TITLE: The Morphology and Ultrastructure of Baboon Muscle Spindles, Including Fusimotor Innervation

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SUMMARY

The work of this study was concerned with looking at baboon muscle spindles both morphologically and ultrastructurally to see if these were not in fact a closer working model for the human than cat spindles, as there is evidence both histological (Cooper and Daniel 1963, Swash and Fox 1972) and electrophysiological (Hagbarth 1981, Vallbo 1981) that there are differences between human and cat muscle spindles.

Seven baboon lumbrical muscles were examined with the light microscope and the general morphology of muscle spindles determined. Twenty-seven of these were studied in more detail, particular emphasis being paid to the elastic fibre distribution within the spindles.

Three spindles were also studied ultrastructurally, two of these having been used in earlier electrophysiological experiments.

The results show that in terms of intrafusal fibre content and size, baboon muscle spindles are similar to those found in humans and cats.

The elastic fibre distribution however, which is a useful means of identifying the static bag2 fibre in the cat, is not a reliable method in baboon spindles and in this respect is more like that found in human spindles.

The M-line distribution in baboon spindles is similar to that found in other mammals and is a useful means of identifying the static bag2 fibre.

The motor endings in baboon spindles are similar to those found in the cat but there may be a species variation in the nuclear chain fibre endings.

INTRODUCTION

Mammalian muscle spindles have been the subject of much research in the past 120 years.

Cat spindles had already been well researched by 1900, and so much more time and effort has been spent in continuing this research, for the simple reason that it is easier to relate new findings to pre-existing work.

Other mammals have been studied, for example rabbits, rats, guinea pigs, dogs, monkeys and humans, but the results of these studies are usually compared to those findings in the cat.

Cat spindles are usually taken as a model for human spindles but there is evidence both histological (Cooper and Daniel 1963, Swash and Fox 1972) and electrophysiological (Hagbarth 1981, Vallbo 1981) that there are differences.

The object of this thesis was therefore to study baboon muscle spindles to see if these were not in fact a closer model for human spindles. The thesis begins with a review of the literature on mammalian muscle spindles in general, followed by a more detailed account of the M-lines, elastic fibre distribution, motor endings, long chain fibres, tandem spindles and one bag fibre spindles. Baboon and human spindles are then described and the specific aims of the thesis are explained.

CHAPTER 1

MAMMALIAN MUSCLE SPINDLES

1.1 Historical Review

Kuhne, in the 1860s, was the first to describe the spindle shaped structures found in skeletal muscle and to give them their name.

In 1894, Sherrington published a full description of the effects of intradural section of both dorsal and ventral spinal roots in cat and monkey spindles. This causes degeneration of motor fibres while leaving peripheral afferent fibres unaffected because the afferent cell body lies in the dorsal root ganglion outwith the spinal cord. He showed that each spindle received at least one myelinated afferent fibre and concluded that the spindle was a sensorial organ.

At around the same time Ruffini (1898) made a detailed study of the appearance of the nerve terminals within the spindle, using gold chloride impregnation. He described the annulo-spiral ending and the secondary flower spray ending which were sensory in function. He also described plate endings which he concluded on histological grounds were also sensory. This was later proven to be erroneous.

The establishment of a motor supply to the spindle was more difficult but was confirmed by degeneration experiments in which dorsal roots were cut distal to the dorsal root ganglion (Hines and Tower 1928). This resulted in degeneration of the equatorial endings but left the plate endings intact.

The systematic studies of B.H.C. Matthews (1931-1933) on frog and cat spindles, established them as highly sensitive mechano- receptors, with the special feature of being powerfully excited by the dynamic stimulus of muscle stretching over and

above their response to maintained stretch.

In 1930 Eccles and Sherrington demonstrated that there were two sizes of motor axons in nerves to muscle but did not suspect that these might have quite different functions. The term α (alpha) for the large motor axon is attributed to Erlanger and Gasser, who had named the first peak of the Bullfrog sciatic compound action potential the α peak. This included all large diameter neurones (12-20 um) both sensory and motor.

In 1945 Leksell termed the smaller axons, γ (gamma) motor neurones and showed that stimulation of these γ axons increased an afferent discharge which he presumed came from muscle spindles.

In 1951 Kuffler, Hunt and Quilliam showed that stimulation of a filament of the ventral spinal root containing a single efferent axon to the muscle being studied, could increase the discharge in a single spindle afferent axon in a filament of the dorsal root. The functional term fusimotor is frequently used for γ efferents, now that they have been shown to be exclusively motor to muscle spindles.

Around the same time Barker (1948) was studying cat and rabbit spindles with silver and gold impregnation techniques and he drew attention to the fact that the intrafusal fibres of the mid-equatorial region contained multiple nuclei. He called this non-striated central region the nuclear bag region and the structure on either side consisting of a single row of nuclei extending along the centre of the muscle fibre, the myotube region. He suggested that the intrafusal muscle fibre consisted of two contractile polar units separated by a non-contractile equatorial zone. He also demonstrated histologically the primary

and secondary sensory endings and motor endings similar to extrafusal end-plates.

In 1956, Boyd in cat and Cooper and Daniel in man, showed that intrafusal fibres could be separated into two morphologically distinct types. In addition to fibres containing a nuclear bag region there were similar fibres which possessed an equatorial region composed of a single row of nuclei within a myotube. Boyd called these two fibre types nuclear bag fibres and nuclear chain fibres respectively.

In 1961, Sybil Cooper showed that the primary endings were much more sensitive to the velocity of stretch than were the secondary endings. She concluded that this was partly mechanical in origin due to the fact that the primary sensory ending lay on nuclear bag fibres and nuclear chain fibres while the secondary endings lay mainly on nuclear chain fibres.

In 1962 Boyd described similar findings.

A typical cat muscle spindle (Fig 1) generally consists of six to eight intrafusal fibres of which there are usually one to three large nuclear bag fibres and larger numbers of nuclear chain fibres. The nuclear chain fibres are smaller in diameter (10-15 um), shorter in length (2-4 mm) and are usually intracapsular, their ends being attached at the apices of the capsule to connective tissue of the capsule or the endomysium of the nuclear bag fibres.

The nuclear bag fibres are of greater diameter (20-30 um), longer in length (4-8 mm) and extend beyong the ends of the capsule, so that the terminal portions of their polar regions are extracapsular at their point of attachment to intramuscular connective tissue or tendon.

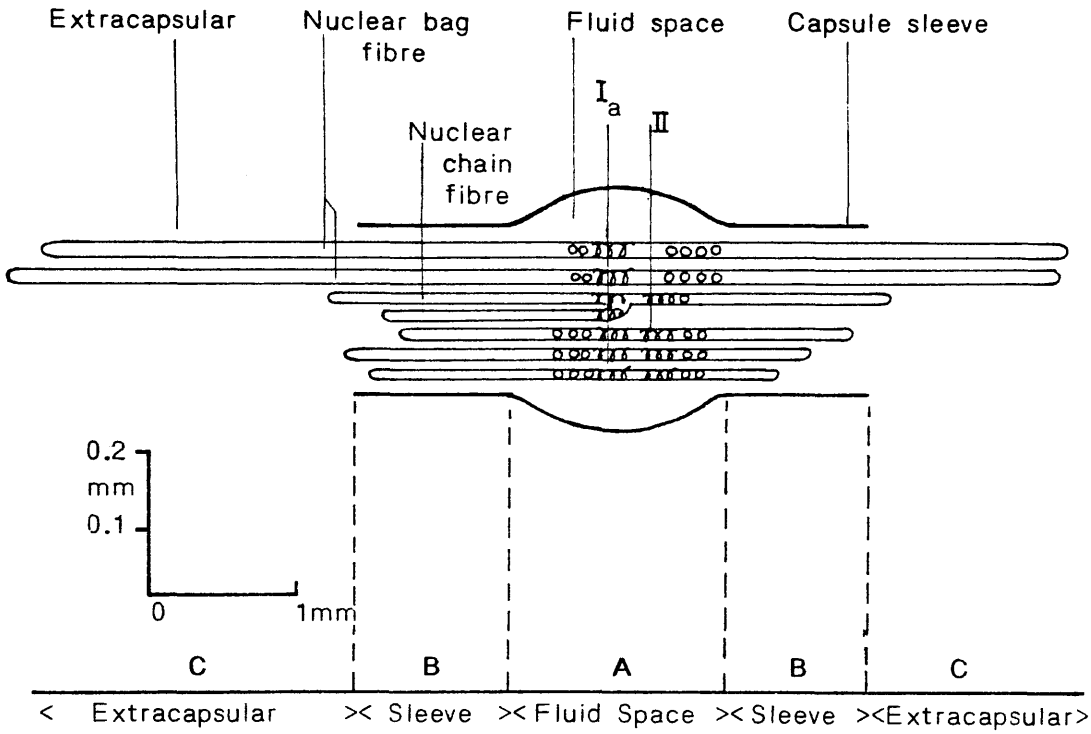


Figure 1 Schematic representation of a typical mammalian muscle spindle showing two nuclear bag fibres and five nuclear chain fibres enclosed in a capsule which is expanded in the centre to form a fluid space.

The primary Ia axon, secondary II axon and the position of the equatorial nuclei are shown.
Region A is the expanded portion of the capsule.
Region B is the capsular sleeve on either side of the fluid space.
Region C is extracapsular.

One large primary afferent (group Ia) fibre supplies the equatorial regions of all the intrafusal fibres of a single spindle whereas 0-5 smaller secondary afferents (group II) supply predominantly nuclear chain fibres on either side of the primary ending.

The motor supply of the spindle has been the subject of much controversy over the years and will be dealt with in detail later.

Before the 1950s the motor innervation of the spindle was considered to consist solely of plate type endings lying predominantly towards the spindle poles, innervated by γ fibres. This simple view has now been greatly modified.

In 1959 Boyd concluded that the larger intrafusal fibres had discrete motor end plates towards the spindle pole while the smaller fibres had endings varying from small plates to diffuse networks. By 1961 he concluded that there were two types of γ axon which supplied the larger and smaller diameter intrafusal fibres and which he called $\gamma 1$, and $\gamma 2$ respectively.

Also at this time Jansen and Matthews (1962) were studying spindle function and they came to the conclusion that the static (length) and the dynamic (velocity) properties of the primary ending were under relatively independent control. They also showed that there were two types of γ axon, one which increased the dynamic response of the primary ending and one which decreased it. In 1964 Crowe and Matthews equated these dynamic and static axons with the γ 1 and $\gamma 2$ axons respectively of Boyd's classification.

In 1966 Barker identified three types of motor ending which he called p_1 plates, p_2 plates and trail endings. The p_1 and p_2

plates commonly lay on nuclear bag fibres but could be found on nuclear chain fibres whereas trail endings were found predominantly on nuclear chain fibres. The trail endings presumably included some of the endings Boyd had previously described as a γ2 network. Barker also demonstrated that the size of the motor axon in no way indicated which fibre or ending type it innervated. He believed that the type of intrafusal fibre movement depended on the motor ending type and not on the muscle fibre type. He also believed that the innervation of intrafusal fibres was non-selective.

The opposite view was taken by Boyd. He believed in selective innervation of intrafusal fibres and that the action of a γ axon on the Ia afferent discharge, whether static or dynamic, depended on the mechanical properties of the intrafusal fibres it innervated.

Evidence to support this view came from experiments on isolated spindles. Nuclear chain fibres were known to contract more rapidly than nuclear bag fibres (Boyd 1966a,b, Smith 1966). Also later studies by Boyd et al (1975) showed that stimulation of known static or dynamic axons in ventral spinal roots resulted in dynamic axons (γD) operating nuclear bag fibres alone, while static axons (γs) could operate nuclear chain fibres alone or nuclear chain fibres and nuclear bag fibres together. The intrafusal fibre contractions were observed in spindles which were isolated from the muscle, but still retained their nerve and blood supply. When nuclear chain and nuclear bag fibres were activated together the resulting effect was static because nuclear chain fibre activity was thought to be dominant.

In 1971a,b Boyd concluded that 90% of all γ axons to cat

muscle spindles were selective in their innervation.

In 1973 Barker et al carried out a detailed histological analysis of thirty spindles whose motor innervation had been reduced to one axon. The function of the surviving axon turned out to be static in each case and it distributed trail endings to both nuclear bag fibres and nuclear chain fibres. In two thirds of cases the innervation was selective supplying either nuclear bag fibres or nuclear chain fibres. However in one third the innervation was non-selective, supplying both nuclear bag fibres and nuclear chain fibres, although it was usually only one bag fibre per spindle.

The answer to this confusing state of affairs came when Ovalle and Smith (1971) discovered that spindles stained for myosin ATPase activity had three different types of intrafusal fibre as reflected by their different staining properties (Fig 2). In both cat and monkey spindles there were two types of nuclear bag fibre which they called bag1 and bag2 fibres as well as nuclear chain fibres. In 1975 Boyd et al proposed that a typical cat spindle (Fig 3) contained two types of bag fibre, one innervated by dynamic axons and the other by static axons. They were subsequently able to show (Gladden 1976) that the dynamic bag fibre and the bag1 fibre of Ovalle and Smith were one and the same fibre while the static bag fibre and the bag2 fibre were also the same. These were therefore subsequently called the dynamic bag1 fibre and the static bag2 fibre.

This clear cut picture of three fibre types with selective innervation was clouded for several years by glycogen depletion studies carried out in 1976 by Barker et al who demonstrated that static γ or dyamic γ axons could innervate nuclear bag fibres or

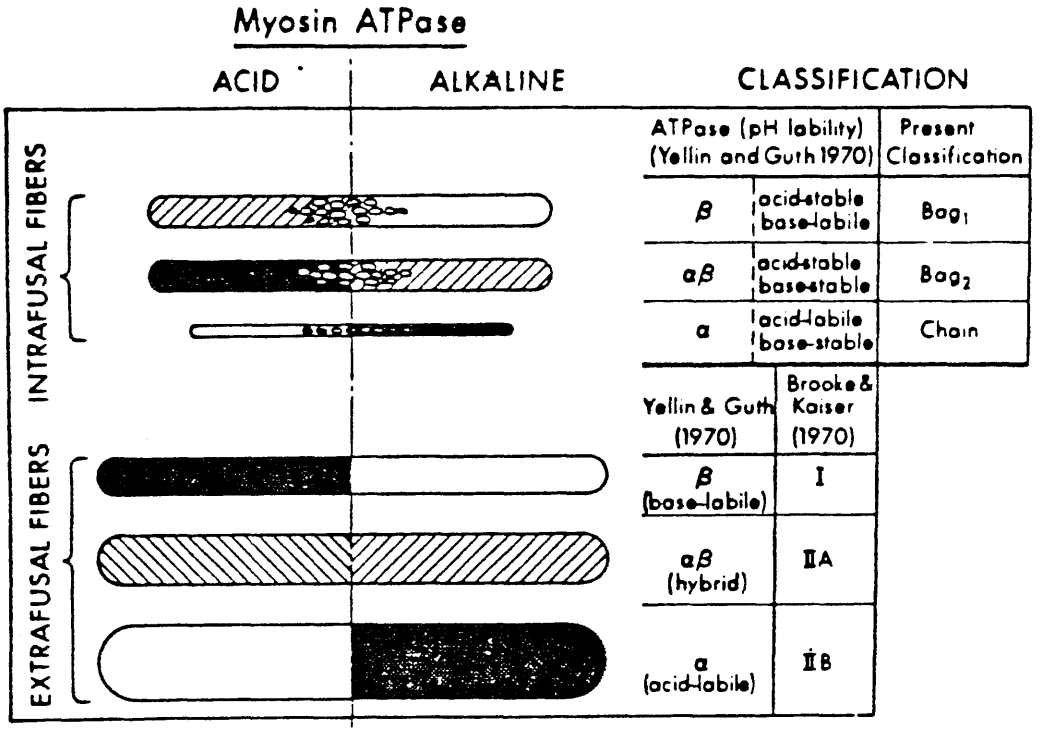


Figure 2 Schematic representation showing the three kinds of intrafusal (above) and extrafusal (below) muscle fibres which are seen in the cat and monkey after staining for myosin ATPase. The staining reactions after acid preincubation (pH 4.35) and alkaline preincubation (pH 10.4) are shown on the left and right respectively. Dark (■), intermediate (▨), light (□). The classifications of Yellin and Guth (1970) and Brooke and Kaiser (1970) are indicated here for the extrafusal fibres. It should be emphasized that the staining reactions of the extrafusal fibres although similar are not identical to those of the intrafusal fibres. (Taken from Ovalle and Smith 1972).

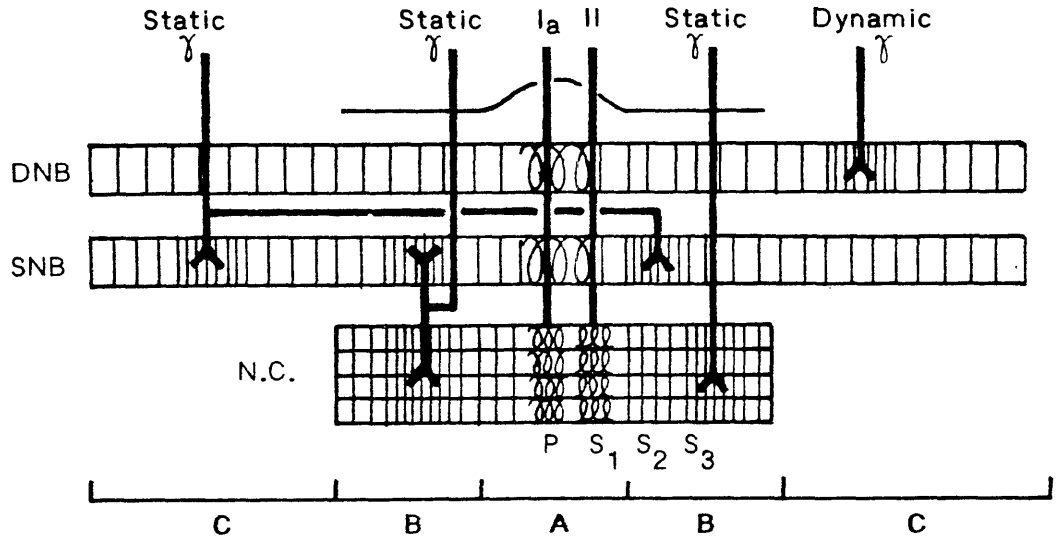


Figure 3 Schematic representation of the motor control of a typical mammalian muscle spindle as depicted by Boyd et al 1975. The spindle receives four fusimotor axons, three selective and one non-selective. DNB, 'dynamic' nuclear bag fibre; SNB, 'static' nuclear bag fibre; NC, nuclear chain fibres; P, primary sensory ending; S_1, secondary sensory ending S_2 and S_3 relative positons of additional sensory endings.

nuclear chain fibres alone, or in any combination.

These results have since been rejected as erroneous. Bessou and Pagès in 1975 showed that stimulation of dynamic γ axons led to contraction in nuclear bag fibres which were never innervated by static axons. Similar results were found by Boyd et al (1975, 1977). Banks, Harker and Stacey (1977) demonstrated that there were three types of intrafusal fibre when studied histochemically and ultrastructurally and Gladden and McWilliam (1977a,b) also supported this when they found that stimulation of different areas of the cats cerebral cortex resulted in three different types of intrafusal fibre contraction. By 1981 Gladden had also shown that dynamic γ axon stimulation gave rise to local responses in bag1 fibres while static γ axon stimulation gave rise to local responses in bag2 fibres or propagated responses in nuclear chain fibres.

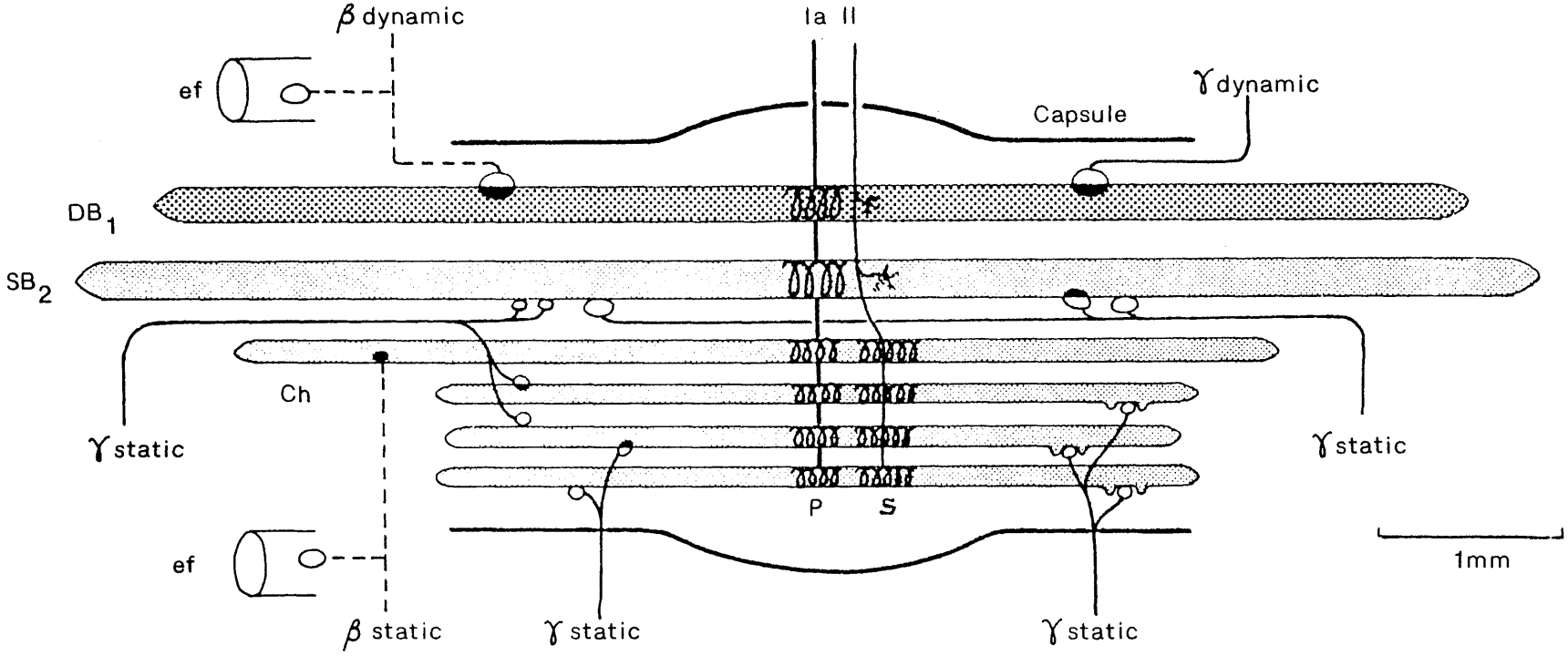
Additional support for selective innervation of intrafusal fibres came from ultrastructural work done by Banks (1981) and Arbuthnott et al (1982) who have shown that static γ axons never terminate on dynamic bag1 fibres.

Fig 4 provides the current agreed view of gamma innervation of the spindle.

β -innervation

It has long been known, and there has never been much controversy about it, that another type of motor axon termed β (beta) which also supplies extrafusal fibres can supply intrafusal fibres. (Fig 4).

These were first demonstrated electrophysiologically by Bessou, Emonet-Denand & Laporte (1963) who demonstrated that



Extracapsular region | Capsular sleeve | Fluid space | Capsular sleeve | Extracapsular region

Figure 4 Schematic representation of β and γ innervation of the mammalian muscle spindle as described by Boyd 1982. DB_1, dynamic nuclear bag fibre; SB_2, static nuclear bag fibre; C, chain fibre; P, primary sensory ending; S, secondary sensory ending.

repetitive stimulation of some axons may elicit both the contraction of extrafusal muscle fibres and an increase in the rate of discharge of spindle primary endings which persisted after selective blockage of extrafusal neuromuscular junctions. The increase in spindle firing could thus be ascribed to the intrafusal fibre contraction.

These axons were identified histologically by Adal and Barker (1965) who traced motor axons from intramuscular nerves to their terminations on both intra and extrafusal fibres.

In 1966 Barker demonstrated that β axons ended in p_1 plates. On nerve section p_1 plates degenerated at the same time as extrafusal end plates. Barker et al (1980) have shown that if a spindle innervated by a β axon is deprived of its γ supply by degeneration, the endings which remain are p_1 plates.

These axons have also been shown electrophysiologically to have a dynamic effect on the primary sensory ending (Bessou, Emonet-Denand & Laporte 1965, McWilliam 1975).

Glycogen depletion studies carried out by Barker et al (1976a) have shown these axons to terminate on dynamic bag1 fibres and in similar studies in 1977 have shown that the extrafusal fibres innervated by these axons are of the slow-oxidative type.

A second type of β axon has also been shown to exist by glycogen depletion studies carried out by Harker et al (1977). They demonstrated that fast conducting motor axons with conduction velocities above 85 m/sec resulted in zones of glycogen depletion in intrafusal fibres after rapid stimulation. Since there were no purely fusimotor fast axons to peroneus tertius, these axons were concluded to be "fast" β axons. They

were found to innervate long chain fibres almost exclusively.

Jami, Murthy & Petit (1980, 1982) have also shown that these fast β axons exert a static effect on the primary and secondary sensory endings, and that they innervate motor units of the fatigue resistant and fast fatiguable types.

