The effect of neonatal nerve crush on adult rat muscle spindles

Elizabeth Laidler

THE EFFECT OF NEONATAL NERVE CRUSH

ON ADULT RAT MUSCLE SPINDLES

TEXT

A thesis presented in candidature for the degree of Master of Science

by

Elizabeth Laidler, B.Sc. (Dunelm)

Department of Zoology, University of Durham.

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CONTENTS OF TEXT

ABSTRACT

A methodology for producing a workable number of chainless/bag-only spindles, (otherwise well developed), in the medial gastrocnemius muscle of the adult rat was explored by means of a series of neonatal nerve crushes. The single-fibre and two-fibre spindles thus produced as permanent structures in the adult were termed " model-adult " spindles. Nuclear bags were well preserved in all spindles designated as model-adult. A histological study was made of these spindles.

Length was the most affected spindle dimension. Most model-adult spindles were shorter than normal, the decrease involving the polar lengths (regions B and C; see Banks et al, 1976) rather than the periaxial lengths (region A).

Apart from some myofibrillar disarrangement and a general increase in sarcoplasm, the fine structure and histochemistry of model-adult bag fibres was comparable to that of control bag fibres (certainly, in the two-fibre spindles). Thus, the bag_1 fibre (Banks et al,'76) lacked an M line throughout its length whereas the bag_2 fibre lacked this feature only in region A. The presence or absence of an M line in many of the single-fibre spindles could not be ascertained because of sarcomere " blurring ", but in some, a double M line could be recognised throughout the entire length of the bag fibre. The identity of this fibre is discussed in the light of its M line structure, its

consistently pale reaction with alkaline ATP'ase and its apparent greater susceptibility to denervation, as evidenced by monitoring the process of degeneration with electron microscopy.

Primary sensory endings were present in all model-adult spindles, but secondary endings occurred in fewer spindles than normal. As regards the form of the primary ending, fewer annulospirals were in evidence. Ultrastructurally, however, the sensory terminals appeared quite normal.

There were fewer fusimotor axons (particularly, trail axons) per model-adult spindle, the reduction reflecting the absence of chain fibres. Of the two types of fusimotor plate recognised in the control spindles, the smaller type remained intact in model-adult spindles. However, the larger type appeared to have been radically altered by the nerve crush; they were reduced to simple, button-like swellings.

The possible physiological implications of the findings are discussed.

INTRODUCTION

In skeletal muscles of the rat, spindles begin to develop in the late foetal stages (three days before birth) and their differentiation is completed during the first two postnatal weeks (Zelená, 1957: Marchand and Eldred, 1969; Landon, 1972; Barker and Milburn, 1972; Milburn, 1973 a, b). Prior to this at the myotube stage of muscle ontogenesis, the myotubes appear to be a homogeneous population of cells (Cuajunco, 1940; Zelená, 1957; Zelená and Hnik, 1963; Milburn, 1973 a). There are no obvious morphological differences to enable one to determine which myotubes are precursors of intrafusal fibres and which are precursors of extrafusal fibres. There is now ample evidence to show that the primary (Group IA) sensory innervation is the critical influence responsible for myotube differentiation along such different pathways, although the precise way in which the influence is mediated still remains unknown.

At birth, the rat spindle consists of two myotubes with developing nuclear bags supplied with sensory terminals, together with a presumptive nuclear-chain myotube. The motor innervation begins to arrive at this stage. By the fourth postnatal day, the normal complement of four intrafusal fibres - two bag fibres and two chain fibres - are present, but are still undifferentiated ultrastructurally. The differences in myofibril architecture as seen in the adult, between the two types of intrafusal fibre, become manifest by twelve days of age.

The first part of this introduction will deal with a more expanded account of normal spindle development in the rat, as defined by electron microscope investigations (Landon, 1966,1970,1971,1972;Barker & Milburn, 1972; Werner, 1972; Levy,1972 thesis;Milburn,1973&thesis,1973)

of such ultrastructural details on this aspect will be borne out in the electron microscope results of this thesis, particularly those analysed shortly after surgery with regard to the sequence of " de-differentiation " and re-differentiation changes that follow denervation.

A resumé of the histochemical development of rat intrafusal fibres will follow, albeit a rather unstaisfactory account, due to the incompleteness of the literature on this and other species of mammal.

The results of various neonatal nerve-lesion experiments will then be described - largely with regard to their effect on intrafusal fibres - in order to show the extent to which normal spindle development depends on normal innervation. Finally, in the light of these experiments, the objective of the present research will be outlined and rationalised.

Normal ultrastructural spindle development in the rat In order to avoid repetition, specific acknowledgements to the author's referred to on page 1, will be omitted in the following section.

At the early primordial stage of spindle development (i.e., at the 18th - 19th day of gestation), each spindle consists of a single myotube which bears simple, sensory nerve terminals - those of the large Group I (Erlanger & Gasser, 1968; Ganong, 1968; Mountcastle, 1974) primary axons. Myelination is still lacking around the axons comprising the spindle nerve trunk. The perineural epithelium of the spindle nerve trunk extends to form the capsule,

which is confined at first to the sensory innervated zone.

By the 20th day of gestation, some of the myoblasts present within the capsule fuse to form a smaller, less diffe differentiated myotube, which shares its basement membrane with the first intrafusal fibre, the apposed membranes sometimes showing areas of specialization that resemble the close junctions described by Kelly and Zacks (1969a). There is an obvious difference in sarcoplasmic maturity between the two myotubes. The myofibrils of the first fibre are well packed, even if still limited to the periphery, whereas those of the second fibre are very sparse.

The sensory terminals have only reached the outer surface of the axial bundle at this prenatal stage of development. They are still immature, being of the overlapping or bundled type (Landon, 1972 b; Milburn, thesis 1973; Uehara, 1973). Unlike the second fibre, the first fibre is no longer myotubular; a bag of nuclei starts to form in its central sarcoplasm in the equatorial region.

By birth, the capsule increases to several layers at the equator. In the polar regions, the intrafusal fibres are covered by but a single layer of similar cells. The pseudopodial interlocking of the two intrafusal fibres disappears as myoblasts begin to separate their apposed surfaces. The second fibre has also matured considerably and contains a small equatorial bag of nuclei as well. In particular, birth is marked by the appearance of a third intrafusal fibre, of about half the calibre of the nuclear-bag fibres. It is, in fact, a nuclear-chain fibre, possessing a central chain of nuclei at the equator. All three fibres are myotubular in the polar region.

The nuclei of the extrafusal fibres have, on the other hand, become peripheral. Even so, immature myotubes may still be encountered in pseudopodial apposition to their more mature neighbours.

The equatorial sensory terminals of newborn rat spindles appear greater in number and size than those of foetal spindles, but they are still confined to the outer surface of the axial bundle. A common basement membrane continues to ensheath all three fibres and their sensory innervation. Only occasional terminals extend between the intrafusal fibres, where the invading terminal is shared between two fibres. Such sensory terminals have also been found in between chain fibres of adult cat spindles (Adal, 1969). Most sensory terminals now adopt a spiral course around the outer surface of the axial bundle, but a few overlapping ones may still be in evidence. The axons of the spindle nerve trunk still lack myelin sheaths and individual Schwann cells, although the number of axons per Schwann cell has decreased.

Besides the appearance of the third intrafusal fibre, birth is also marked by the arrival of γ fusimotor innervation, both polar and juxta-equatorial, on the more mature nuclear-bag fibres. (β innervation and perhaps even γ plate innervation, is thought to have become established one or two days before birth. Refer to Milburn, 1973.). At both levels, the terminals appear as single or multiple small end bulbs (Milburn, Ph.D.Thesis, 1973; Landon, 1972b) that are covered to some extent by Schwann cell cytoplasm.

In common with their extrafusal counterparts, but in contrast to sensory endings, there is always a distinct basement membrane between the axon terminal and the muscle fibre. The post-junctional sarcolemma of the juxta-equatorial and mid-polar terminals is consistently smooth, and even the polar terminals lack any folding of the post-junctional sarcolemma that would suggest the formation of secondary clefts, a feature of (at least some) adult fusimotor end plates. On the other hand, whereas the juxta-equatorial and mid-polar terminals show no sign of sole-plate formation at birth, there is some indication of sole-plate specialisation in the polar terminals.

According to Teräväinen (1968b) and Kelly and Zacks (1969b) extrafusal motor terminals of new born rat skeletal muscles are similar morphologically to fusimotor terminals, apart from their larger size, greater accumulations of synaptic vesicles, their indentation (for telling primary cleft formation) of the wall of the muscle fibre and their more distinctive sole plate.

Some two days after birth, a second chain fibre appears, developing along the same lines as its more mature partner. The full complement of intrafusal fibres is achieved by the fourth post-natal day (4DPN). The second chain fibre may still appear immature. However, the polar myonuclei are now mostly peripheral, as in the adult, but unlike the latter, the polar diameters of the intrafusal fibres are still clearly heterogeneous. There is a distinct M line present in the centre of the pseudo H zone of all four intrafusal

fibres, and each myofibril within the fibres is circled with sarcoplasm. Pseudpodial inpushings are now uncommon and each muscle fibre has its own basement membrane. Moreover, cells from the inner layer of the capsule are in the process of forming individual endomysial sheaths around each fibre at the level of the equator.

The juxta-equatorial and mid-polar fusimotor innervation of 4DPN spindles is much more extensive than at birth as judged by the greater frequency with which the terminals are encountered in transverse sections.

At 12 - 16 DPN, the periaxial space is well developed and the endomysial envelopes of the fibres are more complete. The overlapping sensory terminals that characterise immature spindles are succeeded by the semi-lunar profiles of the adult primary annular-spiral endings. Secondary endings, tentatively identified as such by virtue of their juxta-equatorial position, can be seen on the nuclear-chain fibres at this stage of ontogenesis.

Likewise, motor terminals are more like those of the adult spindle in nature and abundance.

As in the adult, differences in fibre diameters are maintained at the equator, but no longer in the polar regions. Satellite cells adopt a similar position to the myoblasts of developing spindles; they are found beneath the basement membranes of the muscle fibres, whose round profiles are minimally distorted by their presence.

Approximately 2½ weeks after birth, the ultrastructural profiles of the adult have differentiated. Thus, Milburn

(1973) has shown that the second nuclear-bag fibre to develop (which is now identified by Banks, Harker and Stacey (1976) as Barker et al's (1976) " bag_1 " fibre) has lost its M line throughout its entire length, whilst the first nuclear-bag fibre to develop (" bag_2 ") has lost this structure solely in the equatorial region (Barker et al's (1976) region A). The nuclear-chain fibres completely retain a well defined M line. In the nuclear-bag fibres, the loss of the M line sometimes takes the form of two pale lines - a " double " M line. Refer to Table I. As regards myofibrillar content, the nuclear-chain fibres contain more extensive interfibrillar sarcoplasm and larger, more numerous mitochondria than the nuclear-bag fibres.

Electron microscopic studies of sensory innervation in rat spindles from various muscles in the adult have been carried out by several researchers (Hennig, 1969; Merrillees, 1960; Landon, 1966; Rumpelt & Schmalbruch, 1969; Mayr, 1970). Their descriptions are all very similar and will now be outlined briefly.

Spindle sensory endings are characterized by the absence of Schwann cell coverings as well as the absence of basement membrane in the synaptic gap, it being confluent with the one lining the muscle fibre. The inclusion of the sensory endings within the basement membrane of an intrafusal fibre must clearly be a consequence of the fact that during development, these endings make synaptic contact at a stage when the muscle fibres are separating from

themselves and acquiring individual basement membranes (cf motor terminals, below).

Transverse and longitudinal sections have shown that along nuclear bag/chain and myotube regions, sensory endings wrap round the muscle fibres in an annulo-spiral configuration and cause indentations of the intrafusal fibres. The indentations are bound by " sarcolemmal lips ", which often completely engulf the endings within the fibre. The terminals themselves are filled with numerous mitochondria, a few vesicles (200 - 700 Å in diameter), fine branching tubular systems (some 30 μm in diameter), dense bodies and multitubular bodies (20 μm in diameter). The nature and number of these organelles vary from point to point round the spiral. Generally, the larger profiles (round the nuclear bags) contain the bulk, the smaller profiles (in the myotube region) often containing a few vesicles only. The synaptic gap varies from 150 - 200 Å, but at some points where the post-synaptic sarcolemma is thrown into corrugations, the gap may be wider than this. Landon (1966) found no evidence of tight junctions between the synaptic axolemma and the sarcolemma, but Rumpelt and Schmalbruch (1969) differed on this point.

The electron microscopic research on fusimotor endings in the adult rat has not met with as much concord among the various workers (Merrillees, 1960; Landon, 1966; Hennig, 1969; Ovalle, 1972). However, they more or less agree on (i) the existence of three kinds of ending: the multiterminal type at the juxta-equator and two types

of polar plate and (ii) the two major diagnostic features of any motor ending i.e., an attenuated covering of Schwann cell and an intervening basement membrane in the synaptic gap and (iii) the organelle content of the axon terminal, namely, an abundance of clear, round vesicles and small, dense-core vesicles. The former are more prevalent in plate terminals, the latter more so in trail terminals.

Trail endings are characterized by a wide (750 - 1,400 Å), smooth synaptic cleft, i.e., junctional folds are lacking. In addition, there is no sole plate. In bag fibres, the myofibrils are more compactly packed beneath the post-synaptic membrane than in chain fibres. Trail endings are invariably located on the rounded surface of the muscle fibre so that thay do not cause any indentation of the muscle fibre.

Ultrastructural categorisation of the plates has been more problematical. Ovalle (1972) recognises " nuclear-bag " plates and " nuclear-chain " plates which, as their names imply, are located either specifically on the bag fibres or specifically on the chain fibres. The main difference that Ovalle draws between the two lies in the extent of myoneural surface contact and the extent of development of junctional folds. " NB " plates exhibit a small surface of contact and lack junctional folds. The axon terminals themselves are usually quite small and unbranched. The width (300 - 500 Å) of the synaptic cleft is fairly uniform. A sole plate is lacking so that only occasionally are sole-plate organelles present.

In contrast, " NC " plates exhibit a greater area of

myoneural contact and possess a moderate number of unbranched junctional folds. The axon terminals are larger and branched. The primary synaptic cleft is often as twice as wide as for " NB " plates, but twice as narrow as for trail endings. There is a well developed sole plate containing accumulations of mitochondria, vesicles, microtubules and cisternae of rough sarcoplasmic reticulum.

Hennig's (1969) endings of the outer polar regions equate well with Ovalle's " NC " plates. Thus, Hennig describes unbranched junctional folds, terminal bulbs scattered irregularly over the surface of the intrafusal fibre, a Doyere eminence and a sole plate containing nuclei. The other type of ending Hennig located at the " beginning " of the polar region. Like Ovalle's " NB " plates, junctional folds are absent, but the end-bulbs (occurring in a row) are deeply enclosed by sarcoplasm and the synaptic gap is similar to that of trail endings.

Merrillees (1960) sectioned polar fusimotor which he describes as being similar, but simpler, than extrafusal plates. These plates correspond, in large part, to Ovalle's " NC " plates and Hennig's outer polar plates. Infolding was present in some of Merrillees' plates, but he found no Doyere eminence. Moreover, only some had a sole plate with numerous organelles that included Golgi bodies.

The plates that Landon (1966) described were located on the extracapsular (region C) regions of bag fibres. In many respects, these plates appeared very much like Ovalle's " NB " plates and Hennig's outer polar plates.

Table I. Summary of some of the more important features of the three types of intrafusal muscle fibre from hindlimb spindles of three mammalian species: cat, rabbit and rat. Compiled from Banks et al (1976). Key: dM=double M line or none. M=M line. Region A=equator+ juxta-equator. Region B=encapsulated pole. Region C= "naked" pole.

TYPE OF INTRAFUSAL FIBRE		DIAMETER	LENGTH	DEVELOPMENT	ALKALINE ATP'ASE ACTIVITY	M-LINE CONDITION
BAG_1 FIBRES	CAT	medium	shorter than b_2 fibres	*	low	dM switching to M in region B
	RABBIT	similar to chains	usually same length as b_2 fibres	second fibre formed		dM switching to M in region C
	RAT	medium	"			dM
BAG_2 FIBRES	CAT	largest	usually longest	first fibre formed	medium/ high	dM for short stretch adjacent to nuclear bag ie ~ region A. Otherwise M for rest of length.
	RABBIT	"	usually same length as b_1 fibres			
	RAT	"	"			
CHAIN FIBRES	CAT	thinnest	some NCs may be as long as b_1 fibres but always shorter than b_2 fibres	last fibres to be formed	high	M-line present throughout length
	RABBIT	"	shortest			
	RAT	"	"			

* At the equator, the bag_1 fibre of the cat is usually found dissociated from the b_2 fibres and the chains, these latter being closely associated. No such arrangement is found in rabbit or rat spindles.

Thus, a sole plate and junctional folds were lacking. However, a feature less like " NC " plates and more like Hennig's plate was the indentation of the muscle fibre.

Normal histochemical development of rat intrafusal fibres The histochemistry of adult rat spindles will first be described. The literature shows that 3 types of fibre have long been recognised. According to their oxidative/glycolytic activity, they resemble the three extrafusal histochemical types: A (low oxidative/high glycolytic), B (moderate oxidative/low glycolytic) and C (high oxidative/moderate glycolytic). Yellin (1969a) found that the bag fibres fell into the A or B category or a third type (low oxidative/low glycolytic), which was uncommon extrafusally. The chain fibres fell into the C category. Like their extrafusal counterparts, the intrafusal fibres possessed the following diameter-type relationship: A > B > C. Yellin admitted, though, that other intrafusal fibre types appeared to be present (also Arendt & Asmussen, 1974).

James (1971a) also described three fibre types on the basis of oxidative, glycolytic and myosin ATP'ase activities (although he often found it possible to identify the three types in single sections stained only for SDH). Type 1 fibres (chain fibres) had a high oxidative/high glycolytic profile (cp Yellin, 1969a) and reacted strongly with alkaline ATP'ase. They contained many large mitochondria. Type 2 fibres (bag fibres) had a moderate oxidative/low glycolytic profile (cp Yellin, 1969a) and reacted strongly

with alkaline ATP'ase. James found they contained small mitochondria, regularly distributed. Type 3 fibres (bag fibres) possessed a low oxidative/high glycolytic profile (cp Yellin, 1969a) and gave a weak reaction with alkaline ATP'ase. Their mitochondria were variable in size and few in number.

The presence of more than two histochemical types of intrafusal fibre in the rat has also been proposed by Ogata and Mori (1962, 1964) and James (1971b). In a review on muscle spindles, Barker (1974) focusses the evidence for the presence of two distinct types of nuclear-bag fibre in adult spindles of the rat and other mammalian species. More recent experiments (Banks, Barker, Harker and Stacey, 1975; Banks et al, 1976), involving a direct histochemical/ultrastructural correlation of one and the same intrafusal fibre, has served to remove any ambiguities. They surmise that " the three types cannot always be differentiated on the basis of any single technique, whether morphological, histochemical or ultrastructural. Application of any one technique to the muscle spindle usually results in the inclusion of two of the types within a single group. " The following table is taken from Banks et al (1976). It summarizes the earlier attempts to correlate histochemical and ultrastructural properties as well as Banks et al's own classification. References to the rat are underlined. This emphasis is the author's inclusion, together with Yellin's (1974) " classification ".

TABLE taken from Banks et al (1976), showing classifications of intrafusal types i.e., according to one or two of the following histological approaches: morphology, histochemistry or electron microscopy (EM).

AUTHOR, TYPE OF STUDY AND EXPERIMENTAL ANIMAL	ORIGINAL CLASSIFICATION	PROBABLE EQUIVALENT
Boyd (1962), morphology, cat	nuclear bag	(bag_1 (bag_2
	nuclear chain	chain
Yellin (1969), histochemistry, rat	A	bag_2
	B	bag_1
	C	chain
	(one other type)	bag_1
Yellin (1974), histochemistry (ATP'ase), rat:		
Alkaline ATP'ase	dark staining bag fibre	bag_2
	pale staining bag fibre	bag_1
	dark staining chain fibre	chain
Acid ATP'ase	dark staining bag fibre	bag_2
	dark staining bag fibre at distal poles	bag_1
	pale staining chain fibre	chain
Barker & Stacey (1970), histochemistry and morphology, rabbit	nuclear bag	bag_2
	nuclear chain	chain
	intermediate	bag_1
EM	nuclear bag	bag_1
	nuclear chain	chain
	intermediate	bag_2
Barker, Harker, Stacey & Smith (1972), histochemistry and EM, rabbit	nuclear bag	bag_1
	nuclear chain	chain
	intermediate	bag_2
Morphology	nuclear bag	bag_2
	nuclear chain	chain
	intermediate	bag_1
James (1971a), histochemistry, rat; Banks (1971), histochemistry, rabbit	1	chain
	2	bag_2
	3	bag_1

cont.....

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AUTHOR, TYPE OF STUDY AND EXPERIMENTAL ANIMAL	ORIGINAL CLASSIFICATION	PROBABLE EQUIVALENT
Ovalle & Smith (1972), histochemistry, monkey and cat	bag_1 bag_2 chain	bag_1 bag_2 chain
Milburn (1973), histochemistry and morphology, rat	typical bag intermediate bag chain	bag_2 bag_1 chain
EM	typical bag intermediate bag chain	bag_1 bag_2 chain
Arendt & Asmussen (1974), histochemistry, rat, rabbit, cat, guinea-pig	1) 2) 3) 4) 5) 6)	bag_1 bag_2 chain
Banks & James (1975), histochrmistry, EM and morphology, rabbit	1 2 3	chain bag_2 bag_1

As Banks et al (1976) pointed out, those classifications that involve more than three histochemical types of fibres (e.g., Arendt & Asmussen, 1974), might have arisen because of the occurrence of regional variations in intrafusal fibres. Indeed, many workers agree that there are regional variations in histochemical staining pattern and intensity along the length of all three fibre types (e.g., Yellin, 1969a) and that these can be correlated, in large measure, with regional ultrastructural variations in the fibres (Germino & D'Albora, 1965; Banks et al, 1976). Yellin (1969a) found that the histochemical distinction between the intrafusal fibre types was most pronounced at the distal contractile poles. Reactivity was diminished at

the juxta-equatorial level such that in some spindles, the phosphorylase activity of the three fibre types was uniformly moderate. Enzyme activity was minimal at the equator in the bag and chain regions. Foci and " halos " of SDH activity were limited to the central-core cytoplasm between the nuclei of the myotube regions, and to the sensory terminals. In a later study, Yellin (1974) also found three types of intrafusal fibre according to alkaline and acid ATP'ase activity. With alkaline ATP'ase, the large, well developed bag fibre was consistently dark, whereas the other, less well developed bag fibre was consistently pale (James, 1971a). The two chain fibres stained darkly. With acid ATP'ase, the less well developed bag fibre was pale along the more proximal lengths of the poles, but became dark at the distal poles. The reaction was intermediate at the transition zone. The larger bag fibre gave a similar reaction to acid ATP'ase as to alkaline ATP'ase, but the chain fibres stained only slightly. James (1971a) got similar results, apart from the reaction of the smaller bag fibre with acid ATP'ase. He observed just the pale reaction, but perhaps his sections did not include the more distal poles like Yellin's.

Yellin (1970) opined that these results were strongly suggestive of variations in the contractile apparatus along certain intrafusal fibres (the small bag fibre, at any rate, in the light of his ATP'ase results). It may well be that regional variations, as well as the three fibre types themselves, are a reflection of the three morphological/

ultrastructural types of fusimotor innervation (trail, and two types of plates) having qualitatively different regulatory capacities. Certainly, "myofibrillar" ATP'ase is known to be neurally regulated (Guth, Samaha and Albers, 1970; extrafusal muscle). The actual situation, of course, is more complex than this because of the non-specificity of the three types of fusimotor innervation on thebag and chain fibres (Barker, Harker, Stacey and Smith, 1972; cf.Boyd, 1962; Porayko and Smith, 1968; Matthews, 1972), i.e., there is some degree of fusimotor overlap. Moreover, this overlap occurs primarily on one of the two types of bag fibres (Barker, Emonet-Denand, Laporte, Proske and Stacey, 1973; Boyd, Gladden, McWilliam and Ward, 1972; Brown and Butler, 1973). Thus, the three histochemical fibre types and the regional histochemical varitations probably reflect not just specific innervations, but the metabolic consequences of a preponderance or interaction of functionally discrete innervations.

Banks et al.(1977) surmounted the problem of describing longitudinal variation by dividing the spindle into three regions arbitrarily defined by the equatorial nucleation and by the condition of the capsule. Region A extended from the equator to the end of theperiaxial space; region B was that length of thepolar region enclosed by the capsule; and C was the extracapsular length of thepole. Each frozen section was labelled as A, B or C and the staining intensities of each intrafusal fibre was estimated on a scale of 0 (absent), 1 (low), 2 (medium) and 3 (high). In this way, the results from a number of spindles could be easily summed and the average found.

The only studies on the histochemistry of developing intrafusal fibres that have been carried out are by Wirsen and Larsson (1964) on the mouse, and by Ostenda and Strugalska (1971) and Milburn (1973) on the rat.

Wirsen and Larsson showed that the successive generations of intrafusal fibres (F1 to F4; Milburn, 1973) in foetal muscle possessed decreasing glycolytic (phosphorylase) activity, i.e., the large diamter fibre, the first fibre to form (F1), gave the most intense reaction, graduating down to the smallest fibre, the last one to form (F4). Similarly, Ostenda and Strugalska found that in neonatal rats (1 - 5 DPN) the large dimater nuclear-bag fibres had a high glycolytic/low oxidative profile in contrast to the low glycolytic/high oxidative profile of the nuclear-chain fibres.

Of the three neonatal studies, the most extensive in terms of the number of enzymes stained for and the number and spectrum of postnatal stages looked at (foetal - 17 DPN) was Milburn's. In addition, her investigation included the adult, unlike the other two. In 5 DPN muscle, she also found that the large diameter bag fibres exhibited high glycolytic activity, the smaller diameter bag fibre intermediate activity and the small chain fibres low activity. Rather different from the finding of Ostenda and Strugalska (1971), however, was the oxidative (SDH) profile, which Milburn found to be uniform and positive in all these fibre types. The stain for myofibrillar alkaline ATP'ase also gave a uniform positive result.

At 17 DPN, however, histochemical variations became

manifest among the intrafusal fibres, such that the profiles were similar to those of the adult. Thus, with alkaline ATP'ase, the reactions were high, low and moderate - high (but always positive) for the large diameter bag fibre, the smaller diameter bag fibre and the chain fibres, respectively. With SDH, the reactions were low, high (always) and high (always), in the same order, although the large diameter bag fibre occasionally stained intensely for SDH. Milburn feels that this indicates that the differentiation of the oxidative enzyme system is not necessarily complete by 17 DPN.

Normal innervation morphology of rat spindles As far as is known, nothing has been done, to date, on the morphology of developing spindle innervation (i.e., involving silver or gold/tease techniques and the stain for acetylcholinesterase) in any species. In the adult, on the other hand, numerous and extensive morphological studies have been carried out on the spindle innervation of several species of mammal, in particular, the cat (eg Barker, 1973; Barker et al, 1970; Barker et, 1972; Barker and Stacey, 1970; Boyd, 1962), the rabbit (eg Barker & Ip, 1965) and the oppossum (Jones, 1966a,b). The rat, however, has not enjoyed as much attention. Only four morphological studies of spindle innervation are documented for the rat (Porayko & Smith, 1968; Gladden, 1969 & Ph.D. thesis, 1971; Ovalle, 1972; Mayr, 1969). The first two investigations deal with the sensory as well as the motor innervation, the latter two with the motor system

alone.

Both Porayko and Smith (1968) and Gladden (1969) found the primary sensory endings to consist of spirals or annulo-spirals: large-diameter coils on the bag fibres and small-diameter coils on the chain fibres. Gladden also describes their distal branches as terminating as " end bulbs ", which often connect with the rest of the ending by fine filaments. The primary always occupied the central portion of the spindle. According to Porayko and Smith, 50% of the spindles in the plantar lumbrical muscles possessed only a single primary, the remianing 50% containing one or two secondaries in addition. The corresponding values for the intertransverse caudal muscles (Gladden, 1969) were 10%, 10% and 60%, respectively, the remaining 20% containing three secondaries. However, as Gladden pointed out, this is not necessarily a true measure of the number of Group II axons supplying the spindles. since these axons were sometimes seen to branch close to the spindle to produce two secondary fibres.

Porayko and Smith described the morphology of secondary endings as consisting of fine sprays mainly on the juxta-equatorial lengths of the nuclear-chain fibres. Gladden also found them to be located on the chain fibres but occasionally observed small spirals and annulo-spirals (in those secondaries next to the primary) as well as fine beaded filaments (Boyd, 1962) in the more distal regions. Generally, Gladden found more overlap between the primary and the secondary endings than did Porayko and

Smith.

As regards the fusimotor system, Porayko and Smith observed each spindle to receive two motor axons, one specifically innervating the bag fibres and the other specifically the chain fibres. They found no evidence for overlapping innervation. Futhermore, they dsecribe only two kinds of fusimotor innervation, one resembling a"cat plate" and the other consisting of a single filament. It is rather puzzling that these workers did not observe the typical multiterminal " trail " endings characteristic of most mammalian spindles. There should certainly have been no problem over the staining of such terminals with silver. Plate morphology was not specific to a particular type of intrafusal fibre, although their "cat-like" plates occurred more commonly on the bag fibres. Porayko and Smith also witnessed several instances of skeleto-fusimotor (or β) innervation to the bag fibres. In support of this observation, they estimated the number of motor nerve fibres in the muscle nerve and the number of spindles in each muscle. Not only did this indicate there must be some sharing of α motor axons among the spindles, but that γ axons must, too, be shared. Moreover, since Porayko and Smith found the smaller " cat-like " plates to occur more frequently on the bag fibres and since they noted that the instances of β innervation was limited to the bag fibres, it is most probable that their small " cat-like " plates were the terminations of the β axons.

Gladden (1969) classified fusimotor endings into

three morphological types: two kinds of plate and a multi-terminal ending. One plate was confined to the polar regions and had a similar structure to an extrafusal end plate, but was less than half the size. The plate possessed a nucleated sole plate which, however, was not always apparent in silver preparations unless the intrafusal fibre was oriented so that the ending was seen sideways on. Staining for acetylcholinesterase revealed a discrete sub-neural apparatus. There were variations in the complexity of form of this ending (Gladden, Ph.D. thesis, 1971). The axon diameters to these plates were less than half the size of most α axons, although a few of the latter were as small. Gladden noted that a consistent relationship was lacking between axon diameters at the level of the spindle and those in the nerve trunk.

The second type of plate was located in the juxta-equatorial region. It was twice the size of the polar plates and lacked a nucleated sole plate. Morphologically, the ending took the form of several short tapering branches and knobs. The axon diameters to these plates were twice the size of those to the smaller type of plate.

Multiterminal endings in the juxta-equatorial region (bordering the secondary sensory endings) constituted Gladden's third morphological type of fusimotor ending. They were, in fact, the typical trail endings found in most other mammalian spindles. They were characteristically pleomorphic, varying in form from single filaments to numerous ramifying branches of different diameter. With the

stain for acetylcholinesterase, the terminals showed up as a diffuse reaction at the juxta-equator, as Coers (1962) and Coers and Durand (1956) found in rat rectus abdominis spindles.

Ovalle's study (1972) of rat fusimotor endings also involved the use of a silver stain, but his preparations consisted of longitudinal sections rather than whole muscle. Like Gladden (1969), his classification was tripartite: two kinds of plate and a trail or multiterminal type of ending. However, unlike both Porayko and Smith (1968) and Gladden (1969), the plates were specific for either the bag fibres or the chain fibres. Thus, he used the terms " NB " plates and " NC " plates (see EM section, page 150). " NB " plates were usually smaller and more delicate, each ending consisting of one or two filamentous branches ending on a poorly developed sole plate area. One or two sole-plate nuclei were normally present. " NC " plates were larger and more robust, consisting of several thick terminal arborizations resting on a well developed sub-neural apparatus with several sole-plate nuclei. They were usually supplied by a slightly larger fusimotor axon than were the " NB " plates. Clearly, the only discrepancy between Gladden's and Ovalle's observations concerns the relative sizes of the sole plate. In Gladden's terms, the " NB " plate " should " have possessed a well developed sole plate, whereas the " NC " plate " should " not have possessed one as obvious as Ovalle described.

In agreement with Gladden, Ovalle's third type of

fusimotor ending terminated on the juxta-equatorial regions of both intrafusal fibre types. They consisted of a diffuse, irregular network of fine interconnected bulbous terminals which characteristically lacked sole-plate nuclei. Ovalle distinguished between those on nuclear-bag fibres and those on nuclear-chain fibres (" NB " trails and " NC " trails, respectively). " NB " trails consisted of a series of elongated, knob-like thickenings, extending along the surface of the muscle fibre, whereas " NC " trails took the form of a diffuse, grape-like spray, with several punctate terminal knobs, interconnected by ramifying filaments of the parent axon. Each trail fusimotor ending was supplied by a single motor nerve fibre.

The only significant investigation of rat fusimotor endings using cholinesterase staining has been by Mayr (1969). In conjunction with this synaptic stain, he used Sudan Black to stain nerve fibres and muscle fibres. Single spindles were isolated from whole lumbrical muscles. The bag fibres appeared pale whereas the chain fibres gave a dark reaction with the Sudan Black. Like Gladden (1969) and Ovalle (1972), Mayr demonstrated three kinds of fusimotor ending: two types of plate located at the mid-polar level, and an extensive multiterminal ending located in the juxta-equatorial region. Those spindles lying at the proximal or distal end of the muscle had, as a rule, plates only on the pole at the central end. Spindles at the centre had plates on both poles. On the other hand, virtually all poles had trails.

One class of plate was larger than the other and consisted of circular sub-units in loose arrangement; they occurred on pale (bag) muscle fibres only, their number per fibre pole amounting to as many as three. The smaller class of plate showed a more compact arrangement of their sub-units and occurred on dark (chain) fibres only. Their number per fibre pole never exceeded one.

The multiterminal ending was present in both juxta-equatorial zones of every spindle. They were located predominantly on pale (bag) intrafusal fibres.

With regard to the supplying fusimotor axons Mayr observed that those γ fibres to the multiterminal endings were usually very thin, having lost their myelin sheaths long before termination. Each plate type received its own, usually thicker, γ fibre, which lost its myelin sheath only just before terminating as one or other type of plate. Unlike the trail γ axons, the terminal parts of these fibres showed no branching.

Mayr saw the dark (chain) intrafusal fibres that received a single motor end plate and a minority of multi-terminal endings as corresponding to " twitch " fibres. The intrafusal fibres that received more than one end plate and the majority of multiterminal endings corresponded, in Mayr's eyes, to " slow " fibres.

In terms of plate morphology alone, Mayr's classification can be fairly easily resolved with that of previous workers. Thus, his smaller calss of plate compares with Porayko and Smith's " cat-like " plate, Gladden's smaller

" p_1-like " plate and Ovalle's " NB " plate. Similarly, Mayr's larger class of plate would seem to correspond to Porayko and Smith's " long-filament " plate, Gladden's larger " p_2-like " plate and Ovalle's " NC " plate. However, the distirbution of the fusimotor endings to the intrafusal fibres poses a more difficult feature to resolve. Like Porayko and Smith as well as Ovalle, Mayr found no evidence of axonal sharing between bag and chain fibres. However, he is the first to record a difference in the frequency of the two types of plate and also a difference in the proportion of multiterminal endings to the two types of intrafusal fibre.

As hinted at above, Gladden equates her small plate with Barker et al's (1970) p_1 plate in the cat, but whereas p_1 plates were observed to terminate on either bag or chain fibres, Gladden found her small plates only on bag fibres. Gladden witnessed only one instance of skeleto-fusimotor fibres (β) which supplied one of her small plates. On the basis of this observation and from the similarity between her small fusimotor plate and extrafusal end plates, she concluded that the usual termination of a β axon is small, p_1-like plate. Gladden likewise equates her larger plate with Barker et al's (1970) p_2 plate, but again, whereas p_2 plates were located on both bag and chain fibres, Gladden could only identify her large plate with any certainty on bag fibres.

Normal physiological development Investigations into

the physiological development of rat muscle has been limited to extrafusal muscle (Darinskii, 1975; Redfern, 1970; Lewis, 1973; Buller & Lewis, 1965; Buller, 1966). There is no literature on the subject with regard to muscle spindles. The only research on immature spindle physiology is that done on kittens (Skolund, 1960a,b,c).

At birth, Skolund demonstrated a quick-adapting response of both spindle receptors and tendon organs to constant stretch. A maintained response was elicited only later in development. Skolund put forward the following explainations: (i) the refractory period of immature axons is longer than in the adult, the hyper-polarisation being longer-lasting so that it summates during repetetive stimulation or (ii) stretch cannot be maintained in an immature muscle because the tendon " gives " slowly during stretch. On re-stretching the muscle, the response of the spindle afferents was again phasic. Clearly, of the two explainations, the first one bears more weight. To add weight to this conclusion, Skolund tested the effect of succinylcholine on spindle discharge in newborn kittens and older animals. Spindles in the older age group gave a maintained discharge, whereas newborn spindles discharged phasically.

A third experiment involved the excitation of γ axons. Skolund first gave a shock, (maximal for α axons), to the ventral root and then a more intense shock, ten times as great. Since γ axons are present in the muscle nerve of 10 DPN kittens (Skoglund, 1960a), it was predicted that the bigger shock would cause the intrafusal fibres to contract

via γ stimulation. However, Skoglund could get no spindle response to γ excitation until 17 to 20 DPN. The smaller shock (as well as tetanic stimulation at voltages effective only for impulse conduction in α axons) did, however, produce a spindle response during extrafusal contraction, when spindle activity is normally nil. This happened before the 17 DPN stage. Skoglund (1960b,c) was left to conclude that skeleto-fusimotor (" mixed ") innervation is responsible – more specifically, β fusimotor innervation – and that this must develop before the γ supply.

Nerve lesion experiments

The last two decades has seen extensive research into the effects of nerve lesion on the development of muscle spindles. The findings of these experiments have contributed a great deal to our present knowledge of the degree of dependence of intrafusal fibres on their nerve supply during differentiation. The nerve lesions applied were of various kinds: section, crush or extirpation of part of the muscle nerve; extirpation of the spinal cord; ventral root section (de-efferentation); and dorsal root section (de-afferentation). Rats were the main subject of study because of the relative immaturity of their spindles at birth.

Nerve section experiments Zelená (1957, 1959, 1962) pioneered much of the nerve severance work. In her first study, she unilaterally excised part of the sciatic nerve at the level of the mid-thigh in (i) foetal rats (19 - 20 days, or $2\frac{1}{2}$ - 3 days before birth), newborn rats and 20DPN rats, and (ii) also in foetal rabbits (21 days or 7 days before birth).

The hindlimb muscles from the foetal animals were removed three, four, five and seven days after the operation. Those from the neonatal rats were removed five and ten days afterwards.

Spindles did not develop in the rat and rabbit hindlimb muscles which had been denervated in utero; there was no sign of them upon examination at three or seven days after surgery. The rat muscles that were denervated at birth contained spindles with recognisable nuclear bags and equatorial zones five days postoperatively, but by the tenth postoperative day, these features had disappeared with the commensurate atrophy of the intrafusal fibres. There was only the odd spindle remnant left (Zelená, 1957; Hník and Zelená, 1961).

However, spindles from the 20DPN-denervated rats had withstood denervation ten days after nerve severance. The capsule was still intact, whereas the intrafusal fibres had atrophied only in their polar regions and the equatorial nuclei had become reduced. Tower (1932) also observed these changes in adult-denervated rats, although the process occurred more slowly than in developing rats.

From these experiments, it was clear that muscle spindles depend, for their normal development, on an intact, sensory nerve supply, and that this dependence decreases to a nominal level certainly by the third week of life, if not before.

More recently, Zelená and Soukup (1975a) examined two rat hindlimb muscle spindles six hours to ten days after unilateral neurotomy at birth in order to elucidate the character of the denervation changes at the ultrastructural level. They extirpated a section of the sciatic nerve at birth and on the fifth postnatal day, repeated the operation for prolonged intervals.

Degeneration as a whole was rapid, as measured by the presence of "myelin figures" and vacuoles in several intrafusal myotubes as early as six hours after neurotomy. During the first post-operative day, sensory nerve terminals degenerated and were phagocytosed by activated Schwann cells. Intrafusal fibres lost their characteristic nuclear bags during the first and second post-denervation day - as recorded also by Milburn (1973 b) - and subsequently atrophy or degenerate completely.

However, in many other spindles, intrafusal myotubes remained relatively well preserved three days after denervation, although their nuclear bags had disappeared and they sometimes exhibited myofibrillar atrophy together with other minor degenerative changes.

As a rule, by the fifth postdenervation day, the number of intrafusal fibres was reduced to two, in comparison with a normal complement of four fibres from the fourth postnatal day. The spindle capsule thinned to one or two discontinuous layers and no periaxial space was formed. Ten days after neurotomy, only the odd spindle remnant was found, consisting of single, encapsulated myotubes of disarranged ultrastructure.

Zelená and Soukup's work reveals the sequence of cellular events that accompanies denervation. Moreover, it clearly corroborates Zelená's former finding concerning the dependence of spindle differentiation upon sensory innervation (Zelená, 1957, 1964).

A similar experiment had been carried out earlier by Milburn (1973b; thesis, 1973). She sectioned, at birth, the nerve to the flexor halucis longus muscle of the rat, and examined the muscle 1, 3, 5, 7, 9, 12 and 16 days after the operation. In this way, the process of degeneration of the denervated muscle spindles could be monitored.

On the first day after the operation at birth, axons had disappeared, but not the associated Schwann cells. There was a commensurate absence of nerve terminals from the spindles. In comparison, normal spindles possess, by this stage, well-developed sensory endings together with the primordium of a motor component. Two of the 1 DPN experimental spindles were two-fibred (with a nuclear bag in the large-diameter myotube of only one spindle) but the third spindle looked normal except for the presence of an additional nascent myotube. At this early stage of denervation, the interfibrillar sarcoplasm and sarcotubular system appeared no more abundant than normal spindles at the same morphogenetic stage, and the myofibrils themselves remained intact. An abnormal feature common to both intrafusal and extrafusal muscle fibres was

the presence of lipid droplets. The t-tubules of the extrafusal fibres tended to be dilated to a slightly greater extent than normal.

In two of the muscles in which the nerve had shown some signs of regeneration, Milburn found an atypical spindle and a more degenerate spindle. The atypical (12 DPN) spindle contained two small muscle fibres (one larger in diameter than the other) that were myotubular at the mid-length, and enclosed by a bilamellated capsule of flattened cells. A narrow periaxial space was present. A sensory terminal enwrapped both fibres in the myotubular region. It contained numerous vesicles, many of the dense core type. There was no evidence of fusimotor innervation. The spindle nerve trunk appeared degenerate and comprised a few unmyelinated axons enclosed in a single Schwann cell. In the equatorial region, the fibres lacked nuclear bags and individual basement membranes, although the latter were evident in the more polar regions. Peripheral nuclei and distinct M-lines were also observed at the poles. The presence of M-lines suggested a "nuclear-chain-like" nature of the fibres. The myofibrils showed no degeneration but the interfibrillar sarcoplasm, SR tubules and transversely orientated triads appeared more abundant throughout the sarcomere and not only at the I- and Z-band levels. This feature is typical of muscle fibres that have regenerated following nerve lesion experiments (see previous paragraphs, for e.g.).

Milburn's atypical spindle was morphologically very much like the atypical spindle observed in re-innervated rat muscle five months after neonatal nerve crush (see following section: Zelená and Hník, 1960 a; Hník and Zelená, 1961). Zelená and Hník's suggestion that such abnormal spindles are derived from re-innervated

spindle remnants that survive denervation was confirmed by Milburn's observation of the presence of regenerating nerve axons in the spindle capsule and sensory terminals on the intrafusal myotubes.

By the third post-operative day, some axons had regenerated, but there were still no nerve terminals - sensory or motor - present on the muscle fibres. A few large-diameter extrafusal fibres exhibited atrophy in certain peripheral areas that contained sole-plate nuclei, sarcoplasm and shallow post-junctional folds. The atrophy took the form of a disorganization of myofibril orientation and the presence of numerous dense bodies which were presumed to be degenerating Z bands. The denervated spindles, on the other hand, still showed no denervation atrophy, although they contained only two fibres most of which were myotubular. By 3 DPN the normal spindle complement is four intrafusal fibres, two of them having well-developed nuclear bags at the equator. The outer basement membrane of the intrafusal fibres often appeared collapsed. Church (1970 a) and Vracko and Benditt (1972) have described similar basement membrane configurations of basement membrane in degenerating extrafusal fibres of the fruit-bat and the rat and rabbit, respectively.

Myofibrillar atrophy of the intrafusal fibres first became obvious on the fifth post-operative day. The degree of atrophy was extensive in some areas, consisting of disarranged myofibrils of indistinct banding pattern with numerous dilated t-tubules.

Myelinated axons were still absent 2 - 12 days after surgery, but regeneration had gone as far as numerous unmyelinated axons. One very degenerate spindle consisted of mere anucleate fragments of atrophic muscle fibres containing numerous disorganised and disintegrating myofibrils, dense bodied lyosomes characteristic

of neonatally denervated extrafusal muscle fibres (Schiaffino & Hanzlikova, 1972b). In some areas of the capsule only regenerating axons were left.

Goglia and Porfini (1973) carried out an experiment to determine the critical period of development during which muscle spindles depend on the presence of innervation for their normal structural development (cp Werner's (1973b) nerve crush experiment, page 54). Goglia and Porfini cut the sciatic nerves of rats at various ages from birth to 16 DPN and inspected the lumbrical muscles several weeks later. The nerve lesion on rats up to 6 days old caused the arrestment of growth and the degenerat ion and reabsorption of the intrafusal fibres. However, in older rats, the nerve lesion did not markedly affect spindle architecture or growth. The implication was evident: that the histogenic influence/ trophic effect of the muscle nerve on the differentiation of rat intrafusal fibres declines after 6 DPN, by which time the fibres have reached complete maturity.

Schiaffino and Pierobon Bormioli (1976) also agree that spindle maturation is radically altered by nerve lesion in very young animals, apparently as a direct result of inadequate sensory re-innervation. They have also found that there is a rather abrupt transition in this sensitivity to denervation. For this, they severed the sciatic nerve of 4-, 7-, 13- and 22 DPN rats (at the level of the sciatic notch) and sutured the stumps to facilitate subsequent re-innervation. After a recovery period of 6 - 12 months, three hindlimb muscles were examined. Another group

of animals operated on at 7 DPN was killed one to three weeks later in order to monitor the process of re-innervation as Zelená and Soukup (1975) and Milburn (1973b) had done in their respective nerve lesion experiments.

Schiaffino and Pierobon Bormioli found that re-innervated spindles displayed similar features in animals operated on at 4 and 7 DPN but different changes to those operated on at 13 and 22 DPN.

The muscle spindles seen during the period of denervation and early re-innervation (i.e., 1 - 3 weeks following surgery at 7 DPN) displayed obvious signs of impaired maturation of the equatorial region and contained only two to three fibres instead of the normal four fibres. Many of these were, moreover, clearly larger than intrafusal fibres from contralateral muscles. Similarly, spindles examined 6 - 12 months after the 4- and 7- DPN operations, were characterized by a marked hypertrophy and elongation of the intrafusal fibres, a reduction in their number and the absence of a periaxial space. The limiting capsule remained intact and, indeed, was the only spindle feature that could be relied upon for spindle identification. The average number of spindles per section (as counted in the muscle belly region) was always well below the control value. Yet, the ratio of spindles to extrafusal fibres was not significantly reduced (in the 7 DPN group) relative to the control because of a marked decrease in total weight and numbers of extrafusal fibres.

Instances of longitudinal splitting of both intrafusal

and extrafusal fibres were frequently encountered in the re-innervated muscles of the 4- and 7- DPN operated rats. Apart from the presence of a thin capsule and central nuclei, irregularly distributed throughout the entire length of the experimental spindles, the hypertrophic, elongated intrafusal fibres were indistinguishable from their extrafusal neighbours. Nuclear bags and chains were lacking. The length of the capsule itself was variable, yet within the range of normal values, but its diameter was uniform - a consequence of the non-existent periaxial space.

consequence of the non-existent periaxial space.

As regards ultrastructure, the abnormal intrafusal fibres all contained a moderate to large number of mitochondria, but showed the variations in the degree of development of the SR characteristic of normal extrafusal and intrafusal muscle fibres (Schiaffino et al, 1970; Ovalle, 1971). On the other hand, certain features of the SR peculiar to intrafusal fibres in general - namely, peripheral couplings and dilated junctional cisternae (Ovalle, 1971 and 1972; James & Meek, 1973) - were never identified in the reinnervated intrafusal fibres. Neither were those ultrastructural features specific to rat intrafusal fibres in particular: for example, the sarcoplasmic granules, dense cytoplasmic bodies and leptomeric organelles described by Ovalle (1972).

A single motor endplate innervated each intrafusal fibre. These plates were comparable in size and ultrastructure to extrafusal endplates and were frequently located within the intracapsular space. In contrast, sensory terminals were never

encountered.

Muscles re-innervated after surgery at 13- and 22- DPN displayed far less atrophy and hypoplasia compared to the contralateral controls. Indeed, there was, apparently, a progressive tendency towards restoration of normal spindle architecture in re-innervated muscles. Thus, the number of spindles per muscle, the periaxial space, the equatorial nucleation as well as the sensory and motor innervation, were all restored to their normal state. Some features of sarcoplasmic reticulum were also identified. The average number of intrafusal fibres per spindle was not significantly different from the control, although there were a minority of spindles containing 10 or more small intrafusal fibres in re-innervated muscles of the 22 DPN group. Schröder (1974a) mentions similar spindles. Atypical spindles like those in the 4- and 7- DPN groups co-existed with spindles of normal appearance, together with a few spindles of intermediate cha acter, i.e., containing a mixture of hypertrophic and normal hypotrophic intrafusal fibres.

Schiaffino and Pierobon Bormioli's (1976) work also included a histochemical investigation of the intrafusal fibres of both the 4- and 7- DPN group and the 13- and 22- DPN group of rats. They stained for myosin ATP'ase activity (Padykula & Herman, 1955), using pre-incubation at pH 10.4 as well as 4.35 (Guth & Samaha, 1969), succinic dehydrogenase (Nachlas et al, 1957) and mitochondrial α -glycerophosphate (Hess & Pearse, 1961). Sections were also incubated for acetylcholinesterase (Koelle & Friedenwald,

1949).

Major changes in spindle histochemistry were limited mostly to the 4- and 7- DPN-denervated group. In particular, instead of the heterogeneous composition of normal intrafusal fibres, the experimental intrafusal fibres were all of the same histochemical type, probably because of the type-grouping which occurs in re-innervated muscles. Often, type grouping involved neighbouring extrafusal fibres. With acid-incubated ATP'ase, the intrafusal fibres usually exhibited a complete reversal of staining type, i.e., from dark to pale, although some fibres with intermediate staining properties were also observed. Both intrafusal as well as extrafusal fibres revealed high SDH and variable α-GPDH'ase activity.

The serial transverse sections stained for acetylcholinesterase demonstrated a single motor end plate on each intrafusal fibre comparable in size with extrafusal end plates. The plates were frequently located within the intracapsular space. No diffuse reaction, as given by the trail multiterminal innervation of normal spindles, was observed (e.g., Gladden, 1969; Mayr, 1969).

The histochemistry of the 13- and 22- DPN-denervated groups was generally heterogeneous in spindles of normal appearance, but was frequently homogeneous in spindles of atypical structure (i.e., similar to those previously described in the 4- and 7- DPN groups). Schiaffino and Pierobon Bormioli made no mention of the fusimotor innervation, either ultrastructurally or histochemically,

in the two older groups of animals. Presumably, it was similar to normal fusimotor innervation in those spindles of normal aspect.

By this series of experiments, Schiaffino and Pierobon Bormioli supported the earlier finding that the morphogenesis of muscle spindles is profundly affected by nerve lesion during early post-natal development, apparently because of inadequate sensory innervation (Zelená,1964,197 In addition, they showed that the resistance of spindles to degeneration induced by nerve lesion increases with age such that surgery at 13- and 22- DPN produces minimal degenerative changes. Why sensory fibres are apparently unable to form terminals in the spindles of the younger age groups, whereas effective sensory re-innervation does occur in animals operated at later stages of development, is a question that remains to be answered.

The observations on the process of spindle degeneration in the 7- DPN-operated rats indicate that even with animals this young, many spindles survive during the critical denervation period which precedes re-innervation. This effectively rules out the possibility that intrafusal fibres degenerate completely after nerve section, the capsules being later penetrated by extrafusal fibres, or that new capsules are formed around extrafusal fibres. Thus, in concurrence with Zelená's own conclusion (1964), Schiaffino and Pierobon Bormioli regard the encapsulated fibres in re-innervated spindles as intrafusal fibres which survive denervation and are subsequently transformed

by the new innervation.

They also suggested that the increased diameter of intrafusal fibres, even at a pre-innervation stage, may mean that the post-natal growth of intrafusal fibres is actually repressed in normal spindles by their specific innervation. To quote one of their speculations: " The fact that the apparent potential of intrafusal fibres to differentiate like extrafusal fibres is only expressed in animals operated during a short critical period of post-natal development may result from a different pattern of re-innervation or from an irreversible differentiation of the muscle cells which become refractory to re-differentiation. " Quite clearly, their work lends support to the view (Werner, 1973a; Elliott & Harriman, 1974) that intrafusal and extrafusal fibres are not genetically different fibre populations but may, instead, represent extreme types induced by specific patterns of re-innervation of an initially homogeneous fibre population. Certainly, the size and structure of re-innervated intrafusal fibres and of their motor end plates, as well as their frequent histochemical homogeneity occasionally associated with homologous type groupings of extrafusal fibres, suggest that during the process of re-innervation, extrafusal motor axons make connection with the intrafusal fibres and produce a redifferentiation of these fibres towards an extrafusal fibre type. It is known that some innervation by skeleto-fusimotor (β) neurons is a normal feature of mammalian muscle spindles (Adal & Barker, 1965; Bessou et al, 1965; Porayko & Smith 1968). Moreover, Brown and Butler

(1973) have demonstrated that β innervation is markedly increased during early stages of re-innervation in cat muscle spindles, possibly as a result of faster growth of skeleto-fusimotor fibres. " At later stages, however, it appears that the proportion of beta innervation decreases concomitantly with the reestablishment of the full gamma complement. These findings suggest that in the normal spindle during development and in spindles reinnervated after nerve lesion there occurs some form of competition between beta and gamma innervation of intrafusal fibres. Innervation by gamma motoneurons is normally prevalent, possibly under the influence of sensory innervation. In the absence of sensory innervation beta innervation may prevail and influence the differentiation of the intrafusal fibres. " (Schiaffino & Pierobon Bormioli, 1976). This latter statement would be supported if it was found that permanent de-afferentation of spindles that had been temporarily de-efferented (i.e., the operative procedure would involve crushing or sectioning the muscle nerve followed by permanent de-afferentation at the spinal cord level) caused the formation of extrafusal-like intrafusal fibres comparable to those observed by Schiaffino and Pierobon Bormioli (1976).

These workers go on to rule out the possibility that the functional overload suffered by the smaller, re-innervated muscles as a whole contributed significantly towards hypertrophy of the intrafusal fibres. Additional, more recent, research of their own (Schiaffino & Pierobon

Bormioli, in prep.) has shown that increased functional overload imposed on normally innervated neonatal rat muscles by extirpation of synergistic muscles does not significantly affect the differentiation of intrafusal fibres. And similarly, the development of muscle spindles is unaltered by disuse (Zelená, 1963).

Ventral root section experiments De-efferentation provides a means for determining the degree of dependence of differentiating spindles on their fusimotor supply. Zelená, at first on her own (1964, 1965) and later with Soukup (1973, 1974), eliminated skeleto-motor nerve fibres from the hind limb muscles of newborn and neonatal rats by unilateral sectioning of ventral roots or by the extirpation of the lumbrosacral spinal cord. Some 10 - 56 days later, the number of spindles and intrafusal fibres were found to be unaffected by the lesion. Like the normal sequence of events, intrafusal fibres in the experimental muscles increased from two at birth to an average of four at 4 DPN. And the characteristic ultrastructural profiles of the bag and chain fibres develop normally not only at the equator and juxta-equator (Z&S, 1973) but also in the contractile polar regions which become totally denervated after de-afferentation (Zelená and Soukup 1974). Thus, the larger bag fibre (≡ Banks et al's (1975) bag_2 fibre) had confluent myofibrils and relatively few mitochondria at the equator and juxta-equator and a pale M line in its polar regions. The chain fibres possessed, throughout their entire lengths, more delimited myofibrils

concomitant with a better developed sarcoplasmic reticulum, an M line and larger, more numerous mitochondria. With regard to the M line condition of the smaller bag fibre, Zelená and Soukup (1973) could not be decisive since they found it difficult to identify in their random longitudinal sections.

Even though the ultrastructural characteristics of the intrafusal fibres were well preserved, there was still a slow atrophy of the fibres after surgery in the polar zones, as measured by a 25% decrease in diameter compared with the control fibre diameters. Spindle capsules, on the other hand, increased in calibre during post-operative development to attain normal values and, in some spindles, even slightly above normal values.

The findings showed that after complete deprivation of motor impulse activity, intrafusal fibres acquired not only their equatorial nucleation (as predicted from previous work), but also the other ultrastructural fibre-type variations.

On the other hand, Milburn's (1973a,b; thesis, 1973) ultrastructural observations of developing adult rat spindles led her to postulate that the ultrastructural variations of rat intrafusal muscle fibres may, in fact, be an expression of their variable motor innervation. Perhaps the " completeness " of Zelená and Soukup's deefferentation operation should be questioned. Also, Zelená and Soukup (1973, 1975) did not consider the possibility that β innervation may exert some influence before birth.

Nevertheless, Zelená and Soukup's experiments at least serve to confirm Zelená's original belief that

sensory nerve terminals are responsible for the induction and maintenence of spindle development, since she herself had previously shown that intrafusal fibres do degenerate after complete denervation (i.e., deafferentation as well as deefferentation) at birth.

Soukup and Zelená (1975b) repeated the deefferentation experiment on newborn rats in order to compare the progressive effect of the lesion on extrafusal and intrafusal fibres by means of their myofibrillar ATP'ase activity. They inspected the shank muscles at intervals from 4 - 10 weeks after the operation. They found that whereas the intrafusal fibres differentiate anew into distinct histochemical and ultra-structural fibre types, such differentiation was arrested in extrafusal fibres despite the fact that both intrafusal and extrafusal fibres were deprived of motor nerve fibres and their impulse activity during the critical period of post-natal differentiation.

Thus, the extrafusal fibres became atrophic and showed signs of local degeneration such as autophagic vacuoles and myelin figures as in the case of completely denervated muscles (Schiaffino & Hanzlikova, 1972; Shafiq, Asiedu & Milhorat, 1972; Tower, 1932). Such degeneration is easily explainable since, in both cases, the extrafusal fibres were deprived of their motor innervation. In addition, no differentiation into ultrastructural or histochemical types occurred,i.e., the fibres all contained equal numbers and sizes of mitochondria and all exhibited a uniformly high ATP'ase activity.

On the other hand, intrafusal fibres atrophied to a lesser degree and did not usually degenerate. The nuclear bags and nuclear chains still differentiated (Zelená & Soukup, 1973, 1974). Similarly, histochemically, the intrafusal fibres differentiated into distinct types, as in the controls. Both chain fibres demonstrated high ATP'ase activity, whereas one of the bag fibres always had a low ATP'ase activity ($\equiv bag_1$) and the other a low or medium to high ATP8ase profile ($\equiv bag_2$). However, the latter histochemical type apparently occurred less frequently after denervation than normal. Thus, in normal muscle, 25% of the total number of spindles contained two pale bag fibres. In deefferentated spindles, however, 40% of the spindles had both bag fibres pale. It would have been very interesting from the point of view of the present study if Zelená and Soukup had carried out a combined ultrastructural study on the latter spindles in order to discover the M line profiles of the two bag fibre types.

The actual mechanisms involved in the afferent-mediated induction process, on the one hand, and the possible efferent-mediated ultrastructural typing on the other, still remain to be clarified. With regard to the morphogenetic influence of sensory terminals, it is reasonable to assume that it is mediated by the release and uptake of a trophic substance at the synaptic junction. " The occurrence of light and dense-core vesicles in the sensory terminals and of coated invaginations and vesicles at both the axonal and plasma membrane, speak in favour of such a possibility. " (Zelená & Soukup, 1973).

Nerve crush experiments Nerve crush lesion allows ready and rapid regeneration of nerve axons back through their original perineural tubes which were left intact by the operation.

Thus, crushing the sciatic nerve at birth allowed Zelená and Hník (1960 a, b; 1963 a, b, c) to investigate the possibility of muscle-spindle formation following rapid re-innervation in the early postnatal period. In this way, they hoped to discover whether or not the morphogenetic influence of afferent nerve fibres was restricted to the immediate perinatal period only or whether it had the potential to induce differentiation during a later period of muscle growth.

The hindlimb muscles these workers first looked at (5 and 10 months post-operatively) were the tibialis anterior, the soleus and the extensor digitorum longus muscles. Consequent upon nerve degeneration, spindle development was arrested and their disintegration followed within a few days. Re-innervation had begun by the tenth post-operative day, but the disintegrated spindles did not differentiate de novo even after a 10 month recovery period. This clearly was not due to the lack of a sensory nerve supply since de-efferentation of 11 experimental rats revealed that the number of sensory axons had been reduced by a mere 15 - 25 per cent (Zelená & Hník, 1960 a, 1963 c; Zelená, 1964). The occassional atypical spindle was found in the recovered experimental muscles. As the number of these spindle remnants seen 10 days after the nerve crush corresponded closely to the number of atypical spindles 5 months later, Zelená and Hnik concluded that the latter group of spindles had originated from re-innervated spindle remnants that had survived the transitory denervation

period. The atypical spindles themselves contained no more than two intrafusal fibres that lacked a nuclear bag. Some of the fibres approached extrafusal fibre morphology in the polar zones (Hník & Zelená, 1961). Hník (1964) found the spindles to have a resting discharge and a slowly-adapting response to muscle stretch.

In comparison, the extrafusal fibres suffered far less traumatically from the denervation, the majority of them having recovered with little change in diameter and a relatively small total weight loss.

The outcome from these initial nerve crushes was that regenerating sensory nerve fibres in neonatal de-differentiating muscle are apparently unable to induce the re-differentiation of extrafusal muscle fibres into intrafusal fibres. Indeed, Zelena and Hnik found them to terminate extrafusally as free endings.

Crushing the sciatic nerve of 14 DPN rats (i.e., at an age when muscle differentiation is complete) caused much less atrophy. More than 85 per cent of the spindles remained intact. Atrophy of the intrafusal fibres was slow - a great deal slower than in developing rats - and was confined largely to the poles. The equatorial nucleation suffered only a slight reduction (see also, Tower, 1932). Not surprisingly, the extrafusal muscle was even less affected: muscle weight, fibre diameter and number remained relatively unchanged.

In another group of experiments, however, it was found that muscle spindles can and do recover quite fully after nerve crush (Zelená & Hník, 1963 a; Zelená, 1964) at birth. The sciatic nerve was similarly crushed, but the muscle chosen for examination was the medial gastrocnemius, which was anatomically closer to the lesion than the other hindlimb muscles (EDL, soleus and tibialis

anterior) analysed in the previous series of experiments. Because of the difference in proximity to the level of crush, reinnervation of the medial gastrocnemius muscle began by the sixth post-operative day, rather than by the tenth. Consequently, at the onset of reinnervation myotubes were still present in the muscle, together with spindle remnants.

The regenerated muscle nerve contained 60 per cent more sensory fibres compared with the controls and the experimental muscles contained 70 per cent more spindles than the controls five months after surgery. This suggested that apart from re-innervation of disintegrating spindles, differentiation of new spindles had occurred.

However, when the medial gastrocnemius muscle was subjected to prolonged denervation (by repeating the nerve lesion) spindles failed to regenerate after a five month post-surgical period, despite the fact that the regenerated nerves still contained a greater than normal proportion of sensory fibres. The purpose of such prolonged denervation was to delay reinnervation beyond the completion of muscle differentiation. Quite clearly, then, the absence of spindles suggests that the morphogenetic effect of sensory nerve fibres can be exerted only when the muscle cells are "histogenetically flexible" i.e., capable of differentiation.

The first group of temporary denervation experiments carried out by Werner (1973 a) also involved nerve crush at birth. But she chose to crush the tibial nerve 1 mm proximal to the popliteal blood vessels, somewhat more proximal to the level lesioned by previous workers. The muscle examined one or two months later was the medial gastrocnemius. Werner found that the lesion had

produced three major changes in muscle spindle development. Firstly,there was an increase or decrease in the number of spindles. Secondly, there was an alteration in the location of spindles (for example, up to five small, abnormal spindles close together). And thirdly, there was an obvious modification in the number and architecture of the intrafusal fibres.

The increase in the number of spindles on the one hand, and the localization of spindles on the other, may actually have been two different manifestations of the same reinnervation process. Thus, the increase in the number of spindles may have been produced by very early reinnervation by an unusually large number of sensory axons (Zelená, 1964) at a developmental stage when many undifferentiated myotubes remained available for an appropriate innervation to induce intrafusal fibre differentiation. Similarly, spindle localization could have been produced by a large number of unusually closely grouped axons with the ability to induce differentiation of intrafusal fibres. Such axons probably regenerated as a single bundle of fibres to reach a restricted area of muscle at the expense of other areas.

The most striking change, observed in 15 out of a total of 52 experimental spindles, was in the spindle components. The abnormal spindles usually contained only one muscle fibre, which had the morphological characteristics of an intrafusal fibre at one end and in the equatorial zone, but the morphological characteristics of an extrafusal fibre at the opposite end.(cp Hník & Zelená, 1961). These modified spindles were not merely transient, but permanent structures stably maintained in the adult rats.

Not all of Werner's experimental rats contained abnormal intrafusal fibres, perhaps because the pressure and level of crush sufficiently uniform. Alternatively, perhaps the endoneural sheaths were extensively damaged in animals containing the mixed fibres. If this were so, the axons may have regenerated into the muscle in a more disorganised fashion, delaying their progress across the scar. By the time the nerves had actually made contact with the muscle fibres, muscle differentiation had probably been taken a step further.

In the light of her data, Werner tentatively suggested that the peculiar mixed intrafusal fibres may represent a single cell which has differentiated along two different pathways. Her suggestion concurs with that of Schiaffino and Pierobon Bormioli (1976) that the myotubes of muscle histogenesis are a homogeneous population of cells with respect to their future course of differentiation into extrafusal or intrafusal fibres. In other words, these two pathways may not be intrinsically predetermined, but may depend upon their specific pattern of innervation, since disruption of the nerve supply during development modifies the normal course of differentiation of some myotubes.

Still, as Werner so rightly cautions, one should not be too dogmatic at this point in the research. At least two possibilities must be considered when trying to explain the formation of mixed intrafusal fibres. Firstly, two myotubes or muscle fibres (one a potential IF and one a potential EF) could possibly fuse in such abnormal circumstances during early development. It would still be most interesting even so, since fusions like these are not a feature of normal muscle, although bag/chain

fusions have been observed in normal adult cat spindles (Barker et al, 1976). As to the origin of these intrafusal fusions, these workers postulate: " If one of the primary axons were to arrive somewhat later than the other, the sequential development of successive generations of myotubes that it engendered would lag behind such development initiated by its earlier partner. In that event, the bag_1 fibre in the former spindle unit would be starting to develop at a time when the first chain fibre was already forming in the latter. In the subesequant polar fusion of myoblasts in the intercapsular region fusion could occur between the polar extremities of the bag_1 fibre and a chain fibre, especially is this were a long chain fibre. It is unlikely that bag_2 fibres engage in such fusions since they are already well developed in the muscle primordium before the primary axons reach them. "

Secondly, the mixed intrafusal fibres may just be a case of an intrafusal fibre and an extrafusal fibre in close end-to-end apposition, but still separated by their sarcolemmas. Werner did not encounter any instances of apposed membranes herself, but she used light microscopy which would not have provided a sufficiently high resolution for detecting such. In addition, most of the extrafusal fibres comprising the medial gastrocnemius muscle of rat extend all way from one aponeurosis to another (Swett & Eldred, 1960).

Subsequent nerve crush work by Werner (1973b) was to bear particular relevance to the preliminary methodology of

the present research (cp Goglia & Porfini's experiment (1973) and Schiaffino and Pierobon Bormioli's (1976)).

Werner crushed the tibial nerve unilaterally, approximately 1 mm proximal to its entry into the right medial head of the gastrocnemius muscle in 4, 6, 8 and 10 DPN rats. She examined the muscles 2 weeks and 1 month after surgery for the 4 and 6 DPN groups of animals, respectively, and 2 months after surgery for the 8 and 10 DPN groups. She found that the majority of spindles in the experimental muscles of the rats operated on at 8 and 10 DPN contained the usual normal complement of two nuclear-bag and two nuclear-chain intrafusal fibres. Moreover, the number of spindles in these two groups corresponded closely to the number of spindles in the contralateral control muscles. The average lengths of the experimental spindles were slightly greater than the average length of normal two month old spindles (2.88 mm and 2.61 mm as measured from the tips of the nuclear-bag fibres projecting the furthest beyond the capsule at both ends).

On the other hand, the experimental muscles of the 4 and 6 DPN groups gave completely different results. Not only was the number of spindles reduced, but more important from the point of view of the present research, was the finding that most of the spindles did not contain the usual normal complement of two nuclear-bag and two nuclear-chain fibres; they contained three fibres or even fewer, the nuclear-bag fibres being more resistant to temporary denervation than the nuclear-chain fibres. Werner's results are set out more clearly in the table below.

Age of crush	Number of spindles	Number of IFs* per spindle
8	NORMAL	NORMAL
10	NORMAL	NORMAL
6	1st muscle: NORMAL (19) 2nd muscle: 5 (vs 18 in control)	17/19 ABNORMAL 3/5 ABNORMAL
4	1st muscle: 16 2nd muscle: 10	12/16 ABNORMAL ALL ABNORMAL

* IF = intrafusal fibre

From these findings it was obvious that:

(i) nuclear-chain intrafusal fibres are more susceptible to degeneration after early post-natal nerve crush, than nuclear-bag fibres.

(ii) nuclear-chain fibres degenerate more rapidly after such a lesion than do nuclear-bag fibres.

(iii) muscle degeneration progresses more rapidly in neonatal rats than in adults.

Objective

The aim of the preliminary methodology for this thesis was to produce an adult, model muscle that contained as high a proportion as possible of "chainless" (but otherwise, dimensionally well-developed) spindles. Once this was accomplished, bag fibre(s) of the "chainless" or "bag-only" spindles (henceforth referred to as "model-adult" spindles) were studied for their ultrastructure and histochemistry to ascertain their normality.

Against this background data, which served to confirm the

identity(ies) of the bag fibre(s) in single-fibre and two-fibre model-adult spindles, respectively, the innervation of the bag fibre(s) was studied through the medium of teased, whole muscle preparations stained with silver. It was hoped to discover more about the specificity of sensory and fusimotor innervation for the three commonly recognised types of intrafusal fibre, i.e., nuclear-chain fibres and bag_1 and bag_2 fibres. Attempts were also made to answer the following questions:

(i) Do the secondary axons regenerate? If so, what happens to their terminals which, in normal spindles terminate predominately on the chain fibres? Do they fail to connect or do they innervate the remaining nuclear-bag fibres, which could mean an abnormal surplus of static pacemakers on the bag fibres (Hulliger, Matthews & North, 1977; Banks, Barker & Stacey, 1977). If the secondaries do return to the bag fibres, is there any preference for one or other type of bag fibre?

(ii) What is the fate of the static pacemaker branches of the primary ending that once innervated the chain fibres? Do these branches lose their "inducement" to regenerate from the first-order branching point of the primary once the nuclear-chain fibres have degenerated, or do they innervate either or both of the nuclear-bag fibres?

A serial-section approach like that of Banks et al (1977) would have added the detail and greater reliability which the teased, whole muscle approach of the present study lacked, but such approach was not considered at the time.

(iii) What happens to the fusimotor innervation of the eliminated nuclear-chain fibres, particularly the trail axons?

PRELIMINARY METHODOLOGY, MATERIALS AND TECHNICAL METHODS

Choice of Muscle Unfortunately, the medial gastrocnemius muscle (fig.1) is one of the more cumbersome muscles to deal with in the adult rat. Furthermore, the normal number of spindles per muscle is only 18 - 20. Crushing the tibial nerve shortly after birth reduces the number by at least 50 per cent.

Despite the first two obvious disadvantages, the medial gastrocnemius muscle was used primarily because the results of Werner's (1973b) nerve crush work (in which she used the medial gastrocnemius muscle of the rat; see Introduction) were practically relevant to the proposed research.

Ages and number of rats used (Table II)

(i) First series of operations of preliminary methodology. The purpose of the first series of operations was to determine the approximate neonatal age at which to crush the tibial nerve of the rat (at a level just proximal to the popliteal bridge of blood vessels) in order to produce a practically feasible proportion of otherwise well-developed bag-only spindles in the adult medial gastrocnemius muscle.

Three pairs of Sprague-Dawley rats (littermates of similar weight at birth) were subjected to the operation at 2DPN, 3DPN and 4DPN, respectively, and examined two weeks later.

TABLE II Number of rats used for each histochemical procedure

Age when killed	Number of rats: Silver/tease for nerves	electron-microscopy for ultra-structure	histochem-istry for enzyme	paraffin for general histology
(A) Right tibial nerve crushed at $3\frac{1}{2}$ DPN for 1st and 2nd series of operations in preliminary methodology, for study of fibre degeneration process and for study of model-adult spindles (from right limbs) and normal adult spindles				
2DP }		2		
4 "		2		
7 " } see (iv) p.59		2		
10 "		2		
14 " } see (i) & (ii) pp 57, 59		2		19*
1 MPN } see (ii) & (iii) p 59	1	2		
$4\frac{1}{2}$ " }	2	2		
$6\frac{1}{2}$ " } see (iii) p 59	4	$3+\frac{1}{2}$ ‡	$1+\frac{1}{2}$ ‡	
Total number	7	$17\frac{1}{2}$	$1\frac{1}{2}$	25
(B) Right tibial nerve crushed at adulthood (2 MPN and 4 MPN) for study of adult-crush control spindles				
$4\frac{1}{2}$ MPN } see (v) p 60	2			
$6\frac{1}{2}$ MPN }	2			
Total number	4			
(C) Unoperated adult rats to " check " the normal adult spindles in the left limbs of operated animals				
$4\frac{1}{2}$ MPN } see (vi) p 60	1	1		
$6\frac{1}{2}$ MPN }	1			
Total number	2	1		

DPN=days post-natal; MPN=months post-natal

* 10 of these used for "scanning" the 10 litters operated on at $3\frac{1}{2}$ DPN, i.e., one for each litter (see page 81); 9 used for preliminary methodology (see (i) & (ii)).

‡ One animal used in a combined EM/histochemical experiment.

(ii) Second series of operations in preliminary methodology

Once the approximate age of crush had been determined a second series of identical operations were undertaken on another three pairs of littermates at $3\frac{1}{4}$, $3\frac{1}{2}$ and $3\frac{3}{4}$ DPN respectively. The period of recovery allowed was 14 days or one month. In this way, the optimum age of crush for producing the desired model-adult spindles was narrowed to $3\frac{1}{2}$ DPN. (See Results, Part I, page 76).

In order to minimise ambiguous assessment of reinnervation patterns of the model-adult spindles, the post-operative recovery period was usually made lengthy - $4\frac{1}{2}$ to $6\frac{1}{2}$ months - sufficient time fo reinnervation stabilisation of nerve endings.

(iii) Further detailed studies of model-adult spindles The medial gastrocnemius muscles of a total of 16 animals were investigated using electron microscopy ($7\frac{1}{2}$), histochemistry ($1\frac{1}{2}$), one of the latter in a combined EM/histochemical experiment, and two silver/teasing techniques (7). Table II shows the composition of each group - whether normal adult, adult-crush or model-adult rats - and the specific ages at the time of kill.

(iv) Monitoring the process of fibre degeneration Shortly after the operation, 10 rats (from 5 litters) were killed at various intervals (2, 4, 7, 10 and 14 DPO) and processed for electron microscop in order to study the process of intrafusal fibre degeneration (i.e., the degeneration of both chain fibres and sometimes a bag fibre) in the experimental spindles denervated at $3\frac{1}{2}$ DPN. It was hoped that monitoring the process of bag-fibre degeneration in this way might prove useful in the identification (i.e., whether b_1 or b_2) of the bag fibre that was preserved in single-fibre model-adult spindles.

(v) Adult-crush control experiment Apart from the contra-lateral controls, an adult-crush control experiment was set up for the purpose of comparison with the silver/tease study of normal adult spindles and model-adult spindles. Four young rats (two, 2 months old and two 4 months old) were subjected to the unilateral tibial nerve crush operation and allowed 2½ months for the regenerating nerves to " stabalise ".

The reinnervation of these adult-crush control spindles provided a reference background against which the reinnervation patterns of the model-adult spindles were assessed. However, from evidence which suggests that the effect of nerve crush on a young adult rat differs appreciably from that in neonatal rats (Hník & Zelená, 1962 and Romanes, 1946, who notes that nerve regeneration is very deficient in young animals possibly as a result of extensive chromalytic death of nerve cells) it becomes obvious that the perfect control was virtually impossible in the present investigation.

(vi) Unoperated adult controls In order to ensure the normality of the normal spindles from the left limbs of the experimental rats, the medial gastrocnemius muscles of 3 unoperated adult rats were briefly studied for spindle ultrastructure and innervation morphology.

In toto, 10 rat litters were operated on at 3½ DPN. (This excludes 3 litters that were victims of an annual peak of maternal cannibalism.) Of the 10 litters, 4 were rejected when screened for optimum numbers of model-adult spindles (see page 61). That left 6 useable litters,

or 42 rats. As detailed in Table II, 38 of these were used for the thesis research, the remaining 4 being ulti ultimately discarded.

Operative procedure Rapid, whole-body hypothermia was employed as a general anaesthetic. All potential operants from a particular litter were removed simultaneously and placed in stainless steel dishes lined with tissue paper. They were then subjected to a temperature of -5° C for a minimum duration of 10 - 15 minutes. Deep hypothermia was indicated by skin colour, which changed from pink-red to white, as well as insensitivity to tactile stimulation,e.g., a negative response to hind-leg extension. This phase lasted about 5 - 10 minutes at room temperature, ample time for the operation to be carried out.

Each animal was subesequently transferred from the operating table to a padded hot plate at 37 - 40° C until normal skin colour and the tactile response returned ($\frac{1}{2}$ - $\frac{3}{4}$ hour).

The operation itself was carried with the animal's dorsal surface uppermost. A small incision was made in the skin of the posterio-lateral surface of the right thigh in a direction parallel to the length of the femur (see fig. 2). The sheet-like superficial muscles of the thigh were cut through to expose a short length of the tibial nerve just proximal to the popliteal bridge of blood vessels. There was little or no bleeding using this technique. Firm pressure was applied to the tibial nerve

(for a count of 8 seconds) with a pair of fine, smooth-tipped forceps (diameter 0.2 mm). The level of crush was approximately 0.5 mm proximal to the popliteal bridge of blood vessles. Skin suture required a single stitch followed by an even spray of a surgical plastic dressing (Nobecutane, BDH Ltd.).

Removal of nerve sections and muscles The method of killing depended on the age of the animal. Older rats (2 - 7 MPN) were instantaneously killed by breaking the neck, whilst younger ones ($5\frac{1}{2}$ DPN - 1 MPN) were quickly etherized.

To avoid unnecessary damage to the required nerves they were dissected before the muscles. With the help of a Zeiss microscope, a length of some 2 mm was extirpated from the medial gastrocnemius nerve, a few millimetres proximal to its entry into the muscle. The nerve cylinder was kept straight and extended by placement on a small square of card, followed by immediate immersion in glutaraldehyde fixative for subsequent embedding in Epon resin.

The procedure was repeated for the control, left leg. Following removal of the nerve cylinders, each hind limb was severed just above the femoral head, set in a medium contraction

state, (i.e., foot at right angles to calf) and immersed in fixative, be it Bouin, another formalin-based solution (for embedding in paraffin), glutaraldehyde (for embedding in Epon), or formalin with de Castro's fixative (for silver/teasing technique). Severe stretching was avoided as Knappeis and Carlson (1968) have shown that this affects the configuration of the myofibrils. After 10 - 15 minutes, the partially fixed muscle was carefully removed from its in situ position on the limb. Most large medial gastrocnemius muscles were cut lengthways into narrow strips. However, those used in the preliminary methodology for investigation of total number, location and type of spindles, were kept intact. The muscle was stored for two to six days in fresh fixative.

Muscle extirpation for the combined histochemical/electron-microscopic experiment followed a different procedure. The muscle was carefully freed from the intact limb, and the middle-proximal third (which contained the majority of spindles both in normal and model-adult muscle) was dissected out, frozen, and then "glued" onto the metal chocks of the cryostat with 5 per cent tragacanth (Engel and Cunningham, 1963). Freezing was by immersion for at least one minute into a slurry of liquid nitrogen and isopentane at approximately -160°C (Maxwell, Ward and Nairn, 1966). When not required immediately for sectioning, the muscles were stored in sealed containers at -30°C. Prior to sectioning, all frozen material was placed in the cryostat (Slee Ltd.) to equilibrate to -20°C.

