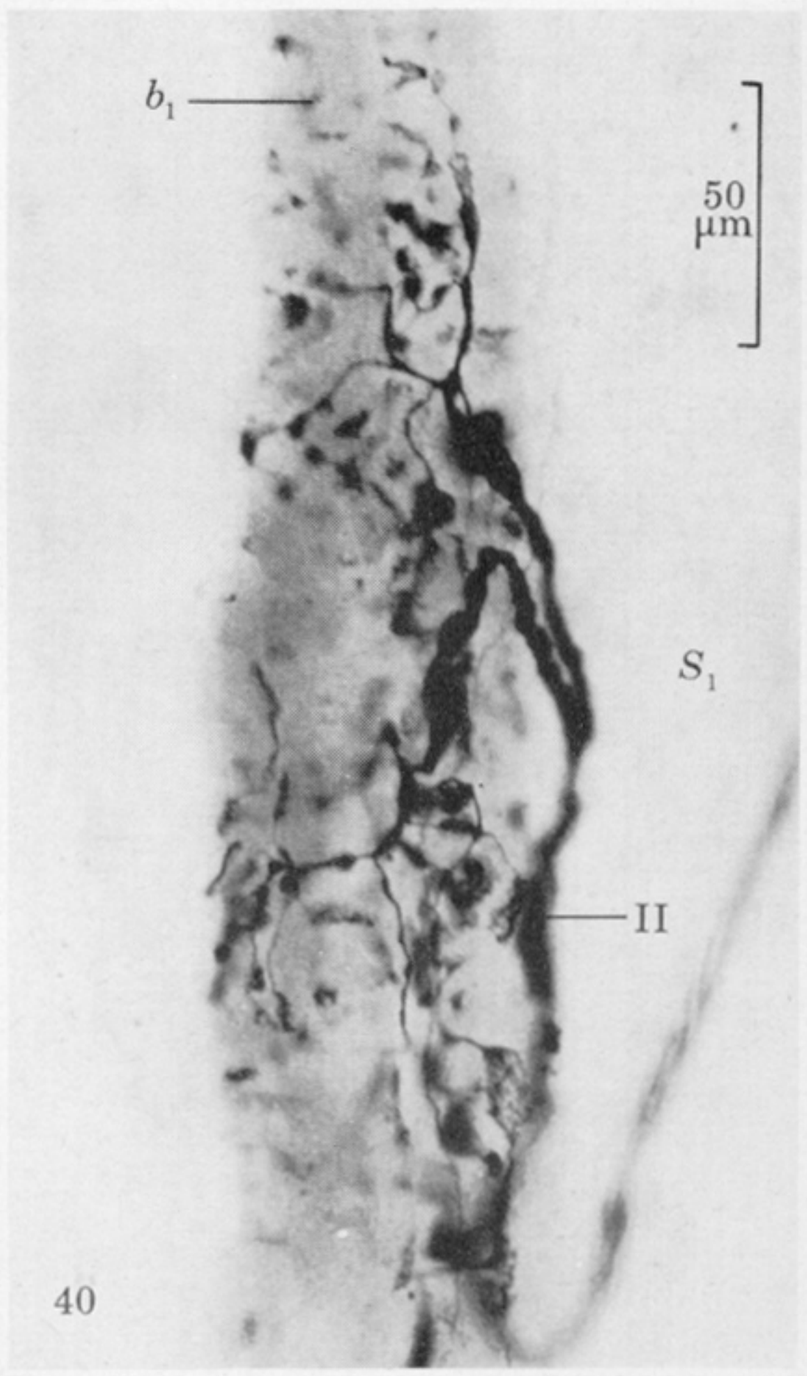












FIGURES 35–40. For description see opposite.