Table 1 summarises the electrophysiological, histological and ultrastructural features of the three types of intrafusal fibre in the cat.

TABLE 1

	Dynamic Bag_1	Static Bag_2	Nuclear Chain	Long Chain
MOTOR SUPPLY	γ_D ; β_D	γ_S	γ_S	γ_S ; β_S
SENSORY SUPPLY	Ia ; II (minimal)	Ia ; II (minimal)	Ia ; II	Ia ; II
MYOSIN ATPASE ACTIVITY	Acid Stable Base Labile	Acid Stable Base Stable	Acid Labile Base Stable	Acid Labile Base Stable
MECHANICAL PROPERTIES	Visco-Elastic		Elastic	Elastic
SPEED OF CONTRACTION	Slow	Intermediate	Fast	Fast
CREEP	Present	Absent	Absent	Absent
RESPONSE TO ACETYL CHOLINE	More Sensitive	Less Sensitive	Insensitive	Insensitive
ELASTIC FIBRE DISTRIBUTION (AT POLES)	Few	Many	Present	Present
M-LINE DISTRIBUTION	Transition from absent to present regions B/C	Transition from absent to present regions A/B	Present along entire fibre	Present along entire fibre
MOTOR ENDING TYPES	p_1 plates; p_2 plates Mb/Mab	p_2 plates + trail endings Ma/Mab	Trail endings + p_1 plates Ma/Mc	p_1 plates Md

Table summarising the electrophysiological, histological and ultrastructural features of the three types of intrafusal fibre in the cat.

1.2 SPECIAL FEATURES

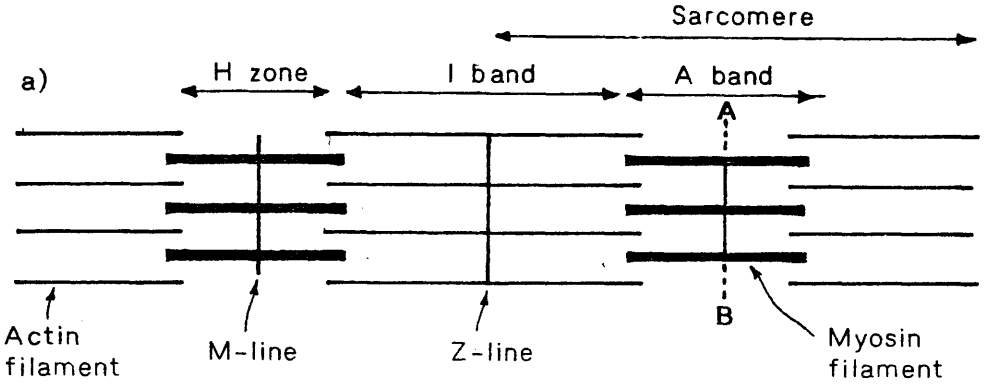
1.2.1

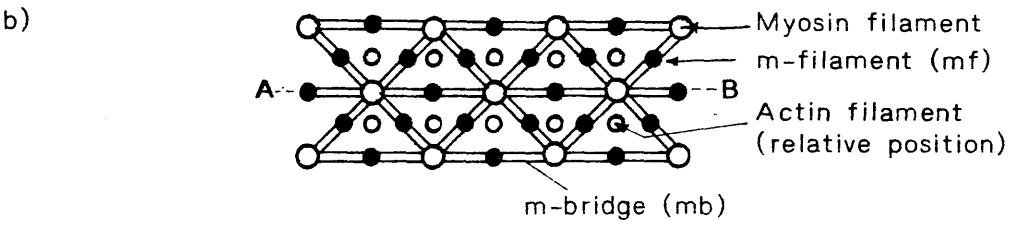
A structure in the middle of the A band of the sarcomere (Fig 5a) in skeletal muscle which differed in transmission from other parts of the A band was observed by Dobie as early as 1849. Since the width of this structure was at the limit of resolution of the light microscope, its relationship to other components in the fibre remained obscure until fairly recently.

Early electron micrographs showed the M-line to consist of an area of high electron density. More detail was only resolved in the 1960s with the introduction of glutaraldehyde as a fixative which enabled better fixation of protein.

The M-line, as described by Knappeiss and Carlsen (Fig 5b,c) consists of bridges mb, connecting adjacent myosin filaments to one another as well as filaments mf, which run in parallel with the myosin filaments and extend for the length of the M region of the sarcomere (70 nm).

Little is known about the chemical composition of the M-line although it does resemble L-meromyosin.





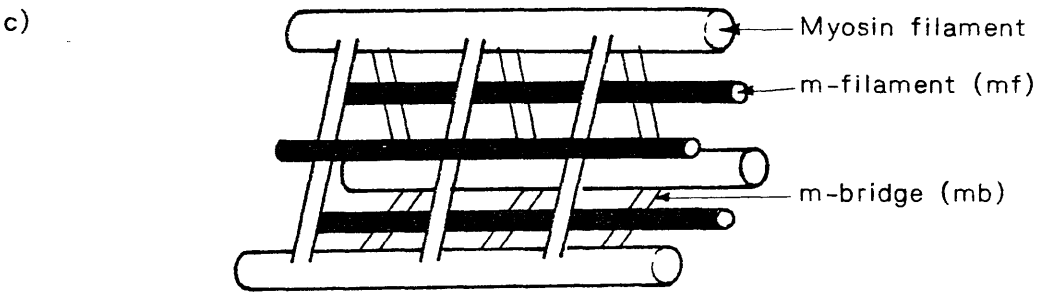


Figure 5 (a) Schematic representation of a sarcomere showing the relative position of the M-line.
(b) Transverse section through A/B of Figure 5a, ie through the M-line showing positions of myosin filaments, M-bridges (mb) and parallel M-filaments (mf) and relative position of actin filaments.
(c) Schematic three dimensional reconstruction of the M-line.

M-Lines of Intrafusal Muscle Fibres

The M-lines of intrafusal muscle fibres were first studied ultrastructurally by Landon in rat muscle spindles in 1966. He showed that nuclear chain fibres possessed shorter sarcomeres than nuclear bag fibres and had well defined H and M zones. The nuclear bag fibres had ill-defined H zones and did not possess M-lines.

In 1969 Corvaja, Marinozzi and Pompeiano studied cat lumbrical muscles ultrastructurally. They demonstrated that nuclear chain fibres had well developed H zones and prominent M-lines. Nuclear bag fibres had ill-defined H zones and absent M-lines.

These two types of intrafusal fibre preserved these differences along their entire length and there was nothing to suggest an intermediate type of fibre.

In 1971 Banker and Girvin in their study of dog muscle spindles also found two populations of intrafusal fibre, based on ultrastructural studies. The nuclear chain fibres possessed well defined A, H, M, I and Z bands which in transverse section appeared as a precisely ordered hexagonal pattern of dots representing the transected myofilaments. This pattern extended the length of the fibres. The myofilaments were grouped together in a single large bundle, unlike skeletal muscle where they are grouped together into discrete myofibrils.

In contrast to this the nuclear bag fibres in the extracapsular and polar intracapsular regions could not be differentiated from extrafusal fibres, possessing A, H, M, I and Z bands and having discrete myofibrils. However as the equatorial region was approached the myofilament arrangement

changed. They were no longer grouped together into discrete myofibrils and there was a gradual loss of the M-line, then the H-zone and finally the I band. In transverse section each myosin filament was surrounded by ten to twelve actin filaments. The chain fibre myofilament arrangement was thought to resemble cardiac muscle while the transitional area of the nuclear bag fibres looked like smooth muscle.

In 1971 and 1972 Ovalle studied rat intrafusal fibres ultrastructurally. In the polar regions of the fibres there were striking differences between nuclear bag fibres and nuclear chain fibres.

Nuclear chain fibres possessed clearly demarcated myofibril units and prominent M-lines. Nuclear bag fibres had ill-defined myofibrils and at low power did not appear to have M-lines. At high power of longitudinal sections however they sometimes had an M-line which was also visible on transverse section.

In the equatorial region similar results were found. The nuclear bag fibres had less conspicuous M-lines composed of two parallel thin densities in longitudinal section. These findings did not agree with Landon's earlier findings in which nuclear bag fibres did not possess M-lines at all.

In 1976b this discrepancy was finally explained by Barker et al who conducted ultrastructural studies on cat, rabbit and rat spindles. By this time Ovalle and Smith 1972 had also shown that there were two types of nuclear bag fibre histochemically. Barker et al (1976b) found that the M-line could be present as a single prominent line in longitudinal sections viewed at low power. They designated this type of M-line, "M".

They also found that some bag fibres had apparently no

M-line at low power but when studied at high power the M-line was present as two parallel lines. They designated this type of M-line, "dM". Thus two types of nuclear bag fibre were described on the basis of their M-line characteristics. Static nuclear bag fibres were mainly "dM" type in region A changing to "M" type in region B in all three species. Dynamic nuclear bag fibres were more variable. In the cat the "dM" picture of region A changed to "M" type at the polar end of region B. In the rabbit the transition occurred in region C and in the rat no transition occurred (See Figs 1 & 3 for regions A, B and C).

They found that chain fibres were of "M" type along their entire length in all three species. Fig 6 summarises the findings of Barker et al (1976). They suggested that since the transition zone of bagl fibres for cats and rabbits occurred at a similar distance from the equator (1 - 1.5 mm) this may be a developmental factor associated with the primary afferent ie that the primary afferent may be responsible for the induction of the M-lines during development. Rat spindles are smaller and shorter than cat or rabbit spindles therefore this could explain the lack of transition. They also found that variations in histochemical profile did not correlate with the ultrastructural variations.

In 1977 Banks et al carried out a combined histochemical and ultrastructural study on cat, rabbit and rat muscle spindles as this was more accurate than the previous work quoted.

The condition of the M-line provided conspicuous differences again between intrafusal fibre types. Two types of M-lines were recognised. In one the M-line was prominent as a single structure on low power electron micrographs or as five parallel lines on higher power electron micrographs. This was designated

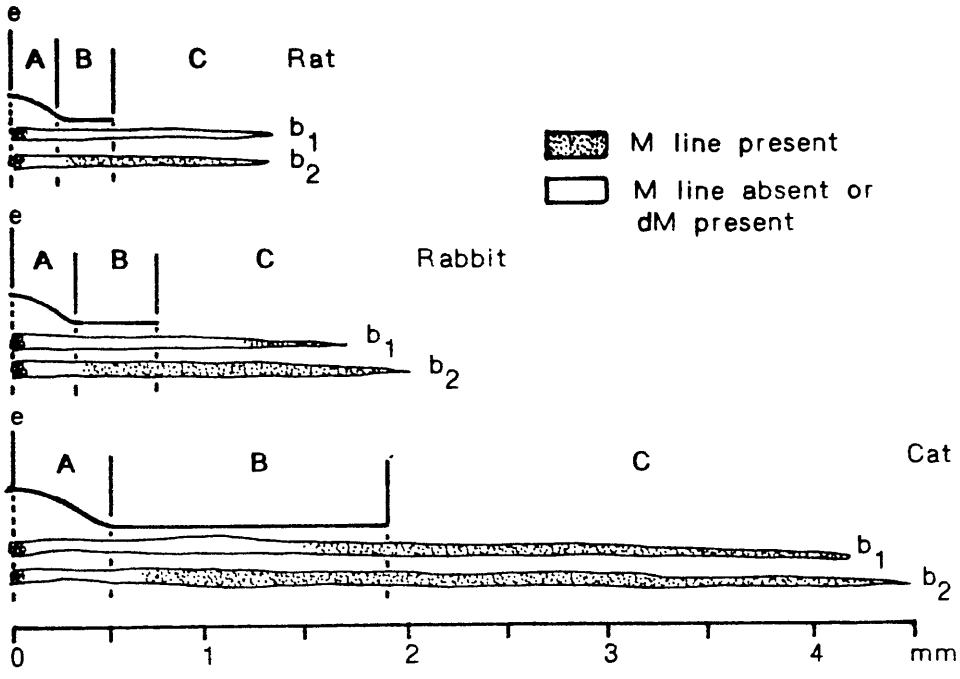


Figure 6 Variation in M-line condition of bag_1 (b_1) and bag_2 (b_2) fibres in regions A, B and C of the poles of typical rat, rabbit and cat spindles (muscle fibre width x 3 that used for length). e equator (Taken from Barker et al 1976b).

type "M". In the other the M-line was seen as two parallel lines on high power electron micrographs only. This was designated type "dM".

Bag1 fibres were of dM type throughout their lengths in rat spindles but in cat and rabbit the dM pattern changed to M type in the extracapsular region.

Bag2 fibres were of dM type in the equatorial region changing to M type in the poles between areas A and B. Chain fibres were of M type throughout their length.

In correlating the ultrastructural features with the histochemical, Banks concluded that there were three different types of intrafusal fibre but that these could not be confidently differentiated on the basis of any single technique. Often two of the three types were included in one group.

In 1979 Kucera and Dorovini-Zis studied human muscle spindles ultrastructurally. They found that the bag1 fibre had absent M-lines in the A region. Some sarcomeres had faint M-lines near the plasmalemmal membrane or nucleus.

In the extracapsular region where the sleeve thins down, well developed M-lines were present.

Bag2 fibres had a similar appearance to bag1 fibres equatorially and juxta equatorially but M-lines appeared sooner in the capsular sleeve region.

Nuclear chain fibres had clearly defined M-lines throughout.

These studies, the findings of which are summarised in Table 2, suggest that M-line distribution can be used to distinguish the dynamic bag1 fibre from the static bag2 fibre providing the fibres end more than 1.5 mm from the equator. Clear differences

TABLE 2

Year	Author	Animal	Nuclear Chain	Nuclear Bag
1966	Landon	CAT	M-lines distinct	Ill defined H zone No M-lines
1969	Corvaja et al	CAT	M-lines distinct	Ill defined H zone No M-lines
1969	During & Andres	CAT	M-lines distinct	No M-lines
1970	Barker & Stacey	CAT	M-lines present	i)Intermediate diameter bag M-lines present ii)Large diameter bag M-lines absent
1971	Banker & Girvin	DOG	M-lines distinct	M-lines in sleeve region
1971/72	Ovalle	RAT	M-lines distinct	M-lines less distinct Present at HP throughout fibres
1975	Banks & James	RABBIT	M-lines distinct	i)Intermediate diameter bag M-lines distinct ii)Large diameter bag No M-lines
1976	Barker	CAT	M-lines distinct	i)Bag_2 - M-lines distinct in sleeve region and outwards ii)Bag_1-M-lines distinct extra capsularly
		RABBIT	M-lines distinct	i)Bag_2 - M-lines distinct in sleeve region and outwards ii)Bag_1 - M-lines distinct extracaps.
		RAT	M-lines distinct	i)Bag_2 - M-lines distinct in sleeve region ii)Bag_1 - No M-lines
1977	Banks	CAT RABBIT	M-lines distinct	i)Bag_2 - M-lines distinct in sleeve ii)Bag_1 - M-lines distinct extracaps.
		RAT	M-lines distinct	i)Bag_2 - M-lines distinct in sleeve ii)Bag_1 - less distinct M-lines
1979	Kucera & Darovini-zis	HUMAN	M-lines distinct	i)Bag_2 - M-lines in sleeve ii)Bag_1 - M-lines extracaps.

Summarising the presence of M-lines in the three types of intrafusal fibre of rat, cat, rabbit, dog and human muscle spindles

in human spindles suggest that M-lines may be used to distinguish the dynamic bag1 fibre from the static bag2 fibre in baboon spindles.

1.2.2 Elastic Fibres

Elastic fibres are rarely seen among the extrafusal fibres of somatic muscle. Much elastic tissue is however found lying along individual intrafusal fibres (Cooper and Daniel 1963). This suggested that elastic fibres had a protective function as intrafusal fibres might be expected to be subjected to greater stress. This is firstly because their contraction, under fusimotor control may occur asynchronously with extrafusal fibre contraction and secondly because the rate of contraction of nuclear bag fibres may be slower than that of extrafusal fibres.

From studies done on various animal spindles it became apparent that there was a variation in the prevalence of elastic fibres in different regions of the spindle. This was of interest because prevalence of elastic fibres might indicate local areas within the spindle subjected to greater stress.

Cooper and Daniel (1967) in their work on human and rat spindles described spindles which had longitudinally running elastic fibres which diminished in number as the equator was approached (Fig 7c).

This extracapsular prevalence of elastic fibres might indicate that they take over the protective role of the capsule extracapsulary. Cooper and Daniel (1967) also found that in transverse sections the nuclear bag fibres had elastic fibres on either side of the nuclear bag region ie in the region of the secondary endings. Nuclear chain fibres had elastic fibres associated with both the primary and secondary endings (Fig 7c).

Cooper and Daniel (1967) proposed that the main function of the elastic fibres in muscle spindles was to ensure that after the spindle had been stretched, it would return rapidly to its

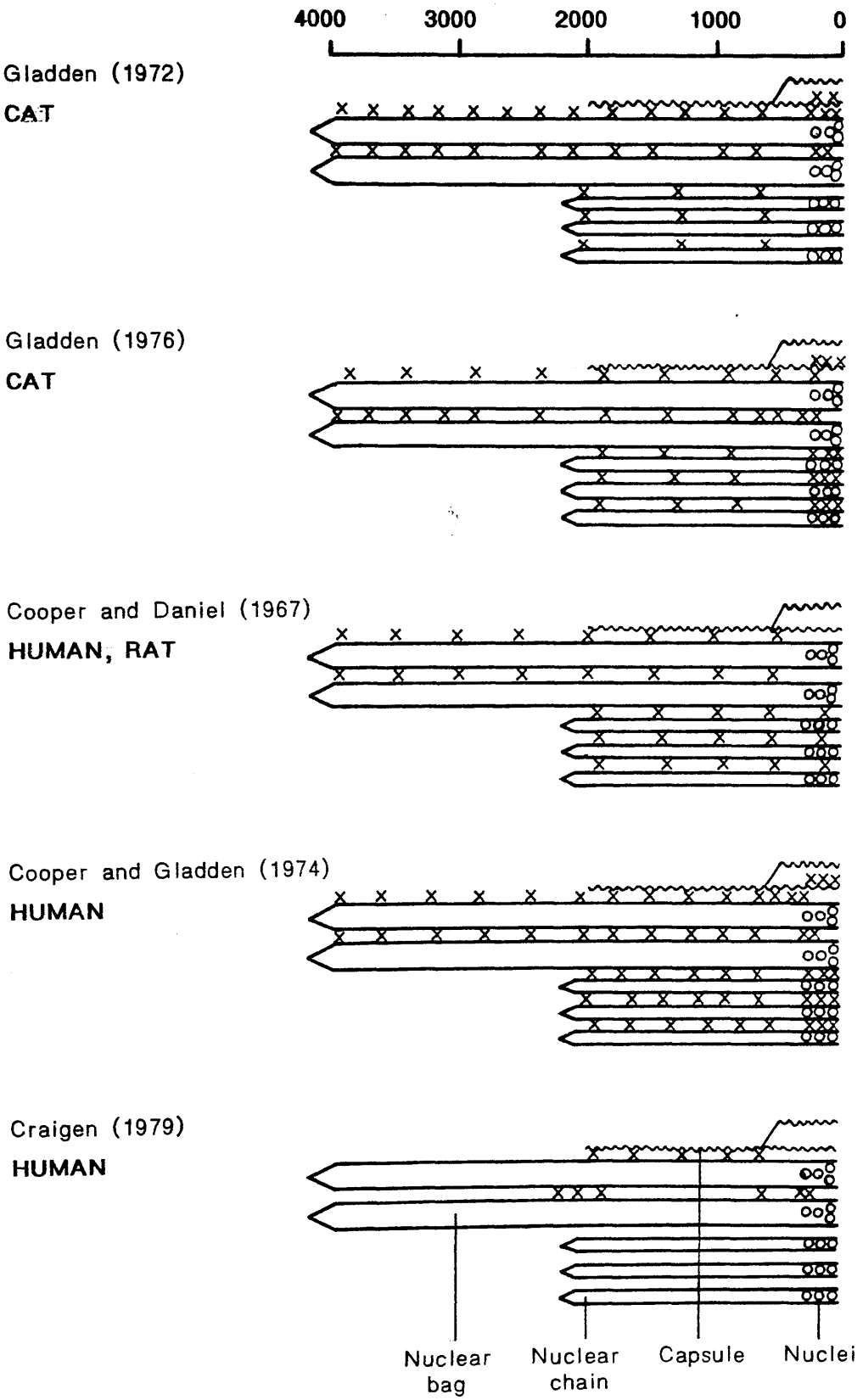


Figure 7 Schematic representation of elastic fibre distribution in mammalian muscle spindles as found by a) Gladden (1972) in cat, b) Gladden (1976) in cat, c) Cooper and Daniel (1967) in human and rat, d) Cooper and Gladden (1974) in human and e) L Craigen (BSc Hons 1979) in human. The presence of elastic fibres is indicated by x.

resting position.

In 1972 Gladden studied the distribution of elastic fibres in cat muscle spindles and described a difference between nuclear bag and nuclear chain fibres. Extracapsularly there were longitudinally running elastic fibres associated with all the intrafusal fibres. Inside the capsule however a network of elastic fibres was formed round the nuclear bag fibres, scarcely any being associated with the nuclear chain fibres. In the equatorial region the elastic fibres increased in number with at least 50% lying among the epithelial cells covering the intrafusal fibres (Fig 7a).

Gladden proposed at this time that the elastic mechanical properties of the nuclear chain fibres (Table 1) and the viscous mechanical properties of the nuclear bag fibres as described by Boyd (1966, 1971) must be innate properties of the intrafusal fibres themselves though the elastic fibres of the nuclear bag fibre might modify its mechanical properties. In 1974 Cooper and Gladden extended the work of Cooper and Daniel (1967) to include cat spindles. This work showed that cat spindles had much elastic tissue extracapsularly and that differences between nuclear bag fibres and nuclear chain fibres only became apparent in the fluid space. Between the beginning of the fluid space and the equatorial region the number of elastic fibres round nuclear bag fibres was greater than around nuclear chain fibres.

At around 250 um from the equator some of the elastic fibres round the nuclear bag fibres divided, some continuing with the bag fibre and some passing outwards in the inner capsule. In the equatorial region the number of elastic fibres round the nuclear bag fibres fell while the number around nuclear chain fibres rose

(Fig 7d).

The distribution of elastic fibres in human spindles was similar to the cat in the extracapsular region and at the equator although the differences in transverse sectioning between nuclear bag fibres and nuclear chain fibres was not so striking. Between the beginning of the fluid space and the equator in human spindles however two types of nuclear bag fibre were distinguishable. One had more elastic than the other for most of its length (Fig 7d).

In 1975 Gladden extended this work on human spindles and demonstrated that as the myoplasmic area of human spindle intrafusal fibres fell there was a mechanical compensatory increase in elastic fibres. The existence of two nuclear bag fibre patterns of elastic fibre distribution was confirmed (Fig 8).

In 1976 Gladden marked the dynamic bag1 fibre and static bag2 fibre intracellularly and found there were differences between the fibres at the poles when studied at least 1 mm from the end of the capsule. The static bag2 fibre had much more elastic than the dynamic bag1 fibre and often ended in an elastic tendon (Fig 7b).

Although the significance of this is not certain this proved to be a very useful way of distinguishing the dynamic bag1 fibre from the static bag2 fibre in cat spindles. Elastic fibres can be clearly seen by both light and electron microscopy (Plate 1, Plate 2).