Method for wax sections Paraffin blocks were employed in the preliminary methodology. The fixatives used were Bouin solution or another formalin-based fixative. The latter consisted of

50 parts tap water, 30 parts 95 per cent ethanol, 10 parts concentrated formalin and 10 parts glacial acetic acid, and gave superior results. The material was dehydrated in an ethanol series, cleared in chloroform and embedded in plasticized paraffin wax (Paramat, George T. Gurr Ltd.). Serial 10μm thick transverse sections were cut on a Spencer A20 microtome and mounted on clean, albumen-coated glass slides. All sections were stained for their general histology with haemalum and counterstained with eosin to emphasize details of spindle components.

Methods for electron microscopy The ultrastructure of three groups of spindles in medial gastrocnemius muscle was investigated: normal adult spindles, model-adult spindles and neonatal-crush developing spindles (see Table II). All muscles were given similar treatment.

The fixative, a 5 per cent solution of glutaraldehyde, buffered at a pH of 7.3 with 0.1 M sodium cacodylate (Mercer and Birbeck, 1966) was maintained at a temperature of 4°C. Larger muscles were cut into long strips (see page 63) and all tissue was usually left overnight in fixative. Post-fixation in one per cent osmium tetroxide (buffered at pH 7.3 with sodium cacodylate) was carried out at room temperature. After dehydration with a series of ethanols, the muscle tissue was treated with propylene oxide and embedded in Epon 812. Polymerisation of the resin took 48 hours at 60°C.

Muscle blocks were sectioned on a LKB or Reichert OMU2 ultra-microtome with glass knives. Thick (1μm) transverse sections were cut, stained with toluidine blue in one per cent borax and scanned for muscle spindles under low power. Spindles

were identified in adult material by the characteristically small diameters of intrafusal fibres and by the presence of a capsule. Spotting spindles in the experimental neonatal-crush material (particularly the 2 days post-op (DPO) and the 4 DPO animals) proved mor difficult due to the "induced" immaturity of the capsule cells and the protracted presence of numerous small-diameter myotubes. Sectioning had often to be continued well into region B (Banks et al,'75' before spindle identity could be confirmed with any confidence.

Once a spindle was located, thin sections were cut and picked up on uncoated copper grids. Their sectioning was alternated with variable lengths of thick sectioning. Most of the thick sections were cut at the poles, and greater lengths were thick-sectioned in adult material as opposed to neonatal material. Diagrammatic notes were made of changes in spindle morphology and location on the block face.

Longitudinal and oblique sections of intrafusal fibres were cut at certain chosen levels along the spindle.

Representative toluidine blue thick sections (both TS and LS) were photographed with a Zeiss Ultraphot II microscope. The grids were stained with aqueous uranyl acetate and aqueous lead citrate and examined and photographed on an AEI 801 electron microscope.

Methods for histochemistry Model adult and contralateral, control muscles from two $7\frac{1}{2}$ MPN rats were frozen for histochemistry. One such pair of muscles was subjected to the combined histochemical/electron microscopic technique by Pierobon Bormioli and Schiaffino (1974) and more recently by Banks et al (1975, 1976). By this means, the histochemistry of each intrafusal fibre could be matched to its particular ultrastructure.

The transverse orientation of the muscle fibres was checked initially under a light microscope with a few frozen sections stained for their general histology with haematoxylin and eosin. Consecutive pairs of 10 µm thick sections were processed for the histochemical stains as well as for H&E. Those sections intended for the PAS/glycogen stain were, however, cut at three times the thickness to maximise the visibility of the stain. In the combined histochemical/EM experiment, a few 60 µm sections were taken of each spindle to confirm the identities of nuclear bag and nuclear chain intrafusal fibres. The sections were flicked directly from the cryostat blade into small vials of freshly prepared glutaraldehyde and processed for electron microscopy.

The four histochemical stains employed were:

(i) succinic dehydrogenase (SDH), an enzyme of aerobic respiration found within the mitochondria of cells. Method used: Nachlas et al's (1957) as modified by Pearce (1961). The sites of activity of SDH were defined by purple diformazan granules scattered in the interfibrillar mitochondria of intrafusal and extrafusal fibres, (Table III).

(ii) phosphorylase (P'LASE), an enzyme of anaerobic respiration located free in the cytoplasm. Method used: Eranko and Palkama's (1961). The presence of phosphorylase was indicated by an amorphous yellow-gold/dark brown/blue-black colouration of the muscle fibre, (Table III).

(iii) periodic acid Schiff reaction (PAS), a reaction that provides a useful indicator of the amount of glycogen storage product present within muscle cytoplasm. Method used: Pearse's 1960. Various shades of pink reflected the amount of glycogen present, (Table III).

Table III. Showing the relationship between the colour/texture of enzyme staining and the intensity of the enzyme reaction of both extrafusal and intrafusal fibres. The intensity of each reaction is rated on a scale devised by Banks et al (1976), whereby negative activity (i.e., no colour) is recorded as 0, minimal activity as 1, intermediate activity as 2, and high activity as 3.

INTENSITY OF ENZYME REACTION	COLOUR AND TEXTURE OF MUSCLE FIBRES AS SEEN IN TS			
	P'LASE	PAS	SDH	ATP'ASE
MINIMAL ACTIVITY (1) — Actual Colour	yellow-gold	pale pink	loosely arranged blue-black streaks of diformazan; no distinct sub-sarcolemmal activity	pale beige
MINIMAL ACTIVITY (1) — B & W Photography	light-grey	light grey	black streaks arranged as above	very light grey
INTERMEDIATE ACTIVITY (2) — Actual Colour	brown	darker pink	more compactly arranged blue-black globules of diformazan; no distinct sub-sarcolemmal activity	brown
INTERMEDIATE ACTIVITY (2) — B & W Photography	medium grey	light grey	black globules arranged as above	light-medium grey
HIGH ACTIVITY (3) — Actual Colour	blue-black	bright pink	masked sub-sarcolemmal activity consisting of large, densely packed blue-black globules	dark brown
HIGH ACTIVITY (3) — B & W Photography	black	medium grey	black globules arranged as above	black

(iv) Calcium-activated actinomyosin adenosine triphosphatase: alkaline-stable, pH 9.6 - 9.8 (Alk. ATP'ase), an essential enzyme for muscle contraction. Its action is responsible for bridge formation between the actin and myosin molecules of a sarcomere. Method used: Guth and Samaha's (1970). The smooth-textured colour of the reaction product ranged from colourless through medium brown to dark brown, (Table III).

The stained sections were studied under a Zeiss GFl light microscope.

Histochemical assessment of staining intensities of intrafusal fibres Histochemical classification of intrafusal fibres, like classification according to their ultrastructure, posed a difficult task because of the variability of enzyme reactivities along the lengths of the fibres (Yellin,1969,1974; Ogata & Mori, 1962(mouse); Jasmin et al,1971(hamster); James,1971). Consequently, no attempt was made to label an extrafusal fibre as fast glycolytic, slow oxidative or fast oxidative-glycolytic (Ariano et al, 1973). The sole purpose of the histological investigation was to make a 'two-dimentional' comparison of the histochemistry of the experimental intrafusal fibres and their normal counterparts. In other words, two parameters were looked at simultaneously: (i) the reactivity of a particular enzyme in (ii) a specific longitudinal region, be it regions A, B or C, respectively (Banks et al, 1975, 1976). Only in this way could any deviation from the norm in the histochemical reactivities of the experimental intrafusal fibres be at all meaningfully evaluated. Following Banks et al's(1976) approach, the staining intensities of the intrafusal fibres (i.e., (i) above) were estimated on a scale of 0 (absent), 1 (low), 2 (medium) and 3 (high). See Table III. This is similar to Banks et al's

(1975) earlier rating system, but allows the results from a number of spindles to be easily pooled so that variations of reactivity can be averaged. Particular attention was paid to the nuclear-bag fibres in normal spindles and in the one- or two-fibre model-adult spindles. As has been shown in other muscles of the rat and other species (eg, Yellin, 1974: rat; Ovalle & Smith, 1972: monkey and cat), the alkaline ATPase reaction in normal spindles of most skeletal muscles serves clearly and consistently to differentiate between the b_1 and the b_2 nuclear-bag fibres; the b_1 fibre gives a consistently pale reaction, while the b_2 fibre always stains darkly. This observation was substantiated during the present study in normal rat medial gastrocnemius muscle (fig. 52 J) the separate identities of the two fibres being confirmed ultrastructurally.

Photography of sections Sections depicting the histochemical characters of both normal and model-adult fibres were selected for clarity and photographed using the Zeiss Ultraphot.II microscope. All photographs of spindles were exposed at a standard (negative) magnification of 250 and enlarged by 4, i.e., a total magnification of 1000.

Methods for silver staining and teasing After removal of the superficial fascia, both model-adult and control muscles from a total of nine young adult rats (see Table II) were subjected to one of the following silver impregnation techniques for nerve axoplasm: (i) Ragab and Tuffery's modification of the methods of Barker and Ip (1963) and Gladden (1969) (6 animals). De Castro's fixative was used in this method. Following initial immersion in fixative (1g chloral hydrate + 45 ml. 95% alcohol + 50 ml.

distilled water + 1 ml. conc. nitric acid) the muscles were cut lengthways into four pieces to aid complete permeation of fresh fixative and subsequent solutions. Incubation in 2 per cent silver nitrate was for five days at 29°C.

The results were generally unpredictable, the degree of staining varying within a muscle itself. Whereas the motor innervation usually stained up well against the muscle fibres (black or dark brown endings on pale brown muscle tissue), the sensory innervation gave altogether poorer results. This insensitivity was attributed to the presence of the spindle capsule.

(ii) A modification by the author of Winklemann and Schmidt's (1957) stain (3 animals). Two other variations were also tested but proved less satisfactory. Details of the modified procedure are given in Table IV where they are compared with Winklemann and Schmit's original method.

With this technique, the nerve terminals generally stained up much darker against a paler, more translucent muscle tissue. This was largely due to the observer's facility to control the development process with the use of the microscope (cf method (i) above) and to the fact that the separation in time of the staining of the nerve axoplasm and the staining of the other structures was reasonably defined. Another advantage of this method over method (i) was the simplicity of the solutions. In some spindles, unidentified black, ovoid spots were observed in the B zone. The main drawback was the tendency of some muscle fibres to appear very pale, and boundaries between two closely apposed fibres were often indistinct. Observations on the size of the nuclear bag(s) and the lengths of intrafusal fibres were consequently rendered difficult.

For both procedures, storage for at least one week in glycerol facilitated subesequent teasing.

A Zeiss dissecting microscope and fine dissecting needles were used for teasing the muscle. Small pieces of tissue were squashed onto to glass slides and examined for spindles under the low power of a Zeiss G.F.I. microscope. Spindles thus located were extricated from excess connective tissue and extrafusal fibres and fresh, permanent mounts made. Dessication was prevented by ringing the coverslips with pitch.

Detailed studies were carried out under oil immersion. Camera lucida drawings were made at various maginifications of all or parts of the spindles' innervation, together with photographic tracings. Various nerve parameters were measured by means of a Zeiss monocular attachment, calibrated beforehand with a 0.1mm and 0.01mm Watson stage micrometer. All measurements were made on similarly processed muscles (namely, method (i)) for valid comparison of normal adult, model-adult and adult-crush spindle innervation.

Table IV Comparison of (i) Winkelmann and Schmit's (1957) simple silver method for staining nerve axoplasm, with (ii) the author's modification of same.

WINKELMANN & SCHMIT'S (1957) PROCEDURE	AUTHOR'S MODIFICATION FOR OPTIMAL STAINING OF SENSORY ENDINGS
(1) Fix in 10% formalin solution without a neutralizing or buffering agent: several days longer (chloral hydrate proves unsatisfactory in this method)	(1) Immediate fixation of whole muscle in 10% formalin. After a day or two, cut muscle into very narrow (approximately 1mm) long pieces and leave for another day or two
(2) Absolute alcohol: 3 x 1 hour minimum	(2) 1 ml concentrated nitric acid + 95 ml distilled water: 2 hours. (suppresses staining of connective tissue, including spindle capsules, as well as nuclei, and enhances staining of nervous elements by the defatting agency of the nitric acid).
(3) Xylene: 1 hour minimum	
(4) Absolute alcohol: 3 x 1 hour minimum	
(Steps (2) to (4) defat, dehydrate and clear the tissue)	Stages (3), (4) and (5) found not to produce any improvement in staining and therefore omitted in modified method.
(5) Distilled water rinse and store in 10% formalin until needed or cut 50μm frozen sections after rinse and store in 10% formalin until needed.	
(6) Thorough rinse in not less than 3 changes of distilled water: approx. $\frac{3}{4}$ hour. (removes excess formalin, which would otherwise cause excessive background staining)	(3) As opposite

Table IV continued

WINKELMANN & SCHMIT'S (1957) PROCEDURE	AUTHOR'S MODIFICATION FOR OPTIMAL STAINING OF SENSORY ENDINGS
(7) 20% silver nitrate in dark: 20 minutes. (impregnation time may be shortened, but it too short, subsequent washing cannot adequately be controlled)	(4) Same solution: 45 minutes
(8) Pieces of muscle immersed, one at a time, in 3 changes of distilled water: approx. 3 seconds each (9 seconds is appropriate for impregnation times longer than 10 minutes)	(5) Repeated distilled water rinse: 10 minutes
(9) 0.2% hydro quinone in 1% sodium sulphite: controlled under microscope. Approx. 10 minutes Solution not to be shaken (reduces or develops the silver nitrate; stopped when background begins to stain up)	(6) As opposite: 60 minutes
(10) Distilled water wash: 2x	(7) As opposite
(11) 0.2% gold chloride: 2 mins.	Stages (11),(12) and (13) omitted in modified procedure The steps serve to increase the contrast between nerve and tissue for photography, and are optional in the actual W&S method itself.
(12) Distilled water rinse	
(13) 5% sodium thiosulphate: 5 minutes then wash	
(14) Dehydrate, clear, mount: 15 minutes - 1 hour	(8) Cleared in glycerol and sometimes left for about 1 week to soften for easier teasing
Total time taken from tissue removal to mounting	
5 days minimum	$2\frac{1}{2}$ days minimum to glycerol immersion

Methods for stem-nerve measurements Control and regenerated nerve trunks from each model-adult and adult-crush rat were removed as described on page 62 and embedded in Epon resin. Because of the lengthiness of axon-diameter counts for histograms, only two pairs of model-adult nerve trunks (i.e. from two model-adult rats) were sectioned together with one adult-crush pair (i.e.from one adult-crush animal).

Several thick (1 µm) transverse sections were cut and stained for 20 seconds with a solution of paraphenylenediamine in 50% methanol, followed by washing in 50% methanol. The sections were mounted on glass slides with DPX.

Photographs were taken at x100 with a Zeiss Ultraphot II microscope and enlarged ten times, giving a total magnification of one thousand diameters. Measurements were taken by means of a glass rule placed directly over the photographs and were made to the nearest 0.5 mm. The total diameter - i.e., including the myelin - of each nerve fibre was found by taking the mean of two measurements at right angles to each other. A few fibres (in no case exceeding 1% of the total) were discarded as unsatisfactory either on account of splitting of the myelin sheath or of close proximity to the node of Ranvier.

Three pairs of histograms - two model-adult pairs and one adult-crush pair - were drawn from the data.

As errors may be introduced at the stages of fixation, staining, photography and measurement, all stages were standardised as far as possible, particularly during the second and third stages, since variations in immersion time in the stain and the exposure time during photography can produce appreciable variations in the measurements of myelin and axon diameters.

Pairs of nerve trunks were also removed from neonatal-crush developing rats (i.e., 2, 4, 7, 10 and 14 DPO) and embedded in paraffin. Serial transverse sections, 5 m thick, were cut and stained with osmium tetroxide or by Holmes silver method for axoplasm. In this way, the general temporal course of axon degeneration and regeneration was recorded by monitoring the changes in nerve axon diameter and axon number.

RESULTS

PART I - RESULTS OF PRELIMINARY METHODOLOGY

(i) First series of operations A consistent decrease - compared with the normal - in the cross-sectional area of the whole muscle of experimental 14 DPO rats was clearly evident. This decrease was due in part to a reduction in the cross-sectional area of the extrafusal muscle fibres as well as to a reduction in their number.

There were also consistently fewer spindles per muscle (See Table V) particularly in the 2 DPN-crush and 3 DPN-crush groups of experimental animals. Except for some spindles in the 4 DPN-crush group muscles, the reinnervated spindles generally looked abnormal in some way, be it the diameter and length of the spindles, the number of intrafusal fibres per spindle, the intrafusal fibre diameters or the size of the nuclear bags, where these were preserved. Quite predictably, reinnervated spindles in the 4 DPN-crush group generally appeared more intact than those in the 2 and 3 DPN-crush groups.

(a) 2 DPN-crush group All spindles in 2 DPN-crush muscle were very foetal in appearance (comparable to 19.5 day foetal normal material). They consisted of a single myotube of small, uniform diameter. No bag of nuclei was ever encountered, only intermittent central and peripheral nuclei along the entire length of the fibre. Unlike 19.5 day foetal muscle, however, a periaxial space was present, but appeared very much reduced in size as did the diameter of the outer capsule layer of cells. Spindle length varied, but was consistently much less than normal.

TABLE V

Number of spindles in medial gastrocnemius muscle of the rat 14 days after nerve crush at 2, 3 and 4 DPN.

E = experimental values; C = contralateral, control values.

Animal No.		Age of crush		
		2DPN	3DPN	4DPN
1	E:	5	10	16
	C:	19	22	22
2	E:	7	9	12
	C:	19	23	26
Mean no. of spindles per muscle	E:	6	9.5	14
	C:	19	22.5	24

(b) 3 DPN-crush group In the two 3 DPN-crush muscles studied, 12 of the 19 spindles (63.2%) contained one fibre, 5 (26.3%) were two-fibred, and 1 (5.3%) contained three fibres. Of the single-fibre spindles only one contained an intrafusal fibre with a normal-sized nuclear bag, yet the capsule was reduced in size. The intrafusal fibre of four of the other single-fibre spindles possessed a smaller than normal nuclear bag, whereas the remaining seven spindles were, in appearance, very similar to the degenerate spindles encountered in the 2 DPN-crush group. Thus, they comprised a single myotube surrounded along the middle third of its length by a capsule which enclosed a very narrow periaxial space.

Of the five spindles containing two intrafusal fibres, only two appeared to be "acceptable" model-adult spindles, i.e. they possessed well-developed nuclear bags - one larger than the other - at the equator, and their periaxial space was well-developed. The remaining three two-fibred spindles were considerably more atrophic, containing two underdeveloped intrafusal fibres which, in all three cases, became closely apposed to each other in the degenerate equatorial region. There, both fibres contained a recognisable bag of nuclei - two nuclei were seen in transverse section in each fibre - and an oblique section revealed that they were arranged in alternate, zig-zag fashion, close together. Some of the polar nuclei were central in position as opposed to being peripheral, which is the normal condition. Both the periaxial space and the total lengths of these spindles were undersized.

The only three-fibred spindle in the 3 DPN-crush group contained a large-diameter bag fibre, a small-diameter bag fibre (both of similar length) and a shorter nuclear-chain fibre. The periaxial space was well developed and the length of the spindle approached a normal value of approximately 1.2 mm. (see page 85. Shrinkage factor (Stickland,1975) not taken into account).

(c) 4 DPN-crush group Similar results to Werner's 1973 nerve-crush work were obtained in the 4 DPN-crush group. Of a total of 28 spindles in the two muscles, 7 (25%) appeared much like spindles in the control muscles. They contained the usual, normal complement of four intrafusal fibres - two nuclear-bag fibres, one of larger diameter than the other, and two nuclear-chain fibres. The parameters of mean spindle diameter and length were similar to the contralateral means. Three-fibred and two-fibred spindles, with otherwise quite normal-looking bags and periaxial spaces, numbered 16 (57.1%) and 5 (17.9%), respectively. Those spindles containing two intrafusal fibres were generally shorter than normal, unlike the three-fibred spindles, whose dimensions approached normality.

(ii) Second series of operations The crucial age of crush for producing bag-only spindles in the medial gastrocnemius muscle of the adult rat clearly lay somewhere between three and four days after birth. Nerve crushes were consequently carried out at $3\frac{1}{4}$, $3\frac{1}{2}$ and $3\frac{3}{4}$ DPN and the animals killed two weeks later.

There was little difference in the organisation of some of the reinnervated spindles among the three experimental groups but, quite clearly, both $3\frac{1}{2}$ DPN-denervated muscles contained the greatest proportion of otherwise well-developed one-fibred and two-fibred spindles (figure 3B). Of a total of 21 spindles counted in the

muscles of the latter experimental group, 6 (28.6%) contained a single fibre with a well-developed nuclear bag; 13 (61.9%) contained two nuclear-bag fibres of different diameters; and only 1 spindle (4.8%) was three-fibred, being composed of two nuclear-bag fibres and a single nuclear chain fibre. The periaxial space was well-developed in all the spindles. Lastly, a tandem spindle was also present in one of the muscles of the 3½ DPN-crush group. Contained within the proximal capsule were two nuclear-bag fibres, whereas in the distal capsule, only the larger diameter fibre was encountered. A bag of nuclei was present at the mid-equator of the latter capsule. The length of the tandem spindle exceeded that of a normal simple spindle. Apart from a general reduction in size, the model spindles appeared otherwise quite normal as far as could be discerned from paraffin sections stained with haematoxylin and eosin.

In the two 3½ DPN-crushed rats, the tibialis postior and the soleus, were also removed after 14 days, from the operated as well as the unoperated legs. The reason for doing so concerned their small size: thus if they were shown to contain a high proportion of otherwise well-developed one-fibre and two-fibre spindles (in the experimental legs) as observed in the medial gastrocnemius muscles, then they would be far more manageable as model muscles - particularly as regard EM and silver/tease preparations - than might be expected using the much larger medial gastrocnemius muscle. As it was, however, the results were poor for the same level of tibial nerve crush, spindles being completely absent (cp. figure 4A, B with 3A(ii), B(ii)). This disparity in spindle number and degree of development can doubtless be attributed to the different distances from level of crush to level of muscle innervation. For the soleus it was 14mm, for the tibialis posterior 10mm, and for the medial gastrocnemius 7 mm.

Having determined the optimum age of crush, a 'production line' of experimental adults was maintained for further investigation of the model-adult spindles. Four to seven months were allowed to elapse after the operation before killing the animals. This period was deemed adequate for the reinnervation process to 'settle down'. It was realistic to predict that not every litter operated on at $3\frac{1}{2}$ DPN was likely to produce model-adult muscles as consistently workable as the two described above for the preliminary methodology. In order to ascertain whether or not an experimental litter did contain a feasible reservoir of 'near-perfect' model-adult spindles, one member of the litter was killed and used as an "indicator". This was done usually one month post-operatively and certainly never less than two weeks post-operatively. Serial paraffin sections were prepared from the entire experimental muscle and an assessment made of the absolute number, as well as the proportion of bag-only spindles. Where the results of the test rat proved unacceptable, (for example, as depicted in figure 4C), the litter from which it was taken was discarded. Even so, in some of the muscles cleared for study feasibility, a few three-fibred spindles and the occasional four-fibred spindle cropped up, particularly towards the proximal ends. Such "impurities" were anticipated owing to the different developmental rates among spindles in any one muscle.

In sum, then, the preliminary methodology of the research showed that, as far as could be gleaned from the rapid scanning, but limited method of paraffin sections, crushing the tibial nerve of $3\frac{1}{2}$ DPN rats at a point just proximal to the popliteal bridge of blood vessels produced bag-only (but otherwise

apparently normal) spindles in the medial gastrocnemius muscle of the adult rat, in other words, these were the desired model-adult spindles. Because the thickness (10 μm) of the paraffin sections and the comparatively poor resolution of the light microscope precluded an absolute confidence in the observation that chain fibres were absent in the experimental bag-only spindles, electron microscopic work was undertaken for confirmation of this observation. At the same time, the normality of the bag fibres(s) was investigated through a study of their ultrastructure and histochemistry by applying a new technique of combined electron microscopy and histochemistry (Pierobon Bormioli,1974; Banks et al,1976). Such an ultrastructural/histochemical study of the nuclear-bag fibre(s) was essential for subsequent observations on their innervation (from silver/teasing methods) to be meaningfully evaluated.

PART II - RESULTS OF HISTOLOGICAL STUDIES ON MODEL-ADULT SPINDLES AND THEIR COMPARISON WITH NORMAL ADULT SPINDLES

(i) General morphology (i.e., light microscopic observations: paraffin sections stained with H and E; thick Epon sections stained with toluidine blue (TB)).

Some of the measurements given in the following two sections have been taken from one or both of the 14 DPN animals used in the preliminary methodology (refer to page 79), since only in this pair of animals were the control and the model medial gastrocnemius muscles closely studied from end to end by means of serial paraffin sections. Refer to Table VI.

Normal adult spindles The mean number of spindles in seven normal 14 DPN medial gastrocnemius muscles was found to be 23.9 (range 19 to 26), with little or no variation between the right and left limbs. This agrees with similar counts made by Werner (1973 a, b), Zelena (1964) and Yellin (1969). With the medial gastrocnemius muscle being a relatively large muscle, this means that its spindle density is low, a feature which characterises any muscle that initiates gross contractions.

The spindles were confined to the axial core of extrafusal fascicles. Yellin (1969) also found this and, in addition, demonstrated that the axial localisation of the spindles corresponded closely with the distribution of the nerve supply. Observations from the present study revealed, as well, that the greatest concentration of spindles occurred in the belly, or middle third of the muscle. Figure 3A depicts the typical localisation of spindles within the normal medial gastrocnemius muscle. It was constructed from serial paraffin transverse sections stained for general histology

TABLE VI Measurement of various spindle parameters in normal "adult" and model-"adult" muscles. With the exception of column two, all data taken from 10 μ_m thick H&E paraffin sections of two week old muscles. Column two includes adult as well as two week old muscles processed by all the various histological methods.

	Mean no. of spindles per muscle	% of spindles that are single-, 2-, 3-, 4-, or 5-fibred	No. of cell layers per capsule	Mean spindle length (mm), = length of bag-fibre	Mean length of peri-axial space (mm)	Mean polar length (mm)	Mean equatorial diameter (μ_m). All capsule cells included	Mean diameter of Ifs at A/B interface (μ_m)
NORMAL "ADULT" MUSCLE	23.9	4-If: 81.4 5-If: 11.1 3-If: 7.4	6	1.28	0.29 Range: 0.21 - 0.37	0.48	52.0 Range: 32.0 - 65.6	b_1: 8.3 b_2: 10.4 NC: 6.4
MODEL "ADULT" MUSCLE	10.5	2-If: 36.8 3-If: 18.4 1-If: 31.6 4-If: 5.3 Anomalous: 7.7	5 - 6	0.68	0.23 Range: 0.13 - 0.30*	0.24	41.2 Range: 20.0 - 54.4*	2-If spindles) b_1: 7.3) b_2: 9.3 1-If spindles) $b_?$: 8.5

* Wide range of values obtained for these two parameters because of variance between two-If and single-If spindles in respect of the parameters.

with haematoxylin and eosin. The mean spindle length of 20 spindles in one two week old muscle was 1.28 mm.

Approximately six cell layers comprised the capsule wall (at the mid-equator) of spindles in the normal muscle. As measured at this level, the mean diameter of 20 two-week old normal spindles was 52.0 μm (range: 32.0 μm to 65.6 μm). The average length of the periaxial space was 0.29 mm (range: 0.21 mm to 0.37 mm) for the same sample.

An average complement of four intrafusal fibres per spindle was found in the normal adult medial gastrocnemius muscle (see also Werner, 1973 a, b and Zelena, 1964; Ovalle, 1972 a, b; Landon, and James, 1971 a). Of a total of 54 spindles, 44 (81.4 %) were four-fibred, whereas only 4 (7.4 %) and 6 (11.1 %) were three- and five-fibred respectively.

The most usual composition of the fibre quartet was two nuclear-bag fibres, of which there were two types, and two nuclear-chain fibres. One of the bag fibres, the bag_2 fibre (Barker et al, 1976) usually contained a larger bag of nuclei at the equator than the bag_1 fibre (2 - 3 nuclei or 3 nuclei in T.S., respectively). Unlike the situation in cat and rabbit spindles (Banks et al.,1977), the size difference between the two types of bag fibre in most normal rat medial gastrocnemius spindles remained obvious at all levels. The mean diameters at the zone A/B interface - from the aforementioned sample of 20 spindles - in normal, two week old muscle were 10.4 μm and 8.3 μm, for the bag_2 and bag_1 fibres, respectively.

Lengthwise, the two types of nuclear-bag fibres were very similar, unlike the situation in cat and most primate spindles, where the larger bag fibre (≡bag_2) always exceeds the smaller bag fibre (≡bag_1) in length (e.g., Barker & Gidumal, 1961: cat).

The nuclear-chain fibres of normal adult medial gastrocnemius spindles commonly extended beyond the capsule for variable distances (Bridgeman, 1969). but were never as long as the bag fibres. The mean diameter of 19 chain fibres at the zone A/B interface of normal two week old muscle was 6.4 μm, roughly two-thirds the value for the bag fibres. However, in region B, at least one of the chain fibres often had a diameter similar in size or sometimes even greater than the bag_1 fibre. Consequently, the surest way of typing an intrafusal fibre as chain or bag_1 in H and E sections, was to follow the spindle serially along most of its length. The shortest fibres that possessed a chain of nuclei at the equator were chain fibres. In some spindles the diameter of one of the chain fibres was two-thirds to one-half that of its neighbour.

Model-adult spindles Predictably, the number of spindles in the model-adult muscles (i.e., those operated on at the optimum nerve crush age of 3½ DPN was usually considerably reduced. As detailed in Part I of the results (page 79), 21 spindles were counted in the two whole muscles serially sectioned in paraffin (10 and 11, respectively), giving a mean of 10.5 spindles per muscle.

The model-adult spindles were similarly confined to the axial core of extrafusal fascicles. And as in the normal medial gastrocnemius muscle, the model-adult spindles were mostly located in the belly of the experimental muscle. But the paucity of spindles in the distal third was much more marked, and the spindles that did regenerate in this region were almost invariably single-fibred or (less frequently) two-fibred. The location of model spindles in one of the 14 DPN experimental muscles is diagrammatically depicted in figure 3B. An example of occasional clumping is shown in figure 4D. The mean spindle length of 11 model spindles in one two week old experimental muscle was 0.68 mm, a 46.9 per cent reduction from the normal contralateral mean. This reduction in spindle length was restricted mainly to the polar lengths. The polar lengths (as measured from the end of the periaxial space towards the poles, i.e., includes much of zone B and all of zone C) of the spindle sample of 11 were measured and the mean (of 22 measurements) was found to be 0.24 mm. The mean for the contralateral, normal muscle was 0.48 mm. Thus, the reduction in the polar lengths of model spindles was some 54.7 per cent, considerably greater than the reduction of their equatorial regions (the length of the periaxial space - region A - being used as a measure of the length of this region, see below).

The number of cell layers constituting the capsule did not differ from the normal adult value of 5 - 6. Whereas the polar lengths were, on average, halved, spindle diameter at the mid-equator was reduced by only 23.7 per cent (mean

of a sample of 11 two week old spindles: 41.2 μm; range 20.0 μm to 54.4 μm), and the length of the periaxial by only 20.7% (mean: 0.23 mm; range 0.13 mm to 0.30 mm). As seen from the wide range of values, the reduction in the values of these two parameters is variable and depends on the number of intrafusal fibres per spindle. In single-fibre spindles, the two parameters generally suffered a greater reduction than in two-fibre spindles. And in three-fibre spindles, the two parameters approached normality, although, of course, these values were not included in the results.

Of a total of 38 model-adult spindles studied by all the methods of histology, 14 (36.8%) contained two nuclear-bag (nb) intrafusal fibres and 12 (31.6%) contained one nuclear-bag fibre. Relatively few - 7 (18.4%) - were three-fibred (2 nb and 1 nc) and only 2 (5.3%) were four-fibred (2 nb and 2 nc). The remaining 7.9% comprised anomalous spindles, i.e., those which were indisputably abnormal with regard to the nature of the intrafusal fibres. One example of such deviation from the " norm " of model-adult spindles was a tandem spindle in which a single nuclear-bag intrafusal fibre was common to both capsules (fig. 67F). Two other fibres arose close to, but outside, the first capsule at its mid-equatorial level and continued to run alongside the common nuclear-bag fibre after the first capsule had disappeared. As the second enclosed all three fibres, the two shorter fibres increased in diameter; one became a nuclear-bag fibre and the other a nuclear-chain fibre.

Anomalous spindles apart, the incidence of tandem spindles was greater among the model-adult spindles than among the normal spindles. Thus, only 1 out of 45 (2.2%) normal adult spindles were tandem compared with 4 out of 32 (12.5%) model-adult spindles. Porayko and Smith (1968) found no tandem spindles in rat lumbrical muscles, but Gladden (1969) found some in rat intertransverse tail muscles.

As regards the number of nuclei in transverse section, the bag_1 and/or bag_2 fibres comprising the one-fibre and two-fibre model-adult spindles were no different from those in normal adult spindles. Exceptions were some single-fibre spindles which lacked a distinct nuclear bag, appearing myotubular at the equator. Mean intrafusal fibre diameters were, in most model-adult spindles, slightly reduced. Thus, in the two week old sample of 13 two-fibre spindles, bag_2 and bag_1 fibre diamters - the two fibre types were distinguished by virtue of their different sized nuclear bags - at the zone A/B interface were 9.3 μm and 7.3 μm, respectively, the range of values being wider than in the control sample. The bag fibres of the 6 single-fibre spindles had a mean diameter at the zone A/B interface of 8.5 μm - in between the normal bag_1 and bag_2 diameters. As in normal spindles, there was usually a consistant difference in the polar (i.e., zone B and C) calibres of the two fibres, particularly in region C. They were equally atrophied by some 25% or actually hypertrophied in a few spindles.

(ii) Ultrastructure

The capsule and axial sheath -

Normal adult spindles Observations on the ultrastructure of the outer capsule cells and the inner axial sheath cells agreed well with those made by other researchers (Landon, 1966; Goglia, 1970; Merrillees, 1960; Milburn, 1973 PhD thesis) who worked on various rat muscles. The description given here will consequently be brief.

Characteristically, the cells of both sheaths were flattened, and zones of close contact were observed between neighbouring cells. Five to seven layers of cells constituted the outer capsule. Within the cytoplasm of the latter were small mitochondria, numerous free ribosomes, together with dilated cisternae of rough endoplasmic reticulum containing densely amorphous material (fig. 5A & B). From sequential sections, the axons comprising the main spindle nerve trunk were seen to run between cell layers of the outer capsule and eventually enter the periaxial space.

The cells of the inner capsule or axial sheath, i.e. the endomysial cells, bore a close resemblance to those of the capsule, but the cytoplasm stained more densely (on account of the higher ribosomal content), the nuclei appeared more compact and, as Shantha and Bourne (1968) pointed out, the basement membrane was characteristically absent from both surfaces. Whilst, at the equator, the endomysial cells almost, or completely, enveloped each intrafusal fibre at the poles, one endomysial cell surrounded several fibres. There was no isolation (in the equatorial region) of bag_1 fibres from the

bag_2 and chain fibres, a condition which obtains in cat tenuissimus spindles (Barker et al., 1976) and one which facilitates identification of bag intrafusal fibres in the cat.

One of the most notable features of the cells of the capsule and axial sheath was the occurrence of numerous, pale vesicles within the cytoplasm, usually lining both sides of the cell membranes (fig.5B). These round, micropinocytotic vesicles appeared in various stages of formation. They were more numerous in the outer capsule cells than in the axial sheath cells.

Model adult spindles There was no obvious effect of neonatal crush on the structure and organisation of the capsule and axial sheath cells. For example, their characteristic flattened form was retained and the number of cell layers at the mid-equater remained the same (five to seven). See fig. 13B. Points of close contact were present and the cell constituents appeared normal. There was a similar profusion of pale, micropinocytotic vesicles lining both sides of the cell membrane (fig.13 B).

The inner axial sheath cells that had enclosed the now degenerated chain fibres at the equator, had long since been absorbed. The only noticeable difference from the norm among the sheath cells enwrapping the bag fibre(s) was the greater dilation of rough sarcoplasmic reticulum, which also contained a greater preponderance of dark, granular material (fig.13 A). There was also more convoluted basement membrane in the periaxial space, and the convolutions appeared more branched and intricately

folded (fig.15A & 24).

The intrafusal fibres -

Normal adult spindles Transverse and longitudinal sections of nuclear-chain intrafusal fibres revealed an abundance of sarcoplasmic reticulum which effectively separated the myofibrils into discrete units. This condition was evident in the outer juxta-equatorial and polar zones, i.e., the equivalent of Barker et al's (1976) inner B and outer C regions (fig.5A & 11B). Milburn (1973) and Ovalle (1971) found the SR to be clearly much better developed at the I and Z band level of the sarcomere in flexor hallucus longus and lumbrical muscles, respectively, but although this condition obtained in the medial gastrocnemius muscle, it was less obvious, i.e. the myofibrils were not completely encircled by a double layer of beaded SR tubules at the I/Z level. Transverse tubules were more prevalent in nuclear-chain fibres than in nuclear-bag fibres (certainly than in the bag_2 fibres), as was the occurrence of triads (fig. 9C). Moreover, chain fibres were characterised by couplings of t-tubules with subsarcolammal cisternae of SR. Equatorially, the myofibrils were reduced to a thin, peripheral cylinder.

Mitochondria were located more frequently at the I band level and were variable in size, although the large, long ones (orientated parallel to the fibre longitudinal axis) tended to outnumber the smaller, rounded ones. The largest mitochondria possessed "loose" (non-ccmpact) cristae and occasionally extended along the length of a sarcomere, sometimes further, (fig.9D), while some of the subsarcolemmal ones produced slight

bulges in transverse section at the periphery of the fibre (fig.5A). Rather smaller, fewer mitochondria characterised the smaller diameter chain fibre.

A thick, clearly demarcated M-line (800 - 900 Å wide) was obvious along the entire length of both chain fibres (fig.9C). It was located in the centre of a well-defined pseudo-H zone and appeased similar in prominence oo the M-band of neighbouring extrafusal muscle fibres (fig. 45A. Cp normal, fig. 45 B & D).

The myofibrils of the bag_1 fibre were indistinctly delineated along its entire length; the absence of interfibrillar SR produced a densely packed pattern (fig.5 & 10B). Fibre delineation was limited to the I and Z band region, since, what little SR there was appeared mostly at this level (fig.9B). In transverse sections the SR was seen as a single broken layer surrounding some of the myofibrils. Compared with the nuclear-chain fibres , the bag_1 fibres possessed few junctional couplings between SR and transverse tubules, so that triads were only occasionally seen. The mitochondria of the bag_1 fibre were substantially smaller and fewer in number than those in the chain fibres (fig.5), but like the latter, they were longitudinally orientated and unbranched and confined largely to the I/Z band level. The cristae appeared long and compactly arranged.

An M-line was never encountered along the length of the bag_1 fibre. At most, a faint double M-line was present (fig. 9B & 10B). Occasionally, a sarcomere with a double M-line lay adjacent to one with no M-line.

Likewise, the bag_2 fibre was seen to possess a faint double M-line or no M-line, but solely in the equatorial and

juxta-equatorial (region A) zones. For the rest of its length an M-line was evident (fig.5D & 9A), although not as thick or as well delineated as in the chain fibres. In region A, where the M-line condition of both bag fibres was the same, there were no marked ultrastructural differences between the two fibres, although, the Z lines of fibre bag_1 were, generally, more clearly defined than those of fibre bag_2. In regions B and C, however, the ultrastructure of the bag_2 fibre switched to a more chain-like nature. In other words, the mitichondria now appeared more numerous (though no larger) and the cristae were arranged less compactly; there was more interfibrillar SR and, concommitant with this, rather more transverse tubules. See fig.5 and 11A. These observations closely parallel those of Barker et al.(1976) in rat soleus and peroneus muscle, as well as cat tenuissimus muscle.

Model-adult spindles The bag fibres of model-adult spindles were sometimes enclosed by a double basement membrane in the capsular region (for example, fig.19B, C, 22A, 26A, B and $_{44}$B). The inner one was of normal thickness and, as normal, lay in close contact with the sarcolemma. However, the outer membrane was thicker and not always complete or distinct. Where intact, the outer membrane wasthrown into numerous folds, usually at two or three points round the circumference of the fibre. A feature confined only to model-adult intrafusal fibres was the odd occurrence of short lengths of thickened sarcolemma (eg,fig.13A,25B&26

At the A/B zone interface of one single-fibre model-adult

spindle, two large multivesicular bodies were observed, one in the periaxial space and the other between the cell layers of the capsule (fig.14B). They contained pale-staining vesicles of similar size and appearance to the pinocytotic vesicles occurring within the capsule cells.

In a couple of model-adult spindles the two bag fibres were closely apposed along part of their circumference in the equatorial region (fig.41A) where normally, and in all other model-adult spindles studied for EM (e.g. fig.33A), they are well separated by the endomysial cells of the axial sheath.

The two most noticeable differences from the normal condition in model-adult intrafusal fibres from the outer A zone to zone C, was the frequent occurrence of oblique myofibrils (especial those at the periphery) in transverse section and a general increase in sarcoplasm (e.g. fig.18B,26B & 34A). . Because of the latter, the myofibril content of the intrafusal fibres (both in TS and LS) sometimes appeared less compact than normal. In these regions, the increased sarcoplasm was not always evenly distributed around the myofibrils as in normal nuclear-chain fibres, but was sometimes patchy and contained a variety of organelles (e.e. fig.43B). These included Golgi bodies and associated clear vesicles which were often seen to be in the process of being pinched off; numerous, free polyribosomes usually evident in circular arrangements; free, dense-core, clear and dark vesicles; myelin figures; and an abundance of rough dilated sarcoplasmic reticulum. In comparison, normal intrafusal bag fibres contained these structures in similar abundance only in the internuclear

cytoplasm (i.e., in the myotubular lengths of zone A) and even there, the sarcoplasmic reticulum was not so extensive. Clearly though, stereological measurements would go a long way in quantifying these differences in ultrastructural content.

In one two-fibre model-adult spindle, cross-striated organelles - quite distinct from the sarcomeres - were seen in the juxta-equatorial region of the larger bag_2 fibre (fig. 26C & 28). These structures, known also as " microladders" or " leptomeric organelles ", have been observed in normal skeletal muscle by other workers (Karlsson and Andersson Cedergnen, 1968; Ovalle, 1972). They had a banded structure of narrow, electron-opaque discs (16 Å wide) that alternated with longer, pale, bands of filamentous structure (188 Å wide). The striations were visible in both TS and LS. The latter sections revealed them to be located at the A-band level. In TS, they were located between the myofibrils in what appeared to be a random fashion, and also as peripheral arcs closely abutting the sarcolemma. Among the myofibrils they were seen as discrete entities (i.e., 5 - 10 dark plus 5 - 10 pale bands in any one section), but beneath the sarcolemma they appeared more like extensive, thin strips.

Ovalle (1972a) identified microladders like these in both bag and chain fibres of both normal lumbrical muscle. He found them most frequently in or near the region of sensory innervation. Whether coincidental or not, the microladders in the bag_2 fibre of this study were never encountered in the same section as sensory endings.

The mitochondria of model-adult bag fibres were similar to

those of normal adult bag fibres (fig. 18B). Thus, they were mostly small - except for a few large ones in the B region of some single-fibre spindles (fig. 16) and those between the myonuclei (fig. 18C) - and were located at the level of the Z lines in both bag fibres (fig. 30, 31 & 32). Moreover, they were few in number, except within the extensive cytoplasmic areas between the myotube nuclei (fig. 18A & C) and in young model-adult rats, i.e., during the recovery period following nerve crush. There also tended to be more in the B region of the bag_2 fibre of some two-fibre spindles (fig. 39A).

Model-adult bag fibres contained t-tubules (mainly in the I region) but seldom in any regular association with SR. In other words, triads and diads were seldom seen (fig. 21B). In some single-fibre model-adult spindles, t-tubules were (in region A) unusually abundant (fig. 14B), but in others, they were less evident.

Like the bag fibres of normal spindles, the Z lines of the bag_1 fibres were sometimes more clearly defined than those of the bag_2 fibres (fig. 44).

A structure not observed in normal nuclear bag fibres, but quite often so in model-adult fibres, was a spherical, densely body, similar to those described by Ovalle (1972b) in both types of intrafusal fibre. Thus, these bodies consisted of a dense homogeneous mass of finely granular material bounded (in contrast to Ovalle's) by a membrane. Their diameter varied from 1 μm to 3 μm (fig. 14A, 21B, 31A & 32B). Unlike the structures described by Ovalle, the dense bodies in this study were firmly wedged between the myofibrils themselves (usually at the Z band level) and not between the equatorial nuclei.

Small and large vesicular bodies, packed with small, dark vesicles, were sometimes present in the post-synaptic muscle and in the sarcolemmal lips that enclosed some of the sensory terminals (fig. 20B, 23A, 25B & 29B). They also cropped among the main bulk of the muscle fibres, but less frequently. None of the sections taken of normal bag fibres showed these structures.

The M-line profiles of the bag fibres in most two-fibre model-adult spindles did not deviate from their normal condition, i.e., a dM or no M-line for both the bag_1 and bag_2 fibres in zone A (fig. 31 & 32); and in zones B and C, a dM or no M-line for fibre bag_1 but an M-line for fibre bag_2 (fig. 40A & B, respectively). In the majority of single-fibre spindles studied in EM, a dM line occurred throughout the entire length of the bag fibre, suggesting a bag_1 identity for this fibre (fig. 21B). This tentative presumption was supported by the reaction for ATP'ase given by two single-fibre spindles in the histochemical study (see page 116). The two bag fibres gave a consistently pale bag_1 type reaction as opposed to the typical dark bag_2 type reaction (fig. 52K). Furthermore, a combined ultrastructural study of one of these spindles revealed a doubles M-line in region B (fig. 21C) as well as region A - a property of normal bag_1 fibres.

For a few of the less well-developed single-fibre model-adult spindles, however, excessive contraction (or, more likely, temporary denervation itself) appeared to have caused a " blurring " of the myofibril striations. Apart from the Z lines, which always remained intact, the rest of the banding pattern was obscured in many of the sarcomeres. The A and I

bands could be identified only as dark and light smudges, respectively, the junction between them being hazy, and the pseudo H-zone was even more obscured (fig. 16).

Particularly interesting in terms of muscle-fibre organisation was one tandem model-adult spindle (7½MPN) sectioned in the combined EM/histochemical experiment. It consisted of two well developed nuclear-bag fibres, but there was also evidence for what appeared, at first, to be remnants of a nuclear-chain fibre. This " appendage " fibre appeared, quite unexpectedly, in the " first " or proximal equatorial region as a small fibre intimately apposed to the bag_1 fibre (fig. 25 & 26A). Then, still within the proximal equatorial region, it proceeded to " cross over " to the bag_2 fibre such that it formed a bridge (strengthened by sensory cross terminals) between the two bag fibres (fig. 27). It finally becamr closely apposed to the bag_2 fibre alone, with the two fibres sharing the same basement membrane (fig. 29). At this point, the tentative chain identity of the appendage fibre was ruled out because it clearly lacked an M line (fig. 30). It is likely that the appendage fibre was a split from either bag fibre that had crossed over and become apposed to the neighbouring fibre, possibly even fusing with it.

In the " second " or distal equatorial region, however, this appendage fibre had disappeared from the circumference of the bag_2 fibre, but a remnant fibre, or " ghost " fibre, was seen quite separate from both bag fibres (fig. 36B & 37). It is not certain whether this ghost fibre and the appendage fibre of the proximal capsule were actually one and the same, as the intercapsular portion of the spindle had already been thick-sectioned for the purpose of histochemistry, or whether the ghost fibre was simply an old "fragmentary" fibre;see overpage.

Whatever the origin of the ghost fibre, its ultrastructure was clearly in a degenerate state. A basement membrane was lacking and only a few residual myofilaments could be identified among the sparse contents, i.e., there was no organisation into discrete fibrils (fig. 36B & 37). Mitochondria, vesicular bodies and dilated rough sarcoplasmic reticulum were abundant. In LS, the banding pattern was virtually non-existent, apart from the Z lines, which still remained sufficiently intact to be identified (fig. 38A).

There was an even more peculiar feature of this tandem model-adult spindle: the occurrence of a small number " fragmentary fibres " lying close to and clearly derived from the bag_2 intrafusal fibre (fig. 33-35). Two to seven could be counted in any one cross-section, always located within the endomysial envelope of the bag_2 or " parent " fibre, and at various stages of detachment from the latter. Once separated from the bag_2 fibre, some of the fragmentary fibres appeared to be engulfed by macrophage pseudopodia (fig. 35). Those " fragmentary " fibres that were on the point of splintering off from the parent bag_2 fibre, but still part of it, contained the best preserved myofibrils. On the other hand, those fragmentary fibres that had actually become detached had a very degenerate myofibril content: a few regularly separated A-filaments surrounded by I filaments contained within a pale, flocculent cytoplasm (fig. 36A). Interspersed among the cytoplasm were broken lengths of dilated sarcoplasmic reticulum, rough as well as smooth. The SR was thickened at several poimts. Longitudinal sections showed that the Z lines were the only banding pattern of the sarcomere to be left intact;

the light and dark pattern of the I and A bands, respectively, was quite unrecognisable (fig. 38B & C).

Most of the fragmentary fibres were pitted and irregular in outline. Like the model-adult bag fibres themselves, they possessed two basement membranes, the inner one being more intact than the outer. But with many fragmentary fibres, even the inner basement membrane was wanting. The sarcolemma itself was absent at certain points yet thickened at others. The These thickened zones of sarcolemma invariably corresponded with zones of intact inner basement membrane.

As already pointed out (see results, part I, page 81), working models are intrinsically perfect, and three-fibre spindles did crop up in several of the model-adult muscles. One such is depicted in figure 42-44. The chain fibre appeared well-developed ultrastructurally, but was considerably shorter in length than most normal chain fibres. This was due to the fact that it extended only from the juxta-equatorial level of zone A. Its other extremity, however, was in no way odd, terminating as it did, in zone B.

The sensory innervation -

Normal adult spindles Primary and secondary terminals could not be reliably distinguished from ultrastructural studies. Certainly, Milburn (1973 PhD thesis)& Banks et al. (1977) conceded this , despite the claims of Corvaja et al. (1969), Hennig (1969), Mayr (1970) and Rudjord & Rommetvedt (1970) to the contrary. The latter workers discerned a difference on the basis of shape. They recognised the secondary endings as

those which, in longitudinal sections, were seen to be in shallow grooves and to present low, flattened profiles. Mayr's electronmicrographs showed one chain fibre encircled by an annulospiral terminal and another supplied with a number of knob-like terminals, identified by him as part of the " flower-spray " type of secondary terminal. To identify this morphologically diffuse ending as a secondary terminal seems reasonable, but the fact that it and the annulospiral ending were actually connected to the same afferent axon, surely emphazizes the unreliability of the shape criterion as a means of distinguishing between a primary and a secondary terminal.

Recently, Banks et al. (1977) carried out a more painstaking and reliable study of the distribution and branching of primary and secondary afferents to cat tenuissimus muscle spindles. They took serial 1/µm transverse sections of three primary and seven secondary afferents and found that each afferent fibre supplied terminals to every muscle fibre. From each of the three primarys, one of the two or three first-order branches terminated exclusively on the bag_1 fibre, the other first-order branches landing on the bag_2 and chain fibres. Four of the secondary afferents were distributed to all three fibre types, two to chain fibres only and one to bag_1 and chain fibres only. In rat lumbrical muscle spindles, however, the secondary endings have been said to innervate chain fibres " almost exclusively " (Merillees,1960; Landon,1966 a, b), but it is obvious that little reliance can be placed on electron-micrographs per se without any attempt to make longitudinal

traces of spindle innervation.

Apart from undertaking a detailed approach like that of Bank's et al. (1977) in order to differentiate between primary and secondary endings at the ultrastructural level, another criterion is sometimes used as a rough and ready guide to the problem: the position of the terminals relative to the zone of equatorial nucleation. Secondary endings are located more or less in the outer myotubular lengths of the intrafusal fibres on one or both sides of the central primary terminations. Gladden (1969) made particular reference to this set up in her teased/silver preparations of rat caudal muscle spindles, but it has long been known as a universal feature among all muscles and all species af animal.

In the present EM study, no attempt was made to differentiate - imprecisely, as it would have had to be - primary from secondary endings. As expected, the shapes/sizes of sensory terminals in the normal adult spindles were found to vary from small circular outlines in transverse section, to an almost complete encircling of the muscle fibre (fig.12). Sensory cross-terminals, like those described by Adal (1968; cat), Merillees (1960; rat), Barker & Stacey (1970), Gruner (1961) were encountered on two occasions and were confined to the chain fibres.

Contained within the axon terminals themselves, were numerous vesicles - some of which Landon (1966) demonstrated as containing small granules, although in the present study, such vesicles were found near the post-synaptic membrane only - many mitochondria, a few neurofilaments and microtubule systems, and lipid droplets(fig 1
In the cytoplasm of the supplying axons, no concentric glycogen

complexes, as described in the cat (Corvaja et al., 1971) were ever seen.

Characteristically, Schwann cell coverings were absent over the outer surface of the sensory endings and common baeement membrane surrounded both ending and intrafusal fibre. The synaptic gap (100 - 200 Å in width) was mostly smooth, although some myonural junctions revealed broad, shallow outpushings that interdigitated with sarcolemmal kinks. Tight junctions were not uncommon (fig. 12). They usually took the form of short lengths of thickened neurilemma and sarcolemma sandwiching a densely-staining amorphous substance. Düring and Andres (1969) described a rather more complex type of tight junction in which the sarcolemma was attached to an adjacent cisternum of SR, but these were not encountered in this study.

Model-adult spindles As in normal adult spindles, the sensory terminals of model-adult spindles were encountered throughout the A region as well as the early B region. They showed a similar range of shapes to those on normal bag fibres, i.e, from small, round endings (often entirely surrounded by sarcolemmal lips, fig., 35 & 41) to horseshoe-shaped terminals which formed a smooth outline with the intrafusal fibre (e.g., fig., 14A, 19, 24, 33 & 36A). The actual axolemmas of the sensory terminals, and to a lesser degree, the sarcolemmas, were finely crenulated (fig., 32B), a well documented consequence of nerve lesion experiments.

An unusual finding in a two-fibre spindle was the presence of cross-terminals between the two bag fibres (fig. 27 & 41).

Cross-terminals have hitherto been found only between chain fibres of normal adult spindles (the control muscles of this study, and Barker and Stacey, 1970; rabbit, etc.).

The presynaptic organelle content appeared more or less normal, apart from a greater number of clear, pinocytotic vesicles - many in the process of being actively pinched off - and dark vesicles, which were two to three times as small as the pinocytotic vesicles. The clear vesicles outnumbered the dark ones. A couple of features apparently unique to model-adult sensory endings were: firstly, the presence of large, flattened vesicles (fig., 22C) and secondly, the small and large vesicular bodies at the base of the sarcolemmal lips that gripped the axon terminals . One such vesicular body was observed in the process of invaginating from the synaptic gap into the muscle fibre (fig., 20B

The width of the synaptic gap varied from normal dimensions (100 - 200 Å) to much greater than normal (400 - 600 Å). At the abnormal end of the spectrum, the nerve-muscle junctions could not really be considered as being in true synaptic contact; indeed, the sensory endings in such situations gave one the impresaion of being partly"torn" off, since they were apposed to the intrafusal only only along a relatively small portion of their circumference (fig.,20, 42 & 43). These widened lengths of the synaptic gap were lined with the common basement membrane that encircled the muscle fibre and the supplying sensory ending. Collagen was frequently observed within these gaps, usually in longitudinal orientation,i.e., parallel to the longitudinal axis of the spindle.

Like the control sensory terminals, the synaptic contact of the more normal model-adult sensory terminals was "reinforced" at various points by tight junctions or at least by specialisation of the synaptic membranes (fig. 14A, 22B, and 31B).

The fusimotor innervation -

Normal adult spindles Ultrastructurally, fusimotor terminals were distinguished from sensory terminals by the presence of Schwann cell coverings, by the possession of basement membranes quite distinct from those of the intrafusal fibres (although fusing with the latter at the synaptic junction); and by a consistently wider synaptic gap (300 - 600 Å for plates; for trails, the gap tends to be wider, measuring some 700 - 1,300 Å). Other less definitive differences were the greater preponderence of pale vesicles, the absence of dense-core vesicles and fewer mitochondria.

Trail endings, as described by previous workers in rat and other muscle spindles (e.g., Milburn, 1973 Ph.D. Thesis) were not encountered in any of the thin sections cut for electron-microscopy. Still, it is well known that locating any motor ending in electron microscopy is a notoriously chancey business when sample sectioning, i.e., when only a couple of thin sections are cut every 100/µm or so of spindle pole length. The following description of trail ending ultrastructure is therefore aken from the literature on rat muscle spindles.

Identification of trail terminals is usually straight forward. Their characteristically juxta-equatorial position - frequently overlapping with the secondary endings - provides the first clue. The endings themselves show up as small, round outlines in transverse section, closely abutting the

intrafusal fibres. In any one cross-section, there are commonly three or four of these round terminals per fibre.

Both bag and chain fibres are innervated by trail terminals, their ultrastructure being no different between the three types of intrafusal fibre. A Doyere eminence and sole plate are totally lacking in trail endings, as are post-junctional folds and cytoplasmic organelles. The presynaptic cytoplasm contains numerous flattened, pale vesicles but comparatively few dense-core vesicles.

Plate endings were confined mainly to the outer encapsulated B region and, less frequently, to the extreme poles, i.e., the non-encapsulated C region. A plate in cross-section appeared usually as a large, discrete prominence in contrast to the several round profiles of trail endings. The presynaptic content of the plates did not differ markedly from those described for trail endings.

The plates encountered were put into two classes according to three postsynaptic ultrastructural criteria: (a) the size of the sole plate, (b) the presence/absence of junctional folds, (c) the structural complexity of the latter, i.e., whether long, branched/unbranched and narrow (such that the basement membranes lining both sides of a fold fuses in the middle) or whether short and wide with the lining basement membrane remaining unfused. In effect, (c) is a measure of synaptic contact in any one section.

The two classes of plate were:

(i) those that had a large Doyere eminence, a commensurately

substantial sole plate - for example, at least one sole-plate nucleus was in evidence - and a smooth synaptic junction, i.e., no post-junctional folds (fig. 5A, B & 6A, C).

(ii) those that sometimes had a less obvious Doyere eminence and a thinner sole plate, but always numerous, fairly long, unbranched post-junctional folds that were sufficiently wide to prevent fusion of the lining basement membrane (fig. 7 & 8).

Both types of plate contained abundant postsynaptic organelles, i.e., accumulations of mitochondria, cisternae of rough sarcoplasmic reticulum, sarcoplasmic vesicles and microtubules.

In the comparatively limited study encompassed by this thesis, the ultrastructural type (i) plates were found only on bag_2 bag fibres, while the ultrastructural type (ii) plates were observed only on chain fibres. It is quite obvious, though, that a more intensive EM study is needed for quantification purposes in order to see whether or not this plate/fibre dualism can be substantiated. Unfortunately, none of the sections revealed a fusimotor plate on the bag_1 fibre, even though they most certainly exist (see silver/tease results, p. 124).

With careful serial sectioning, it would also have been possible and useful to estimate the proportion of plates on the bag_1 fibres and the proportion on the bag_2 fibres, and to compare these values with those derived from teased, whole muscle preparations. The lengthiness of this technique (requiring as it would have, a sample size of at least a dozen spindles) precluded its application in the present research.

In the absence of a combined morphological/ultrastructural

experiment on one and the same spindle, any correlation of the two ultrastructural types of plate described here with the two morphological types of plate distinguished in the silver-stained whole muscle preparations (see page 93) was, at most tentative. An attempt was made at finding a technique (by partly immersing the muscle in oxygenated methylene blue according to Boyd's (1957) method followed by rapid photography before the stain faded, and finally, fixing with glutaraldehyde and processing for Epon), but it met with difficulty, particularly in actually finding the spindles themselves.

However, because of their obvious Doyere eminence, the ultrastructural type (i) plates probably correspond to the morphological " short " plates while the absence, or at best, the small size, of a Doyere eminence in the type (ii) plates points to their correspondence to the morphological " long " plates.

The ultrastructural and morphological findings of this study are discussed later in the light of previous data on the subject, for example, Ovalle (1972 b) and Gladden (1969).

Model-adult spindles Fusimotor endings were very seldom encountered in the EM preparations of model-adult spindles, even though the sampling procedure was more intensive than in normal adult spindles, i.e., thin sections were cut every 50 m instead of every 70 - 100 μm. In one two-fibre spindle, a type (ii) plate was found on the smaller diameter (b_1) bag fibre (fig. 40). The plate was covered by a thin process of Schwann cell. The Doyere eminence was not prominent,

but there was a distinct sole-plate nucleus, long junctional folds (but not so narrow as to cause fusion of the basement membrane lining them) and abundant post-synaptic organelles namely, numerous mitochondria, as well as numerous vesicles congregated immediately beneath the synapse. The axon terminal itself contained numerous vesicles - a couple of the dense-core type - and neurofilaments localised in one spot.

Normal adult and model-adult extrafusal fibres and skeletomotor end plates compared

In contrast to the control muscles, many of the model-adult muscles sectioned for EM contained abnormally small extrafusal fibres, henceforth referred to as " dwarf " extrafusal fibres (fig. 67A & 48). Some of these were no bigger in diameter than intrafusal fibres, but possessed more or less all the ultrastructural characteristics of normal extrafusal fibres, such as peripheral nucleus, thick M-lines and triads (cp. fig.49 with 45A & with control Efs fig.45B,D). However, the contents themselves were invariably disarranged. This may have been due to the possible lack of attachment at one or both ends of the fibres or, perhaps, to the apparent absence of motor end plates (see below), which play a vital part in normal muscle histogenesis. Indeed, the dwarf fibres may well have resulted from the inability of α motor axons to reinnervate some of the de-differentiated extrafusal fibres. Dwarf extrafusal fibres were also identified in histochemical and silver/tease preparations (see page 86 & 99 & fig. 67B & C).

On a few of the normal-sized extrafusal fibres in one four week old model-adult muscle, macrophages were seen breaking up the peripheral muscle mass (fig. 45C). In the same preparation,

numerous free macrophages were also located between the extrafusal fibres (fig. 47). Some of the fibres contained lysosomes and appeared degenerate, as if in the latter stages of autolysis (fig. 46A). Others contained lipid droplets near their periphery, and a few were closely abutted by activated satellite cells, or myoblasts (fig. 46C & B, respectively).

Motor end plates were not encountered on the dwarf fibres in any of the sections taken from the end-plate band. On the other hand, motor end plates were sectioned on many of the surrounding (more or less normal-sized) extrafusal fibres (fig. 51). Apart from some thickening of the post-synaptic membrane, there was no obvious ultrastructural difference between model-adult end plates and those of the control (fig. 50). Thus, they possessed a Doyere eminence and a substantial sole plate, containing nuclei, mitochondria and polyribosomes. Like all motor endings, the basement membrane of the axon terminal and the one round the muscle fibre were fused at the synapse. There were numerous, long post-junctional folds, some of which were branched. The folds were narrow, causing the lining basement membrane to fuse in the middle (cf fusimotor plates: the type (ii) plate, to be precise). Each axon terminal possessed a Schwann cell covering and contained mitochondria and numerous pale vesicles.

(iv) Histochemistry

The histochemistry of normal adult spindles and model-adult spindles compared

Table VII gives details on the number of spindles sectioned and the number studied in the belly portions of the two pairs of medial gastrocnemius muscles that were used . A combined histochemical/EM experiment (see page 19+65) was carried out on the second animal only. The freezing treatment precluded optimum ultrastructural results, but the condition of the M line was certainly sufficiently recognisable for typing the intrafusal fibres. Only some time after the experiment was it realised that the excessive contraction of muscle sarcomeres that occurred during fixation might have been checked by first immersing the frozen sections in acold solution of a chelating agent such as E.D.T.A,.

One of the total of 6 model-adult spindles sectioned was three-fibred and was therefore excluded from the histochemical results, although it is briefly described in the context of anomalous spindles encountered in EM (page 101).

Thus, in all, 5 model-adult spindles were studied for their histochemistry, although only two of them had their histochemistry directly correlated with their ultrastructural However, as the bag_2 fibre of rat spindles is fairly consistently larger in diameter than the bag_1 fibre and is also characterized by a bigger nuclear bag (page 86), the bag fibres of the other 2 two-fibre spindles could be quite reliably typed. Consequently, their histochemical profiles are included in the results (Table VIII b). The only doubt as regards fibre-type identity lay in the

Table VII. Details on the number of spindles studied for histochemistry alone and for histochemistry combined with EM. The medial gastrocnemius muscles from two experimental adult rats were used.

	No. of spindles:	Normal adult (left side)	Model-adult (right side/ operated)
Belly portion from first musc.- histochem. alone	Sectioned	6	3
	Studied	3(all 4-If)	3(two 2-If, one 1-If)
Belly portion from second musc.- histochem./EM	Sectioned	4	3
	Studied	2(all 4-If)	2(one 2-If, one 1-If)

Table VIII Histochemical staining reactions in two* regions of intrafusal muscle fibres. (a) normal adult fibres, classified into bag_1, bag_2 and chain types. (b) model-adult fibres, classified as bag_1 and bag_2 in two-fibre spindles and tentatively, as bag_1 in single-fibre spindles. The numbers of levels sectioned in region A and region B are given in brackets after the bag_1 rows of values and apply to all three fibre types.

	Intrafusal fibre type	Reg.	M-line (2 spd)	P'ase	PAS	SDH	ATP'ase
(a)	BAG_1	A	dM	1.3(5)	1.5(3)	2.0(5)	1.0(5)
		B	dM	1.3(5)	2.0(9)	1.0(7)	1.5(7)
		A	dM	1.3	2.0	2.0	2.5
		B	M	2.0	1.0	2.0	3.0
		A	M	2.5	2.5	3.0	2.5
		B	M	3.0	2.8	3.0	3.0
(b)	Two-fibre spindles (one spindle)						
		A	dM	1.5(4)	1.0(4)	3.0(3)	1.0(5)
		B	dM	2.0(5)	1.5(6)	2.0(4)	1.5(6)
		A	M	1.5	1.0	3.0	1.0
		B	M	2.0	1.5	2.0	3.0
	Single-fibre spindles (one spindle)						
		A	dM	1.0(3)	1.0(2)	1.5(2)	1.0(2)
		B	dM	1.0(2)	1.0(2)	1.0(2)	1.0(4)

* The number of sections cut in region C was insufficient for inclusion in the table.

Sectioning scale according to Banks et al (1976); see intro'.

single-fibre spindle sectioned in the first muscle. However, its histochemical profile was found to be very similar to that of the other single-fibre spindle in the second muscle studied, and so these two sets of assessment values were also combined (Table VIII b).

Similarly, for comparison, 5 normal adult spindles were chosen for study on the basis of their lengthwise intactness. Intrafusal fibre typing was confirmed by EM in 2 of these spindles. However, the histochemical reactions of the other three spindles were included in the results (Table VIII a) because, as explained above, rat intrafusal fibres can be fairly reliably typed by monitoring the progress of the fibres from the outer C region (where only the bag intrafusal fibres are present) through to the equator (p85 & 86). Thus, the bag fibres are differentiated from each other by their different diameters and by their different sized nuclear bags, and the chain fibres - which in the rat are often similar in diameter of even bigger, than the bag_1 fibre at the poles - are easily typed by virtue of their shorter length and absence of a nuclear bag at the equator.

Both types of nuclear-bag fibres in normal adult spindles reacted similarly to each other with stains for P'lase, PAS and SDH (fig. 52 A,D & G, respectively). With P'lase, the reaction was usually pale (rating: 1), darkening to intermediate (rating: 2) in region B and C. The nuclear-chain fibres were always darker (rating: 3). The response of all three fibre types to the PAS stain was very variable (0-3) throughout the length of every spindle, although there was a tendency

for the chain fibres to be darkest (2-3). With SDH, the chain fibres stained consistently dark (3), whereas both types of bag fibres usually gave a pale reaction (1-2) although, occasionally either the bag_1 or the bag_2 fibre darkened (2-3) at the poles,i.e., the outer Bregion plus the Cregion. The stain for myofibrillar ATP'ase was the only one that distinguished between the two bag fibre types for virtually the entire length of spindle (cp Efs,Farrell&Fedde,'69). Thus, the bag_2 fibres gave a dark (3) reaction in contrast to the pale reaction (1) of the bag fibre (Jasmin et al, 1971). The bag fibres were consistently dark, like the bag_2 fibres (fig. 52J).

Generally, all three fibre types gave paler reactions to all four stains in region A (Table VIII(a)).

The two nuclear-bag fibres of two-fibre model-adult spindles gave similar responses to normal bag_1 and bag_2 fibres with P'lase, PAS and ATP'ase stains (fig. 52C,F & L, respectively). However, the reaction to SDH (fig. 52I) was noticeably darker (2-3) in region A and B as well as region C. Two of the three two-fibre spindles were subjected to a combined EM study. Like those two-fibre spindles sectioned purely for EM (page ??), their M-line character was similar to normal bag fibres (fig. 40 A & B) i.e., dM throughout for bag_1, and for bag_2, dM in region A only, being M in regions B and C.

The single nuclear-bag fibre of single-fibre model-adult spindles gave a pale reaction with all four stains for most of its length (fig. 52B,E,H & K). Thus, the histochemistry of these lone bag fibres could not be said to deviate from the norm. However, their pale response to myofibrillar ATP'ase was interesting in so far as it suggested a bag_1 identity.

Because only the second pair of muscle discs were combined with an ultrastructural study, only one of the two single-fibre spindles that were studied histochemically could be looked at under EM. The well-nucleated bag fibre possessed a double M-line in both region A and region B (fig. 21C), which also suggested a bag_1 nature. In the light of Milburn's (1973) and Landon's (1972) results on the ontological development of rat spindles, this finding came as something of a surprise. Milburn found that the bag_2 fibre was the first to form, followed sequentially by the bag_1 fibre and then the chain fibres. Since the chain fibres are the first to distintegrate after neonatal nerve crush, it was reasoned that if degeneration was taken a stage further (i.e., to the formation of single-fibre spindles), then the bag_1 fibre would be the next to disappear, leaving just the bag_2 fibre intact. In other words, on the basis of 'the first to be formed the last to degenerate after denervation' pattern, the bag_2 fibre should be the least susceptible of the three fibre types to denervation.

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Extrafusally, the most obvious histochemical difference between the normal adult and the model-adult medial gastrocnemius muscles was the occurrence of fibre clumping in the latter. This feature is commonly found in reinnervated muscles where the muscle nerves have been crushed neonatally or in the adult (Jaweed et al, 1975; Yellin, 1967) and is a manifestation of motor-unit localisation. Fibre clumping is most marked in regenerated muscles whose nerves have been severed as opposed to being crushed (e.g., Schiaffino & Pierobon Bormioli, 1976).

As recorded in both EM and silver/tease histological preparations, abnormally small-diameter extrafusal fibres (or " dwarf " Efs) were also encountered in frozen sections (e.g., fig. 678).

(v) Morphology of muscle innervation (i.e., observations on silver-stained, teased material)

The sensory innervation - Refer to Table IX.

Normal adult spindles All five normal spindles intensively studied in teased marerial were of the " complex " type, i.e., besides a primary axon, they each possessed at least one secondary axon (mean:1.8) as well. According to Boyd's (1962) terminology, the configuration of the sensory endings was as follows: (S_1P) two spindles, $S_2(S_1P)$ two spindles and $S_2(S_1P)S_3$ one spindle. The brackets are the author's, and represent extensive overlap of the primary and secondary ending. See fig. 51A,B & C. Together, the Group IA and Group II axons usually entered the spindle in the mid-equatorial zone, along with 0 - 5 trail axons (mean:2.4). Shortly before entry into the spindle capsule, the mean diameters of the primary and the secondary axons were 4.1 μm and 2.9 μm, respectively.

In the region of sensory innervation, the sarcomere banding pattern of the intrafusal fibres was not apparent. The primary terminals consisted of wide and narrow spirals on the bag and chain fibres, respectively (fig. 54). The mean distance between four spirals on the bag_2 fibre was 16.1 μm. At the extremities of the rings and spirals, the primary ending sometimes appeared irregular. The secondary endings were identified by tracing the Group II axon from its point of entry into the spindle capsule. The characteristic thickness and pronounced nodes of Ranvier of the secondary axons (like those of the primary axons) helped to distinguish

TABLE IX. Measurements of some sensory parameters from each experimental group of muscles, i.e., normal adult (NA), adult-crush (AC) and model-adult (MA).

Experimental Group	NA	AC	MA
Mean distance between spirals of primary (m)	16.1	16.0	17.2
Mean no. of secondaries per spindle	1.8	1.4	1.0
Mean diameter of IA axon (μm)	4.1	3.9	3.0 range:2.0 - 4.1
Mean diameter of II axon (μm)	2.9	2.8	2.3 range:2.1 - 2.6
Mean length of sensory region (mm)	0.35 mid 0.28= prim.	0.32 mid 0.29= prim.	0.18 mid 0.10 = prim.

them from those trail axons entering the spindle capsule with the sensory axons. The secondary ending(s) lay beside, and overlapped with, the primary ending to a greater or lesser degree, (although compared with the cat, the degree of overlap was more pronounced), and gave out irregular, root-like branches. Seldom were there any rings or spirals. Except for one spindle (fig. 54C), it was very difficult, if not impossible, to tell whether or not the terminals were confined mainly to the chain fibres as in cat spindles (Barker et al,1970) on account of the poor delineation of intrafusal fibres at the equator, although in the $S_2(S_1P)$ spindle, a small sprig from one of the secondaries was clearly observed to terminate on a bag fibre (fig.54B).

The length of the equatorial region covered by sensory innervation was 0.35 mm, and about 0.28 mm of this (80 per cent) wastaken up by the primary endings .

Adult-crush spindles From a study of five adult-crush spindles , it was clear that the sensory innervation had suffered little from the temporary denervation (fig.58 A, B & C). The spirals of a primary ending were present in all the regenerated spindles together with at least one secondary axon (mean: 1.4; cf 1.8 for the control mean). The mean separation distance between four spirals of the primary ending was just the same as for the control, i.e. 16.0 μm. The normal morphology of the secondary endings also appeared to have remained intact.

In the sample of adult crush spindles studied, there was no deviation from the normal complement of four or five intrafusal fibres. Unlike model-adult spindles, the nuclear-

chain fibres had not degenerated, although in several of the adult spindles, their regions showed signs of atrophy - central nuclei each covered by a thin sphere of cytoplasm that interconnected the nuclei. This condition also occurred in some single-fibre model-adult spindles (fig. 67D).

Model-adult spindles As in the other two groups, measurements were taken from a sample of five (out of a total of ten) model-adult spindles obtained in silver/teased preparations.

A primary axon was always present, but two of the ten spindles (20 percent) had no secondaries - which made them " simple " spindles (fig. 59C) - while two other spindles had two secondaries (fig. 59A). The remaining eight were innervated by a single secondary (fig. 59B), giving a mean of 1.0 secondary per spindle, i.e., 0.8 fewer than a normal contralateral sample of the same size. The primary and secondary axons of the model-adult spindles entered the spindle together at the equator and were usually accompanied by 0 -5 trail axons (or a mean of 1.8, 33.3 per cent fewer than the contrals).

The mean diameters of the primary and secondary axons just outside the spindle capsule were 3.0 μm (range 2.0 - 4.1 μm) and 2.3 μm (range 2.1 - 2.6 μm) respectively, i.e., approximately three-quaters the normal values.

In most of the model-adult spindles, the primaries did no end as regular spirals for most of their length, but instead gave simpler irregular branches with occasional bulbus swellings, and sometimes ended distally as incomplete rings (fig. 60A-D, 62B & C). Where rings and spirals were identified,

they were less extensive than normal, apart from one spindle, whose primary spirals appeared quite normal (fig. 61 B). But the mean separation distance of 17.2 µm between two spirals was similar to the contralateral mean.

Where secondary axons were present, the endings were rarely spiral. They consisted of one or two simple branches, which were variably robust (fig. 60 B & E) or delicate and root-like (fig. 60A & 61 B). But multiple branching, typical of secondary endings on chain fibres in normal spindles, was never encountered.

In three of the model-adult spindles, the entire secondary ending, or a branch of it, appeared to weave itself into a loose knot-like configuration in the endomysial tissue immediately alongside the bag fibre(s). The form and actual point of termination of the ending was obscured by inadequate impregnation of the silver stain (fig. 61A).

The mean length of model-adult spindle covered by sensory endings was 0.18 mm, roughly 51 per cent of the control value. Most of this (0.10 mm or 55.6 per cent) was taken up by the primary ending. In other words, the mean length of the primary ending in model-adult spindles was reduced to 36 per cent of the control mean value.

In one model-adult spindle studied in silver, there was nothing to indicate the presence of primary or secondary endings (fig. 59D & 61 C). Neither were any large diameter axons seen to enter the capsule in the equatorial region. The spindle was covered in the A and B regions with long, trail-like ramifications.

Another rather odd model-adult spindle was a tandem,

single-fibre one (fig. 59C & 62). Both capsules were undersized, and in one of them, a primary ending was positively identified (fig. 62B & C)., but the identity of an adjacent ending as a secondary or trail was obscured because of the difficulty in tracing the overlapping axons. At the level of the other equator, three axons entered the capsule (fig. 62 A). One (" s. ax. "? in the fig.) was more than three times thicker than the other two and was tentatively identified as a IA axon. The ending itself, not surprisingly, lacked spirals. The two smaller diameter axons were possibly trail axons, one of which (" tr. ax. "1) left the capsule after a short distance without terminating. However, the other trail axon ("tr. ax." 2) ran along the length of the A region and finally terminated in the inner B region.

The fusimotor innervation - Refer to Table X.

Normal adult spindles In a sample of five normal adult spindles, 36 out of a total of 50 fusimotor axons (72 per cent) were trail axons, whilst seven axons (14 per cent) ended as " short " plates and another seven (14 per cent) ended as " long " plates. (The latter terms are those of the author and are defined overpage.) See Table Xa.

Trail axons embraced the widest spectrum of fusimotor axon diameters, ranging from 0.3 m to 1.4 m immediately before entry into the spindle. The thickest fusimotor axons were trail axons at the maximum diameter end of the scale (Table Xb).

Branching from any one trail axon was multiple and of variable configuration, ranging from single, fine tapers

Table X Measurements of some fusimotor parameters from each experimental group of muscles.

(a) The mean number per spindle of each type of fusimotor axon.

Experimental Group	NA	AC	MA
Mean no. of trail axons per spindle	7.2	9.0	5.2
Mean no. of "short" plate axons per spindle	1.4	1.2	1.8
Mean no. of "long" plate axons per spindle	1.4	0	0
Therefore, mean no. of fusimotor axons per spindle	10.0	10.2	7.0

(b) The mean diameters of fusimotor axons as measured shortly prior to entry into the spindle.

M = mean diameter in ; R = range of measurements in ; N = number of axons measured in each five-spindle sample.

Mean diam. of trail axons	M: 0.9	0.8	1.2
	R: 0.3–2.4	0.2–2.2	0.8–2.4
	N: 36	45	28
Mean diam. of axons ending as "short plates	M: 0.7	0.7	0.9
	R: 0.4–1.5	0.5–1.3	0.6–1.2
	N: 7	6	7
Mean diam. of axons ending as "long" plates	M: 0.8	–	–
	R: 0.2–1.2	–	–
	N: 7	0	0

(c) The mean number of "short" and "long" plates. (The non-discrete nature of trail innervation made it impossible to identify the actual points of synaptic contact by the silver/tease technique).

M = mean no. per spindle; N = total no. of plates counted in each five-spindle sample.

Mean no. of "short" plates per spindle	M: 1.8	1.6	2.0
	N: 9	8	10
Mean no. of "long" plates per spindle	M: 1.8	–	–
	N: 9	0	0

(fig. 55 A, D & F) to thick knobs and brushes (fig. 55E) which were often in the form of a short, robust ladder. Axonal swellings were of two types: the common type (fig. 55 G), which have been described by other workers and which form part of a multi-branched trail ramification, and the " occasional " type (fig. 55C). These appeared as discrete, button-like swellings - about 3.5 μm in diameter - at the end of long, unbranched axons, very similar to those that end as plates. Only two of the 36 trail axons ended in such " occasional " swellings, and these were both located in the outer B zones, i.e., roughly the polar limits of normal trail innervation. One of the axons entered the spindle capsule at the equator with the sensory innervation and the other entered in the mid-B region.

The ramifications were spread throughout the B zone, frequently overlapping with the sensory endings (fig. 53A, B & C). The mean middle length of spindle between the two polar limits of trail innervation was 1.2 mm. Clearly, though, because of the infrequency with which trail endings are encountered in (sample section) EM, only short lengths of the complex ramifications visible in silver, must actually make synaptic contact with the muscle fibres (see also, Boyd, 1962). The diffuse reaction given with the stain for cholinesterase (e.g., Mayr, 1969; Gladden, 1969) could not therefore be an accurate measure of synaptic contact. Perhaps, though, the proximity of the various ramifications to the intrafusal fibres induces a widespread sensitivity to

acetylcholinesterase.