Preliminary work done by L Craigen (BSc. Hons. project) in 1979 on human spindles did not however confirm this extracapsular difference in elastic fibre distribution between the dynamic bag1

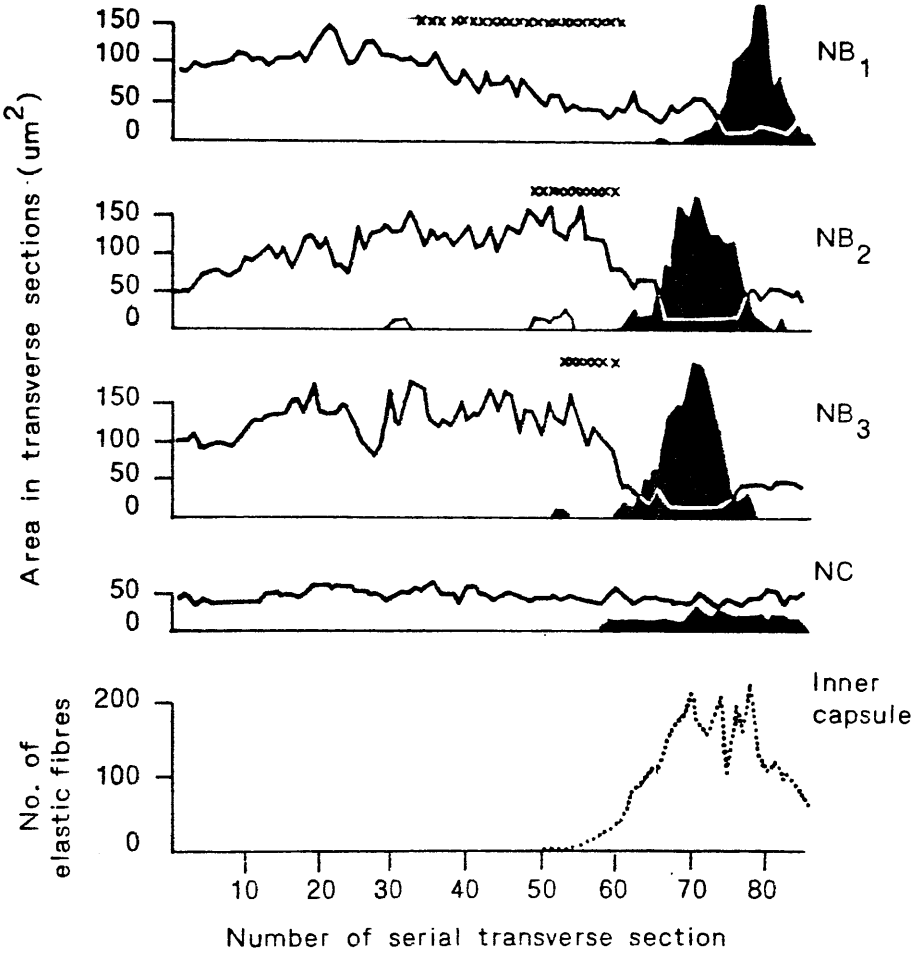
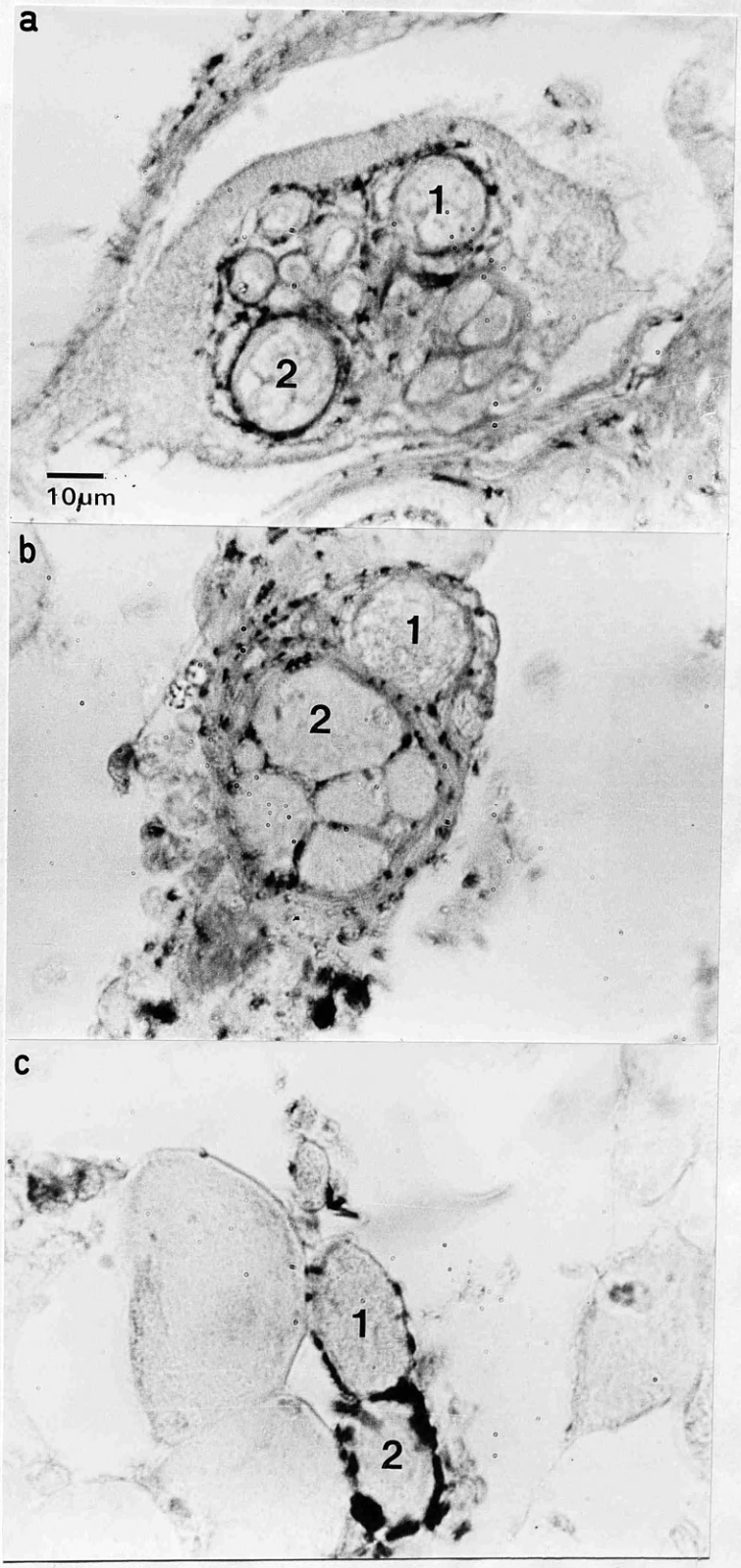


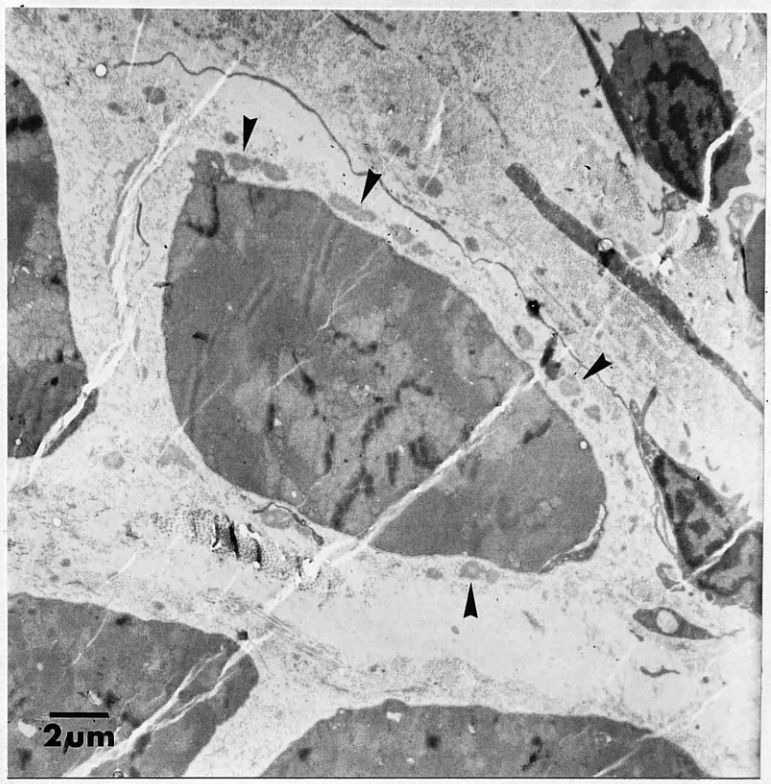
Figure 8 Diagram showing the number (dotted line) and position of elastic fibres (xxx) in human muscle spindles relative to the myoplasmic area of intrafusal fibres (solid lines). The periaxial space began at section 0. The equator occupied sections 55-85. (Taken from Gladden 1975).

Plate 1



Transverse sections of cat muscle spindle stained for elastic fibres with resorcin-fuchsin a) at equator b) 750 μm from equator and c) 2000 μm from equator.
1 - Dynamic bag_1 fibre; 2 - static bag_2 fibre.

Plate 2



Ultrathin transverse section of a static bag$_2$ fibre from a cat tenuissimus muscle spindle. Elastic fibres are arrowed.

and static bag2 fibres. One type of bag fibre did appear to have more elastic at the beginning of the inner and outer capsules while the other had more in the sleeve region (Fig 7e).

In view of the usefulness of elastic fibres in distinguishing the two types of nuclear bag fibre in cat spindles, elastic fibres in baboon spindles may be of similar value.

1.2.3 Motor Endings

The light microscopy and electron microscopy of mammalian motor endings will be considered separately.

Light Microscopy

In 1962 Boyd, using gold chloride stained teased muscle spindles, demonstrated that there were two sizes of fusimotor axon. The larger axon was 3-5 um in diameter, did not branch much and terminated in motor end plates on the nuclear bag fibres. These motor end plates were usually found in the S3 region of the nuclear bag (Fig 3) although an odd one did occur further out. The endings were not unlike extrafusal endings. He labelled the supplying axon, $\gamma 1$.

The smaller axon was 2-3 um in diameter in the intramuscular nerve, branched extensively until it was less than 1 um in diameter on approaching the motor endings. These were narrow elongated delicate structures linked together by fine axon branches and arranged in a row along the muscle fibre which was usually a nuclear chain fibre although occasionally it was a nuclear bag fibre. The feeding axon was labelled $\gamma 2$ and the endings were described as a $\gamma 2$ network since one ending often received several fine axons. Most of those endings were found on

the S2 region of the nuclear chain fibres.

Barker and lp 1965 using a new method of staining teased spindles with silver showed that any intrafusal fibre may receive one or both types of ending and that the different endings were not innervated by axons of any particular size. They described the two types of ending as plate endings and trail endings. The latter were so called because they were not seem to link up or branch as Boyd had described the γ2 network.

In 1966 Barker extended his classification to include type 1 plates and type 2 plates.

Type 1 (later called p_1) were identical to extrafusal end plates. They were typically found near the ends of spindle poles. In 1970 Barker et al demonstrated that p_1 plates degenerated at the same time as extrafusal end plates when their motor supply is severed. They hypothesised that β axons innervated p_1 plates.

Type 2 (later called p_2) were longer than type 1, had no nucleated sole plate or Doyere eminence, consisted of irregular knobs and rings and were found typically in a mid polar region. They were innervated by γ axons.

Trail endings were diffuse multiterminal endings extending over several hundred um. They were found juxta equatorially.

Both nuclear bag fibres and nuclear chain fibres could be innervated by any of these endings which in turn could be supplied by axons of varying diameter.

Barker also suggested at this time that the trail endings were similar to the "en grappe" endings of various vertebrates. These endings produce slow local contractions initiated by non propagated potentials. He also suggested that p_1 and p_2 plates

were capable of producing fast twitch contractions.

These results are summarised in Table 3.

TABLE 3

AUTHOR	MOTOR ENDING	FIBRE TYPE	POSITION ON FIBRE	AXON DIAM.	AXON TYPE	AXON BRANCHING	SIZE OF END PLATE	FEATURES OF ENDING
BOYD (1962)	END PLATE	NUCLEAR BAG	S_3 REGION	3-5 um	γ_1	LITTLE	50-75 um	SIMILAR TO EXTRAFUSAL ENDINGS
	γ_2 NETWORK	NUCLEAR CHAIN	S_2 REGION	1 um	$\delta 2$	EXTENSIVE	50 um	NARROW ELONGATED, FREQUENTLY IN ROWS
BARKER (1966)	p_1 PLATE	NUCLEAR BAG OR NUCLEAR CHAIN	MID POLAR OR EXTREME POLAR	LARGE	β	LITTLE	13-62 um	SIMILAR TO EXTRAFUSAL ENDINGS
	p_2 PLATES	NUCLEAR BAG (occasionally nuclear chain)	MID POLAR OR EXTREME POLAR	SMALL	γ	LITTLE	27-120 um	TERMINALS CLOSE TO MUSCLE NO DOYERE EMINENCE
	TRAIL ENDINGS	NUCLEAR BAG OR NUCLEAR CHAIN	INTRA CAPSULAR JUXTA-EQUATORIAL	SMALLER	δ	LITTLE	940-1580 um	TERMINALS CLOSE TO MUSCLE. NO SUBJUNCTIONAL FOLDS

Summary of light microscopy findings of cat spindle motor endings.

Electron Microscopy

The first ultrastructural picture of motor endings came from Landon in 1966. He found a motor ending extracapsularly on a nuclear bag fibre. It lay in a depression on the surface of the muscle and contained mitochondria and numerous small vesicles. The post synaptic membrane had no folds and there was very little sole plate.

In 1967 Adal and Barker looked at p_2 plates and trail endings ultrastructurally. The p_2 plates consisted of knob like axon terminals which lay in shallow depressions of thin sole plate. Junctional folds were usually present but different from extrafusal end plates in that the folds were unbranched and about half the depth.

Thirteen p_2 plates were found on nuclear bag fibres and eight p_2 plates were found on nuclear chain fibres. (Fig 9).

Trail endings were applied to the surface of the muscle overlying a thin sole plate. Junctional folds were absent.

Nine trail endings were found on nuclear chain fibres and five were found on nuclear bag fibres (Fig 9).

In 1969 Corvaja et al described two types of ultrastructural fusimotor ending in cat spindles.

The first type was found on nuclear bag fibres and nuclear chain fibres, contained numerous vesicles, no subjuctuional folds and minimal sole plate which sometimes contained mitochondria and a nucleus. These endings were found near the end of the spindle capsule but were observed extracapsularly. They were described as trail endings (Fig 10a).

The second type was found on the extracapsular polar region of the nuclear bag fibres. It appeared as a group of thin

Figure 9 Electron micrograph tracings of cat myoneural motor junctions (peroneal muscle) showing axon terminals of A, a trail ending in transverse section; B, a p_2 plate in longitudinal section C, an extrafusal plate in longitudinal section and D, a p_1 plate in longitudinal section. j.f., junctional fold; m., mitochondrion; m.f., myofibrils; pt. a., preterminal axon; S.c., Schwann cell; s.p., sole plate. (Taken from Adal and Barker 1967 and Barker et al (1970).

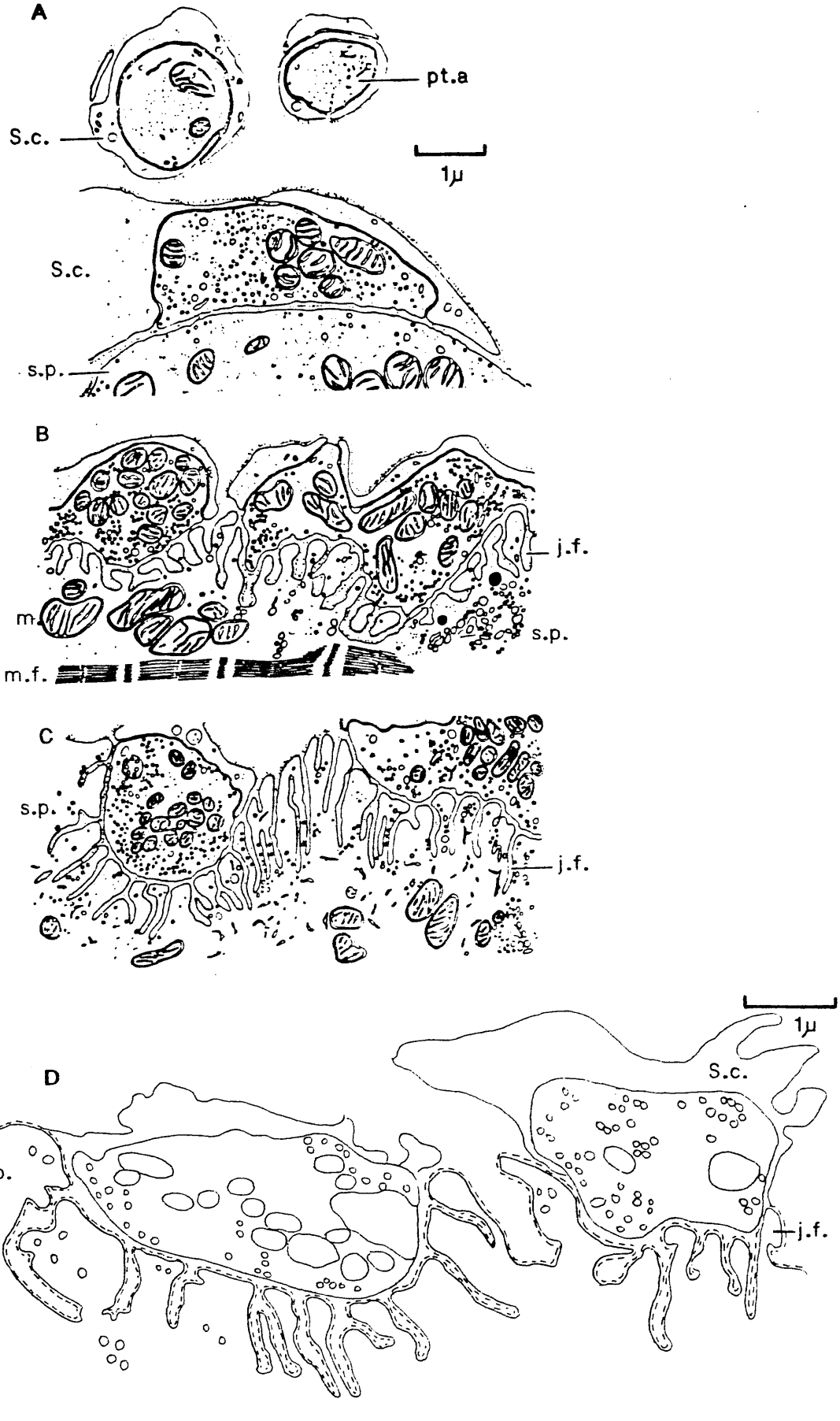
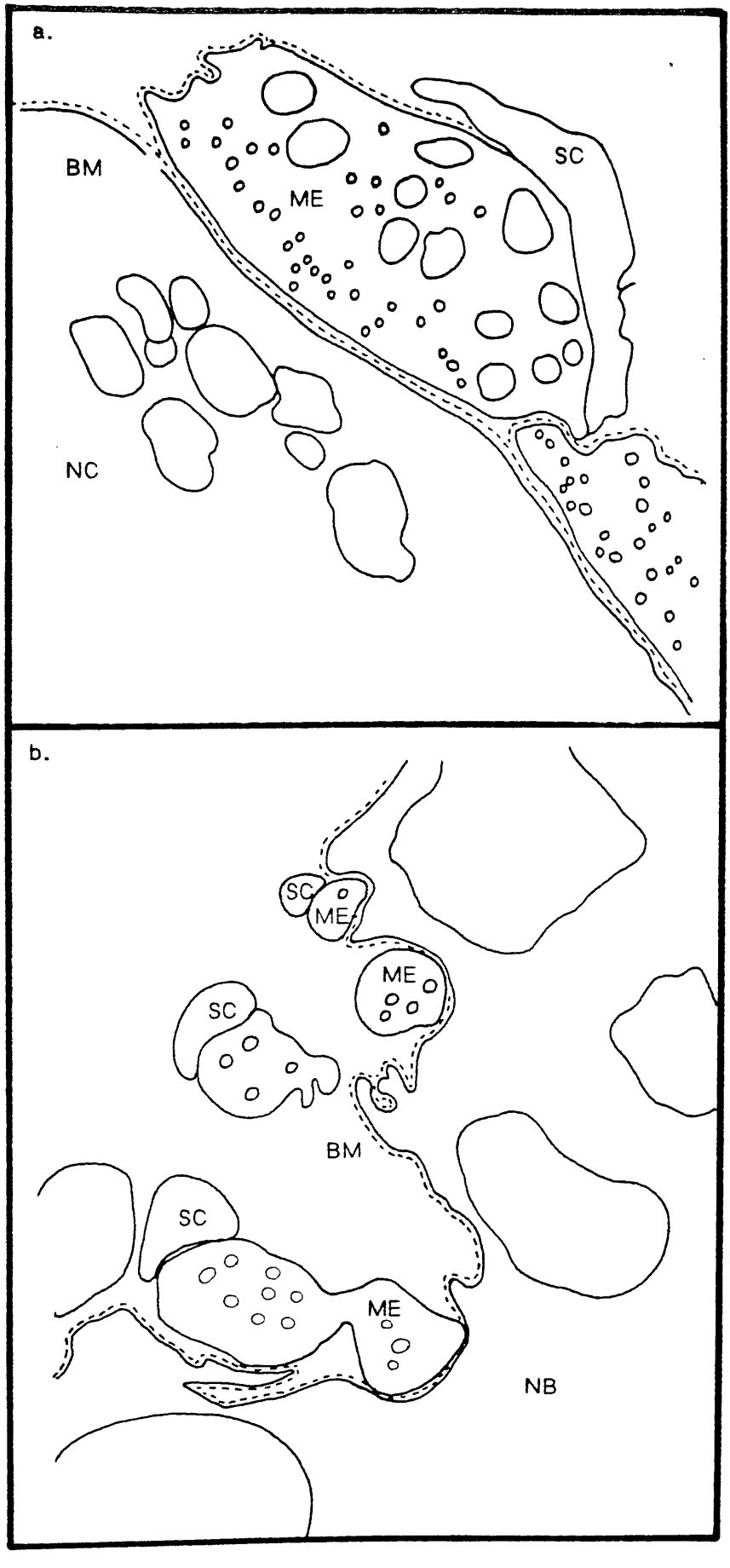

A
pt.a
S.c.
1μ
S.c.
s.p.
B
j.f.
m
s.p.
m.f.
C
s.p.
j.f.
1μ
D
S.c.
s.p.
j.f.

Figure 10 Electron micrograph tracing of a) a cross section through the polar end of a cat spindle capsule illustrating a trail ending on a nuclear chain fibre x 17,500 and b) a longitudinal section of intrafusal muscle fibres extracapsularly, illustrating numerous small motor endings on a nuclear bag fibre x 7,000. N.C., nuclear chain fibre; N.B., nuclear bag fibre; B.M., basement membrane; S.c., Schwann cell; M.E., motor ending. (Taken from Corvaja, Marinozzi and Pompeiano 1969).


a.
BM
ME
SC
NC
b.
SC
ME
ME
SC
BM
SC
ME
NB

endings penetrating the intrafusal fibre deeply. The sole plate contained an accumulation of nuclei and mitochondria and there was some rudimentary folding (Fig 10b).

In 1971 Banker and Girvin studied dog spindles ultrastructurally. They observed that trail endings were more numerous on chain fibres but were frequently observed on nuclear bag fibres. Only one plate ending was found. This was on a nuclear bag fibre extracapsularly.

The trail endings had small subjunctional folds whereas the plate endings had larger branched subjunctional folds (Fig 11).

Banker and Girvin noted however that the size of the trail ending subjunctional folds and mitochondria varied depending on the section. The post-synaptic specialisations were present only at the core of the ending.

In 1978 an ultrastructural study of motor endings by Barker et al showed that static γ axons supplied bag2 fibres and nuclear chain fibres. The lengths of the endings found were 16-100 um. They possessed both smooth and folded post synaptic membranes but the deepest and most regular folds occurred on nuclear chain fibres (Fig 12a-f). Terminals with smooth junctions were sometimes present in the same sections as those with folded junctions therefore post synaptic folding was not a good marker for distinguishing trail endings from plate endings.

Dynamic γ axons supplied dynamic bag1 fibres in seven out of nine fibres. The endings were 15-50 um long and all had smooth post synaptic membranes (Fig 12g, h).

In 1981 Banks studied cat motor endings histologically to try and clarify the problem of selective innervation of intrafusal fibres.

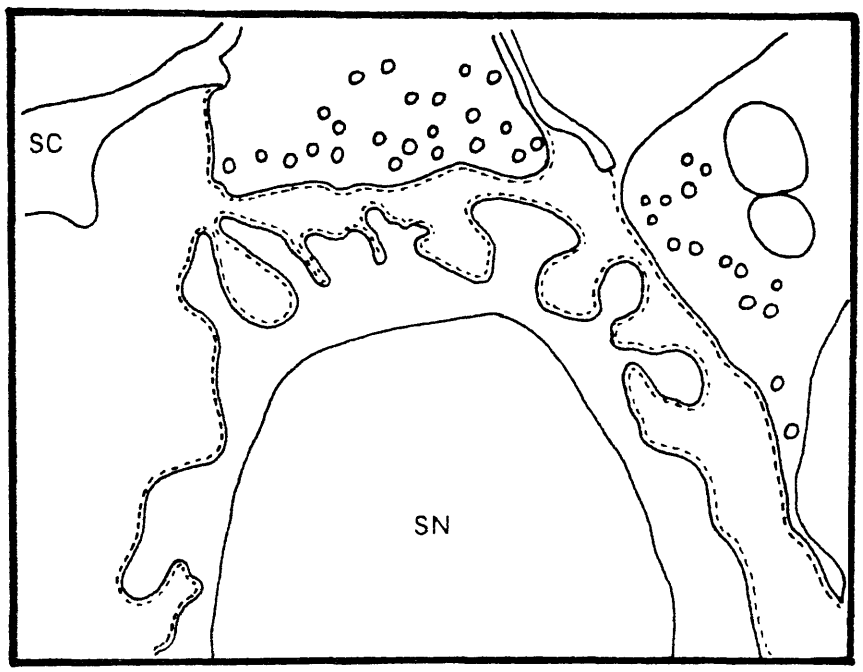


Figure 11 Electron micrograph tracing of a transverse section through a trail ending found on a nuclear chain fibre in a dog spindle (x 27,280) as observed by Banker and Girvin 1971. S.C., Schwann cell; S.N., Sole plate nucleus.