Parts of the trail endings appeared similar to type (ii) plates, but that the latter were a separate entity could be shown by the fact that their axons only branched to supply large plates. Similarly, long unmyelinated branches of trail axons which ended as small plate-like terminations, could also have been confused with type (i) plates but (a) there was no obvious nucleated sole plate and (b) if the were traced back to their origin, they were seen to be part of a multiterminal ending.

From a study of the silver/tease preparations, plate endings seemed to fit into two fairly distinct categories according to size and complexity:

(i) short, compact plates (fig. 56), consisting of a single short taper or sometimes, a few short terminal branches. A Doyere eminence was visible in those short plates that happened to be in profile view on the fibre, and

(ii) long, " loose " plates (fig. 57), consisting either of a single long taper or a few long " feathery " branches. A long, low Doyere eminence could sometimes be identified.

"Short" plates were approximately 16μm or less in length, whereas "long" plates were generally more than 16μm long, measuring as much as 45μm. Unfortunately, the small sample (18) precluded the feasibility of a frequency histogram, which might have been used as evidence for the above proposal. Using the criterion of length and the relative size of the Doyere as a guide, 9 of the 18 plates were placed in the "short" plate category and 9 in the

"long" plate category (Table Xc).

Two of the nine axons ending as short plates and two of the nine axons ending as long plates each branched to give two plates (fig. 56D, E & 57D & E, respectively). One of the two branching short-plate axons gave both plates to bag fibres, the other gave one plate to a bag fibre and one to a chain fibre. A similar condition obtained for the two branching long plates.

Short plates were more often seen on bag_2 fibres than on bag_1 fibres, although the data could not be quantified because of the difficulty in telling the two types of bag fibre apart in the silver preparations due to overlap. Long plates were observed on both bag fibre types.

As far as the present study went, there appeared to be no correlation between the diameters of axons (immediately prior to entry into the spindle) ending as plates and the type of plate (Table X), although there was a trend for the large diameter axons to end as trails or as long plates. The smallest diameter axons also ended as trails.

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The extrafusal motor end plates of normal medial gastrocnemius muscle were confined to a band about a third of the total length of the muscle from its origin. Each muscle fibre was innervated by one end plate. Preterminal branching into two plates was quite often seen, but the plates were always on separate fibres (comprising

the motor unit , fig. 65B, never on the same fibre - a phenomenon that occurred in model-adult extrafusal muscle. The plates themselves consisted of several axonal arborizations usually claw-like in shape (fig. 65B), raised on a prominent Doyere eminence (fig. 65A). One plate, however, appeared straight and simple, without any obvious arborizations (fig. 65C).

Adult crush spindles The mean number of fusimotor axons per adult crush spindle was 10.2 (sample number of spindles:5) of the total fusimotor supply of 51 axons, 45 axons (86 per cent) were trail axons and 6 (12 per cent) axons were short-plate axons. Thus, whereas the number of short-plate axons remained more or less normal, there was a noticeable increase in the number of trail axons and an apparent absence, altogether, of axons ending as long plates. See also Table Xa;also,Xb for values of axon diameters.

For the most part, the morphology of the trail ramifications of adult crush spindles was not very different from normal (fig. 58F). The main difference was the greater number of discrete button-like swellings that have already been described in normal spindles as " occasional " swellings. They were certainly not as " occasional ",though, in adult crush spindles as in normal adult spindles: 6 compared with 2 in the respective five-spindle samples. Yet, because of a two-fork and a three-fork branching, the axons themselves numbered only 3, just one more than in the normal sample. There was also the tendency for the trail innervation, as a whole, to extend further towards the poles. In other words, the mean middle length of spindle between the two polar limits of trail innervation was 1.7 mm (cp 1.2 for normal adult).

The morphology of the short plates was similar to normal short-plat morphology (fig.58D): a Doyere eminence was visible together with a few short terminal branches. Two short-plate xaons branched to give two plates each, making a total of 8 short plates in the five-spindle sample (Table Xc). Long plates, like those described in the control spindles, were not identified in any of the five adult-crush spindles

Extrafusally, the end-plate:muscle fibre ratio was always 1:1, as is the case in normal extrafusal muscle. Likewise, there was sometimes preterminal branching into two plates, but in the muscle sample teased, the plates always innervated separate (though closely adjacent) muscle fibres. Morphologically, the motor end plates of adult crush muscle were similar, though smaller, than normal (fig. 58G).

Model-adult spindles In a sample of five model-adult spindles, the mean number of fusimotor axons per spindle was seven compared with the normal mean of 10.0 per spindle,i.e., a reduction of 30 per cent.

Out of a total of 35 fusimotor axons, 26 (74.3 per cent) were trail axons, the remaining 9 (25.7 per cent) ending as short plates. As found in adult crush spindles, no large plates like those observed on normal adult spindles could be identified in the sample of model-adult spindles, although what appeared to be a plate in one spindle was tentatively identified as such (fig. 64H). See Table Xa; also Table Xb for values of axon diameters.

The data showed that even though the number of trail axons per spindle was reduced from 7.2 (inthe normal adult) to 5.2 (in the model-adult), the proportion of trail fusimotor

axons was slightly greater than in normal adult spindles. On the other hand, the mean number of short-plate axons per spindle remained the same, but their proportion among the fusimotor supply also increased.

Morphologically, trail endings in model-adult spindles appeared quite similar to those in normal adult spindles (fig. 63). Thus, some ramifications consisted of fine tapers, while others were much thicker and brush-like. Unlike normal trail endings, however, they tended to extend, unbranched, for longer distances, sometimes well into the polar zones; themean middle length of spindle between the two polar limits of trail innervation was 1.7mm (cp 1.2 mm for normal adult and 1.7 mm for adult-crush). In addition, the frequency of axonal swellings, both the " common " and the " occasional " type, was noticeably increased (fig. 61D, E, F & B, respectively) - a finding similar to that described for adult-crush spindles.

As regards the morphology of model-adult short plates, 7 out of a total of 10 plates appeared as normal (fig. 64B, D & G), but the remaining three were unlike any of the 9 normal adult short plates encountered. They stained up as simple pear-shaped distensions of their axons (fig. 64A, C & F), all of which approached the spindle in region C.

In model-adult spindles, branching of short-plate axons at the level of the spindle occurred with no greater frequency than in the control sample of spindles: one of the nine short-plate axons in model-adult spindles bifurcated (fig. 64D & E) in comparison to two out of seven short-plate axons in normal adult spindles. However, the bifurcation was peculiar in that

the two branches were very short and both plates terminated on one and the same intrafusal fibre (the model-adult being, in fact, single-fibred).

It must be mentioned that one model-adult muscle (6½MPN) contained an aberrant six-fibre spindle (fig. 67E), a finding similar to Schiaffino and Pierobon Bormioli's (1976; see page of introduction). The intrafusal fibres did not appear to be products of fibre splitting; they were separate from one extreme pole to the A/B interface of the opposite side. The B and C region of the latter side were missing in the preparation, but it does not seem likely that branching might have occurred there.

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As in the control, contralateral muscles, the skeleto-motor end-plate band in model-adult medial gastrocnemius muscles was located at the proximal third transverse level of the muscle. There was abundant collateral branching(into two, three or four branches, each terminating as an end plate) of the extrafusal motor axons (fig. 65E,G & H) and, in the silver/teased preparations, this was particularly evident at the more distal nodes of the axons. The type of preparation made it impossible to tell whether or not branching occurred more proximally,i.e., at the level of the intramuscular nerve trunk. The branches were either derived from one and the same node, or from separate nodes, and sometimes a branch itself gave off other branches (fig. 65H). The plates usually terminated on different extrafusal muscle fibres – in other words, a 1:1 plate to muscle fibre ratio was still maintained – although in a few instances,

two plates terminated on the same muscle fibre (fig. 66B & C). Then, the two plates lay close together on the fibre, very much like the small plate described on page that was found in one single-fibre spindle.

Compared with the normal adult, the morphology of model-adult extrafusal end plates was more variable. Some had a simple "hook-like" form, like some normal adult end plates (cp fig. 65A, B with 63E), whereas others appeared more complex than normal, consisting of several terminal axon branches, extending over a larger area (fig. 65D). One branch of an α motor axon ended in a simple end bulb (fig. 65H). Another extrafusal end plate stained up as a compact structure with thick, short axon terminals (fig. 65F).

A feature of the model-adult extrafusal muscle itself, was the abundance of abnormally thin muscle fibres (fig.67 C), i.e., some 6.3 - 10.0 per cent of the diameter of the largest and smallest extrafusal fibres, respectively; infact, of the order of intrafusal fibre thickness. They stood out as a distinct group, clearly absent from normal adult and adult crush muscles, and tended to be more numerous in areas of abundant connective tissue. These fibres were also observed in Epon sections (fig. 67A &48 & 49) and frozen sections (fig.67 B). It was difficult to tell whether they were any different in length from normal extrafusal fibres. Some of the dwarf fibres were seen to taper amid the normal mass (fig. 67C. lower photograph). Motor end plates were never observed on them.

<u>An evaluation of the adult crush experiment as a second control</u>

As explained on page 60 of 'Materials and Methods', the adult crush experiment was carried out in an attempt to determine which of the morphological changes in the model-adult spindles were produced by the nerve crush lesion <u>per se</u> and

which were produced as a consequence of temporarily suspending muscle innervation at a critical stage of development. The value of this additional control will now be assessed in the light of the preceeding results.

The fact that, in the adult crush spindles, the mean number of trail axons per spindle was 9, compared with the normal adult value of 7.2 and the model-adult value of 5.2 - reveals an inadequacy in the adult crush experiment as a second control. Indeed, one expects any neonatal nerve crush to produce some different effects on the neuromuscular system compared with nerve crushes in mature adults, for the simple reason that neonatal rate are still actively undergoing differentiation and growth. But despite this intrinsic shortcoming, the adult-crush controls did prove useful in evaluating many other observations in the model-adult:

(i) the finding that recognisable long plates were not located in the adult crush spindles or the model-adult spindles suggests that nerve crush <u>per se</u> (whether neonatal or in the adult) may in some way selectively eliminate " long " plate axons or, at any rate, prevent the re-formation of long plates on intrafusal fibres or even perhaps, merely alter the morphology of long plates so radically that they become unrecognisable.

(ii) likewise, the finding that trail axonal swellings occurred more frequently in both adult crush and model-adult spindles suggests that it is a consequence of nerve crush at any age;

(iii) on the other hand, the finding that the morphology of " short " fusimotor plates was virtually unchanged in the adult crush spindles but so only in some of the model-adult spindles, suggests that short-plate morphology depends, to some extemt,

on an intact nerve supply during early postnatal development. This is plausible because, at this age of nerve crush, i.e., 31/2 DPN, the fusimotor innervation is still incompletely differentiated;

(iv) the observation that " dwarf " extrafusal fibres were peculiar to model-adult muscle - having never been seen in adult crush muscle - indicates the involvement of an age-dependent factor.

(v) similarly, the occurrence of multiple collateral branching of α axons and variable morphology of end plates in model-adult extrafusal muscle but not in adult crush extrafusal muscle, suggests that these changes, like (iii) and (iv) are consequences of interrupting the nerve supply of an immature neuromuscular system.

PART III - MONITORING THE PROCESS OF INTRAFUSAL FIBRE DEGENERATION AND REGENERATION WITH ELECTRONMICROSCOPY

Spindles were looked at 2,4,7,10 and 19 days after the operation (DPO) at 31/2 DPN.

2DPO (i.e.,51/2 DPN): There was no sign of nerve axons or endings even in the equatorial regions. This was expected, since neural degeneration is known to occur rapidly after nerve lesion (Zelena,1964; Milburn, thesis '73). The Schwann cells were however, well preserved and similarly, the four-layered capsule remained quite normal (fig.68). The intrafusal fibres, consisting of two bag fibres, very different in diameter from each other, and two chain fibres, were immature compared with their normal 51/2 DPN counterparts. Indeed, they were only as mature as 31/2 DPN intrafusal fibres. Central nuclei were often still present in the polar regions and one of the chain fibres (the last one to form) was still closely apposed to the large diameter bag fibre, although pseudopodial inpushings were lacking (fig. 68B). In this fibre (Milburn's F4 fibre), the myofilaments were not organised into discrete myofibrils as in the other chain fibre (Milburn's F 3). Myoblasts were still encountered within the axial bundle, usually in close association with the chain fibres. Another immature feature was the underdevelopment of individual basement membranes which, certainly in the equatorial region, are well formed by the 5th. - 6th. postnatal day. The only indication of fibre separation at the equator were short lengths of individual basement membranes in the centre of the axial bundle along the sectors of fibre circumference that

were not in contact (fig. 68B).

Early postdenervation changes were most obvious in the chain fibres (cp Boyd,1962) and the extrafusal fibres. From general observation of the bag fibres - i.e., a qualitative as opposed to a quantitative approach - the initial signs of atrophy seemed more prevalent in the large diameter bag fibre than in the small diameter bag fibre. Thus, even though both fibres contained a few dilated t-tubules, lipid droplets and degenerating Z bands, the bag_2 fibre also contained Golgi bodies (often two per cross-section), myelin figures, vesicular bodies and generally more sarcoplasm (fig. 68B).

The extrafusal fibres possessed many peripheral nuclei on the one hand but, on the other, some fibres still had basement membranes in common with closely applied myotubes (fig. At this stage of denervation, degenerative changes were just beginning to manifest themselves, as in the bag intrafusal fibres. Compared with the myofibrils of the latter fibres, however, those of the extrafusal fibres were more intact and better aligned.

4DPO (i.e.,71/2 DPN): There was evidence of axonal regeneration in intramuscular nerve trunks and spindle nerves as early as 4 days following nerve crush at 31/2 DPN, although neuromuscular endings were clearly still absent. In both spindles sectioned, the chain fibres had completely degenerated (fig. 69A). Both bag fibres were still surrounded by a common basement and some of their polar nuclei remained central. The axial bundle also included myoblasts; flattened projections from

these cells frequently formed a bridge between the two bag fibres. The large diameter fibre remained twice the size of the small diameter fibre. Myofibril architecture of both the intrafusal and the extrafusal fibres was not much different from the 2DPO stage (fig. 69B).

7DPO (i.e., 10½ DPN): Nerve regeneration was well underway 7 days after the operation since immature sensory endings were observed in one single-fibre spindle (fig. 70). They were "layered" and clearly had not yet assumed the spiral course typical of normal adult and many model-adult spindles. However, myelination had not yet commenced, at least not at the level of the spindle nerve trunk. In region A, at the myotubule level, the M line was still present in the single bag fibre (fig. 70B), as it is in all intrafusal fibres of normal 10½ DPN spindles (Milburn, 1973).

10DPO (i.e. 13½ DPN): Myelination had reached many of the larger intramuscular axons by this stage of reinnervation. In one and the same nerve, the larger diameter myelinated axons were present alongside smaller diameter unmyelinated axons (fig. 73). Nerve trunks containing all unmyelinated axons are depicted in fig. 71A,C and 72C, and those containing axons in the early stages of myelination are depicted in fig. 72B. Still unmyelinated were the axons that comprised the spindle nerve trunk (fig. 71 & 73). Indeed, some of these axons were bunched together in such a wa that the central ones were not in contact with the Schwann cell.

The intrafusal fibres of both single-fibre spindles sectioned still showed

some signs of atrophy (i.e., myelin figures and irregularities of some of the Z bands), but the polar myofibrils filled the fibres more completely (fig. 71B&73). It was impossible to recignise the M-line character of either fibre because the sarcomere striations were too blurred. However, a dilated, more complex t-tubule system was still present as were polyribosomes, and Golgi bodies. In the polar regions, myoblasts were seen apposed to the single fibres for about half their cross-sectional circumference (fig. 72A). Clearly, the intrafusal fibres were still undergoing active growth.

The ultrastructure of the extrafusal fibres was now very similar to that of normal adult muscle. The myofibrils were compactly arranged, with little separating sarcoplasm, so that they were no longer easily delineated from one another (fig. 72A). The only indication of temporary denervation was the occurence of a more complex, dilated system of t-tubules.

2WPO (i.e. 17½ DPN): By this stage of reinnervation, the spindle nerve trunk of one of the two two-fibre spindles sectioned containedmyelinated axons (fig. 76C). Groups of non-myelinated axons were no longer evident and sensory terminals enwrapped the bag fibres. Both fibres (fig. 75C) had lost their M lines in the central length of region A but whereas the smaller diameter fibre (bag_1:fig. 76B) had also lost it at the myotubular level (outer region A) and in regions B and C, the larger diameter fibre (bag_2) had retained it in this region (fig. 76A) as well as in regions B and C. In normal adult spindles and in model-adult two-fibre spindles, the M line of the bag_2 fibre is present only in regions B and C.

In the other two-fibre spindle, however, no sensory endings were sectioned in the equatorial region (fig. 74). Yet both fibres contained small bags, comprising two nuclei per cross-section, although it must be said that

in much younger, developing spindles. Along a length of region A (towards region B), the basement membranes of the two fibres fused to form a common basement membrane which also enclosed a row of myoblasts between two fibres (fig. 75A). The bag_1 fibre had, by this stage, lost its M line throughout its length and now possessed a double M line (fig. 75A), but the b_2 fibre retained its M line from the myotube level (outer region A , fig. 75A & B) outwards to include B&C. Unlike normal bag fibres in region A , there was little difference in their diameters, the larger fibre being no more than two-thirds the size of the smaller fibre (fig. 74).

Both capsules were 5 - 7 cells thick, the cells having flattened to adult appearance.

Inference: The above observations quite clearly indicate that the two-fibre, model-adult spindles were truly chainless spindles, i.e., spindles in which the temporary denervation had caused the developing chain fibres (still very dependent on their innervation) to rapidly degenerate before reinnervation had taken place. The two fibres left were the original bag fibres whose M-line characters corresponded to those of normal bag_1 and bag_2 fibres.

As far as helping to elucidate the identity of the bag fibre in single-fibre, model-adult spindles, the above experiment is not conclusive. But, indeed, it was not expected to provide much more than clues. The only significant clue was the apparent greater prevalence of denervation changes in the larger bag fibre ($F1/b_2$) compared with the smaller bag fibre ($F2/b_1$) during the early denervation period in three of the four spindles sectioned at this stage. Perhaps this indicates a greater susceptibility on the part of the bag_2 fibre to

denervation. This susceptibility might become manifest in those spindles destined to be single-fibred.

All that can be said about this identity question from the research *in toto* is that (1) the two single-fibre model-adult spindles stained for alkaline ATP'ase gave a pale b_1-type reaction and (2) in the 7 single-fibred model-adult spindles investigated under EM for much of their length, M line character was unrecognisable in 4 of them, but in the other 3 spindles, a double M line occurred throughout the lengths of the bag fibres, i.e., they possessed b_1-type M line profiles.

DISCUSSION

On the basis of nerve crush experiments at birth (Zelená & Hník, 1960a,b; 1961; 1963a,b,c; Zelená, 1964; Werner, 1973a) and more recent ones on slightly older rats (Werner, 1973b), a methodology was found in this investigation for experimentally producing chainless/bag-only spindles as permanent structures in adult medial gastrocnemius muscles. Deprived of their chain fibres, these " model-adult " spindles contained either two bag fibres or a single bag fibre. The methodolgy itself consisted of crushing the tibial nerve about 0.5mm proximal to the popliteal bridge of blood vessels in 3½DPN rats.

At 3½ days of age, rat muscle spindles contain their normal adult complement of four intrafusal fibres (Landon, 1972; Barker & Milburn, 1972; Milburn, 1973), although the last chain fibre is still immature. Differences still exist in the polar fibre diameters, which in the adult are minimal or absent. An M line is still present throughout the length of all four fibres and they contain a similar arrangement of abundant interfibrillar sarcoplasm and small mitochondria. Even at this early stage of development, the sensory innervation is well developed; indeed, its trophic influence is already on the wane (Werner, 1973b; Schiaffino & Pierobon Bormioli, 1976). On the other hand, the fusimotor innervation is still immature, having just reached the spindle at birth (Landon, 1972; Milburn, 1973). Milburn located intracapsular motor terminals in 4DPN spindles. They all possessed smooth

post-junctional membranes. Unfortunately, no acetylcholin-esterase or axon impregnation techniques have been employed in studies of developing spindles.

In the results of this thesis, model-adult spindles have been extensively defined in terms of their general morphology, ultrastructure, histochemistry and innervation morphology - and compared with normal adult and adult-crush controls. It is now necessary to view these findings in the context of other research on abnormal, denervated spindles.

The model-adult spindle; its structure in perspective - General morphology Predictably, the nerve lesion caused a decrease in the normal complement of spindles (approximately 23) in the medial gastrocnemius muscle. More or less 50% of the spindles failed to regenerate. However, the other 50% that survived the 6 - 7 day denervation period (Zelena, 1962; Milburn, Ph.D thesis, 1973), consisted largely of model-adult spindles. Two-fibre model-adult spindles always slightly outnumbered single-fibredones, which tended to be located more distally. In addition, most model-adult spindles included a minority of three-fibre, four-fibre and anomalous spindles.

Apart from a decrease in length and intrafusal-fibre diameter - both primarily in the polar regions - a " typical " model-adult spindle was otherwise quite well developed. Thus, the mid-equatorial diameter and periaxial space was only slightly smaller than normal, the capsule was structurally intact with no difference in the number of cell layers

and the intrafusal fibres possessed normal-sized nuclear bags.

Werner (1973b) also found a reduction in the number of spindlesand intrafusal fibres of rat medial gastrocnemius muscles temporarily deprived of their innervation 4 and 6 days after birth. Of the two major types of intrafusal fibre, the bag fibres were clearly the more resistant to tibial nerve crush. The nuclear bags were well maintained as was the periaxial space. However, Werner did not measure spindle length or diameter or the diameter of the intrafusal fibres.

The results of Schiaffino and Pierobon Bormioli (1976) were quite different as regards the normality of experimental spindles temporarily denervated half a day later than the ones in the present study, i.e., on the fourth post-natal day. True, they also found (after a period of recovery of 6 - 12 months) two-fibre spindles, but of quite abnormal character. The reinnervated intrafusal fibres had hypertrophied and elongated to extrafusal fibre proportions and although the capsule had been retained, it was thin and did not enclose a periaxial space. Nuclear bags or chains were entirely lacking. All myonuclei were central and irregularly distributed throughout the entire length of the experimental spindles. In addition, the innervation was completely abnormal; sensory terminals were never encountered and each intrafusal fibre was innervated by a single motor end plate of comparable size and ultrastructure to extrafusal end plates. They were frequently located

within the intercapsular space.

Intrafusal fibre ultrastructure The M line profiles of the bag_1 and bag_2 fibres in two-fibre model-adult spindles were similar to their normal counterparts, i.e., dM for bag_1 throughout its length and dM for bag_2 in region A, switching to M in regions B and C. These findings agree with those of Barker et al (1976) and Banks et al (1976) on normal adult rats. The bag fibre of single-fibre spindles possessed a dM line throughout its length. It must be said, though, that in several other single-fibre spindles, the M line could not be recognised in the B and C regions because of sarcomere " blurring ". Such indistinction of sarcomere banding pattern was also seen in a normally occurring (atypical) single-fibre spindle in guinea-pig lumbrical muscle (author, second year undergraduate project). The presence of a dM line in region A pointed to a bag fibre identity, although the nuclear " bag " was merely myotubular in size. Sarcomere blurring may well be a consequence of aberrant or inadequate fusimotor innervation induced by nerve lesion (or in the case of the normal guinea-pig spindle, by a chance abnormality) during the critical period when fusimotor innervation is being established.

The author's observations are essentially similar to Zelená's (1964; 1965) and Zelená and Soukup's (1971) own observations on the polar regions of rat spindles permanently de-efferented at birth. They found that the characteristic ultrastructural profiles of the bag and chain fibres, (all

four fibres having survived), developed more or less as normal, but that in a minority of spindles, they were less discernable, being blurred by disarranged cross-striations. In all the experimental spindles there was one conspicuous aberrant feature: the rarity of triadic and diadic junctions in both types of intrafusal fibre (cp the bag fibres of model-adult spindles).

The large, extrafusal-like intrafusal fibres in the 4- and 7- DPN experimental groups of Schiaffino and Pierobon Bormioli (1976) were described by these workers as possessing M lines. However, in one of their micrographs of an intrafusal fibre (from the 7- DPN experimental group) in the capsular region, revealed the presence of a double M line.

As regards the relative abundance of interfibrillar sarcoplasm, mitochondria and sarcoplasmic reticulum, the bag fibres of model-adult spindles did not deviate noticeably from the norm. Thus, the mitochondria were generally small and moderate in number and the sarcoplasmic reticulum did not delineate the myofibrils. However, it was noticed that the polar regions of some bag_2 fibres had a myofibril architecture tending towards a chain-like profile (cp Barker et al, 1976) particularly with respect to mitochondrial size and the amount of general sarcoplasm encircling the myofibrils.

The main aberrations in fine structure of model-adult spindles were the presence of disorientated, often disorganised myofibrils and patchy areas of sarcoplasm which frequently contained a variety of organelles.

Intrafusal fibre histochemistry Except for a trend towards higher oxidative activity two-fibre model-adult spindles exhibited a normal profile. No two-fibre spindles were encountered in which both bag fibres gave a pale reaction with alkaline ATP'ase (cf Zelená & Soukup, 1975: four-fibre spindles de-efferented at birth; Schiaffino & Pierobon Bormioli, 1976: two-fibre spindles denervated at 4 and 7 DPN). These author's found that the experimental fibres stained rather uniformly (pale) with alkaline ATP'ase. As in most rat spindles, however, the bag_1 fibre of two-fibre model-adult spindles consistently gave a pale reaction to the stain for alkaline ATP'ase, whereas the bag_2 fibre consistently stained darkly. The explaination for the histochemical normality of model-adult spindles as opposed to the experimental spindles of Zelena and Soukup (1975) and Schiaffino and Pierobon Bormioli (1976), very probably lies in the nature of the nerve lesions - nerve crush, permenent de-efferentation and nerve section/ suture, respectively. Nerve crush is the least traumatic and can be regarded as the equivalent of temporary denervation, although, of course, other influences come into play, not least the fact that changes in skeletal muscle have a formative influence on spinal cord organisation (e.g., Prestige,1967; Eccles, Eccles, Shealy and Willis,1962). All the same, perhaps a larger sample of spindles might have revealed low levels of alkaline ATP'ase in both bag fibres of model-adult spindles. This is a reasonable presumption, since a minority of spindles in normal rat muscle contain all pale-staining bag fibres Zelená & Soukup, 1975).

The pale reaction with alkaline ATP'ase of the bag fibres of single-fibred model-adult spindles can be interpreted

in two ways. Simplistically, it could mean that these fibres are bag_1 fibres, a proposal that is supported by the EM observation of a double M line throughout its length. This would, in turn, imply a greater resistance on the part of the bag_1 fibre (in comparison to the bag_2 fibre) to temporary denervation. But Milburn (1973) and Landon (1972) have shown that the bag_2 fibre is the first intrafusal fibre to form during ontological development, followed sequentially by the bag_1 fibre and then the chain fibres. Extrapolating from the knowledge that the last-formed fibres - the chain fibres - are the first to degenerate following nerve lesion, it was reasonable to predict that the next fibre type to degenerate would be most likely the bag_1 fibre. In other words, it was thought that the bag_2 fibre would prove the most resiliant.

The alternative interpretation of the low myofibrillar ATP'ase activity in single-fibre spindles is perhaps more realistic and is based on the fact that myofibrillar ATP'ase is neurally regulated (Guth, Samaha & Wayne Albers, 1970). Because of this, it would be expected that any alterations in innervation (see page 11) from the norm - the pattern of fusimotor innervation of single-fibre spindles being, certainly, more deviant than that of two-fibre spindles - would be bound to cause , at the very least, operational changes in the myofibrillar enzyme. Consequently, the single bag fibre of single-fibre model-adult spindles may actually be considered to be the bag_2 fibre which has suffered a reduction of myofibrillar ATP'ase activity as well as a loss

of M line in regions B and C in addition to its normal loss in region A. Futhermore, not only must the altered pattern of fusimotor innervation in single-fibre spindles be taken into account, but also the fact that <u>two</u> fibre types are missing (i.e., the chain fibres and, for the purpose of the present argument, the bag_1 fibre). As yet, no one knows how a change in the intrafusal fibre environment affects the functional influence of fusimotor innervation on fibre ultrastructure and metabolism.

Before embarking on the discussion of spindle innervation, the origin of " dwarf " muscle fibres in many of the model-adult muscles must be considered. Are they peculiar intrafusal fibres? If they are, then it would be expected that remnants of a capsule should have remained (Schiaffino & Pierobon Bormioli, 1976) since the spindle capsule is a stable structure, independant of sensory innervation for its maintenance. However, in none of the histological preparations (Epon/toluidine blue, EM, histochemical or silver/tease) was a capsule discerned. It is far more likely that the dwarf fibres are abnormal extrafusal fibres which lack an end plate (cf Schiaffino & Pierobon Bormioli, 1976). Their α axons could have failed to regenerate after the nerve lesion, so that subsequent growth was severely retarded. Or, perhaps, these muscle fibres were formed anew from myoblasts (which are still present at the $3\frac{1}{2}$ DPN stage and, moreover, proliferate after nerve lesion) and failed to be reinnervated by regenerating α axons. This hypothethis seems quite plausible when the

nature of nerve crush is considered. It involves the regeneration of axons back into their original endoneural sheaths to the original sites of termination. Either way, the absence of an end plate at such an early post-natal stage of differentiation and growth was obviously responsible for the diminutive size of these muscle fibres.

Spindle innervation (fine structure and surface/synapse morphology) Before the innervation of model-adult spindles can be evaluated with any sort of meaning, the ambiguites surrounding the innervation of normal adult rat spindles (in particular, their fusimotor innervation) must first be clarified. What follows (to page 170) is therefore a comparison of the present author's observations, including plate typing, with those contributed by previous workers (surface morphology: Porayko & Smith, 1968; Gladden, 1969; Ovalle, 1972; Mayr, 1969; ultrastructure: Merrillees, 1969; Landon, 1966; Hennig, 1969; Rumpelt & Schmalbruch, 1969; Mayr, 1970; Ovalle, 1972; Milburn, 1973). From this analysis, a ' typical ' model of spindle innervation in the normal adult rat has been constructed (fig. 79A & 80A) - parallelling that depicted for the cat (Barker et al, 1970, for example) - thereby providing a firmer basis for the subsequent evaluation of model-adult and adult-crush spindles. The various descriptions of sensory ending will be looked at first.

There is relatively little controversy concerning the morphology and ultrastructure of sensory endings in the

rat. As in other mammalian spindles, (see Barker, 1974 review), the primary endings are annulospiral in form, as evidenced in EM as well as from silver/tease preparations. The wider spirals indicate bag fibre innervation, the few narrow spirals, chain fibre innervation. Those ramifications of the primary that extend into the early reaches of the juxta-equator, appeared as irregular sprays. Bulbous terminations, similar to those described by Gladden (Ph.D. thesis, 1971) in rat intertransverse tail muscles, were identified in the medial gastrocnemius muscle. They were sometimes seen to be connected with the rest of the ending by fine filaments.

According to Karlsen (1965: jaw muscles), Gladden (1969: tail muscles), Porayko and Smith (1968), Mayr (1969), Merrillees (1960) and Landon (1966; lumbrical muscles in the latter cases), the primaries predominantly supply the bag fibres whereas the secondary (s) exclusively supply the chain fibres. The same could not be said with as much certainty about spindles in the medial gastrocnemius muscle on account of the poor delineation of intrafusal fibres at the equator. Certainly, the primaries appeared to supply the chain fibres with fewer branches, if the infrequency of narrow spirals was anything to guage by. Only in one normal adult spindle were the bag fibres and chain fibres separated well enough for the secondaries and primaries to be seen exclusively innervating the chain and bag fibres, respectively. This preparation was

fortuitous; in silver/tease preparations of rat muscle, applying pressure to the coverslip does not induce separation of the bag and chain fibres as it often does in cat spindles (see also Gladden, Ph.D. thesis, 1971). Efforts to accomplish this merely ended in transverse splitting of the spindle at the equator (fig. 54 A & B). The reason must lie in the fact that, in the rat, the bag and chain fibres appear more bound together than in the cat, where the chain fibres and bag_2 fibre(s) are grouped together in a connective tissue sheath that is separate from the sheath surrounding the bag_1 fibre(s). The observation that, in the rat, secondary sensory endings are prevalent on the chain fibres, gains support from Milburn's electron microscopic studies on developing spindles. She located sensory endings at the juxta-equatorial level (i.e., most probably secondaries) only after the chain fibres had formed. Moreover, the endings lay on the chain fibres themselves. It is reasonable to assume that the few secondary sprays to the bag fibres branch out subsequently.

Banks et al's (1977) recent electron microscopic study of the distribution of primary and secondary axons to cat intrafusal fibres

demonstrated that each afferent fibre supplie terminals to bag (both types) as well as chain muscle fibres.

The silver/tease preparations of the present study demonstrated a substantial degree of overlap between the position of the primary and secondary endings at the equator. In effect, the secondaries did not consistently extend as far out into the juxta-equatorial regions as in other mammalian species, for example, the cat (Barker et al, 1970) and as in rat lumbrical muscles, according to Porayko and Smith (1968). However, from Gladden's description (Ph.D. thesis, 1971) of spindles in rat tail muscles, she also found substantial overlap between the two types of sensory ending.

Secondary endings appear as irregular, root-like sprays and branches, which bear a fairly close resemblance to Barker et al's " flower spray " secondary endings in the cat. Only some spindles possessed a few spirals at the mid-equatorial level. With the electron microscope, small endings, perhaps of secondary origin, were seen at the juxta-equatorial level closely applied to the surface of the intrafusal fibre. The profiles contained a pale, flocculent cytoplasm, which was invariably bereft of the mitochondria and vesicles that are found in the spirals of the primary (cp Landon, 1966).

Mayr (1970) describes two electron microscopically different forms of secondary ending on two neighbouring chain fibres in a rat spindle. The two endings were connected to the same Group II axon and exhibited a similar organelle

content. One took on an annulospiral form and the other consisted of a number of axons lying close together between the sarcolemma and the neurilemma. Not all of the latter axons appeared to be in direct contact with the intrafusal fibre. Mayr suggests that this ending is a correlate of the flower spray type of endings seen with axon impregnation techniques. His photographs and description fit those for immature sensory endings both in normal developing rat spindles (Landon, 1972: " overlapping "; Uehara, 1973: " bundled "; Milburn, 1973) as well as in the regenerating spindles of this study (fig. 70).

The proportion of spindles in the rat that are normally without secondary endings varies considerably between different muscles. Porayko and Smith (1968) found that as many as 50% of the spindles in the plantar lumbrical muscles possessed a single primary, the remaining 50% containing one or two secondaries in addition. The intertransverse tail muscles (Gladden, 1969), on the other hand, contain only 10% " simple " (Boyd, 1962) spindles, 90% being " complex ". The latter is composed of 10% one-secondary spindles, 60% two-secondary spindles and 20% three-secondary spindles.

Unlike the happier position of cat spindles, there has been no serious attempt to resolve the various inconsistencies of description of fusimotor innervation in the rat. This has called for the following effort in that direction.

Let us begin with the two observations that <u>are</u> held in common concensus and are recognised as fact: (a) the presence of three types of fusimotor innervation and (b) the

identification of multiterminal endings (the " trails " of Barker & Ip, 1965, Barker, 1967a & Barker et al, 1970; the " γ_2 network " of Boyd, 1962) as one of the three types (exception: Porayko & Smith, 1968, who make no reference at all to multiterminal fusimotor endings). However, the various descriptions of rat fusimotor plates are more difficult to resolve into a coherent and reliable picture. It is hoped that the observations of the present study have helped clear up at least some of the ambiguities and put rat spindle research on a footing with that of the cat.

Trail innervation consists of a " tangle " of numerous branches that cover the juxta-equatorial lengths of a spindle. Every trail axon divides into a variable number of such terminations. In preparations stained for acetylcholinesterase, the presence of trail innervation is manifest as a diffuse reaction on either side of the sensory endings (Gladden, 1969: tail muscles; Coers, 1962; Coers & Durand, 1956: rectus abdominis muscle; Mayr, 1969: lumbrical muscle). The actual form of the endings varies from simple fine tapers and axonal swellings to thick knobs and brushes. Ovalle (1972) is alone in making a morphological distinction between two types of trail endings: those to bag fibres " NB trails " and those to chain fibres " NC trails " . He sees NB trails as consisting of a series of knob-like thickenings, extending along the surface of the muscle fibre, whereas NC trails take the form of a more diffuse, grape-like spray, with several punctate terminal knobs, interconnected by ramifying filaments of the parent axons. Each type of trail ending

is supplied by a single fusimotor axon.

At the ultrastructural level, trail endings of rat spindles are no different from trail endings in other species. Thus, they appear as rounded profiles on the surface of the muscle fibre, making little or no indentation. The synaptic gap is smooth, there being no junctional folds. A sole plate as well as sole-plate organelles are absent, so that myofibrils occur close to the post-synaptic membrane. Ovalle (1972) found them to be more tightly packed beneath the bag fibre than beneath the chain fibre. The synaptic gap (700 - 1,300Å) is variable in width but always wider than that of fusimotor plates. Like other motor endings, trail pre-synaptic organelles consist of fewer mitochondria and many more vesicles than are found in sensory axon terminals.

All in all, descriptions of trail-ending morphology are very similar, but a basic difference of opinion exists in relation to the two major intrafusal fibre types - bag fibres and chain fibres. Mayr (1969) claims that multiterminal endings predominate on bag fibres, but he admits that in his experiment, judgement was made difficult by overlapping intrafusal fibres. Indeed, it was for this very reason that attempts to identify such preferences were precluded in the present study. In his early morphology studies on the cat, Boyd (1962a; 1966) also found his " γ_2 network " almost exclusively on chain fibres and Jones (1966) made a similar statement about oppossum spindles. However, Barker and Ip (1965) and Barker et al (1970) observed no such specificity in the same species; they clearly demonstrated trail endings

on both bag and chain fibres. Gladden (1969) made no mention of trail/fibre type specificity in her work on rat tail muscles.

In the present study, two types of plate have been indentified in normal adult rat spindles on the basis of their ultrastructure as well as their morphology (refer to Table XI). To avoid confusing the issue on plate typing in rat spindles, inventive labels were avoided. Thus, the terms " type (i) " and " type (ii) " simply refer to the two paragraphs describing the ultrastructural criteria of the two categories of plate and similarly, the terms " short " plate and " long " plate are simple descriptive terms used to distinguish one morphological category from the other. As explained in the text and as reiterated below, the ultrastructural type (i) category was tentatively equated with the morphological " short " category and the " type (ii) " category with the " long " category. However, a combined EM/ morphological experiment would provide a far more reliable method of resolution.

A plate was regarded as belonging to the morphological short category if appeared short (usually less than 16μm) and compact and consisted of a short taper or a swelling with a few, short terminal branches, and if a discrete Doyere eminence happened to be visible. On the other hand, a plate was regarded as belonging to the long category if it appeared simply as a long, single filament or, more frequently, as a long (usually more than 16μm, up to 45μm) branched structure taking up the entire width of the intrafusal

TABLE XI Histological correlations of the two types of fusimotor plate described in normal adult rat muscle spindles. Comparison is also made with the two classes of cat fusimotor plate which are generally more complex in appearance (Barker et al, 1976). The literature consistently agrees on a dual classification. Discrepencies from the " typical " description are given in brackets under the name of the relevant author. Refer also to Landon (1966), Merrillees (1960) & Milburn (1973) in text.

Author → / "Typical" description ↓	Porayko & Smith (1968); Ag impreg.; rat lumbrical	Gladden (1969) Ag impreg. & Ach; rat inter-transverse.	Ovalle (1972); Ag impreg. & EM ; rat lumbrical	Hennig (1969); EM; rat lumbrical	Mayr (1969); Ach & Sudan Black; rat lumbrical	Present author; Ag impreg & EM; rat medial gastroc.	Barker et al (1970); Ag impreg., Ach & EM;cat tenuissimus
A) Ag impreg studies: smaller of the 2 types; few, short, filamentous axon terminals; Doyere eminence. EM studies: several round profiles on Doyere eminence; well dev. sole plate & s-p organelles including round nuclei; smooth syn.gap,ie., no post-junc.folds. Ach: Compact sub-units not clearly separable. All 3 techniques: Always found at more extreme poles.	"cat-like" plate	small, compact plate	NB plate (EM: poorly developed sole-plate)	smooth-synapse plate	small, compact plate (Ach:junctional folds, as indicated by by fringe of reaction product)	short/ type (i) plate	p_1 plate (EM: long junc. folds)
Fibre type on which found, ie. either bag or chain.	both types but more numerous on bag fibres	no comment	bag fibres only	no comment	chain fibres only	both types, but predom. on bag fibres	both fibre types, but predom.(75%) on bag fibres
B) Ag impreg.studies: larger of the 2 types with several axon terminals of variable length, but occasionally consisting of a single long filament; Doyere eminence low or absent.	Single filament plate	large, branched plate	NC plate	plate with junctional folds	large, loosely branched plate	type (ii) plate	p_2 plate
EM studies: Usually 1 or 2 wide profiles; Doyere eminence low or absent; numerous sole-plate organelles, but flat s-p nucleus; short, unbranched junc.folds with unfused basement membrane. Ach: large non-compact sub-units. All 3 techniques: located at more extreme poles as well as fairly close to trails.			(EM: well developed sole plate		(Ach: junctional folds absent)		(EM: short junc. folds. More recently, though (Barker et al,1976), p_2 plates described with a smooth synapse
Fibre type on which found	both bag and chain fibres	no comment	chain fibres only	no comment	bag fibres only	both bag & chain fibres	both fibre types, but predom. on + on bag fibres.

* The recently confirmed dichotomy of bag fibres into bag_1 and bag_2 types (Banks et al 1975; Banks et al 1976; combined EM/Histochemical study) was not taken into account in these early studies

\+ Boyd (1962) took this condition a step further and observed " " plates (= p_1 + p_2) exclusively on bag fibres of cat tenuissimus spindles.

fibre, and if a low Doyere eminence, or none, happened to be visible. Sole-plate nuclei could not be used as a criterion for distinguishing between the two morphological types of plate because nuclei generally stained poorly with the silver impregnation methods employed. Gladden (Ph.D. thesis, 1971) also encountered this problem with her morphological studies on rat tail muscle spindles.

The two classes of ultrastructural plate were (i) those with a recognisable Doyere eminence, a well developed sole plate containing at least one round sole-plate nucleus per thin section and various organelles, and a smooth synaptic junction that lacked post-junctional folds, and (ii) those with no Doyere eminence or a less obvious one, a thinner sole plate lacking round sole-plate nuclei and sarcoplasmic organelles (so that the myofibrils are effectively closer to the post-synaptic membrane than in (i)), and numerous, unbranched post-synaptic folds, sufficiently wide to prevent fusion of the lining basement membrane. The synaptic gap was consintently wider in the type (ii) plates than in the type (i) plates. There were abundant organelles present in the post-synapse of both plates. On the basis (admittedly uncertain) of the presence/absence of a Doyere eminence and the fact that both ultrastructural type (ii) plates sectioned appeared extensive in length and on a chain fibre, it is not too unreasonable to correlate the ultrastructural type (i) and type (ii) plates with the morphological short and long plates, respectively.

Both types of plate were most frequently located at

more or less the same polar level, i.e., near the junction of region B with region C. However, several long/type(ii) plates were seen closer to the trail terminals, around the mid-B level. Short/type (i) plates were never encountered this close " in ".

Gladden's description and photos of the two types of plate she found in rat tail muscles with a silver impregnation technique, fits well with the description of the two types of morphological plate recognised in this study (Table XI). Thus, she describes a small, discrete plate having a similar structure to an extrafusal end plate but being less than half the size and having a rather more variable complexity. A nucleated sole plate was seen on those intrafusal fibres that, by chance, were orientated in such a way that the ending was seen sideways on. Staining for acetylcholinesterase revealed a discrete sub-neural apparatus. These compact plates of Gladden's correlate well with the morphological short category of plate identified in medial gastrocnemius spindles. As in the latter, Gladden observed that such plates were confined largely to the outer polar regions. Their equivalent in the cat are the (morphological) p_1 plates of Barker et al (1970). Certainly some of the simpler p_1 plates depicted in their photographs look very much like Gladden's small plates and the short plates found in the medial gastrocnemius spindles.

Gladden located the second type of plate in the juxta-equatorial region (cp intracapsular region of the present study). Twice the size of the polar plates and

lacking a nucleated sole plate, the ending took the form of several short tapering branches and knobs. It compared easily with the long category of plate described for medial gastrocnemius muscles and Barker et al's (1970) p_2 plate in cat spindles although, like many of the p_1 plates, these all tended to be more complex structures, possessing many more axon terminals.

Ovalle's (1972) " NB plates " and " NC plates " are also distinct correlates of the author's short and long plates, respectively (Table XI), Ovalle describes his NB plates as being the smaller and more delicate of the two, each ending consisting of no more than a couple of filamentous branches. However, unlike Gladden's small, compact plates and the short plates of this study, NB plates apparently have a poorly developed sole plate (containg one or two nuclei). NC plates are larger and more robust, being made up of several thick terminal arborizations that rest on a well defined sub-neural apparatus containing several sole-plate nuclei. Again, except for the latter feature, Ovalle's NC plates seem very much like Gladden's large plates and the long plates of this study.

Ovalle also carried out an ultrastructural study as part of the same experiment. He describes NB plates as having a smooth post-synaptic membrane with no junctional folds (cp type (i)/ short plates of the present study). The axon terminals appear comparatively small and unbranched. NC plates possess a moderate number of unbranched junctional folds, the axon terminals being larger and branched

(cp type (ii)/long plates of the present study). In addition to this basic difference regarding the extent of myoneural contact, Ovalle also points out a wider synaptic gap under the NC plates. The only observation which contrasts with the findings of this study concerns the relative abundance of post-synaptic organelles; Ovalle failed to record any in NB plates, although NC plates contained an extensive variety.

Porayko and Smith (1968) likewise observed two types of plate in rat lumbrical muscle spindles: one resembling a cat plate (they do not specify which type but, presumably, mean a p_1 plate) and the other consisting of a single filament (as were a few of the long plates observed in this study). Unfortunately, their description ends there, leaving much to be desired, but it is still possible to correlate them with the two types of plate variously labelled in the literature (Table XI).

Using a combined stain for intrafusal fibres and the sub-neural apparatus of fusimotor endings (i.e., Sudan Black with acetylcholinesterase), Mayr (1969) defined two classes of plate in rat lumbrical spindles. The smaller class of plate (13 - 25 μm long) showed a compact arrangement of sub-units. These were not clearly separable and were often surrounded by a halo of reaction product, which Mayr regards as being a sign of sub-synaptic folding. There were certain similarities between these small fusimotor plates and extrafusal end plates. (See Table XI for correlation with those of authors previously mentioned.)

According to Mayr, their number per fibre pole never exceeds one. The larger class of plate (15 - 40 µm long) consists of circular sub-units in loose arrangement (Table XI). Their number per fibre pole was usually one but sometimes there were as many as three. Both classes of plate were located at the mid-polar level.

Apart from Ovalle's (1972), other ultrastructural studies have been carried out on rat fusimotor endings by Hennig (1969), Merrillees (1960), Landon (1966) and Milburn (1973).

At the " beginning " of the polar region (presumably the more equatorial level of region B) Hennig describes a type of plate consisting of a longitudinal row of end bulbs enclosed in deep inlets of sarcoplasm. Her electron micrograph shows smooth synaptic gaps. The other type of plate, located at a more polar level resembled an extrafusal plate in having a Doyere eminence, a sole plate containing nuclei and post-junctional folds, though the neither branched nor long were as numerous as in extrafusal plates. The terminal bulbs did not lie behind one another in a row, but were scattered irregularly over the surface of the intrafusal fibre. The fine structure of this extrafusal type plate of Hennig's is largely similar to that of the type (ii)/long plate and its correlates (Table XI). However, a feature in discord with the type (ii)/long plate ultrastructure (see also Table XI) but in agreement with Ovalle's (1972) NB plates, was the presence of a Doyere eminence.

In respect of the smooth-surfaced post-synaptic membrane, Hennig's first plate finds its equivalent in the type (i)/

short plate and its correlates (Table XI). But, in addition to the absence of a Doyere eminence, the first is deeply enclosed by sarcolemmal lips. In contrast, the type (i) plates of this study are located on the surface of the muscle fibre, causing no indentation whatever; in fact, a Doyere eminence gives it quite the opposite appearance.

As regards fusimotor innervation, Merrillees' (1960) electron microscopic study of lumbrical spindles was incomplete, extensive lengths of the spindles having been lost through technical drawbacks. Nevertheless, he sectioned a number of plates at the poles which he compares with those on extrafusal fibres (Palade, 1951, 1957). Thus, junctional folds were present, though not as extensive as in extrafusal end plates. Unlike the latter, and in keeping with type (ii)/long plates in the medial gastrocnemius muscle, a Doyere eminence was lacking. Some of Merrillees' plates possessed next to no sole plate, but others contained the usual accumulation of organelles also identified in the present study. Merrillees mentions that in some plates, there were no junctional folds, but fails to give any more structural details. One is left to wonder whether or not his data revealed two types of fusimotor plate.

Landon (1966) sectioned plate endings on the extra-capsular regions of bag fibres. The plate depicted in his micrograph shows a smooth post-junctional membrane like that of the type (i)/short plate in figure 6 on the bag_2 fibre of this study. However, quite different from the latter, was the absence of a Doyere eminence in Landon's plate, which

compares more closely to Hennig's (1969) smooth-synapse plate; it actually lay in a depression n the bag fibre, being invested at its edges with sarcolemmal lips. Refer to Table XI. Landon equates these plates with Hess' (1965) endings on the " slow " extrafusal muscle fibres of the garter snake and with Pages (1965) on those of the frog.

In her electron microscopic study of rat spindles, Milburn (Ph.D. thesis, 1973) sectioned a single fusimotor plate in the polar region of a nuclear-chain fibre. There is little difference in appearance between Milburn's plate and the type (ii) plate of this study (Table XI). It possessed an extensive area of myoneural contact with short, unbranched post-junctional folds. Milburn also describes a well developed sole plate, in which the sub-neural sarcoplasm is rich in mitochondria and glycogen. However, the Doyere eminence could not be said to be as " well-developed " as in the type (i) plates of medial gastrocnemius spindles. Milburn sectioned a different type of plate in 12 DPN muscle. She compares it with Ovalle's NB plate. Thus, it terminated on a bag fibre and was considered to lack post-junctional folds since where they were present, they were wide and shallow. As with Ovalle's NB plate, the only feature that was different from the author's type (i) plate was the thinly spread sole plate, i.e., presumably there was no Doyere eminence, but her micrograph certainly shows sole-plate organelles in abundance.

To summarise the fusimotor plate situation in the rat (Table XI), there seems to be general agreement not

only on the recognition of two histological types of plate (applying common criteria) but also on their description - ultrastructural as well as morphological. The two most obvious discrepencies (Ovalle, 1972; Milburn, 1973; both EM studies) concern the relative sizes of Doyere eminence and sole plate . However, with respect to the presence/ absence of junctional folds, practically all of the workers on rat spindles concur with the findings of this study (apart from Mayr, 1969; acetylcholinesterase technique. See Table XI). It may be that the degree of development of the Doyere eminence and sole plate varies along different parts of one and the same motor terminal and may even depend on the longitudinal location of the plate on the particular intrafusal fibre. In other words, these two features may not be constant, reliable criteria for typing fusimotor plates. Perhaps the most accurate approach to the problem would be to carefully serial (thin) section, both transversely and longitudinally, each of a number of plates in order to construct complete and accurate pictures of their ultrastructure.

Opinions on the specificity of the two plate types in the rat for the bag fibres or the chain fibres suffers from the same variability among researchers. At one end of the spectrum are the contenders of ultimate specificity, i.e. Mayr (1969) and Ovalle (1972). Mayr found his small compact plates only on chain fibres (always one per fibre) and his large, loose plates only on bag fibres (usually one per fibre, but sometimes two or three). In reverse, Ovalle observed his large, NC plates innervating chain fibres only, and his small, NB plates

innervating bag fibres only.

Most other workers do not support the existence of such specificity. Porayko and Smith (1968) located both types of plate on bag as well as chain fibres, although the majority of "cat-like" plates supplied bag fibres. The present study depicted short and long plates on both types of fibre; however, most of the short plates were confined to the bag fibres (indeed, only one - from a dual branching - could be identified on a chain fibre), whereas long plates were more or less equally distributed to bag and chain fibres alike. Gladden (1969) does not mention any differential distribution of her two morphological types of plate. The only fact that emerges from her description on this subject is the preponderance of the small, compact plates on the bag fibres. This was gleaned from circumstantial evidence: the confinement of her small plates to " the more extreme polar regions " where, in the rat, chain fibres do not usually extend.

Hennig (1969), Merillees (1960) and Landon (1966) do not remark on the subject of plate/fibre distribution. Landon merely mentions that the smooth-synapse plates that he sectioned were all located on bag fibres (see Table XI for corresponding observations on the cat, Barker et al., 1970).

The only study in the rat on the number of plates and the number and type of fusimotor axons per spindle, comparable to the one undertaken for this thesis and to Boyd's (e.g., 1962) and Barker et al's (1970) in the cat, is Mayr's (1969).

As noted in the present study, Mayr too, found no evidence of mixed motor innervation, i.e., one axon supplying

two (or all three) different types of fusimotor endings. Indeed, the lack of such mixed motor innervation in any mammalian spindle can be looked on as an undisputed fact. As regards axonal branching between different fibre types, Porayko and Smith (1968) and Ovalle (1972) record no branching of any one axon to supply a bag fibre and a chain fibre with the same type plates. However, two instances of axonal branching between bag and chain fibres were observed in spindles from themedial gastrocnemius muscles. In one instance, the axon was a long-plate axon and in the other a short-plate axon.

The number of plates per spindle pole varied from 0 - 5, depending on the position of the spindle in the muscle (cp. a mean of 1.8 for the medial gastrocnemius muscle). Both types of plate often lay at virtually the same level next to one another, a situation that obtains in medial gastrocnemius spindles as well.

Mayr counted 2 - 7 axons per spindle (cp a mean of 10.0 in medial gastrocnemius spindles). All of these he referred to as γ, there being no evidence of β innervation, aswas the case in this study. (Other workers, namely, Porayko and Smith (1968) and Gladden (1969) have, however, observed skeletofusimotor innervation in their preparations. In every instance, the axons ended in a short/type (i)-like plate,) The number of trail fibres per spindle Mayr put at 1 - 2 (cp mean of 7.2 in present study), only one of which generally entered at the equator (cp mean of 2.4). The consistent discrepancy between Mayr's values for

lumbrical spindles and the ones found in the medial gastrocnemius muscle, may well be a reflection of the different anatomical muscles. Indeed, in support of Mayr's low values, Porayko and Smith (1968) record only 2 fusimotor axons for any one lumbrical spindle, one specifically to the chain fibres and one to the bag fibres. Unfortunately, Gladden (1969) makes no reference to fusimotor axon number per spindle. Her account of fusimotor axons was limited to their relative diameters.

Mayr noted that those fusimotor axons ending as plates usually approached the spindle at the poles rather than at the equator.

Except for Boyd's (1962b; Boyd & Davey, 1962) and Adal and Barkers(1965) study on the cat, there have been no statistical approaches to the relationship between axonal diameter and fusimotor plate type in other mammalian spindles. Boyd tried to prove a distinct correlation between thicker "γ_1" fibres and plates and thinner "γ_2" fibres and his "γ_2 network". However, Adal and Barker were not convinced and in a subsequent analysis, found that the relationship between stem axon diameter and type of fusimotor plate was not statistically significant. Gladden (1969) and Mayr (1969) felt that this was also true of rat spindles, but they still noticed certain trends at the level of the spindle. Thus, Gladden observed that axons to small plates were usually less than half the diameter of most α axons, although some of the latter were as narrow. Axons to large plates tended to be twice the diamter of those to small plates, i.e., approximately equal to α axons. In a similar vein, Mayr reported that axons to <u>both</u> types of plate were usually equal in

diameter to the terminal portions of α axons and that trail axons were frequently the thinnest. However, in agreement with Adal and Barker (1965), Mayr found that this diameter relationship was sometimes reversed. The observations of this study were virtually no different; the trail group of fusimotor axons contained the thinnest and thickest fibres, and long-plate axons were often thicker than short-plate axons.

When comparing the number and distribution of fusimotor axons to spindles of different mammalian species one must obviously exercise care, since the relative proportion of bag and chain fibres is different in each species. For example, the bag fibre:chain fibre ratio is 2:2 in the rat, 3 or 4:0 in the rabbit and 2:4 or 5 in the cat (Barker and Hunt, 1964). Taking the problem a step further, the fact that bag fibres can now for certain be sub-divided into two distinct types on the basis of their combined histochemical/ultrastructural profiles (see Banks et al., 1977), complicates matters and demands caution even at the intra-specific level.

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Having defined the innervation pattern of normal adult rat spindles in the medial gastrocnemius muscle (see fig. 79A & 80A) and found support for it from previous research, we are now in a position to evaluate the innervation pattern of model-adult and adult-crush spindles and to distinguish which features are normal and which features are not.

The three composite questions posed at the onset of this study (page 56) have, in large part, been answered. Thus, question (i)a: Do secondary axons regenerate in chainless/bag-only spindles? The axon diameter histograms (fig. 77) constructed for the medial gastrocnemius nerve show that all of the sensory and motor axons regenerate at this level. The figure records that the number of regenerated axons is similar to the number of axons in the control nerve and the assumption is then made that the axon make-up of the model-adult nerve (i.e., the proportion of primary, secondary and motor fibres) is probably similar to that of the control nerve at the level of the muscle nerve. In other words, it seems fairly safe to assume that all the Group II sensory axons do, in fact, regenerate. On the other hand, the silver/tease studies showed that the mean number of secondary axons per spindle was reduced from a mean of 1.8 in the normal adult to a mean of 1.0 in the model-adult, a loss of some 44%. Thus, more model-adult than normal adult spindles had no secondary; most had single secondary whereas most normal adult spindles had two. It can be seen that a discrepancy exists between the number of Group II axons at the level of the muscle nerve and the number of secondary axons at the spindle level. There are two possible explanations, both of which could have operated simultaneously. Firstly, the "missing" secondaries may well have ended freely among the connective tissue of the main muscle mass (Zelena & Hnik, 1960a, 1963c); none were actually seen but then, they were not specifically looked for. Secondly, there may have been a reduced incidence of intramuscular branching of the Group II axons; this state of affairs may have been induced by complete degeneration of some 50% of the spindles.

Question (i)b: Assuming that at least some, if not all, of the secondaries do regenerate, is there any preference for one or other type of bag fibre in two-fibre spindles? And what happens to their terminals which, in normal spindles

predominantly supply the chain fibres? Let us look, first of all, at the two-fibre spindles. The morphological studies revealed that the secondaries in those two-fibre spindles that possessed them, ended on both bag fibres as one or two simple terminals, variably robust or delicate. Spirals (present in the more equatorial part of normal secondary endings on chain fibres) and extensive, spray-like branching (forming the major part of normal secondary endings on chain fibres) were absent entirely. In effect, the model-adult bag fibres appeared to be innervated by the secondaries to no greater extent than in normal spindles, suggesting that the temporary denervation prevented the chain secondary branches from regenerating, leaving the few bag branches intact. Thus, there was no evidence whatever to indicate that normal adult bag fibres ended up with an abnormal surplus of secondary terminals. The sparseness or complete absence of secondary endings in model-adult spindles also lends support to the observation in normal spindles that the secondaries predominate on the chain fibres. In one single-fibre model-adult spindle, a secondary axon had regenerated, entering the spindle capsule with the primary axon and one trail axon. The secondary axon looped several times in the periaxial space close to the nuclear bag, but contact with the fibre could not be ascertained because of inadequate impregnation. Even so, the extensive weaving was itself aberrant.

Question (ii): What is the fate of the branches of the primary ending that once innervated the chain fibres?

The only evidence available was circumstantial. None of the model-adult spindles appeared to possess a surplus of primary endings; rather, the contrary obtained. It is very probable that these branches lost their ability to regenerate from the first-order branching point of the primary axon after " finding " that the chain fibres had degenerated. Figure 79 depicts " typical " models of sensory innervation for normal adult, adult-crush and model-adult spindles.

More directly answerable was the last question, question (iii): What happens to the fusimotor innervation of the eliminated chain fibres? Fusimotor innervation returned to the model-adult spindles, but there were abnormalities, both qualitative as well as quantitative. There was a reduction in the number of trail axons and a total (apparent, see later speculations) absence of long plates. Long plates, as recognised in normal adult spindles, were also apparently lacking in adult-crush spindles. However, there was an increase (from 1, in normal adult spindles, to 6) in the number of axons per adult-crush spindle ending as " occasional " axonal swellings, (designated mid-polar " trails " in the results), which corresponded well with the number of long-plate axons per normal adult spindle. Certainly, the axons ending the " occasional " swellings were unbranched (cf trail multiterminals) and were located at the more polar level than trail endings. The implication is clear: that occasional axonal swellings, both in model-adult and in adult-crush spindles, are actually aberrant long plates. Temporary denervation (neonatal and adult) seems to cause

an alteration in the morphology of long plates. Fewer occasional axonal swellings were encountered in model-adult spindles, suggesting that the long plates that once innervated the chain fibres had failed to regenerate. On the other hand, the frequency of short-plate axons per spindle remained virtually unchanged after the neonatal as well as the adult nerve crushes. The number in two-fibre model-adult spindles was particularly normal, which supports the observation that short plates predominate on bag fibres.

Compared with two-fibre spindles, the fusimotor innervation of single-fibre model-adult spindles had obviously suffered a greater reduction in terms of the number of axons, particularly the trail axons. If it is accepted that those axons that ended as occasional axonal swellings were, indeed, long-plate axons and not trail axons, then the number of trail axons per single-fibre spindle was about 2 as opposed to about 4 per two-fibre spindle.

Put simply, the findings suggest that the neonatal nerve crush effectively " knocked out " the trail axons and long-plate axons that normally supplied the chain fibres, and that the few long-plate axons that regenerated were the ones that had previously innervated the bag fibres. In other words, the model-adult bag fibre(s) do not receive a surplus of innervation. Indeed, the opposite could be said to be true of single-fibre spindles. In addition, temporary denervation caused a radical change in the morphology of long plates in both model-adult and

adult-crush spindles. It is therefore reasonable to deduce that long/type (ii) plates are more susceptible to nerve crush at any age than are short/type (i) plates, at least as far as their morphology is concerned (Figure 80 depicts " typical " models of fusimotor innervation for normal adult, adult-crush and model-adult spindles.

Since there was no change in the number of axons in the two representative model-adult muscle nerves (fig. 77), the reduction in the fusimotor axons at the spindle level must be a reflection of reduced intramuscular branching of γ axons. An estimate was made of the number of intramuscular branches that must have degenerated. Taking into account the fact that about 50% of the spindles had themselves failed to regenerate (and therefore their entire fusimotor supply), the estimate came to some 137 γ intramuscular axons.

To date, very little research has been undertaken into the effect of neonatal or adult nerve lesions on spindle innervation morphology in any species of mammal. Most studies have concentrated on the innervation of extrafusal muscle (
Spindle studies on nerve section at birth (Zelená, 1957, 1959, 1962; Zelená & Soukup, 1975;
Tower, 1932) generaly involved permanent denervations for assessing the effect on the intrafusal fibres. However, some of Milburn's operations on newborn rats, which were intended to be permanent, actually resulted in some spindle reinnervation. With electron microscopy, she discovered sensory endings in a few spindles but did not

encounter any fusimotor terminals. Many of the temporary denervations at birth (Zelena & Hnik, 1960a,b; 1963a,b,c; Hnik & Zelena, 1961; Zelena, 1964; Werner, 1973a) and a few days after birth (Werner, 1973b; Schiaffino & Pierobon Bormioli, 1976) centred largely on the general morphology - using paraffin sections - of spindles. The only information to be gleaned on the regeneration of spindle innervation was the fact that the sensory endings returned, as evidenced by the retention of nuclear bags.

Werner's (1973b) work on nerve crush at 4, 6, 8 and 10 DPN was the most relevant to to this study, but apart from circumstantial evidence, (the presence of nuclear bags), which showed that the sensory endings had returned, the nature of the histological technique precluded collecting any useful data on spindle innervation.

Schiaffino and Pierobon Bormioli (1976) did, at least, stain for acetylcholinesterase in their nerve lesion experiment, which consisted of nerve section followed by resuture of the nerve stumps to facilitate nerve regeneration. Spindles in the 4- and 7 DPN-denervated groups were grossly abnormal; they lacked sensory endings (as seen by EM) and were shown to possess a single motor end plate with a sub-neural apparatus very similar to that of a normal extrafusal fibre. Normal looking spindles in the 13- and 22 DPN-denervated groups were reported as having sensory and motor innervation approaching normality. The description of spindle innervation went no further than this.

Investigations into the effect of adult nerve lesions on spindle innervation morphology are virtually non-existent. As with neonatal nerve lesions, there have been no studies on a par with those of normal cat spindles by Boyd (1962), Barker et al (1970), etc., or even with those of normal rat spindles (e.g., Porayko & Smith, 1968; Gladden, 1969; Ovalle, 1972). However, there have been a few physiological studies on cat spindles that have provided some useful information on the functional presence/absence of sensory and fusimotor endings after temporary denervation Brown & Butler, 1973; Thulin & Blom, 1975; Takano, 1976).

Brown and Butler demonstrated that, following a complete section or crush of the nerve to either the tenuissimus or the peroneus longus muscles, γ axon re-innervation is not haphazard; each type of γ fibre regenerates to to the original specific sites within the spindle. Thus, as found in normal cat spindles (Brown & Butler, 1973; Barker et al, 1972; Boyd et al, 1973), individual γ dynamics always re-innervate bag fibres and individual γ statics always re-innervate chain fibres, but may re-innervate bag fibres as well. However, during the re-innervation period, Brown and Butler (1973; 1974) observed a difference between the two muscles in the sequence of γ and β axonal regeneration. In tenuissimus spindles during the re-innervation period, a high proportion of spindle innervation may be due to β fibres. This was not found in peroneus longus muscle.

Thulin and Blom (1975) obtained similar results to

Brown and Butler for tenuissimus spindles. After sectioning and resuturing a peripheral muscle nerve, they found that the Group I, II and III afferent fibres and the α efferents became re-established far earlier than the small-diameter γ fusimotor fibres. Thus, for a period of time, the muscle was deprived of a functional γ innervation system. They do not specify the actual regeneration time.

Takano (1976) recorded a very similar sequence of events following re-innervation. He subjected the sciatic nerve to local freezing, an operation equivalent to nerve crush (Gaster, Davidson, Rand & Fonkalsrud, 1971) and made recordings over a period of 6 months. Even at the end of this time, Takano still found only phasic spindle responses to ramp and hold stretches and only a few of them exhibited a pause during muscle contraction. None showed any sign of γ innervation, as tested by several independant γ-activating or γ-blocking procedures. The conduction velocities of regenerated afferent and efferent fibres were below normal values after 6 months. Fibre histograms showed that Group I, II and III afferents had regenerated but of the efferent fibres, only the α group had done so. Clearly, during the 6 months of post-operative observation, γ fibres either had not returned or were not functioning. Takano coined the term " alpha muscles ".

Such differential growth of axons is also seen during neuromuscular development (Skoglund, 1960b,c; see introduction, page 28). Skoglund's experiments demonstrated that β fusimotor innervation develops (or more precisely,

functions) several days before γ innervation.

Parallels can be drawn between certain human diseases and nerve lesions. For example, leprosy is recognised as a bacterial disease that affects both muscle and nerve. Pandya and Chulawala (1973) carried out an axon impregnation study of lepromatous spindles and extrafusal fibres. The primary endings of some spindles appeared irregular and one fusimotor fibre ended in a bulb (cp fig. 64 A & F , two aberrant short plates in two model-adult spindles) and others appeared enlarged. Extrafusally, there were thin (presumably unmyelinated) preterminal and ultraterminal sprouts from some α axons similar to those described by Tuffery (1971: Tuffery's " contribution ") in cat hindlimb muscles. An occasional sprout ended as a separate end plate (i.e., with a separate sub-neural apparatus) adjacent to the parent end plate on the same muscle fibre (cp Tuffery's " duplex " ending).

The model-adult spindle; physiological speculations -

It may seem unwise to speculate on something that can be readily elucidated by simple experiment, but in the light of recent physiological experiments on cat hindlimb muscles (Brown & Butler, 1973, 1975; Barker, Emonet-Denand, Harker, Jami & Laporte, 1976), some speculation is justified on the physiology of chainless spindles in so far as the latter's use as potential models.

The aim of the physiological experiments referred to was to throw light on the distribution of fusimotor

innervation to the three fibre types (bag_1, bag_2 and chain fibres). The procedure involved the depletion of glycogen in cat tenuissimus intrafusal fibres by prolonged tetanic stimulation of single fusimotor axons. Having done this, each spindle preparation was fixed and transversely sectioned and the sections then stained for glycogen with periodic acid Schiff reagent.

Barker and co-workers (1976) found that the dynamic γ axons depleted bag_1 fibres almost exclusively (chain fibres being occasionally depleted simultaneously) whereas static γ axons depleted both bag and chain fibres. Bag_1 and bag_2 fibres were depleted about equally, although the slower conducting γ statics (19 - 23m/sec.) depleted only the bag_1 and chain fibres whereas the faster γ statics (28 - 45m/sec.) depleted mostly bag_2 fibres. Thus the bag_1 fibre of cat tenuissimus spindles is innervated by γ dynamics (p_2 plates being considered their morphological terminals; see Barker et al, 1976) as well as γ statics (trail multiterminals being considered the morphological terminals; see Barker et al, 1973). This finding agrees with morphological studies, namely those employing axoplasmic impregnation techniques: p_2 plates and trail endings have been observed on one and the same bag (bag_1) fibre (Barker et al, 1970) and, moreover, 90% of p_2 plates were located on bag fibres and only 10% on chain.

Not all workers agree on dual dynamic/static innervation of bag_1 fibres. Bessou and Pages (1973, 1975), Boyd, Gladden, Mc William and Ward (1975) and Boyd and

Ward (1975), using cinematographic analysis of contractions elicted by stimulation of single fusimotor axons, suscribe to a concept of selectivity in which dynamic/slow bag fibres ($\equiv bag_2$) are stimulated to contract by γ dynamics only and static/fast bag fibres ($\equiv bag_1$) by γ statics only.

From their glycogen depletion findings, Barker et al (1976) also deduced that bag_2 fibres and chain fibres are innervated largely by γ statics; certainly, they found no bag_2 fibre depleted by γ dynamic excitation. The results of previous physiological/morphological experiments involving single γ static axons (Barker et al, 1973) also uphold this finding from the glycogen depletion work.

In attempting to reconcile the disparity between the two schools of thought regarding bag_1 innervation (see above), Barker et al (1976) suggest that perhaps cinematography cannot detect the contractions elicted in bag_1 fibres by static axons (see also, Bessou & Pages, 1975). But in acknowledgement of a dual innervation of bag_1 fibres, they are left with even greater problems to resolve. " If the activation of bag_1 fibres by static axons has a static effect on the primary-ending response, we have to accept that these muscle fibres, depending on the way they are activated, can produce either a static or a dynamic effect. It is difficult to see how this could occur. Static and dynamic axons do not selectively activate the two ultrastructuralally different regions of

bag_1 fibres, their sites of activation overlap; and there is no evidence to encourage the idea that morphological differences between their terminals might be responsible. The alternative is that bag_1 fibres produce a dynamic effect whether activated by static or dynamic axons, and that during static activation this effect is either swamped by contractions occurring simultaneously in other types of fibre, or manifests itself by adding some particular feature to the static response. If bag_1 fibres always produce dynamic effects, it would follow that, as an occasional aberration, a static axon could produce a dynamic response from a spindle if it activated the bag_1 fibre only. "

Barker et al (1976) maintain that there were three possible instances of this in their sample of γ static stimulations, but that the additional activation of chain fibres could not be excluded because of their suspected partial depletion in each case. On the other hand, a particular fusimotor axon has been shown to produce the same afferent response from all the spindles it supplies (Crowe & Matthews, 1964; Brown et al, 1965; Bessou et al, 1966). It may be relevant to the issue to heed a word of caution by Gladden (Ph.D. thesis, 1971): " It may be that the inclusion of different structures under the third fusimotor ending described here (i.e. trails) covered by the umbrella description " pleomorphic " is really wrong, and that these are quite different functional entities."

Assuming that we can extrapolate from cat to rat muscle spindles, the model-adult spindles of rat medial gastrocnemius muscle, particularly the single-fibre ones, may well assist in dispelling the confusion, since there would be no chain fibres to taint the results of glycogen depletion experiments. If the identity of the bag fibre in single-fibre model-adult spindles could be confirmed as bag_1, and if it is accepted that the innervation of these fibres consists of trails only (see fig. 80 D), then the spindle response to γ static stimulation would be very telling. A dynamic afferent response would indeed mean that bag_1 fibres could produce a dynamic effect whether activated by static or dynamic axons.

For the same reason, it would also be interesting to see the response of the two-fibre model-adult spindles to γ static stimulation, particularly if both bag fibres are stimulated by the same trail fibre. In this situation, could γ static stimulation possibly elicit a dynamic response from the bag_2 afferent supply?

Even if the bag fibre of single-fibre model-adult spindles was considered a bag_2 fibre, the fact that its alkaline ATP'ase reaction and the M line condition in regions B and C were aberrant, is sufficient to prompt a physiological investigation. It is believed that the alkaline ATP'ase activity of an intrafusal fibre as well certain ultrastructural features are related to the pattern of fusimotor innervation (e.g., Guth et al, 1970) and therefore any histochemical or ultrastructural

aberrations would be expected to be a reflection, at least in part, of aberrant fusimotor innervation. However, since morphologically, the fusimotor innervation of single-fibre spindles was not found to be drastically abnormal, this points a finger in the direction of physiological abnormalities. So, whatever the type of bag fibre present in single-fibre model-adult spindles, it would be of considerable interest to discover the response to γ stimulation. Physiological experiments may even reveal the presence of γ dynamics, which would, in turn, indicate the presence of long-plate axons to these spindles after all. Of course, if such a discovery were made, it would mean that the bag fibre of single-fibre spindles was definitely a bag_1 fibre (Barker et al, 1976), assuming the accuracy in extrapolating from cat to rat spindles.

Bearing in mind the altered morphology of long plates, what sort of response would γ dynamic excitation produce? The greatly reduced size of long plates means that they must obviously extend over many fewer sarcomeres and this, in turn, might be expected to affect the local contraction (Boyd, 1976) of myofibrils. Contraction would probably be weaker on account of its being limited to a few sarcomeres.

Likewise, consider the reduction in the number of primary spirals in many model-adult spindles. Would the altered morphology affect the velocity of stretch of the spirals? Boyd has shown that in cat tenuissimus spindles,

the velocity of stretch is 3 - 8 times greater for static/fast fibres ($\equiv bag_1$) than for dynamic/slow fibres ($\equiv bag_2$).

The results of potential physiological experiments on model-adult spindles would be made more meaningful by similar stimulation experiments on adult-crush spindles, in which long plate morphology is also affected, and on normal adult spindles. Perhaps one would then be able to answer the ultimate question: How necessary <u>are</u> chain fibres for normal spindle function?

The model-adult spindle; its possible effect on gross muscle function -

In addition to physiological studies on model-adult spindles, it would be interesting and certainly medically relevant, to go a step further and study the effect of chainless/bag-only spindles on the normal efficiency of whole limb movement.

First of all, a series of tests would heve to be devised and carried out on normal adult and adult-crush muscles. With the control baselines set, model-adult muscles could be"screened" for abnormalities in mechanical operation. It would be best to carry out the nerve crush operation bilaterally instead of unilaterally so as to obviate any influence from healthy contralateral muscles. Any deviation on the part of model-adult muscles from the baseline could then be attributed in large part (i) to the absence of chain fibres and their innervation

and (ii) to the possibly anomalous physiological behaviour of the remaining bag fibres. Prior physiological study would help explain any anomalies at the level of whole muscle operation.

The only test carried out in the present study on the hindlimb was the plantar (stretch) reflex, which was found to return 11 days after neonatal nerve crush and 9 days after the adult operation. Stretch reflexes, which include the knee-jerk as well, are indicators of muscle tone. While they fail to provide any information on physiological or possible morphological abnormalities of spindle innervation, they do show whether or not the circuit of afferent and efferent axons are intact.

Concluding Remarks

To a large extent, it seems that the future of spindle research lies in the refinement of present technical methods. More correlative studies are needed to be carried out on one and the same spindle, rather like Banks et al's (1976) combined EM/histochemical study and Barker et al's (1973) physiological/EM study. Indeed, the ideal approach to spindle research would be a combined physiological, silver/tease, electron microscopic, histochemical study on one and the same spindle preparation.

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THE EFFECT OF NEONATAL NERVE CRUSH ON ADULT RAT MUSCLE SPINDLES

PLATES

A thesis presented in canditature for the degree of Master of Science

by

Elizabeth Laidler, B.Sc. (Dunelm)

Department of Zoology, University of Durham.

Durham, March 1978.

CONTENTS OF PLATES

ABBREVIATIONS OF LABELS ON PLATES

DPN	days post-natal
DPO	days post-operation
CAP	spindle capsule
dM	double M line or no Mline
Ef	extrafusal fibre
dEf	" dwarf " extrafusal fibre
G	golgi body
If	intrafusal fibre
LG	lateral gastrocnemius muscle
LS	longitudinal section
M	muscle
Mf	myelin figure
MG	medial gastrocnemius muscle
N	nucleus
P	primary ending
PL	plantaris muscle
S	secondary ending
SOL	soleus muscle
T	tendon
TS	transverse section
Z	Z line

IA	Group IA sensory fibre
II	Group II sensory fibre

a	myelinated axon
bm	basement membrane
c	collagen
cap	blood capillary
cg	cytoplasmic granule (small)/body (large)
cv	coated vesicle
dbm	double basement membrane
dv	dense-core vesicle
ec	endomysial cell
ff	fragments of intrafusal fibres
g	glycogen
gf	" ghost " fibre
fv	flattened vesicle
icc	inner capsule cell
lvb	large vesicular body,i.e., a vesicle, usually with a double-membrane wall, containing other vesicles
li	lipid droplet
m	mitochondrion
mb	myoblast
mf	myofibril
mfl	myofilament
ml	microladder
mp	macrophage
mt	motor terminal
mvb	multivesicular body,i.e., a " frothy-looking " membrane-bound extrusion from the capsule cells
na	non-myelinated axon
nb_1	nuclear-bag_1 intrafusal fibre
nb_2	nuclear-bag_2 intrafusal fibre

nc	nuclear-chain intrafusal fibre
nf	neurofilaments
nt	neurotubules
occ	outer capsule cell
pl(i)	type(i) plate
pl(ii)	type(ii) plate
pr	polyribosomes
pas	periaxial space
r	free ribosomes
rbc	red blood cell
rer	rough endoplasmic reticulum
rsr	rough sarcoplasmic reticulum
s. ax.	sensory axon
sc	Schwann cell
sct	sensory cross-terminal
sg	synaptic gap
sh. pl.	" short " fusimotor plate
spdl	spindle
spl	sole plate
spn	sole-plate nucleus
sr	smooth sarcoplasmic reticulum
st	sensory terminal
svb	small vesicular body,i.e., a relatively small vesicle, with a single membrane wall, containing tiny vesicles
tj	specialisation of synaptic membranes,e.g., simple thickening or close apposition sandwiching electron-dense material (i.e., a " tight junction ")
tr	trail terminal
tr. ax.	trail axon
tt	transverse tubule
v	vesicle - pale, round and clear

FIGURE 1 THE POSITION OF THE MEDIAL GASTROCNEMIUS MUSCLE IN RELATION TO NEIGHBOURING MUSCLES IN THE HINDLIMB

A. TS of calf muscles extirpated from the right leg of 3½DPN rat. Paraffin section, 10 µm thick, stained with H and E. The medial gastrocnemius was marked (asterisk) during dissection in order to ensure its identity.

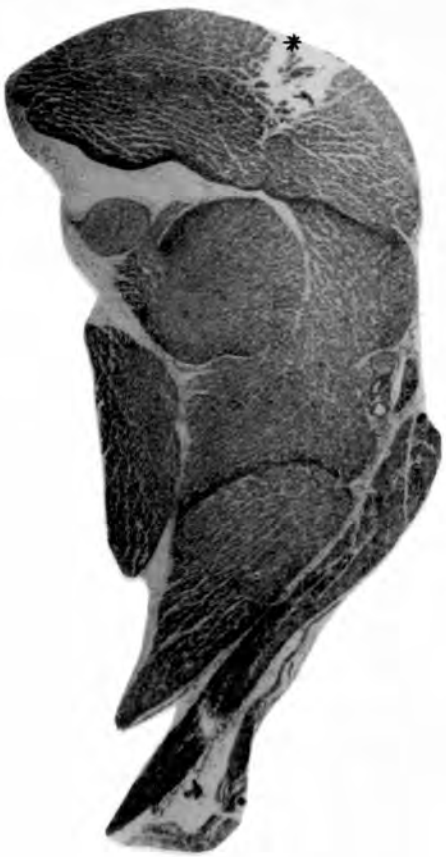
× 640

B. Diagrammatic tracing of muscle outline. The soleus and the medial gastrocnemius insert together into the Achilles tendon. The plantaris inserts nearby.

Length of bar:

= 0.63mm

A



B

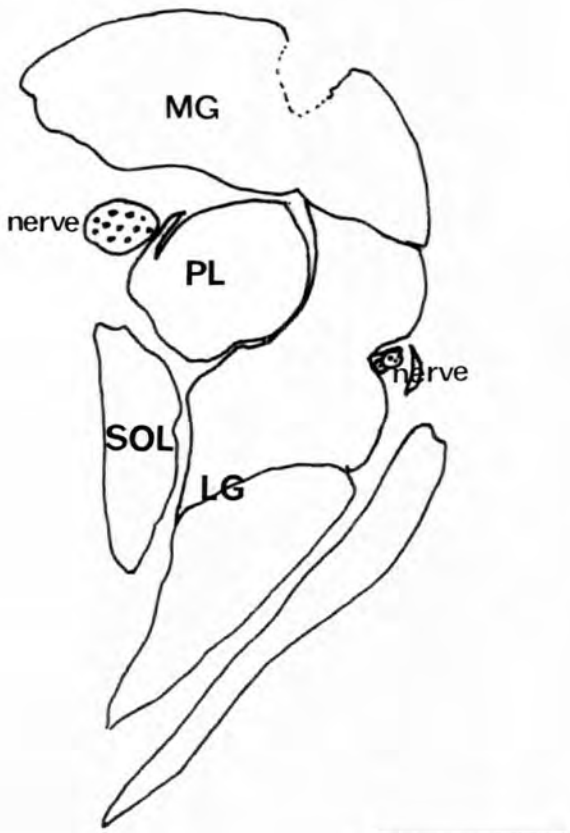


FIGURE 2 DIAGRAM ILLUSTRATING THE OPERATIVE PROCEDURE FOR CRUSHING THE RIGHT TIBIAL NERVE

A. The level and actual extent of the incision.

B. High-power drawing of the incision to reveal the position of cut through the sheet-like thigh muscles underlying the skin.

C. Comparable high-power diagram of the wound to show the precise level of nerve crush. The nerves crushed included the sural and all of the tibial.

There are three branches of the tibial:

(i) the first branch innervates the soleus and plantaris muscles and lateral head of the gastroc..

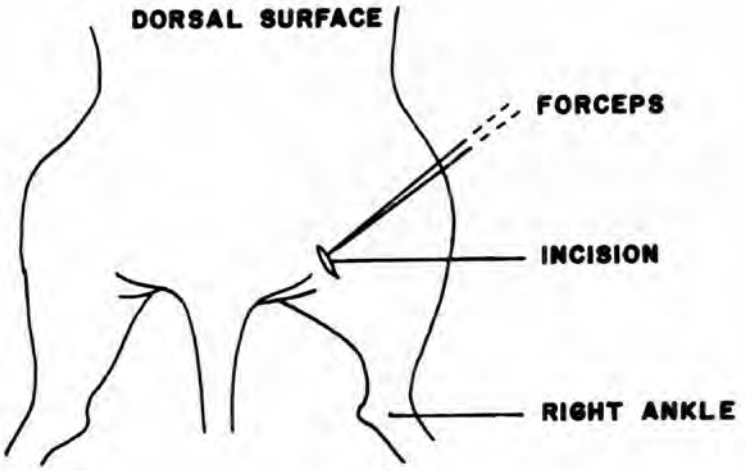
(ii) the second branch innervates the medial head of the gastrocnemius.

(iii) the third branch innervates the flexor hallucis longus, the flexor longus digitorum and the tibialis posterior.

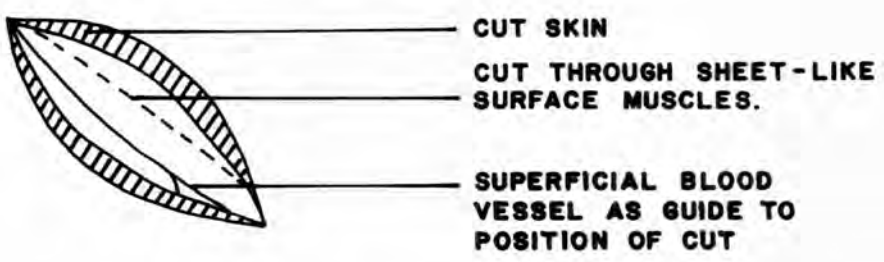
The tibial continues through the calf between the plantaris and the medial head of the gastrocnemius and divides just above the ankle into the lateral and medial plantar nerves.

The sural nerve branches from the common peroneal nerve in the hip and shares the same sheath with the latter and with the tibial for a variable distance in the thigh.

A.


DORSAL SURFACE
FORCEPS
INCISION
RIGHT ANKLE

B.


CUT SKIN
CUT THROUGH SHEET-LIKE SURFACE MUSCLES.
SUPERFICIAL BLOOD VESSEL AS GUIDE TO POSITION OF CUT

C.

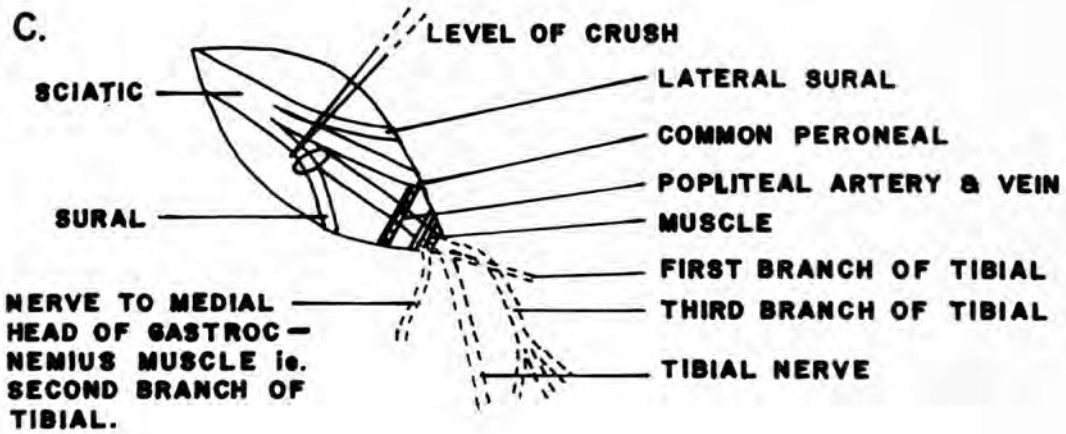

LEVEL OF CRUSH
SCIATIC
LATERAL SURAL
COMMON PERONEAL
POPLITEAL ARTERY & VEIN
SURAL
MUSCLE
FIRST BRANCH OF TIBIAL
NERVE TO MEDIAL HEAD OF GASTROC-NEMIUS MUSCLE ie. SECOND BRANCH OF TIBIAL.
THIRD BRANCH OF TIBIAL
TIBIAL NERVE

FIGURE 3 SCHEMATIC DIAGRAM (AT BOTTOM) TO SHOW THE NUMBER, RELATIVE SIZES AND DISTRIBUTION OF MUSCLES IN A PAIR OF 14DPN MEDIAL GASTROCNEMIUS RAT MUSCLES, COMPOSED FROM SERIAL 10 m THICK PARAFFIN SECTIONS STAINED WITH H AND E.

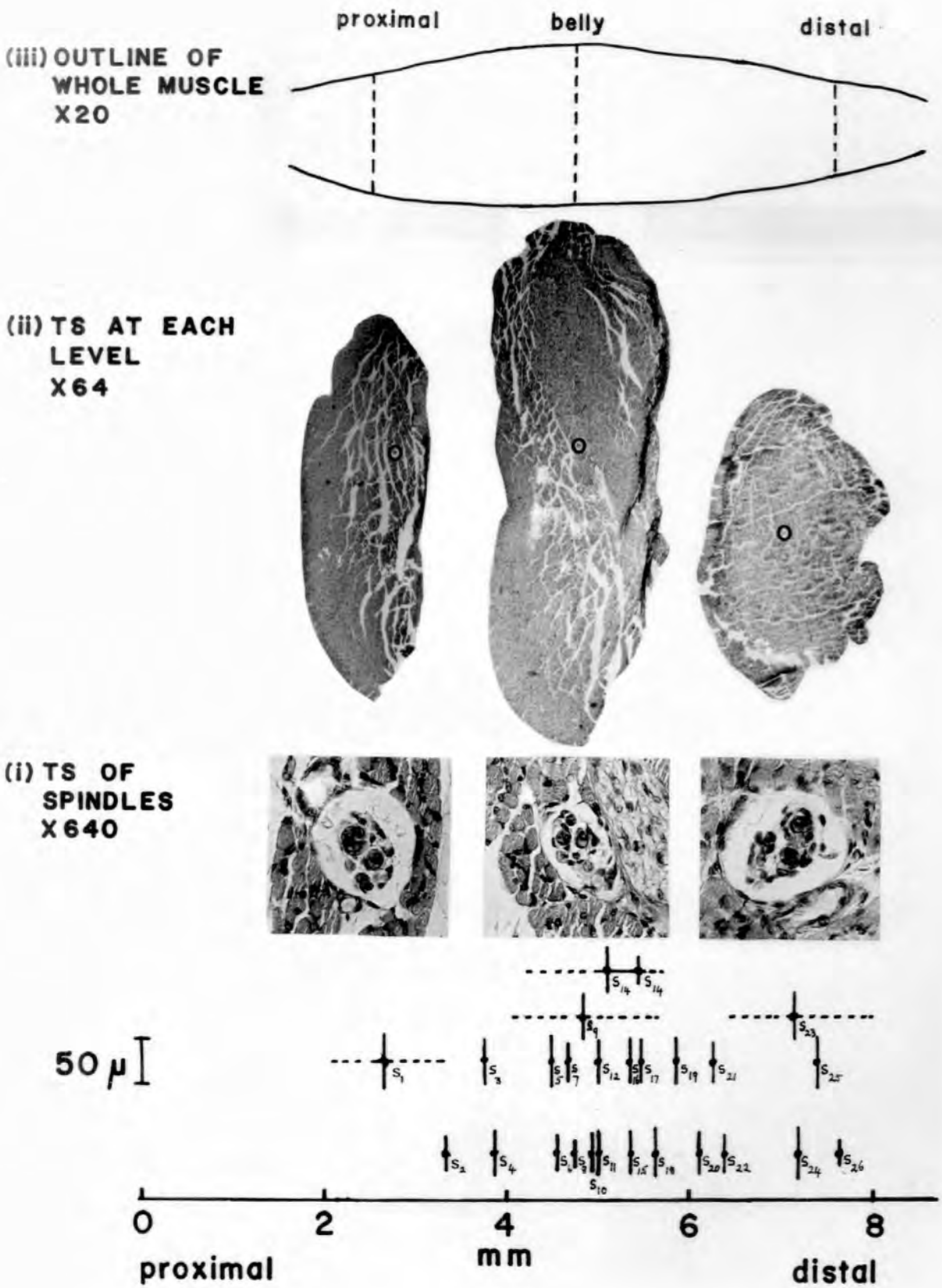
A. Control, contralateral muscle (26 spindles, $s_1 - s_{26}$; s_{14} tandem; s_{13} omitted to avoid congestion of the diag.).

B. Model-adult muscle (11 spindles, $s_1 - s_{11}$). The vertical bars, drawn to scale, represent the mid-equatorial diameters of the spindle capsules.

Three spindles have been chosen from each muscle (plus the tandem s_{14} from the control) to depict the relative lengths of their periaxial spaces (solid horizontal lines bisecting the mid-equatorial bars) and their polar lengths (dotted horizontal lines). Of necessity, the horizontal scale is ten times as small as the vertical scale. Shrinkage was not taken into account in any of the measurements.

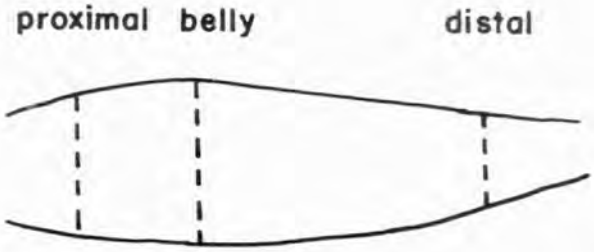
The photographs show: (i) the intrafusal fibre component, in TS, of the three spindles in each muscle. The three depicted from the control muscle are all four- fibred. The three depicted from the model-adult muscle are, from left to right, single-fibred, two-fibred and two-fibred. (ii) the location (indicated by circles) of the spindles shown in (i).

The drawing shown in (iii) is a longitudinal outline of each muscle, using measurements of their largest transverse diameters. It is at the same mag. as the horizontal axis (i.e., x 20) and serves to show the relative sizes of the two muscles.

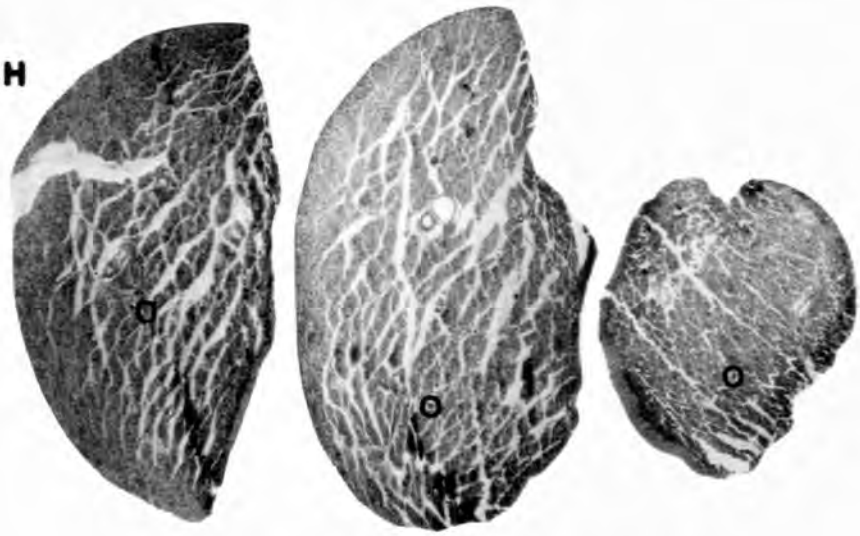


A. CONTROL MUSCLE

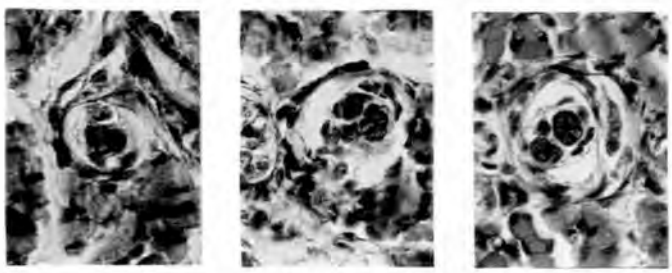
(iii) OUTLINE OF WHOLE MUSCLE. X20

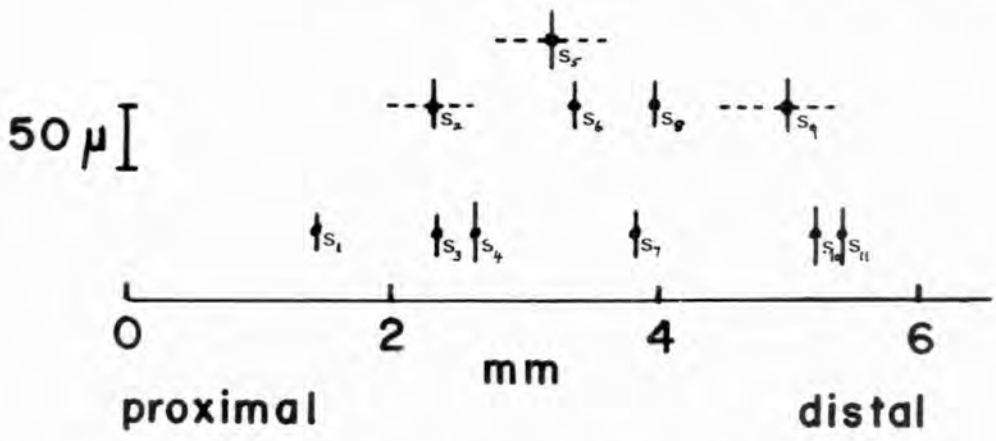


(ii) TS AT EACH LEVEL X64



(i) TS OF SPINDLES X640





B. MODEL ADULT MUSCLE.

FIGURE 4 EFFECT OF NEONATAL 3½DPN TIBIAL NERVE CRUSH ON THREE HINDLIMB RAT MUSCLES

Transverse sections shown taken from belly of muscle. Paraffin, H and E.

A. Soleus muscle. Normal left leg (left photo) shows well developed spindles. Experimental right leg (right photo) contains no spindles

× 100

B. Tibialis posterior muscle. Normal left leg (left photo) contains well developed spindles as opposed to experimental leg in which spindles are entirely absent (right photo).

× 100

Compare A and B above with fig. 3.

C. Medial gastrocnemius muscle. An experimental right leg muscle from a rat used for scanning a litter. Model-adult spindles poorly represented, so litter was discarded.

× 25

D. Medial gastrocnemius muscle. Illustrates clumping of spindles.

× 500

Arrowheads all indicate spindles.

Length of bar:

In A & B = 0.25mm

C = 1.00 "

D = 0.05 "

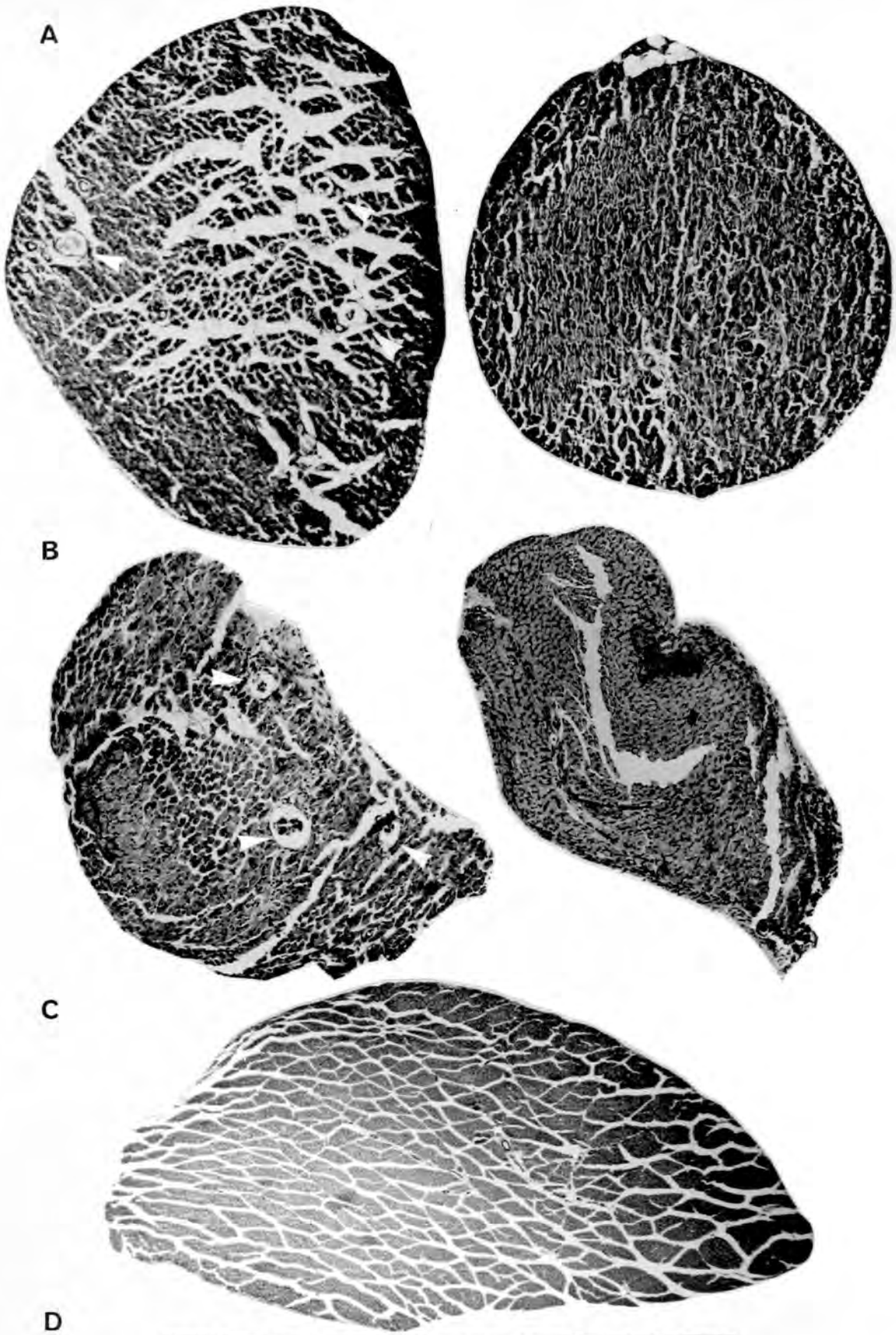

A
B
C
D

FIGURE 5 ULTRASTRUCTURE OF NORMAL ADULT SPINDLE

A. TS through region B. On bag_2 fibre is a type (i) plate, with Doyere eminence, substantial sole plate and sole-plate nucleus, and no junctional folds.

x 3,200

B. TS of capsule cells in region B. Vesicles in various stages of pinocytosis. Basement membrane lines both sides of inner cell.

x 17,100

C. High-power TS near level shown in A. Vacuolated cell in periaxial space contains pinocytotic vesicles. Note the relatively large mitochondria of the bag_1 fibre, helping to separate the myofibrils. Many of the mitochondria are similar in size to those in the chain fibres. However, in this preparation, the cristae in the bag_1 fibre are much more compact than in the chain fibres.

x 5,000

D. LS through region B. On one chain fibre is a type (ii) plate with no Doyere eminence, a small sole plate and sole-plate nucleus, and short junctional folds.

x 3,200

Length of bar:

In A & D = 7.81 μm

B = 1.47 "

C = 5.00 "

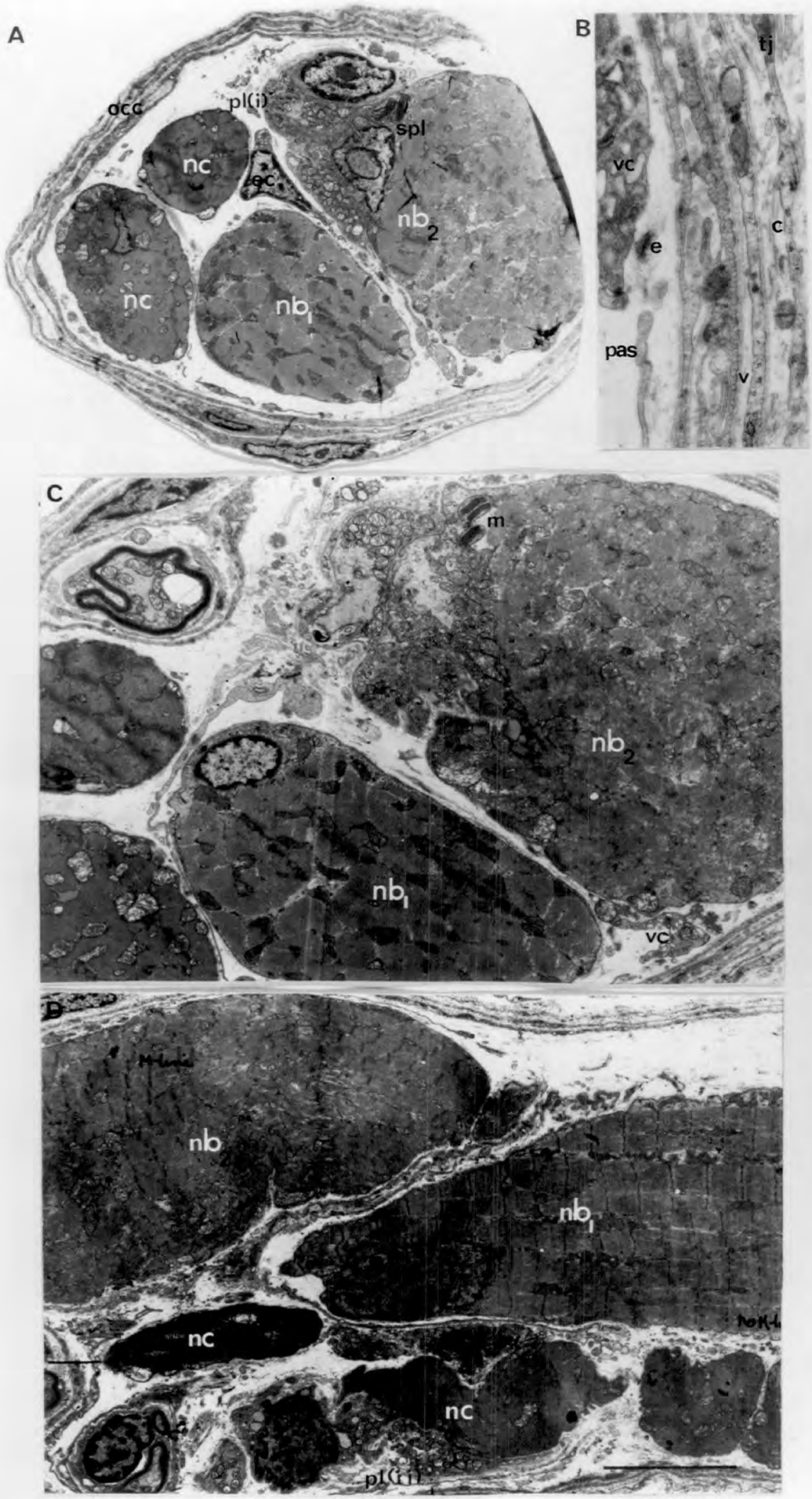

A
dcc
pl(i)
spl
nc
ec
nb2
nc
nb1
B
tj
vc
c
e
pas
v
C
m
nb2
nb1
vc
D
nb
nb1
nc
nc
pl(i)

FIGURE 6 ULTRASTRUCTURE OF NORMAL TYPE(i) FUSIMOTOR PLATE

A. TS. Arrowheads point to filaments of nucleic acid in nucleus.

× 32,000

B. Two large sole-plate mitochondria with crystallised cristae.

× 32,000

C. Large vesicular body in nerve terminal. Note trilamellate membrane.

× 32,000

Length of bar:

In A,B & C = 0.78 μm

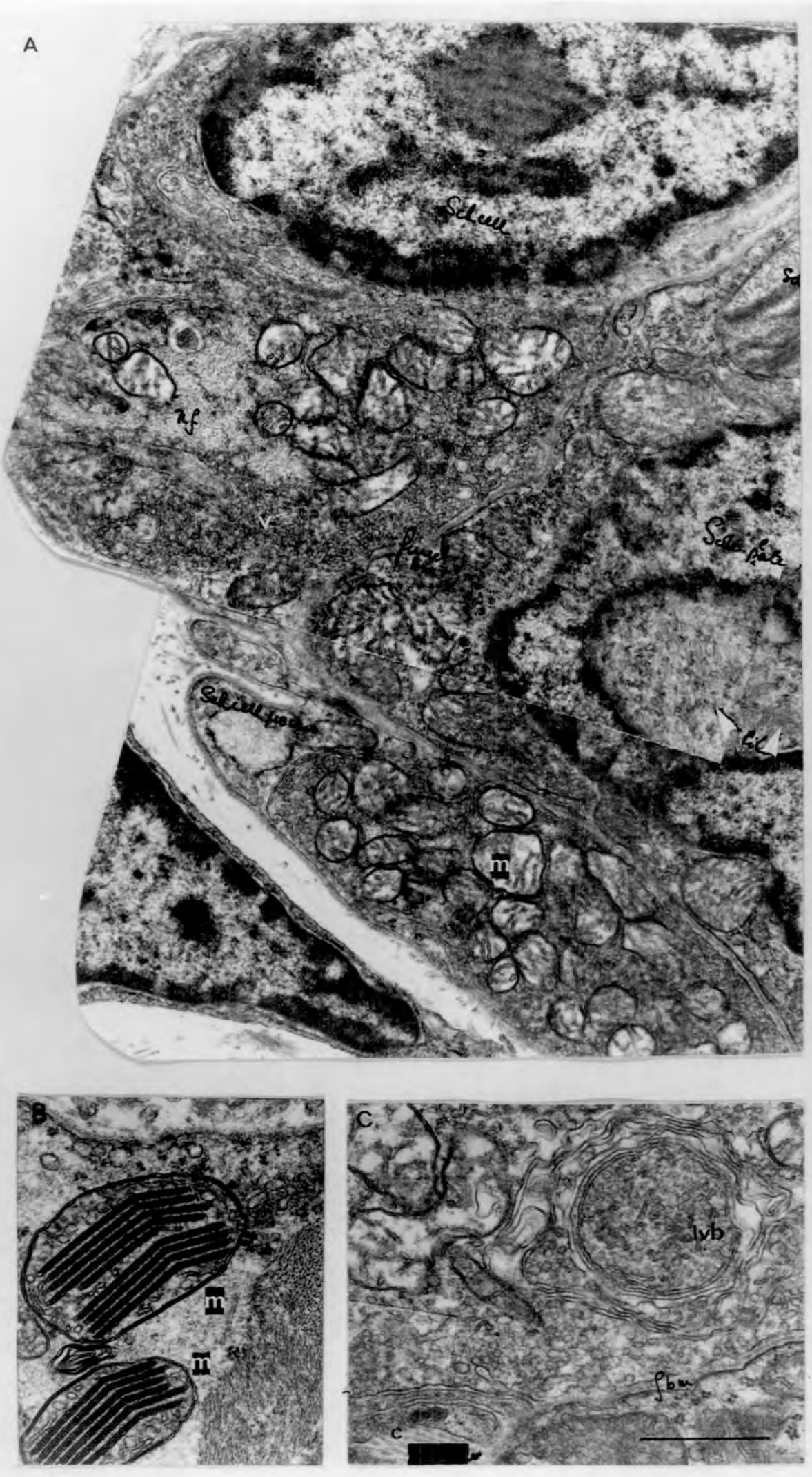

A
Sclm
nf
V
Sclm plate
m
B
m
m
C
lvb
fbm
c

FIGURE 7 ULTRASTRUCTURE OF NORMAL TYPE (ii) FUSIMOTOR PLATE

A. LS of a type (ii) plate on a chain fibre. Arrows point to short junctional folds. Note the absence the latter along a short length of the synapse depicted in this section.

x 12,600

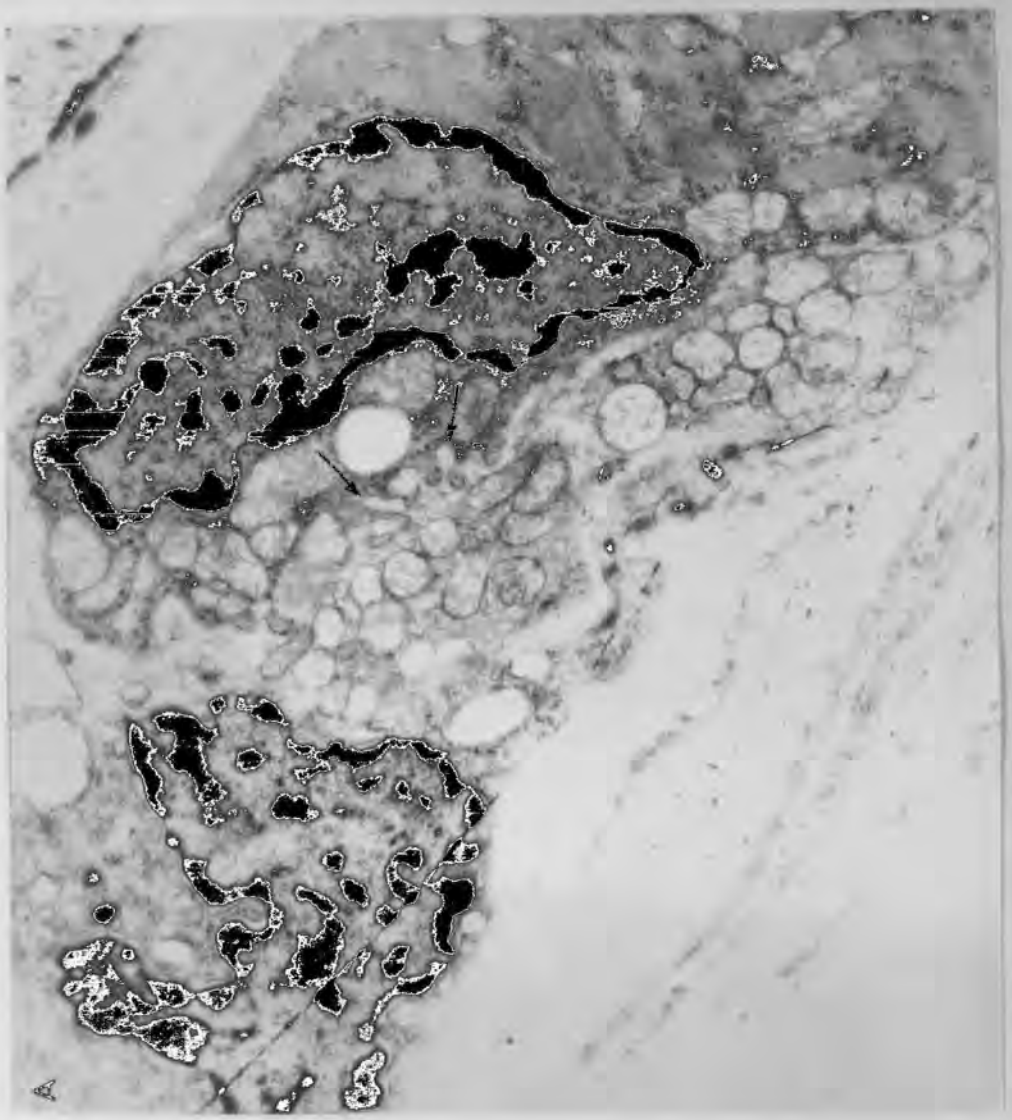
B. LS. Different section. Arrows point to short junctional folds lined with unfused basement membrane.

x 29,700

Length of bar:

In A = 2.00 μm

B = 0.83 "


A

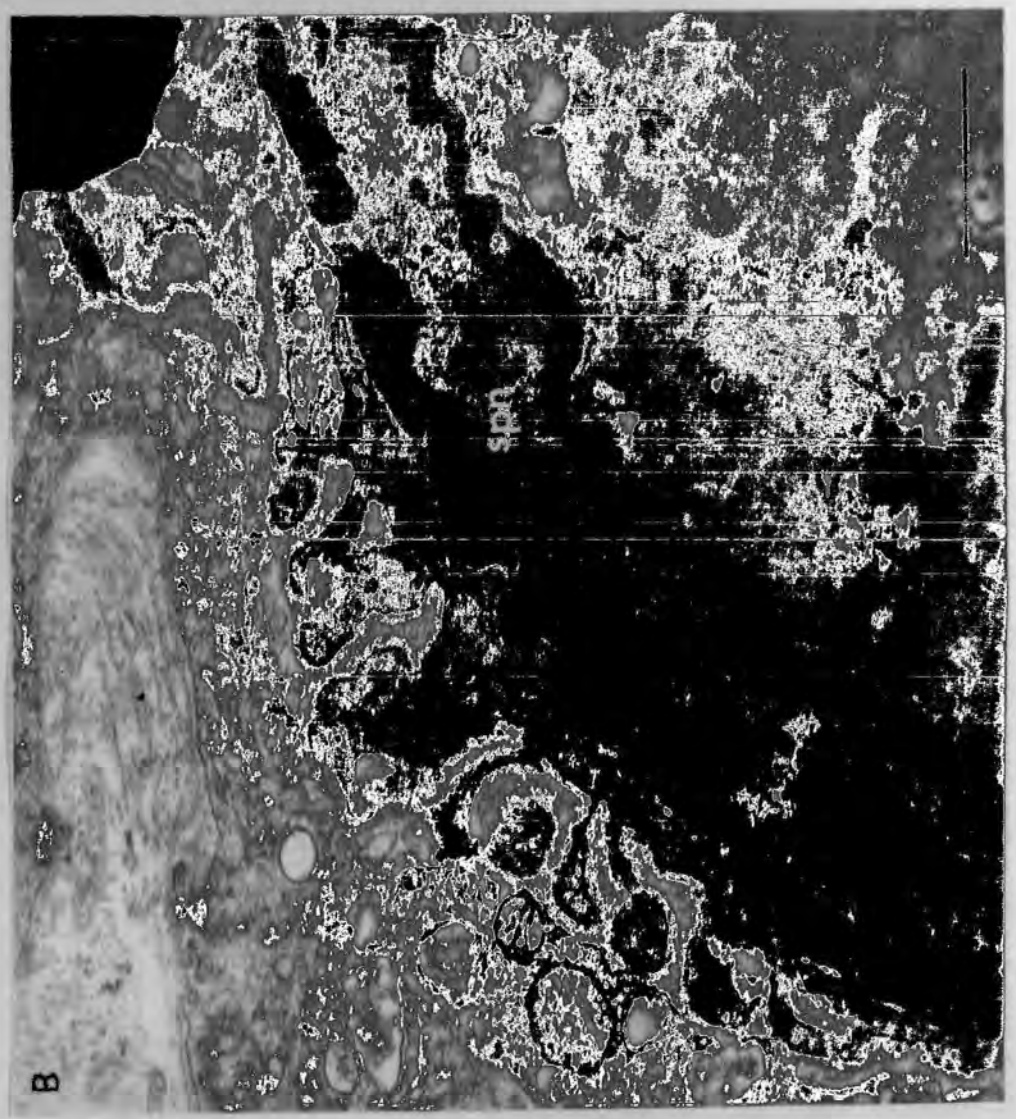

B
spn

FIGURE 8 ULTRASTRUCTURE OF NORMAL TYPE(ii) FUSIMOTOR PLATE

LS. Note numerous sole-plate mitochondria.

× 20,000

Length of bar:

= 1.25 μm

Granular
Sebaceous
Sebaceous
Sebaceous

FIGURE 9 M LINE CHARACTERISTICS OF THE THREE TYPES OF NORMAL ADULT INTRAFUSAL FIBRE IN REGION B

A. LS of bag_2 fibre with distinct M line

x 32,000

B. LS of bag_1 fibre with double Mline or no M line.

x 32,000

C. LS of chain fibre with distinct M line.

x 32,000

D. Topmost: LS of chain fibre, featuring a long mitochondrion spanning more than four sarcomeres. Arrow points to centriole in endomysial cell.

x 20,000

Length of bar:

In A, B & C = 0.78 µm

D = 1.25 "

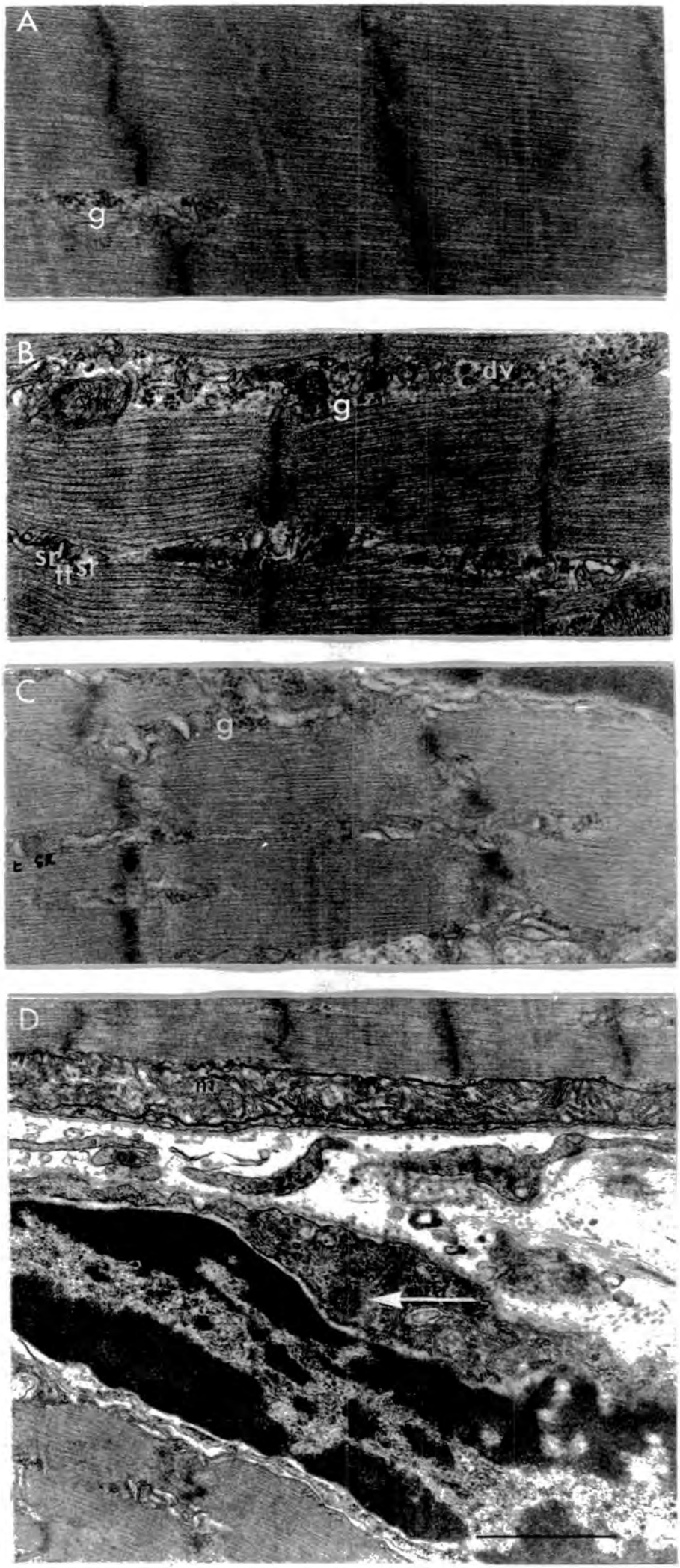

A
g
B
dy
g
sr
tt
st
C
g
t SR
D
m

FIGURE 10 ULTRASTRUCTURAL APPEARANCE OF NORMAL ADULT MUSCLE AFTER PROCESSING FROZEN 60 m SECTION FOR EM IN COMBINED HISTOCHEMICAL/EM EXPERIMENT

A. TS of normal muscle spindle in region B. 1 µm Epon section stained with toluidine blue. Pale markings in intrafusal and extrafusal muscle fibres are points where ice crystals formed during freezing.

x 1,250

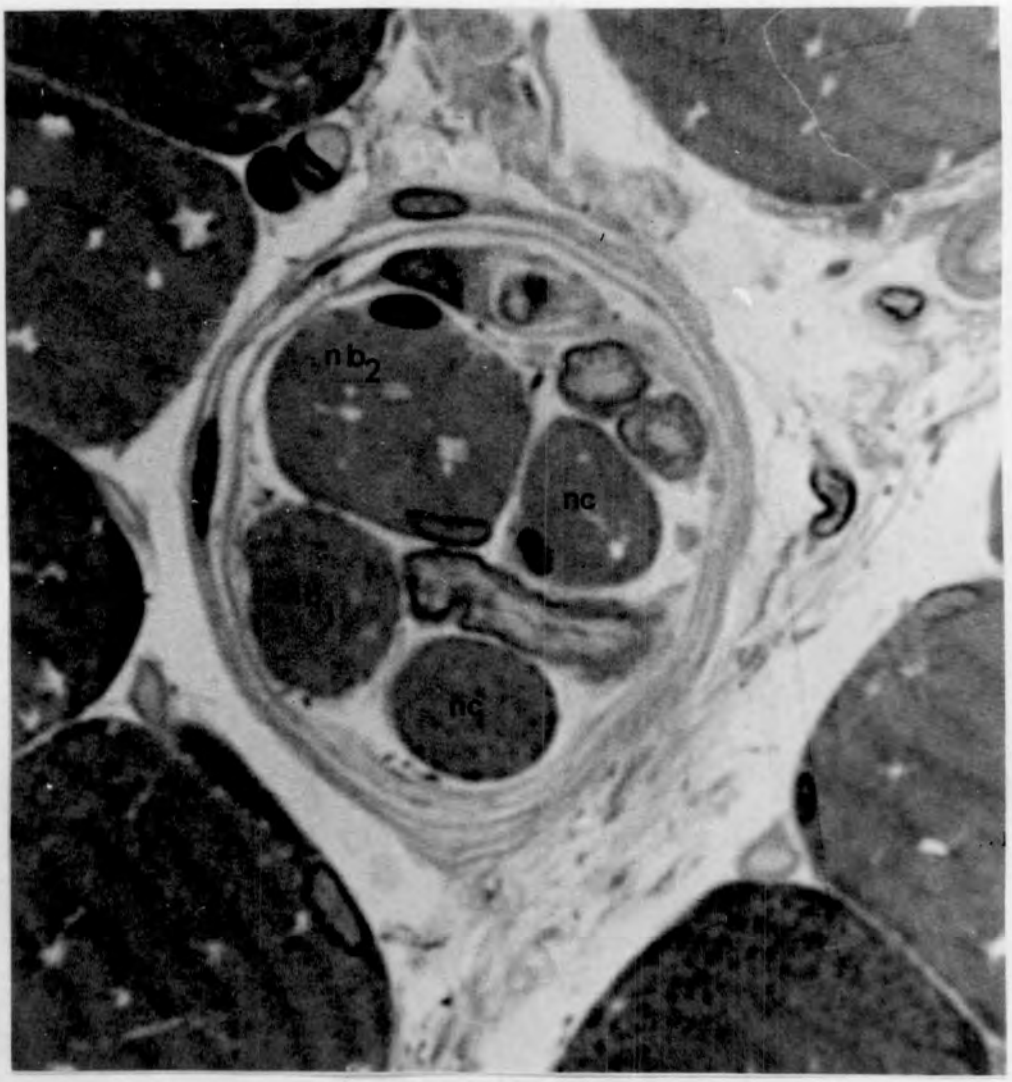
B. LS of bag_1 fibre. Areas of ice crystallisation seen as pale speckled areas (x).

x 12,600

Length of bar:

In A = 20.0 µm

B = 2.00 "


nb2
nc
nc

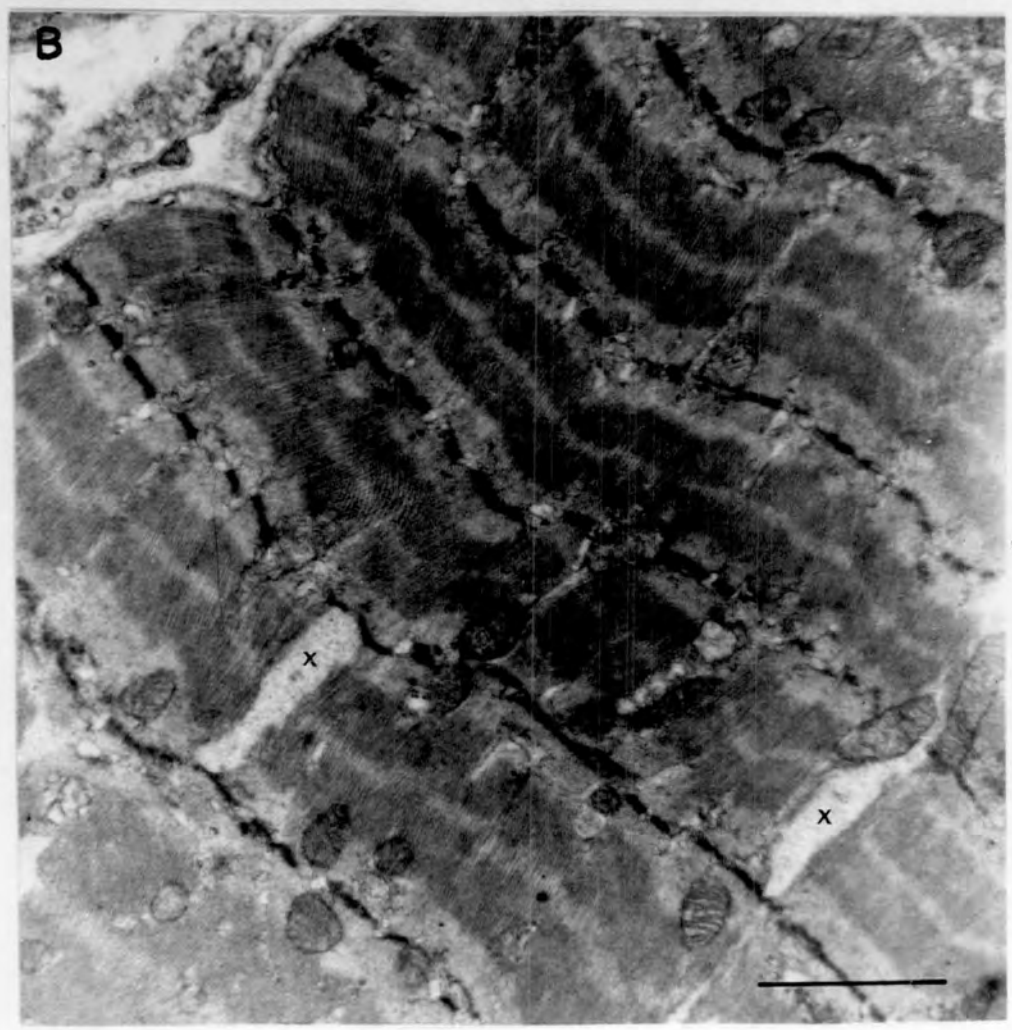

B
x
x

FIGURE 11 ULTRASTRUCTURE OF BAG_2 AND CHAIN FIBRES. PREPARATION FROM COMBINED HISTOCHEMICAL/EM EXPERIMENT

A. TS of bag_2 fibre.

× 12,600

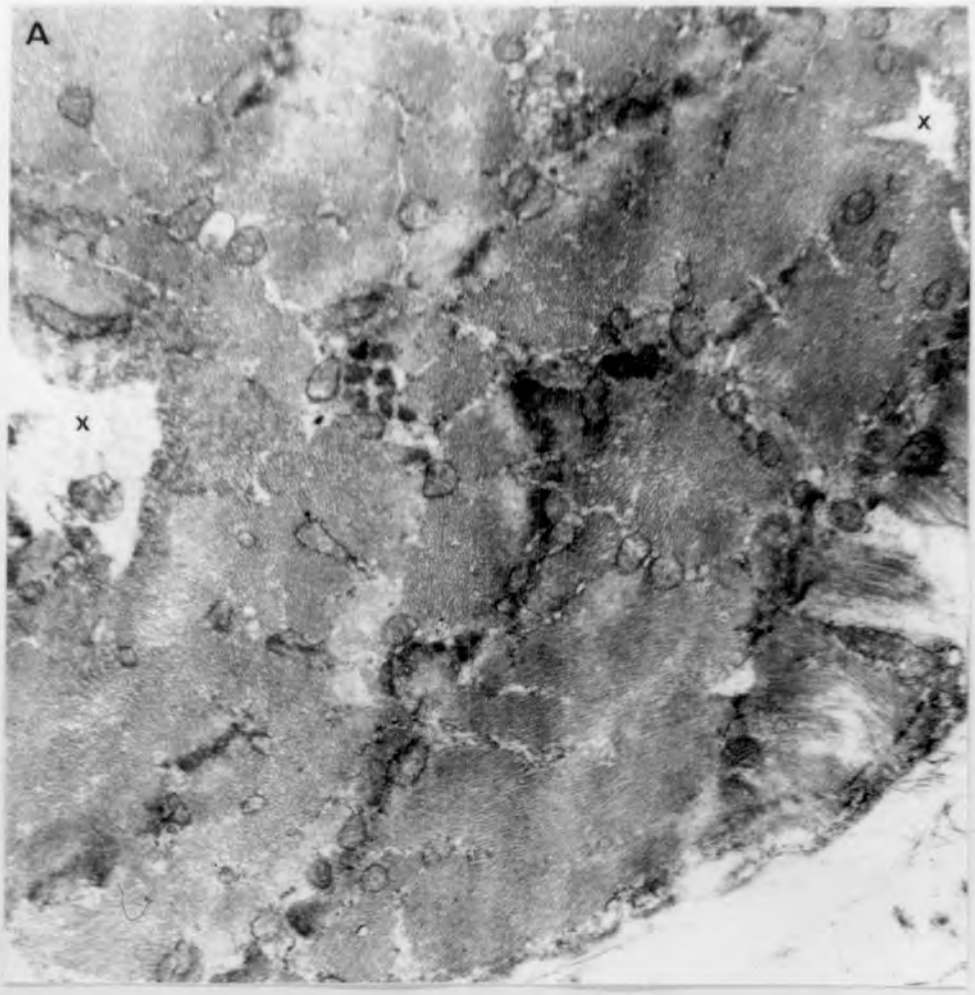
B. TS of chain fibre. Mitochondria and sarcotubular system quite well preserved. The abundant sarcotubular system at the I band level is indicated by arrowheads.

In both plates "x"s mark areas of ice crystallisation in frozen section.

× 12,600

Length of bar:

A & B = 2.00 μm


A
x
x


x
x

FIGURE 12 ULTRASTRUCTURE OF SENSORY ENDINGS OF A NORMAL ADULT SPINDLE

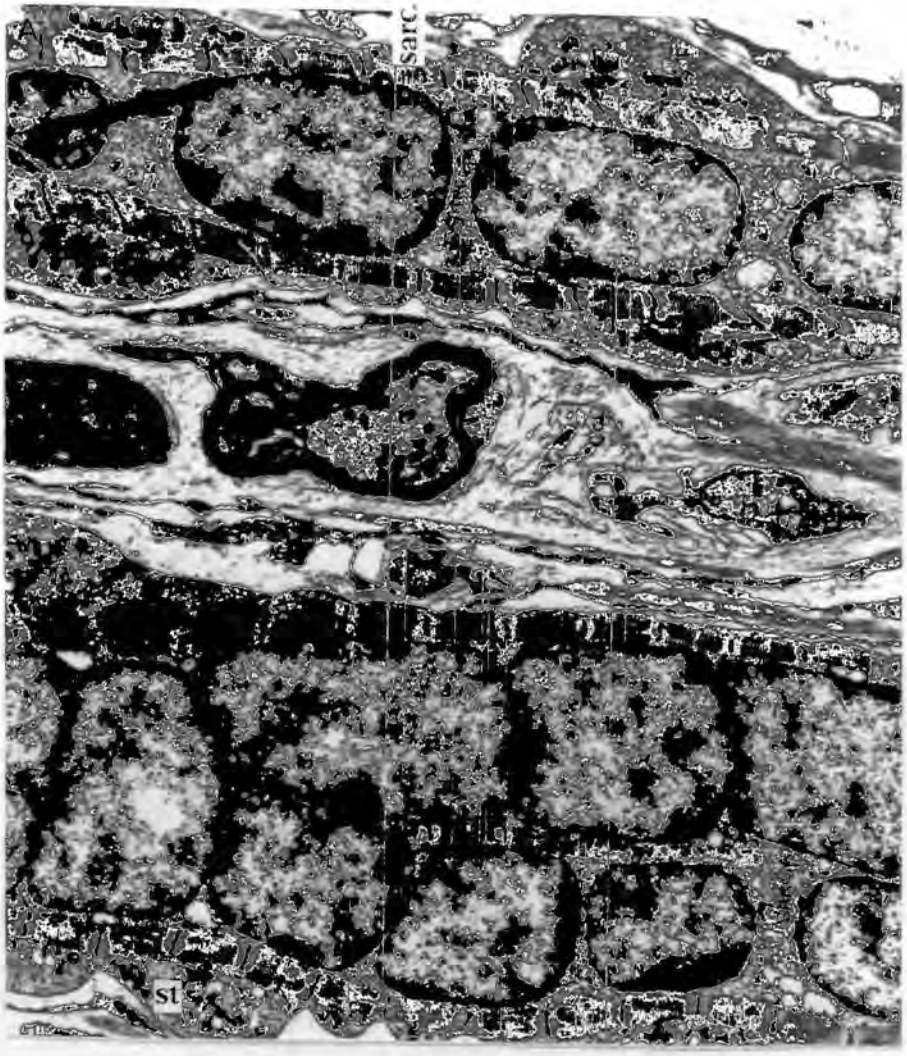
A. LS through a bag fibre (with double Z line) and a chain fibre (with M line) at mid-equator. sarc. = sarcomere. × 5,000

B. High-power LS of a sensory terminal on a bag fibre. × 32,000

Length of bar:

In A = 5.0 μm

" B = 0.79 "


A
sarc.
st

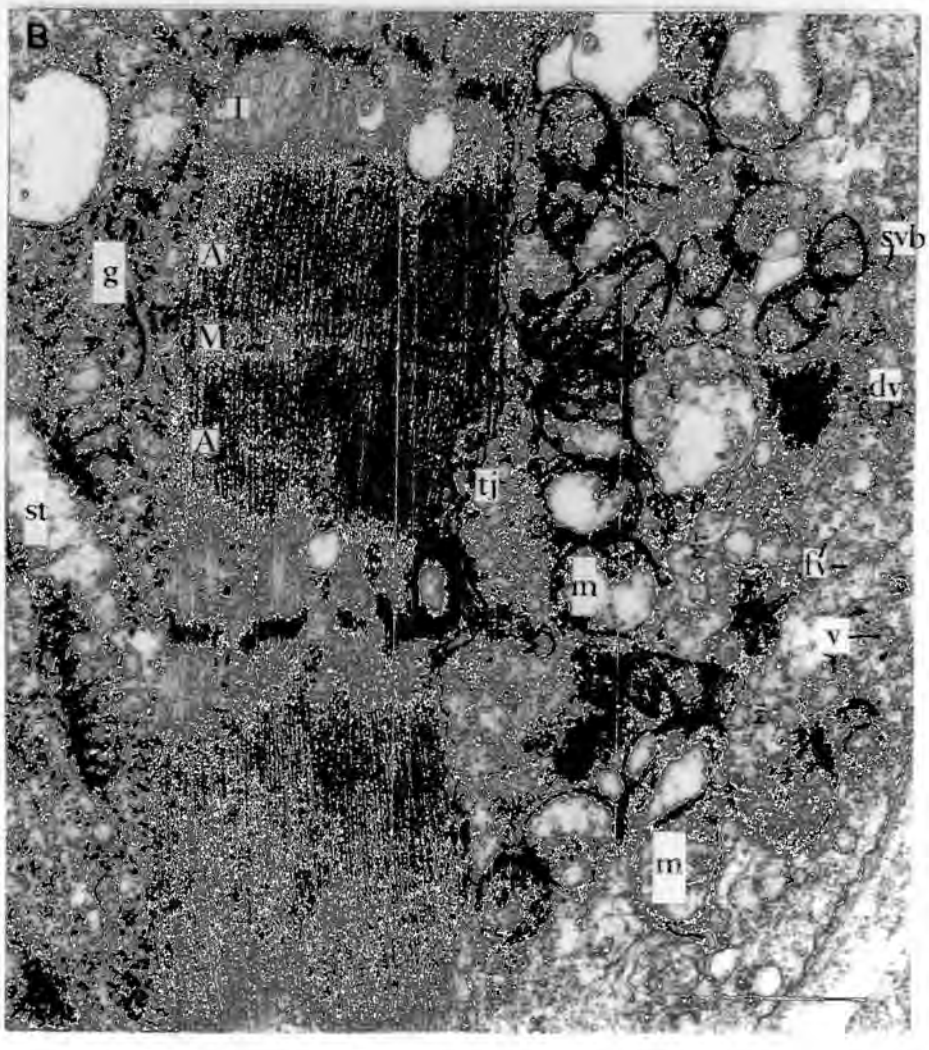

B
I
A
M
A
g
st
tj
svb
dv
m
v
m

FIGURE 13 SINGLE-FIBRE MODEL-ADULT SPINDLE. REGION A (MYO-TUBULAR LEVEL)

A. TS. White arrowhead points to thickened sarcolemma.

x 12,600

B. TS of capsule cells. Arrow head points to vesicle in process of pinocytosis. Basement lines both surfaces of each cell.

Compare with fig. 5B.

x 17,000

Length of bar:

In A = 2.00 μm

B = 1.41 "

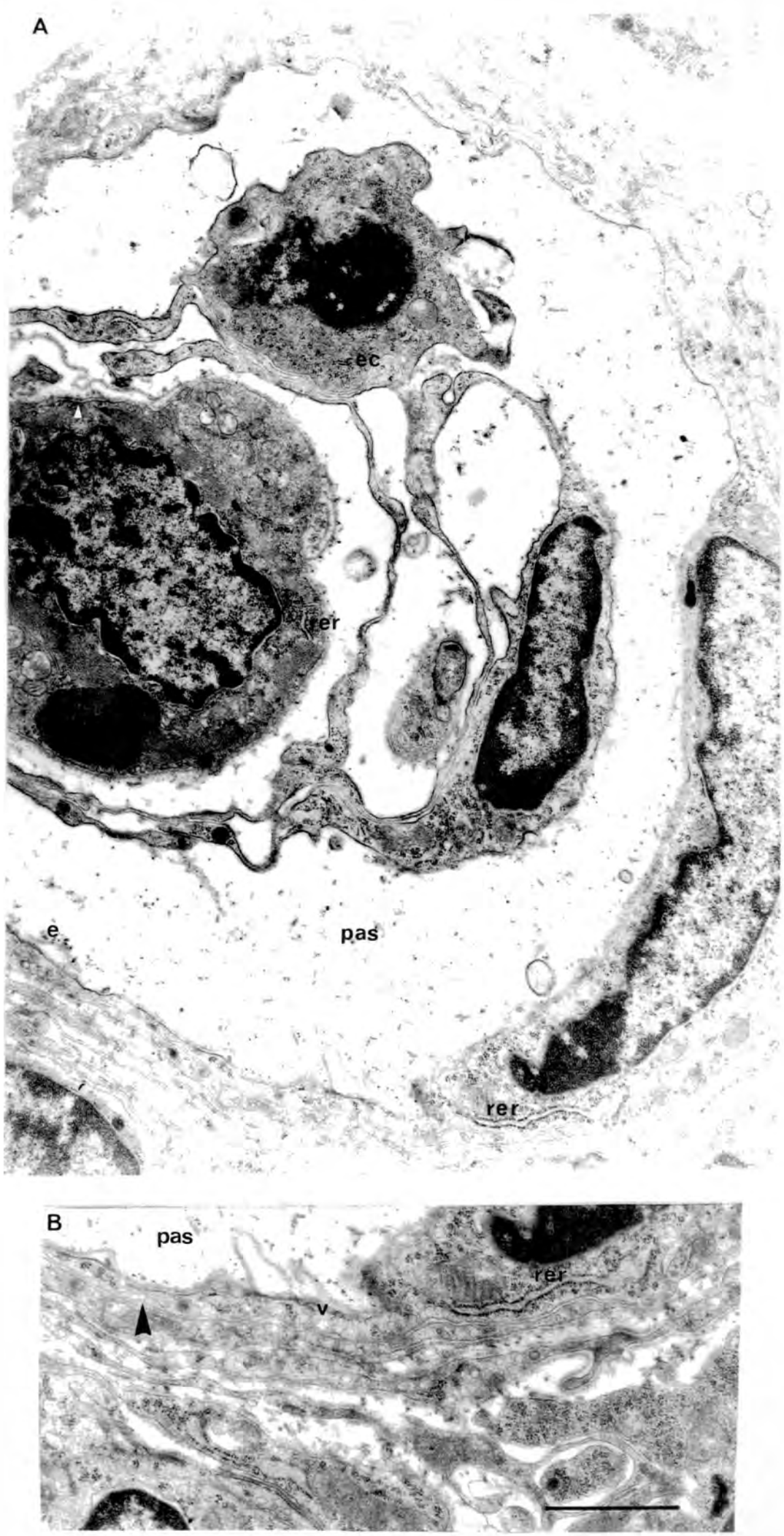

A
ec
rer
pas
e
rer
B
pas
rer
v

FIGURE 14 ULTRASTRUCTURE OF THE BAG FIBRE IN SINGLE-FIBRE MODEL-ADULT SPINDLE. REGION A (MYOTUBE LEVEL)

A. Enlargement of fig. 13A

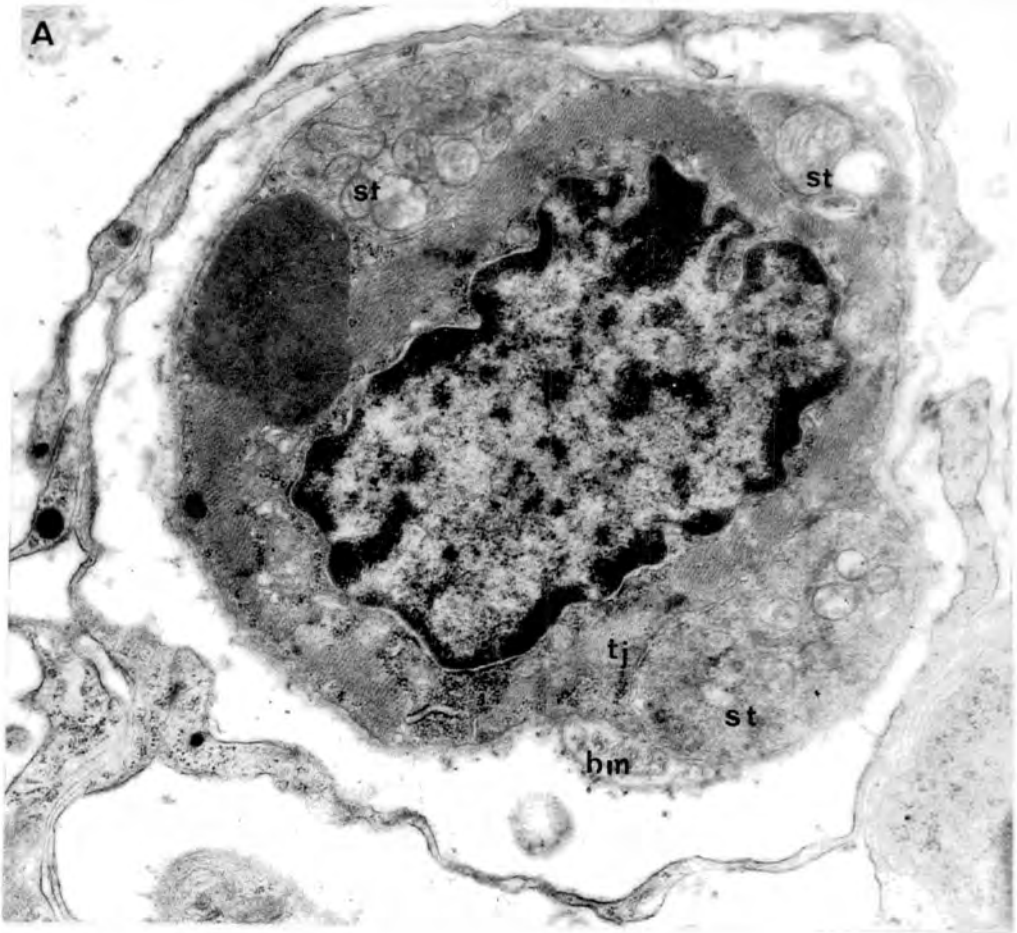
× 12,600

B. TS of fibre in different section, showing multivesicular body among capsule cells. Note the more numerous, dilated t-tubules at the periphery of the fibre.

× 12,600

Length of bar:

= 2.00 µm


A
st
st
tj
st
bm

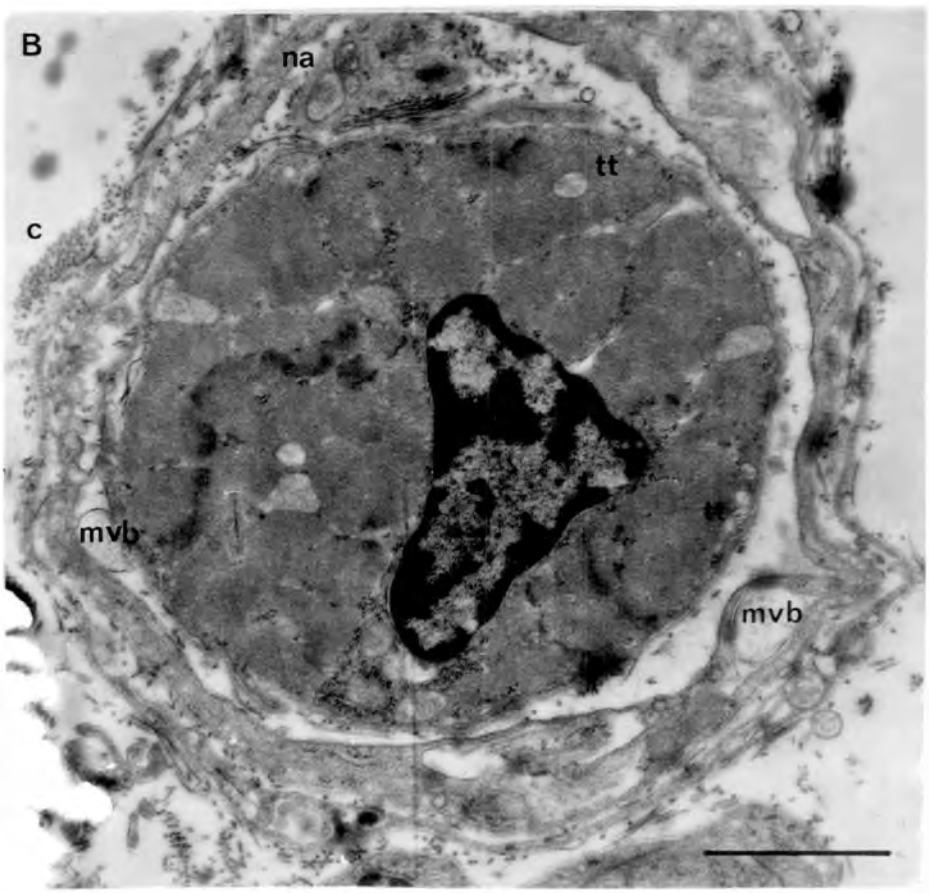

B
na
tt
c
mvb
mvb

FIGURE 15 ULTRASTRUCTURE OF THE BAG FIBRE IN A SINGLE-FIBRE MODEL-ADULT SPINDLE. REGION A (MYOTUBE LEVEL)

A. TS of fibre, showing extensive, convoluted basement membrane between endomysial cells. Arrowhead points to dense-core vesicle in sensory terminal.

× 12,600

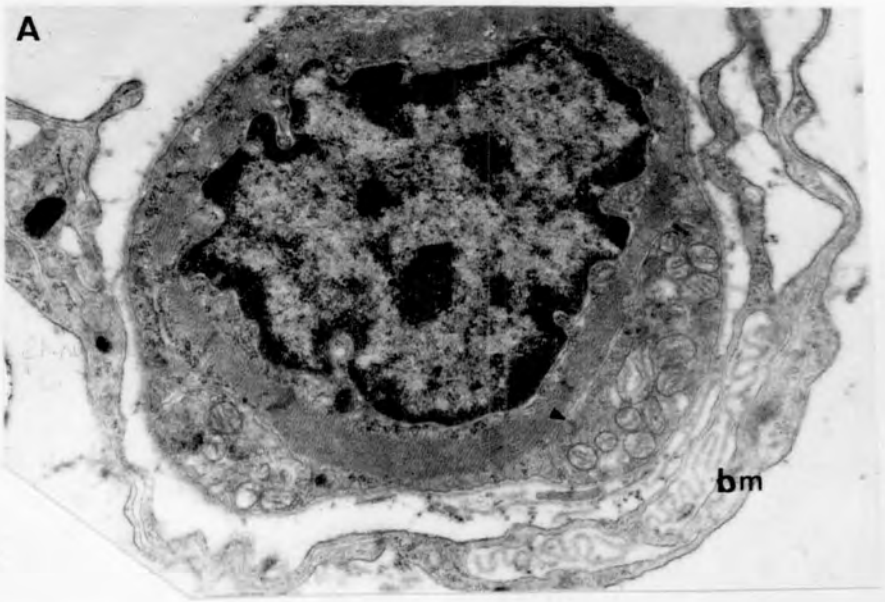
B. TS of fibre, showing various organelles. Arrowhead points to Z bands.

× 17,000

Length of bar:

In A = 2.00 μm

B = 1.41 "


A
bm

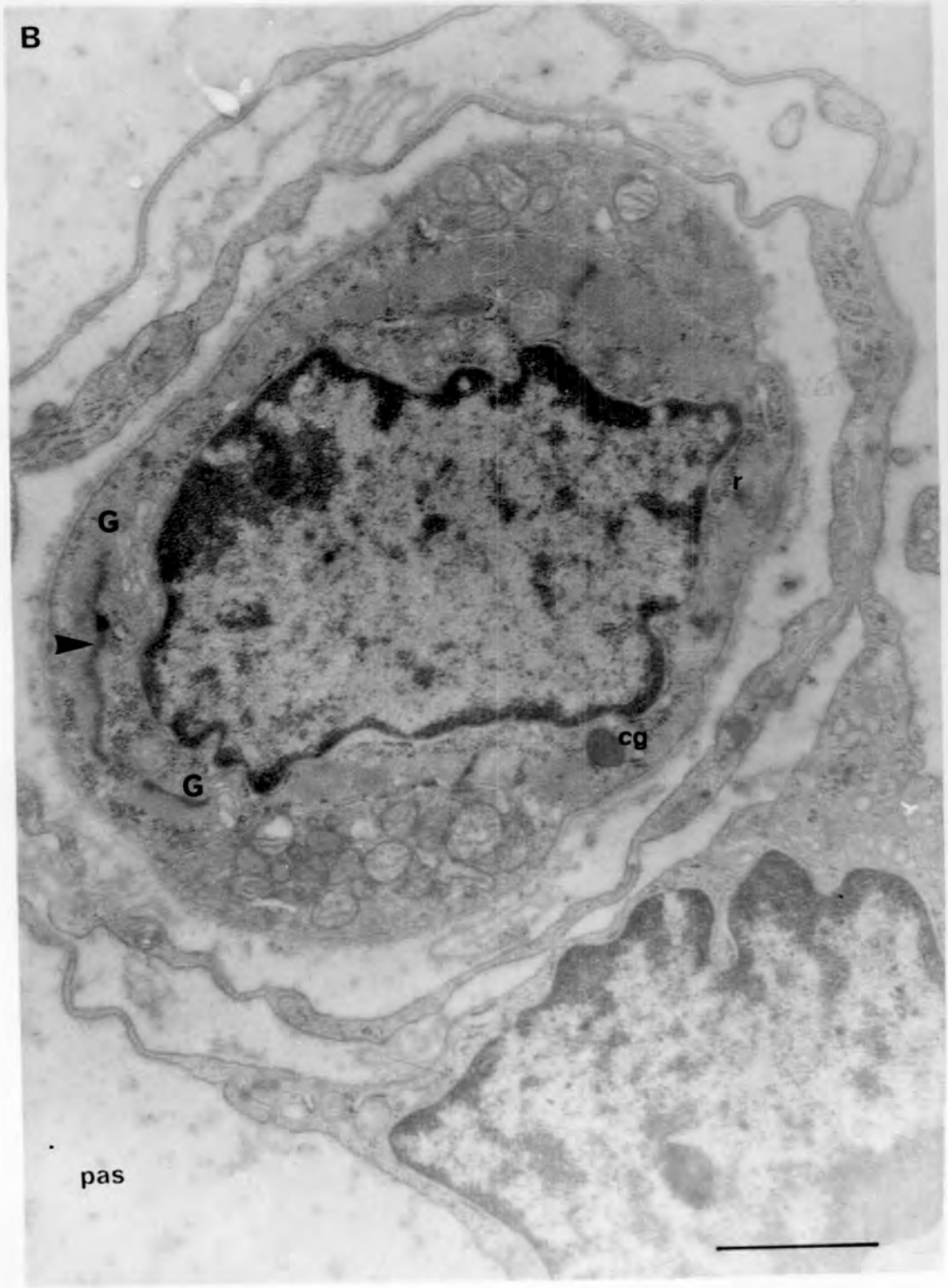

B
r
G
cg
G
pas

FIGURE 16 SARCOMERE BANDING PATTERN OF A SINGLE-FIBRE MODEL- ADULT SPINDLE. REGION B to C.

A. LS. Note " loose " cristae of mitochondria and compare those of normal chain fibres in the same region (fig. 5B).

x 5,200

B. LS of different section.

x 5,200

C. LS of different section. Note t-tubules ("tt") without any association with SR.

x 17,000

Arrows in B and C point to dark line in H zone. Because of sarcomere blurring, the identity of the M line - either dM or M - is not possible.

Length of bar:

In A & B = 4.81 µm

C = 1.41 "

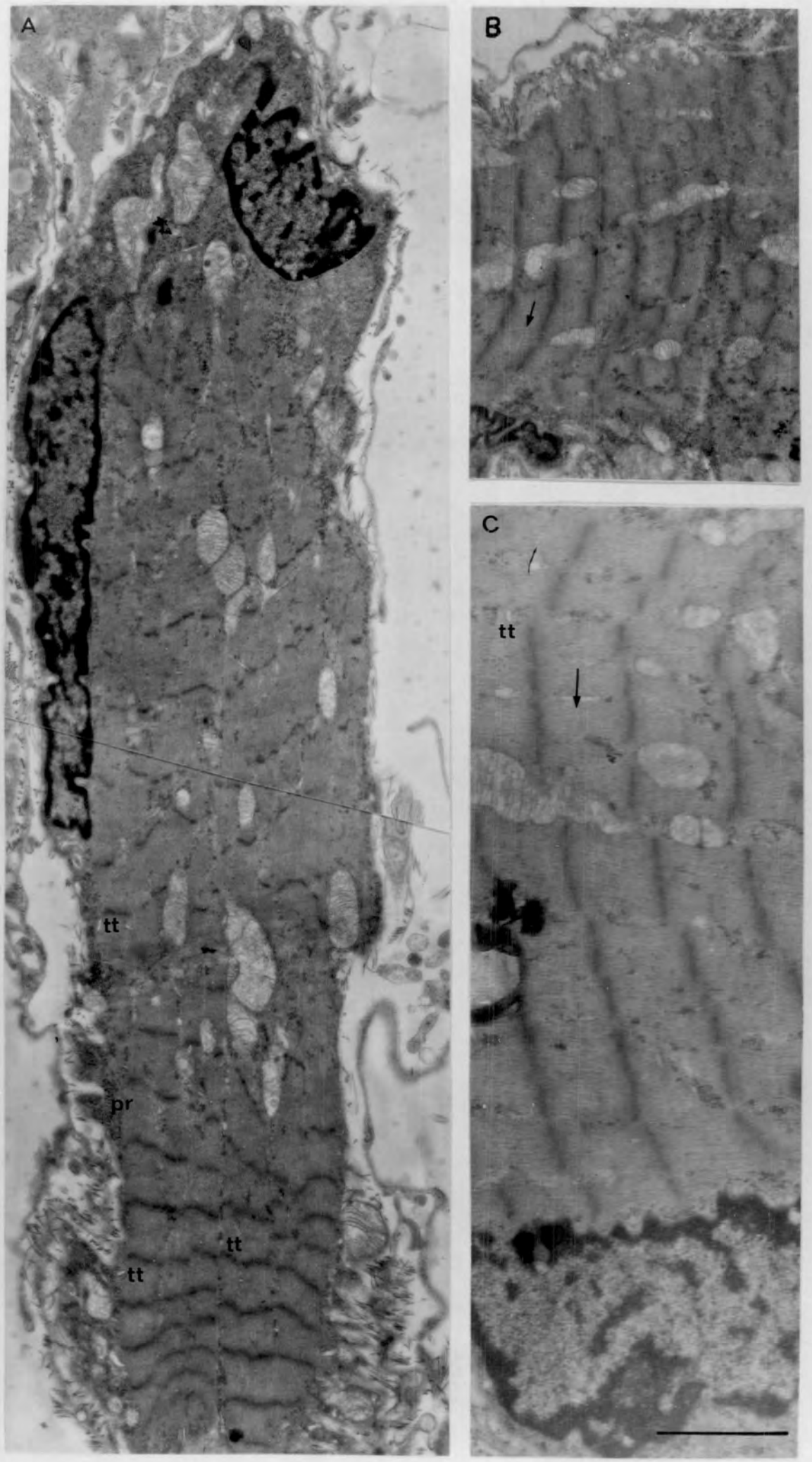

A
B
C
tt
pr
tt
tt
tt

FIGURE 1.7 SINGLE-FIBRE MODEL-ADULT SPINDLE. REGION A (MID-EQUATORIAL LEVEL)

A. TS through mid-equator. Note nuclear bag and sensory terminals.

x 2,000

B. TS through myotube region, internuclear section. Note central accumulations of cytoplasmic organelles in fibre. Arrowheads point to axons of spindle nerve entering capsule.

x 2,000

C. TS through myotube region, section through nucleus. Arrowheads point to process of satellite cell. These cells are more usually found in the polar regions.

x 5,000

Length of bar:

In A & B = 12.5 μm

C = 5.00 "

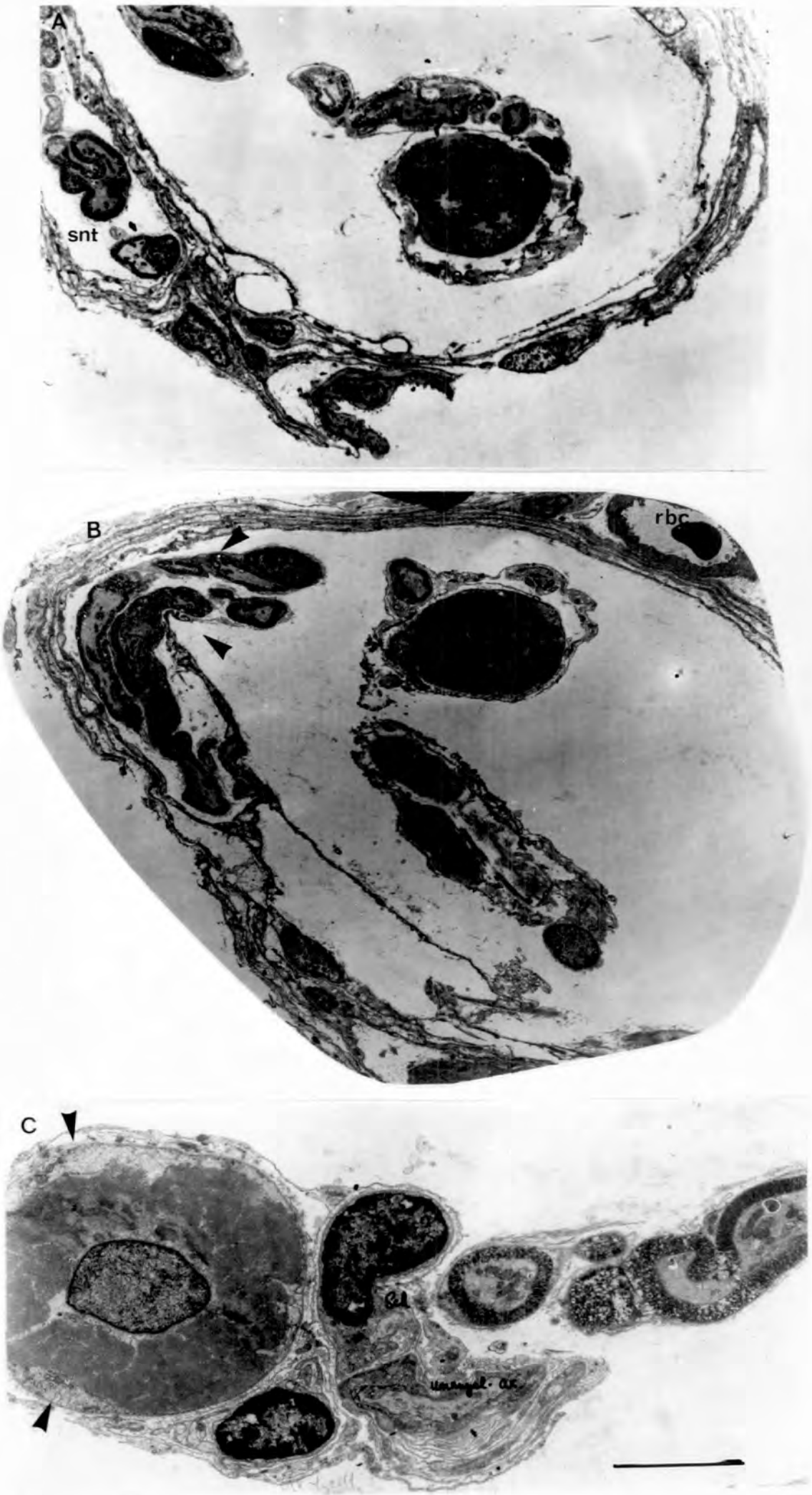

A
snt
B
rbc
C

FIGURE 18 ULTRASTRUCTURE OF SINGLE-FIBRE MODEL-ADULT SPINDLE. REGION A (MYOTUBE LEVEL).