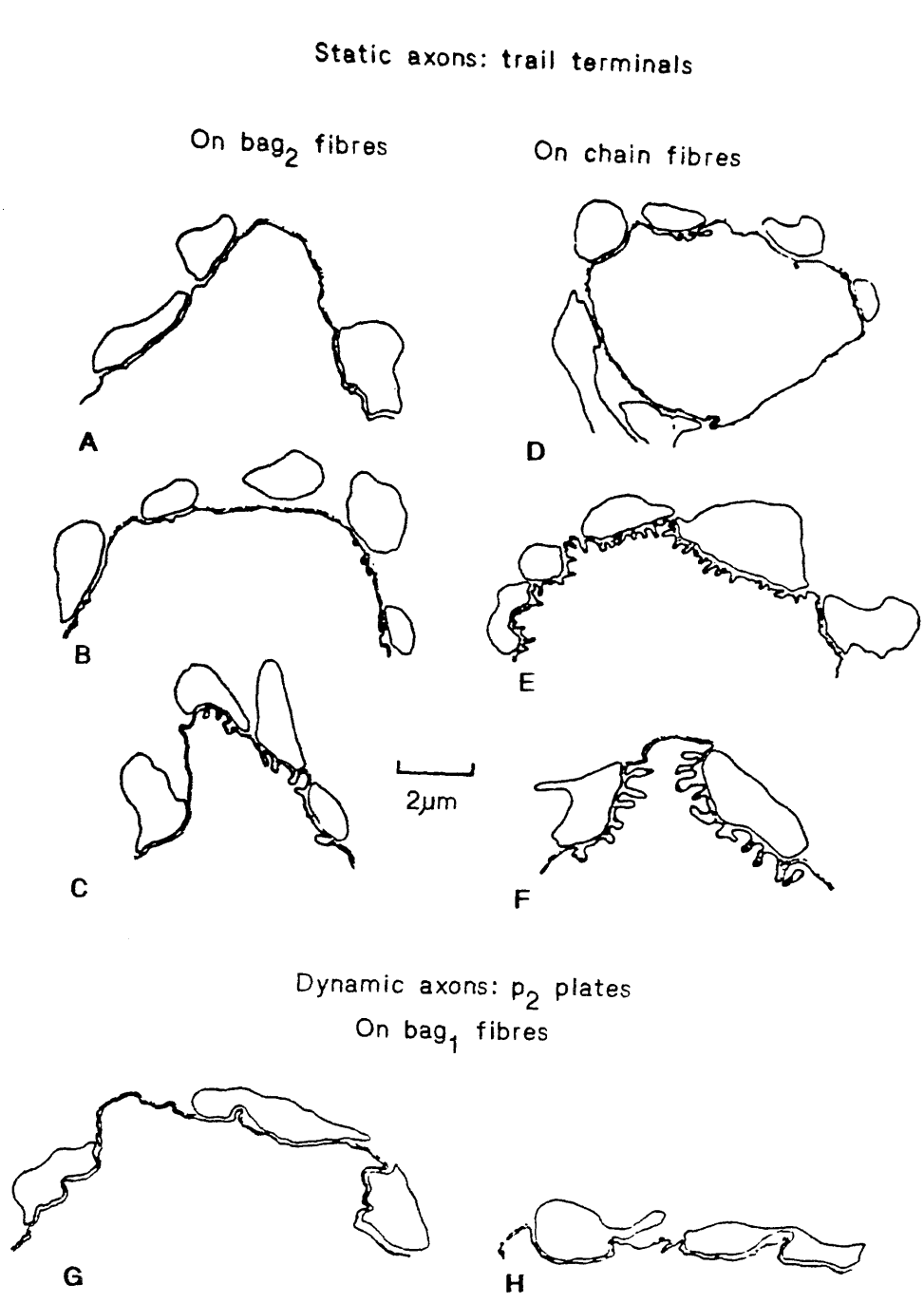


Figure 12 Electron micrograph tracings of transverse sections through the myoneural junctions of static (a-f) and dynamic (g,h) axons. (Taken from Barker et al 1978).

He found that the majority of dynamic γ axons innervated dynamic bagl fibres but one also supplied a nuclear chain fibre. Although this did not support the "typical spindle" proposed by Boyd et al 1975 it did not support Barker's view of non selective innervation in which dynamic bagl fibres could be innervated by static γ axons. Banks did not find this.

Twenty four motor endings were reconstructed from 1 um sections. They exhibited a range of characteristics none of which made it possible to identify any of the endings as being appropriate to a particular type of intrafusal fibre. Axons known to be skeleto-fusimotor innervated endings which were not obviously different from other endings on the nuclear bag fibres.

Nuclear chain fibre endings tended to have more prominent sole plates, post-junctional folding and more profuse branching of pre-terminal axons (Fig 13).

In 1982 Arbuthnott et al followed up their electrophysiological studies supporting selective innervation with an ultrastructural study.

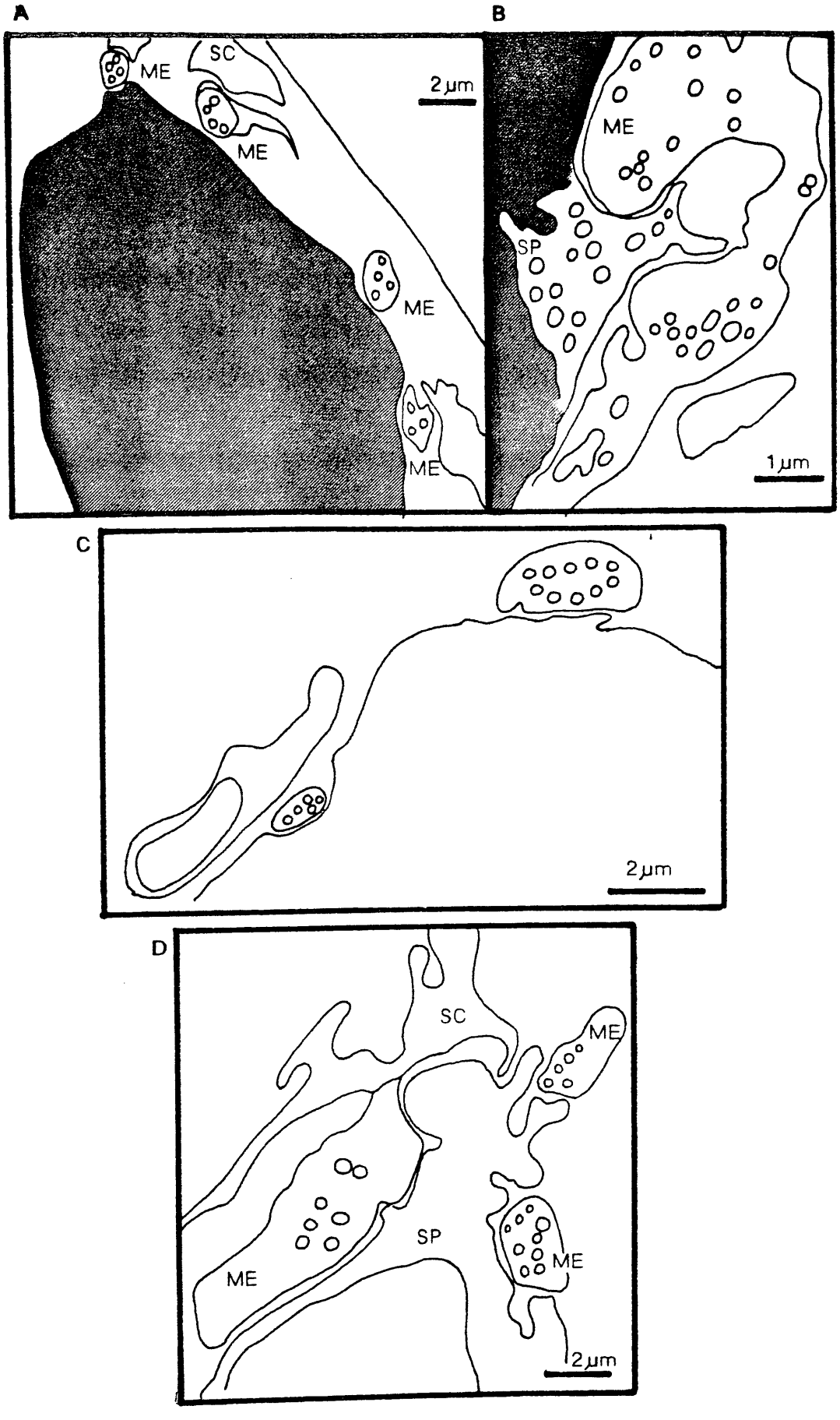
This showed that static γ axons never terminated on dynamic bagl fibres. However one dynamic γ axon did terminate on a nuclear chain fibre.

They classified the motor endings they found into five types dependent mainly on the degree and manner of the indentation of the axon terminals into the muscle surface (Fig 14).

Type Ma - Lay superficially (not more than 25% indented).

Mb - Indented more than 25% into muscle surface

Figure 13 Electron micrograph tracings of transverse sections through motor endings found by Banks 1981 in cat spindles.
a) probably a p_2 plate found on a bag_1 fibre
b) probably a p_1 plate found on a bag_1 fibre
c) probably a trail ending found on a bag_2 fibre
and
d) probably a trail ending found on a nuclear chain fibre.
M.E., motor ending; S.c., Schwann cell; s.p., sole plate.


A
B
ME
SC
ME
2 µm
ME
ME
ME
SP
1 µm
C
2 µm
D
SC
ME
SP
ME
ME
2 µm

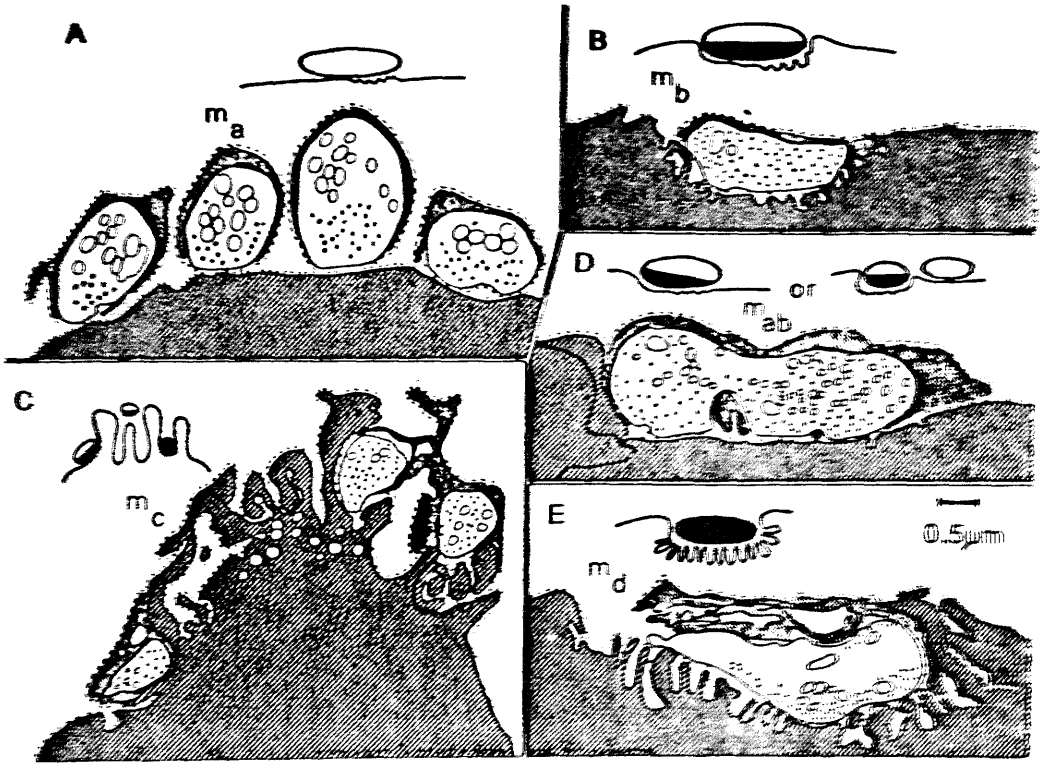


Figure 14 Electron micrograph tracings of axon terminals of each of the five ultrastructural types together with a diagrammatic representation of each type as described by Arbuthnott et al 1982. Intrafusal fibres hatched. Schwann cell lid, light stipple.

appreciably affecting contour.

Mab - Intermediate between Ma and Mb.

Mc - Plates with finger-like processes protruding from the muscle surface between the terminals. Several sections required to identify this type.

Md - Fully indented plate with complex subjunctional folds.

Type Ma had smooth post-synaptic membranes or a few wide, shallow subjunctional folds.

Type Mb had variable wide, shallow subjunctional folds.

Type Mc had wide shallow folds but occasionally narrow subjunctional folds with fusion of the basement membrane in between were seen.

Type Md had numerous deep, narrow folds with fusion of the basement membrane in between in more than 50%.

The number of sole plate nuclei was generally greater in Mb plates than in the others but seemed to be related more to plate length than plate type.

With the exception of the Md plate which lay extracapsularly on a long chain fibre and one Ma and Mc plate which lay near the end of the fluid space, all the endings were found in the sleeve region.

Ma endings and Mab which lay on static nuclear bag fibres were supplied by static γ axons.

Mb endings and Mab which lay on dynamic nuclear bag fibres were supplied by dynamic γ axons.

Mc endings were supplied by static γ axons except one which was supplied by a dynamic γ axon.

Md endings were assumed to be supplied by static β axons.

They equated Ma terminals on static bag2 and chain fibres with Barkers trail terminations but showed that some were not true trail ramifications but plate endings. The Mb endings equated with p_1 and p_2 plates of bag1 fibres. The Mc endings equated partly with the trail endings and partly with p_1 plates because the protrusion of muscle could correspond to the Doyère eminence of light microscopy. However the wide shallow subjunctional folds resembled more closely the p_2 plate.

Md endings were most like p_1 plates in their subjunctional folding.

Arbuthnott et al correlated the type of fusimotor ending with the function of the supplying axon.

Static γ axons ended in Ma or Mc plates.

Static β axons ended in Md plates on long chain fibres.

Dynamic γ axons ended in Mab or Mb plates on dynamic bag1 fibres.

Dynamic β axons ended in Mab or Mb plates on dynamic bag1 fibres.

This work suggested that it is the type of intrafusal fibre which influences the form of the motor endings.

Mb and Md plates satisfy this hypothesis. However in order for Ma and Mc endings to fit, additional qualifications are imposed. They hypothesise that there are two types of static

axon, one innervating Ma endings and one innervating Mc endings.

Thus both the motor axon type and the intrafusal fibre type together determine the motor ending type.

The motor endings of long chain fibres are considered in the next section.

1.2.4 Long Chain Fibres

Long chain fibres are a well defined subtype of cat nuclear chain fibres.

In 1976b Barker described long chain fibres as being similar in length to, or even longer than, dynamic bagl fibres. They end 1 mm or more beyond the end of the capsule and were present in 29.4% of spindle poles studied in the tenuissimus muscle.

Kucera has done much work on the histochemistry, topography and motor endings of long chain fibres. In 1980 he studied histochemically the long nuclear chain fibres and found that out of a total of 309 spindle poles from the cat tenuissimus muscle, 11.6% contained a long chain fibre and 0.6% contained two long chain fibres. Only 1.2% of spindles had long chain fibres in both poles. The mean polar length of the long chain fibres was 2988 um and they extended for a mean distance of 1624 um beyond the end of the capsule. Twenty five percent of long chain fibres were longer than the longest nuclear bag fibre.

In region A and the inner part of region B the long chain fibres had diameters comparable to other chain fibres but in the outer part of region B and in region C the diameter was equal to or greater than the adjoining nuclear bag fibre.

ATPase and NADH-TR reactions displayed a profile in the long

chains which was histochemically unlike the typical chains. The profile suggested that in the outer B and C regions the long chains have less mitochondria and sarcoplasmic reticulum than the other chain fibres. Also the extrafusal fibres which were innervated by a branch of the same axon as the long chain fibres were different histochemically from the long chain fibre.

In 1982a Kucera showed that long chain fibres tend to assume a particular position within the axial bundle of the intrafusal fibres. This position may stem from the manner in which the spindle develops (Milburn 1973). In this study the chain fibres were often observed to form two or more layers round a portion of the bag2 perimeter. This arrangement may reflect the sequential formation of several generations of chain fibres with the older ones separating progressively from the bag2 fibre.

The fact that the longest chain fibre was always located in the outermost layer of the chain fibre bundle suggests that it was among the earliest formed.

The long chain fibre also faces the bag1 fibre in the A region, is adjacent to it for much of its length in the poles and sometimes has a small bag of nuclei at the equator rather than a single row of nuclei. This also supports the early development theory as Milburn 1973 suggested that equatorial nucleation of intrafusal fibres reflects the decreasing morphogenetic effect of afferent innervation.

It is almost certain now that all long chain fibres in cat tenuissimus muscle are innervated by skeletofusimotor axons.

In 1978 Jami et al studied the motor supply of the spindle with respect to fast β axons using the glycogen depletion method. They showed that fast β axons almost exclusively

innervated long chain fibres. This was in agreement with Harker et al (1977).

In 1979 Jami et al showed that the extrafusal muscle fibres depleted of glycogen by the same technique were of the fast oxidative glycolytic type. Physiological correlates of this histochemical profile are fast contraction associated with resistance to fatigue. Long chain fibres were often found to be similar in this. The morphological resemblance of long chain fibres to extrafusal fibres is variable and when present is most apparent in the outer extracapsular region often beyond the site of the motor endings.

In 1983 Kucera & Hughes studied the motor innervation to long chain fibres in cat muscle spindles. They found in toluidine blue stained sections that long chain fibres had dense dark blue granules in various sizes scattered singly or in clusters. The intensity of the fibre background staining and the apparent size and number of granules tended to decrease as the distance from the equator increased. Close to the equator the intrafusal fibres all had similar staining characteristics. Further from the equator the encapsulated polar region of the long chain fibre did not match the other fibres although in occasional sections the long chains and bagl fibres looked alike. This staining resemblance was especially apparent in the case of a long chain fibre which was adjacent to a bagl fibre in a separate compartment of the inner capsule.

Each long chain fibre pole displayed one motor ending of plate-type situated extracapsularly. The endings were supplied by myelinated motor axons that originated from intramuscular nerve fascicles containing motor axons to extrafusal fibres. One

of the endings was supplied by a collateral from a motor axon that innervated an extrafusal end-plate. Ultrastructurally the long chain endings resembled extrafusal end plates (Fig 15).

Thirty-six additional endings were present on intrafusal fibres other than the long chain fibres. None of these were similar to the long chain endings neither by light nor electron microscopy. This was true for a bag1 fibre which had an ending extracapsularly close to endings on adjacent long chain fibres.

The other endings had less prominent sole plates and were longer. Subjunctional folding was less marked with shallow wide folds on bag2 fibres and chain fibres or absent entirely, most bag1 fibre endings. Mitochondrial clusters in the post-synaptic area of some endings especially bag1 fibre endings were more numerous than in long chain fibres.

A spindle which had a long chain fibre at both poles had motor endings at each pole. They were of comparable appearance. However another spindle had endings at each pole of a long chain fibre which were dissimilar. The longer pole contained the more complex ending.

It is noteworthy that almost every tandem spindle including those with linked one-bag fibre capsules had at least one chain fibre which was a long chain.

1.2.5 Tandem Spindles and One Bag Fibre Spindles

The most common type of tandem spindle is that which has two encapsulated sensory regions occurring in linear succession. Swett and Eldred (1960) estimated that 11% of spindles in cat soleus muscle and 24% in medial gastrocnemius were tandem spindles. Barker and lp (1961) found that 16-20% of spindles in cat rectus femoris were tandem, a similar number in soleus,

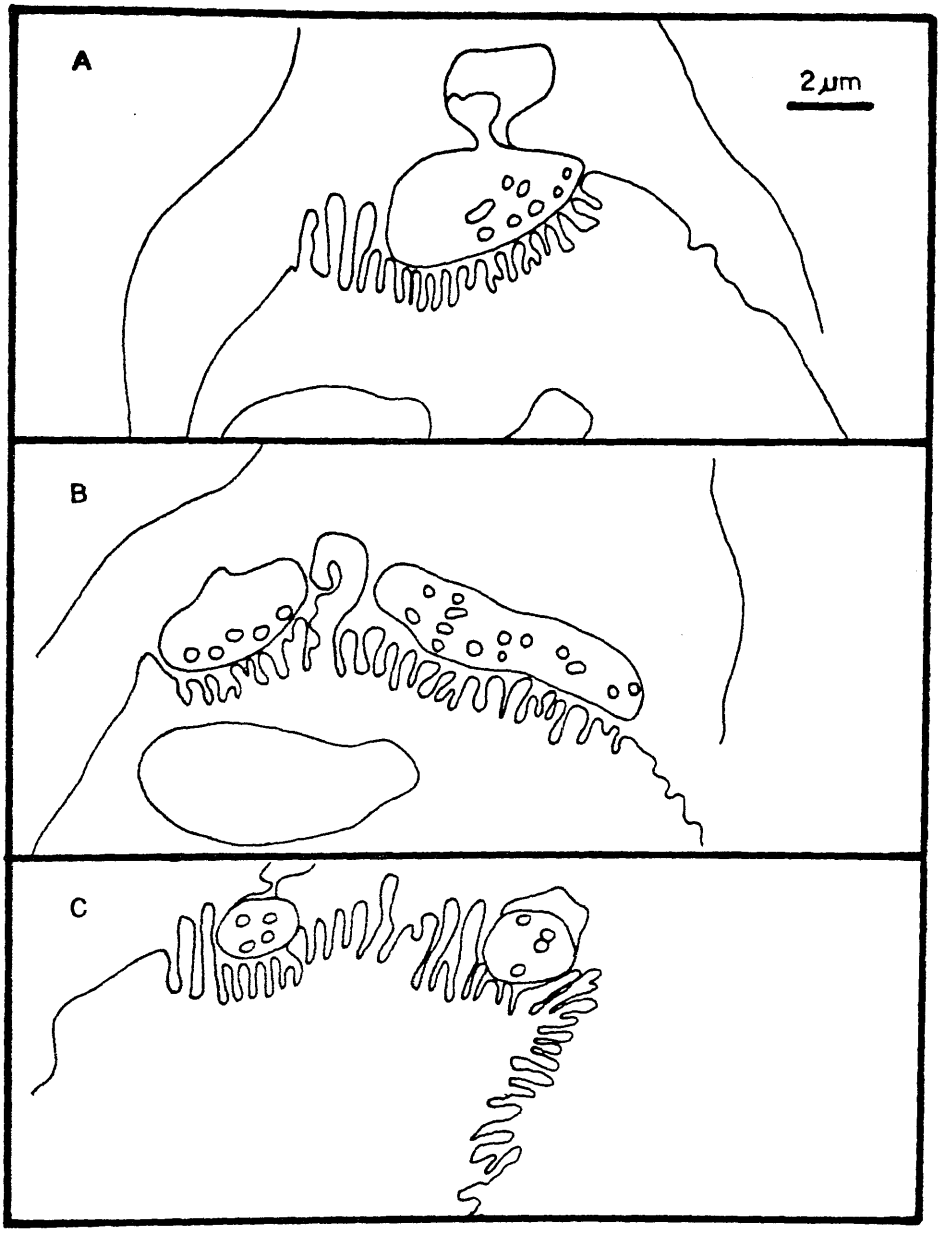


Figure 15 A.B. Electron micrograph tracings of transverse sections through two separate regions of a long chain fibre motor ending described by Kucera and Hughes (1983).
C. Transverse section through an extrafusal fibre motor ending.

higher in semitendinosus and flexor digitorium longus and lower in pes interossei.

Boyd (1962) found very few tandem spindles in cat tenuissimus muscle.

Of the 64 tandem spindles in cat rectus femoris studied by Barker and Ip (1961) 88% were double, 9% triple and 3% quadruple. The lengths of these spindles ranged from 8.43 to 22.3 mm (mean = 13 mm). The length of single spindles in the same muscle range from 2.4 to 13.68 mm (mean = 7.02).

Some of the tandem spindles extended for the entire length of the extrafusal muscle bundles.

Barker and Ip (1961) found that typically a large capsular unit receiving a primary axon and one or more secondaries was linked with one or two small capsules that received a primary axon only.

Kucera in 1982b studied single bag fibre spindles in the tenuissimus muscle of the cat and found that each capsular unit of a tandem spindle had one primary axon and at least one of the two capsules received secondary innervation also, always a two bag capsule.

He found nineteen complete or almost complete tandem spindles each of which had the two capsular units arranged linearly along the bag2 fibre. In one spindle another fibre, a long chain fibre, was found to pass through both equators in addition to the bag2 fibre.

Nine spindles possessed both bag1, bag2 and several chains per capsule.

In some tandem spindles, one capsule contained fewer chain fibres.

Each of the remaining ten tandem spindles had one large capsule containing a bag1 fibre and a bag2 fibre and a smaller capsule which had only the bag2 fibre.

Every two bag capsule contained at least one chain which qualified as either an intermediate or long chain in one or both poles.

Only two of the one bag capsules had intermediate chain fibres and none had long chain fibres.

The motor innervation pattern of these double spindles was comparable to that of the single spindles in terms of the appearance and distribution of cholinesterase plates.