A. Oblique section, depicting central-core cytoplasm containing numerous mitochondria. Double M line present at this level. Arrow indicates small sensory terminal. Note lack of mitochondria, a feature of some of the smaller sensory endings in both model-adult and normal adult spindles.

× 8,000

B. TS through myotube nucleus. Note sensory ending similar to the one in A (arrow).

× 8,000

C. Higher power TS, showing sensory terminal (with the more usual prevalence of mitochondria) closely abutting satellite cell (arrow). Note absence of outer basement membrane; and cleft in muscle fibre (arrowhead) as if in the initial stages of splitting. However, this spindle was sectioned from pole to pole and the fibre was never seen to split.

× 12,600

Length of bar:

In A & B = 3.13 µm

C = 2.00 "

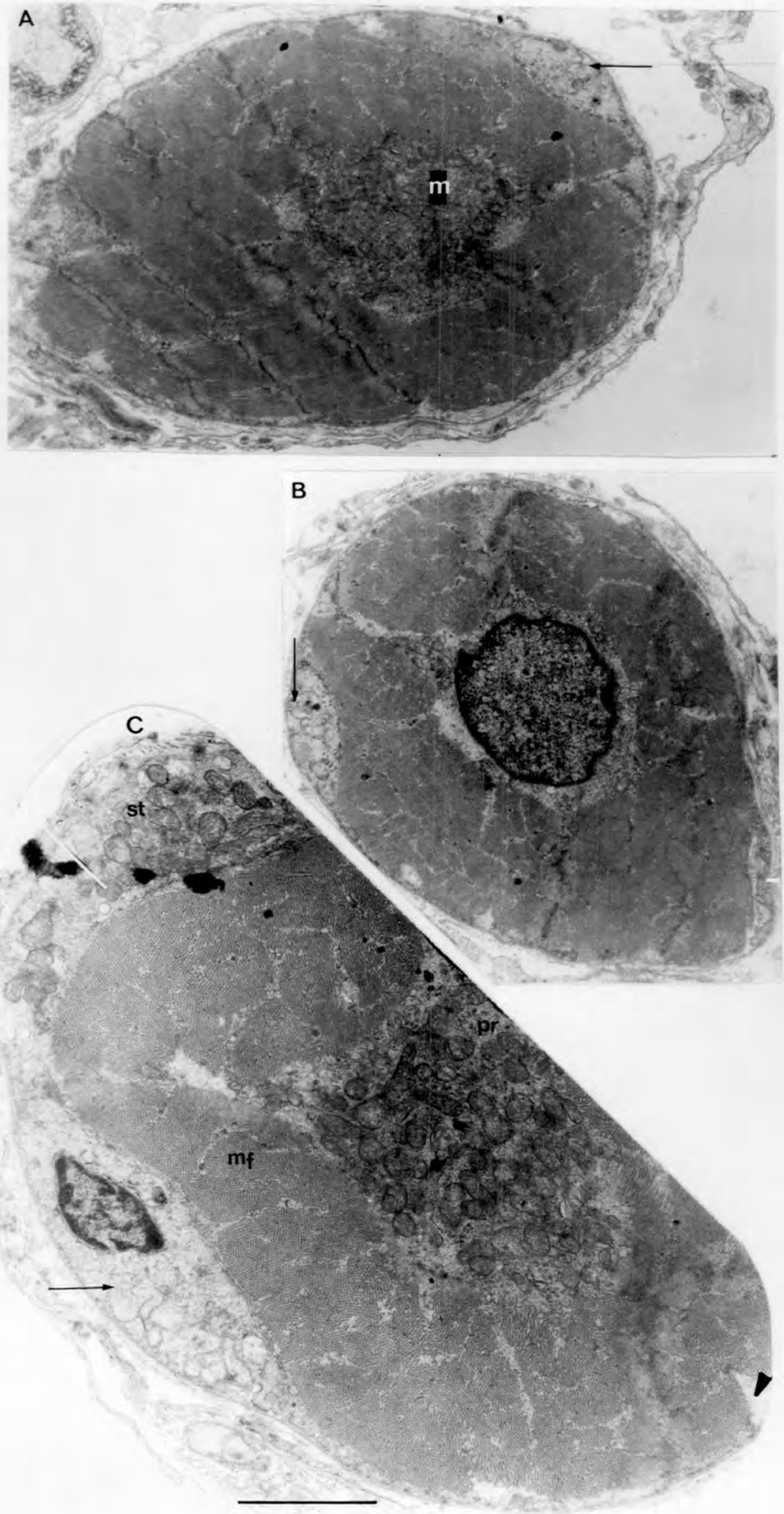

A
m
B
C
st
pr
mf

FIGURE 19 ULTRASTRUCTURE OF SENSORY ENDINGS IN A SINGLE-FIBRE MODEL-ADULT SPINDLE

A. Oblique section shows normal spiral configuration of sensory (probably primary) ending.

× 3,200

B. TS at nuclear bag level. Headed arrow points to specialisation of synaptic membranes along an outpushing of the muscle fibre into the sensory ending. Non-headed arrow points to a pinocytotic vesicle at periphery of sensory terminal.

× 8,000

C. Similar section to B, showing crenulated axolemma and double basement membrane surrounding both sensory terminal and intrafusal fibre. Arrowheads indicate pinocytotic vesicles at periphery of sensory terminal. Arrow indicates a length of abnormally wide synaptic gap.

× 8,000

Length of bar:

In A = 7.81 μm

B & C = 3.13 "

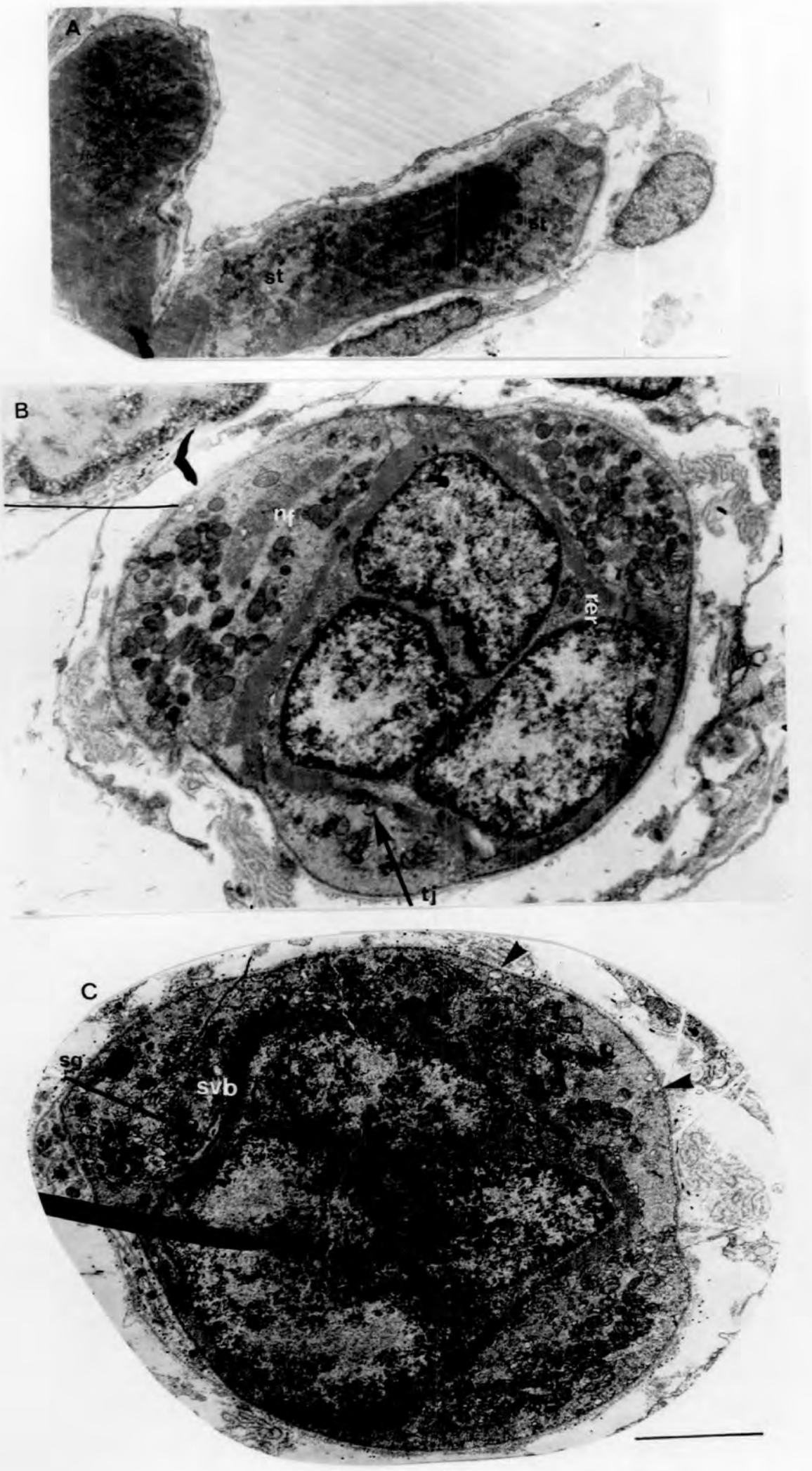

A
st
st
st
B
nf
rer
tj
C
sg
svb

FIGURE 20 ULTRASTRUCTURE OF SENSORY ENDINGS OF ABNORMAL " TORN OFF " APPEARANCE IN A SINGLE-FIBRE MODEL- ADULT SPINDLE

A. TS of two such round, abnormal terminals adjacent to two normally apposed terminals.

x 5,980

B. TS of abnormally apposed terminals seen to be part of normally apposed terminal. As in fig. C, a mvb is located at junction of neuromuscular " split ". Note collagen in wide " synaptic gap " in both A and B. White arrowhead indicates inner basement membrane (ibm) of muscle fibre being shared with the abnormal sensory terminal but not the outer basement membrane (obm). Black arrowhead points to vesicle undergoing pino- cytosis.

x 8,000

C. TS shows nerve ending and muscle fibre with separate inner basement membranes (arrowheads) but common outer basement membrane.

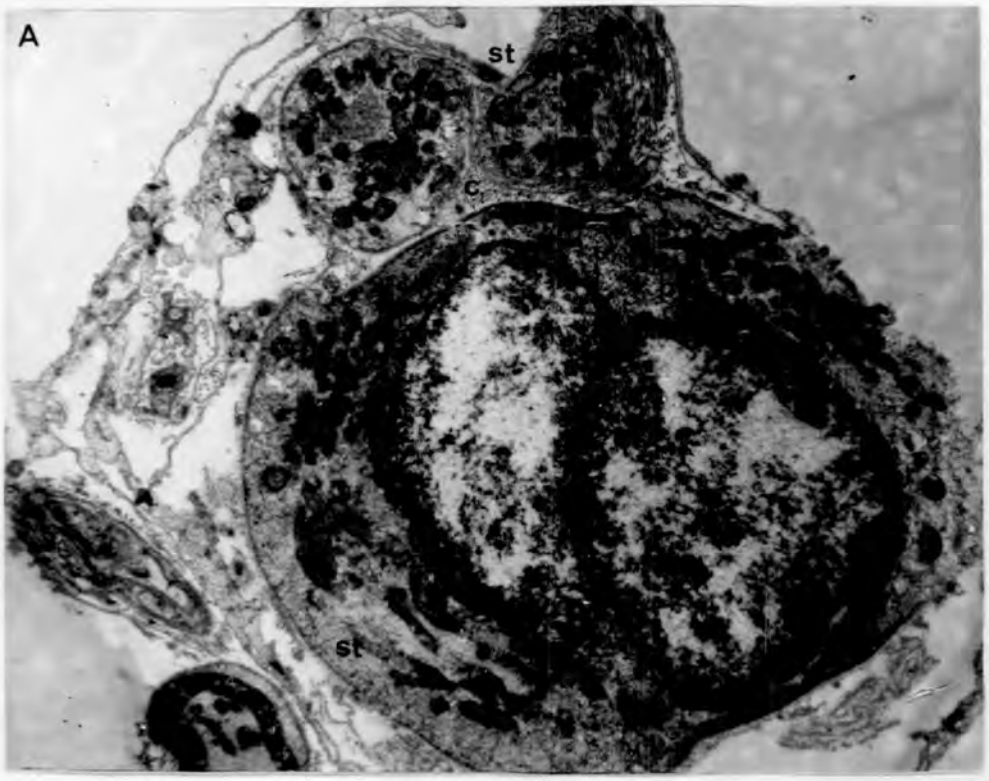
x 9,660

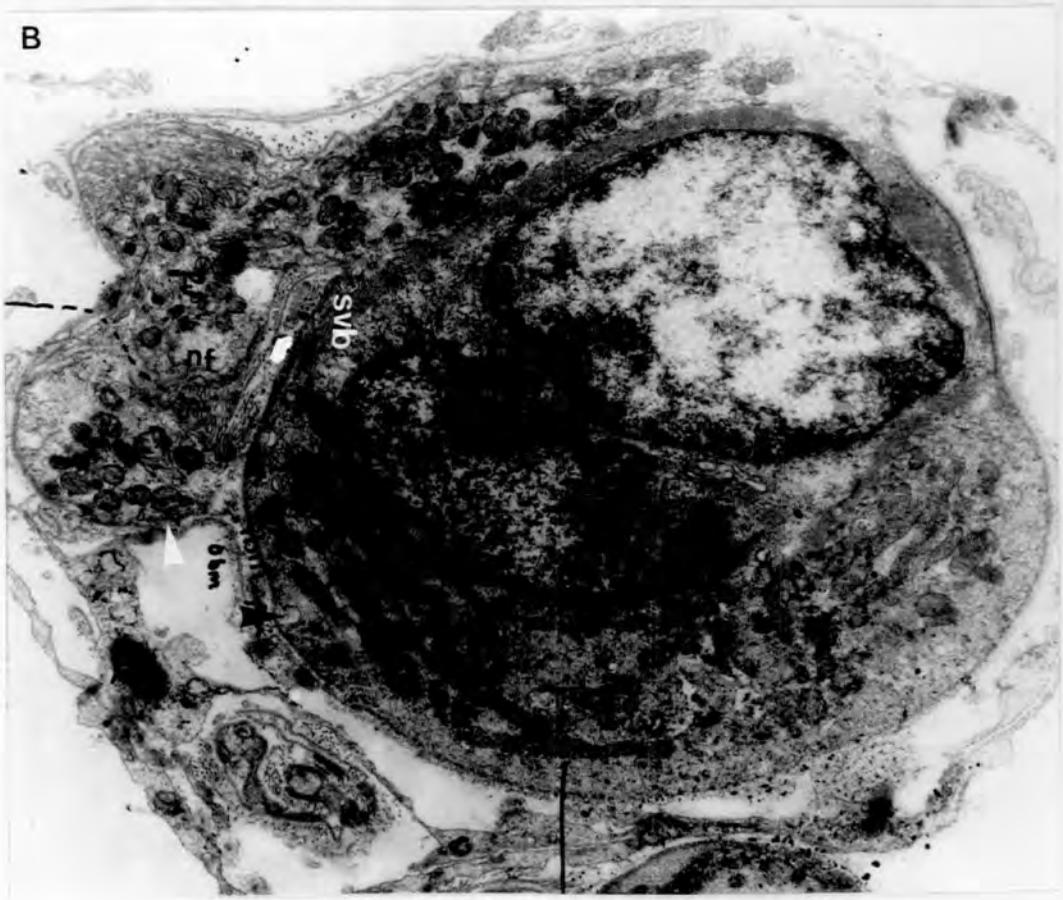
Length of bar:

In A = 4.67 µm

B = 3.13 "

C = 2.54 "


A
st
c
st


B
svb
nf
bm

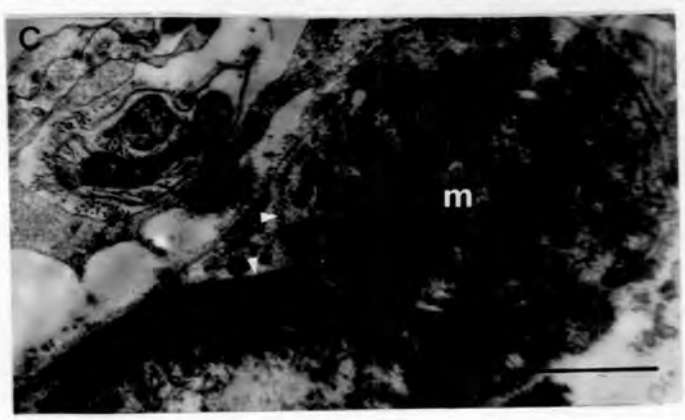

C
m

FIGURE 21 SARCOMERE BANDING PATTERN OF SINGLE-FIBRE MODEL-ADULT SPINDLE

A. LS of region A.

x 8,000

B. LS of region B. Arrowheads indicate double M line. Note diad (i.e.,"tt" and adjacent cisternum of SR to the left).

x 32,000

C. LS of region B. From frozen section in the combined histochemical/EM experiment.

x 32,000

Length of bar:

In A = 3.13 μm

B & C = 0.78 "

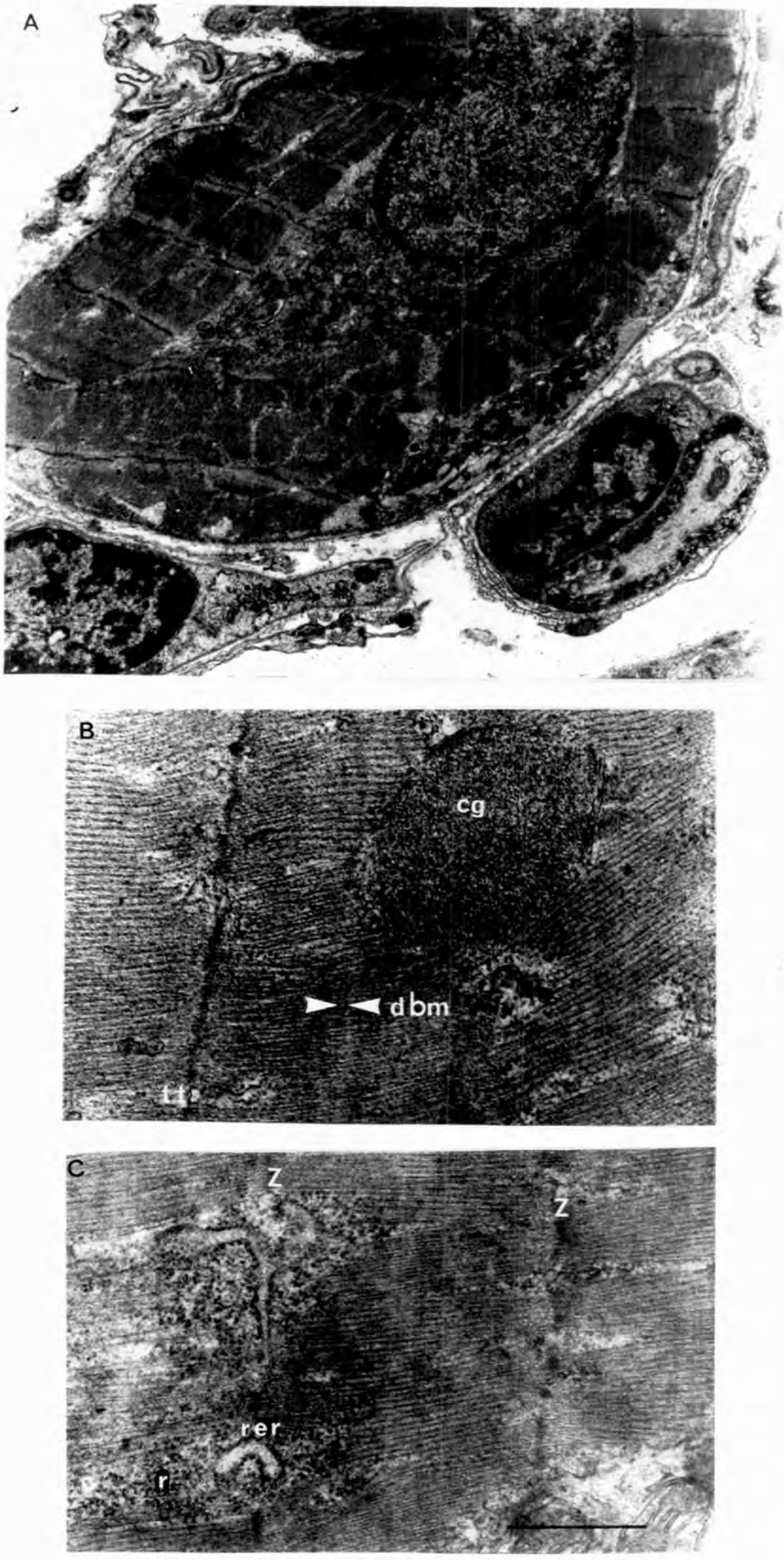

A
B
cg
dbm
tt
C
Z
Z
rer
r

FIGURE 22 ORGANELLES IN MODEL-ADULT SENSORY ENDINGS (NORMALLY APPOSED)

A. TS showing tightly packed, round mitochondria. Note basement membrane (arrowheads) surrounding nerve ending and muscle fibre. Collagen fibres between the two basement membranes.

x 20,000

B. TS showing neurotubules, pale vesicles, coated vesicles and dense-core vesicles. Note specialisation of synaptic membranes (arrow).

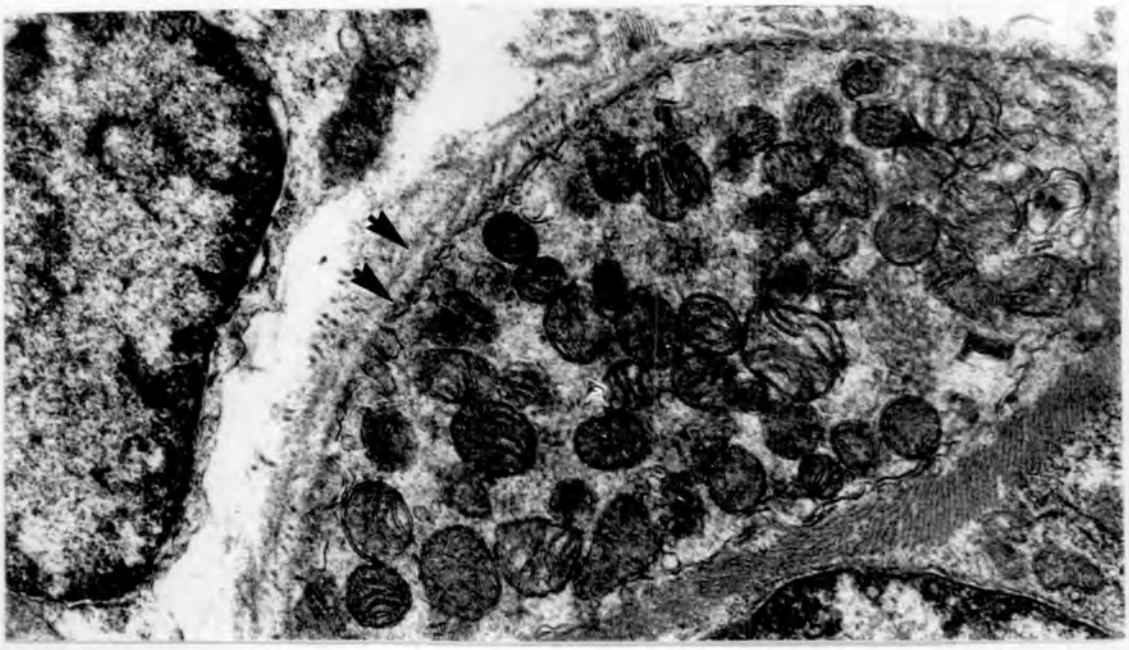
x 20,000

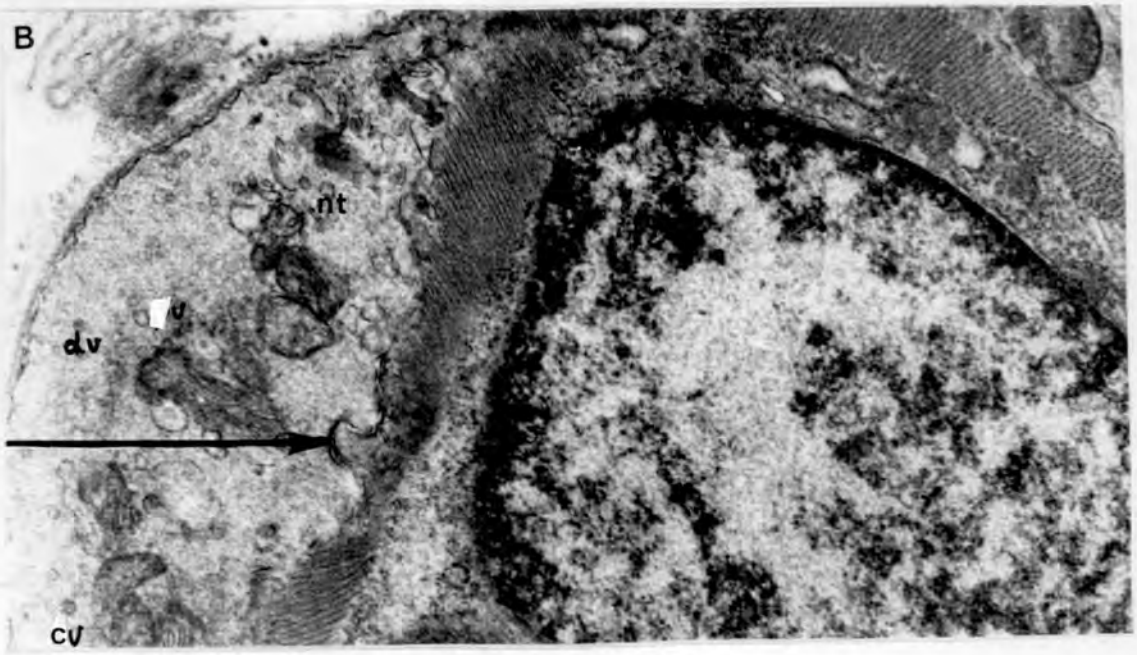
C. TS showing vesicular body in nerve ending

x 20,000

Length of bar:

A,B & C = 1.25 μm




B
nt
v
dv
cv

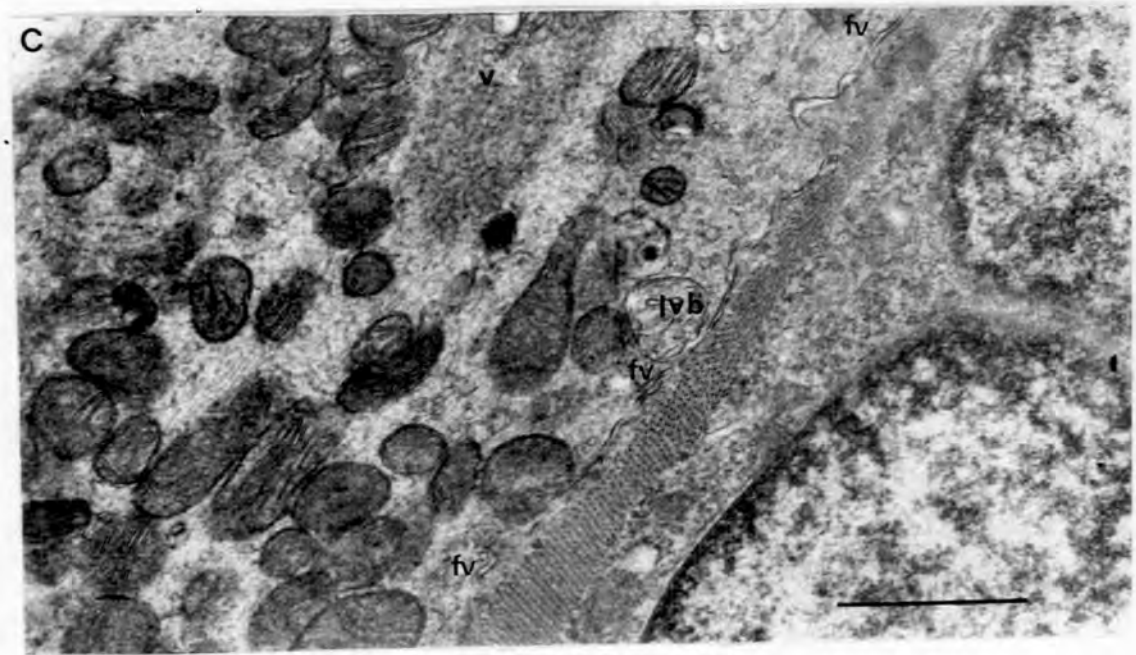

C
v
fv
lvb
fv
fv

FIGURE 23 ULTRASTRUCTURE OF SENSORY TERMINALS OF SINGLE-FIBRE MODEL- ADULT SPINDLE

A. TS organelle content of endings continued from overpage. Arrow points to large vesicular body.

x 20,000

B. Oblique TS in juxta-equatorial region, showing myotube of nuclei.

x 2,000

Length of bar:

In A = 1.25 µm

B = 12.5 "

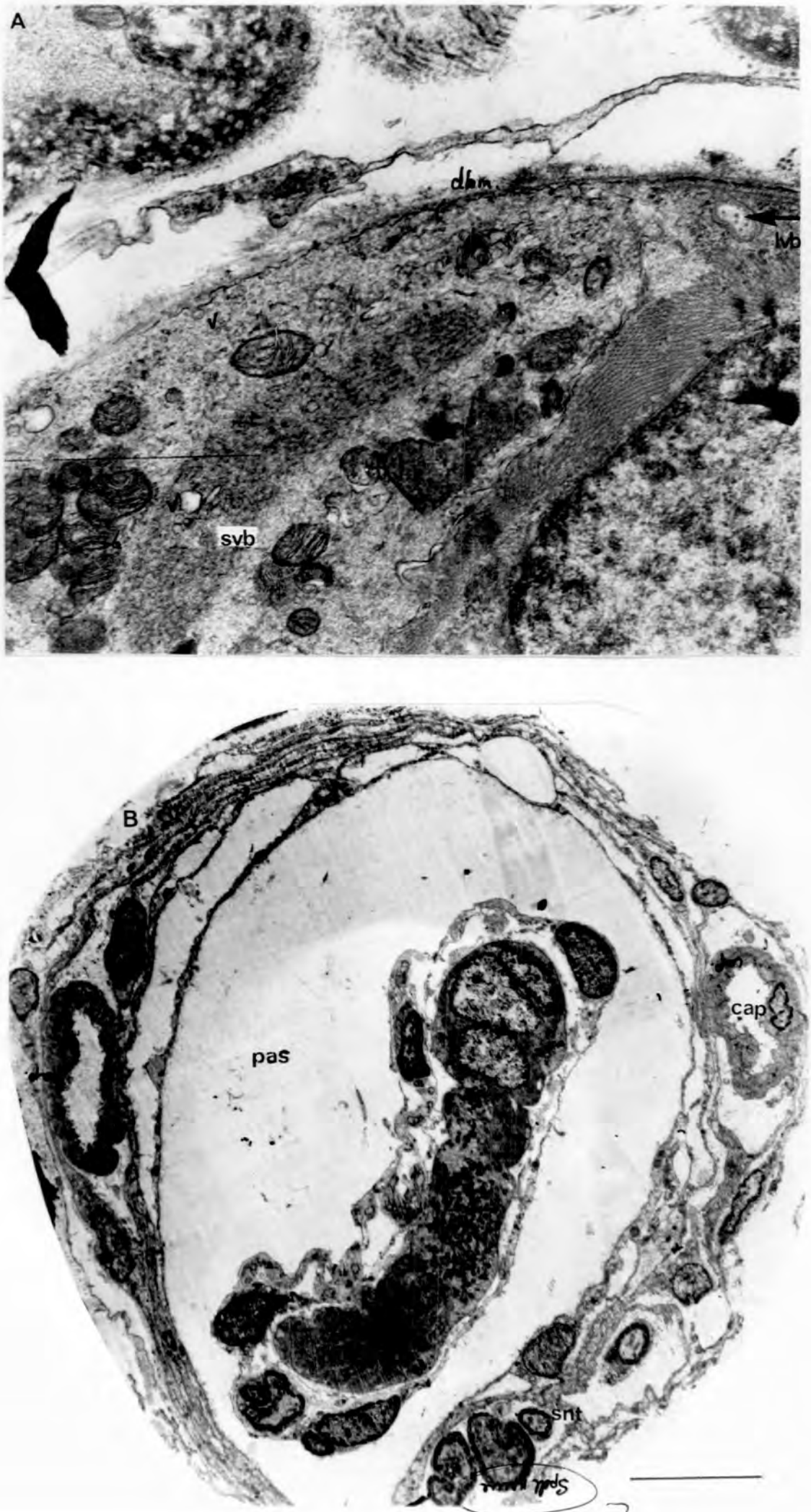

A
dbm
lvb
v
svb
B
pas
cap
snt

FIGURE 24 HIGH POWER OF FIGURE 238

Arrow points to unmyelinated axons in periaxial space.

× 5,000

Length of bar:

= 5.00 μm

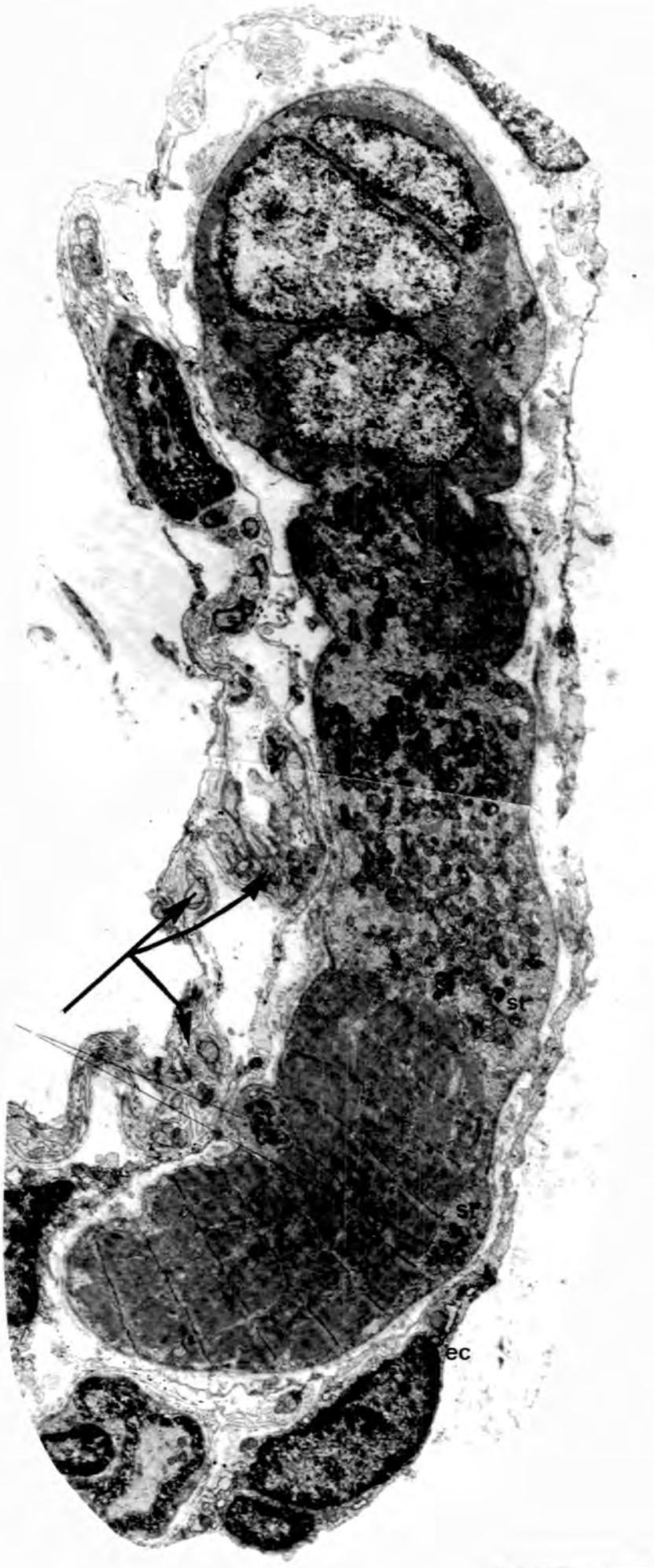

st
st
ec

FIGURE 25 ULTRASTRUCTURE OF A TWO-FIBRE, TANDEM, MODEL-ADULT SPINDLE SECTIONED IN THE COMBINED HISTOCHEMICAL/EM EXPERIMENT. PROXIMAL CAPSULE.

A. TS of " appendage " fibre (asterisked) apposed to bag_1 fibre. Note common basement membrane and sensory cross-terminal. " Appendage " fibre sectioned for first time in equatorial region. Larger bag fibre (bag_2) also shown. x's indicate artifacts caused by ice crystals. The odd appearance of the myelin of the spindle axons is also a consequence of the combined technique.

x 3,200

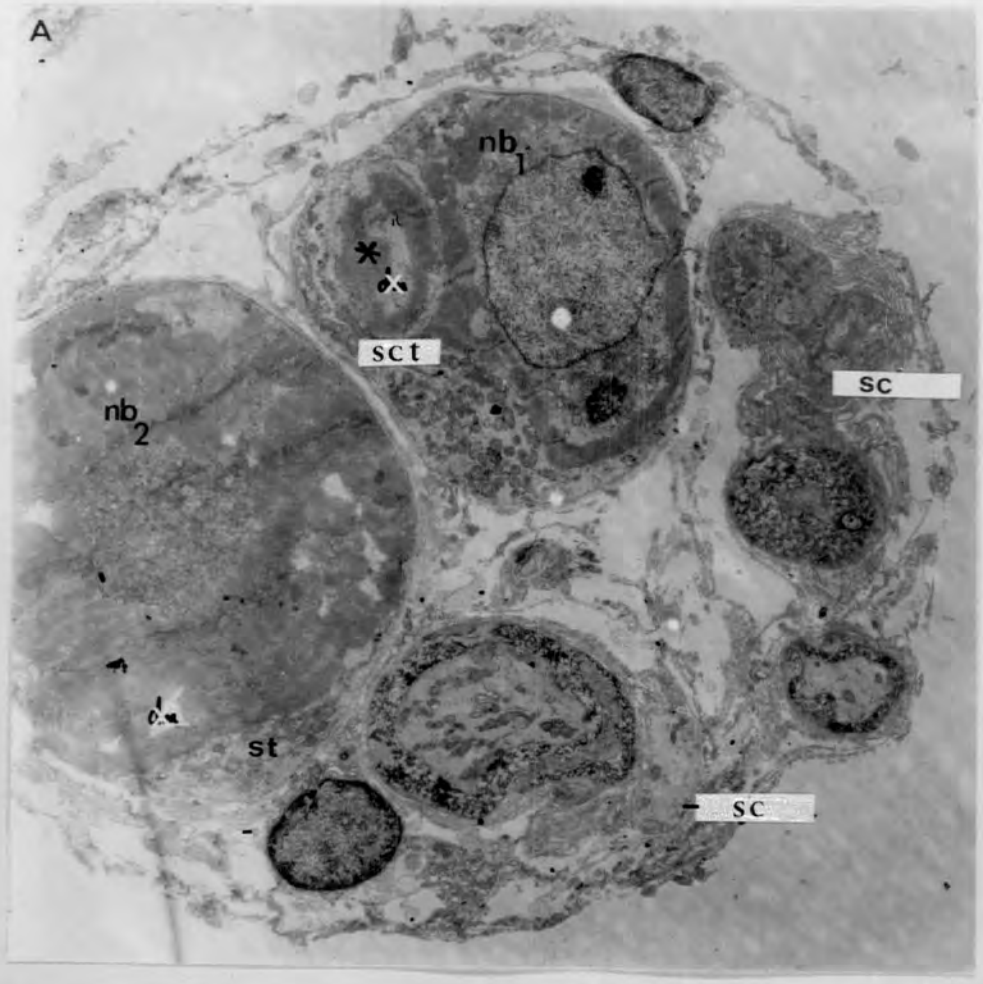
B. High power TS of bag_1 fibre and appendage fibre. Arrowhead points to thickening of sarcolemma. ibm = inner basement membrane.

x 8,000

Length of bar:

In A = 7.81 µm

B = 3.13 "


A
nb1
sct
sc
nb2
st
sc

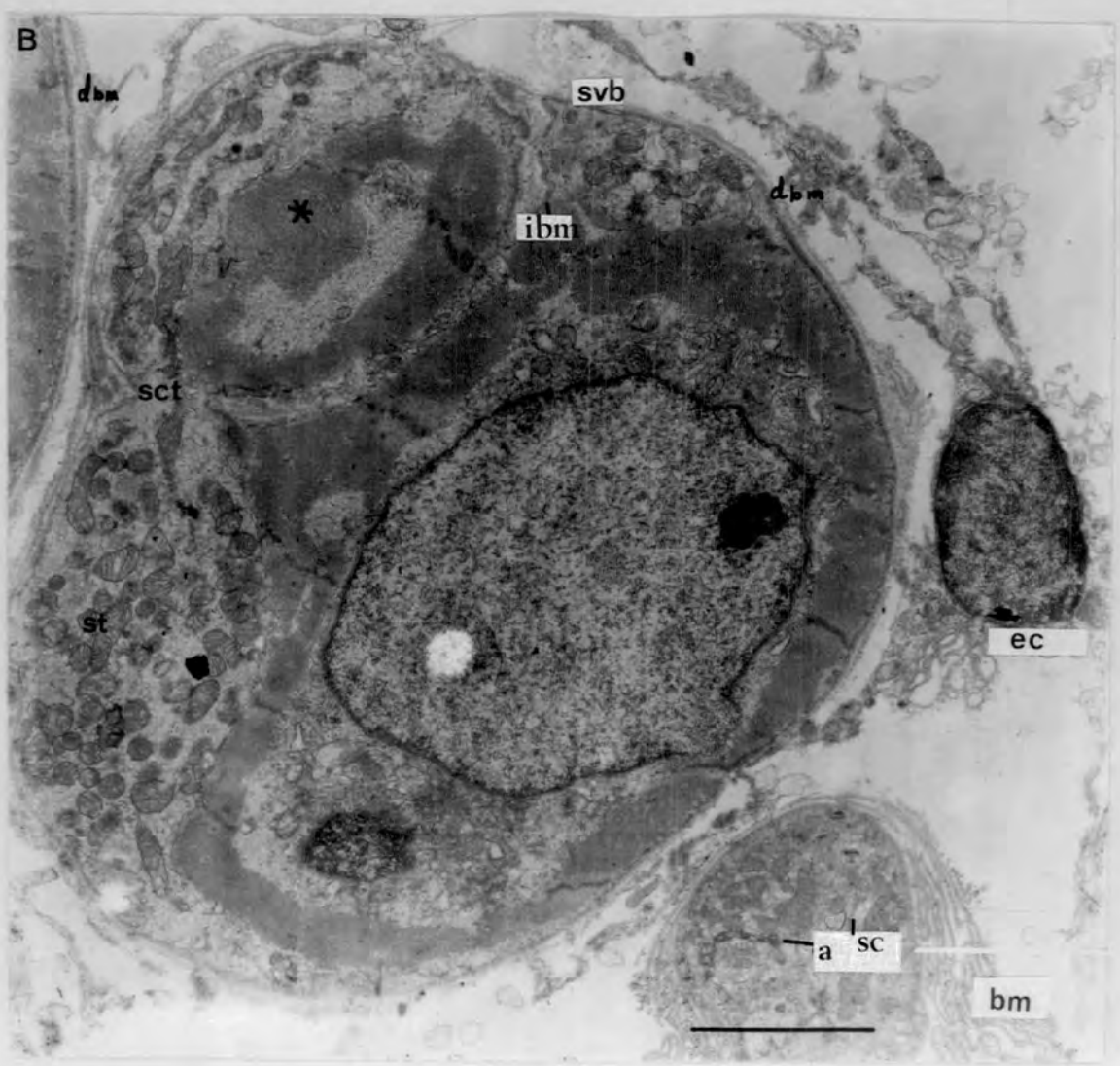

B
svb
ibn
sct
st
ec
a
sc
bm

FIGURE 26 ULTRASTRUCTURE OF THE INTRAFUSAL FIBRES OF THE TWO-FIBRE, TANDEM, MODEL-ADULT SPINDLE SHOWN IN FIG. 25. PROXIMAL CAPSULE.

A. TS of appendage fibre showing details of sensory cross-terminal with bag_1.

x 12,600

B. TS of bag_1 fibre showing oblique myofibrils with double M line

x 8,000

C. TS of bag_2 fibre showing microladders among myofibril mass and beneath sarcolemma. Arrowhead points to thickened sarcolemma.

x 8,000

Length of bar:

In A = 2.00 µm

B & C = 3.13 "

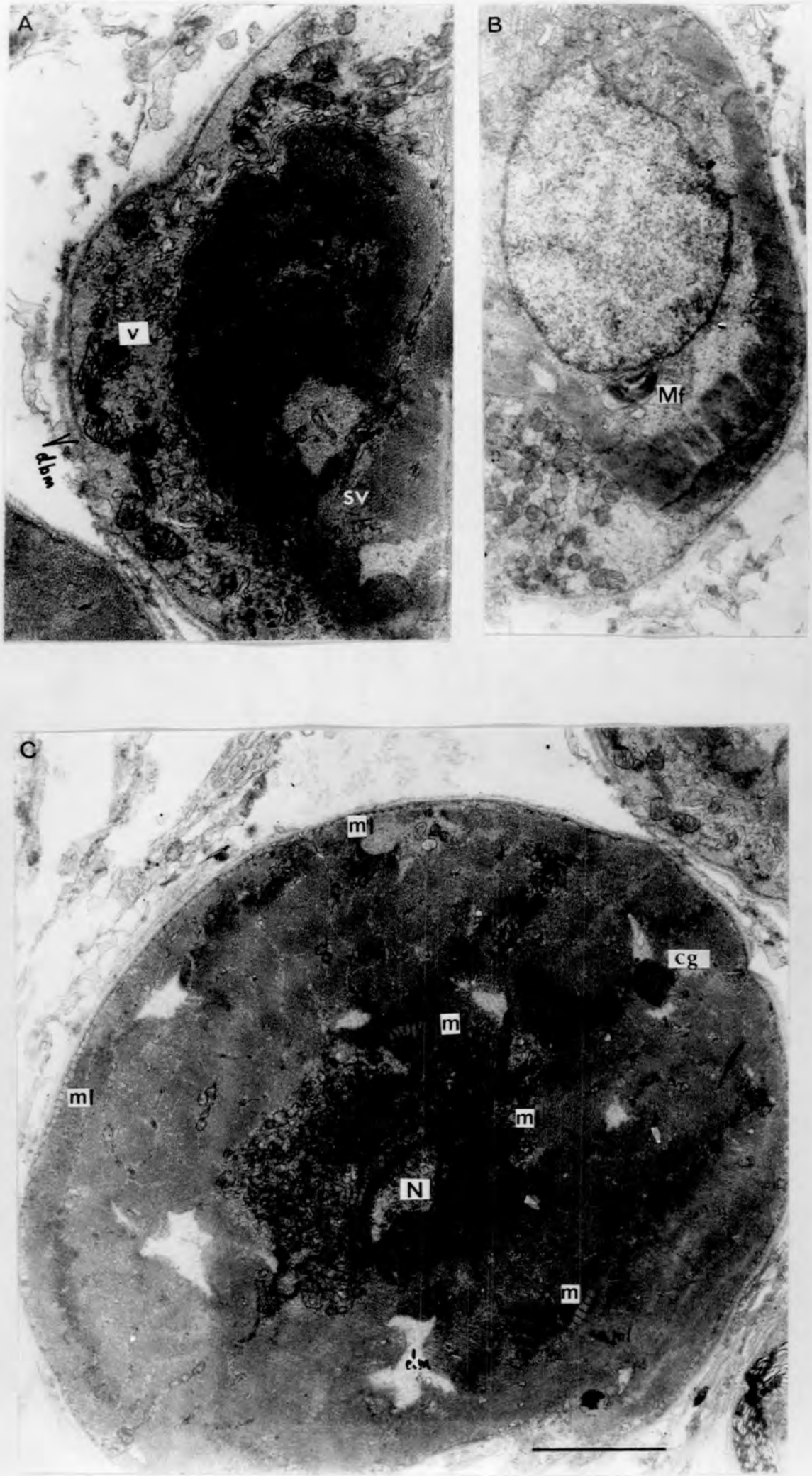

A
v
SV
B
Mf
C
ml
cg
m
ml
m
N
m

FIGURE 27 ULTRASTRUCTURE OF THE TWO-FIBRE, TANDEM, MODEL-ADULT SPINDLE SHOWN IN FIG. 25 AND 26. PROXIMAL CAPSULE.

A. Oblique section of appendage fibre apposed to both bag fibres. Note sensory cross-terminal and common basement membrane.

× 5,000

B. High power of A.

× 12,600

Length of bar:

In A = 5.00 μm

B = 2.00 "

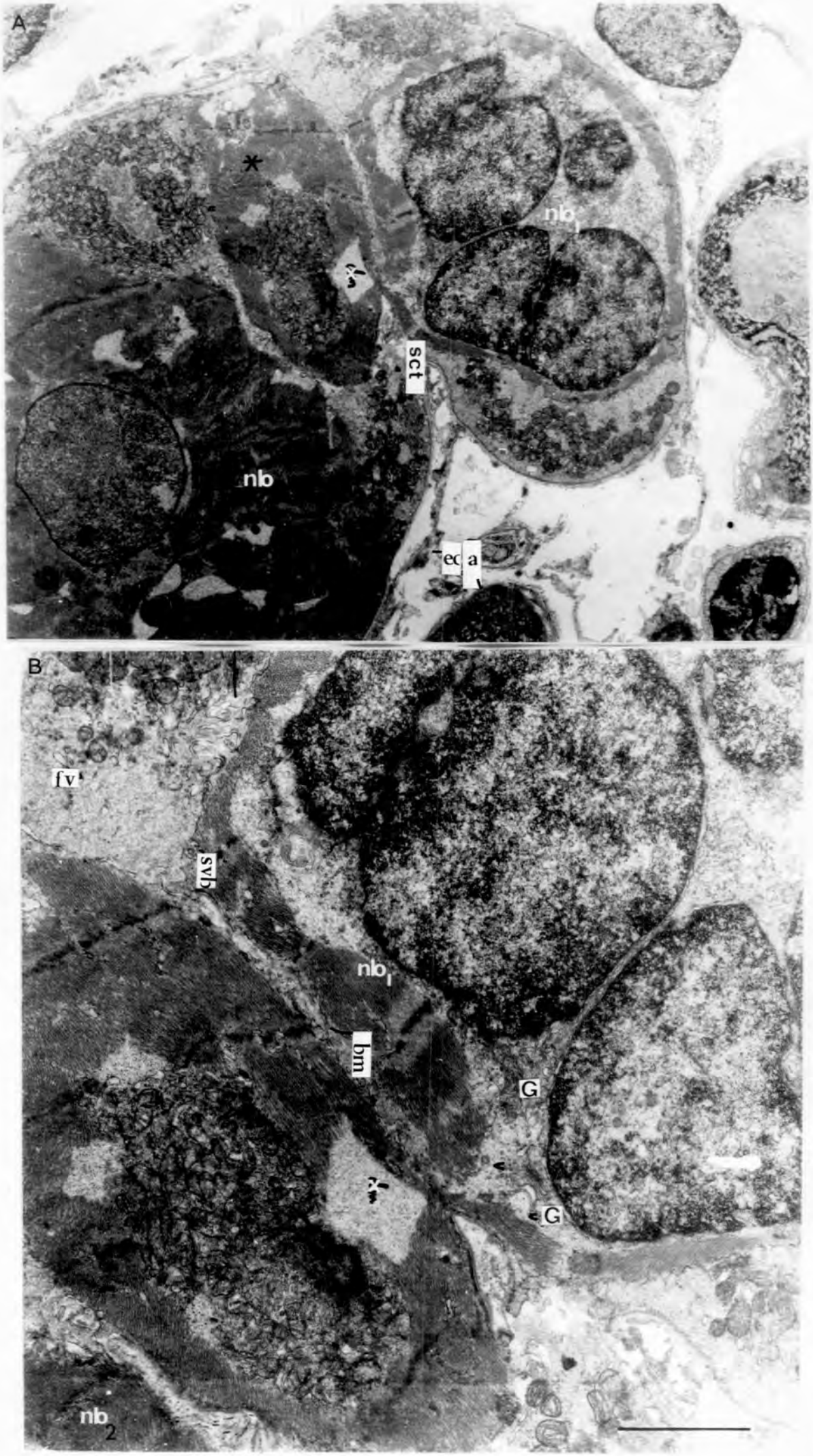

A
nb1
sct
nb
ec
a
B
fv
svb
nb1
bm
G
G
nb2

FIGURE 28 ULTRASTRUCTURE OF MICROLADDERS IN BAG$_2$ FIBRE OF A TWO-FIBRE MODEL-ADULT SPINDLE. REGION A.

A. TS of microladder near central core cytoplasm.

× 32,000

B. TS of microladder in main muscle mass.

× 32,000

C. TS of microladder adjacent to sarcolemma.

× 32,000

Length of bar:

In A, B & C = 0.78 μm

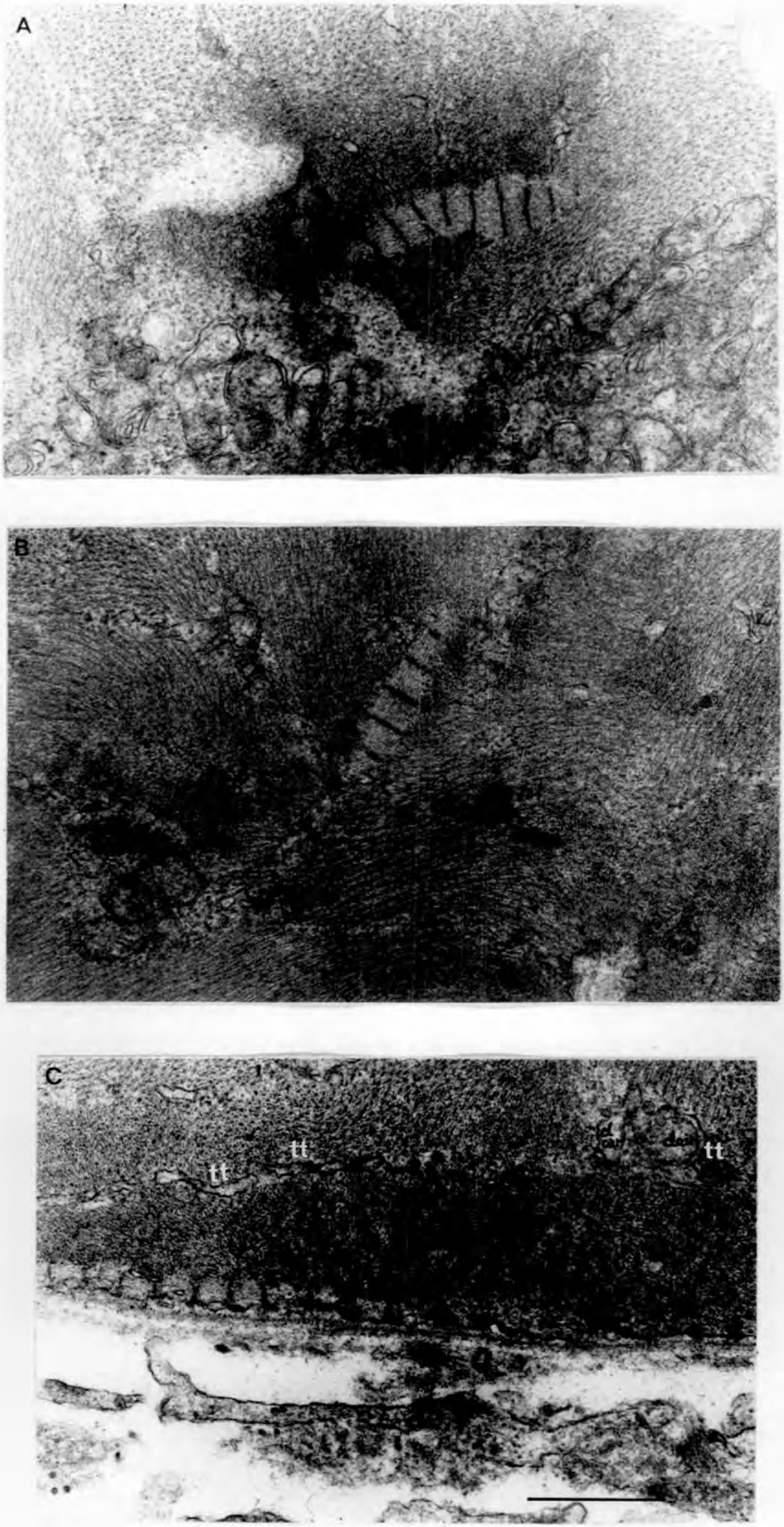

A
B
C
tt
tt
tt

FIGURE 29 ULTRASTRUCTURE OF TWO-FIBRE, TANDEM, MODEL-ADULT SPINDLE. PROXIMAL CAPSULE.

A. Oblique section of appendage fibre now completely apposed to bag_1 fibre.

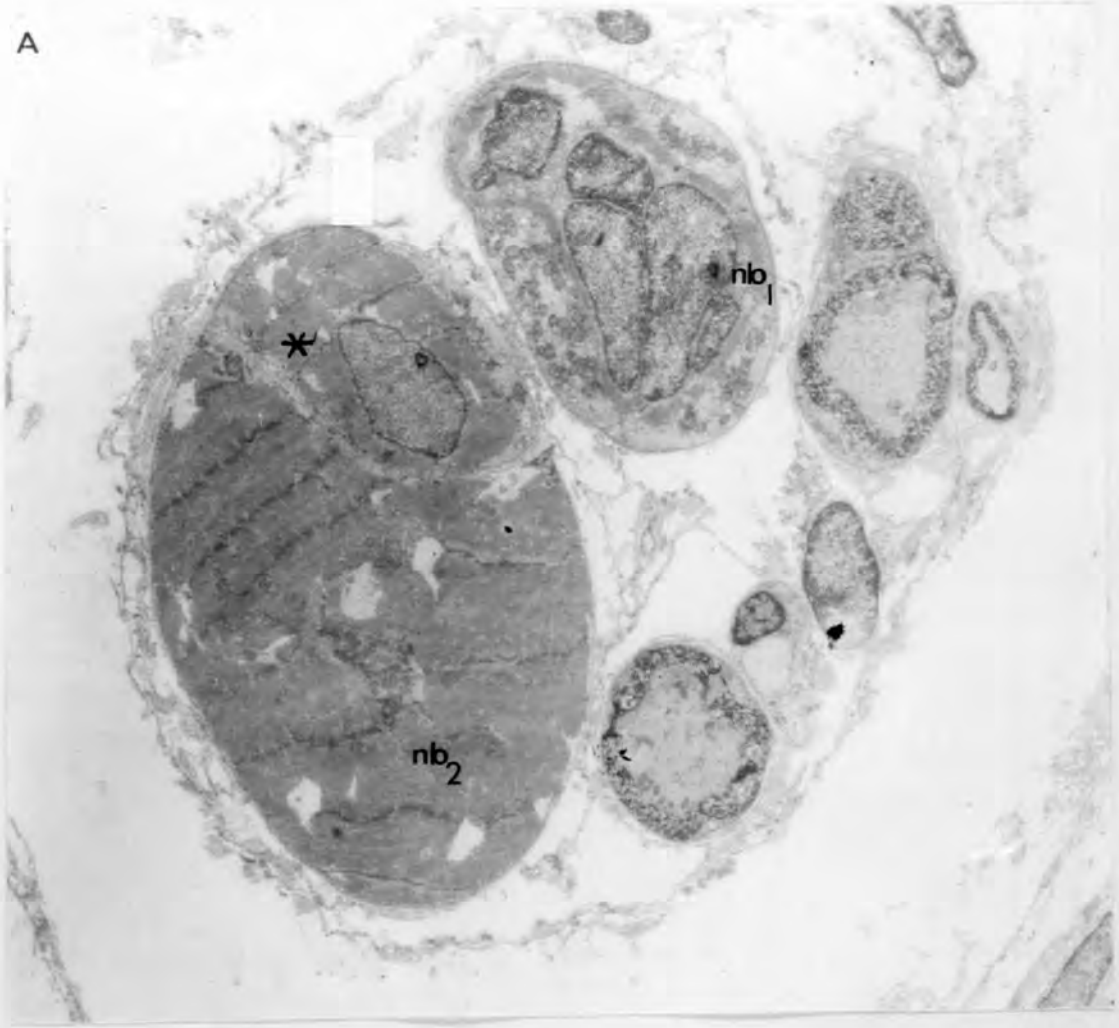
× 3,200

B. High power of bag_1. Arrow points to obliquely sectioned myofibril with double M line. A double M line was also present in region A of the distal capsule. Double arrowhead points to small vesicular body in the process of forming. Forked arrow indicates separate basement membrane for short length of junction between bag_2 and the appendage fibre. ibm & obm = inner and outer basement membrane, respectively. × 8,000

Length of bar:

In A = 7.81 μm

B = 3.13 "


A
nb1
nb2

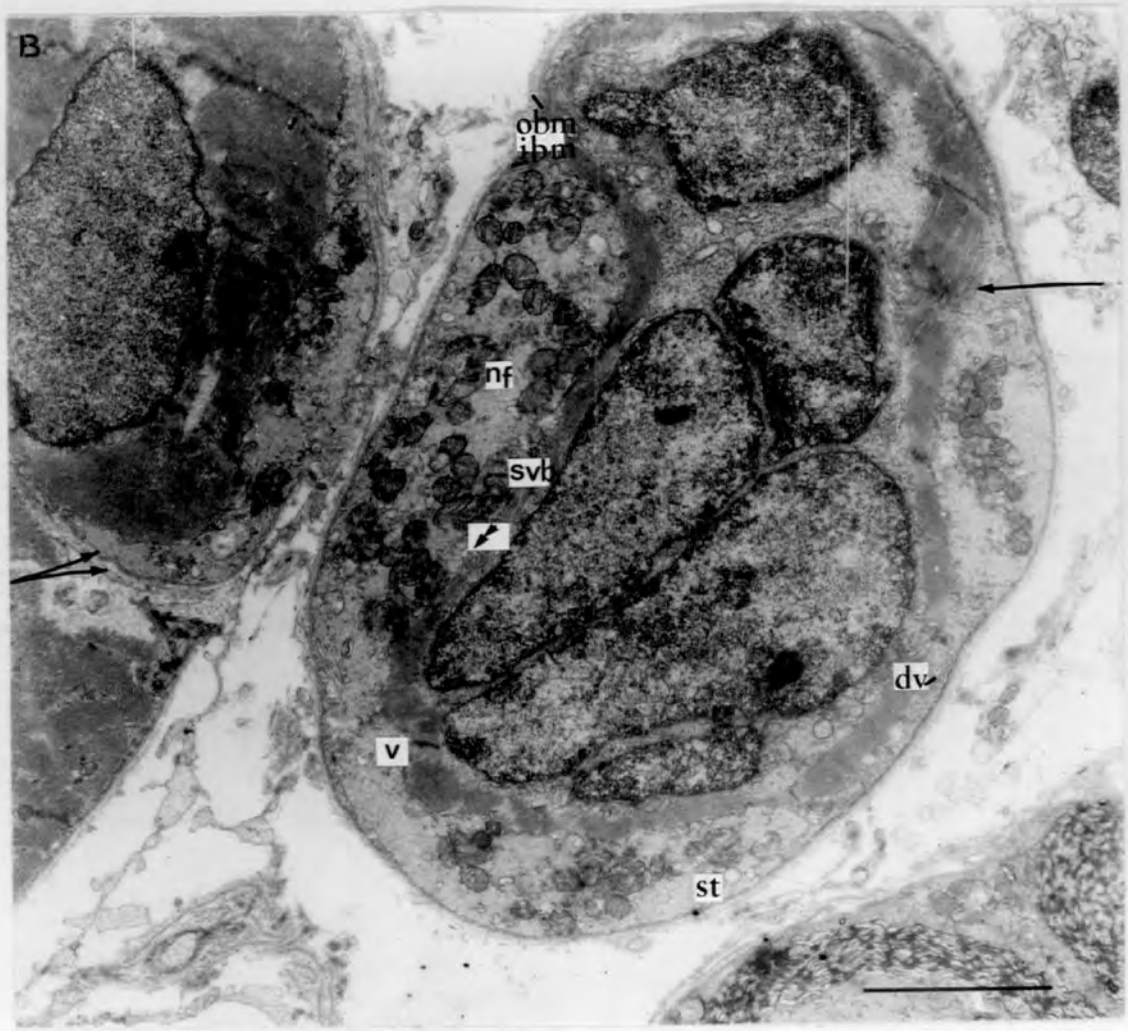

B
obm
ibm
nf
svb
dv
v
st

FIGURE 30 ULTRASTRUCTURE OF BAG_2 FIBRE AND APPENDAGE FIBRE

High power from fig. 29A. Oblique section reveals absence of M line. The M line was also absent in region A of the distal capsule.

× 8,000

Length of bar:

= 3.13 μm


tt
dv
v
dM
fv
bm
mfl
tt

FIGURE 31 SARCOMERE BANDING PATTERN OF BAG$_1$ FIBRE IN A TWO-FIBRE MODEL-ADULT SPINDLE. REGION A.

A. LS depicting double Mline, cytoplasmic granule among myofibril mass and coated vesicle in the process of invagination at the post-synapse (arrowhead).

x 20,000

B. LS through sensory ending, depicting dense-core vesicles and specialisation of synaptic membranes. Note single peripheral myofibril.

x 20,000

Length of bar:

In A & B = 1.25 μm

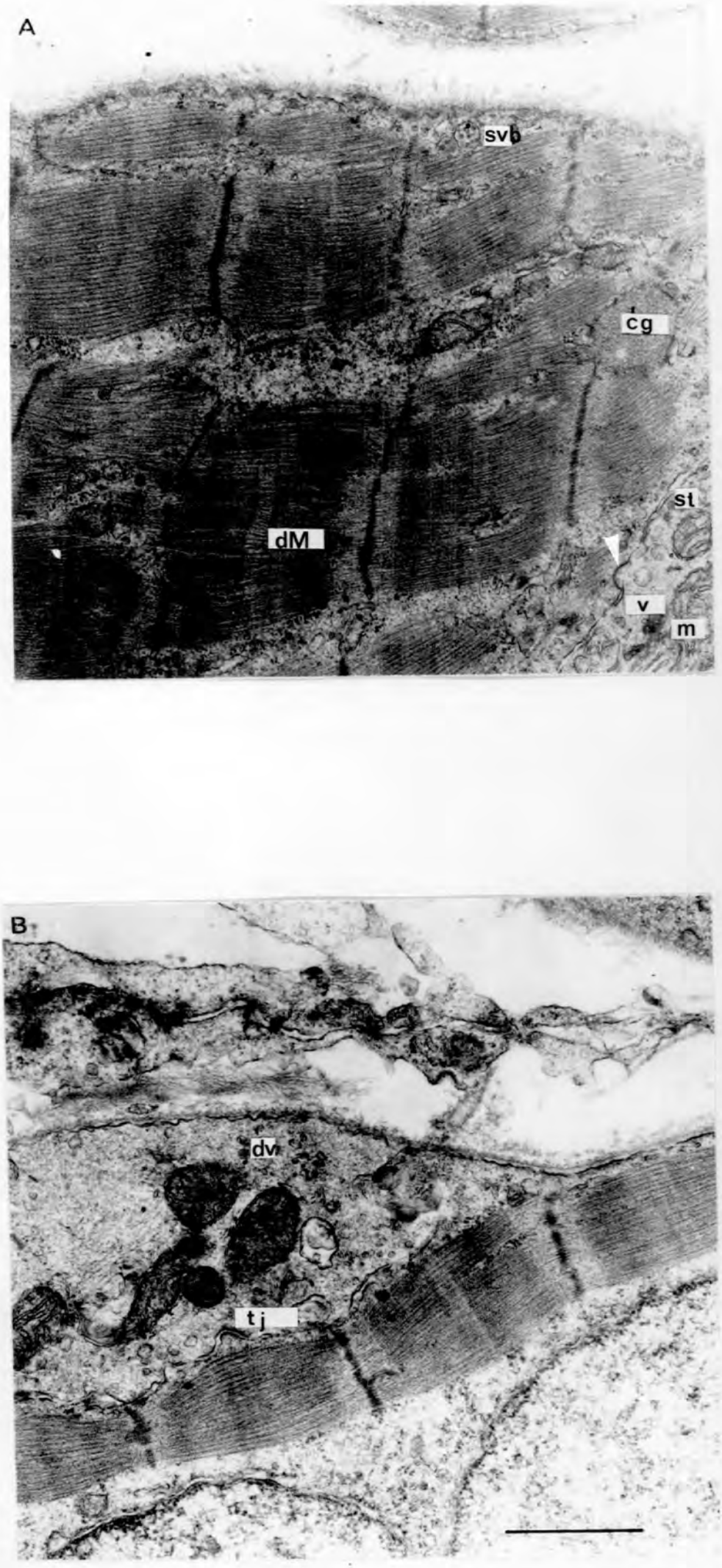

A
svb
cg
st
dM
v
m
B
dv
tj

FIGURE 32 SARCOMERE BANDING PATTERN OF BAG_2 FIBRE IN A TWO-FIBRE MODEL-ADULT SPINDLE. REGION A.

A. LS depicting absence of M line.

x 20,000

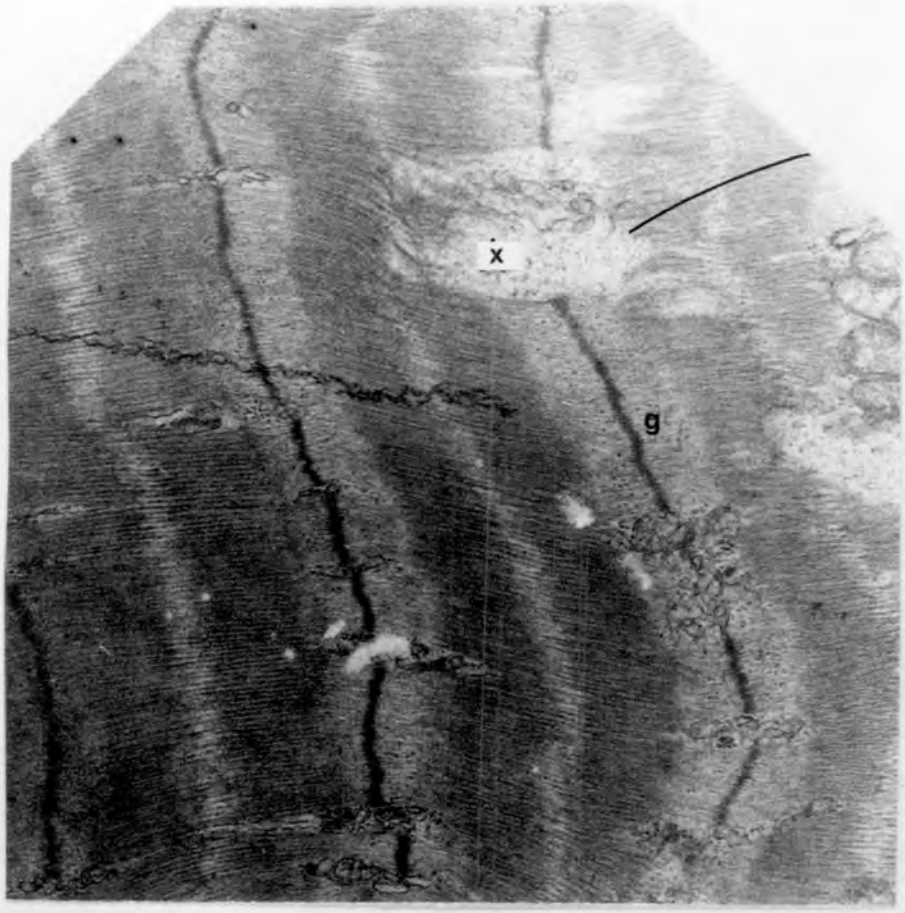
B. LS depicting cytoplasmic granule and microladder which has a similar appearance in TS.

x 20,000

Length of bar:

In A & B = 1.25 μm

A



B

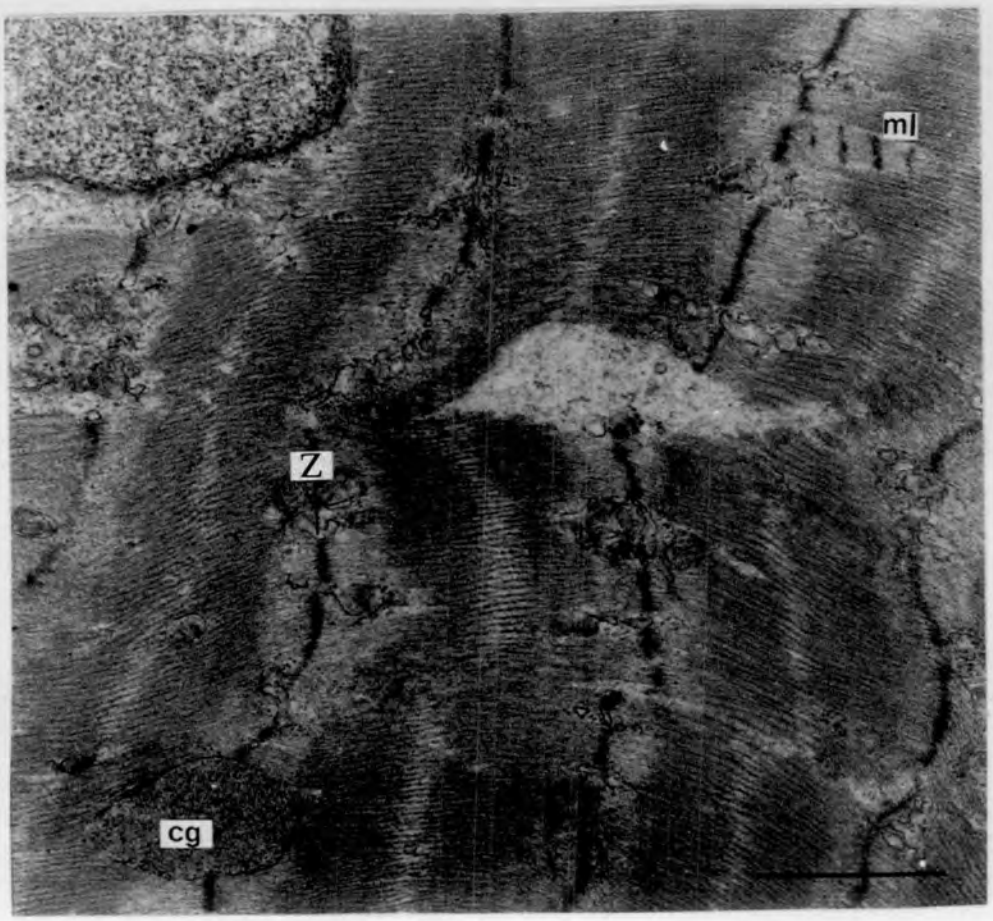


FIGURE 33 ULTRASTRUCTURE OF TWO-FIBRE, TANDEM, MODEL-ADULT SPINDLE, DISTAL CAPSULE.

A. TS of axial bundle in region A, composed of larger diameter bag_2 fibre, smaller bag_1 fibre " ghost " fibre and two to three " fragmentary " fibres close to bag_2. Note well developed sensory endings.

x 5,000

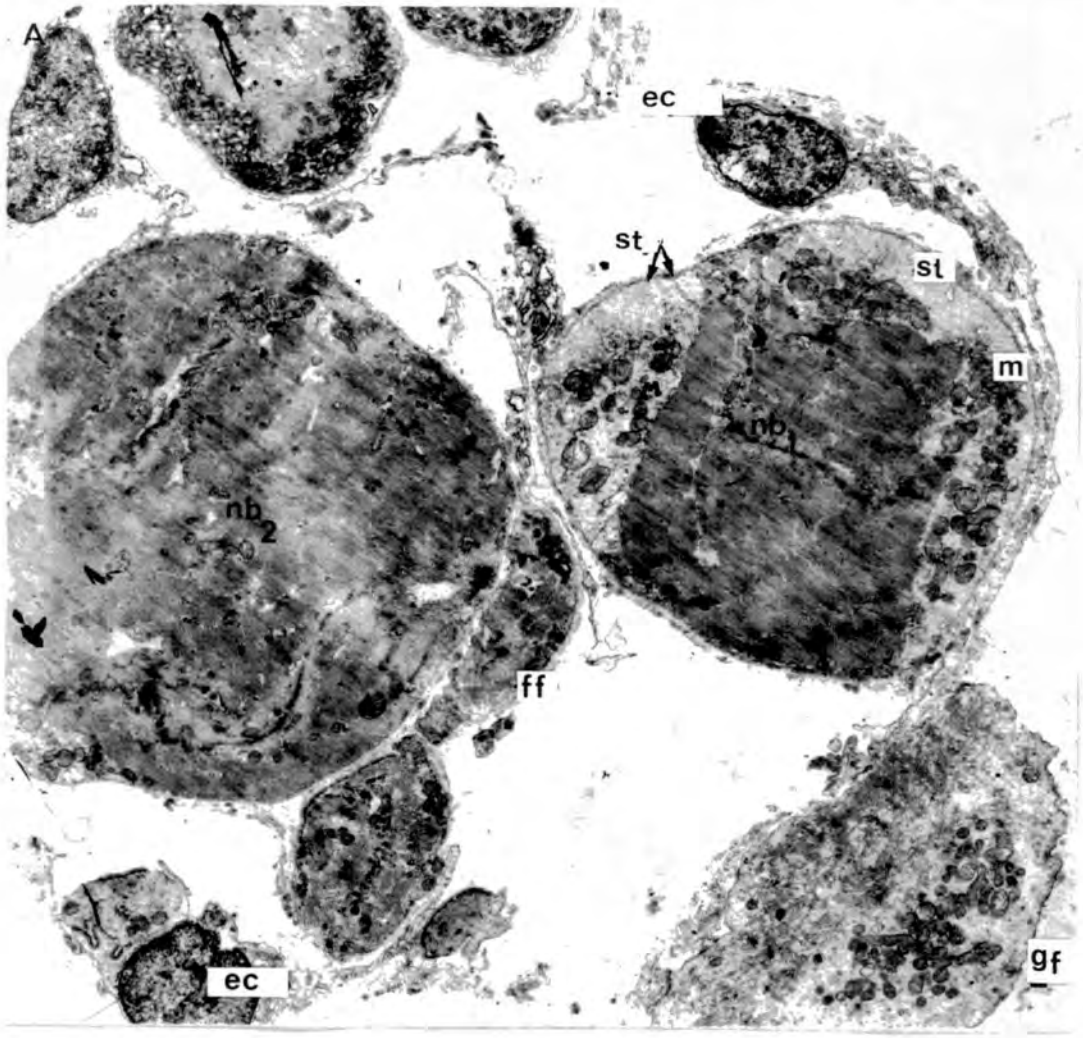
B. High power TS of bag_1 fibre. Note invagination (double arrowhead) of peripheral axolemma and thicker but incomplete, less dense outer basement membrane.

x 8,000

Length of bar:

In A = 5.00 µm

B = 3.13 "


A
ec
st
st
m
nb1
nb2
ff
ec
gf

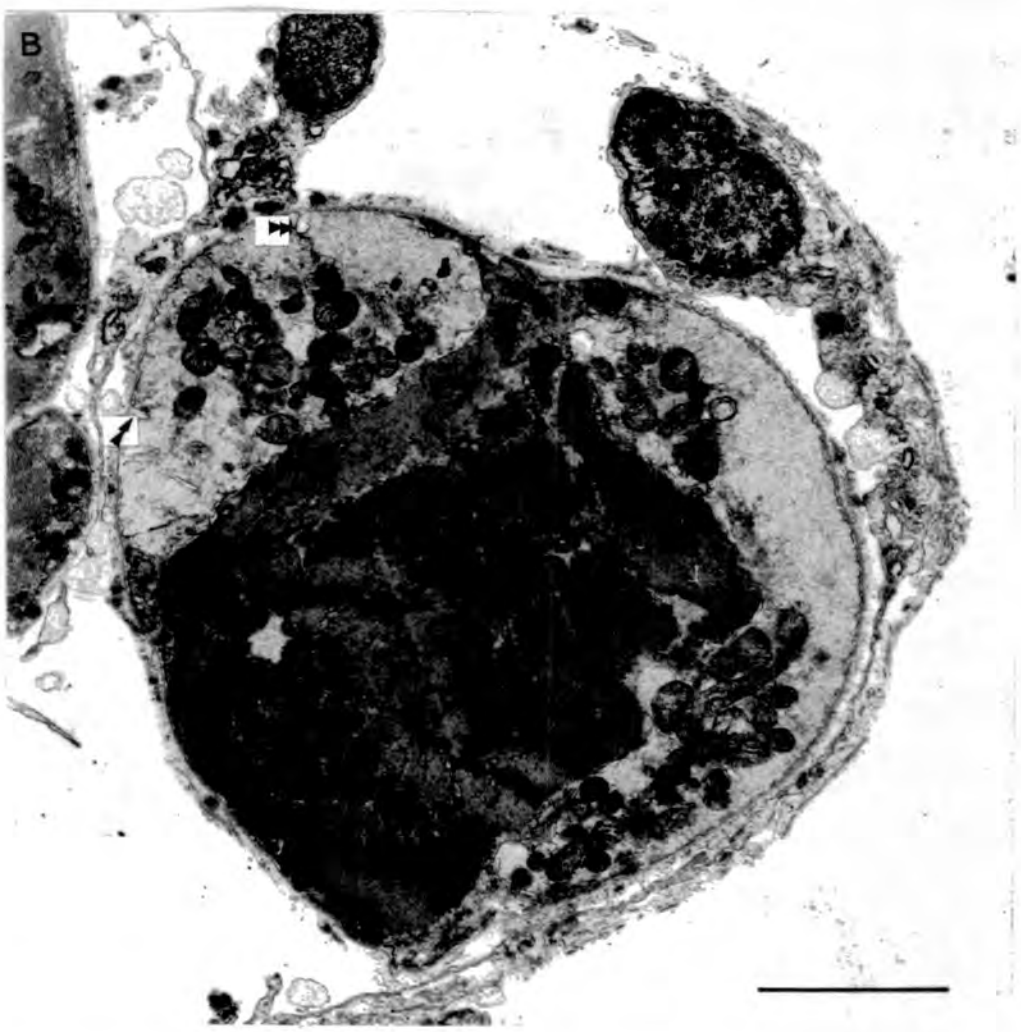

B

FIGURE 34 ULTRASTRUCTURE OF BAG_2 FIBRE IN TWO-FIBRE, TANDEM, MODEL-ADULT SPINDLE. DISTAL CAPSULE.

A. High power TS.

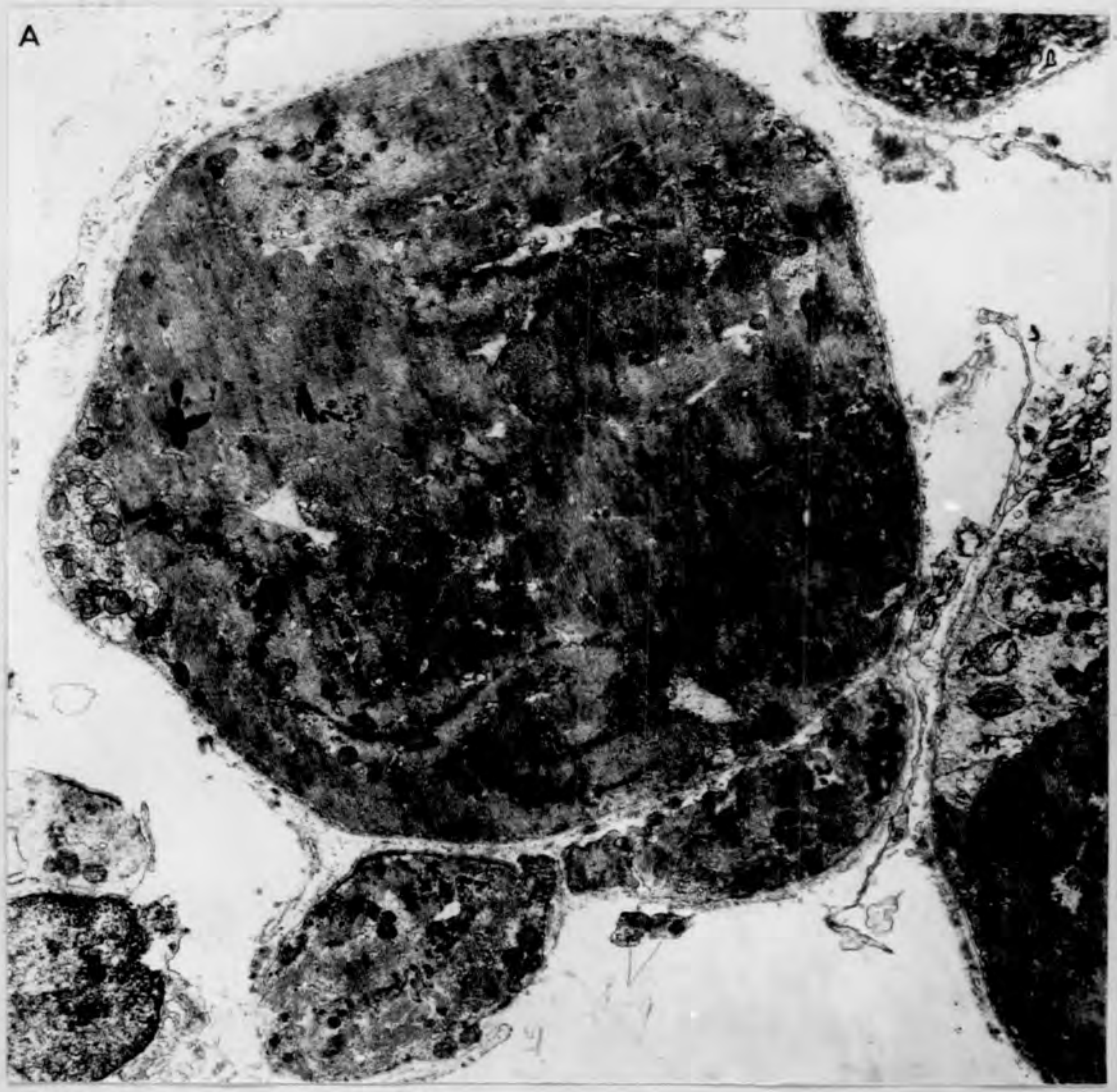
x 8,000

B. & C. show " fragmentary " fibres close to bag_2. In C, the fragmentary fibres can be seen to have a distinct inner basement membrane. It is quite separate from that of the bag_2 fibre, but is lacking along parts of the circumference. Where it is present, the sarcolemma beneath is unusually thickened.

x 8,000

Length of bar:

In A & B = 3.13 µm


A


B
nb
nb

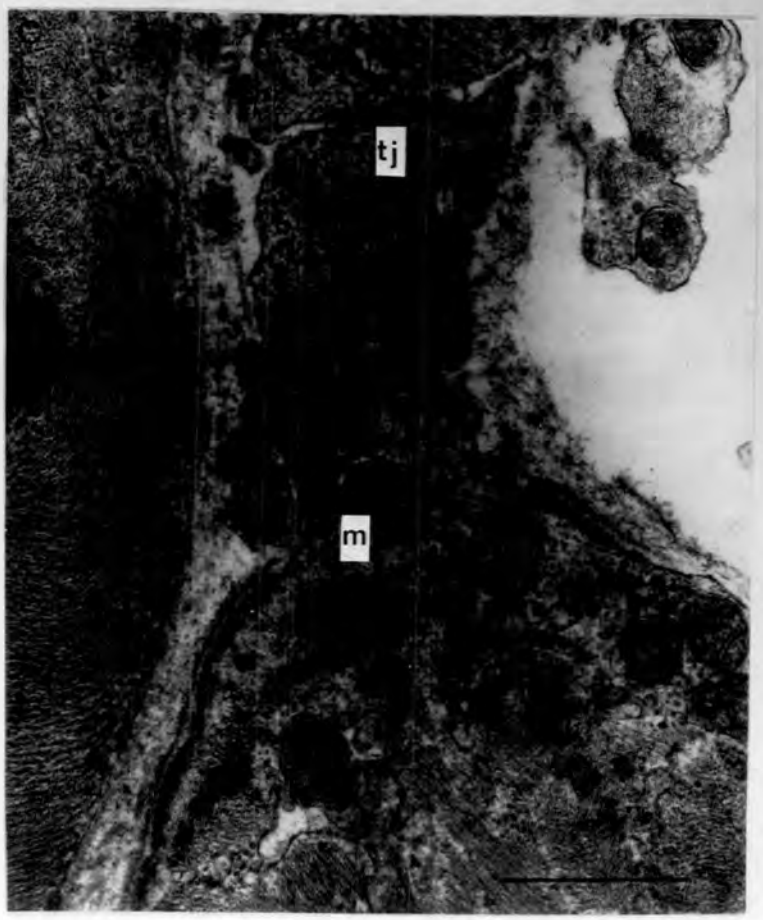

tj
m

FIGURE 35 ULTRASTRUCTURE OF FRAGMENTARY FIBRES

More polar section than fig. 34. Note macrophage, from which pseudopodia surround a potential fragmentary fibre (" pot. ff ").

× 8,000

Length of bar:

= 3.13 μm

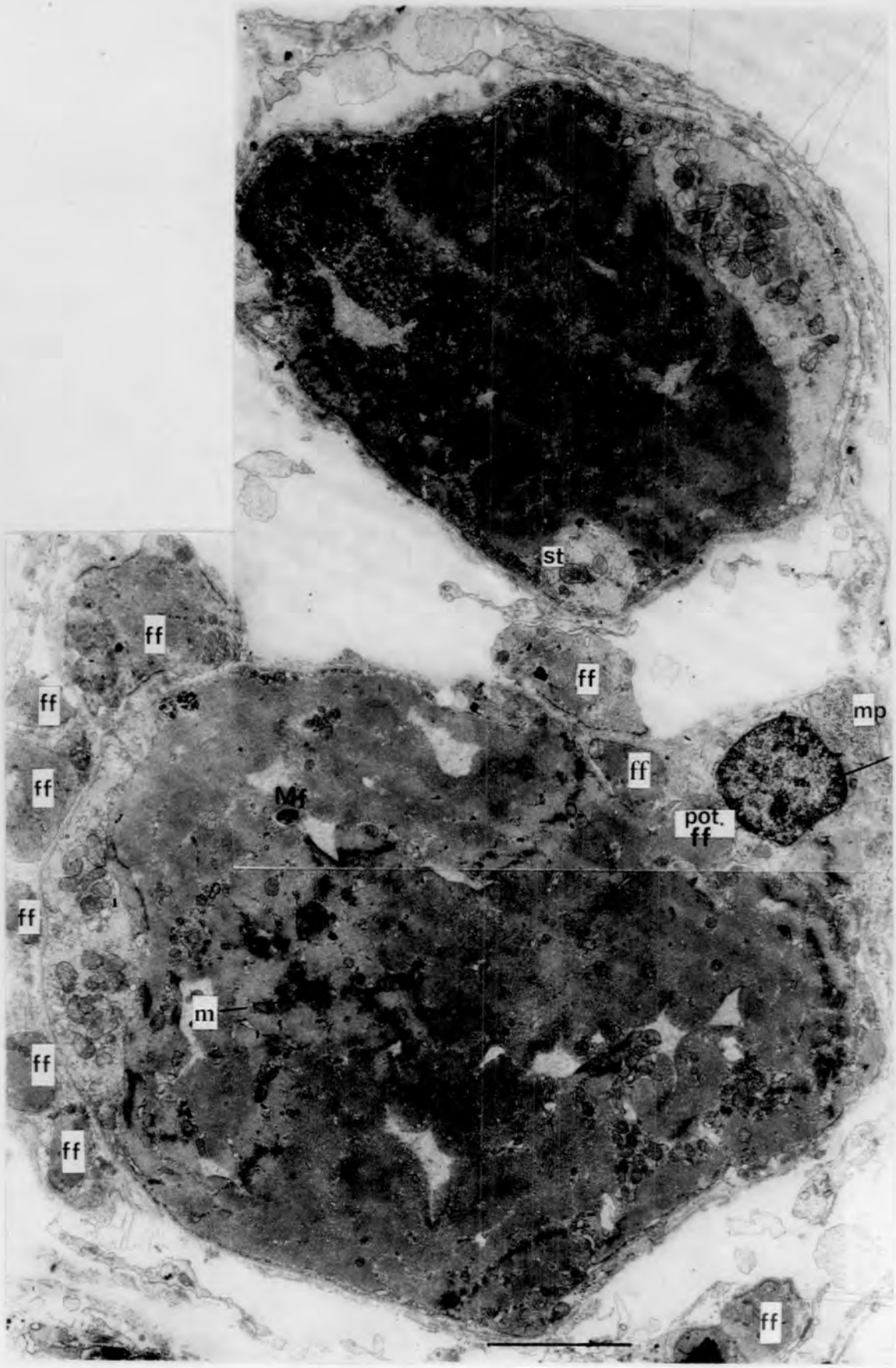

st
ff
ff
ff
ff
ff
mp
Mf
pot.
ff
ff
m
ff
ff
ff

FIGURE 36 ULTRASTRUCTURE OF " GHOST " FIBRES IN TWO-FIBRE, TANDEM, MODEL-ADULT SPINDLE

A. TS region A. A few myofibrile still present in " ghost " fragmentary fibre. Arrowhead points to extruding sarcoplasm.

x 8,000

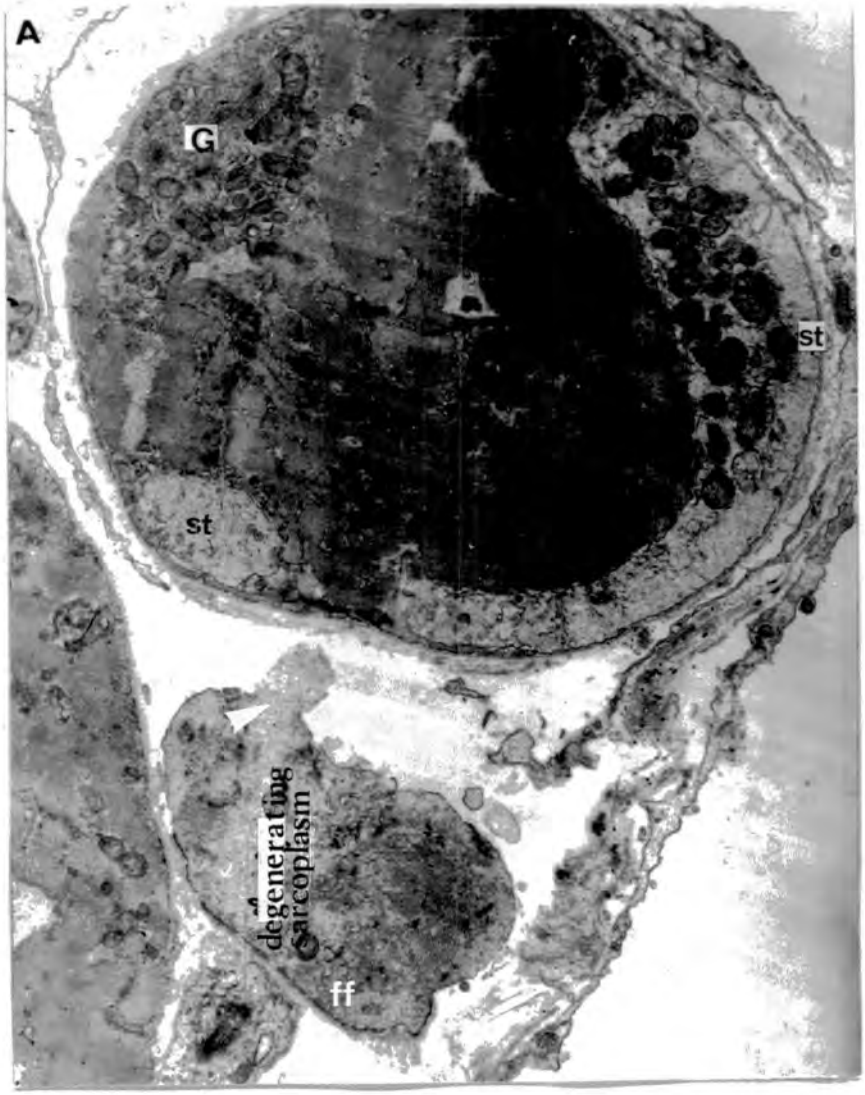
B. TS region A. The myofibrils of this " ghost " fibre have almost completely atrophied. Inner as well as outer basement membrane are lacking and in some parts, the sarcolemma appears to be absent. Note macrophage on bag_2 fibre. Arrowhead points to thickened sarcolemma.

x 12,600

Length of bar:

In A = 3.13 µm

B = 2.00 "


A
G
st
st
degenerating sarcoplasm
ff

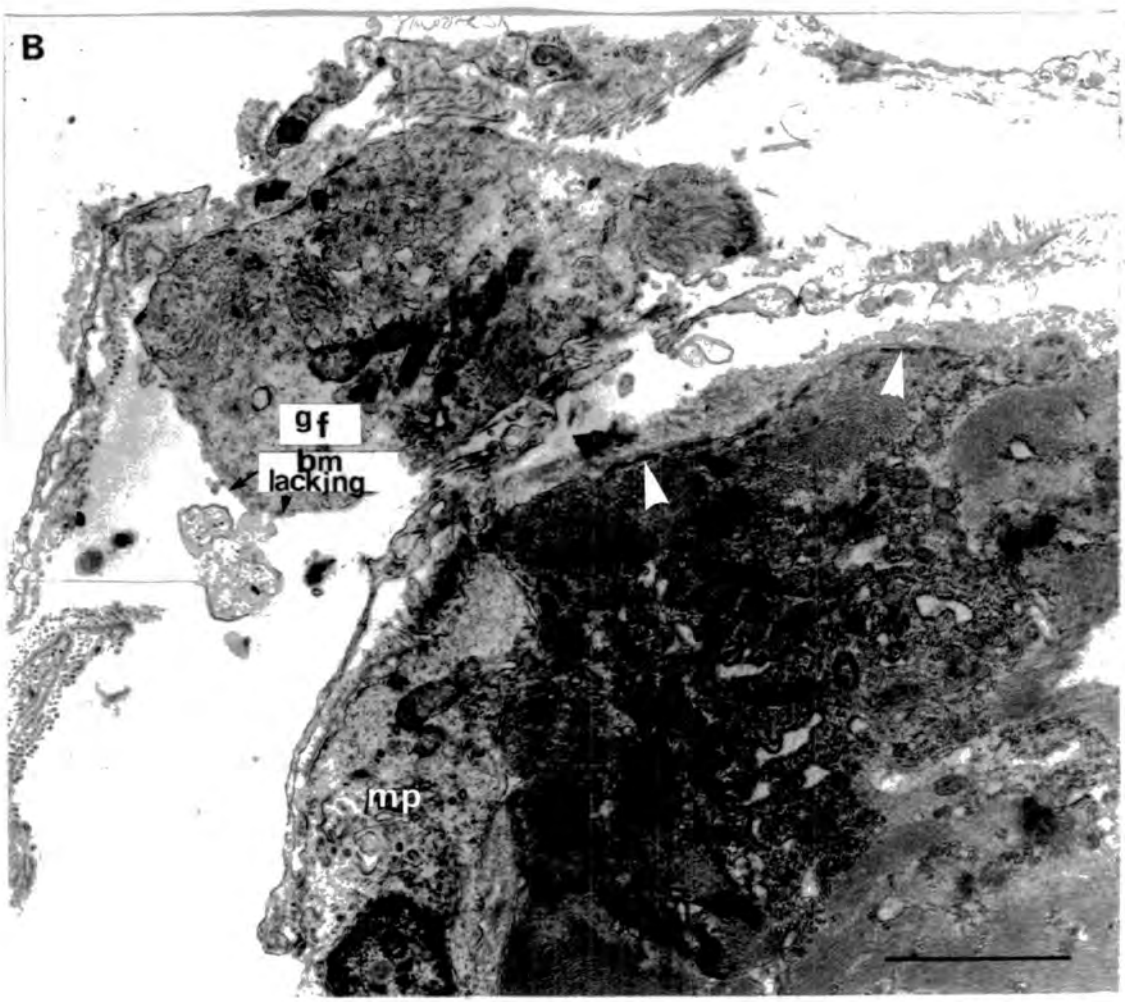

B
gf
bm lacking
mp

FIGURE 37 ULTRASTRUCTURE OF A RELATIVELY LARGE GHOST FIBRE

A. TS. The ghost fibre lies near the bag_1 fibre. Note central accumulation of non-aligned mitochondria and sparse myofilaments, which are lacking any organisation into myofibrils.

x 8,000

B. High power of A. Note small vesicular body packed with densely staining granules. The membrane of this body is incomplete.

x 32,000

Length of bar:

In A = 3.13 μm

B = 0.78 "

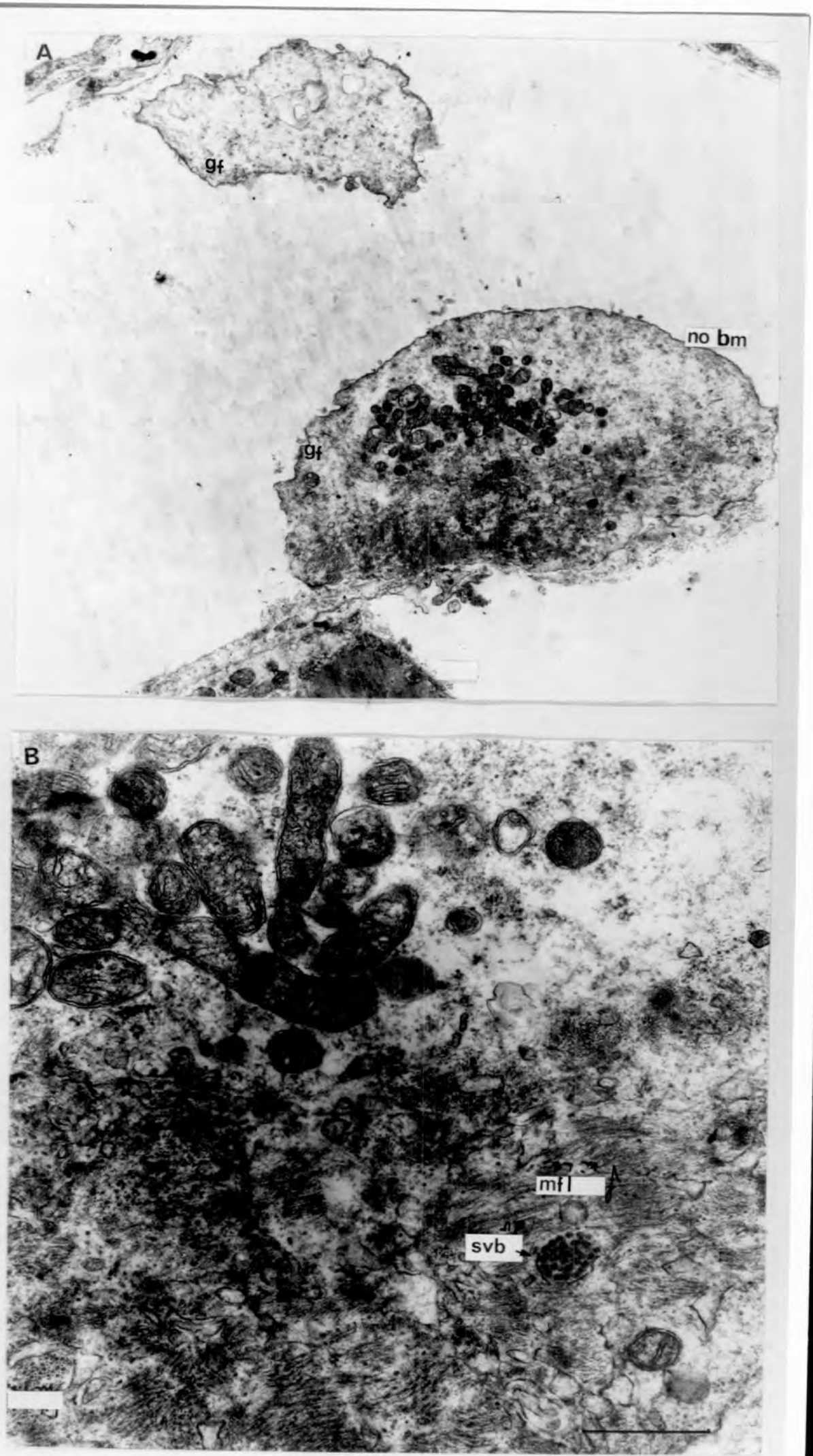

A
gf
no bm
gf
B
mfl
svb

FIGURE 38 SARCOMERE BANDING PATTERN OF A GHOST FIBRE AND A FRAGMENTARY FIBRE

A. LS ghost fibre. Remnant Z lines are the only vestige of a sarcomere banding pattern. Note dilated smooth sarcoplasmic reticulum. Where inner basement membrane is intact, the sarcolemma is thickened.

x 20,000

B. LS fragmentary fibre adjacent to bag_2 fibre on left. Note abundant glycogen.

x 32,000

C. LS fragmentary fibre. Compared with ghost fibre, the myofilaments are more intact. Note, though, the separation of the myofibrils.

x 50,000

Length of bar:

In A = 1.25 μm

B = 0.78 "

C = 0.50 "


A
dil.
dil.
svf
rsr
fv
pr
ff
C
Mf
Mf
mf
v

FIGURE 39 ULTRASTRUCTURE OF A TWO-FIBRE MODEL-ADULT SPINDLE ILLUSTRATING FIBRE SPLITTING. REGION B.

A. TS. Smaller diameter bag_1 fibre appears to have a more compact myofibrillar architecture than bag_2. Note peripheral nucleus; and flattened sensory terminal on bag_2.

x 5,000

B. TS, more polar section. The bag_2 fibre has split into two fibres of equal diameter.

x 5,000

Length of bar:

In A & B = 5.00 μm

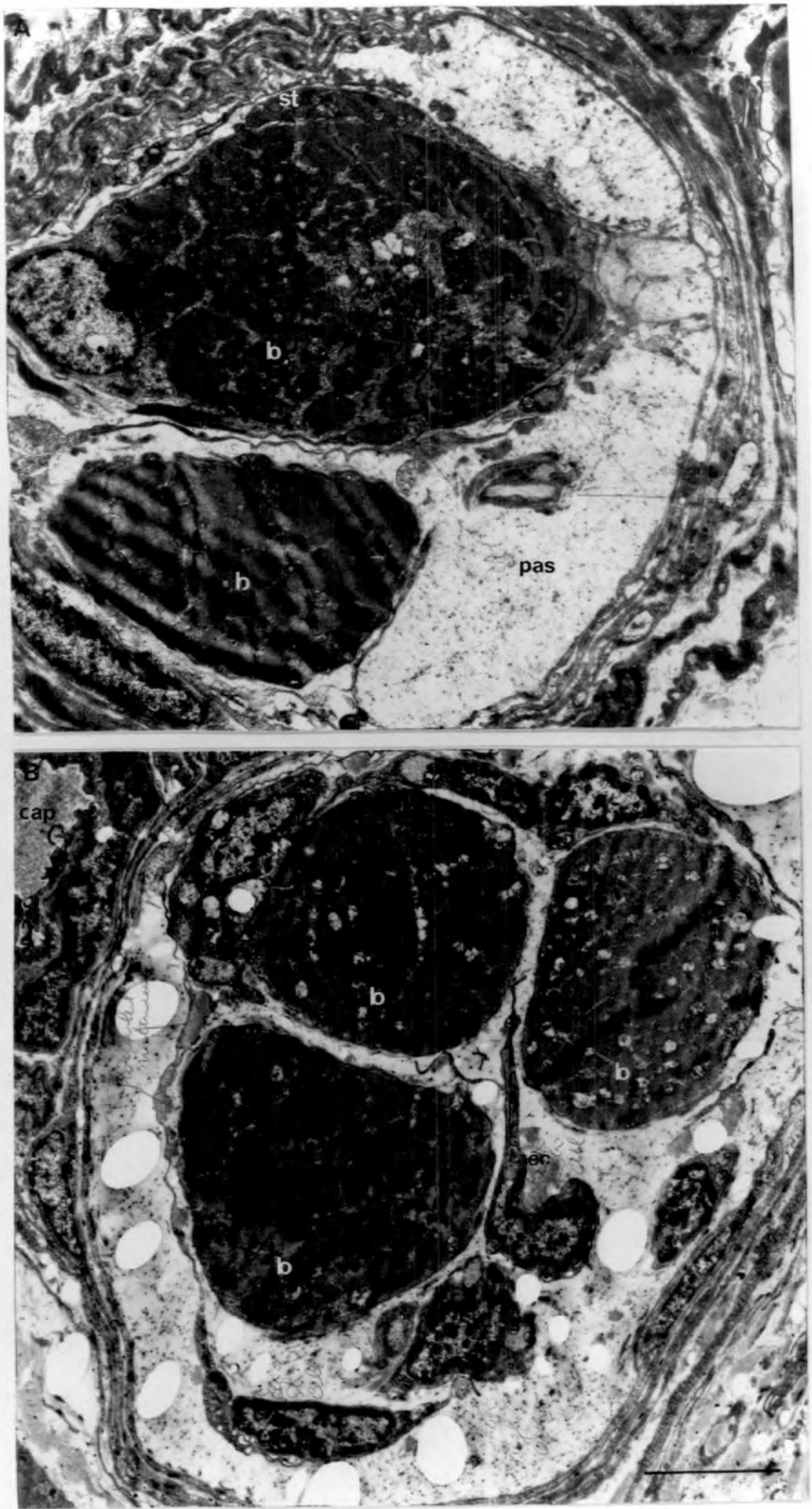

A
st
b
b
pas
B
cap
b
b
b
en

FIGURE 40 ULTRASTRUCTURE OF A TWO-FIBRE MODEL-ADULT SPINDLE

A. LS of bag_1 fibre in region B, depicting a faint double M line in leftmost sarcomere and complete absence of an M line in the other two sarcomeres.

x 17,100

B. LS of bag_2 fibre in region B, depicting the presence of an M line.

x 17,100

C. TS through type (ii) fusimotor plate on the bag_1 fibre at the mid-polar level (region B). Note post-junctional folds (arrowheads), the the lining basement membrane of which does not fuse in the middle; sole-plate nucleus; and post-synaptic as well as pre-synaptic vesicles. Many of the latter are flattened.

x 50,000

D. TS through juxta-equator of spindle in fig. 39 and fig. 40A, to show well-developed, normal looking periaxial space at this level. Inset shows nucleation of the fibres.

x 8,000

Inset : x 2,000

Length of bar:

In A = 0.50 µm

B = 3.13 "

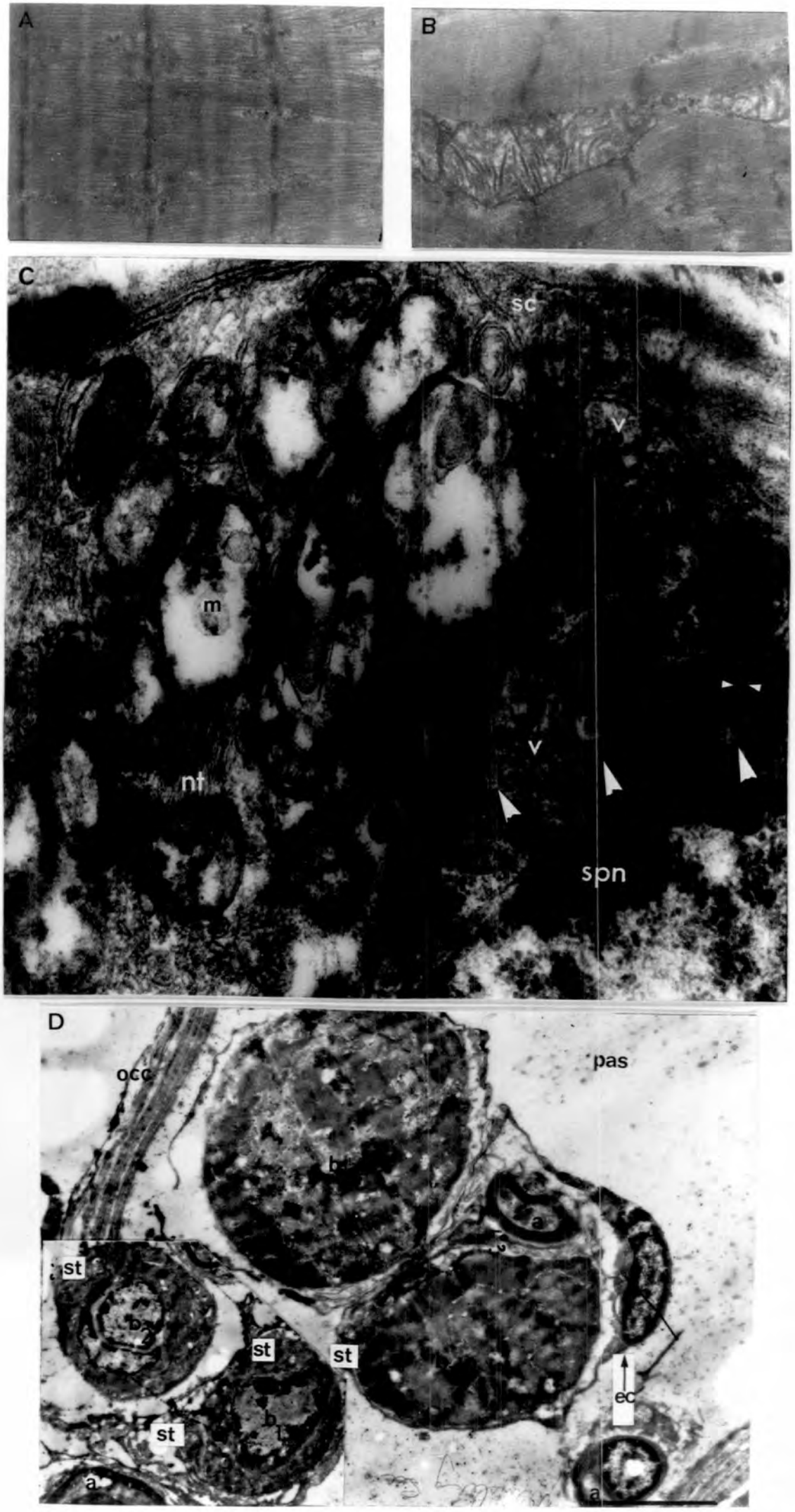

A
B
C
sc
v
m
v
nt
spn
D
occ
pas
b
a
st
b
st
st
st
b
ec
a
a

FIGURE 41 ULTRASTRUCTURE OF AN ANOMALOUS EXPERIMENTAL SPINDLE. REGION A (JUXTA-EQUATORIAL LEVEL)

A. Oblique TS. Two bag fibres share a basement membrane and a sensory terminal. Large forked arrow points to unmyelinated axons in periaxial space. Small arrow points to myoblast on bag_1 fibre.

×5,000

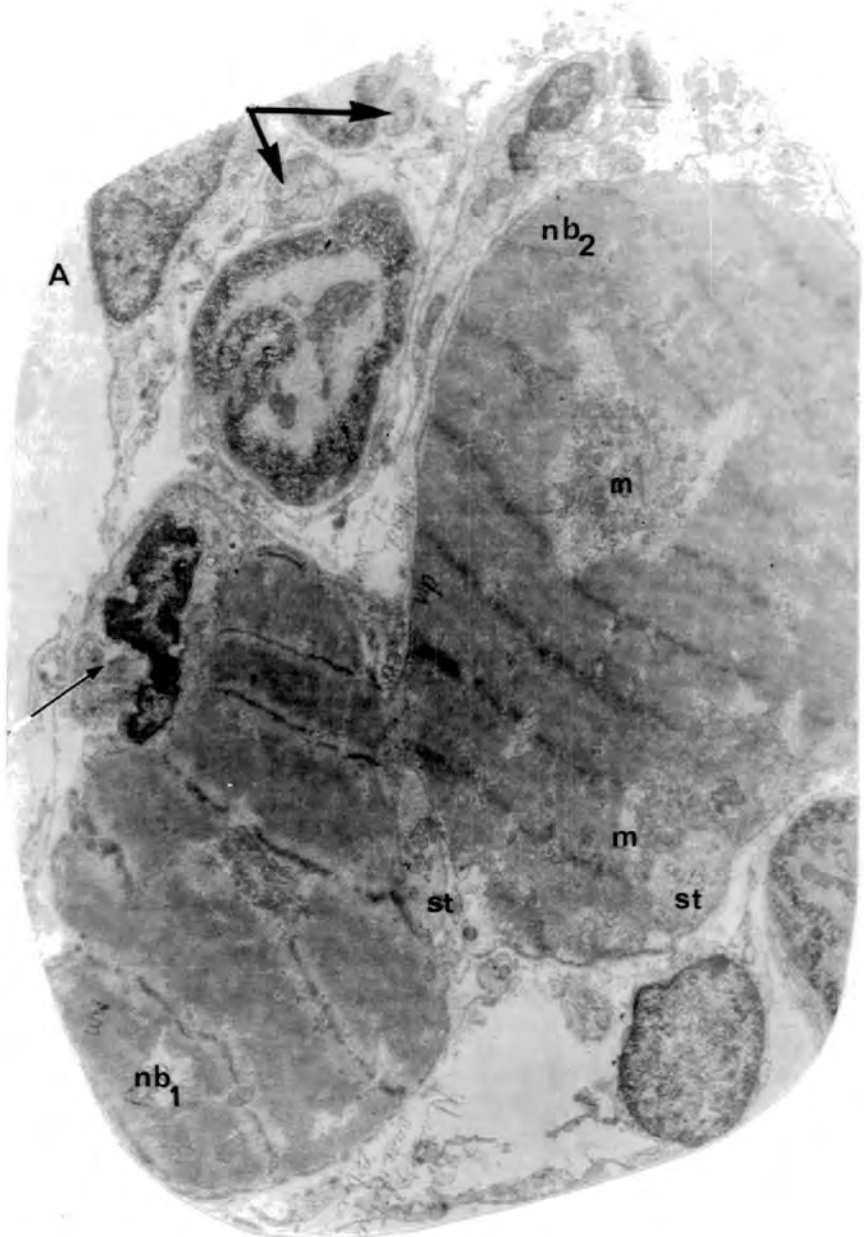
B. High power of A, showing absence of M lines in both bag fibres.

× 5,980

Length of bar:

In A = 5.00 μm

B = 4.67 "


A
nb2
m
m
st
st
nb1

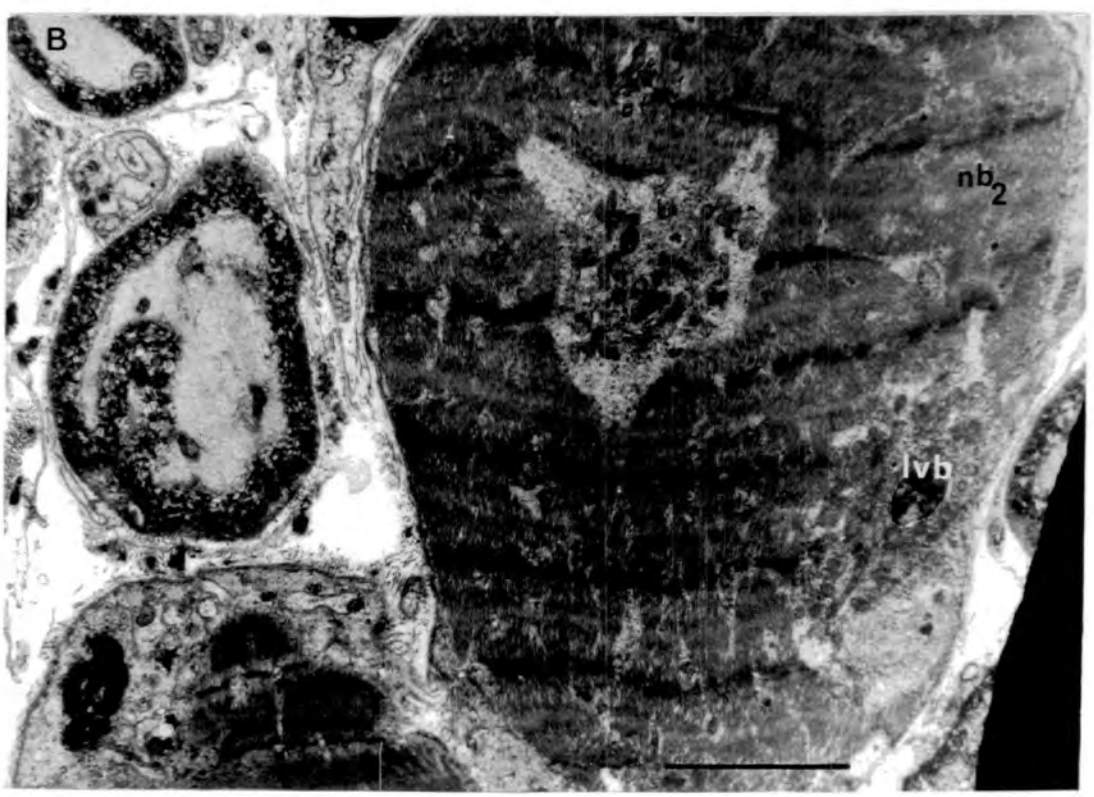

B
nb2
lvb

FIGURE 42 ULTRASTRUCTURE OF AN ANOMALOUS EXPERIMENTAL SPINDLE

A. TS, showing presence of chain fibre. Note well-developed sensory terminals. Also, sensory pre-terminal axon in centre of periaxial space.

× 5,000

B. TS of bag_2 fibre with abnormal sensory ending of " torn off " appearance (st). Arrowheads point to sarcolemmal lips completely encircling a round sensory terminal.

× 8,000

C. TS of bag_2 fibre, showing normally apposed sensory ending. Double-headed arrow points to extrusion of some substance from the fibre into what appears to be an invagination of the sensory terminal. Single arrowhead points to a peripheral myofibril in TS, i.e., at a distinct angle to the other myofibrils.

× 8,000

Length of bar:

In A = 5.00 μm

B & C = 3.13 "

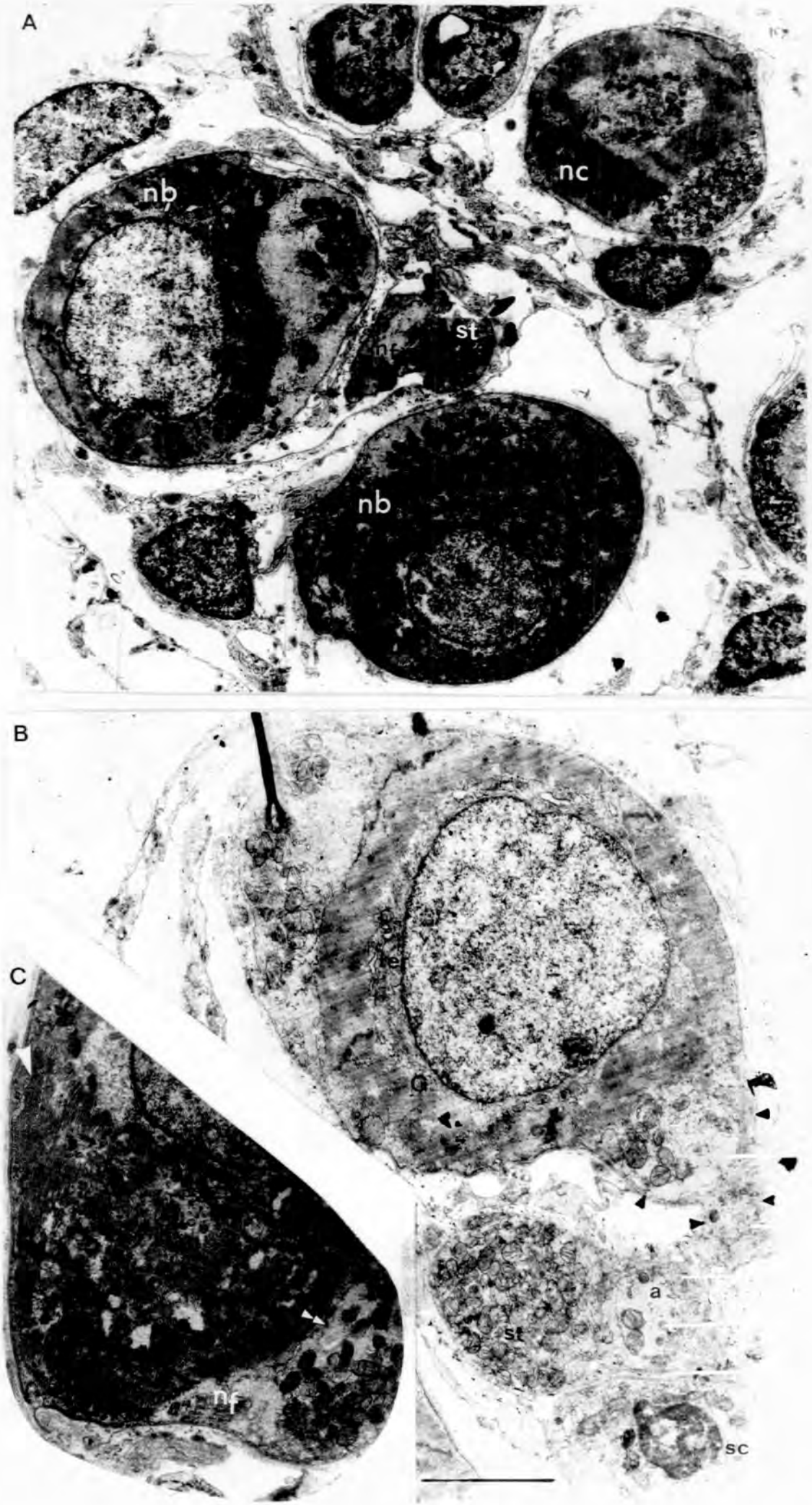

A
nb
nc
st
nf
nb
B
C
nf
a
st
sc

FIGURE 43 ULTRASTRUCTURE OF AN ANOMALOUS EXPERIMENTAL SPINDLE

A. TS of abnormal sensory ending of " torn off " appearance, showing that it is part of the round terminal that is normally apposed to bag_2 . Note neurotubules.

x 20,000

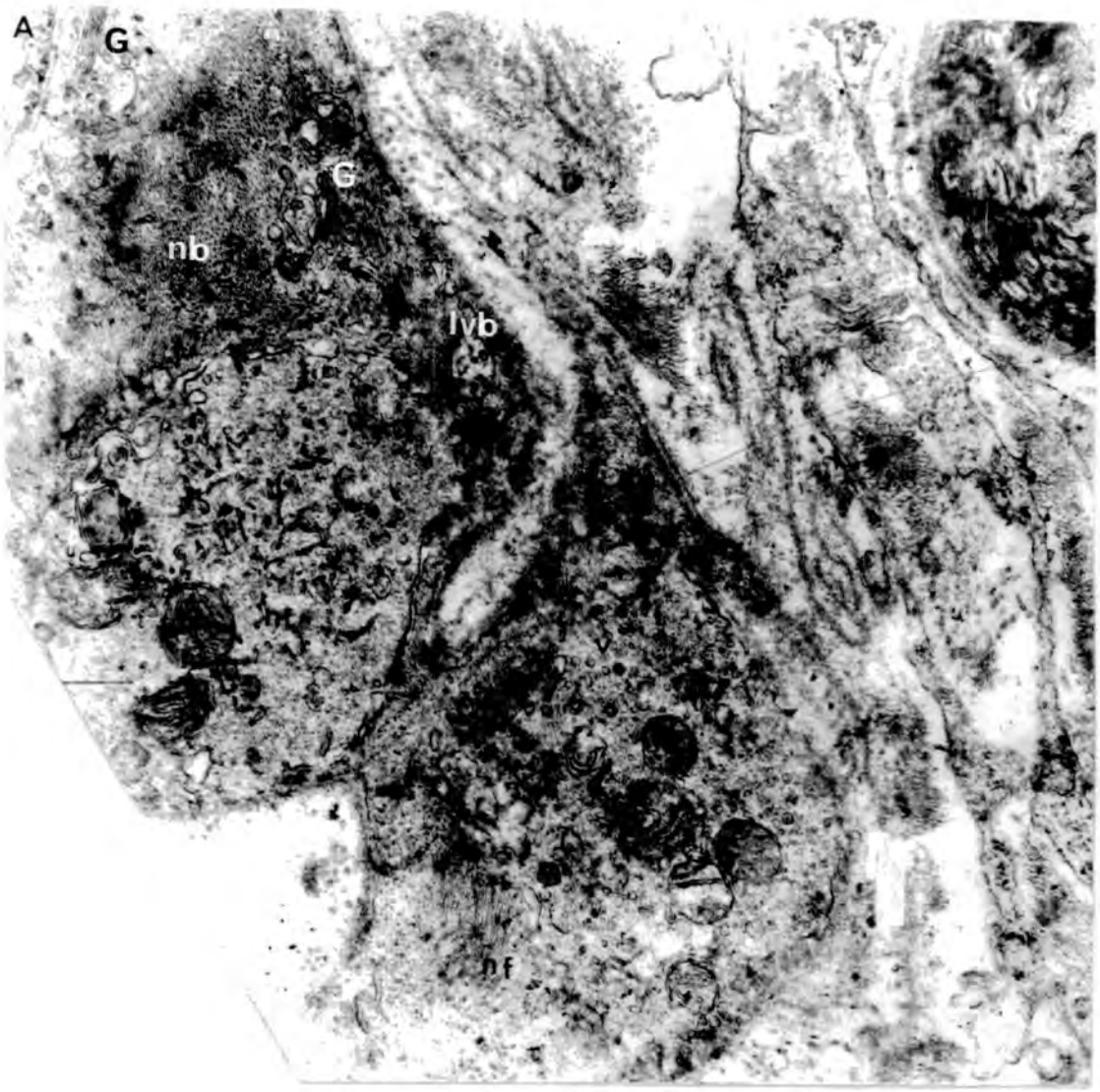
B. TS of bag_1 fibre, showing a sensory terminal that is partly normally apposed. Note surface TS through a Z band.

x 8,000

Length of bar:

In A = 1.25 μm

B = .13 "


A
G
G
nb
lvb
nf

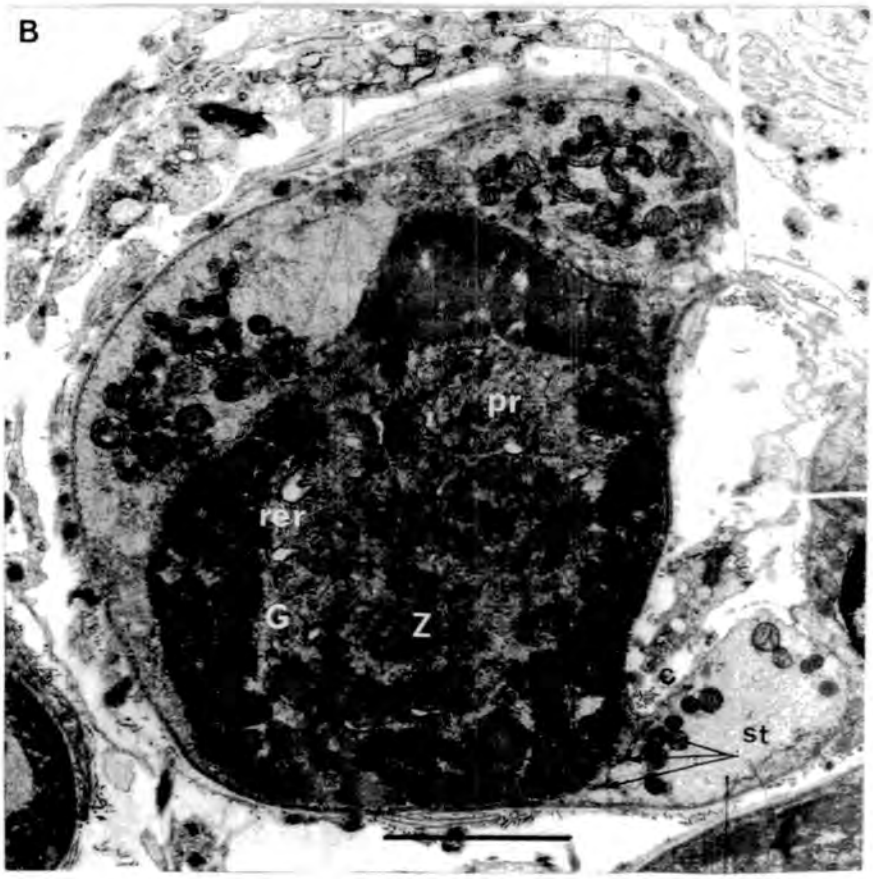

B
pr
rer
G
Z
st

FIGURE 44 ULTRASTRUCTURAL OF AN ANOMALOUS EXPERIMENTAL SPINDLE

A. High power of fig. 43B, showing dilated rough sarcoplasmic reticulum within central core cytoplasm.

x 32,000

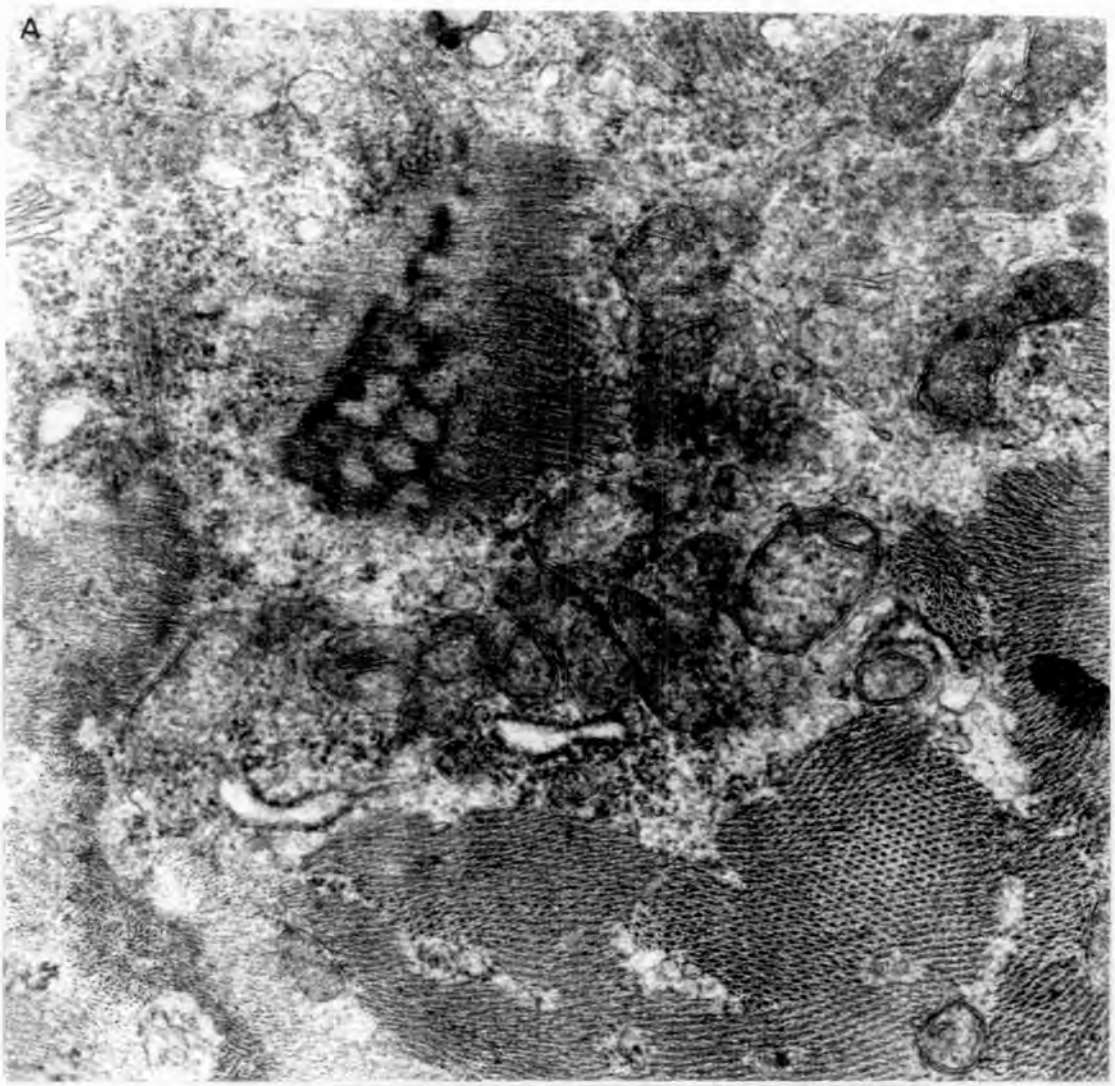
B. Another TS of bag_1 fibre, showing abnormal abundance of sarcoplasmic organelles, i.e., vesicles (pale: "v", dark: arrowheads, and dense-core: arrows, types), dilated rough sarcoplasmic reticulum and polyribosomes in circular arrangements.

x 12,600

Length of bar:

In A = 0.78 µm

B = 2.00 "


A

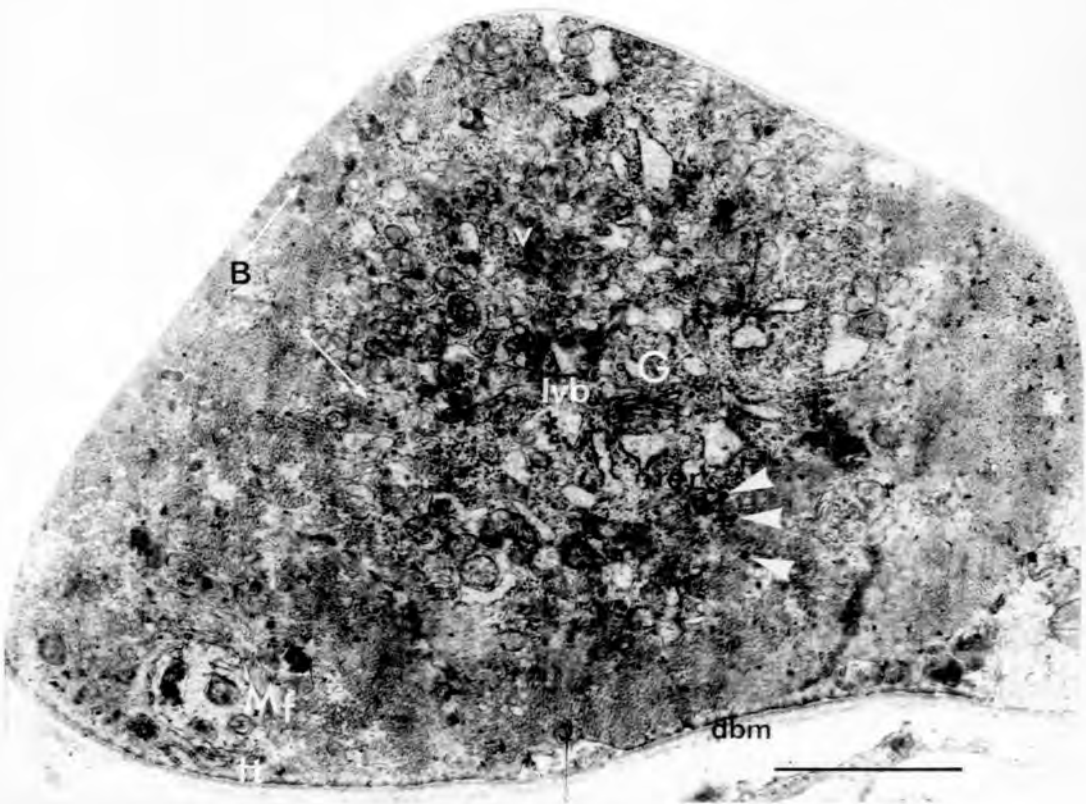

B
V
lvb
G
Mf
dbm

FIGURE 45 ULTRASTRUCTURE OF EXTRAFUSAL MUSCLE, BOTH NORMAL ADULT AND MODEL-ADULT

A. LS of model-adult muscle. Note preponderance of glycogen at the I and Z band level.

x 32,000

B. LS of normal adult muscle.

x 32,000

Transversely orientated t-tubules featured in both muscle groups.

C. TS of (1MPN) model-adult muscle, showing invasion of extrafusal fibre by a macrophage. Arrow-heads point to pseudopodia from the macrophage cell. x's indicate islets of disintegrating muscle, akin to the " fragmentary " fibres observed in the bag_2 fibre of one ($6\frac{1}{2}$MPN) model-adult spindle.

x 5,980

D. LS of normal adult muscle, illustrating the effect of freezing on fibre ultrastructure,i.e., this is muscle from the combined histochemical/ EM experiment. Note disrupted mitochondria and interfibrillar sarcoplasm. Arrowheads indicate cisternae of sarcoplasmic reticulum forming triads with t-tubules.

x 17,100

Length of bar:

In A & B = 0.78 µm

C = 4.67 "

D = 1.47 "

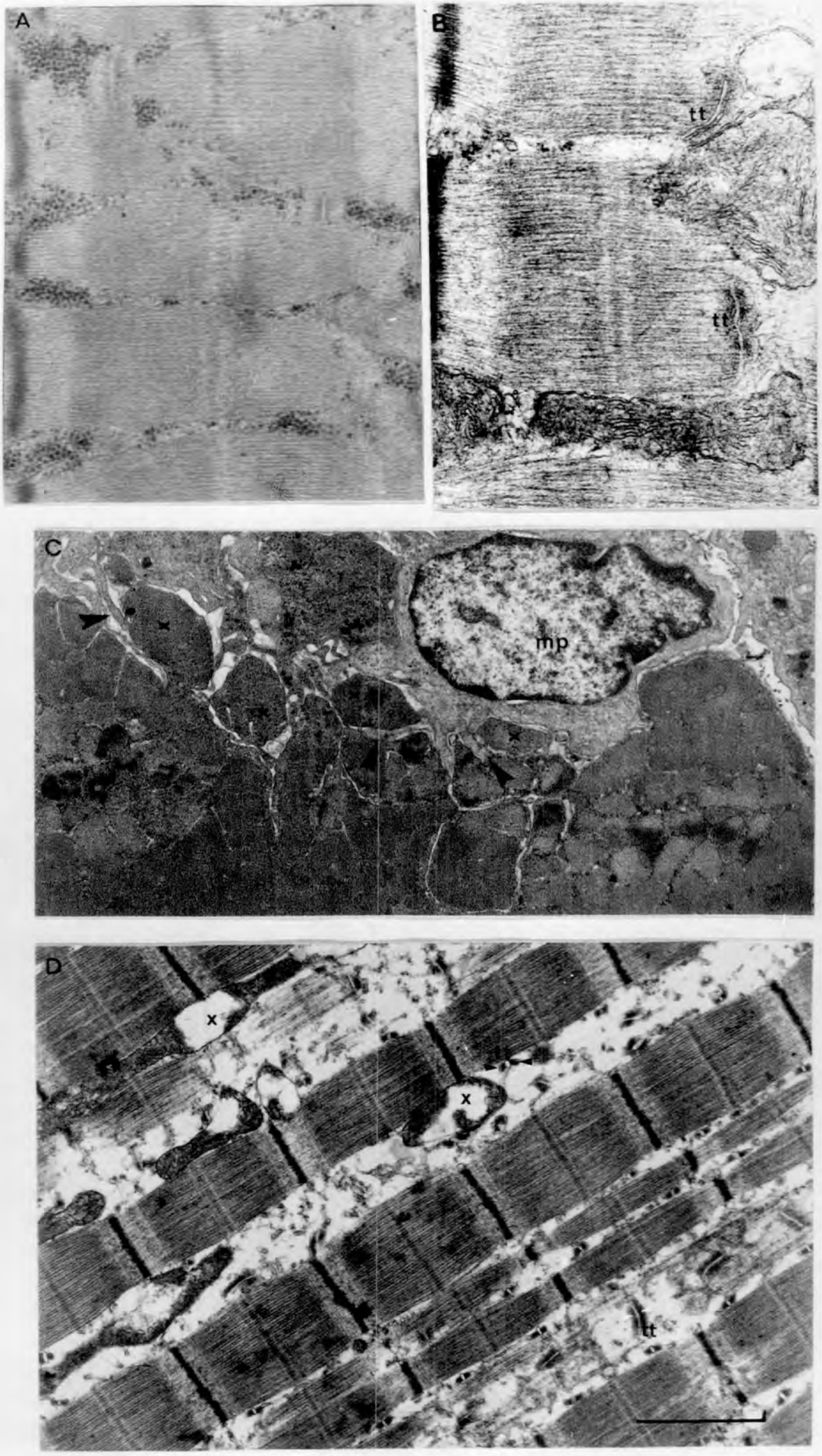

A
B
tt
tt
C
mp
D
x
x
tt

FIGURE 46 ULTRASTRUCTURE OF 1 MPN MODEL-ADULT EXTRAFUSAL MUSCLE, SHOWING THE EFFECT OF DENERVATION ON SOME FIBRES

A. TS of an affected fibre (labelled Ef), containing dense-bodied lysosomes (double arrowheads) and with sparse myofibril architecture, adjacent to two unaffected fibres.

x 13,110

B. TS of myoblast adjacent to one fibre. Nucleus appears to be in state of mitosis.

x 17,100

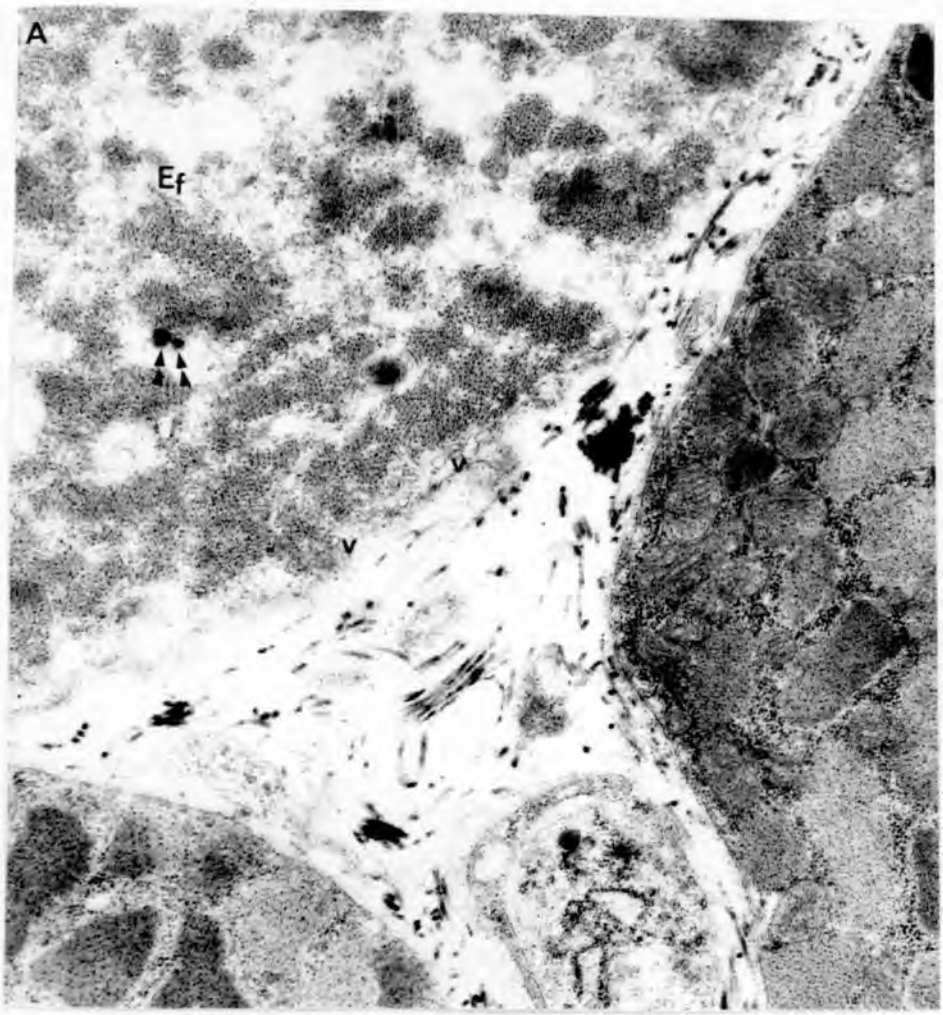
C. TS to show lipid droplets at periphery of a fibre.

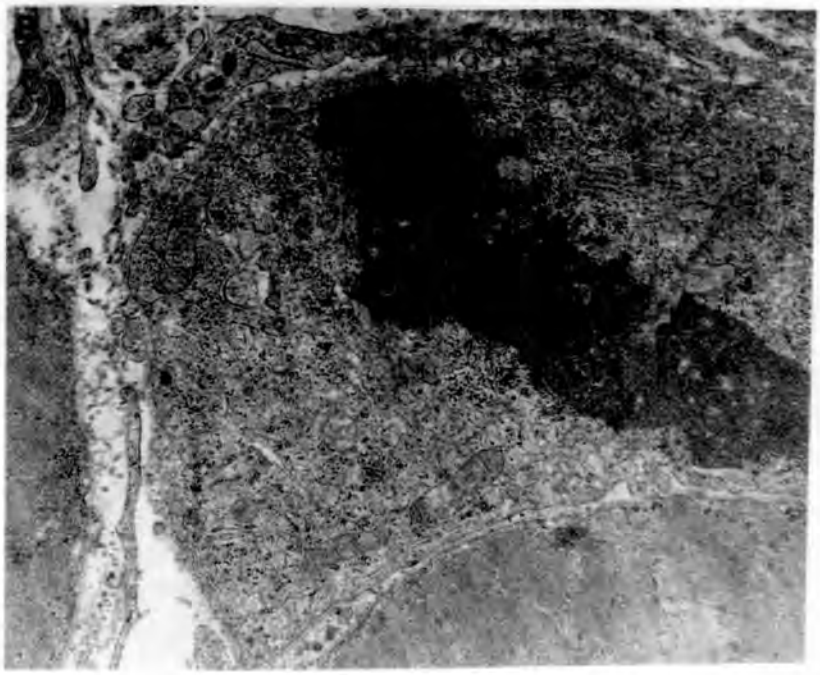
x 13,110

Length of bar:

In A & C = 1.91 μm

B = 1.47 "


A
Ef
v
v




C
I
II

FIGURE 47 ULTRASTRUCTURE OF VACUOLATED MACROPHAGES AMONG 1 MPN MODEL-ADULT EXTRAFUSAL MUSCLE

A. Arrowheads indicate vacuoles containing electron-dense material.

x 12,600

B. High power TS. Note the variable size of the vacuoles and their different staining intensities and granularity. Several of the vacuoles are in various stages of pinocytosis.

x 13,110

C. Note particularly large vacuoles, all containing pale-staining substance.

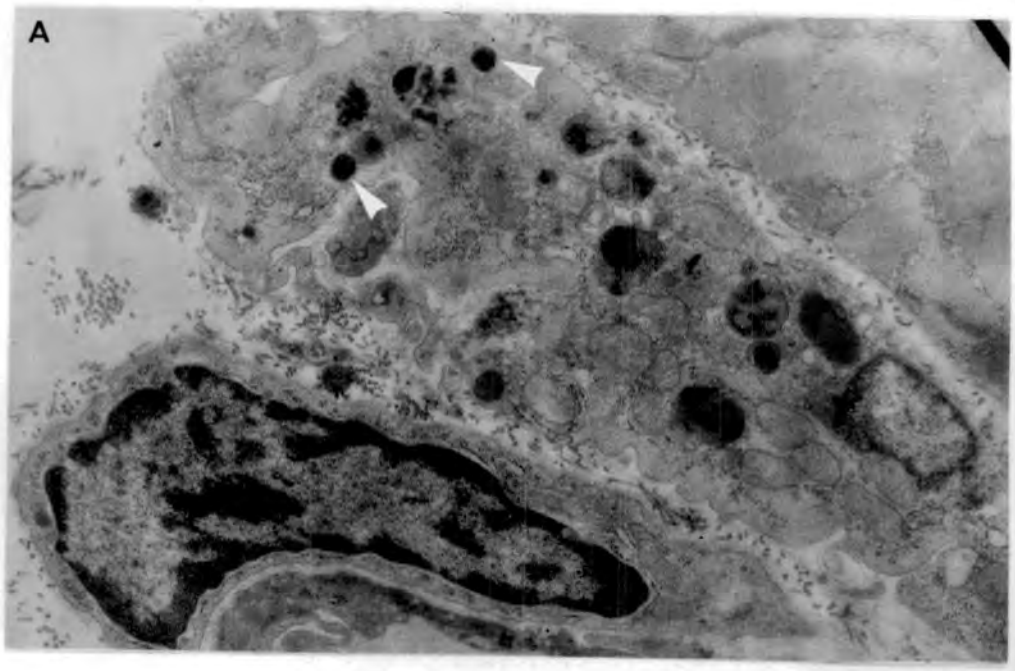
x 5,980

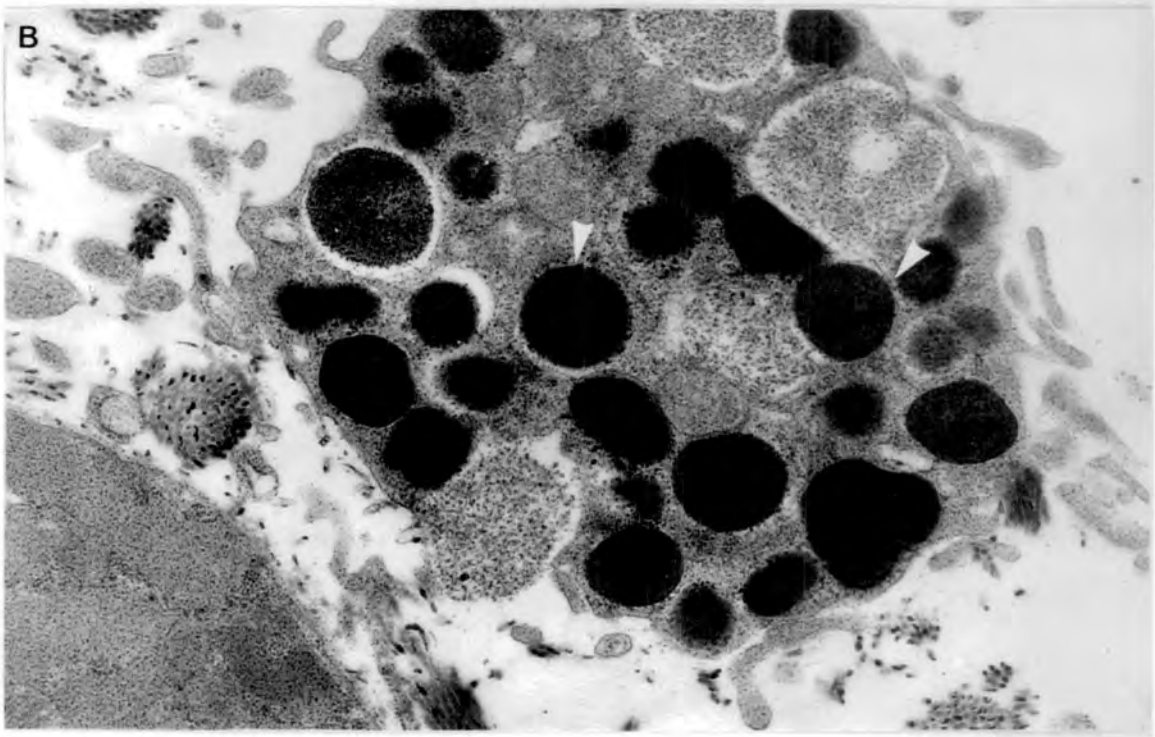
Length of bar:

In A = 2.00 μm

B = 1.91 "

C = 4.67 "


A


B

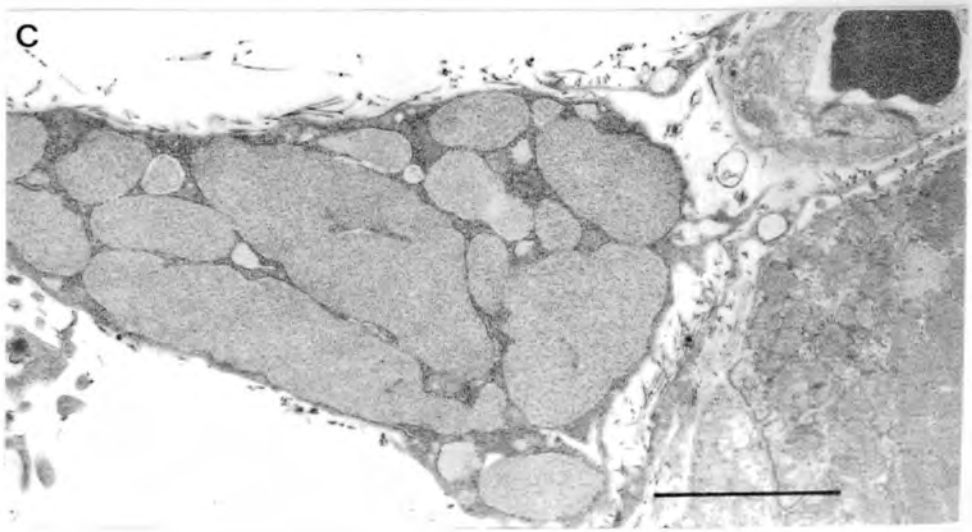

C

FIGURE 48 ULTRASTRUCTURE OF " DWARF " EXTRAFUSAL FIBRES IN MODEL-ADULT MUSCLE

A. Oblique TS, showing peripheral nuclei.

× 5,000

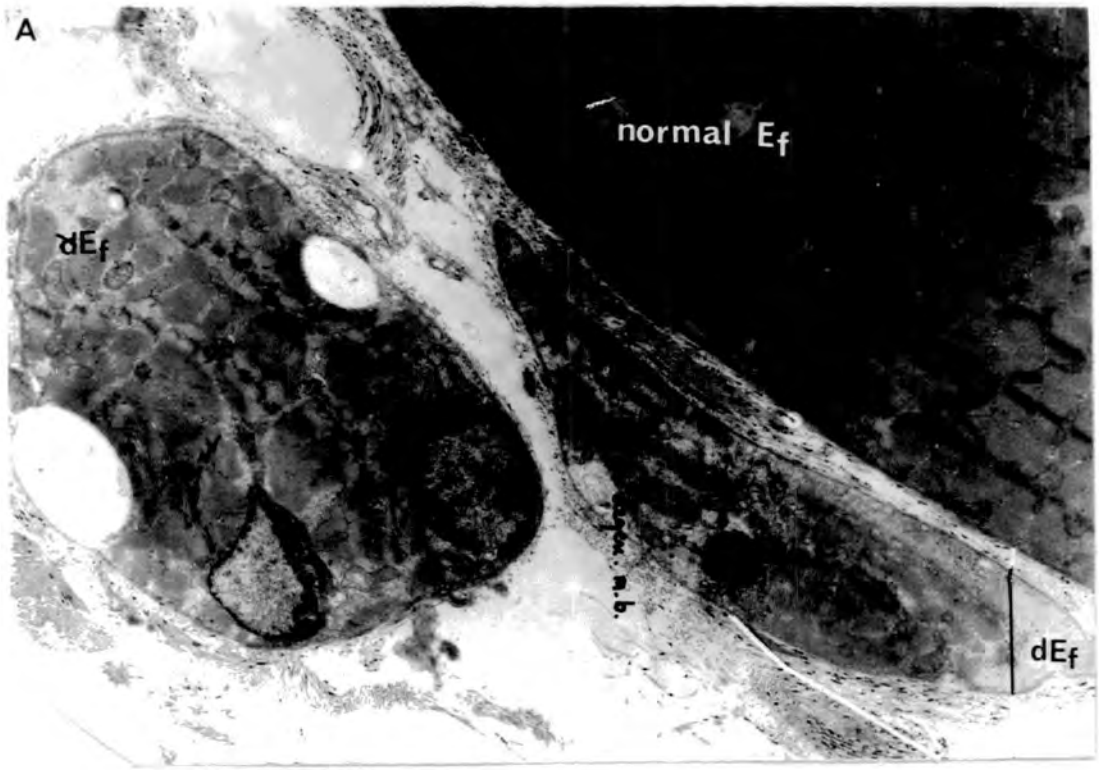
B. TS, showing abundance of collagen around a " dwarf " fibre.