The bag1, bag2 and chain fibres of the double tandem spindle with one bag capsules had the usual histochemical properties (Kucera 1981, Table 1).

In tandem spindles with widely separated equatorial regions the extracapsular bag2 fibre that linked the capsules had low alkali stable ATPase activity.

In tandem spindles with closely linked equators the corresponding bag2 fibre had high alkali stable ATPase activity.

Bakker and Richmond (1981) studied muscle spindles in cat neck muscles and found that 50% of single bag spindles could be traced into tanden linkages.

Single bag fibre spindles were found in one third of spindles studied. Each spindle was found to have a bag2 fibre and nuclear chain fibres.

In the tandem linkages studied the bag1 fibre was confined to the larger capsule so that the smaller spindle contained only a bag2 fibre.

Usually the number of nuclear chain fibres was smaller in

the single bag fibre spindles and Bakker and Richmond (1981) never found long chain fibres in the complement.

The single bag fibre spindles were frequently found at tendinous inscriptions.

In a study of over 150 complete and 139 incomplete, single spindles, Kucera (1982b) found only two spindles which lacked a nuclear bagl fibre altogether. In contrast they were much more commonly found as part of double or tandem spindles. He also found that long chain or intermediate chain fibres were a feature of single one bag spindles and tandem linked one bag spindles.

CHAPTER 2

PRIMATE MUSCLE SPINDLES

2.1 General Morphology

In 1963 Cooper and Daniel described the general morphology of human muscle spindles found in the lumbrical muscles and deep muscles of the neck.

They found as many as fourteen intrafusal fibres inside the capsules of large spindles but as few as two fibres in short spindles. They also found two distinct types of intrafusal fibre. One type of fibre was larger in diameter and longer, passing out of the capsule at the poles to lie among the extrafusal fibres. In the equatorial region this type contained numbers of large closely packed nuclei which swelled the fibre and formed a nuclear bag. This type of fibre was labelled the nuclear bag fibre. The other type of fibre was of smaller diameter, rarely extended beyond the capsule and contained an equatorially placed chain of nuclei. These smaller fibres were labelled nuclear chain fibres.

The nuclear bag fibres were 15 um or more wide, continued for an average distance of 0.8 mm beyond the end of the capsule at either pole and averaged 5 mm in length. The nuclear chain fibres were 10-15 um wide and averaged 3.5 mm in length.

The capsule varied in length from 1 mm to 5 mm, mean 3.5 mm. At the equator the capsule stood away from the intrafusal fibres which thus lay in the fluid space. The width of the spindle here depended on the amount of stretch of the muscle spindle at the time of fixation but rarely exceeded 200 um. The capsule decreased in diameter at the poles so that the lamellae were closely wrapped round the intrafusal fibres. Elastic tissue was particularly marked at the poles. A lateral extension of the capsule was sometimes seen near the equator so that the capsule

passed round 10-50 extrafusal muscle fibres for a distance of 750 um or more.

Kennedy 1970 described nuclear bag and nuclear chain fibres rather crudely. Nuclear bag fibres being of greater diameter and length than nuclear chain fibres. The capsule was found to be 250 um at its widest point and occasionally surrounded two spindles. A few tandem spindles were isolated. Kennedy noted that sometimes the capsule extended laterally to enclose one or several extrafusal fibres. He also reported seeing single extrafusal muscle fibres which penetrated the capsule near the equator and received motor innervation by gamma axons.

Kucera and Dorovin-zis (1979) studied human external intercostal muscle spindles and found on average 2-3 nuclear bag fibres per spindle with 2-7 nuclear chain fibres. They found that some nuclear chain fibres were very short, not extending beyond the A region of the spindle. They also noted that the capsule could surround extrafusal fibres.

2.2 Sensory Innervation

Cooper and Daniel (1963) found that a single spindle received one large afferent nerve fibre to the primary ending on the nuclear bags and nuclear chain fibres and one, two or more nerve fibres, usually slightly smaller in diameter to secondary afferent endings. They noted that the large afferent axon divided several times dichotomously and that the final branches ended in non-myelinated axons which lay on both nuclear bag fibres and nuclear chain fibres, making up the primary ending. The non-myelinated portion consisted of fine fibrils running directly or in a somewhat irregular spiral fashion towards the

poles. The very regular spiral form seen in cat spindles was uncommon in human spindles but they had noted it in baboon and monkey spindles.

The secondary afferent endings were found on one or both sides of the primary ending with possible additional endings nearer the poles. They lay predominantly on nuclear chain fibres as in the cat.

In 1970 Kennedy studied human intercostal muscle spindles and found that the primary axon supplied all the intrafusal fibres but lacked the regular annulo-spiral form seen in the cat. He noted secondary endings on both nuclear bag and nuclear chain fibres.

Swash and Fox (1972) studied post-mortem material from human subjects. The spindles were studied in a number of muscles including hand, forearm, chest, abdomen and leg muscles.

The primary axon was found to supply only nuclear bag fibres and some did not have regular spirals but more of a looped appearance. They noted that the nuclear chain fibres did not receive a well developed primary ending although some received a few short unmyelinated spirals from a branch of the group Ia fibre.

Secondary endings were situated juxtaequatorially and were predominantly distributed to nuclear chain fibres. They were usually more extensive on one side of the equatorial region. As in other mammals additional secondary endings were sometimes found in the mid-polar region of nuclear chain fibres.

2.3 MOTOR INNERVATION

2.3.1 Light Microscopy

Cooper and Daniel (1963) noted that human spindles contained groups of nerve fibres ranging in size from medium to very small and thought they were probably all motor in function. They entered the spindle at the equator travelled towards one or both poles and remained unbranched until they neared motor end-plates. Occasionally a group of similar motor nerve fibres entered the spindle at a pole. They noted that if one nerve fibre was slightly larger in diameter it nearly always continued beyond the capsule to supply a single motor end plate on each of the nuclear bag fibres. These same nuclear bag fibres were also found to have at least one more motor end plate within the capsule. The other pole of the spindle did not have extracapsular innervation. Motor endings were not seen on nuclear chain fibres.

This pattern of innervation was noticed to be different in tandem spindles. In tandem spindles each capsule received an independent set of sensory endings. Nuclear bag fibres which passed through both capules were found to have a motor end plate inside the capsule of the bigger spindle, in between the two nuclear bag regions and also another ending in the inter-capsular region. There were no motor end plates on these fibres when they entered the second capsule. The usual motor end plates were seen at the proximal and distal poles of the double organ.

The only reference Cooper and Daniel (1963) make to the form of the motor end plates is to say that the nuclear bag fibres had motor end plates resembling extrafusal motor end plates.

In 1970 Kennedy using a modified version of Barker's classification of motor endings described his findings in human spindles. He noted motor axons whose intrafusal fibre course was

similar to that of cat beta axons. They proceeded directly from their point of entry into the spindle distally towards the poles where they doubled back before ending on single or paired plates resembing extrafusal end plates. These endings were located predominantly on nuclear bag fibres (Fig 16).

He also described multibranched fusimotor axons which could innervate only nuclear bag fibres or nuclear chain fibres selectively but frequently together. The endings were situated in an intermediate zone between the pole and the equator. Each major sub-branch of a multibranched axon paralleled its assigned intrafusal fibre and at intervals deposited pairs of motor endings on that fibre. Some of the larger endings resembled extrafusal end plates while the smaller endings were "simple" in form. The endings on chain fibres were similar to those on bag fibres (Fig 16). Trail endings were also described.

In 1972 Swash and Fox described their findings of the motor innervation of human spindles.

They were using both serial sections and teased preparations made by gold chloride and silver nitrate block impregnation techniques.

They found motor end plates on both nuclear bag fibres and nuclear chain fibres in the juxtaequatorial region, in the mid polar region and on the poles of the spindle extracapsularly. They described the motor endings using the terminology and classification adopted by Barker et al (1970).

The p_1 plate endings (Fig 17) consisted of motor end plates situated on a typical Doyère eminence in which sole plate nuclei were usually seen. They were morphologically indistinguishable from motor endings in extrafusal muscle. They were usually found

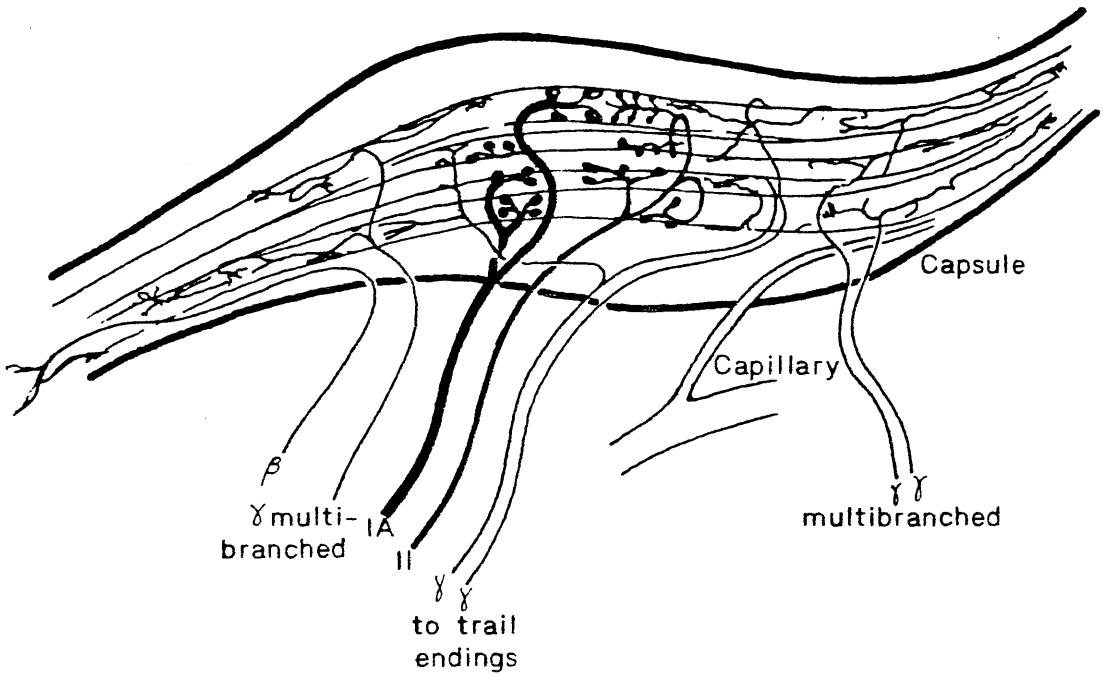
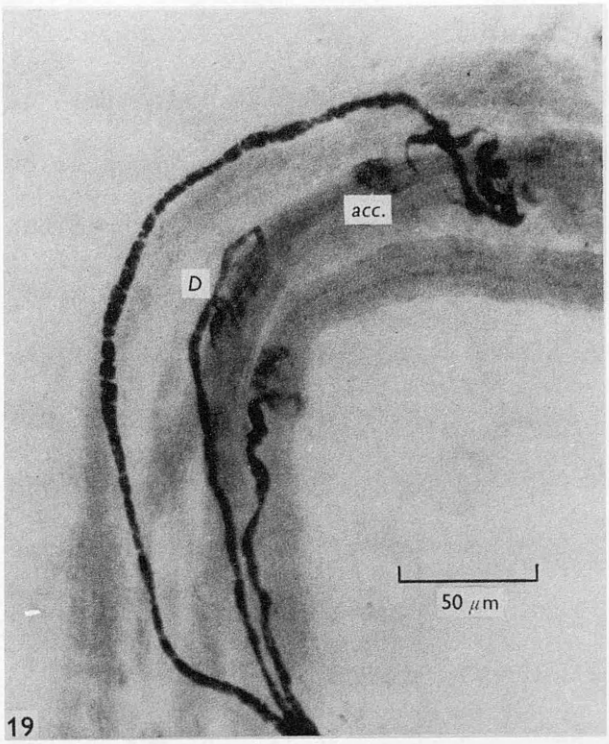
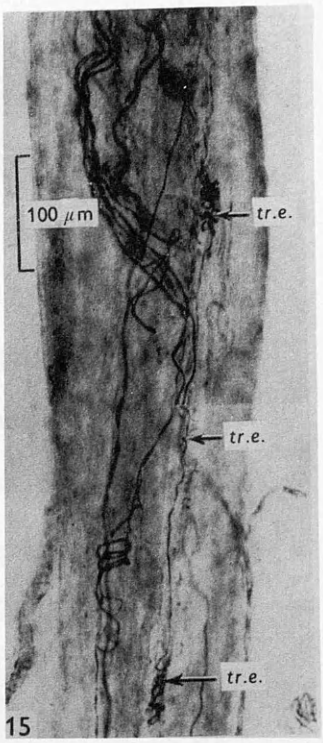


Figure 16 The drawing shows the types of nerve fibres and nerve endings found in normal human muscle spindles as described by Kennedy 1970. Only 2 bag and 2 chain intrafusal muscle fibres are represented. The number of entering nerves, particularly the degree of branching and number of endings, has been limited to a few of each type for clarification. The transverse diameter of the spindle has been greatly exaggerated.





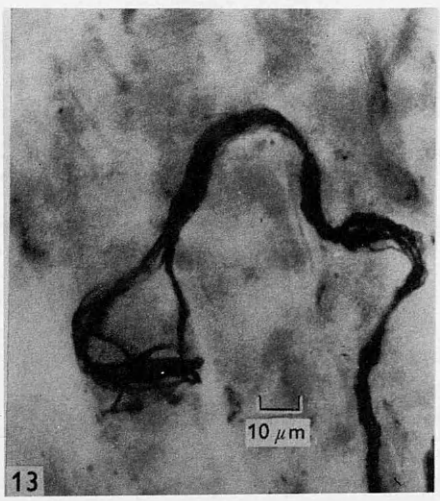


Figure 17 a) Tracing of a human gold chloride preparation showing three p_1 motor endings, two of which are situated on a single intrafusal fibre, as found by Swash and Fox 1972 (see text).

b) Barker and 1p silver preparation showing the trail endings (tr.e.) found by Swash and Fox in human spindles.

c) Barker and 1p silver preparation showing the p_2 motor ending found by Swash and Fox in human spindles.

at the poles of nuclear bag fibres, often extracapsularly. Both poles could be innervated and frequently there were two p_1 endings at a single pole. They were rarely found on nuclear chain fibres. The p_1 ending was innervated by a short thinly myelinated axon which was a branch of a thickly myelinated fibre (10-18 um diameter). The fibre usually entered the spindle in the mid polar region and pursued a straight course to the pole without branching. Before entering the spindle however it could give off collateral branches to supply extrafusal motor endings.

The p_2 plate endings (Fig 17) were located in the mid polar region and in most spindles occupied a zone 300-600 um in length on both sides of the equatorial region. They were sometimes found in close relation to the secondary endings but in most spindles there was a gap, occupied by trail endings.

The p_2 ending consisted of several loops or rings, occasionally forming two to five short, tight spirals from which branched unmyelinated, fine axons were given off. These ended as knobs, tapers and round end plates which were closely applied to the muscle surface. There was no Doyère eminence, but the muscle fibre underlying the ending usually expanded fusiformly. Sole plate nuclei were not conspicuous. The endings were larger on nuclear bag fibres than nuclear chain fibres and nuclear bag fibres usually received more p_2 endings than the chain fibres. The endings varied in length from 47-108 um. They were innervated by thinly myelinated fibres 1.5 -4.5 um in diameter (α motor neurones). Each nerve fibre typically innervated one or two endings which could be located on different intrafusal muscle fibres.

The trail endings described by Barker (1966) were not easily

recognisable by Swash and Fox (1972). When seen (Fig 17) they were located juxtaequatorially and extending towards the mid polar region. They were closely applied to the surface of the muscle and found predominantly on nuclear chain fibres. They were supplied by thinly myelinated nerve fibres (1 - 3 um in diameter) and fine unmyelinated nodal branches of these fibres often ran for long distances before ending in a leash of multibranched axons bearing synaptic knobs, beads or rings. Sole plate nuclei were not found. This type of ending could also appear as a more discrete but still multibranched tangle of end plates and axons which though similar to the p_2 plate could be differentiated from it by the absence of fusiform swelling of the underlying muscle fibre.

Swash and Fox further described accessary motor endings which consisted of a group of three to six small end plates usually situated in close relation to a p_1 or p_2 ending. They were supplied by fine unmyelinated axons (0.5 - 2.0 um in diameter). They were distinguishable from trail endings, by their small size and irregular shape and by the very fine dimater of their unmyelinated nerve fibres (Fig 17).

Table 4 summarises the light microscopic findings of primate muscle spindle motor endings.

2.3.2 Electron Microscopy

A systematic study on the ultrastructure of primate motor endings has never been done.

2.4 HISTOCHEMISTRY

In 1972 Ovalle and Smith described three types of intrafusal fibre based on myosin ATPase activity (Fig 2) in cat and monkey

TABLE 4

AUTHOR	ENDING TYPE	FIBRE TYPE	POSITION ON FIBRE	AXON	FEATURES
COOPER AND DANIEL (1963)	END PLATE	NUCLEAR BAG	EXTRA-CAPSULAR	LARGE DIAMETER LITTLE BRANCHING	SIMILAR TO EXTRAFUSAL
	END PLATE	NUCLEAR BAG	INTRA-CAPSULAR	SMALL DIAMETER LITTLE BRANCHING	SIMILAR TO EXTRAFUSAL
KENNEDY (1970)	END PLATE	NUCLEAR BAG	EXTRA-CAPSULAR	SIMILAR TO B AXONS LITTLE BRANCHING	SIMILAR TO EXTRAFUSAL
	END PLATE 'SIMPLE'	NUCLEAR BAG AND/OR NUCLEAR CHAIN	BETWEEN POLE AND EQUATOR	MULTI-BRANCHED	LARGE END-PLATES SIMILAR TO EXTRAFUSAL SMALL END-PLATES, SIMPLE
	TRAIL ENDING	NUCLEAR BAG AND/OR NUCLEAR CHAIN	JUXTA-EQUATORIAL		
SWASH AND FOX (1972)	P_1 PLATE	NUCLEAR BAG	EXTRA-CAPSULARLY	10-18 um B AXON LITTLE BRANCHING	SIMILAR TO EXTRAFUSAL WITH DOYERE EMINENCE
	P_2 PLATE	NUCLEAR BAG AND/OR NUCLEAR CHAIN	MID-POLAR	1.5-4.5 um ∝ AXON	NO DOYERE EMINENCE MUSCLE EXPANDED
	TRAIL ENDING	NUCLEAR CHAIN	JUXTA-EQUATORIAL	1-3 um MULTI-BRANCHED	NO MUSCLE EXPANSION

Light microscopic findings of primate spindle motor endings.

muscle spindles.

In 1975 Harriman, Parker and Elliot studied human intrafusal fibres histochemically. They found that there were two types of nuclear bag fibre based on acid and alkali ATPase activity. One had little or no acid and alkaline stable ATPase activity while the other had both varieties fairly uniformly from pole to equator. Nuclear chain fibres had alkali stable myosin ATPase activity throughout their length with some acid stable activity at the poles.

In 1979 Kucera and Dorvini-zis found two types of human nuclear bag fibre based on acid and alkali ATPase staining. The difference in staining of the fibres was most obvious in the intracapsular B region of the spindle, when stained for acid stable myosin ATPase. The bag1 fibre stained lightly, the other bag fibre darkly. In the extracapsular region and juxtaequatorially both fibres stained similarly.

When stained for alkaline stable myosin ATPase activity bag1 fibres stained lightly throughout their length, while bag2 fibres stained moderately in the B region, but lightly in regions A and C. Nuclear chain fibres stained darkly throughout their lengths.

2.5 ELASTIC FIBRE DISTRIBUTION AND M-LINE DISTRIBUTION

These two special features of primate muscle spindles have already been covered in sections 1.2.1 and 1.2.2.

AIMS OF THESIS

Differences and similarities between cat a primate spindles have been described. These are:-

1) There are more intrafusal fibres per spindle in human spindles (Cooper and Daniel 1963) than in cat spindles (Boyd 1962). Baboon spindles have not been studied morphologically.

2) Sensory endings have been studied extensively histologically (Cooper and Daniel 1963, Kennedy 1970, Swash and Fox 1972) in human muscle spindles. Histologically they are less complex than cat sensory endings.

3) Motor endings have been less extensively examined.
Swash and Fox 1972 have identified p_1, p_2 and trail endings in human spindles at light microscopy level. These were similar to those described in the cat. They have not been studied in the baboon and a systematic comprehensive study of the ultrastructure of primate fusimotor endings has not yet been done.

4) There are differences histochemically between the dynamic bag1 fibre and the static bag2 fibre in human spindles (Kucera and Dorvini-zis 1979). Myosin ATPase activity suggests that the dynamic bag1 fibre contracts more slowly than the static bag2 fibre as in the cat. However there are

regional differences.

5) There are also differences in the M-line distribution in the two types of human nuclear bag fibres as in the cat although these differences occur over only a short segment of the sleeve region.

6) Elastic fibre distribution seems from preliminary work to be less useful in distinguishing the bag1 fibre from the bag2 fibre in human spindles.

Histochemistry has demonstrated that there are two types of nuclear bag fibre in monkeys (Ovalle and Smith 1972) and in humans (Harriman, Parker and Elliott 1975, Kucera and Dorovini-zis 1979) but electronmicroscopy gives a wealth of additional information about structure eg motor endings, M-line distribution and elastic fibre distribution.

The main objectives of this thesis were therefore to study baboon muscle spindles both morphologically and ultrastructurally to see if in fact these were not a closer working model for the human than the cat. Particular attention was paid to:

1) General morphology of baboon muscle spindles.

2) Elastic fibre distribution at both light and electron microscopy levels to see if this was a useful means of identifying the static bag2 fibre as in the cat.

3) M-line distribution at electron microscopy level to see if there were differences between intrafusal fibres

and

4) The form and pattern of the fusimotor innervation to baboon spindles.

CHAPTER 3

MATERIALS AND METHODS

3.1 ELECTROPHYSIOLOGICAL STUDIES

Baboon hand lumbrical muscles were used. These were obtained from another department on completion of cerebral blood flow studies on the baboons. The muscles were removed within minutes of the animals death and transported quickly in an oxygenated Kreb's solution (Table 5) to the Department of Physiology, Glasgow University where Dr M. H. Gladden dissected out the spindles using a dissecting microscope.

Procedure

Once removed from the animal, the muscle was pinned out on to dental wax and placed immediately in the buffered solution which was made up freshly before each experiment. The solution was gassed continuously with 100% O_2 until completion of the experiment and the pH was maintained at 7.4.

The dissection involved exposing the intrafusal fibres sufficiently so that they could be visualised on a video screen. Two spindles were successfully isolated from two different lumbrical muscles - spindles B and C. Each spindle responded electrophysiologically on stimulating a nerve branch by the method of Gladden, Smith and Wilson (1982). These spindles were transferred from the dissecting petri dish to the small bath specially designed for studying isolated spindles. They were bathed continuously in oxygenated buffer (Fig 18) and a binocular microscope connected to a videomonitor was used to visualise the spindles.

A gated pulse generator controlled by a digitimer provided stimuli of varying frequencies and intensities to the stimulating electrodes which were placed on a nerve branch to the spindle.