× 5,980

Length of bar:

In A = 5.00 μm

B = 4.67 "


A
normal E_f
dE_f
dE_f

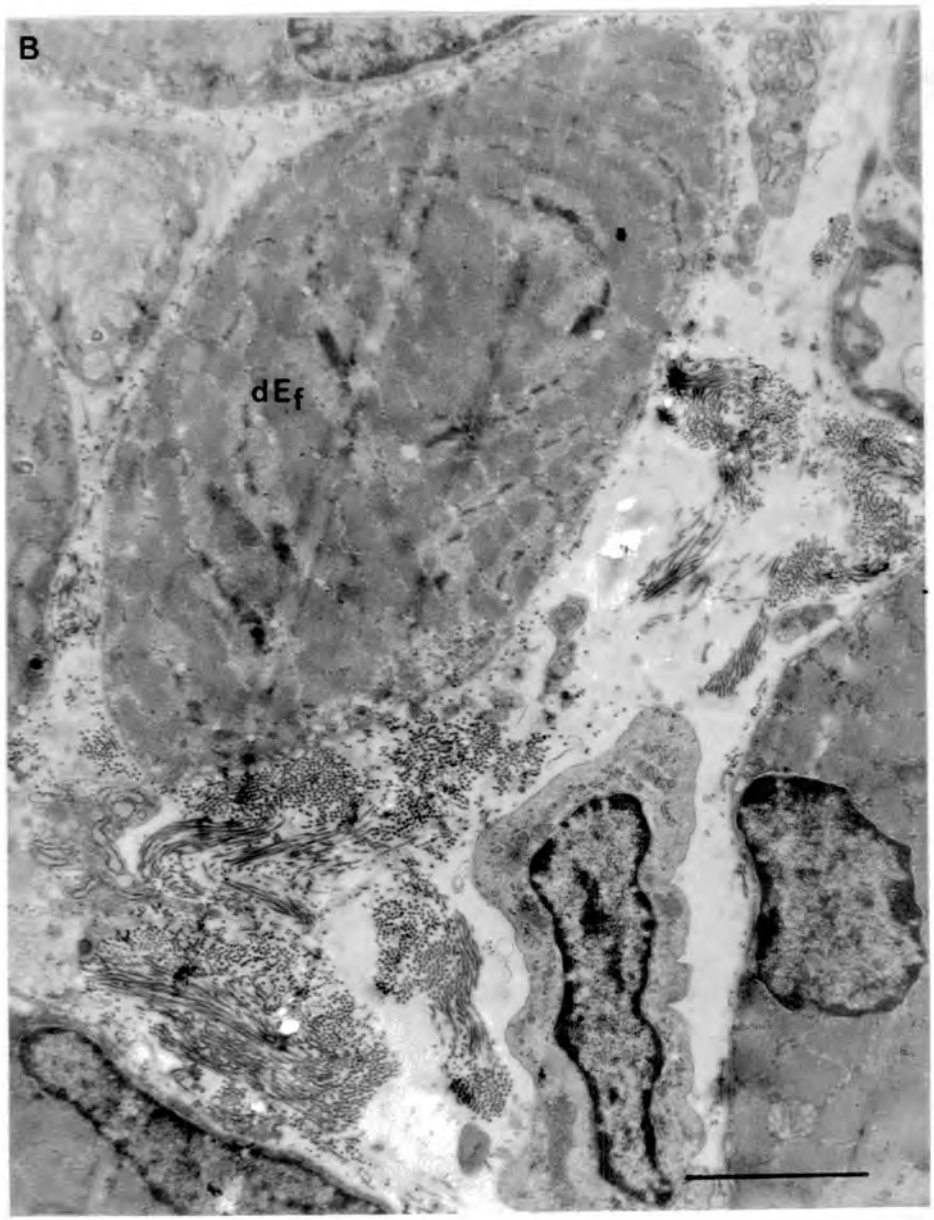

B
dE_f

FIGURE 49 SARCOMERE BANDING PATTERN OF " DWARF " EXTRAFUSAL FIBRES IN MODEL-ADULT MUSCLE

A. LS. Note size difference between the two fibres. Note also the M line.

x 5,980

B. Illustrates extensive contraction, a common feature of " dwarf " extrafusal fibres as opposed to normal extrafusal fibres. Note again, the conglomerates of collagen fibres.

x 9,660

Length of bar:

In A = 4.67 µm

B = 2.54 "

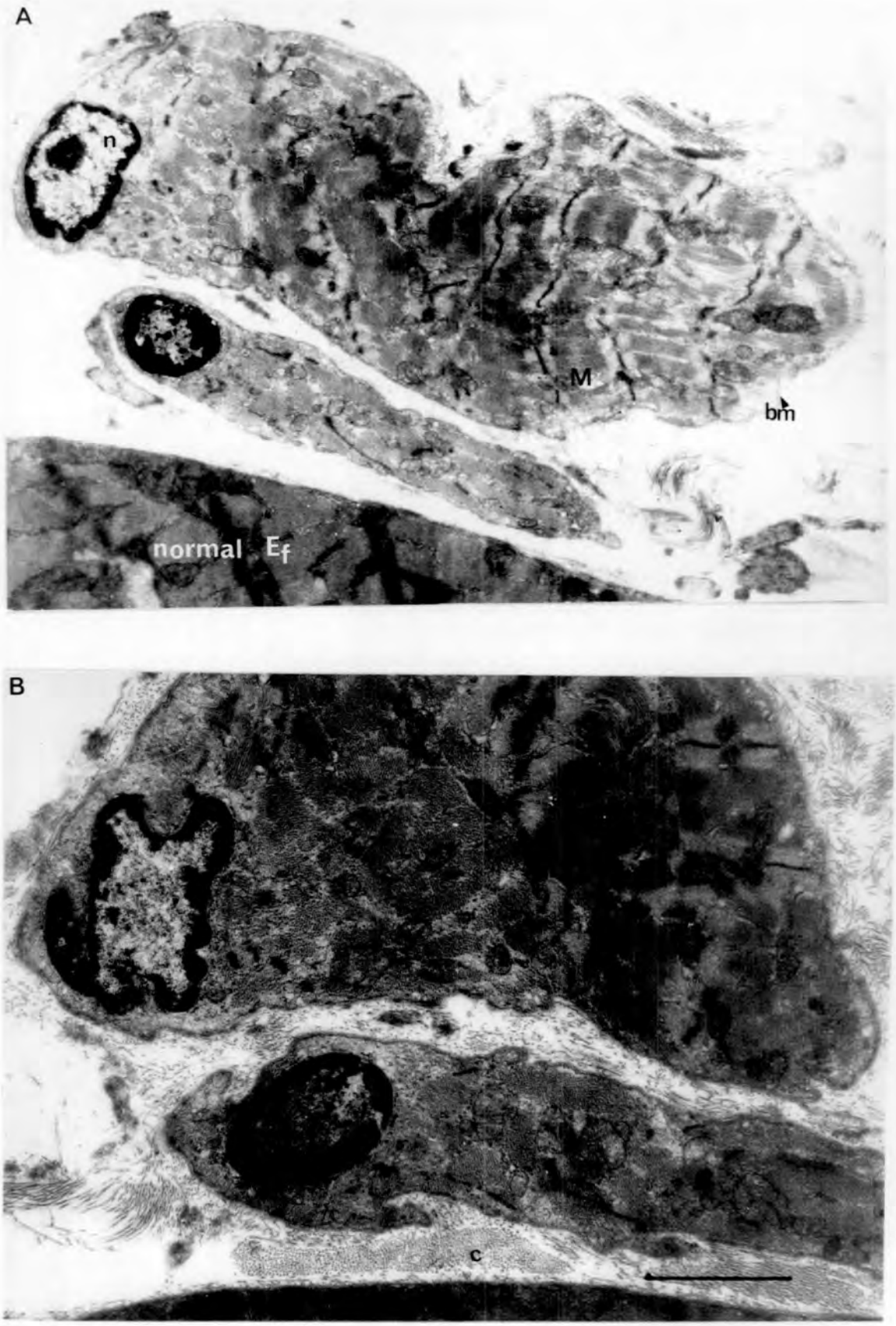

A
n
M
bm
normal Ef
B
c

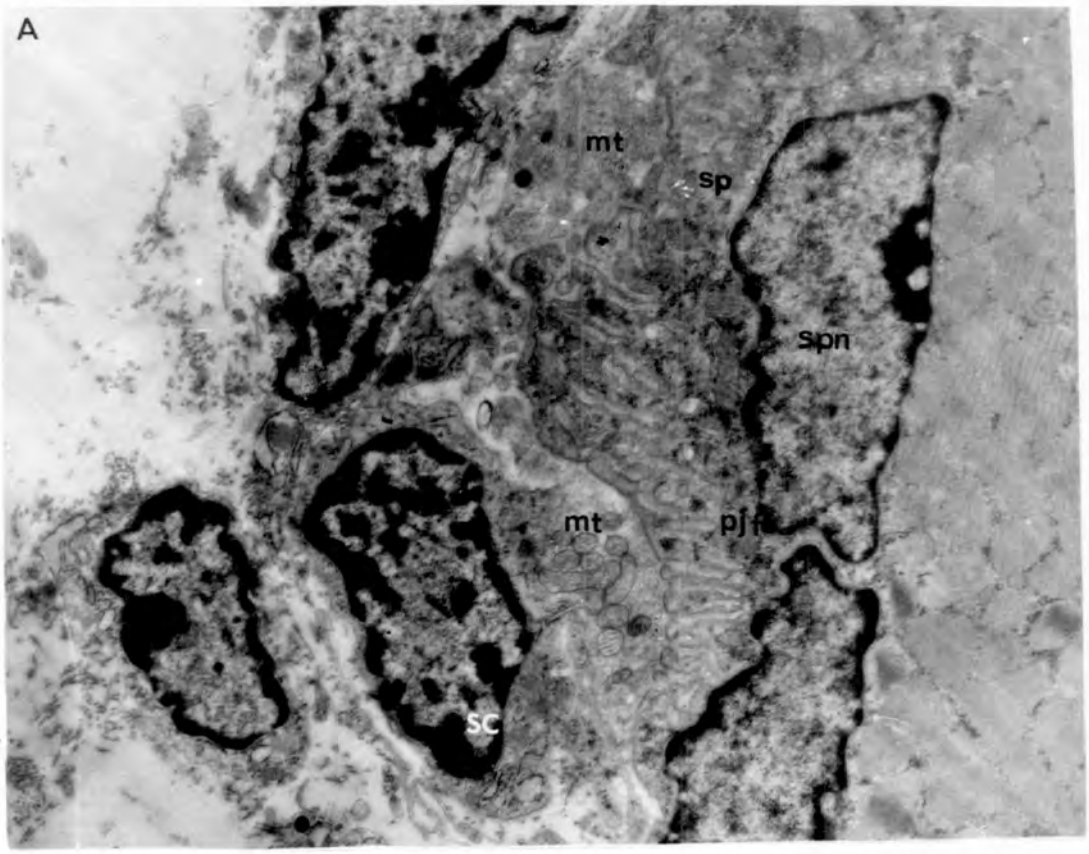
FIGURE 50 ULTRASTRUCTURE OF NORMAL ADULT EXTRAFUSAL MOTOR END PLATES

A. & B. TS's. Note substantial sole plate, long post-junctional folds lined with fused basement membrane and discrete motor terminals with Schwann cell covering.

× 9,660

Length of bar:

= 2.54 μm


A
mt
sp
spn
mt
pjf
sc

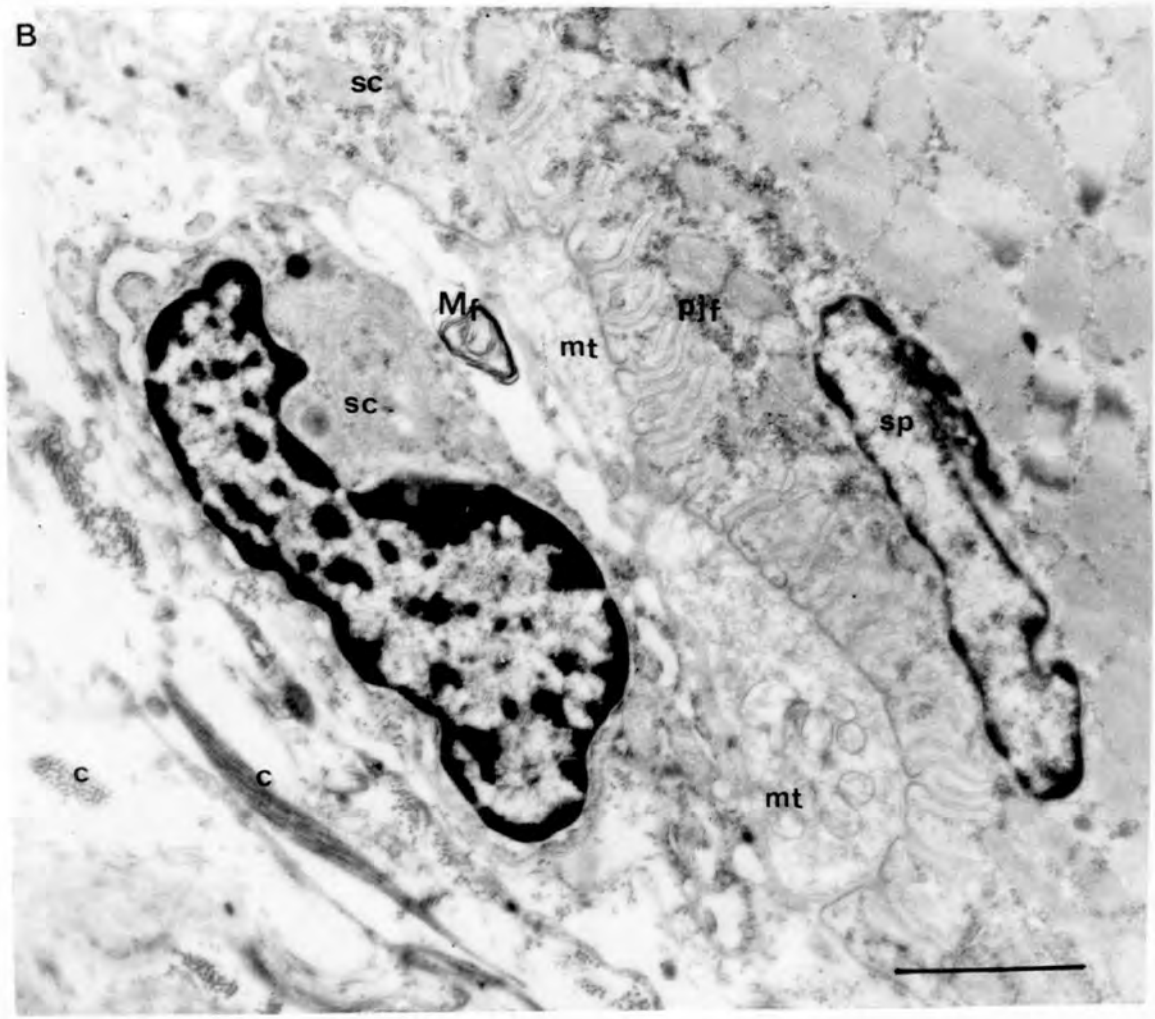

B
sc
Mf
pjf
mt
sc
sp
c
c
mt

FIGURE 51 ULTRASTRUCTURE OF MODEL-ADULT EXTRAFUSAL MOTOR END PLATES

A. TS, showing large Doyère eminence and other features similar to those in normal adult extrafusal plates. Note interstitial cell with dilated cisternae of rough endoplasmic reticulum, Golgi bodies and coated vesicles (arrowheads).

x 12,600

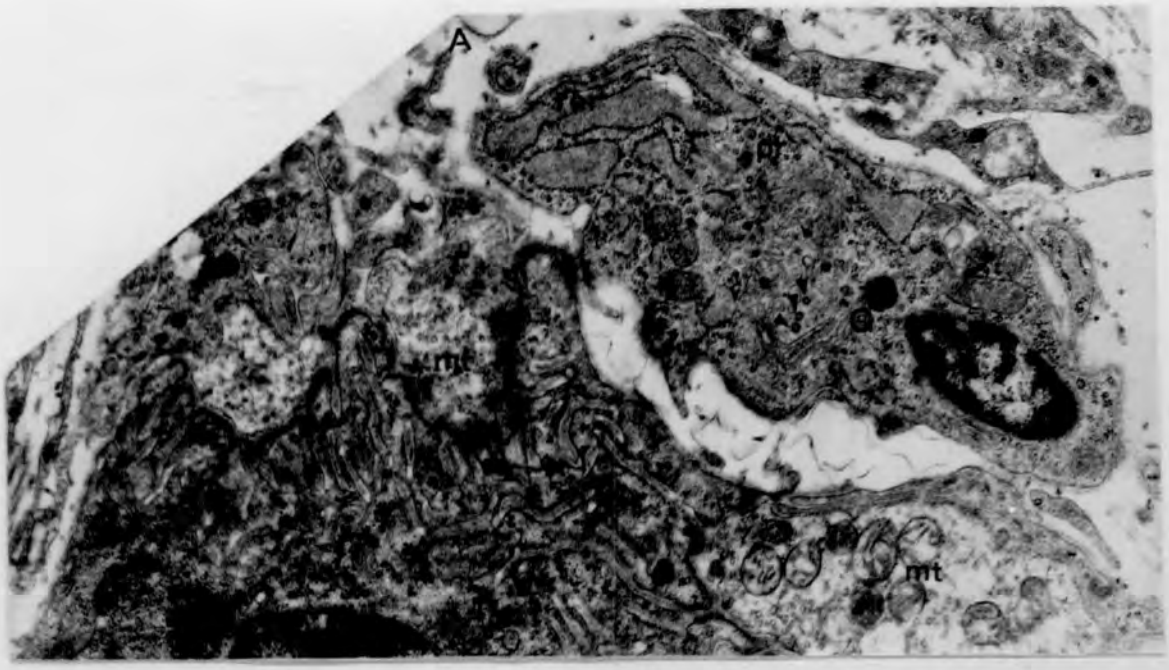
B. High power TS. Double arrowheads point to thickened post-synaptic membrane.

x 13,110

Length of bar:

In A = 2.00 μm

B = 1.91 "


A
mt
mt

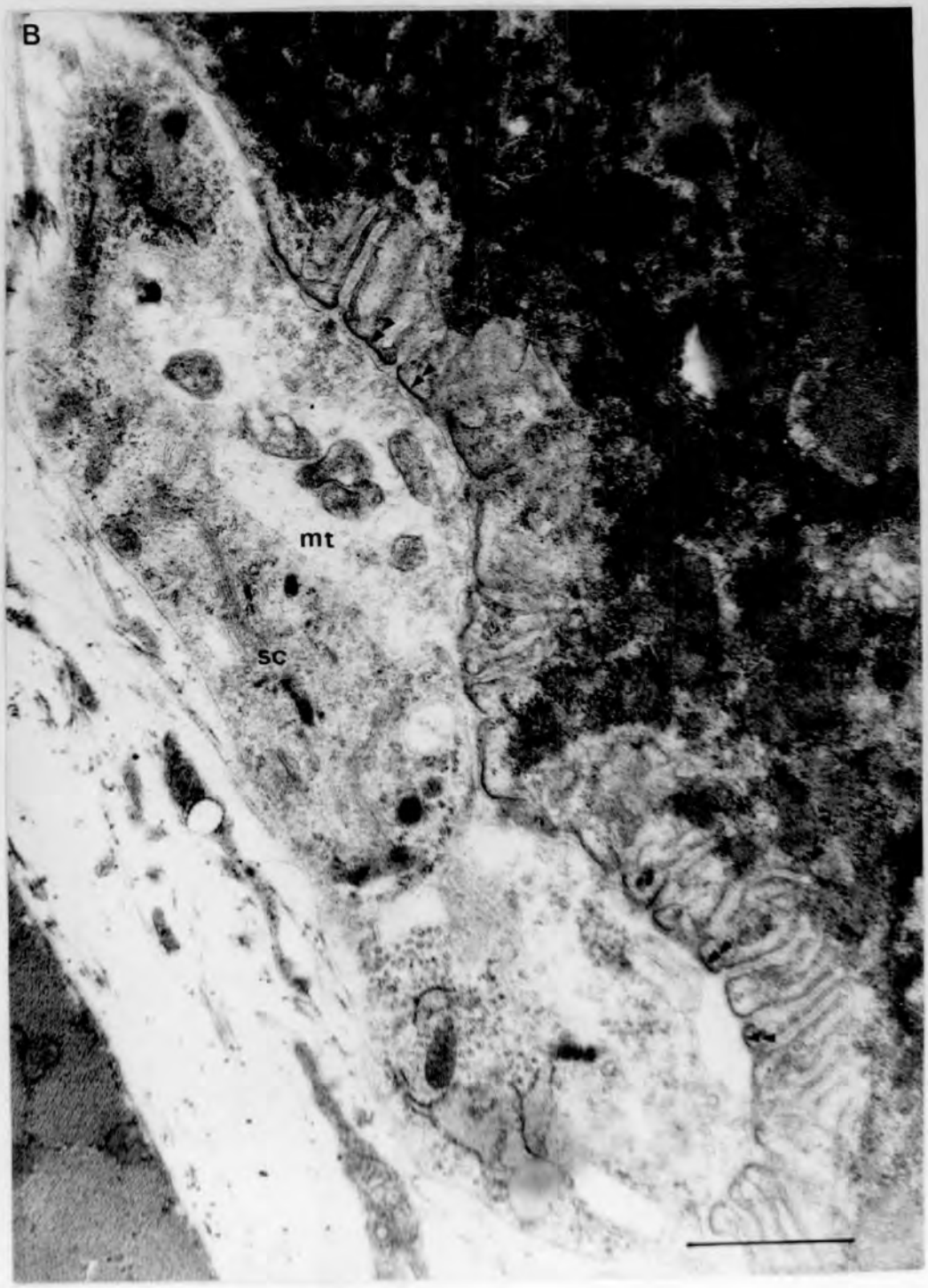

B
mt
sc

FIGURE 52 HISTOCHEMISTRY OF NORMAL ADULT SPINDLES COMPARED WITH MODEL-ADULT SPINDLES

B region depicted in all the photographs.

A. - C. P'lase. A: normal adult spindle
B: single-fibre model-adult spindle
C: two-fibre model-adult spindle

D. - F. PAS. A: normal adult spindle
B: single-fibre model-adult spindle
C: two-fibre model-adult spindle

G. - I. SDH. A: normal adult spindle
B: single-fibre model-adult spindle
C: two-fibre model-adult spindle

J. - L. ATP'ase A: normal adult spindle
B: single-fibre model-adult spindle
C: two-fibre model-adult spindle

All : x 1,000

Length of bar:

= 25.0 μm

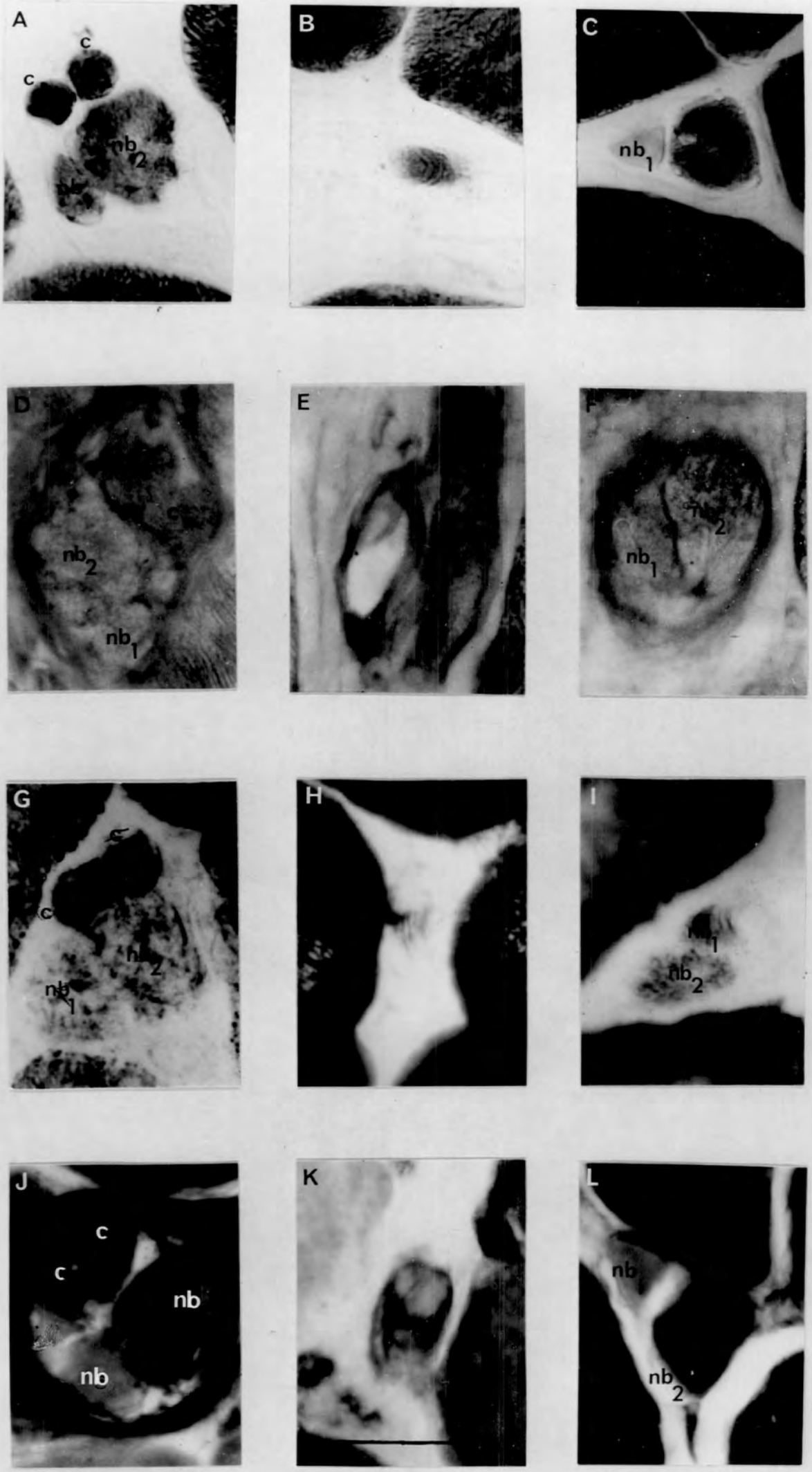

A
c
c
nb2
B
C
nb1
D
nb2
nb1
E
F
nb2
nb1
G
c
c
nb2
nb1
H
I
nb1
nb2
J
c
c
nb
nb
K
L
nb
nb2

FIGURE 53 MORPHOLOGY* OF NORMAL ADULT SPINDLE INNERVATION

A. - C. Low powers of three spindles showing the general pattern of innervation.

A. Depicts a type SP spindle.

B. Depicts a type $S_2(S_1P)$ spindle.

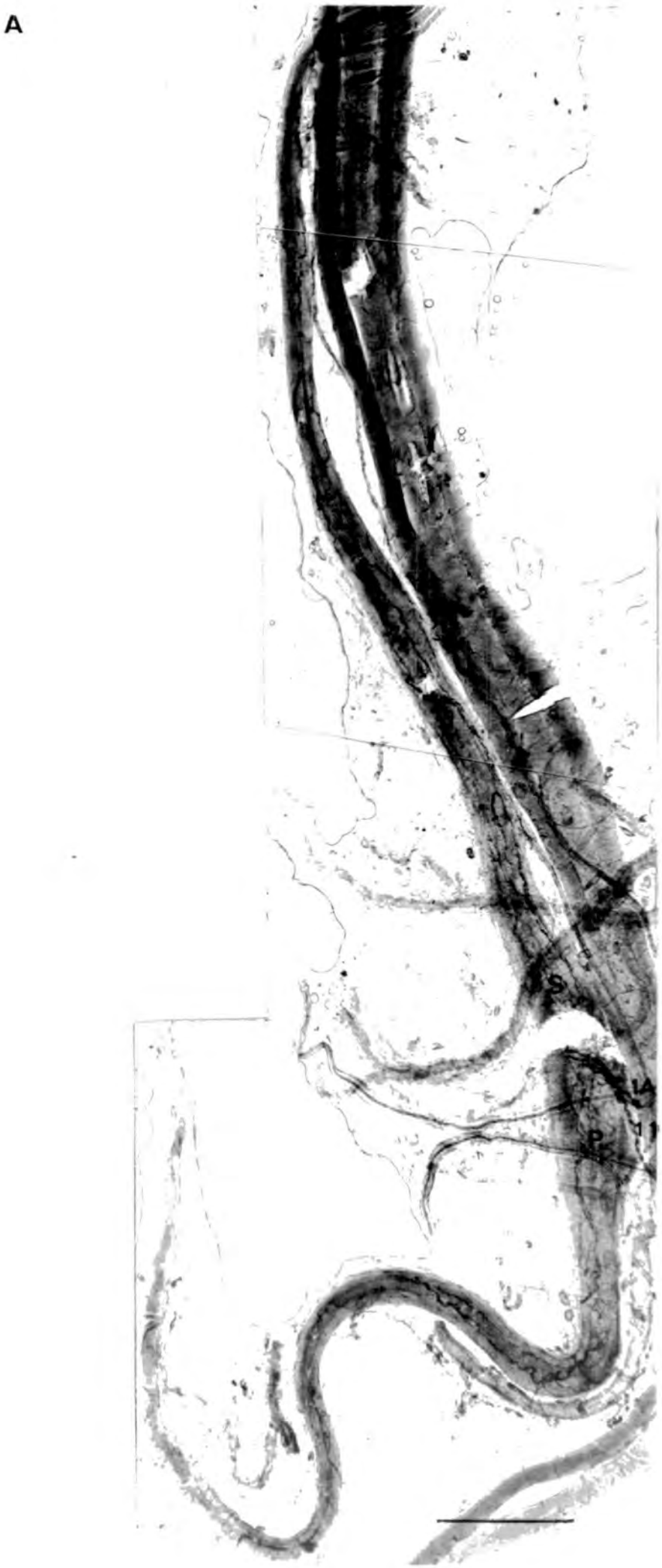
C. Depicts another (SP) spindle. Note secondary ending quite distinct from primary ending and landing specifically on the chain fibres.

All : x 160

Length of bar:

= 0.16 mm

* Teased, whole muscle preparations stained with silver. The following photographs are of preparations stained with Ragab and Tuffery's modication of the silver methods of Barker and Ip (1963) and Gladden and Kidd (1969) for nerve axoplasm:
53 - 57, 59C & D, 61B & C, 62, 63A,B & C, 64D,E & H, 65B,G & H.
The rest of the photographs (listed below) are of preparations stained with the author's modication of Winkelmann and Schmit's silver stain for nerve axoplasm:
58, 59A & B, 60, 61A, 63D,E & F, 64 A,B,C,F & G, 65A,C,D,E & F.


A
S
P


B
1A
11
11
S
S P
C
1A
11
S
P

FIGURE 54 MORPHOLOGY OF SENSORY INNERVATION IN NORMAL ADULT SPINDLES (EXCEPT E)

A. SP configuration. The IA axon branches (first-order) into two before entering the spindle. Note teasing damage, due to deliberate pressure on the coverslip in an attempt to separate the intrafusal fibres (see text, page , and contrast cat spindle preparations).

B. $S_2(S_1P)$ configuration. Each of the three sensory axons gives off two first-order branches within the capsule. The montage is imperfect because the spindle had completely split at the mid-equator (for the same reason given in A) and the two halves had to be photographed separately.

C. SP configuration. In this preparation, the two bag fibres (" NB's ") have been fortuitously separated from the two chain fibres (" NC's "), revealing the irregular endings of the secondary axon to be confined largely to the latter fibres.

D. $S_2(S_1P)$ configuration. The IA axon branches (first-order) into two within the capsule. Free arrowheads point to spirals of primary ending. Large arrowhead in B points to a branch of S_1 that lands on one of the bag fibres.

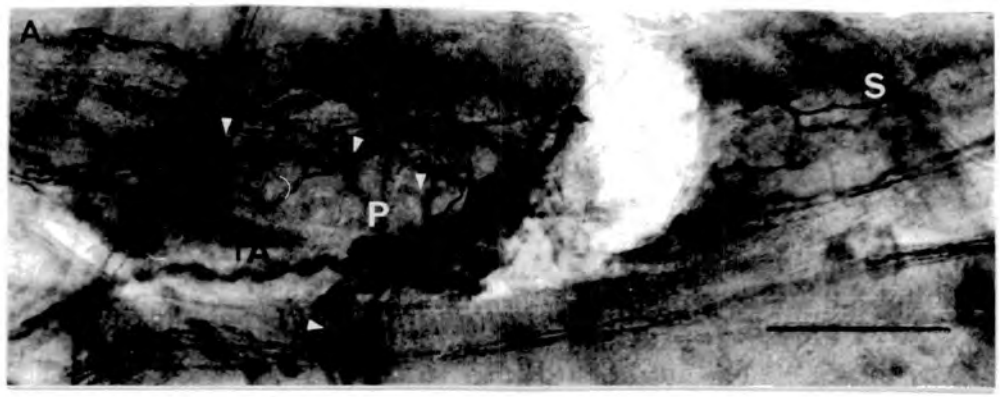
All : x 400

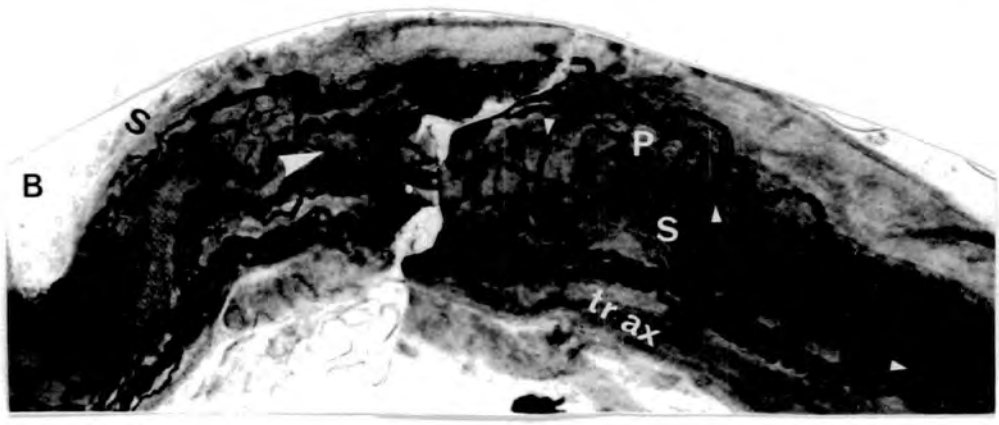
E. Tendon organ in normal muscle.

x 252

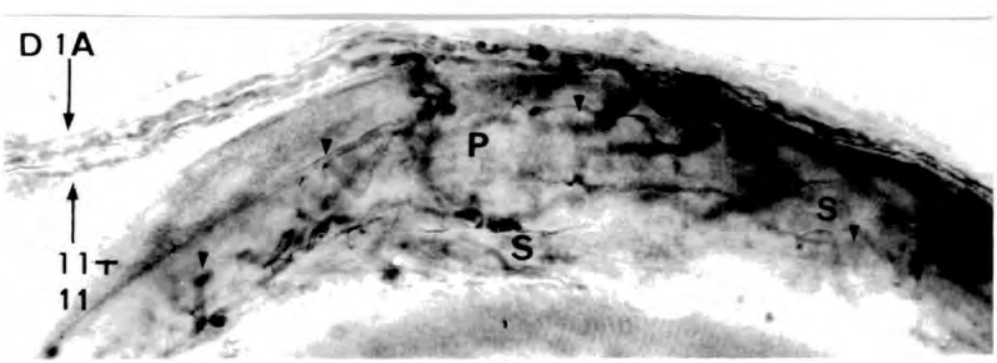
Length of bar:

In A – D = 62.5 μm


A
S
P


B
S
P
S
tr ax


C
NB
P
NC's
S
cap


D 1A
P
S
S
11
11

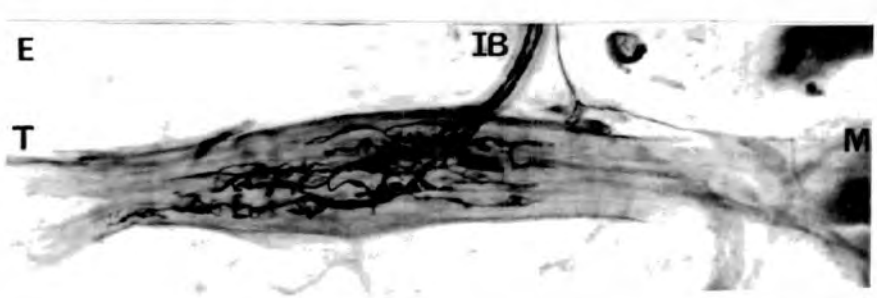
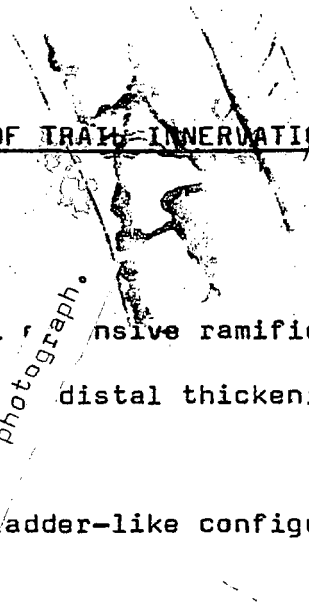

E
IB
T
M

FIGURE 55 MORPHOLOGY OF TRAIL INNERVATION IN NORMAL ADULT SPINDLES



A. Typical r nsive ramifications. Arrowhead points distal thickening.

x 400

B. Thi adder-like configuration.

x 800

C. Button-like axonal swelling of the " occasional " type.

D. Fine, twig-like branches (arrowheads).

E. Thick knob-like ending (arrowhead) and brush-like ending (double arrowhead).

F. As for A.

G. " Typical " button-like axonal swellings (arrowheads).

C - G : x 640

Length of bar:

In A = 62.5 µm

B = 31.3 "

C - G = 39.1 "

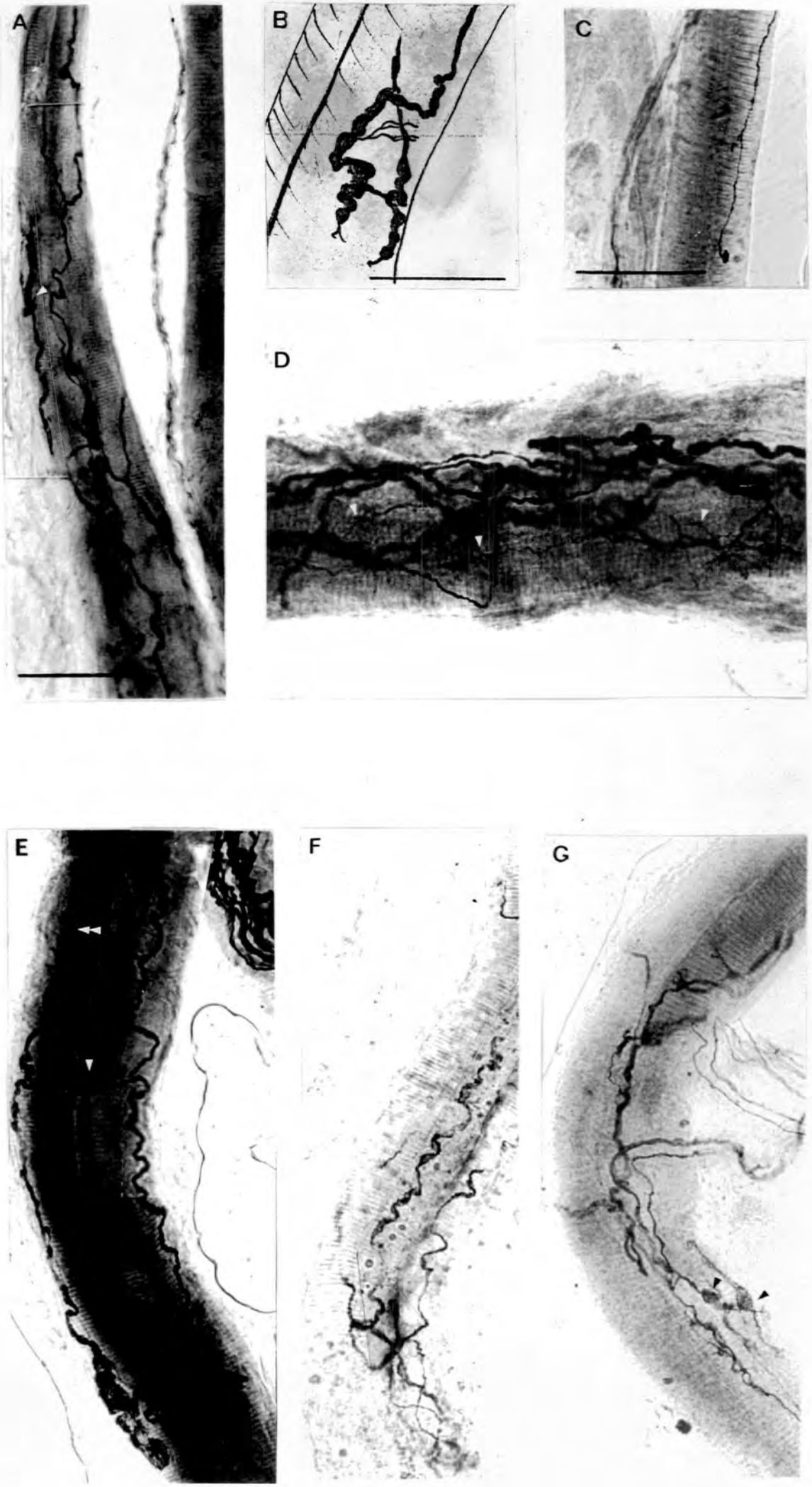

A
B
C
D
E
F
G

FIGURE 56 MORPHOLOGY OF " SHORT " FUSIMOTOR PLATES IN NORMAL ADULT SPINDLES

A. " Short " plate on a bag fibre in region B/C. Note discrete morphology. Arrowheads point to axon terminals.

B. Note more brush-like axon terminals (arrowheads) on this other " short " plate.

C. " Short " plate on bag_2 fibre. Note Doyere eminence in profile view.

D. Bifurcation of fusimotor axon (arrow) to give two " short " plates (arrowheads) each of which innervates a bag fibre.

E. Bifurcation of fusimotor axon (arrow) to give two " short " plates (arrowheads), one of which innervates a chain fibre and the other a bag fibre.

All : x 640

Insets : x 1,600

Length of bar:

In A - E = 39.1 µm

Insets = 16.0 "

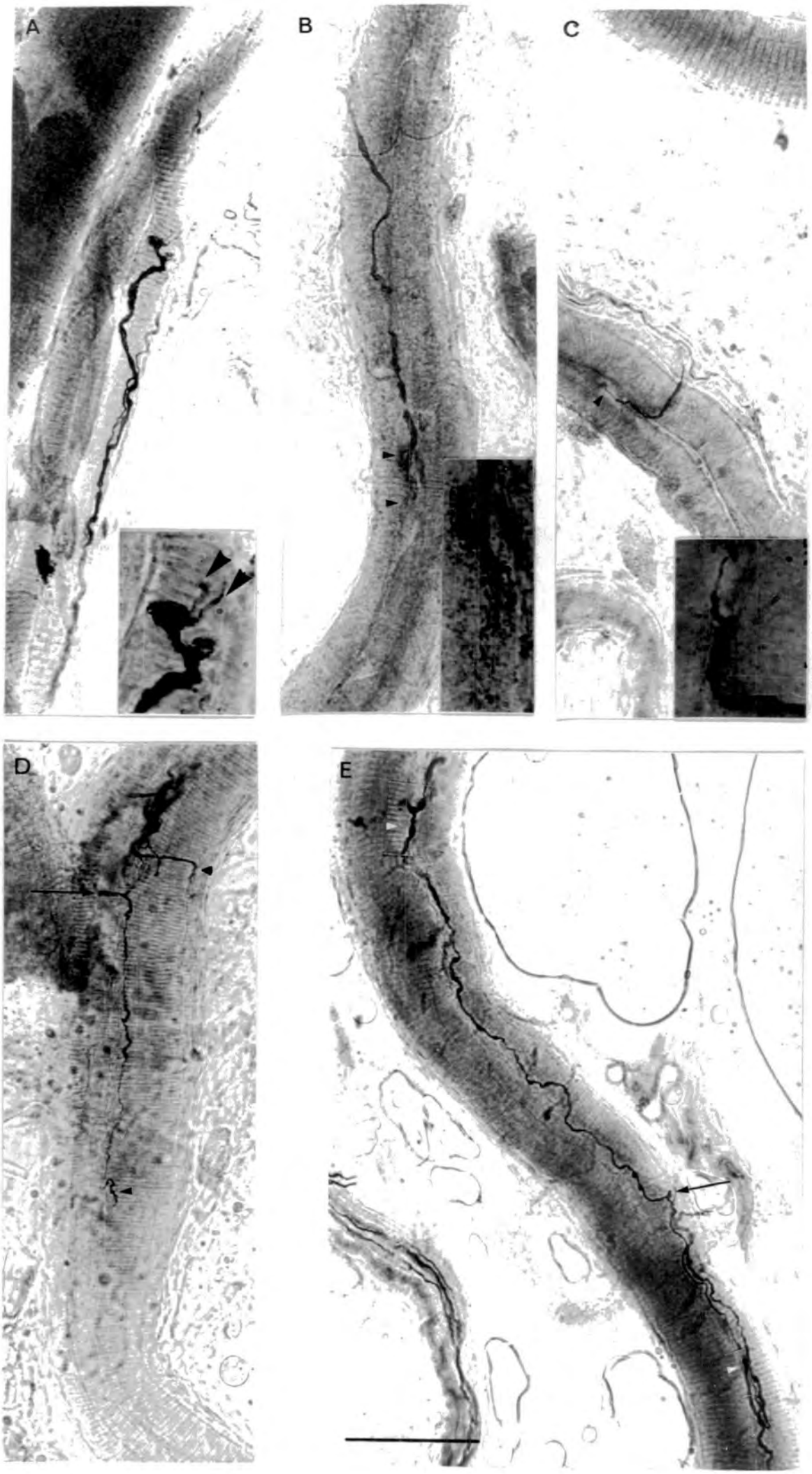

A
B
C
D
E

FIGURE 57 ULTRASTRUCTURE OF " LONG " FUSIMOTOR PLATES IN NORMAL ADULT SPINDLES

A. Note low profile, suggestive of a small Doyere eminence.

B. Arrowhead points to thick, indiscrete termination.

C. Branched " long " plate (arrowhead) on a bag fibre.

D. Bifurcation of axon (arrow) to give two plates, both on bag fibres, one of which is clearly in focus (" Nb "). The chain fibre did not extend as far polar as this.

E. Bifurcation of axon (arrow) to give two plates, one on a chain fibre and one (questionably) on a bag fibre.

All : x 640

Inset (A) : x 800

Inset (C) : x 1,600

Length of bar:

In A - E = 39.1 m

Inset A = 31.3 "

" C = 16.0 "

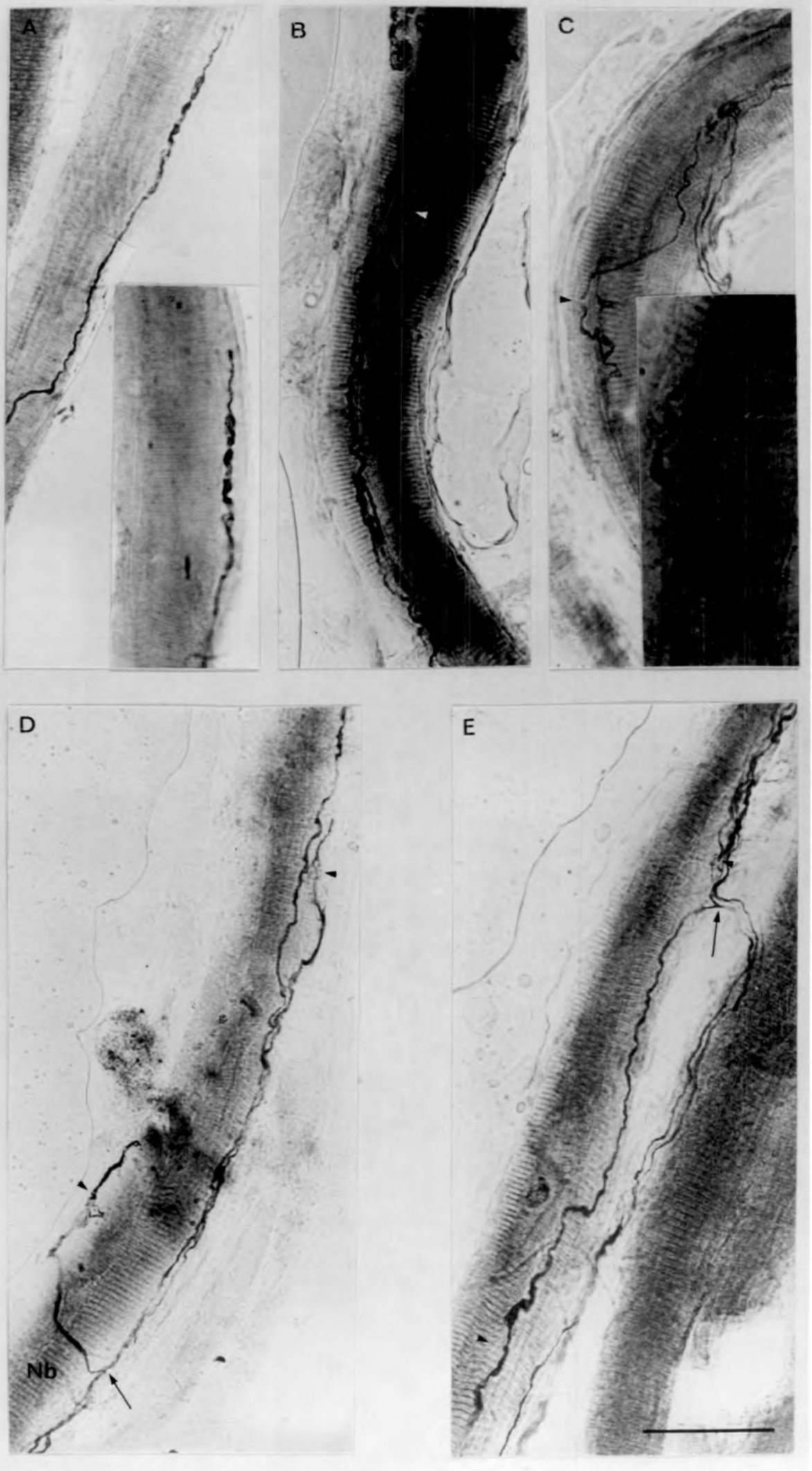

A
B
C
D
Nb
E

FIGURE 58 MORPHOLOGY OF ADULT-CRUSH SPINDLE INNERVATION

A. Low power, depicting a " simple " spindle, i.e., there are no secondary endings, only a primary ending. By Boyd's (1962) terminology, it is a spindle with a P configuration. Only one such spindle was seen in the sample studied.

x 160

B. High power of A.

C. Sensory region of a type $S_1(PS_2)$ spindle.

D. " Short " plate on a bag fibre.

E. Trail innervation. Button-like axonal swellings (arrows) of the " occasional " type. These two are part of a tripartite bifurcation of a trail axon that entered the spindle in the inner B region. The third axonal swelling is not included in the photo.

F. Trail innervation. Typical extensive ramifications.

G. Extrafusal motor end plate, of normal appearance.

B - G : x 500

Length of bar:

In A = 0.16 mm

B - G = 50.0 µm

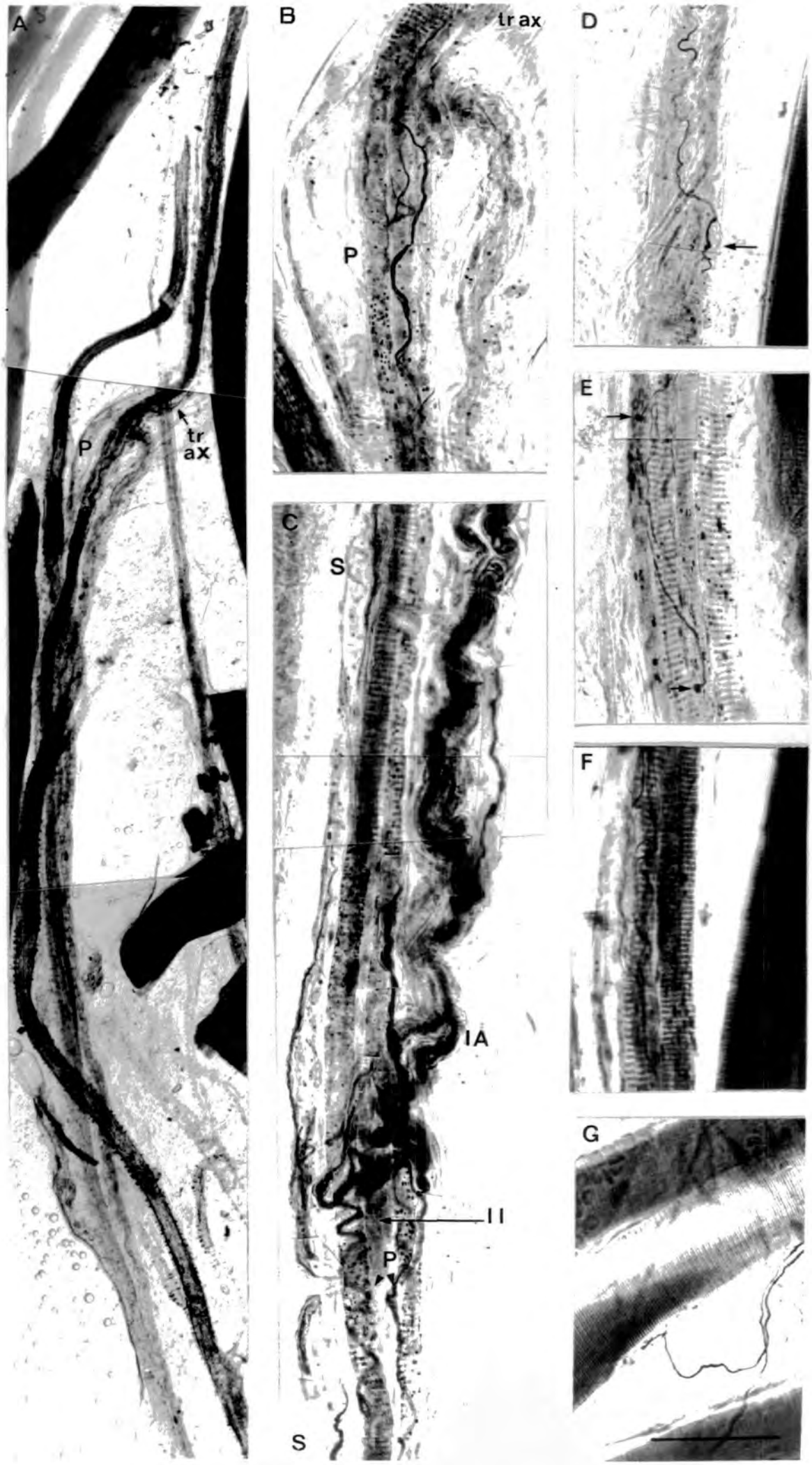

A
P
tr
ax
B
trax
P
C
S
IA
II
P
S
D
E
F
G

FIGURE 59 MORPHOLOGY OF MODEL-ADULT SPINDLE INNERVATION

A. – D. Low powers of four spindles, showing the general pattern of innervation.

A. Depicts a $S_2(S_1P)$ two-fibre spindle.

B. Depicts a (SP) single-fibre spindle.

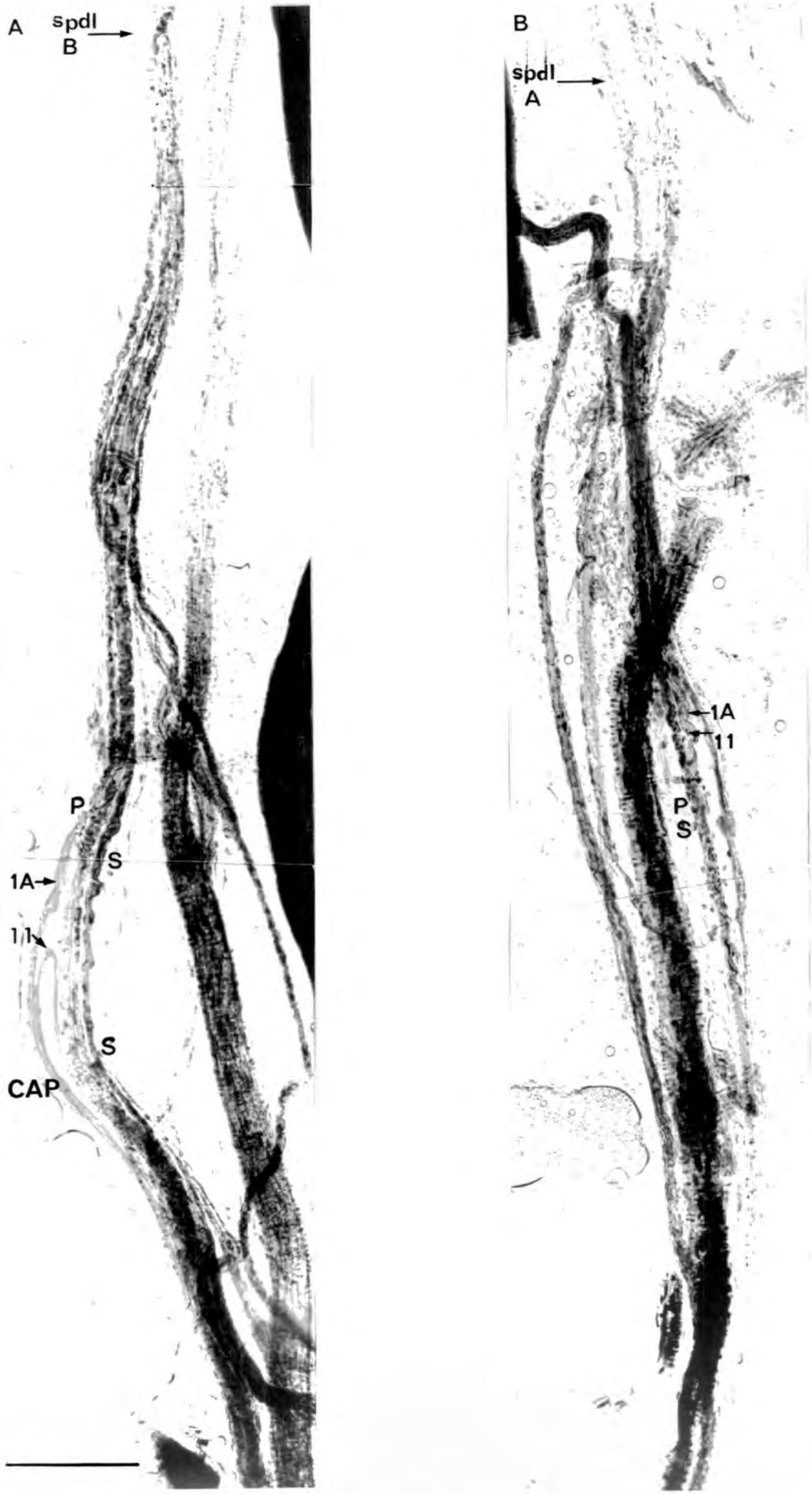
C. Depicts a " simple " (i.e., no secondary axons) tandem, single-fibre spindle.

D. Depicts an anomalous two-fibre experimental spindle with no apparent sign of sensory innervation. A periaxial space and nuclear bags are also lacking.

All : x 160

Length of bar:

= 0.16 μm


A
spdl
B
P
S
1A
11
S
CAP
B
spdl
A
1A
11
P
S

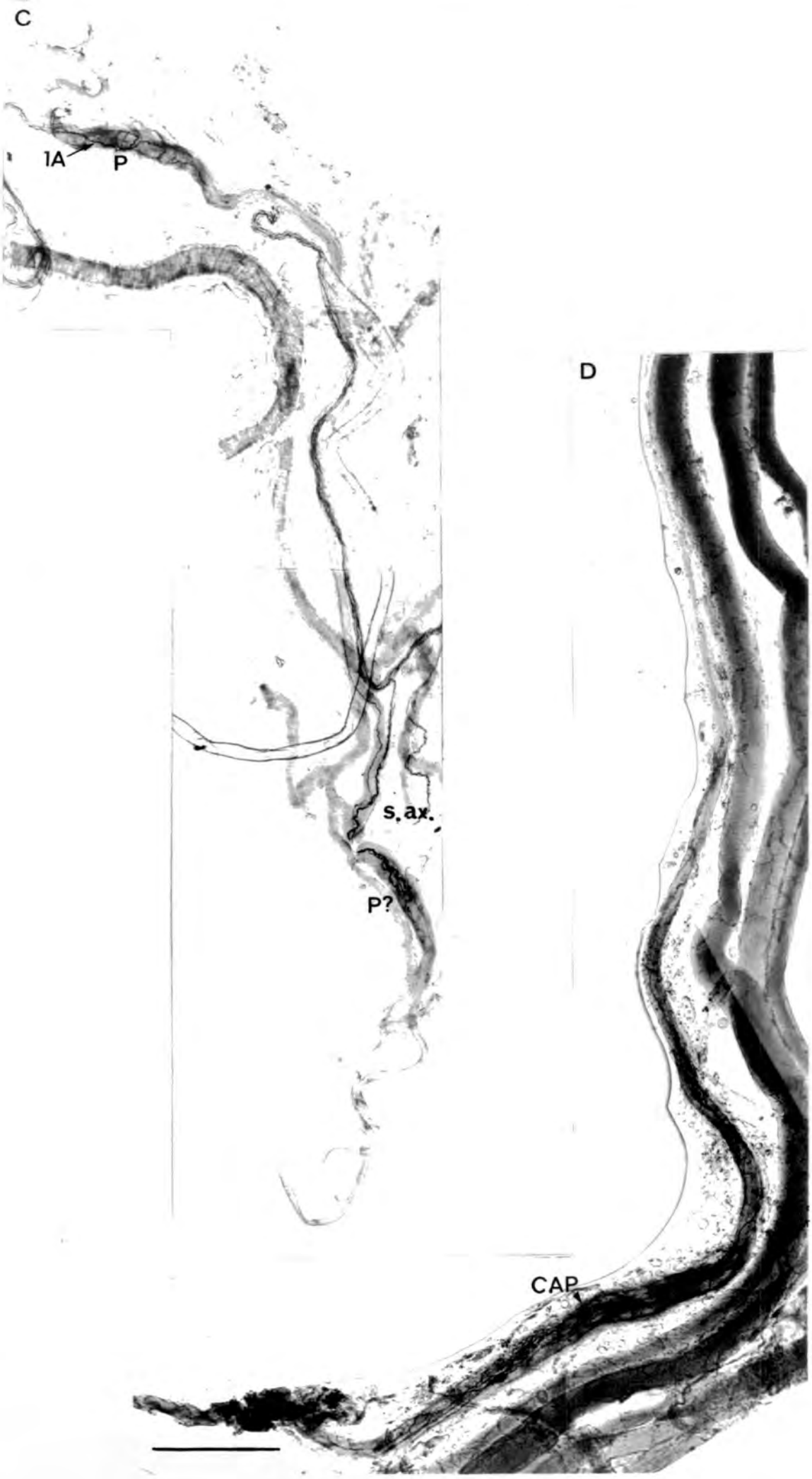

C
1A
P
s. ax.
P?
D
CAP

FIGURE 60 MORPHOLOGY OF SENSORY INNERVATION OF MODEL-ADULT SPINDLES

A. & B. Two different foci of a two-fibre spindle. A short " appendage " fibre (leftmost) arises as a small-diameter " splinter " from the bag_1 fibre in region B (nearest region A). At the mid-equatorial level, it fuses with the bag_2 fibre abruptly (arrowhead in B). This situation is similar to the one in another two-fibre sectioned for EM (figs. 25 & 29). Arrowhead in A indicates a couple of half-ring configurations of the primary ending. Arrow in A indicates blunt termination of the primary. Note delicate secondary ending in A (S_1) and compare with more robust ending (S_2) seen in B and in inset, E.
See also low power, fig. 59A.

A, B & E : x 400

C. High power of a few spirals of the primary ending on bag_2. Spiral configurations are not as extensive as in normal spindles.

D. A bulbous termination of the primary on the bag_2 fibre.

C & D : x 640

Length of bar:

In A, B & E = 62.5 µm

C & D = 39.1 "

C
B
D
A
S_1
II
IA
CAP
II
S_2
E
S_2

FIGURE 61 MORPHOLOGY OF SENSORY INNERVATION OF MODEL-ADULT SPINDLES

A. Single-fibre spindle, consisting of a bag fibre with a distinct, but small nuclear bag. Sensory with (SP) configuration. As the (intracapsular) axons in the preparation stained up rather pale with the silver stain, their outline has been accentuated in ink. This was done directly from the microscope over many changes of the fine focus.
The primary ending on the single bag fibre is limited to a few spirals and fine branches. The secondary axon is seen to " weave " itself close to the fibre. Its actual termination is questionable because of particularly in inadequate staining at this point. The trail axon seen entering the spindle with the two sensory axons, continues into the B region where it ends as a few typical ramifications. The two arrowheads point to a " dwarf " extrafusal fibre alongside a normal extrafusal fibre. See low power, fig. 59B.

x 400

B. Sensory innervation of a two-fibre spindle with SP configuration. Black arrowheads point to spirals of the primary ending. White arrowheads point to root-like endings of the secondary ending.

FIGURE 61 continued.

C. Sensory region of an anomalous two-fibre spindle experimental spindle in which there were no obvious signs of sensory axons entering the spindle at or near the mid-equator. The capsule was not " distended " as in normal adult and other model-adult spindles, indicating the likely absence of a periaxial space. Nuclear bags were not apparent in either fibre. Trail axons were seen to traverse the middle region of the spindle and to end in the adjacent B regions. Tracing from photograph.

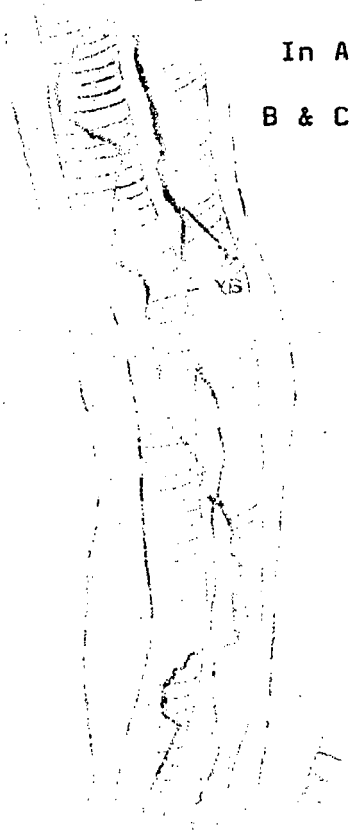
See low power, fig. 59D.

B & C : x 640

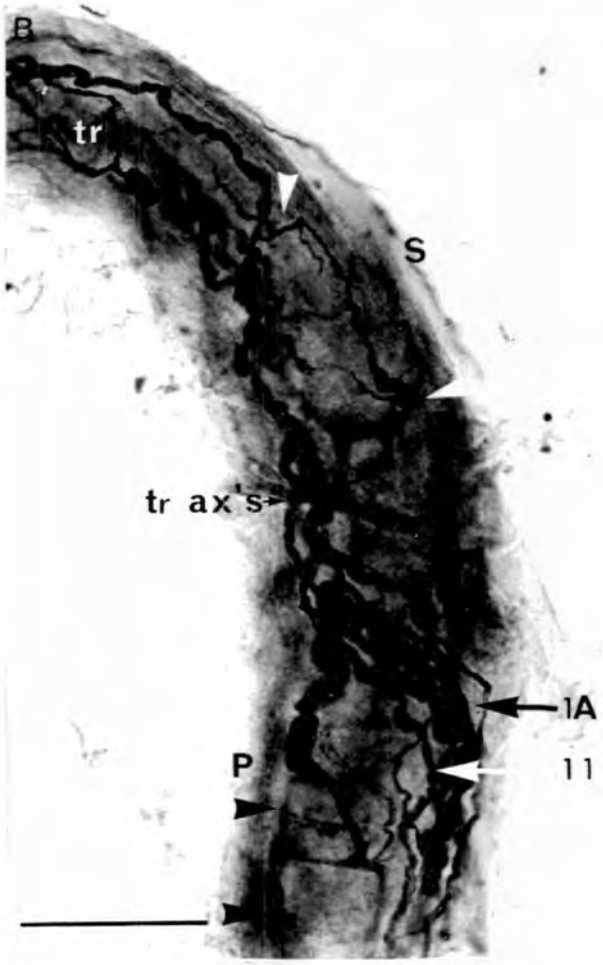
Length of bar:

In A = 62.5 μm

B & C = 39.1 "




A
cap
1A
tr ax
S
tr ax
tr ax


B
tr
S
tr ax's
1A
P
11

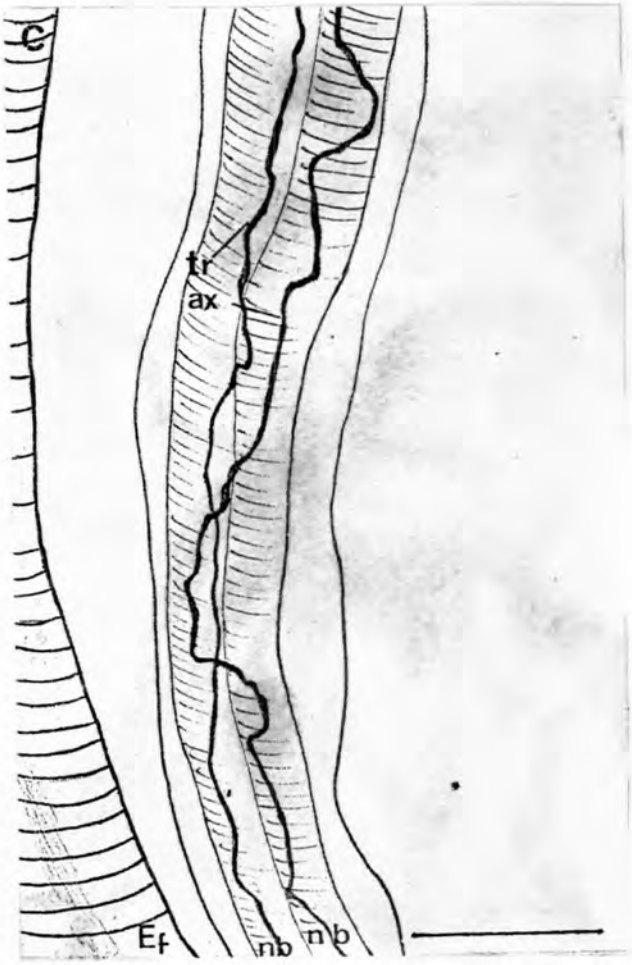

C
tr
ax
Ef
nb
nb

FIGURE 62 MORPHOLOGY OF SENSORY INNERVATION OF A SINGLE-FIBRE, TANDEM, MODEL-ADULT SPINDLE

See low power, fig. 59C.

A. In this capsule, the striations of the single fibre are obvious at the equator, unlike normal intrafusal fibres. Two trail axons are seen entering the capsule with a single primary axon (the latter is questionable).

B. &C. Other capsule at two different focuses. The sarcomere banding pattern of the fibre is absent at the equator, suggesting the presence of equatorial nuclei with only a thin peripheral cover of myofibrils. Arrowhead in B points to a bifurcation of the primary ending. The two first-order branches each form a half ring before extending on either side of the fibre to give a long, more or less straight termination. The identity of the ending labelled as trail (tr) or secondary sensory (S) in B, was impossible to ascertain. One trail axon that entered the spindle with the primary axon, made an S-like twist within the periaxial space (see C), then left the spindle without terminating.

All : x 400

Length of bar:

= 62.5 μm

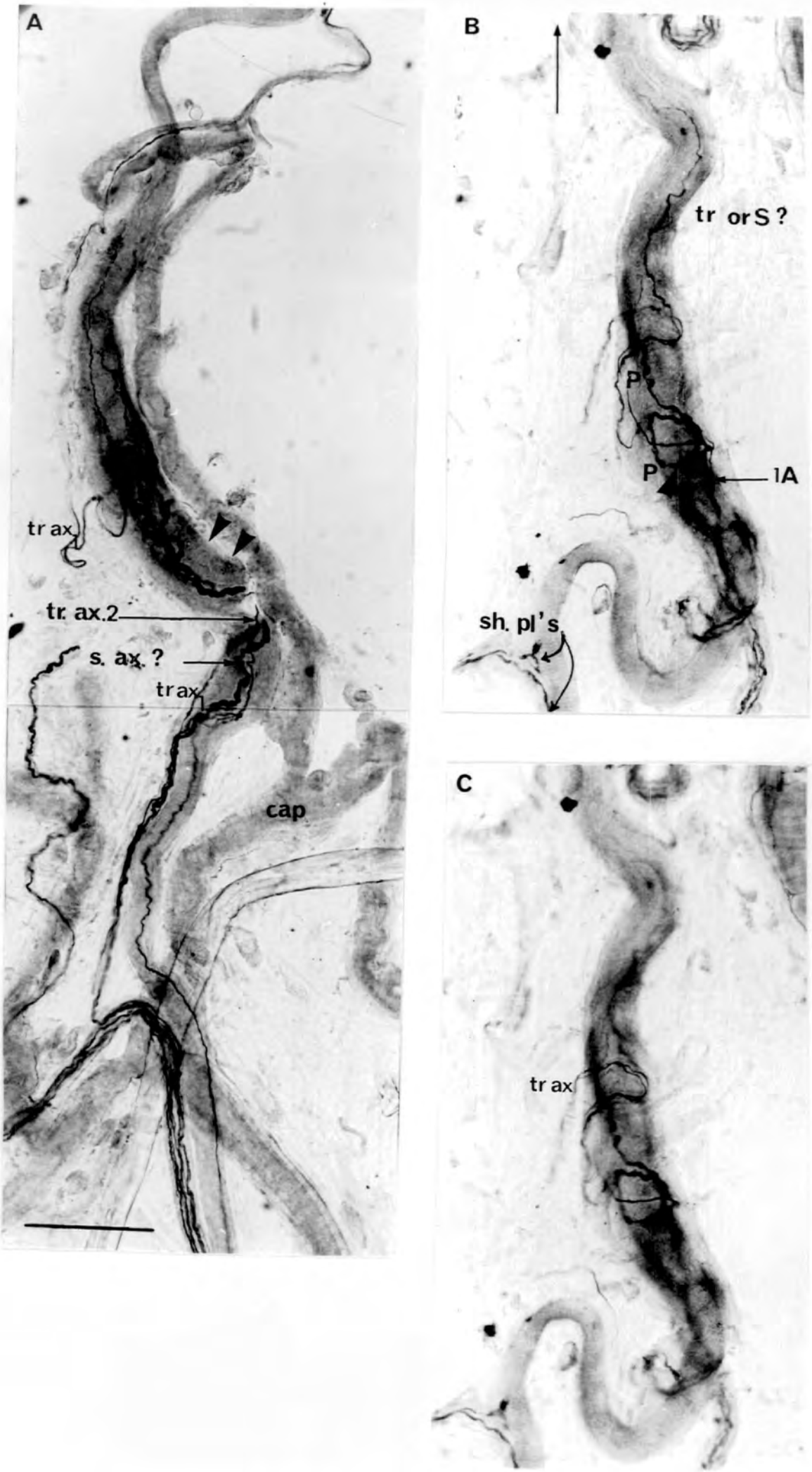

A
tr ax
tr. ax.2
s. ax. ?
tr ax
cap
B
tr or S ?
P
P
lA
sh. pl's
C
tr ax

FIGURE 63 MORPHOLOGY OF TRAIL INNERVATION OF MODEL-ADULT SPINDLES

A. Normal looking robust termination in a two-fibre spindle.

B. Button-like axonal swelling (arrow) of the " occasional " type on the bag fibre of a single-fibre spindle.

C. Questionable trail ending on the bag fibre of a single-fibre spindle. Arrow points to single, long, delicate termination.

D. Two-fibre spindle. Fine, root-like branches (arrowheads) of trail ramifications comparable to some normal configurations. Arrow points to typical axonal swelling.

E. Different level of focus to D to show thick, bulbous trail termination (arrowhead) in the outer B region.

A - E : x 640

F. Inner B region of a two-fibre spindle. Arrow points to a particularly large, typical axonal swelling.

x 800

Length of bar:

In A - E = 39.1 µm

F = 31.3 "

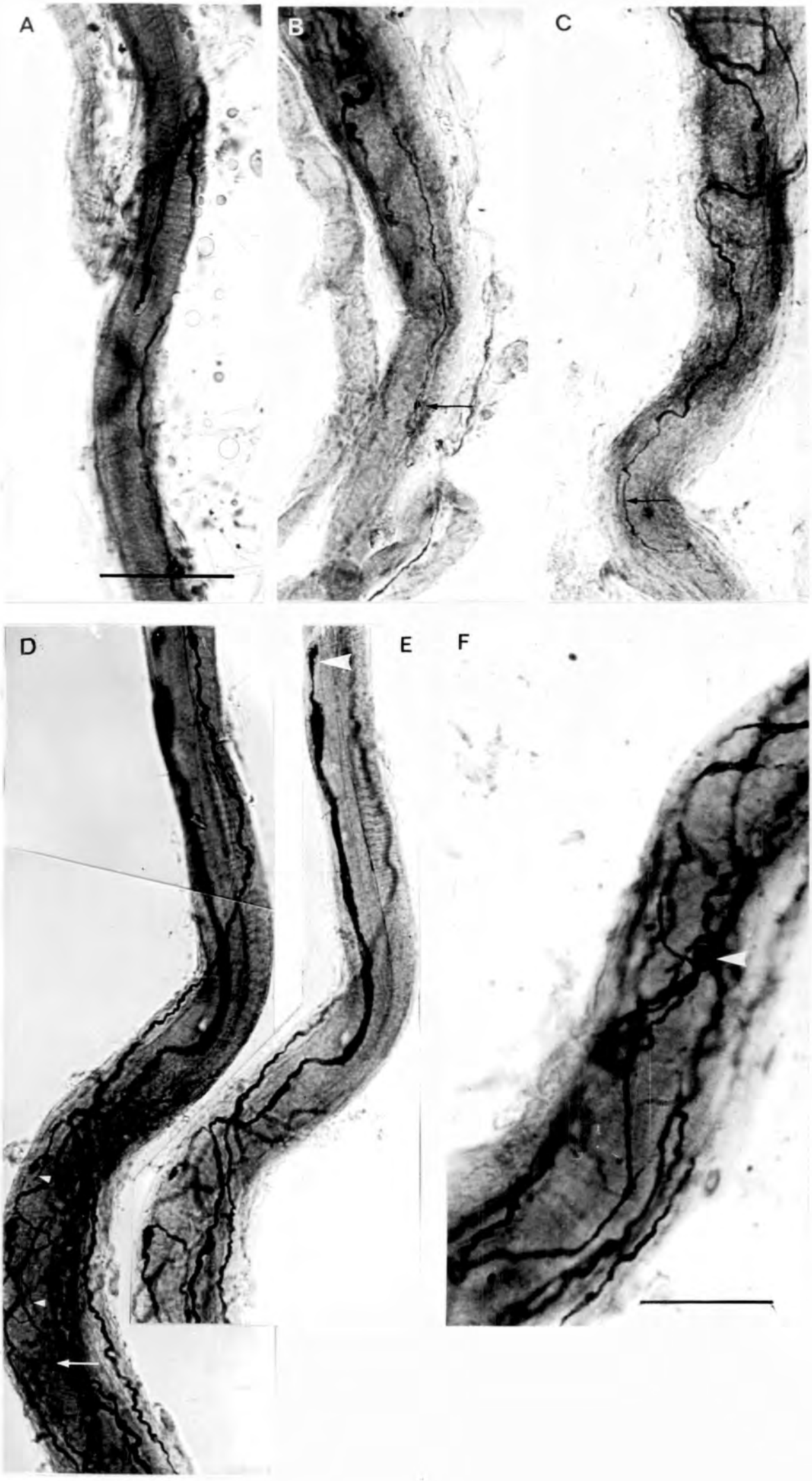

A
B
C
D
E
F

FIGURE 64 MORPHOLOGY OF " SHORT " FUSIMOTOR PLATES OF MODEL-ADULT SPINDLES

A. B/C region of a " two-fibre " spindle. The " short "-plate axon entered the spindle at this polar level. The plate itself is indicated by arrowhead.

x 640

Inset : x 1,600

B. Similar region of another two-fibre spindle. " Short " plate (arrowhead) with a simple hook-like configuration.

x 640

Inset x 1,600

C. Mid-B region of a two-fibre spindle. Arrow points to bifurcation of " short "-plate axon. Black arrowhead points to a simple bulbous " short " plate. White arrowhead indicates other branch. The ending of the latter (on the other bag fibre) is obscured by an irregular deposit of the silver stain.

x 640

D. & E. Mid-B region of a single-fibre spindle. Two " short " plates on the bag fibre close together. Arrow in E points to the bifurcation of the " short "-plate axon. Note the preter-minal axons of one of the plates.

D : x 640

E : x 1,600

FIGURE 64 continued

F. Intercapsular region of an anomalous, tandem single-fibre spindle. Anomalous, because it consists of a single bag fibre in the first capsule, while in the second capsule, two other fibres are present with it: another bag fibre and a chain fibre. The latter fibres arose, as very small-diameter fibres, at the mid-equatorial level of the " first " capsule, but outside and quite apart from it. They became enclosed only by the second capsule.
The axon of the ending shown here (arrowhead) entered the spindle in the " first " (single-fibre) capsule. It is not certain whether the ending is a " short " plate or, perhaps, a trail axonal swelling of the " occasional " type.

x 640

G. " Short " plate in the inner C region of a two-fibre spindle.

x 1,600

H. The only instance of a plate which could be regarded as a " long " plate. Two-fibre spindle in region B/C. Axon entered spindle at this level.

x 640

Insets : x 1,600

Length of bar:

In A - F & H = 39.1 µm

Insets & G = 16.0 "

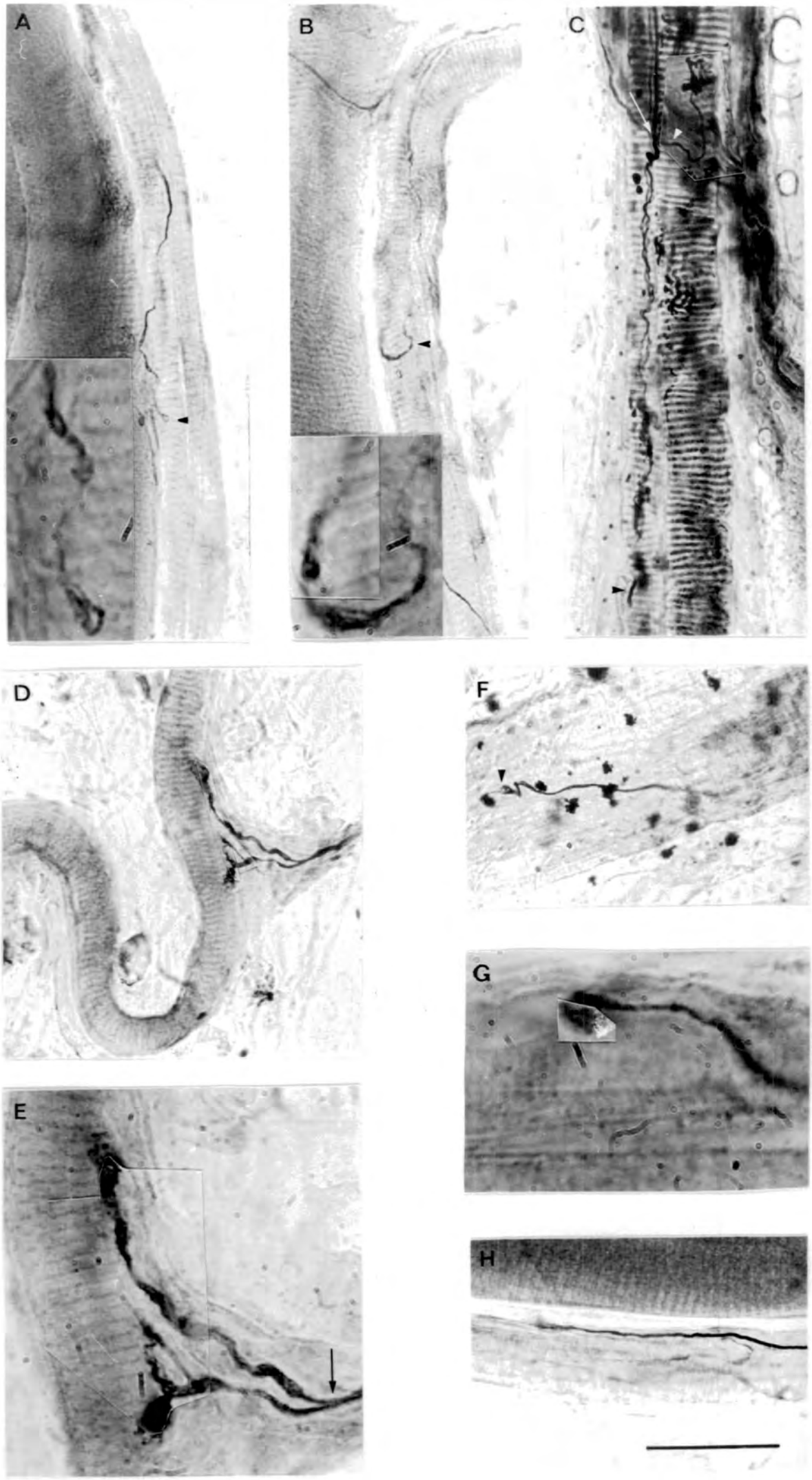

A
B
C
D
F
G
E
H

FIGURE 65 MORPHOLOGY OF EXTRAFUSAL FIBRE INNERVATION OF NORMAL ADULT AND MODEL-ADULT MUSCLE

A. Normal adult muscle. Arrowhead indicates branching of an α axon. One of the two plates from this bifurcation is shown in the figure (top right). Note its prominent Doyere eminence, which manifest because of its profile view on the muscle fibre.

Another plate is shown in the bottom right hand corner.

B. Normal adult muscle. Arrowhead indicates branching of an α axon. The two plates land on adjacent fibres.

C. Normal adult muscle. Atypical, long end plate.

D. Model-adult muscle. Two end plates on adjacent fibres. The plates stem from the same α axon, although the bifurcation is not included in the photo. These particular plates extend over a larger area of the fibre than normal, and appear to have more axon terminals.

E. Model-adult muscle. Branching of an α axon ; one of the plates from this bifurcation is shown here.

Another plate is also depicted in the inset.

F. Model-adult muscle. A long end plate. Arrowheads indicate axon terminals.

G. Model-adult muscle, illustrating collateral

FIGURE 65 continued

branching of an α axon. A tripartite branch is depicted.

H. Model-adult muscle, illustrating collateral branching of an α axon: first-order, second-order and third-order. Arrowhead points to bulbous swelling of one distal/third-order branch.

All : x 640

Length of bar:

= 39.1 µm

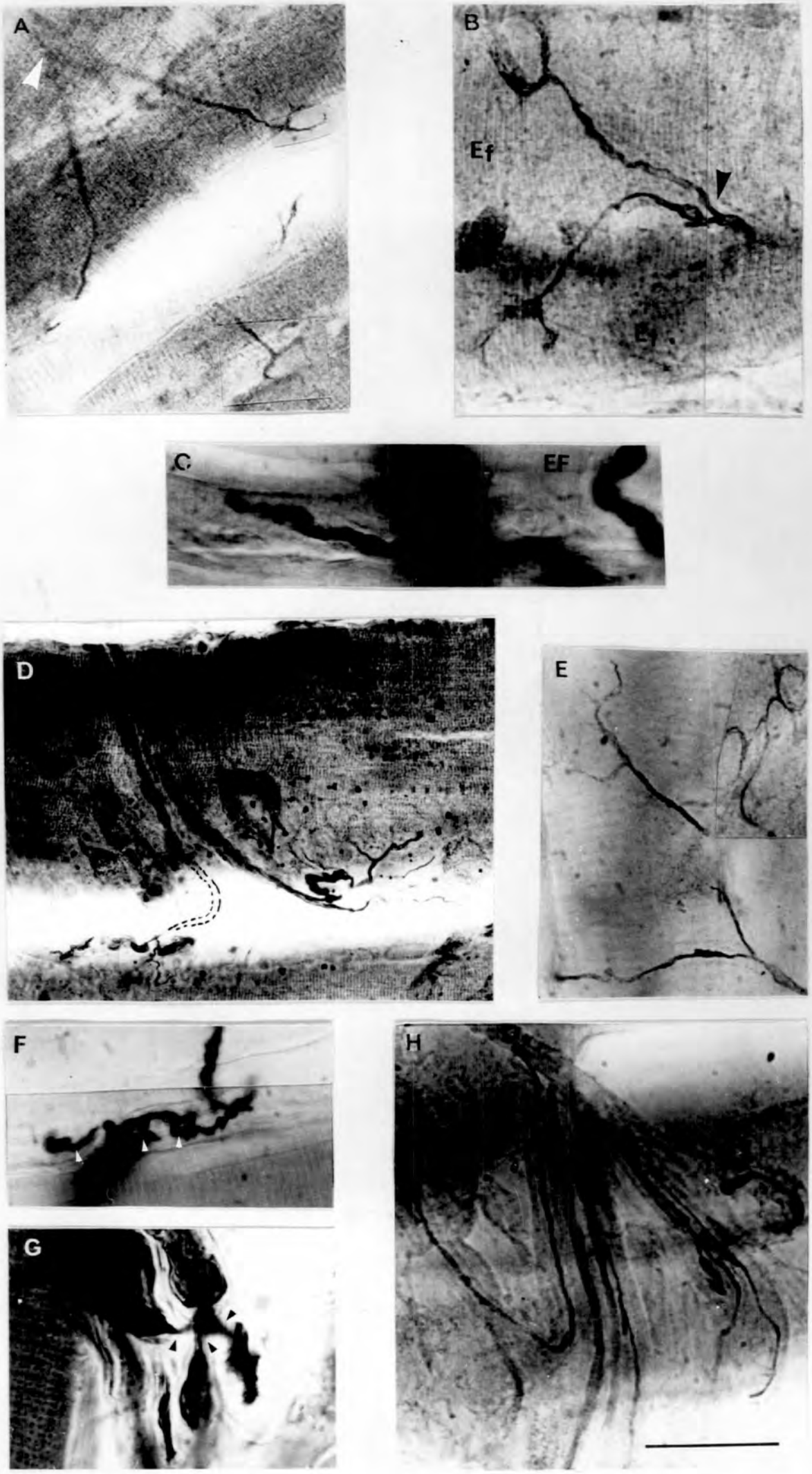

A
B
Ef
C
EF
D
E
F
G
H

FIGURE 66 CAMERA LUCIDA DRAWINGS OF SOME ANOMALOUS MODEL-ADULT EXTRAFUSAL MOTOR END PLATES

All three diagrams of plate morphology from silver/tease preparations.

A. Two α axons shown (arrowheads), each branching at the pre-terminal node to innervate different fibres. The axon branch (arrow) of one of the plates appears unmyelinated.

B. Illustrates double innervation: two plates close together on the same fibre. Both are derived from the same α axon (arrowhead).

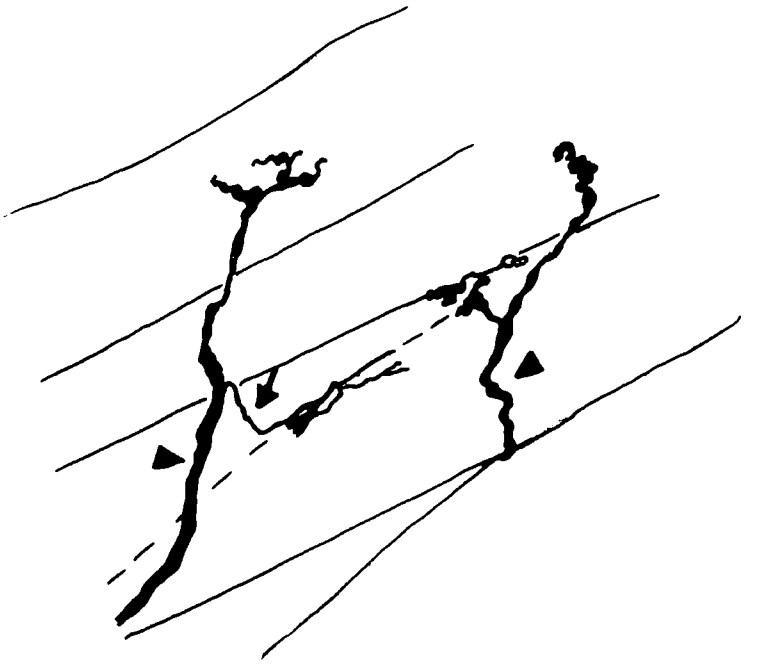
C. Illustrates double innervation: two plates close together on the same muscle fibre. One of the plates is much smaller than the other, being derived from a distal branch (arrowhead) of the common α axon.

All : x 640

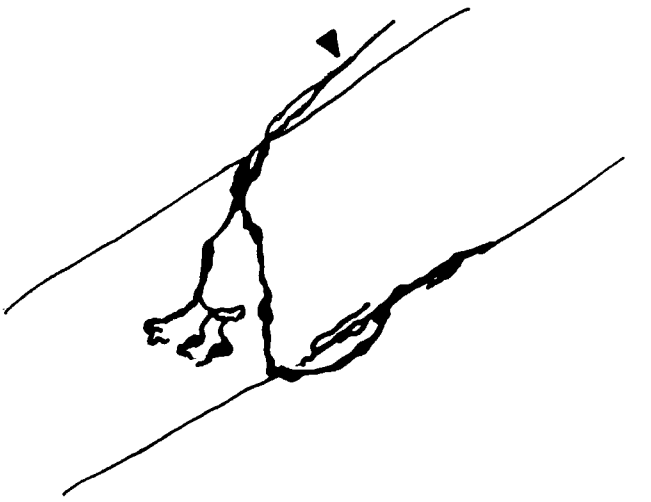
Length of bar:

= 39.1 µm

A



B



C

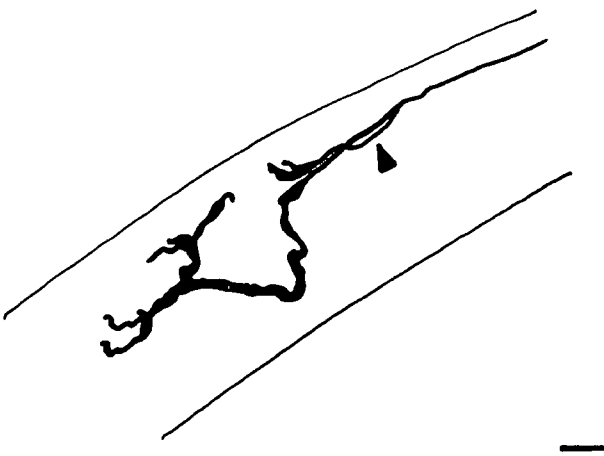


FIGURE 67 MODEL-ADULT MUSCLE: " DWARF " EXTRAFUSAL FIBRES AND SOME ABERRANT SPINDLE FEATURES

A. TS thick (1 m) Epon section stained with toluidine blue. " Dwarf " extrafusal fibres (arrowhead) amongst other fibres of normal diameter (Ef).

x 480

B. TS 10 m frozen section stained for P'lase. " Dwarf " extrafusal fibres (arrowheads) with their normal neighbours (Ef).

x 1,000

C. Longitudinal orientation of a silver/tease preparation. Arrowheads point to " dwarf " extrafusal fibres, several of which can be seen ending with little attachment. This apparent absence of a strong attachment, certainly at one end at any rate, might account for the contracted appearance of dwarf extrafisal fibres in EM (fig. 48 & 49).

x

D. Extreme pole of a single-fibre spindle, showing thinning of fibre and end to end attachment of satellite cells at termination. Silver/tease preparation.

x 400

E. The mid-equatorial region of a six-fibre experimental spindle, which co-existed with two-fibre and single-fibre in one and the same experimental

FIGURE 67 continued

muscle. silver/tease preparation. The sensory endings failed to stain up.

× 252

F. Diagrammatic lay-out of a tandem model-adult spindle (see caption of fig. 64F for description). Shaded fibre is a nuclear-bag fibre common to both capsules.

~ × 160

Length of bar:

In A = 50.0 m

B = 25.0 "

C =

D = 62.5 "

E = 0.16 mm

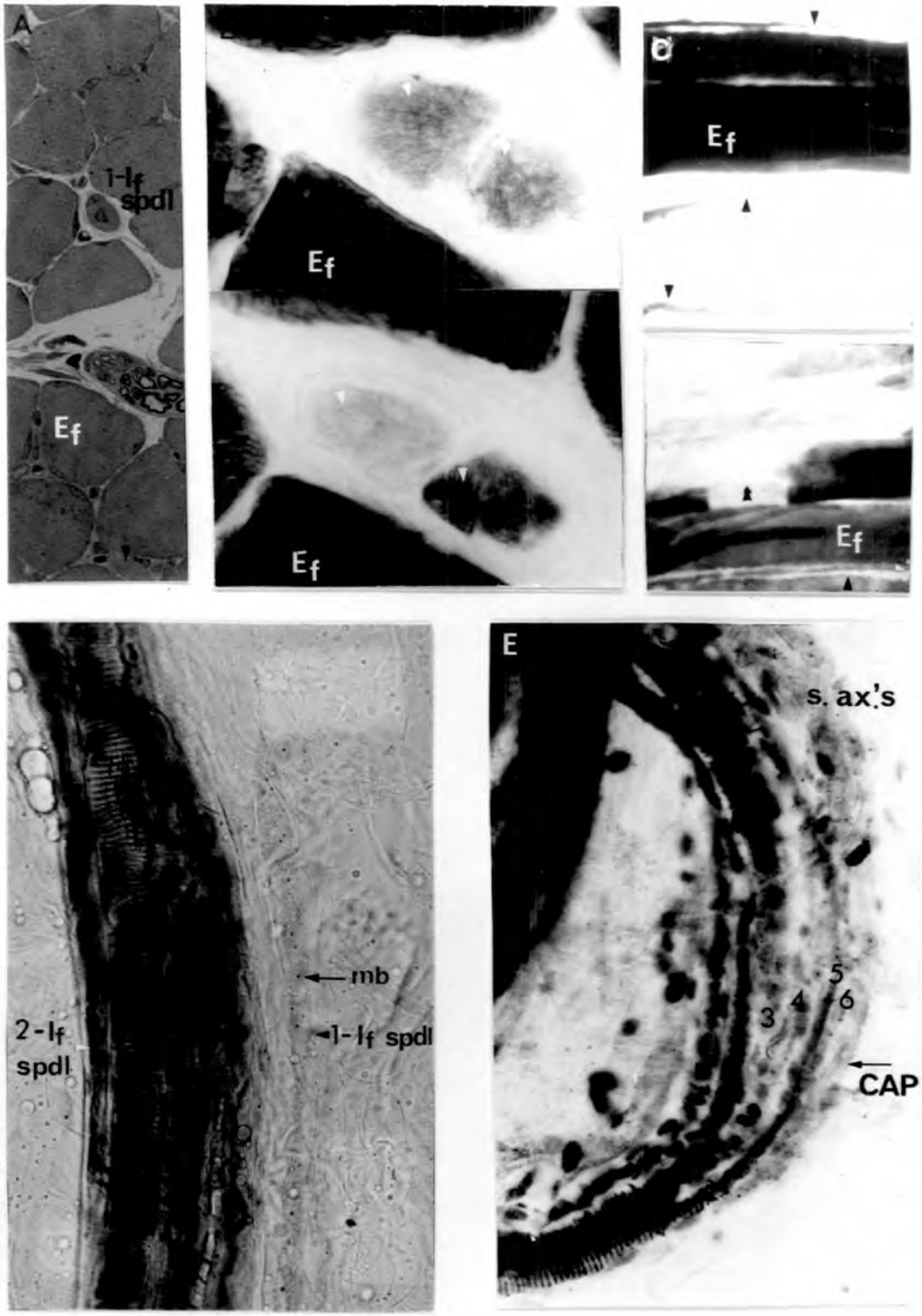

A
1-If spdl
Ef
Ef
Ef
D
Ef
Ef
2-If spdl
mb
1-If spdl
E
s. ax's
3
4
5
6
CAP
F
nb
nc
nb

FIGURE 68 MUSCLE-SPINDLE ULTRASTRUCTURE TWO DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 5½DPN

A. TS. Chain fibres still present, but spindle appears underdeveloped for 5½ days of age, e.g., F_4 barely formed and F_3 immature.

x 5,000

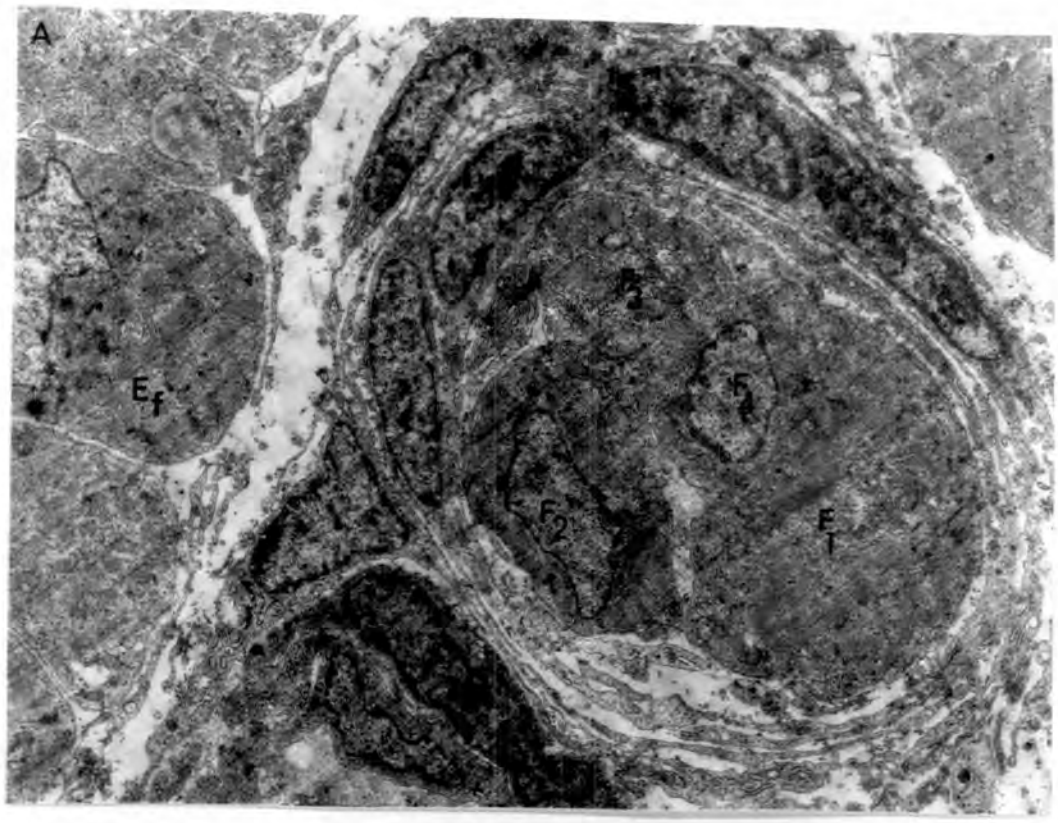
B. High power of A. Note close apposition of F_4 to the largest bag fibre (F_1); myoblast lining F_2, F_3, and F_4; central nuclei; common basement membrane enclosing all four intrafusal fibres, although individual basement membranes are beginning to form between the two large diameter fibres (arrowhead); dark blotches of Z band (?) ma terial; and complex, dilated t-tubules, many of them peripheral.

x 12,600

Length of bar:

In A = 5.00 μm

B = 2.00 "


A
E_f
F_3
F_2
F_1


B
mF
G

FIGURE 69 MUSCLE-SPINDLE ULTRASTRUCTURE FOUR DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 7½DPN

A. TS. Chain fibres completely degenerated, leaving two bag fibres. Note large difference in their diameters and their central nuclei.

x 5,000

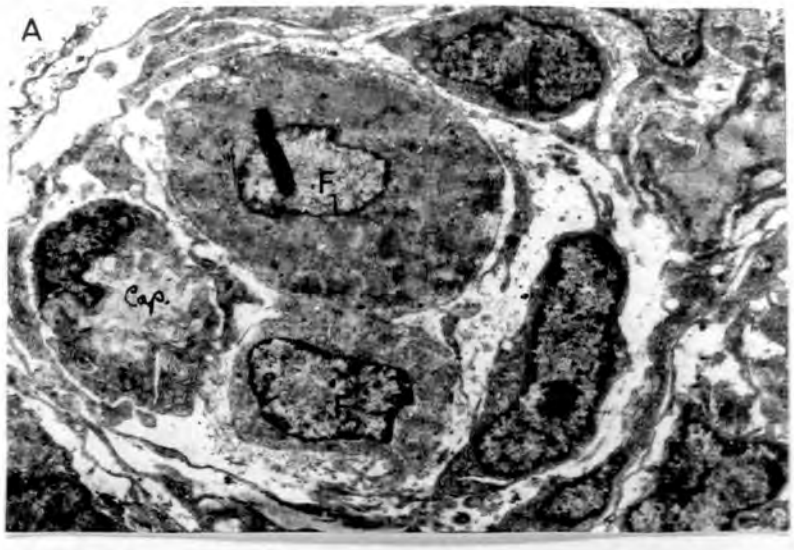
B. High power of A. Note more complex, dilated t-tubules; basement membranes almost separate (arrowheads); and process of myoblast cell bridging the two fibres along a small part of their circumferences.

x 12,600

Length of bar:

In A = 5.00 µm

B = 2.00 "


A
F
Cap


B
tt
tt
tt
tt

FIGURE 70 MUSCLE-SPINDLE ULTRASTRUCTURE SEVEN DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 10½DPN

A. TS. Chain fibres and one (assuming the spindle was a 4-If one) bag fibre absent. Myotubular region with sensory endings. Myoblasts still present at this post-denervation stage.

× 5,000

B. High power of A. Note small, round, layered sensory endings containing neurofilaments in TS and LS; expanded t-tubule system, in particular, the t-tubule network adjacent to the largest nucleus; and M line.

× 12,600

Length of bar:

In A = 5.00 µm

B = 2.00 "

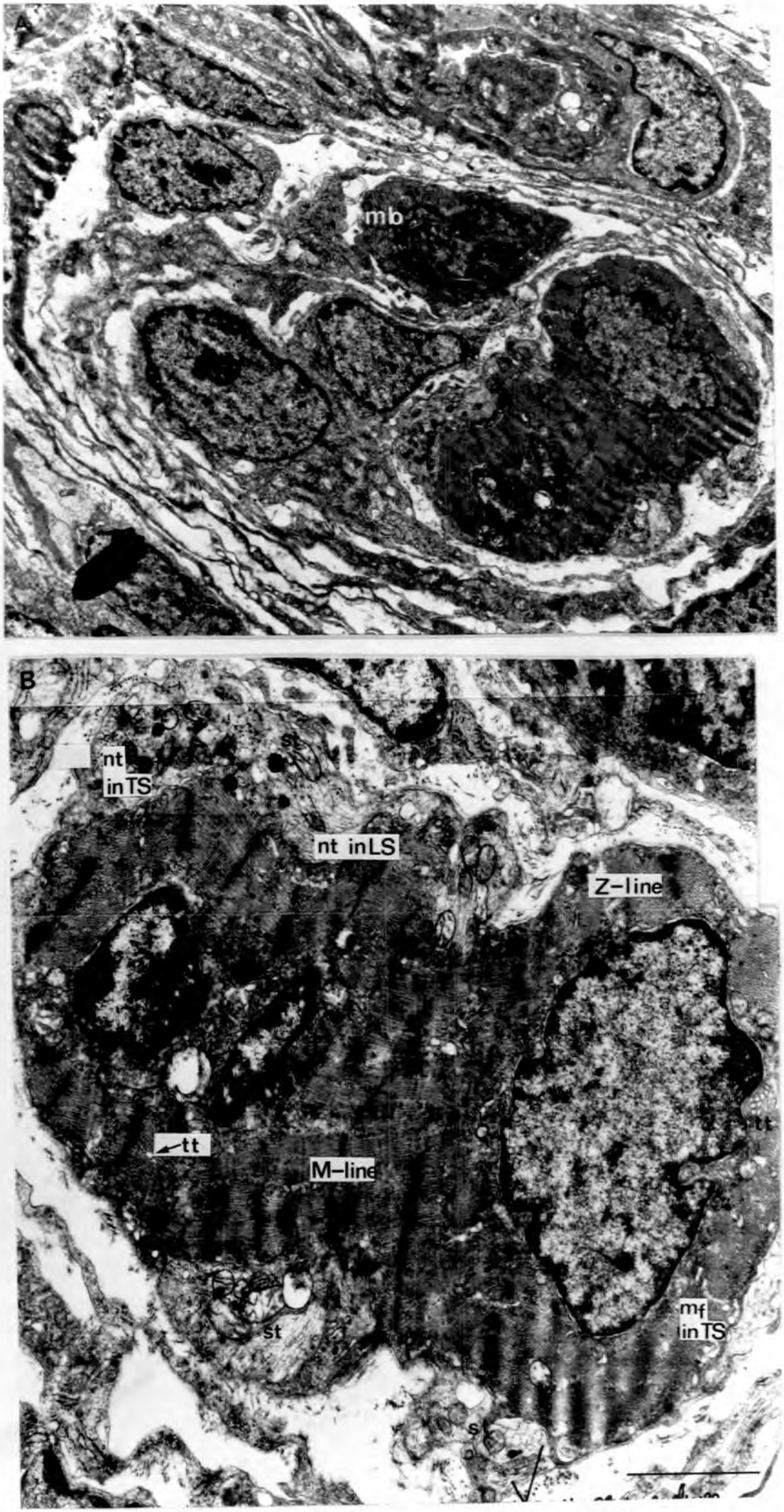

A
mb
B
nt
in TS
nt in LS
Z-line
tt
M-line
st
mf
in TS

FIGURE 71 MUSCLE SPINDLE ULTRASTRUCTURE TEN DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 13½DPN

A. TS of single-fibre spindle, with unmyelinated spindle nerve trunk close to the fibre. Note intramuscular nerve lined by sheath cells of endoneurium.

x 5,000

B. High power of spindle. Note expanded t-tubule system; polyribosomes; and degenerating Z bands (arrowheads). White arrowhead points to centriole/cilium structure in outer capsule cell.

x 6,300

C. High power of intramuscular nerve seen in A. Note variable diameters of axons. Some axon bundles are separated by processes of common Schwann cell.

x 12,600

Length of bar:

In A = 5.00 µm

B = 2.00 "

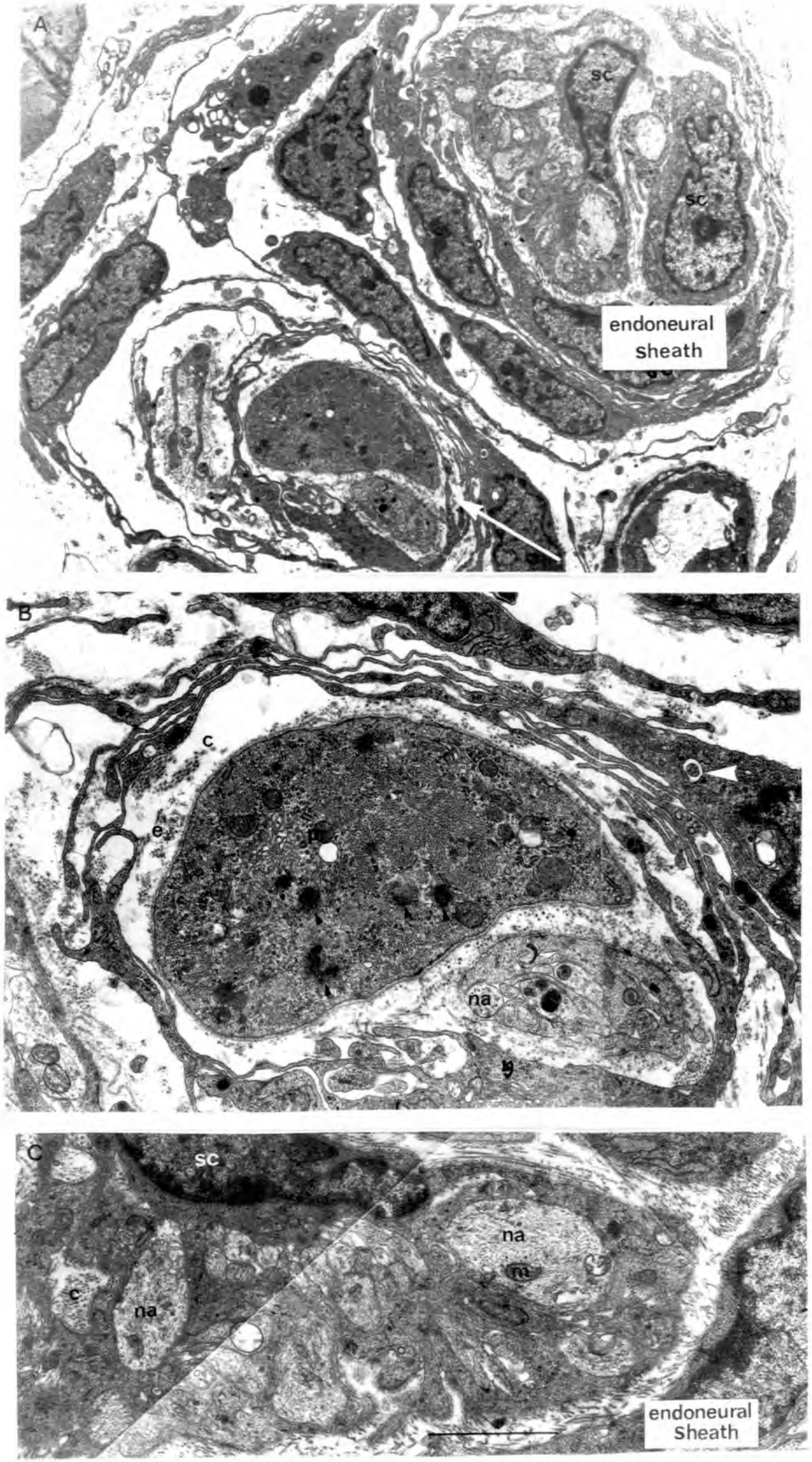

A
sc
sc
endoneural
sheath
B
c
e
na
C
sc
c
na
na
m
endoneural
Sheath

FIGURE 72 MUSCLE SPINDLE ULTRASTRUCTURE TEN DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 13½DPN

A. Section, at different level, of spindle shown in fig. 71. Note myoblast in close apposition with fibre, i.e., within common basement membrane (white arrow).

x 5,000

B. High power of smaller intramuscular nerve shown in A. Note first signs of myelination of both the small diameter and the large diameter axons.

x 12,600

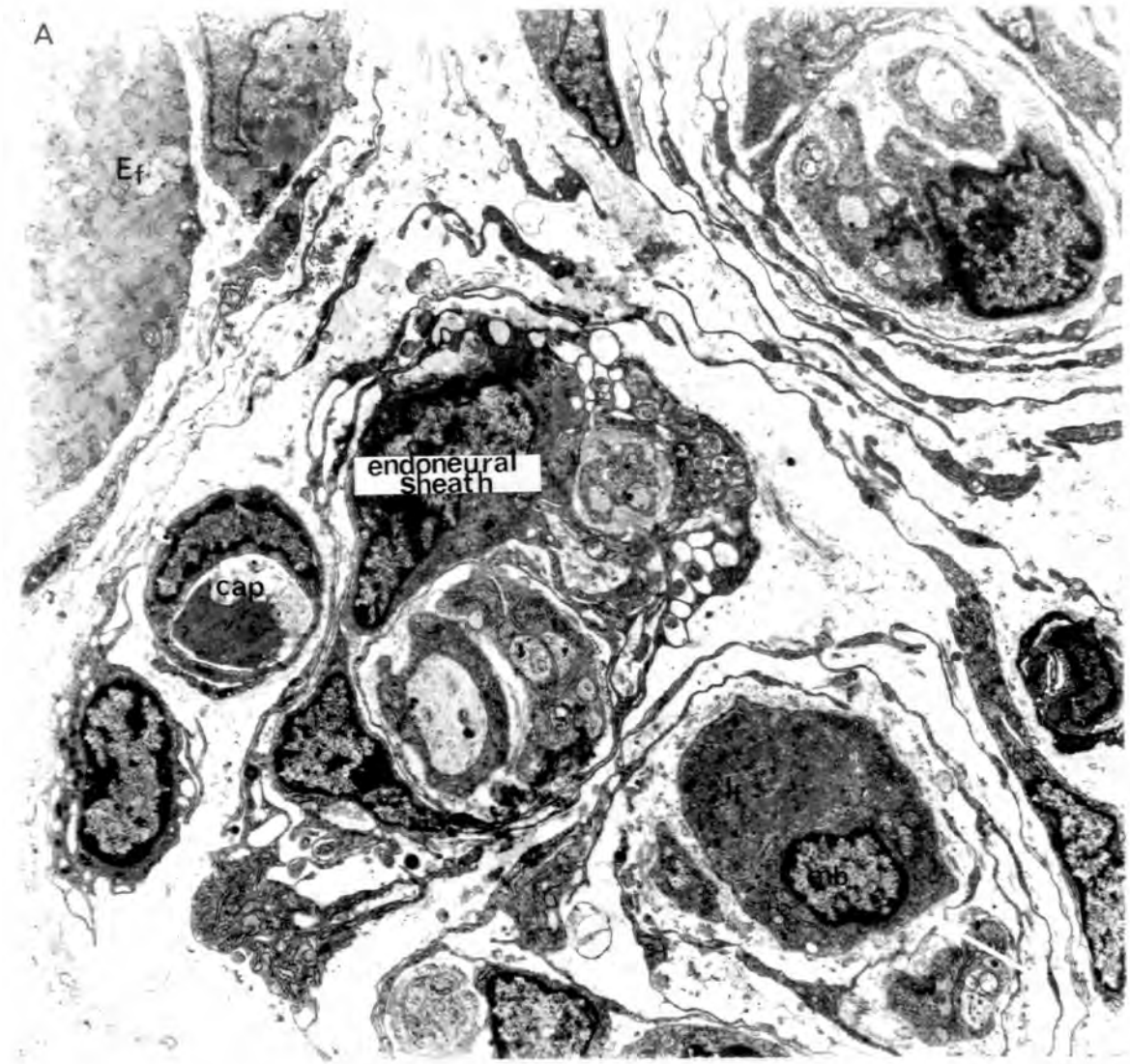
C. High power of the larger intramuscular nerve shown in A. Note central axon bundle surrounded by Schwann cell pseudopodium and longitudinal collagen fibres.

x 12,600

Length of bar:

In A = 5.00 μm

B & C = 2.00 "


A
Ef
endoneural sheath
cap
nb

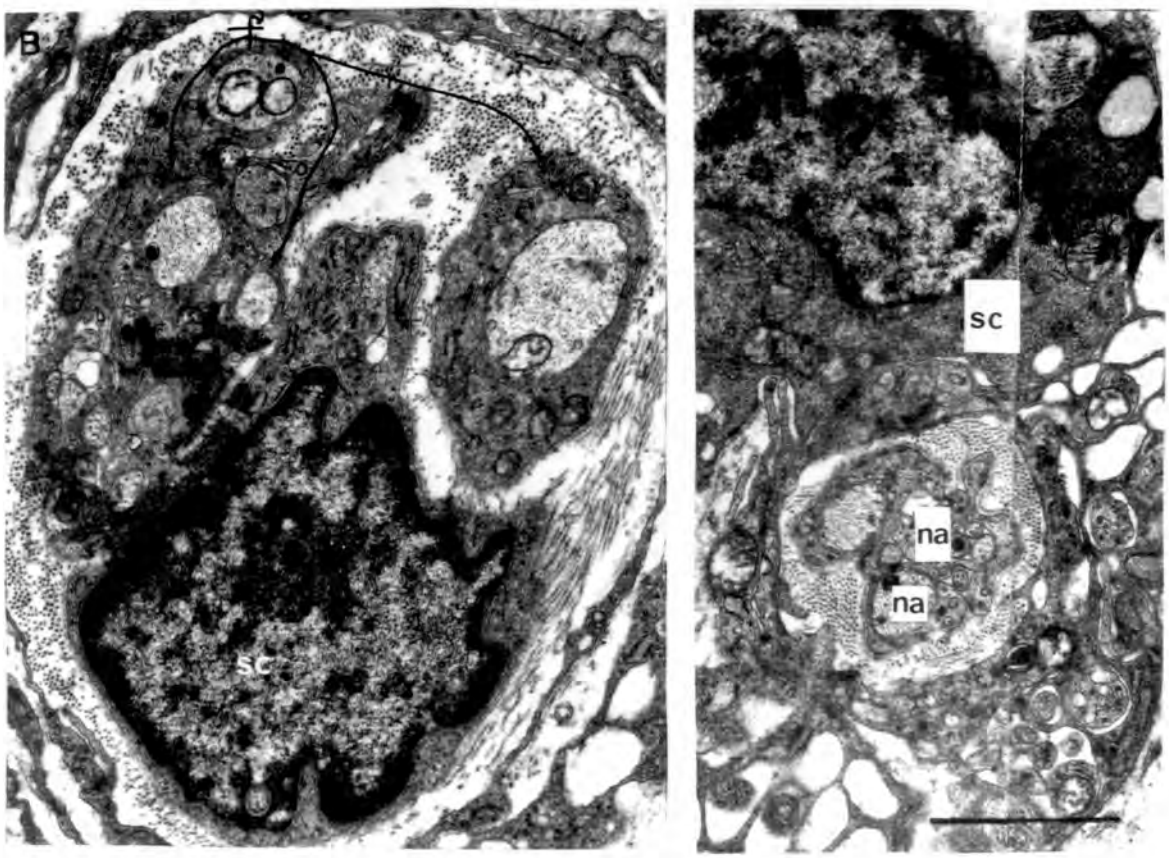

B
sc
sc
na
na

FIGURE 73 MUSCLE-SPINDLE ULTRASTRUCTURE TEN DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 13½DPN

TS of another single-fibre spindle, depicting myelinated axon (with its own Schwann cell) in intramuscular nerve. Note spindle nerve trunk, consisting of still unmyelinated axons; expanded t-tubules; and myelin figure.

× 12,600

Length of bar:

= 2.00 μm

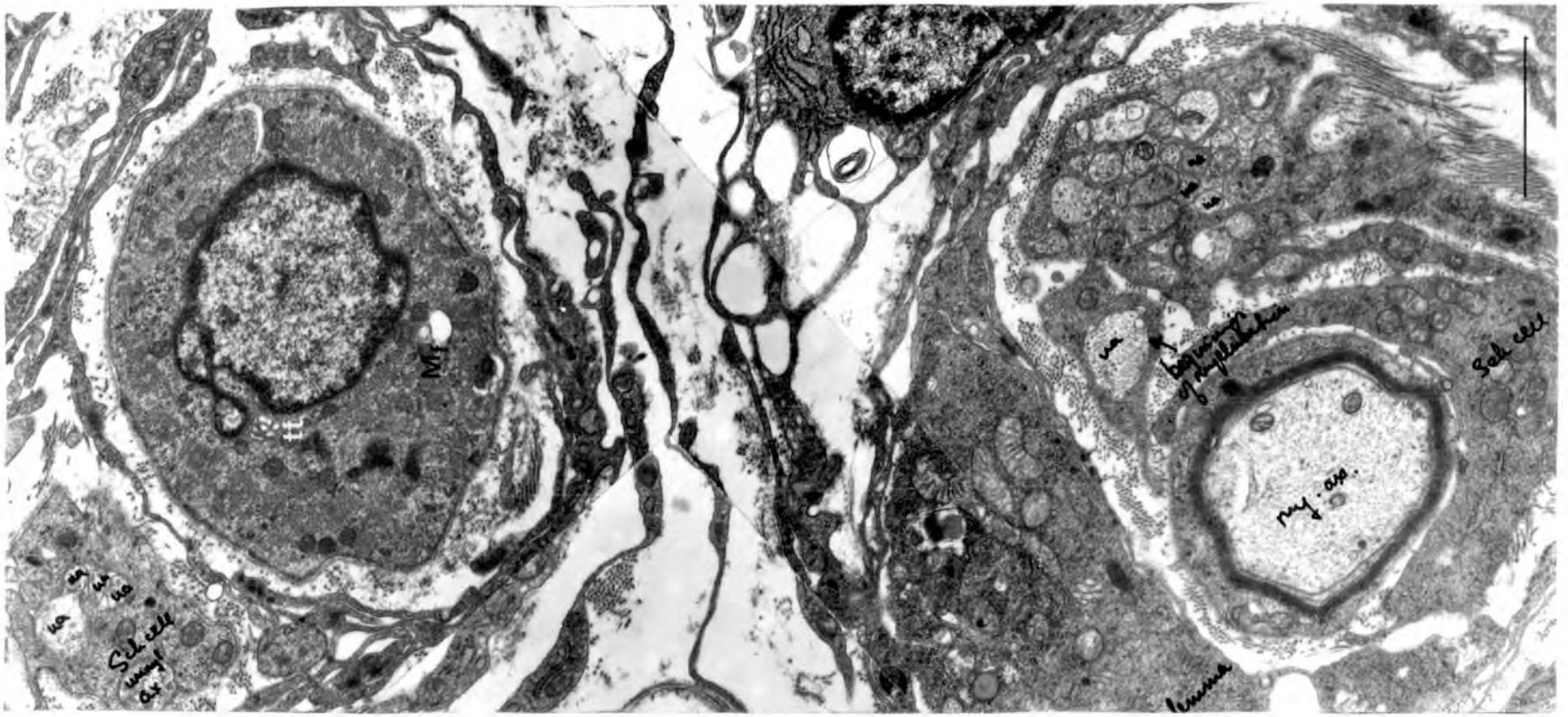

Mit
Sch. cell
myel. axis
myel. axis
Sch. cell
lumen

FIGURE 74 MUSCLE-SPINDLE ULTRASTRUCTURE FOURTEEN DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 17½DPN

TS of two-fibre spindle in equatorial region. Note separate basement membranes; small nuclear bags; well-developed periaxial space; and small difference in diameter between the two fibres. Neither of the nuclear bags increased beyond two nuclei per cross-section. Moreover, even though this spindle was carefully sectioned from one pole to the extremity of the opposite A region, no sensory terminals were encountered. This was the only model-adult spindle, sectioned for EM, that was characterised by the absence of sensory endings (cp spindle in silver/tease preparation, fig. 59D).

× 12,600

Length of bar:

= 2.00 μm

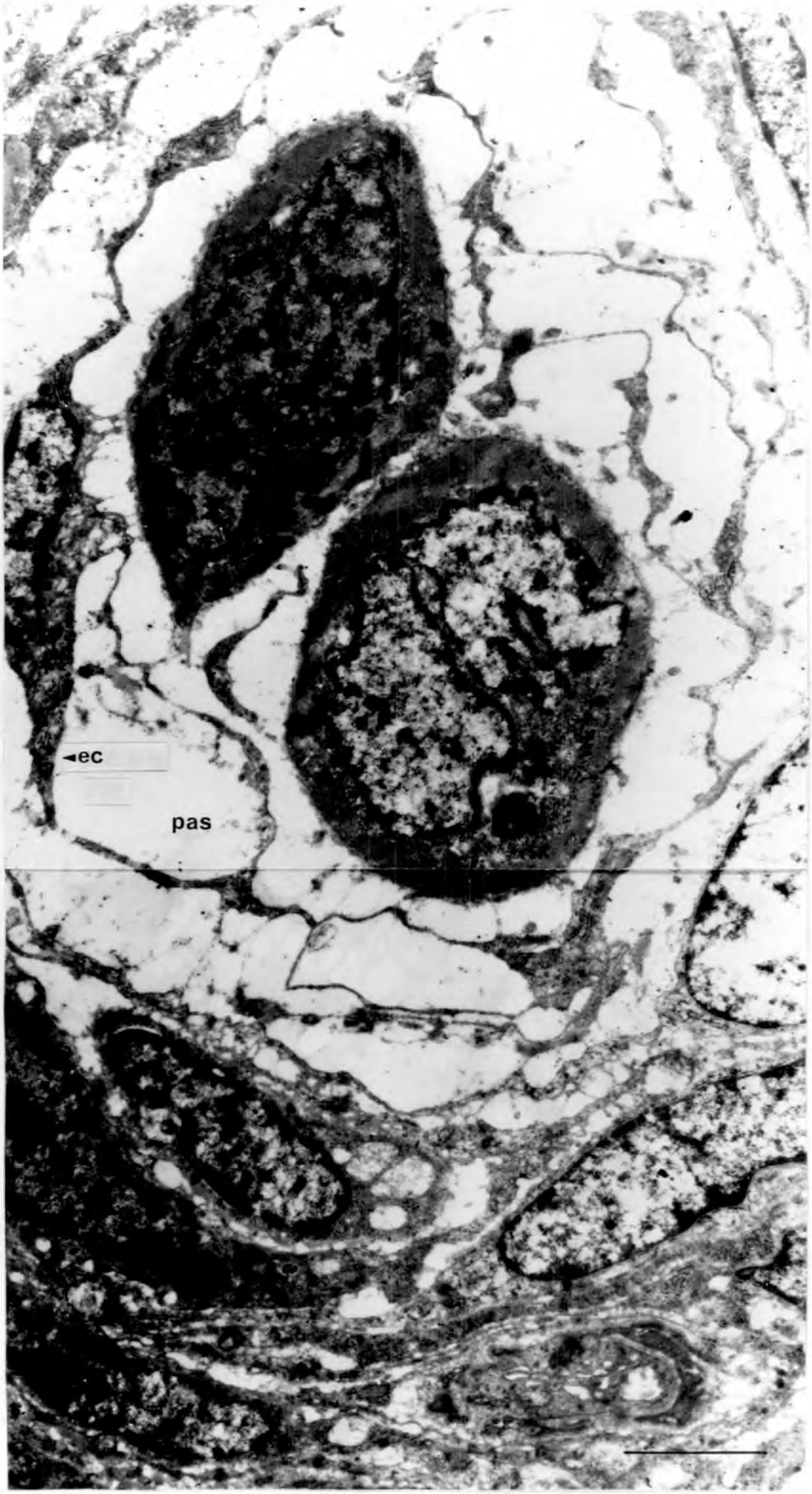

ec
pas

FIGURE 75 MUSCLE-SPINDLE ULTRASTRUCTURE FOURTEEN DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 17½DPN

A. LS through the outer myotubular zone (region A) of the spindle shown in fig. 74. The two intrafusal fibres are closely apposed via a myoblast. The smaller bag fibre (left) features a double M line. The larger bag fibre possesses an M line (arrow) which, in normal adult spindles, is absent at this myotubular level.

x 12,600

B. Adjacent sarcomeres to those shown in A, depicting the M line of the large bag fibre.

x 12,600

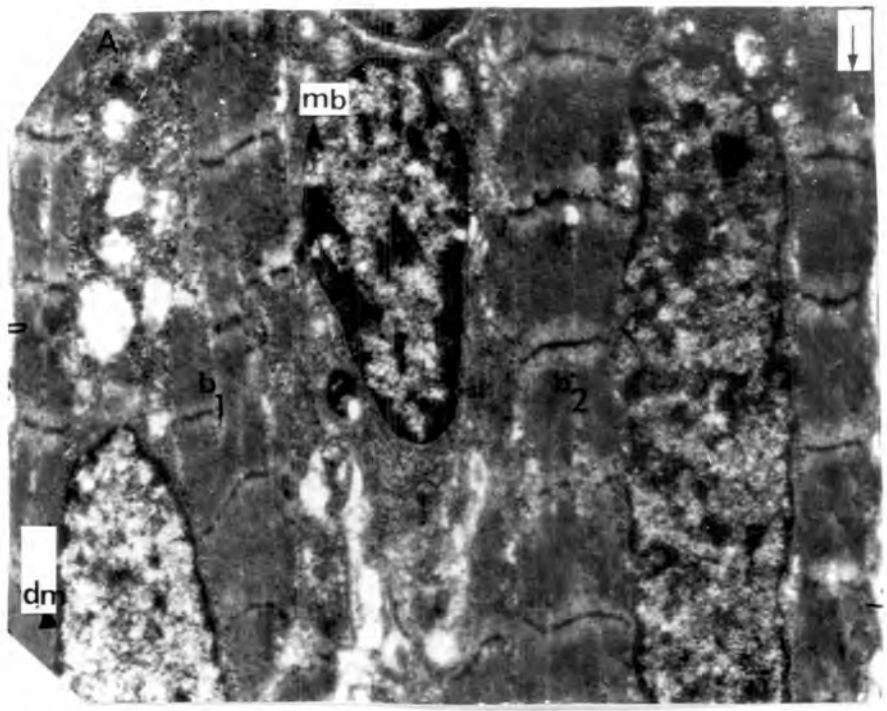
C. Another two-fibre spindle, sectioned transversely at the myotubular level (outer region A). Sensory innervation present, as illustrated by the large terminal on the bag_2 fibre. Note M line of fibre bag_2; and well developed periaxial space.

x 5,000

Length of bar:

In A & B = 2.00 μm

C = 5.00 "


A
mb
b1
b2
dm

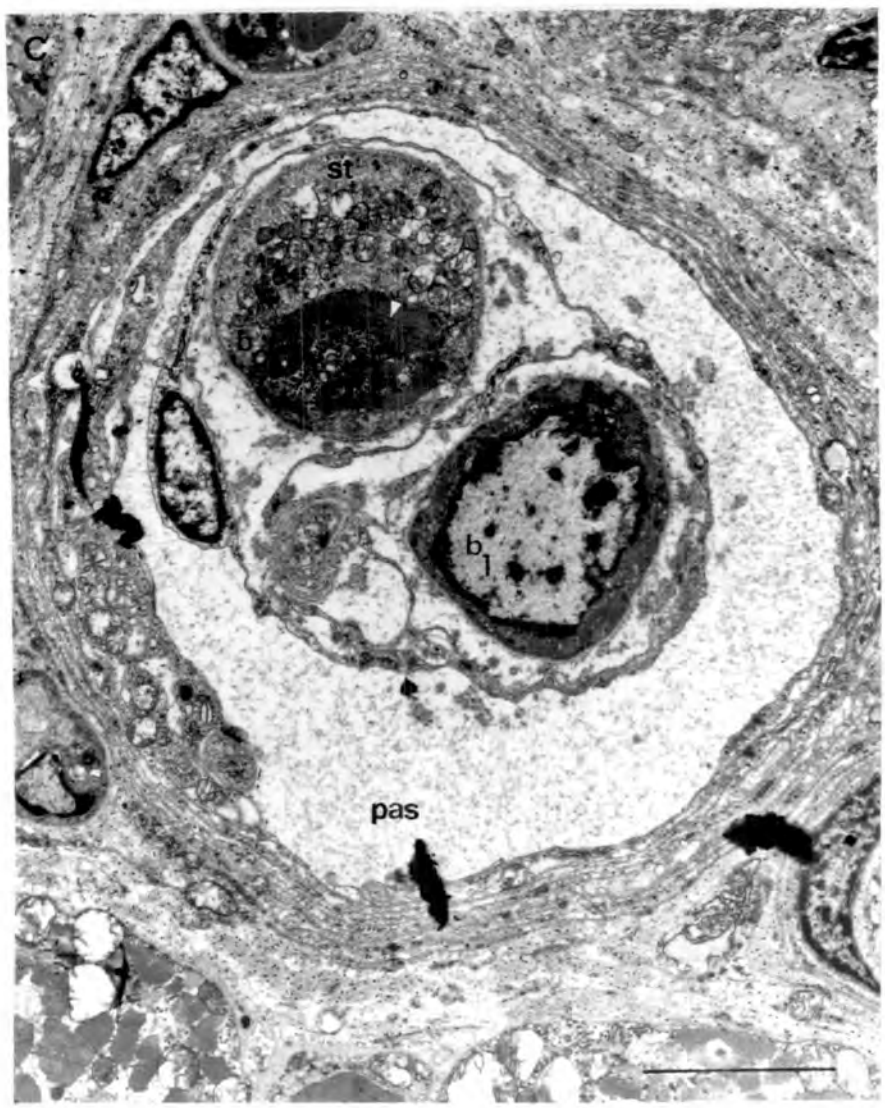

C
st
b
b1
pas

FIGURE 76 MUSCLE-SPINDLE ULTRASTRUCTURE FOURTEEN DAYS AFTER NERVE CRUSH AT 3½DPN, i.e., AGE = 17½DPN

A. High power of fig. 75C: bag_2 fibre. M line clearly seen in oblique myofibril. Note that the sensory ending completely encircles the fibre. Arrowheads point to inner (new) and outer (old) basement membranes. The outer is more intact at this relatively early post-denervation stage than it is in the model-adults.

x 12,600

B. High power of fig. 75C: bag_1 fibre. Arrowheads indicate same feature as in A. Note central myotube nucleus. This fibre also possessed sensory endings, even though this section does not show them.

x 12,600

C. Illustrates myelinated sensory axon in periaxial space spindle in fig. 75C. (More equatorial section. Note large Schwann cell nucleus.

x 12,600

Length of bar:

= 2.00 µm

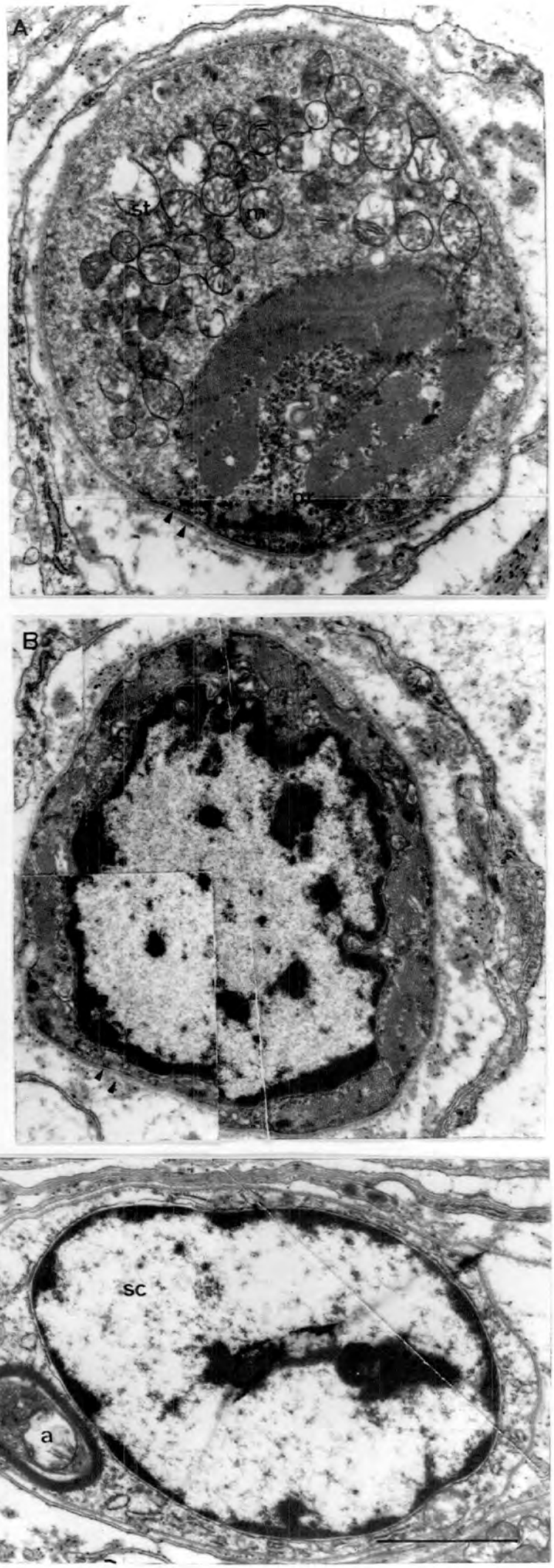

A
st
m
B
C
sc
a

FIGURE 77 FREQUENCY HISTOGRAMS OF AXON DIAMETERS IN THE MEDIAL GASTROCNEMIUS NERVE

A. & B. Model-adult animal, 2½MPN. A depicts the left contralateral (control) profile and B the right experimental profile.

C. & D. Model-adult animal, 6½MPN. C depicts the left contralateral (control) profile and D the right experimental profile.

E. & F. Adult-crush animal, 6½MPN. Nerve crushed at 4½MPN, allowing 2½ months for regeneration. E depicts the left contralateral (control) profile and F the right experimental profile.

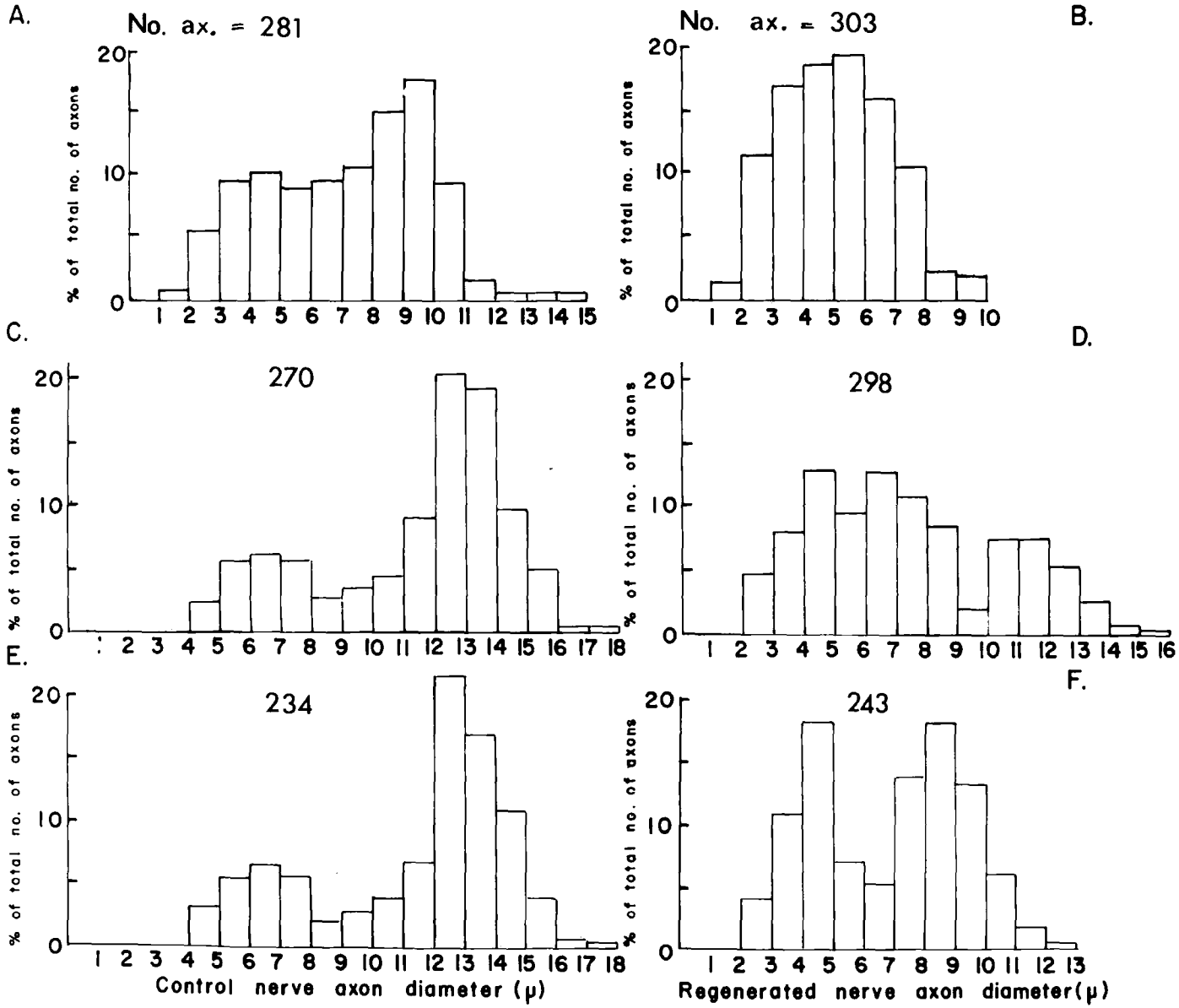

A.
No. ax. = 281
% of total no. of axons
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
B.
No. ax. = 303
% of total no. of axons
20
10
0
1 2 3 4 5 6 7 8 9 10
C.
270
% of total no. of axons
20
10
0
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
D.
298
% of total no. of axons
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
E.
234
% of total no. of axons
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Control nerve axon diameter (μ)
F.
243
% of total no. of axons
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Regenerated nerve axon diameter(μ)

FIGURE 78 SECTIONS THROUGH TIBIAL AND MEDIAL GASTROCNEMIUS NERVES

A. Section through the tibial nerve, distal to level of crush, 1 month after nerve crush at $3\frac{1}{2}$ DPN. Paraffin section, 8 µm thick, stained according to Holmes silver method for axoplasm. A_l depicts the left contralateral (control) nerve and A_r the right experimental nerve.

x 100

B. Section through the tibial nerve, distal to level of crush, $3\frac{1}{2}$ months after nerve crush at $3\frac{1}{2}$ DPN. Paraffin section, 8 µm thick, stained with osmium tetroxide for myelin. B_l depicts the left contralateral (control) nerve and B_r the right experimental nerve.

x 100

The degree of shrinkage in A and B is similar, since the embedding procedure was identical in each case.

C. Section through the medial gastrocnemius nerve, $2\frac{1}{2}$ months after nerve crush at $3\frac{1}{2}$ DPN. Epon section, 1 µm thick, stained with paraphenylenediamine. C_l depicts the left contralateral (control) nerve and C_r the right experimental nerve.

FIGURE 78 continued

These sections were used for making axon measurements for the histograms shown in fig. 77 A & B.

x 250

Length of bar:

In A & B = 0.25mm

C = 0.10"

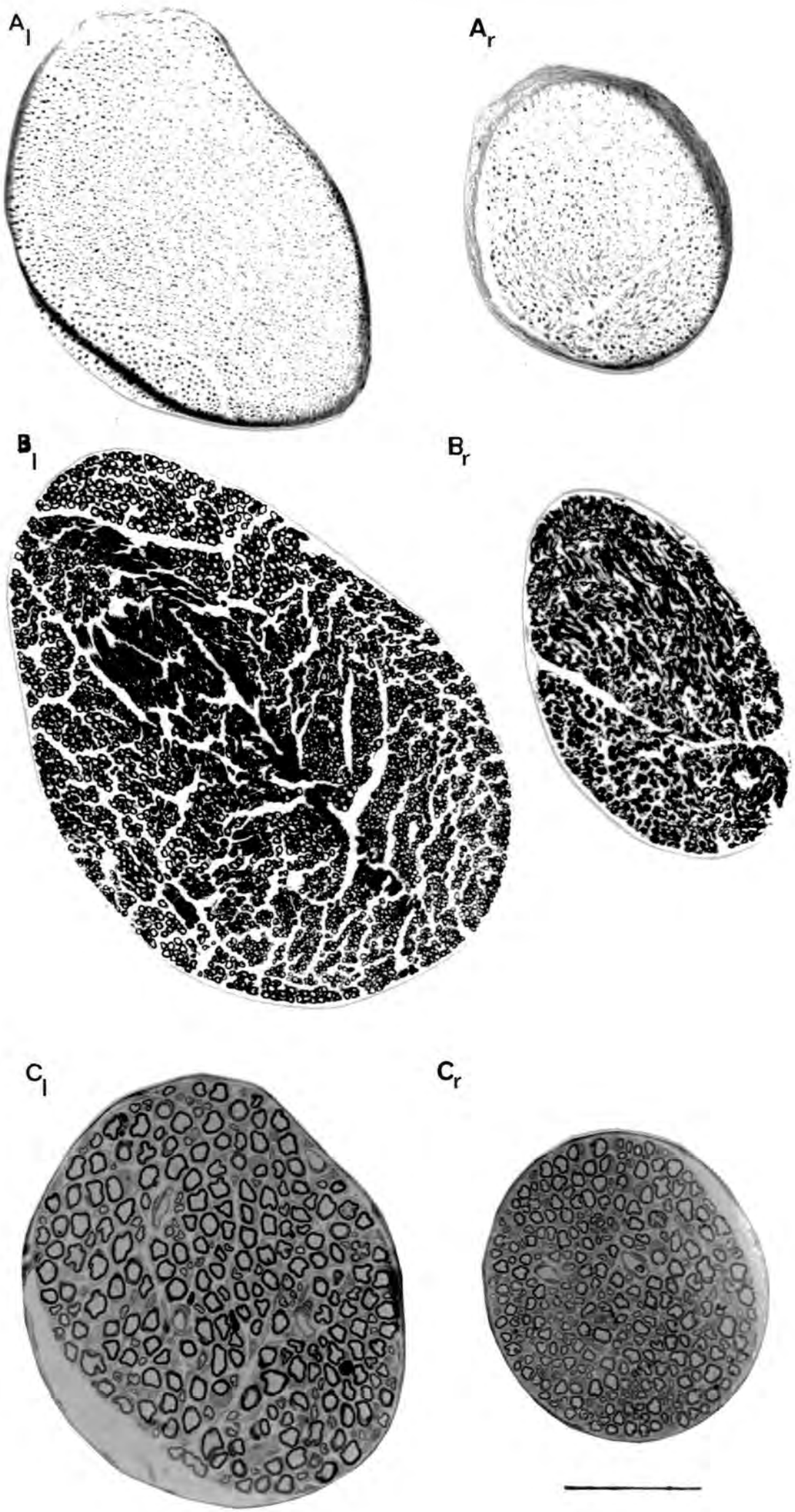

A l
A r
B l
B r
C l
C r

FIGURE 79 " TYPICAL " MODELS OF SENSORY INNERVATION IN NORMAL ADULT, ADULT-CRUSH AND MODEL-ADULT SPINDLES

Region A (equator plus juxta-equator) depicted. Drawn only very roughly to scale, approx. x 500. Those axons and endings drawn as broken lines are present in some spindles but not in others, i.e., in terms of a mean value for a " typical " spindle, they represent a fraction of an axon.

A. Normal adult spindle. The primaries are shown predominating on the bag fibres (" b_1 " and " b_2 "), the secondaries mostly on the chain fibres (" c " and also shaded for clarity). A single primary axon (Group " IA ") and two secondary axons (Group " II ") comprise the sensory supply.

B. Adult-crush spindle. Virtually no different from the normal adult condition. From the findings of this thesis, the mean number of secondary axons per spindle was slightly reduced, and so one of the secondaries is depicted with broken lines in the diagram. However, it is possible that a larger spindle sample would have revealed no difference at all.

C. Model-adult two-fibre spindle. Only one sensory axon is shown but, as in A and B, it must be remembered that this is a simplistic representation; some two-fibre model-adult spindles had no

FIGURE 79 continued.

secondaries whereas others had two. The endings of the primary consist of fewer spirals.

D. Model-adult single-fibre spindle. The primary ending is sometimes more spiral than shown here (see EM results). Ideally, there should be more silver/tease data on the presence/absence and form of the secondary endings. The dotted outline of a secondary axon with question mark termination is based on observations from two silver/tease preparations. In one spindle (fig. 62B), a terminal was clearly seen but its identity as trail or secondary was questionable. In the other spindle (fig. 61A) a secondary axon was clearly present but the termination was obscured by inadequate staining.

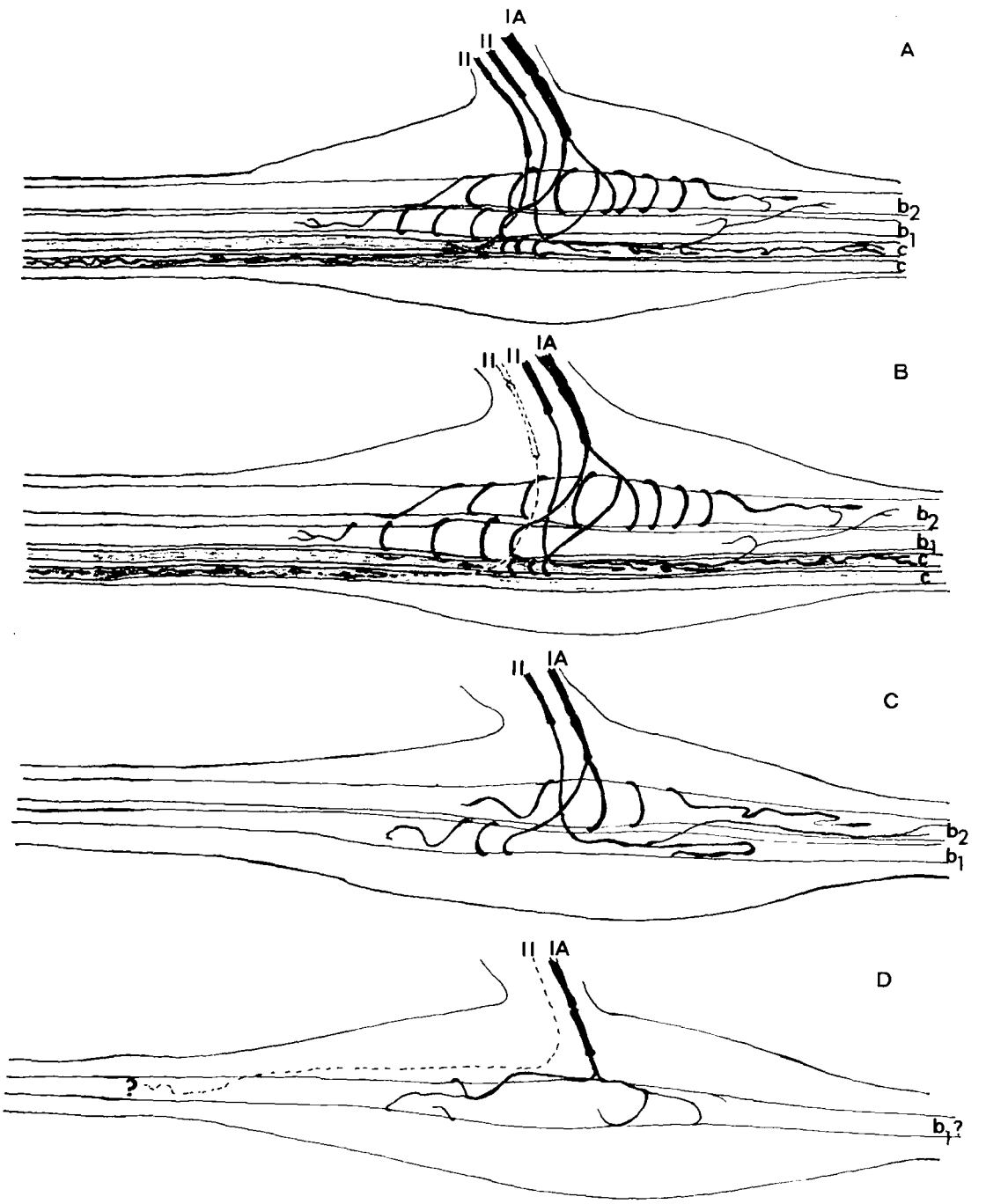

IA
II
II
A
b2
b1
c
c
IA
II
II
B
b2
b1
c
c
II
IA
C
b2
b1
II
IA
D
?
b1?

FIGURE 80 " TYPICAL " MODELS OF FUSIMOTOR INNERVATION IN NORMAL ADULT, ADULT-CRUSH AND MODEL-ADULT SPINDLES

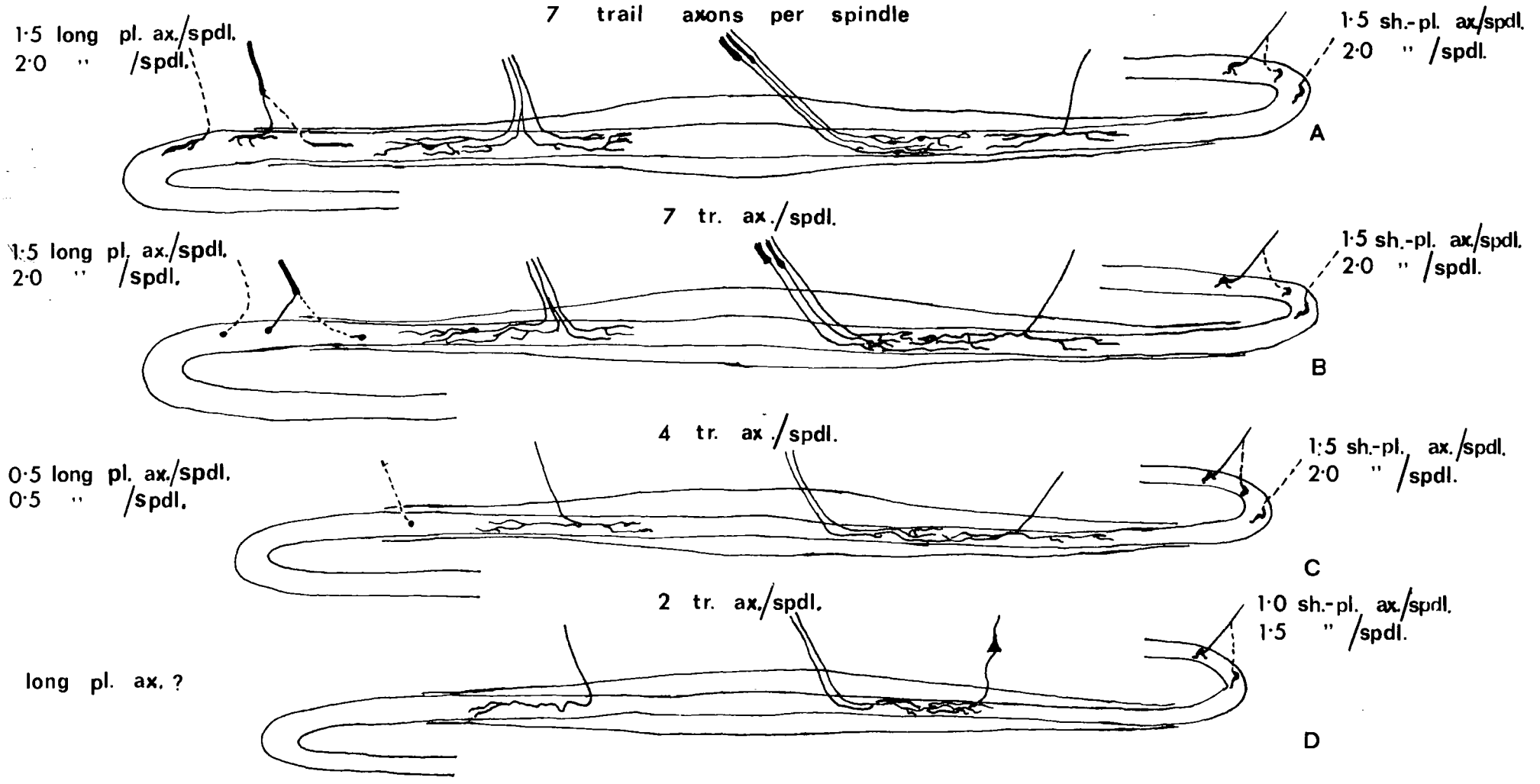
Regions A and B and part of region C depicted. Not drawn to scale; region B particularly telescoped. Same explaination as in figure 79 for broken line drawings. Individual intrafusal fibres not drawn, for the sake of clarity, but one should bear in mind that (i) the short plates predominate on the bag fibres, (ii) the long plates terminate on both the bag and chain fibres and (iii) trail/fibre specificity remains to be resolved in the rat. The long, unmyelinated preterminal trail axons that traverse longitudinally through the periaxial space for variable distances in all three spindle groups, are not depicted in the diagram. The two plate types have been drawn at opposite poles merely to simplify the labelling. In actual fact, both types can be found on one and the same spindle pole.

A. Normal adult spindle.

B. Adult-crush spindle. Note the change in morphology of the long plates.

C. Model-adult two-fibre spindle. Note the change in morphology of the long plate.

D. Model-adult single-fibre spindle. One of the two trail axons shown entering the spindle at the equator, leaves in region B without terminating.


7 trail axons per spindle
1·5 long pl. ax./spdl.
2·0 " /spdl.
1·5 sh.-pl. ax./spdl.
2·0 " /spdl.
A
7 tr. ax./spdl.
1·5 long pl. ax./spdl.
2·0 " /spdl.
1·5 sh.-pl. ax./spdl.
2·0 " /spdl.
B
4 tr. ax./spdl.
0·5 long pl. ax./spdl.
0·5 " /spdl.
1·5 sh.-pl. ax./spdl.
2·0 " /spdl.
C
2 tr. ax./spdl.
long pl. ax. ?
1·0 sh.-pl. ax./spdl.
1·5 " /spdl.
D