TABLE 5

Human Buffer Solution

Na Cl	128 ml
Mg SO_4	1.4 ml
Ca Cl_2	1.3 ml
KCI	4 ml
Hepes	5 ml
Glucose	1 g

0.1 m $Na_2 HPO_4$ 18 ml) 1 ml
0.2 m $Na H_2 PO_4$ 5 ml)

Make up to pH 7.4 using M Na OH

Make up to 1000 ml with distilled water

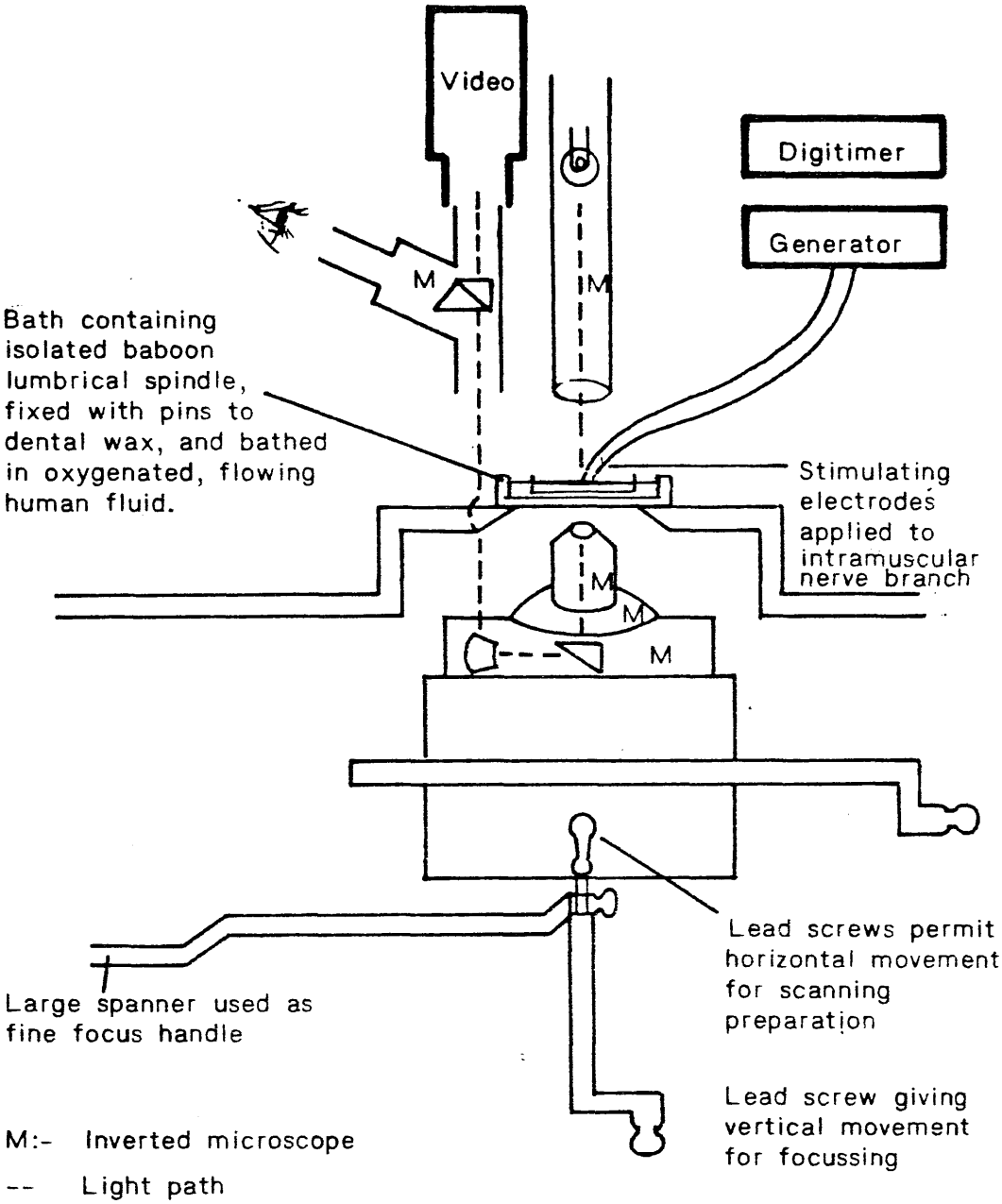


Figure 18 Showing diagrammatically the apparatus used in studying the baboon muscle spindles electrophysiologically.

The responses of the intrafusal fibres were observed on the video screen and particular attention was paid to the site of muscle fibre contraction, the speed of contraction and the fatiguability of the fibres. The spindle position on the screen could be varied by means of a manual stage control which was calibrated in mms. Distances along the intrafusal fibres could be measured when referred to particular landmarks such as nerve axons, fluid space or fat cells.

3.2 ULTRASTRUCTURAL ANALYSIS

Following the electrophysiological experiments spindles B and C were processed for electron microscopy. In addition a third spindle, spindle A was similarly processed. This spindle was isolated by the technique already described but was not studied electrophysiologically. The three spindles were fixed and processed using the following solutions and method.

3.2.1 Fixation

STOCK SOLUTIONS

SOLUTION A = 2.26% Na H PO 2HO

SOLUTION B = 2.52% Na OH

SOLUTION C = 25% Glutaraldehyde

SOLUTION D = 5.4% Glucose

PRIMARY FIXATIVE (Sabbattini, Bensch and Barrnett 1963)

SOLUTION A = 64 ml

SOLUTION C = 16 ml

Adjust pH to 7.2 - 7.4 with solution B.

Bring to 100 ml with distilled water.

BUFFER WASH (Millonig 1961)

SOLUTION A = 83 ml

Adjust to pH 7.2 - 7.4 with solution B.

Bring to 100 ml with distilled water.

Remover 10 ml and add 10 ml solution D.

POST FIXATIVE (Millonig 1961)

Buffer wash = 50 ml

OsO_4 = 0.5 g

Prepare 24 hours before use.

Allow OsO_4 to dissolve.

3.2.2 Method

The whole muscle spindes which were pinned out on dental wax were processed as follows:

1) Primary Fixative = One and a half hours at 37c.

2) Buffer Wash = 3 x 10 minute washes, left overnight at 4°c.

3) Post Fixative = One and a half hours.

4) Buffer Wash = Rinse.

5) Dehydrate with graded alcohols.

6) Propylene oxide as intermediate solvent.

7) Propylene oxide/araldite = overnight (Glauert, Rogers and Glauert 1956, Glauert and Glauert 1958).

8) Fresh unpolymerised resin for 3-4 days.

9) Embed and polymerise in flat embedding trays.

10) Sectioning regime.

The three spindles were sectioned, with glass knives on a Reichart OM2 ultratome, into 1 um sections. These were transferred on to gelatin chrome alum coated glass slides. Ultrathin sections 40-50 nm thick were cut every 20-100 um depending on whether the part of the spindle cut was of particular interest. The ultrathin sections were placed on formvar coated one hole mounts with a 0.8 mm diameter aperture (Arbuthnott 1974). Spindle B was photographed at stage 9 of the above procedure and a montage (Fig 27) mounted.

This was very useful because features which had been identified while it was in the living state could be seen in the photograph and any shrinkage due to electronmicroscopy processing could be assessed. Zero on the montage was taken as being the position of the Ia afferent in the second fluid space.

3.2.3 Ultrathin Section Staining

After sectioning, the ultrathin specimens were then stained with one of the following:

i) 2% uranyl acetate in 50% methanol or ethanol (Stempak and Ward 1964) followed by Reynold's lead citrate (Reynolds 1963). This is a good general electron microscopy stain.

ii) Palladium chloride 1% aqueous (Morris et al 1978) followed by Reynold's lead citrate.
This is a stain specific for elastic fibres and when used on cat spindles which were known to have elastic fibres round the static nuclear bag fibre extracapsularly, the elastic fibres were clearly demonstrated (Plate 2). This stain

was used on alternate ultrathin sections.

iii) Ruthenium Red 0.5% in 0.1 m ammonia solution (Hayat 1970).

This is a stain which is specific for polysaccharide material. It was however discarded after several attempts at staining of sections as they were either overstained or understained and the results were inferior to either of the other two stains.

3.2.4 Light Microscopy

The unstained 1 um sections were visualised with phase contrast light microscopy and the spindles reconstructed from the serial sections.

Photomicrography was carried out using a Zeiss Ultraphot for phase contrast microscopy and a leitz orthoplan for bright field microscopy.

3.2.5 Electron Microscopy

The stained ultrathin sections were examined with a J.E.O.L. JEM-100 C electron microscope at 80 KV. Electron micrographs were taken on Ilford E.M plates and developed with Ilford P.Q. universal developer.

3.2.6 Analysis of Motor Endings

A semi-automatic planimeter (Moss 1981) which was linked to a digital computer was used to measure pre and post synaptic membrane lengths. The digitising tablet incorporates a stylus or curser that is moved by the operator round the perimeter of the object to be quantified. The positon of the stylus on the table can be detected by the computer at regular intervals and is

accurate to the nearest 0.1 mm.

3.2.7 Analysis of Elastic Fibres

Electron micrographs were taken of the nuclear bag fibres in spindles A and B and of the long chain fibre in spindle B at varying distances from the equator.

Elastic fibres showed up clearly at a magnification of x 4,000 (Plate 2) in cat spindles but since baboon spindles appeared to have less elastic fibres at light microscopy level micrographs were examined at x 12,500.

A quantitative analysis of the amount of elastic fibres round the muscle fibres was done by measuring the circumference of the elastic fibres using a manual planimeter at varying distances along the fibres and comparing this with the circumference of the muscle fibre at each level.

3.2.8 Analysis of M-lines

All three spindles were examined for the presence or absence of M-lines. Each ultrathin level sectioned and stained was examined at 20,000 for M-lines.

3.3 LIGHT MICROSCOPY

Seven baboon hand lumbrical muscles were dissected out from seven different animals within minutes of the animals death (see electrophysiological methods). These were processed and stained for light microscopy.

METHOD

After removal of the lumbrical muscles from the baboons they were stretched out to their normal resting length and tied to a

perspex frame. The frame with the muscle attached was then placed in Petrunkovitch Fixative, Petrunkovitch (1933) for varying periods of time (15-21 hours) depending on the muscle thickness. The fixed tissues were then dehydrated with graded alcohols and embedded in paraffin wax.

Serial transverse sections of 10 um thickness were cut and mounted on glass slides.

These sections were subsequently stained for elastic fibres with either resorcin fuchsin (Hart 1908) Table 6 and counterstained with orange G or they were stained with Millers stain (Table 6).

The stained sections were then viewed with a binocular microscope.

Twenty seven spindles were studied with the light microscope. These were spindles selected at random from the seven muscles.

All the muscles, except muscle VI, were stained with resorcin fuchsin and counterstained with orange G. Resorcin fuchsin is a good stain for demonstrating elastic fibres.

Millers stain, which stains fine reticular fibres very clearly, was used to stain muscle VI.

TABLE 6

Stains for Light Microscopy

Resorcin Fuchsin

Dissolve 0.4 g resorcin function in 100 ml of 70% alcohol by boiling for approximately 3 hours. Make up to 100 ml with 70% alcohol.

Staining Solution

80 ml of above stock solution

20 ml acid alcohol (1% HCI in 70% alcohol)

2 ml concentrated HCI made up just before use.

Milers Stain

Victoria blue 4R - 1 g

New fuchsin - 1 g

Crystal violet - 1 g

Dissolve in 200 ml of hot distilled water.

Add in following order,

Resorcin 4 g

Dextrin 1 g

30% ferric chloride - 50 ml (fresh)

Boil for 5 minutes, then filter while hot.

Transfer the precipitate plus filter paper to original beaker and re-dissolve in 200 ml 95% alcohol.

Boil for 15-20 minutes

Filter and make up to 200 ml with 95% alcohol.

Add 2 ml concentrated HCI.

CHAPTER 4

RESULTS - GENERAL MORPHOLOGY OF BABOON MUSCLE SPINDLES

4.1 DISTRIBUTION AND ARRANGEMENT OF MUSCLE SPINDLES WITHIN LUMBRICAL MUSCLES OF THE BABOON

Fig 19 shows the position of muscle spindles with seven lumbrical muscles taken from seven baboons.

4.1.1 Numbers of Spindles

The number of spindles found in each muscle was 12, 25, 5, 2, 23, 22 and 16.

It is likely that muscles III and IV are fourth lumbricals since they have only two muscle spindles and the fourth lumbrical is involved in less complex finger movements. During a dissection of lumbricals from another baboon the fourth lumbrical had very few spindles compared with the first lumbrical. Muscles III and IV are also the shorter which would be in keeping with them being fourth lumbricals but their length would also depend on the size of the baboon.

4.1.2 Arrangement of Spindles

The muscle spindles were found lying in parallel with extrafusal muscle fibres. They were often associated with intramuscular nerve branches so that several spindles were found in the same muscle plane along its length.

The majority of muscle spindles were found in the proximal half of the muscles.

Many spindles overlapped at their ends without being continuous. Other spindles were arranged in parallel with as many as four appearing in transverse section (Fig 19 muscle II).

The spindles were found to be more or less symmetrical with the fluid space occuring in the middle.

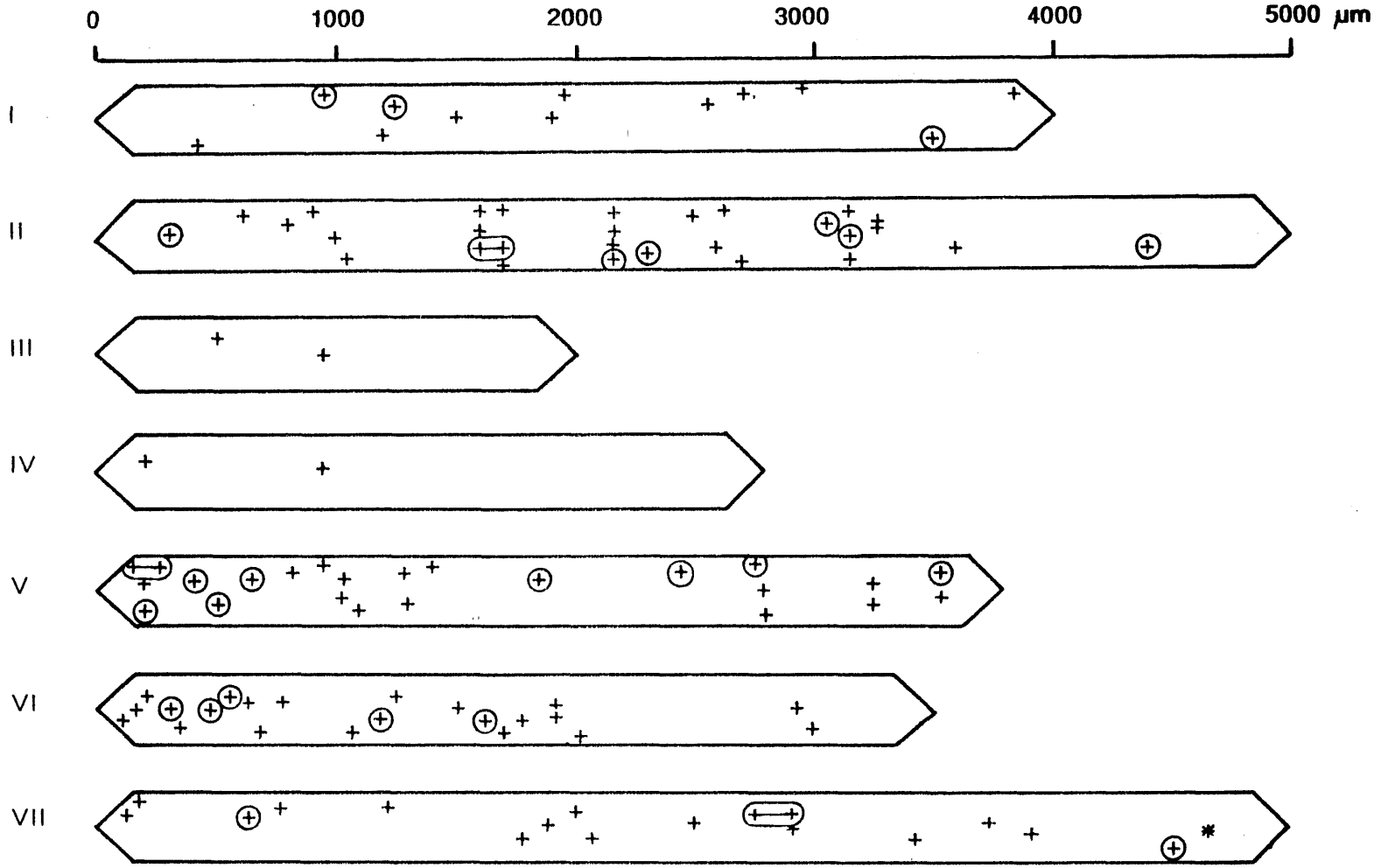


Figure 19 Diagrammatic reconstruction of the seven lumbrical muscles examined by light microscopy showing the relative positions of muscle spindles. Those encircled were studied in more detail by L.M. The spindles in muscles VII (*) and II (‡) are reconstructed in Figures 20 and 24 respectively.

4.2 Structure of Muscle Spindles in Baboon Lumbrical Muscles

Each muscle had to be cut up into 1 cm lengths to enhance fixation. Consequently it was not always possible to trace a spindle from end to end.

Twenty seven of the 103 muscle spindles of the seven lumbrical muscles were selected at random for more detailed analysis. These are encircled in Fig 19. Three spindles were studied ultrastructurally making a total of 30 spindles.

10 spindles were complete at both poles.

13 spindles were complete at one pole.

7 spindles were incomplete at both poles.

Each spindle was reconstructed from serial sections as illustrated in Fig 20. This spindle (asteristed in Fig 19) was thought to be typical of the majority of spindles examined with regard to the number, type and length of intrafusal fibres.

4.2.1 Identification of Nuclear Bag and Nuclear Chain Fibres

All spindles were found to contain two types of intrafusal fibre distinguisable both by their nuclear arrangement and by their diameter at the equator. This is illustrated in Plate 3a which shows a transverse section through the equatorial region of a baboon lumbrical spindle.

The three nuclear bag fibres (A, B and C) can be distinguished clearly from the nuclear chain fibres (numbered 1-8).

The nuclear bag region was normally 303 um + 25.4 um (S.E.M.) in length.

The nuclear chain region was 272 um + 39.7 um (S.E.M.).

4.2.2 Number of Intrafusal Fibres

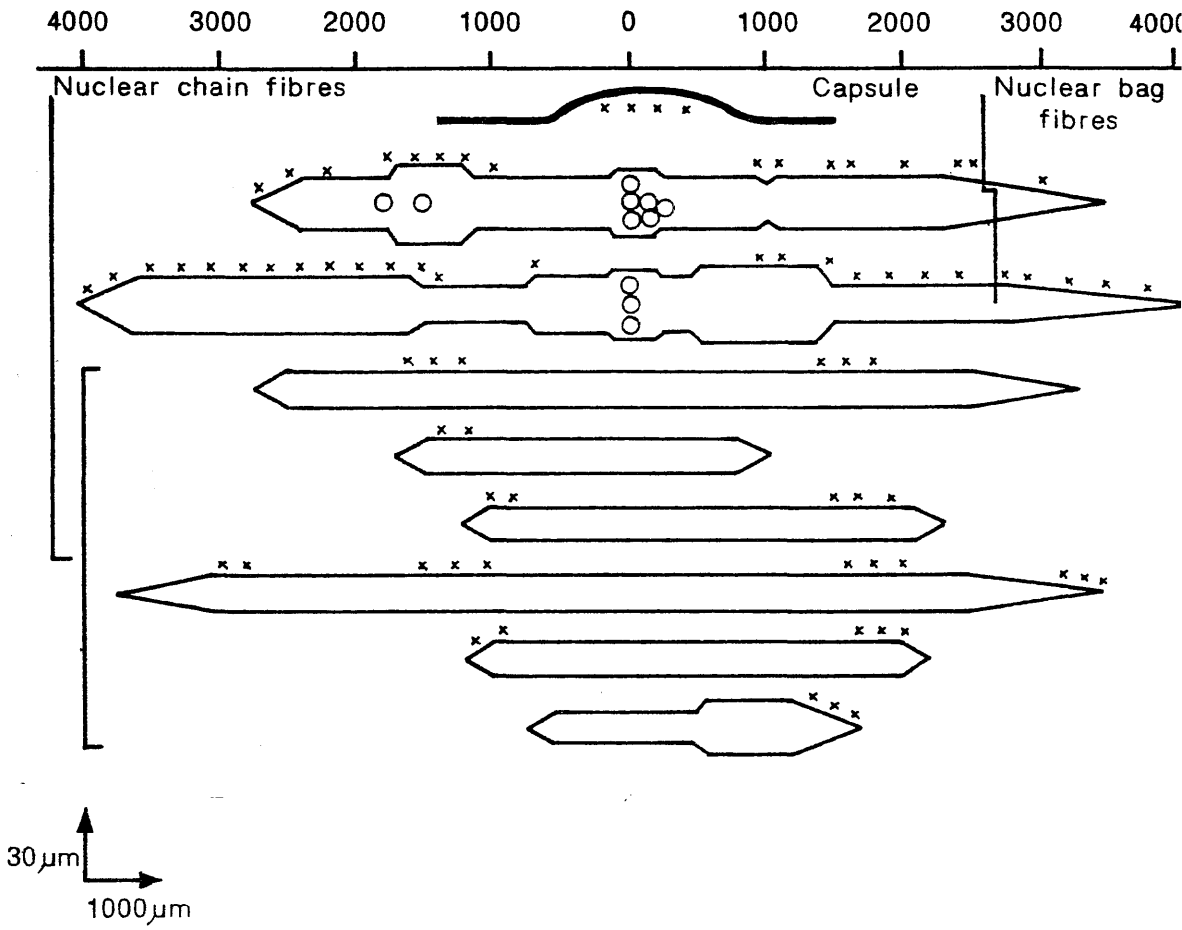
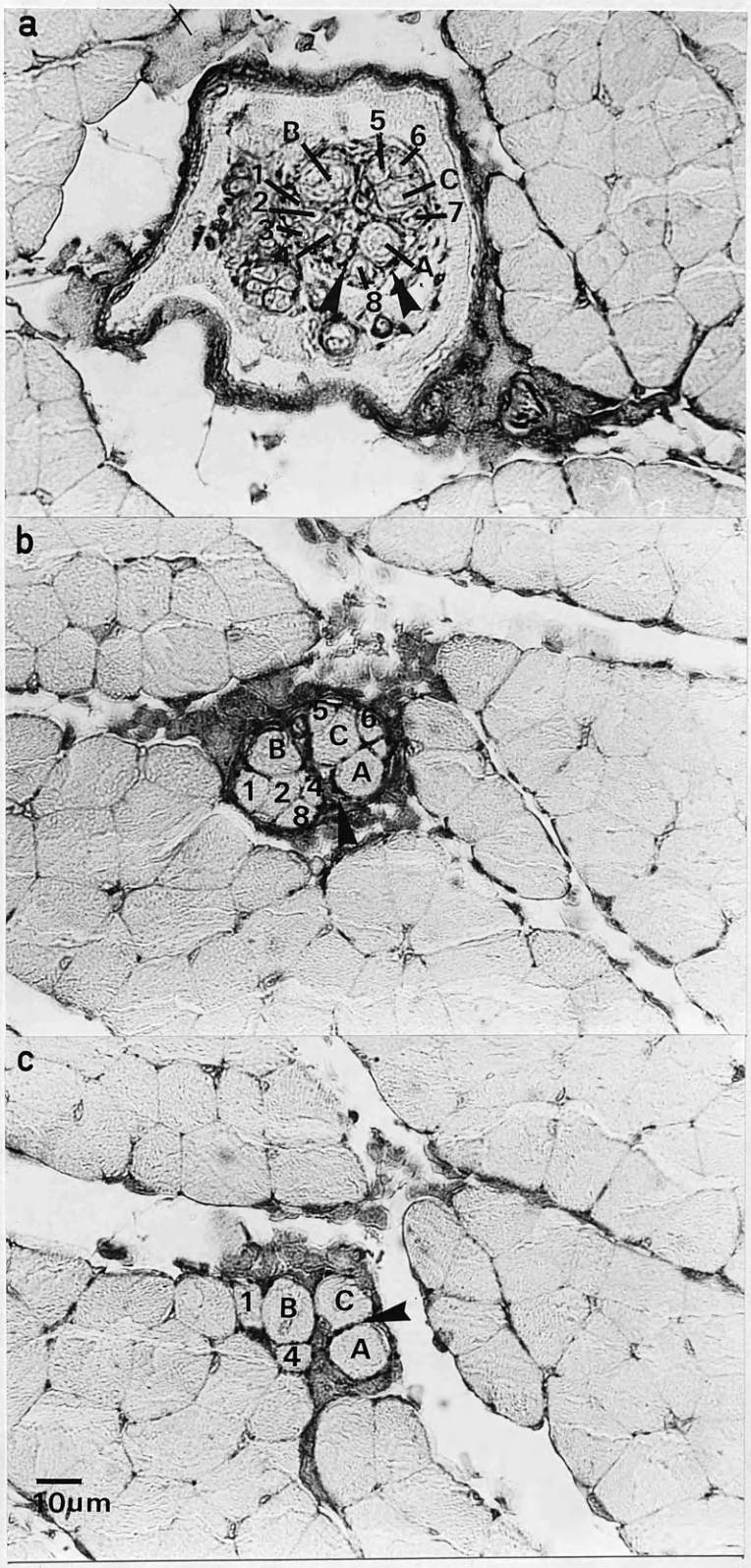


Figure 20 Diagrammatic reconstruction of muscle spindle asterisked in Figure 19. The spindle contains two nuclear bag fibres A and B and six nuclear chain fibres. The presence of elastic fibres is indicated by (xx).

Plate 3



Transverse section through a) the equator of a baboon lumbrical muscle spindle found in muscle II Fig 19 and reconstructed in Fig 26. The three nuclear bag fibres A, B and C and the nuclear chain fibres numbered 1-8 are clearly visible, b) and c): the same spindle 800 µm and 1700 µm from the equator. The elastic fibres are arrowed.

The complement of nuclear bag fibres and nuclear chain fibres per spindle was variable.

Fig 21 shows the distribution of intrafusal fibres per spindle in the twenty seven spindles examined at light microscopy level plus the three spindles studied ultrastructurally.

The minimum number of fibres per spindles was four, the maximum, fifteen. The mean number was 7.13 + 0.276.

Each spindle possessed at least one nuclear bag fibre, the maximum encountered was five. The mean number was 2.29 + 0.15.

One spindle had no nuclear chain fibres while one had ten. The mean number was 4.94 + 0.4.

4.2.3 Intrafusal Fibre Length

Nuclear bag fibres were usually longer than nuclear chain fibres. They varied in length from 3,600 um to 9,500 um. Their mean length was 5935.17 um + 512.55 um.

Nuclear chain fibres varied in length from 1,000 um to 7,400 um. Their mean length was 3401.31 um + 216.51 um.

4.2.4 Long Chain Fibres

Chain fibres which extended for more than 1,000 um beyond the end of the capsule were found in 8 out of 26 single spindles examined ie 30% of single spindles. Seven of these spindles had one long chain fibre at one pole only. One spindle had two long chain fibres extending through both poles. The total number of long chain fibres per spindle pole is therefore 11 out of 52 or 22%.

One tandem spindle out of four examined had one long chain fibre at one pole and two long chain fibres at the other pole. The total number of long chain fibres per tandem spindle pole is

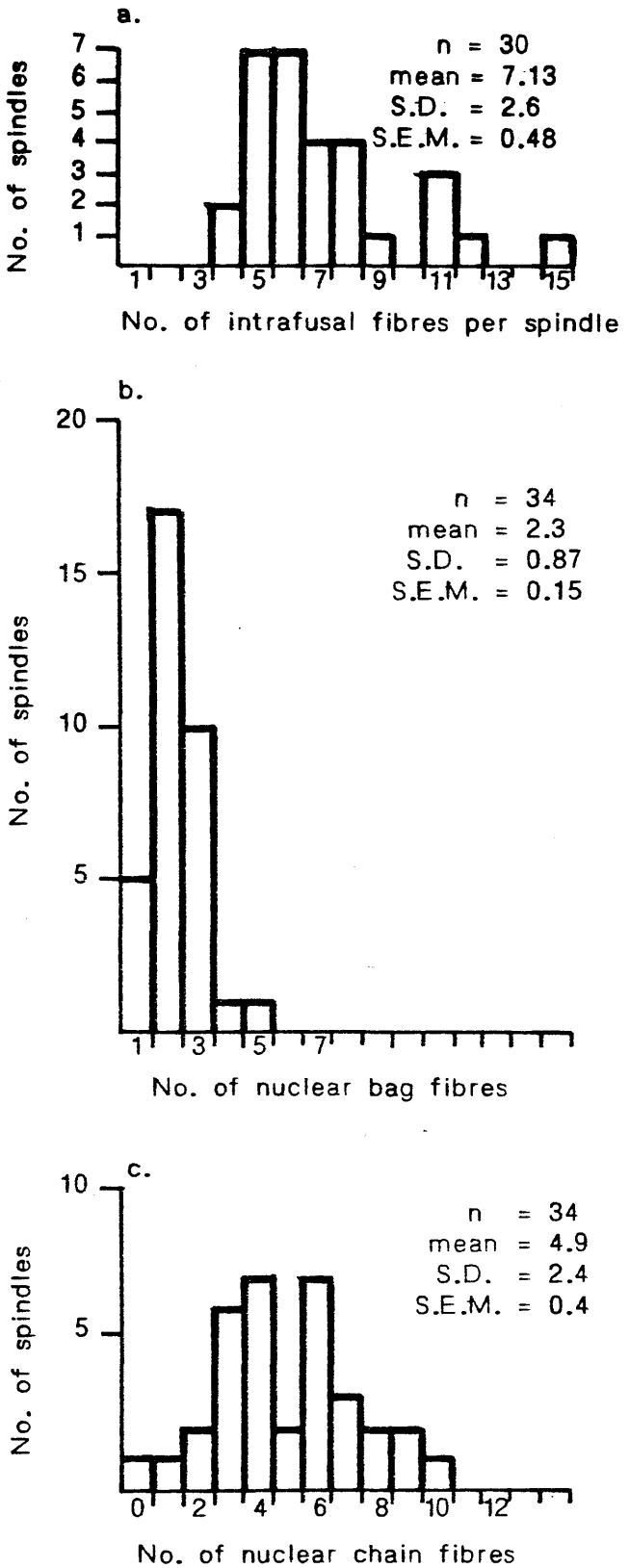


Figure 21 Histograms showing a) the number of intrafusal fibres per spindle, b) the number of nuclear bag fibres per spindle and c) the number of nuclear chain fibres per spindle.

therefore 3 out of 8 or 37.5%.

4.2.5 Intrafusal Fibre Diameter

The diameter of the intrafusal fibres was not accurately measured. It was variable along the length of the fibres with the nuclear chain fibres tending to be wider at the poles than at the equator. Usually the nuclear bag fibres were larger than the nuclear chain fibres but this was not a reliable criterion by which to identify them.

4.2.6 Capsules of Muscle Spindles

The capsule length varied from 850 um to 4,100 um. The mean length was 1,977 um. The capsule expands in the centre of the spindle to form a fluid space the length of which varied from 300 um to 1,300 um. The mean length was 797 um (Fig 22b).

The sleeve region on either side of the fluid space varied from 200 um to 1,500 um in length with the mean length being 680 um (Fig 22a).

4.3 GENERAL MORPHOLOGY OF MUSCLE SPINDLES STUDIED ULTRASTRUCTURALLY

4.3.1 Spindle A

This spindle had two nuclear bag fibres and six nuclear chain fibres (Fig 23). It was not always possible to be sure of the nuclear chain fibre topography as the quality of the muscle fibre architecture was poor. The nuclear bag fibres were easier to trace as they did not vary much in position and were usually the larger fibres. The nuclear bag fibres were labelled A and B with fibre B being larger in diameter for most of its length than fibre A.

4.3.2 Spindle B

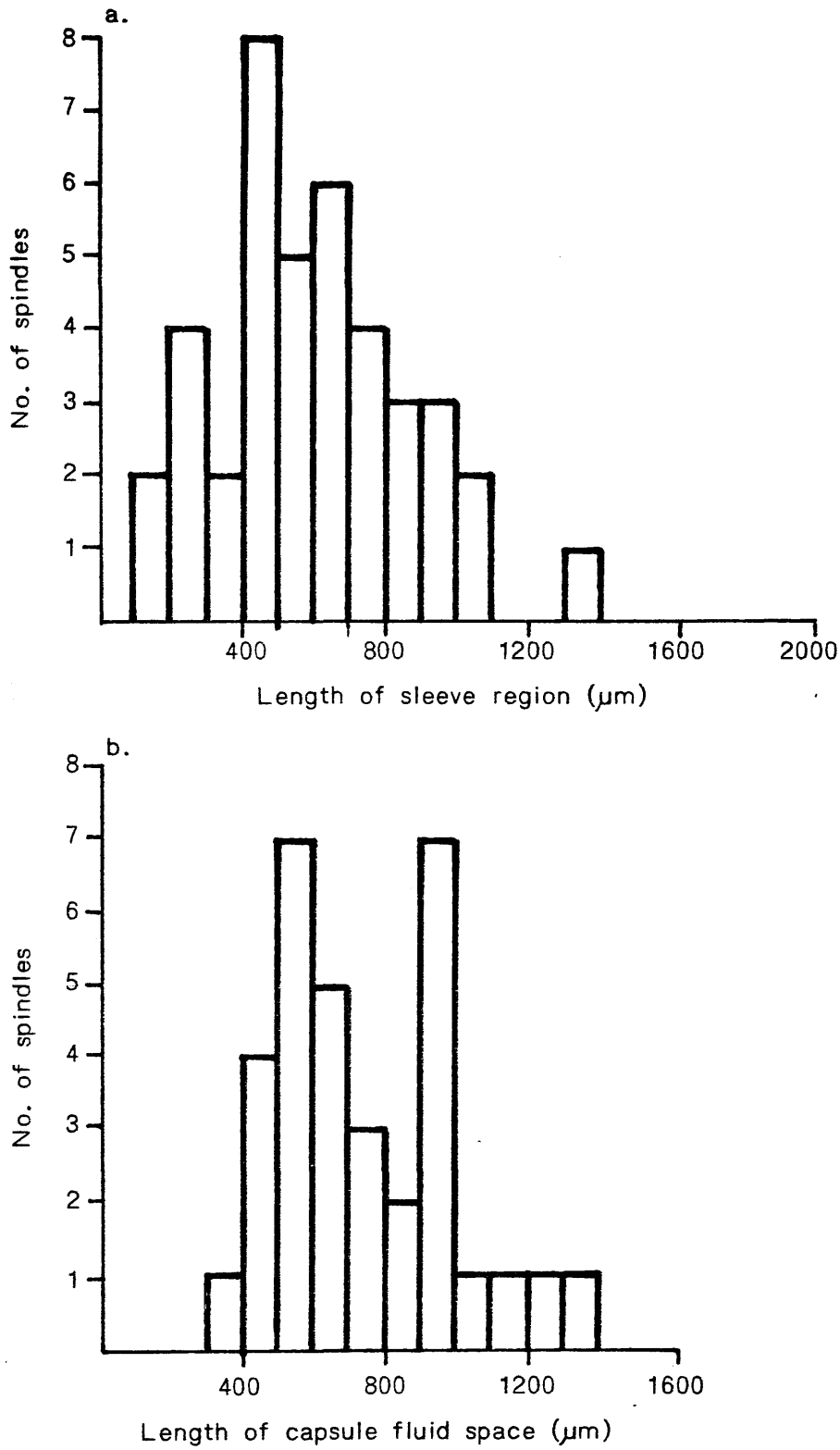


Figure 22 Histograms showing a) the lengths of the sleeve regions and b) the lengths of the capsule fluid spaces of the spindles examined.

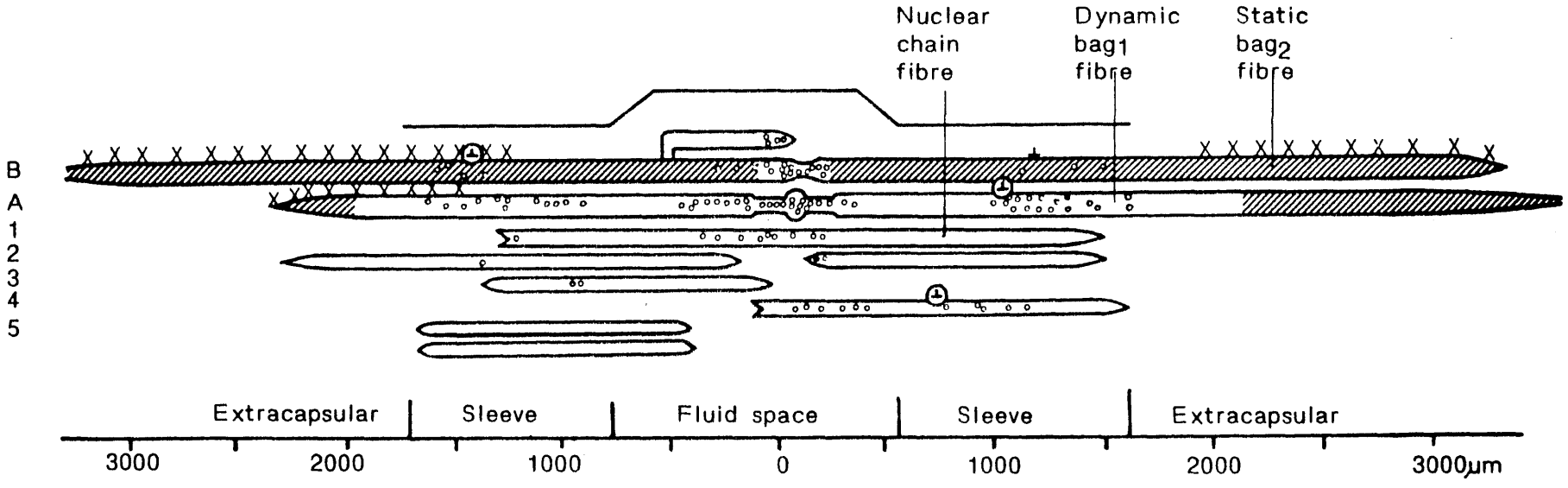


Figure 23 Baboon spindle A reconstructed from serial 1 um sections. The spindle contains two nuclear bag fibres and probably six nuclear chain fibres. Elastic fibres are indicated by (xxx), M-lines by (////) and motor endings by 1. Those motor endings encircled are illustrated in plates 13, 14 and 15.

This spindle was broken in the region of the second fluid space before sectioning was commenced. The break occurred in the region of the equatorial nuclei and it appeared in retrospect that a small piece of the spindle may have been lost at the point of the break as very few nuclei were found in this fluid space. However the spindle was a tandem spindle so by tracing the fibres into the first fluid space it was possible to identify each fibre with certainty.

The spindle contained one nuclear bag fibre (Fibre A) and ten nuclear chain fibres (Fig 24). Fibre B was a long chain fibre at both poles and was thought initially on the basis of its size to be a nuclear bag fibre. This was later rejected for the following reasons:

i) It did not contain a nuclear bag region in the first or second fluid spaces.

ii) It was longer than the nuclear bag fibre which was believed to be a static bag2 fibre and this is a feature of long chain fibres (Kucera 1980c).

iii) It had very little elastic tissue associated with it, unlike static bag2 fibres in general.

iv) The form of its motor endings were similar to those on other chain fibres.

v) Long chain fibres are more common than dynamic bag1 fibres in passing through both fluid spaces of a tandem spindle (Kucera 1982).

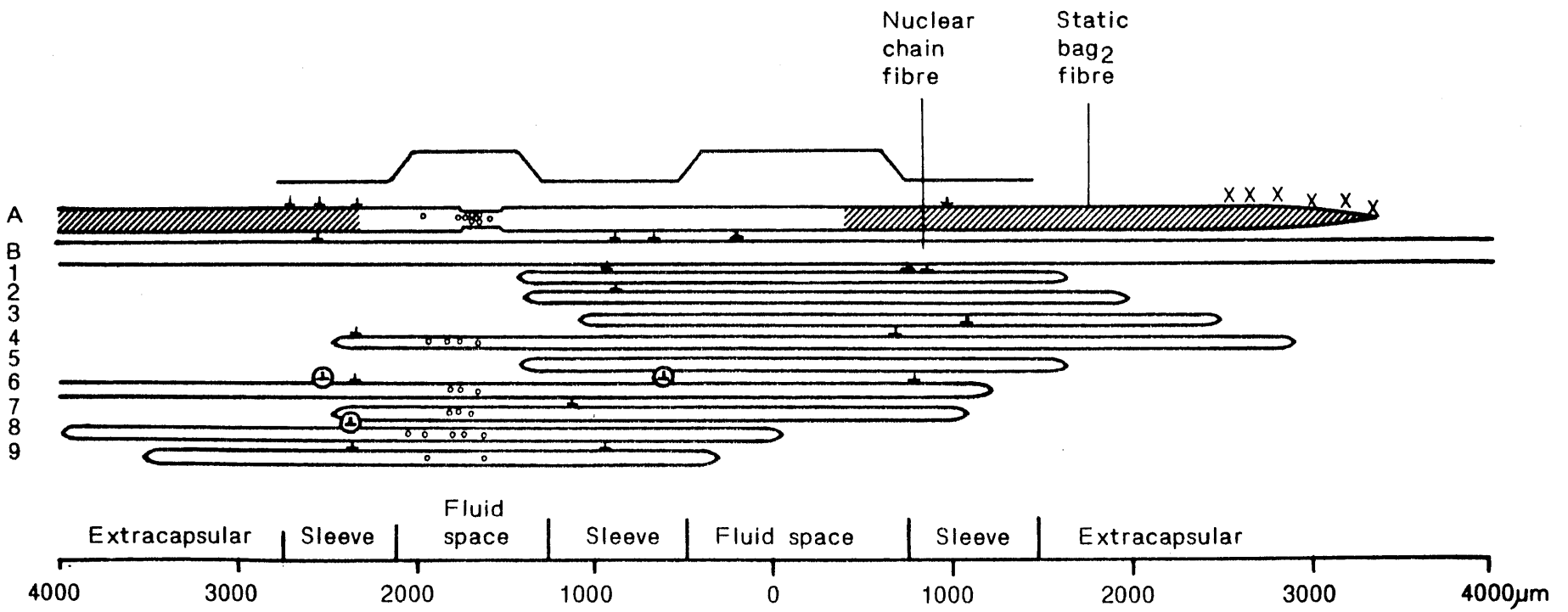


Figure 24 Baboon spindle B reconstructed from serial 1 um sections. The spindle contains one nuclear bag fibre A, one long chain fibre B and nine nuclear chain fibres numbered 1-9. Elastic fibres are indicated by (xxx), M-lines by (////) and motor endings by 1. Those encircled are illustrated in plate (18).

The other fibres were easily identifiable as nuclear chain fibres.

4.3.3 Spindle C

This spindle had two nuclear bag fibres and three nuclear chain fibres and was reconstructed from 1 um sections as in Fig 25.

This spindle was studied electrophysiologically.

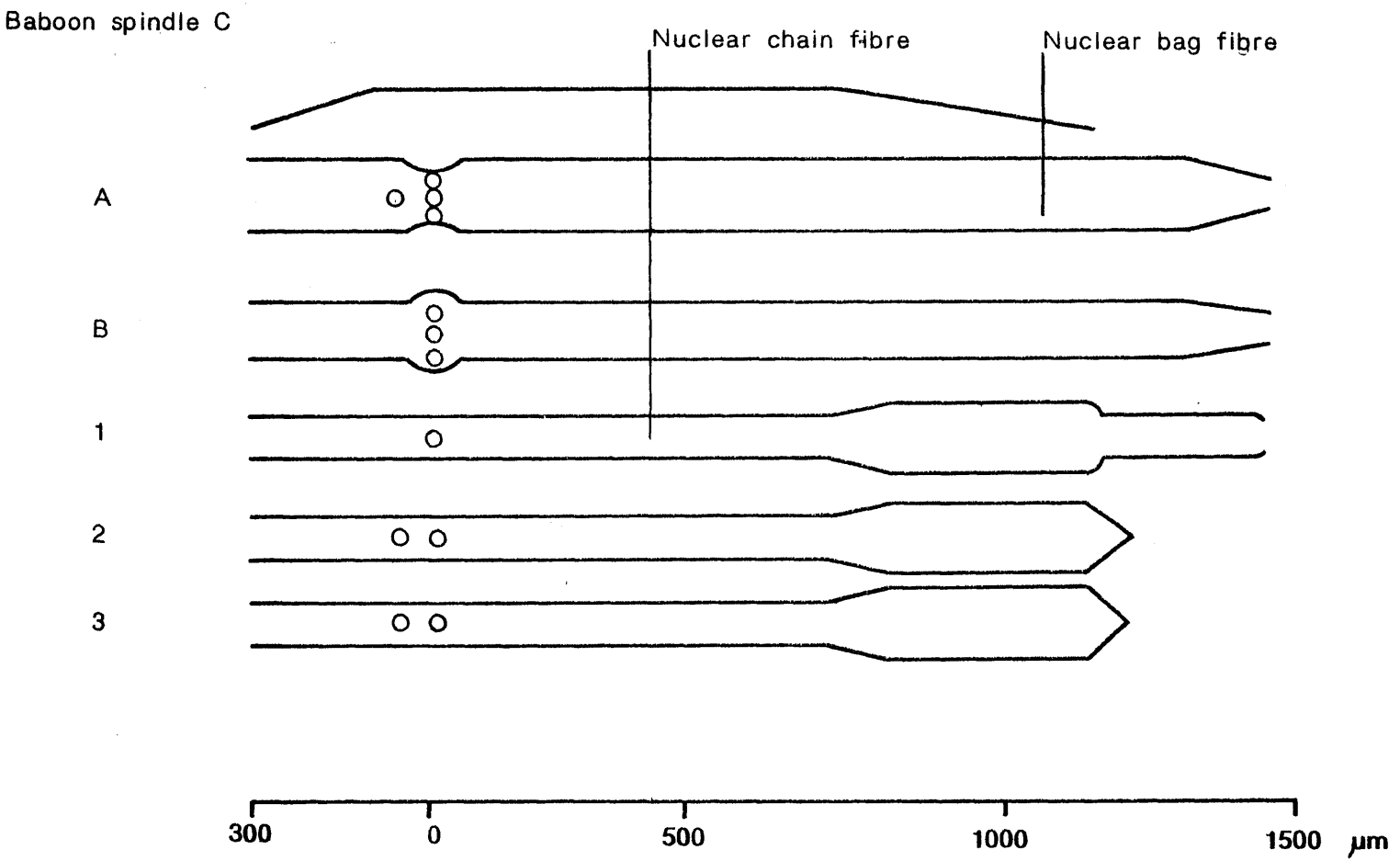


Figure 25 Baboon spindle C reconstructed from serial 1 um sections. The spindle contains two nuclear bag fibres and three nuclear chain fibres.

CHAPTER 5

RESULTS - M-LINE DISTRIIBUTION IN BABOON MUSCLE SPINDLES

5.1 Spindle A

M-lines were found in nuclear bag B at the following levels:-

100 um

200 um

707 um

1636 um

2141 um

2242 um

2343 um to the right of the equator

M-lines were found in nuclear bag A at 2141 um to the right of the equator and 2268 um to the left of the equator.

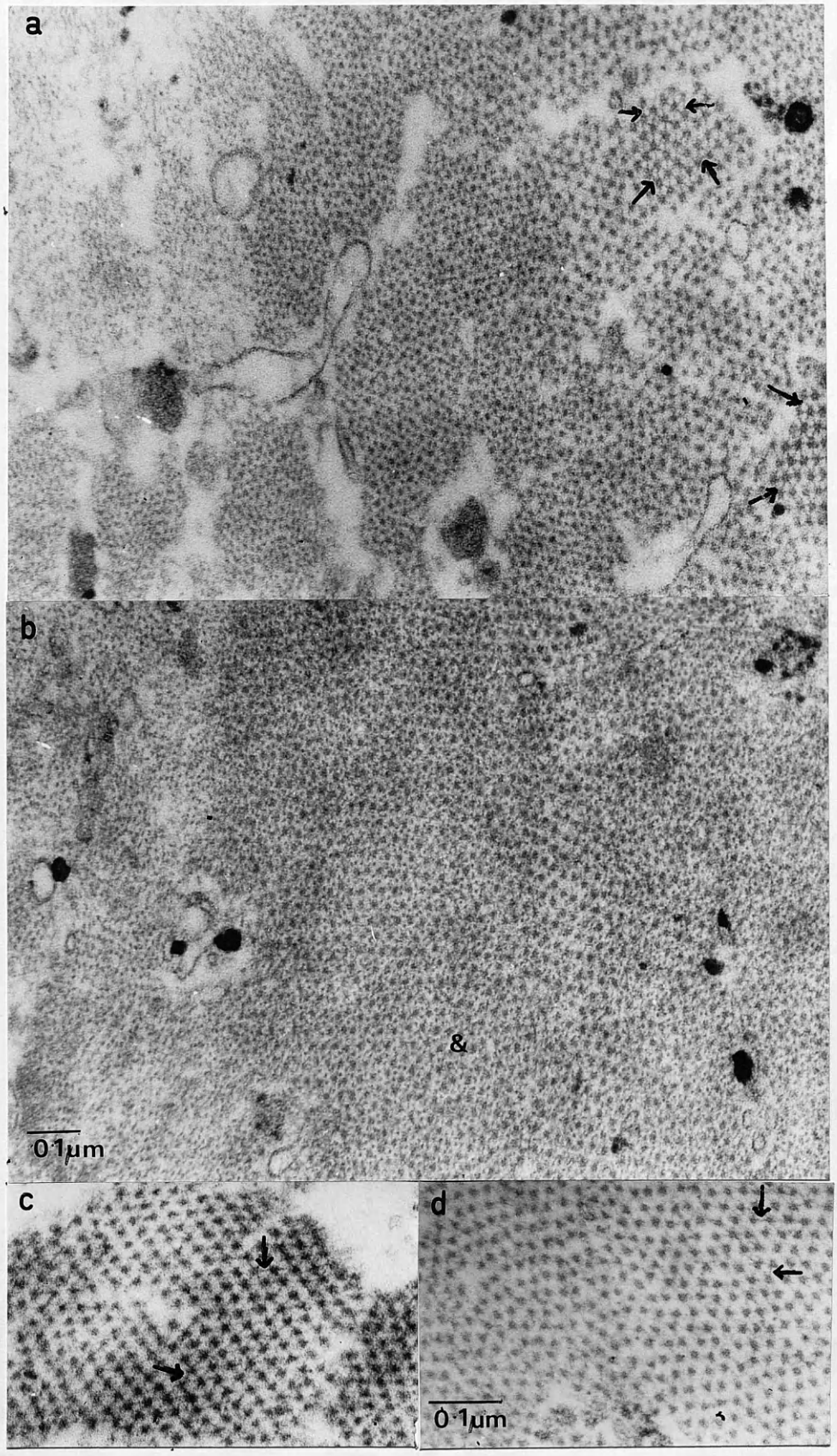
Plate 4 shows high power electron micrographs of transverse sections of the two nuclear bag fibres of spindle A, showing M-lines (arrowed) in nuclear Bag B at 120 um and 2150 um um from the equator and in nuclear bag A at 2150 um only.

5.2 Spindle B

M-lines were found in the following fibres in spindle B at each of the levels described.

a) Nuclear Bag Fibre A at 361 um, 381 um, 451 um,
471 um, 492 um, 501 um, 521 um, 561 um, 581 um,
621 um, 641 um, 721 um, 88 um, 901 um, 921 um
1601 um, 1651 um, 1751 um, 1851 um, 2201 um,
2303 um, 2404 um, 2606 um, 2707 um, 2808 um,
2909 um and 3010 um from the equator toward the
right pole and at 2441 um, 2761 um, 2781 um,
2801 um, 2881 um, 2941 um, 3001 um, 3051 um,
3251 um, 3271 um, 3321 um, 3341 um, 3361 um

Plate 4



High power electron micrographs of transverse sections of the dynamic bag_1 fibre and static bag_2 fibre of spindle A. M-lines (arrowed) are visible in the static bag_2 fibre at 120 um (a) and 2150 um (c) from the equator. There are no M-lines in the dynamic bag_1 fibre at 120 um (b) but they are obvious at 2150 um (d).

3381 um and 3401 um towards the left pole.

b) Long chain Fibre B at 2101 um, 2404 um, 2601 um, 2909 um and 3010 um towards the right pole. No M-lines were visible at the left pole.

c) Chain fibre 1 at 641 um, 681 um, 881 um and 911 um towards the right pole..

d) Chain fibre 2 at 641 um, 681 um, 881 um, 901 um and 921 um towards the right pole.

e) Chain fibre 3 at 641 um and 881 um towards the right pole.

f) Chain fibre 4 at 681 um, 921 um, 1851 um 2303 um and 2404 um towards the right pole.

g) Chain fibre 5 at 641 um, 681 um and 901 um towards the right pole.

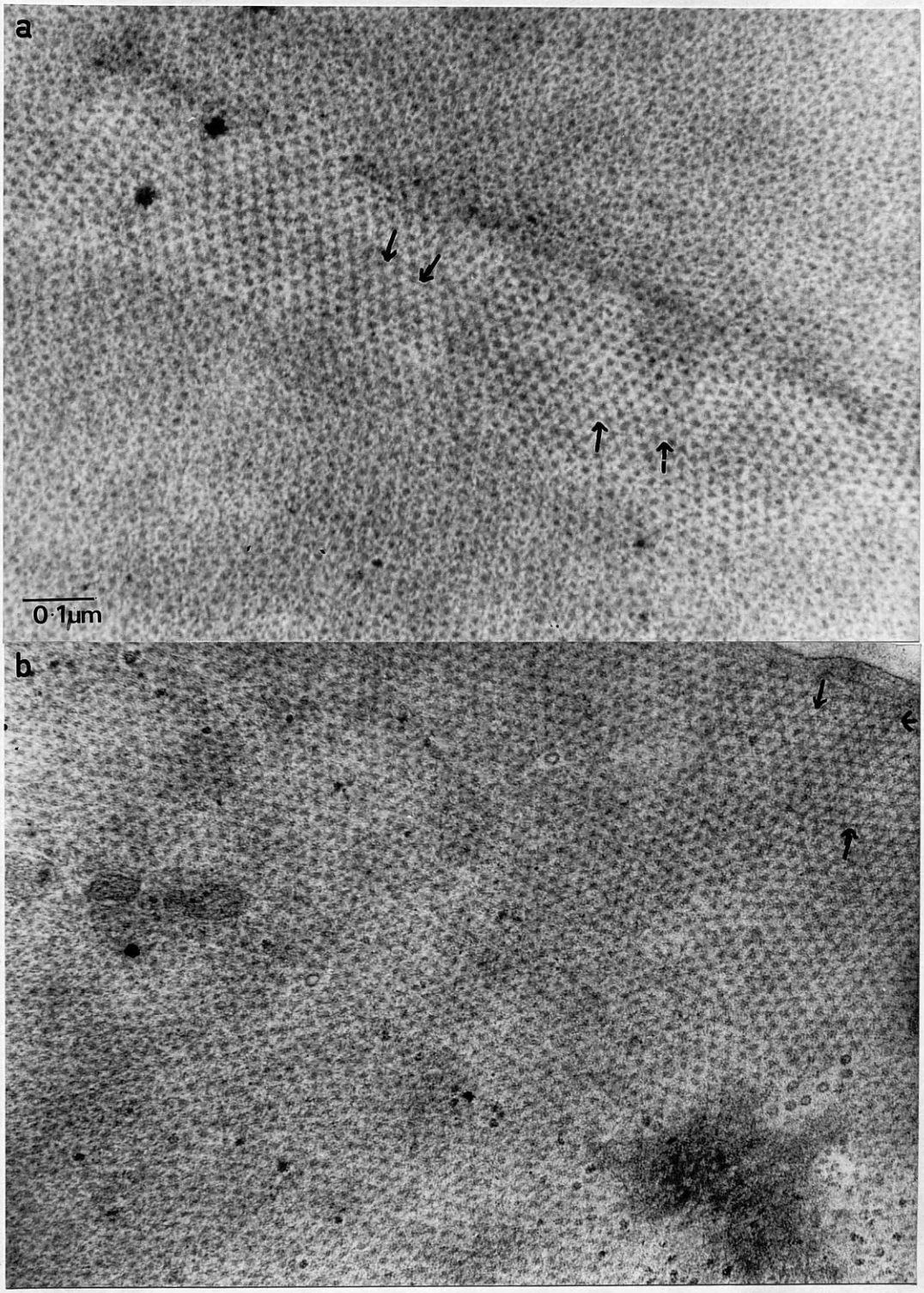
h) Chain fibre 6 at 681 um and 802 um towards the right pole.

i) Chain fibre 7 at 802 um at 921 um towards the right pole.

j) Chain fibre 9 at 2441 um and 2481 um towards the left pole.

Chain fibre 8 was not observed to have M-lines. Plate 5 shows M-lines clearly visible at 360 um and 2404 um from the equator in the nuclear bag fibre.

Plate 5



High power electron micrographs of transverse sections of the static bag$_2$ fibre of spindle B. M-lines (arrowed) are visible at 360 um (a) and 2400 um (b).

5.3 Spindle C

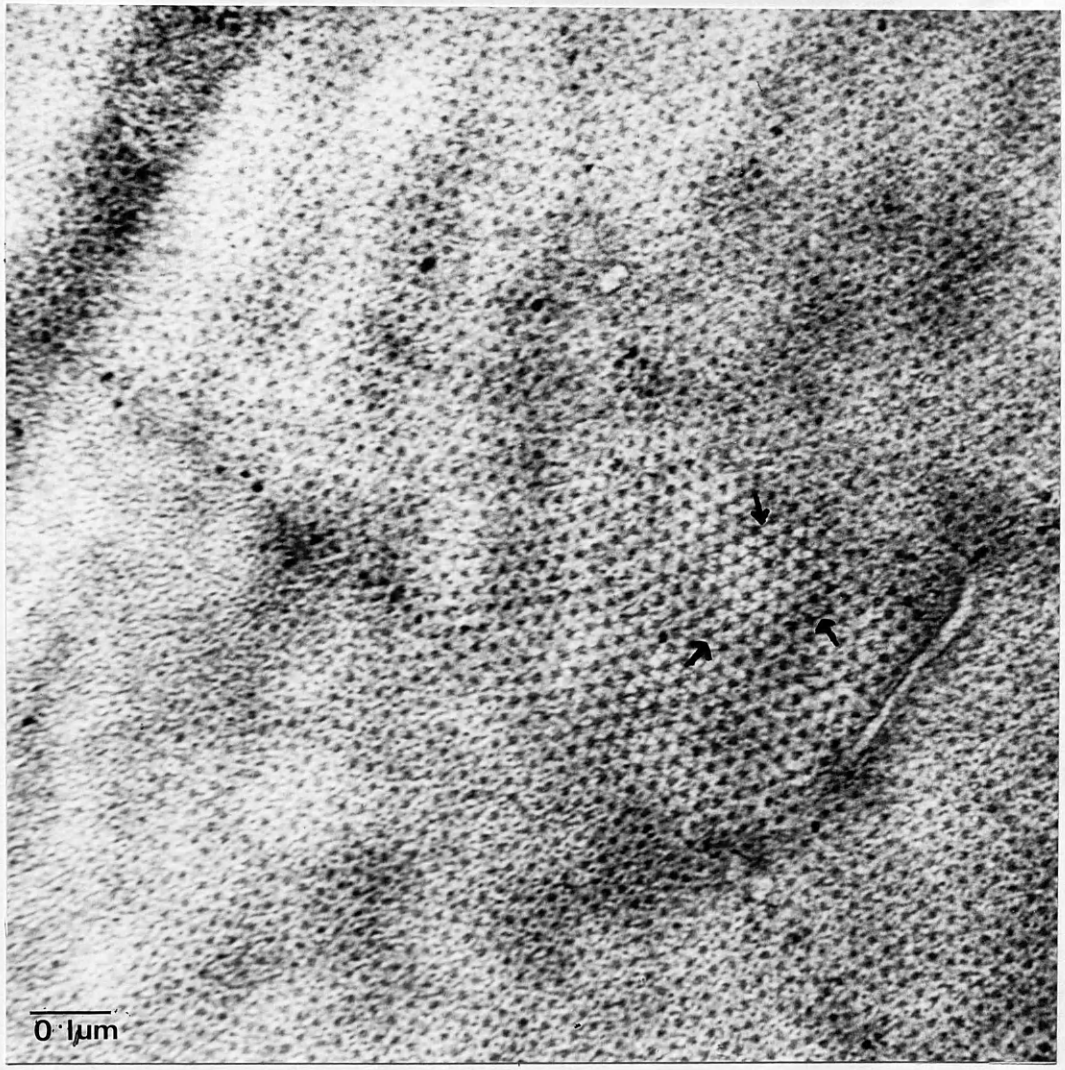
M-lines were observed in transverse section in nuclear bag fibre B at 124 um, 306 um, 848 um, 949 um, 1050 um, 1151 um and 1252 um towards the right pole from the equator.

Nuclear bag fibre A did not have M-lines at any of these levels.

Nuclear chain fibres 1 and 3 had M-lines at 949 um from the equator towards the right pole.

Plate 6 shows M-lines clearly visible in nuclear bag fibre B of spindle C.

Plate 6



High power electron micrographs of transverse section of nuclear bag fibre B of spindle C cut 1000 um from the equator. M-lines are arrowed.

CHAPTER 6

RESULTS - ELASTIC FIBRES IN BABOON MUSCLE SPINDLES

6.1 LIGHT MICROSCOPY OF 27 MUSCLE SPINDLES

The most striking feature seen in all spindles examined was that nuclear bag fibres had more elastic fibres associated with them for a greater proportion of their lengths than nuclear chain fibres.

This is illustrated in Figs 20 and 26 in which the crosses (x) indicate where elastic fibres were seen in transverse sections with the light microscope. Plate 3 is a photograph of transverse sections of a baboon muscle spindle stained with resorcin fuchsin. The elastic fibres round the nuclear bag fibres are clearly seen (arrowed).

Nuclear chain fibres had small amounts of elastic associated with them along their entire length but mainly at the end of the capsular sleeve region (Figs 20, 26).

In 19 out of the 30 spindles examined ie including the three studied ultrastructurally, there were differences in elastic fibre distribution between nuclear bag fibres within spindles.

In the extracapsular region of the spindles one bag fibre had much more elastic associated with it (nuclear bag B, Fig 20). This was the case at both poles in only 4 of the 19 spindles. Of the remaining 15, however, 14 were incomplete due to the fact that the lumbrical muscle was cut into 1 cm divisions during fixation and it was difficult to trace a spindle from one block to another. It was not possible therefore to say whether this difference between nuclear bag fibres was symmetrical. In one spindle (studied ultrastructurally) there was a difference between bag fibres at the right pole extracapsularly but not the left pole (Fig 23, plate 7). Plate 7 shows how elastic fibres are obvious even in unstained transverse sections.

Figure 26 Diagrammatic reconstruction of a baboon spindle containing three nuclear bag fibres A, B and C and eight nuclear chain fibres. This spindle is also identified (‡) in Figure 19 and a transverse section through it is shown in Plate 3

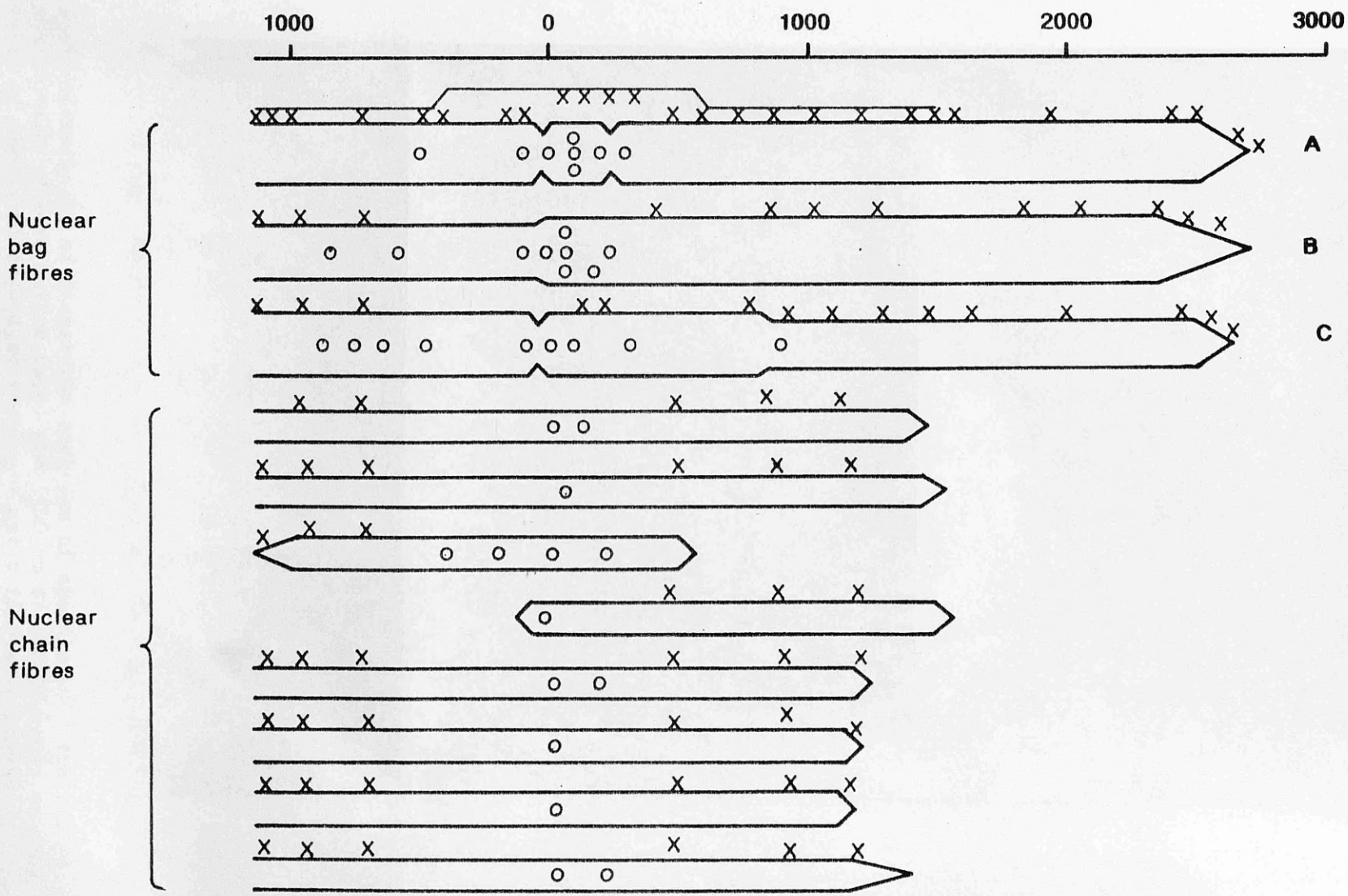
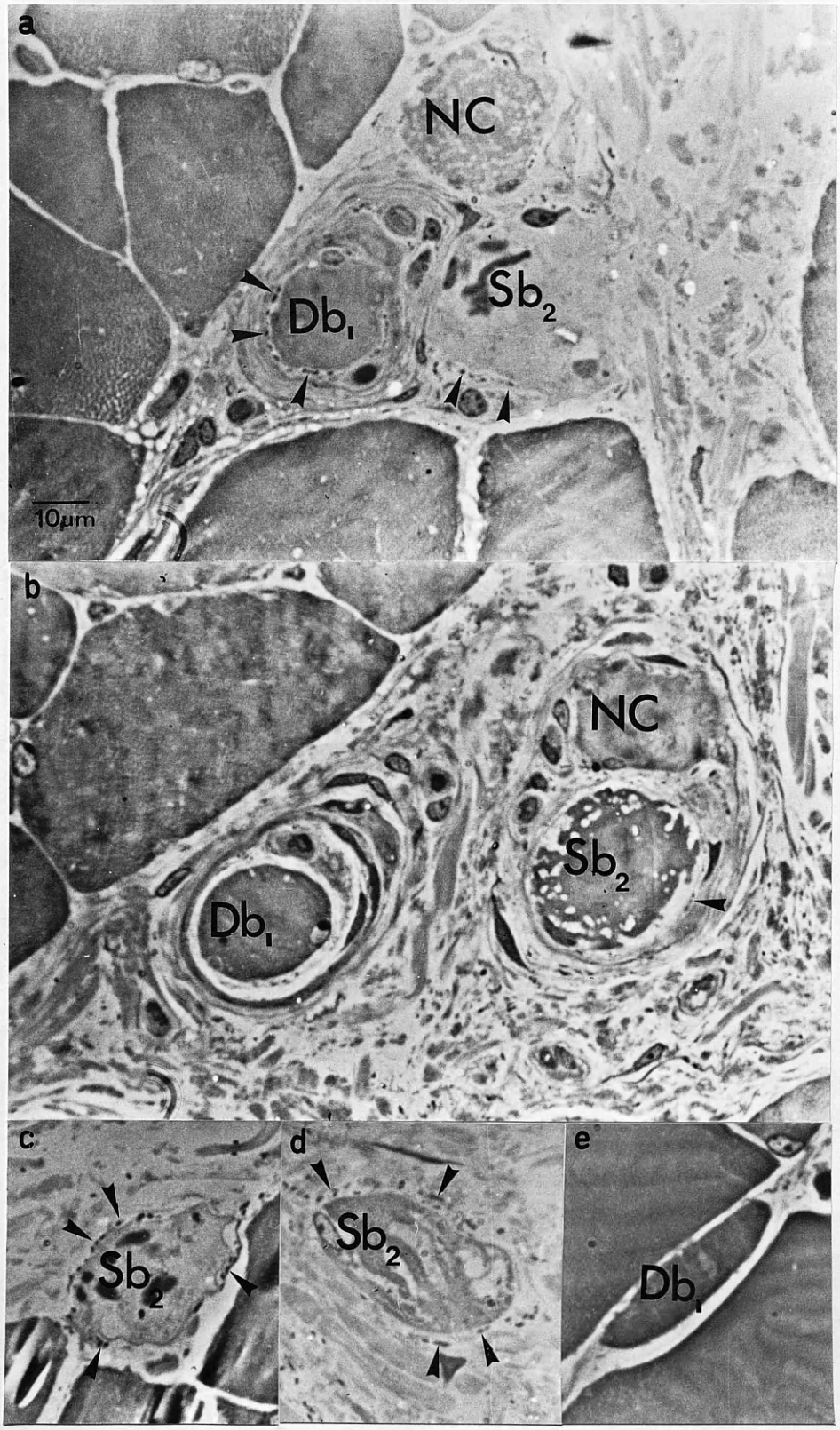

1000
0
1000
2000
3000
A
B
C
Nuclear bag fibres
Nuclear chain fibres

Plate 7



Light micrographs of transverse sections of spindle A at the end of the capsular sleeve region (a,b) and 1000 um extracapsularly at both poles of the spindle (c,d,e) showing elastic fibres (arrowed) associated with both the dynamic bag_1 fibre (Db_1) and static bag_2 (Sb_2) fibre at the left pole (a,c) but associated with only the static bag_2 fibre at the right pole (b,d,e). NC: nuclear chain fibre.

The remaining 11 spindles examined showed no obvious difference extracapsualrly in the elastic fibre distribution round nuclear bag fibres (Fig 26).

Within the sleeve region differences in elastic fibre distribution were found between nuclear bag fibres in 14 of the spindles in which extracapsular differences were noted. Again it was the same nuclear bag fibres which had more elastic fibres.

Seven of the 14 spindles had differences in elastic fibre distribution which were symmetrical in both sleeve regions. Two of the remaining 7 were asymmetrical (Fig 20) and 5 were incompletely sectioned in the opposite sleeve region.

There was no obvious pattern of elastic fibre distribution around nuclear bag fibres near the equator although they appeared to have more elastic than nuclear chain fibres.

All 30 spindles had elastic fibres present in small amounts in the outer capsule while 20 spindles also had elastic fibres in the inner capsule.

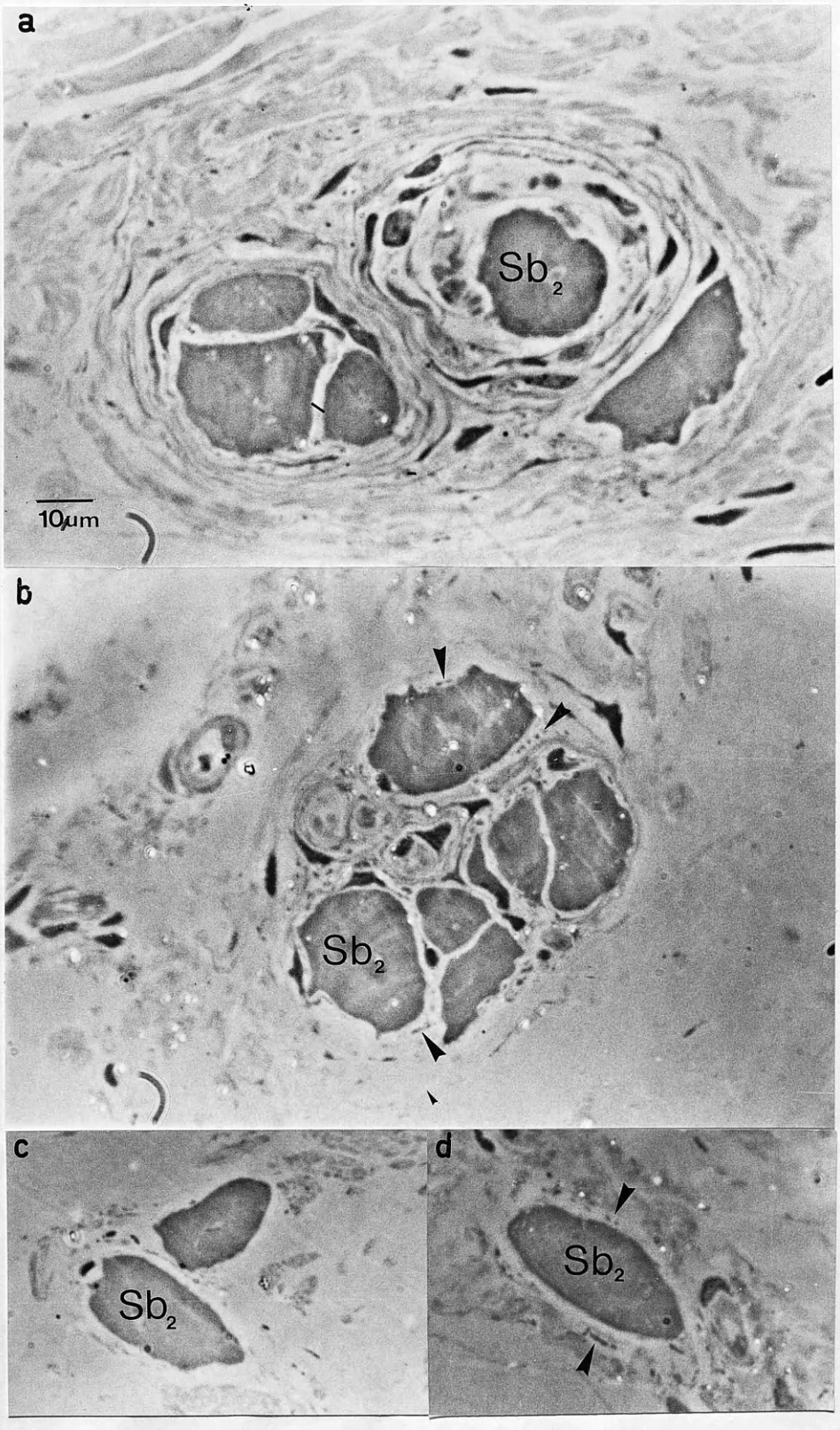
Five spindles had only one nuclear bag fibre so no comparison could be made between bag fibres. Plate 8 shows light micrographs of transverse sections of spindle B (Fig 24) through the end of the capsular sleeve region and 1000 um extracapsularly at both poles. Elastic fibres are arrowed.

6.2 QUANTITATIVE ANALYSIS OF ELASTIC FIBRES IN TWO SPINDLES STUDIED ULTRASTRUCTURALLY

The two spindles used for quantitatve analysis are reconstructed in Figs 23 and 24.

They were originally dissected out from the first lumbrical muscle of two different baboons.

Plate 8



Light micrographs of transverse sections of spindle B at the end of the capsular sleeve region (a,b) and 1000 um extracapsularly (c,d) at both poles showing very few elastic fibres associated with the static bag_2 fibre more being present at the right pole (b,d).

6.2.1 Spindle A

Fig 23 shows that the spindle had two nuclear bag fibres and six nuclear chain fibres.

Plate 7, shows that elastic fibres could be clearly identified in unstained 1 um sections at light microscopy level. Plate 9 shows the elastic fibres in ultrathin sections stained post-fixation with palladium chloride and lead citrate.

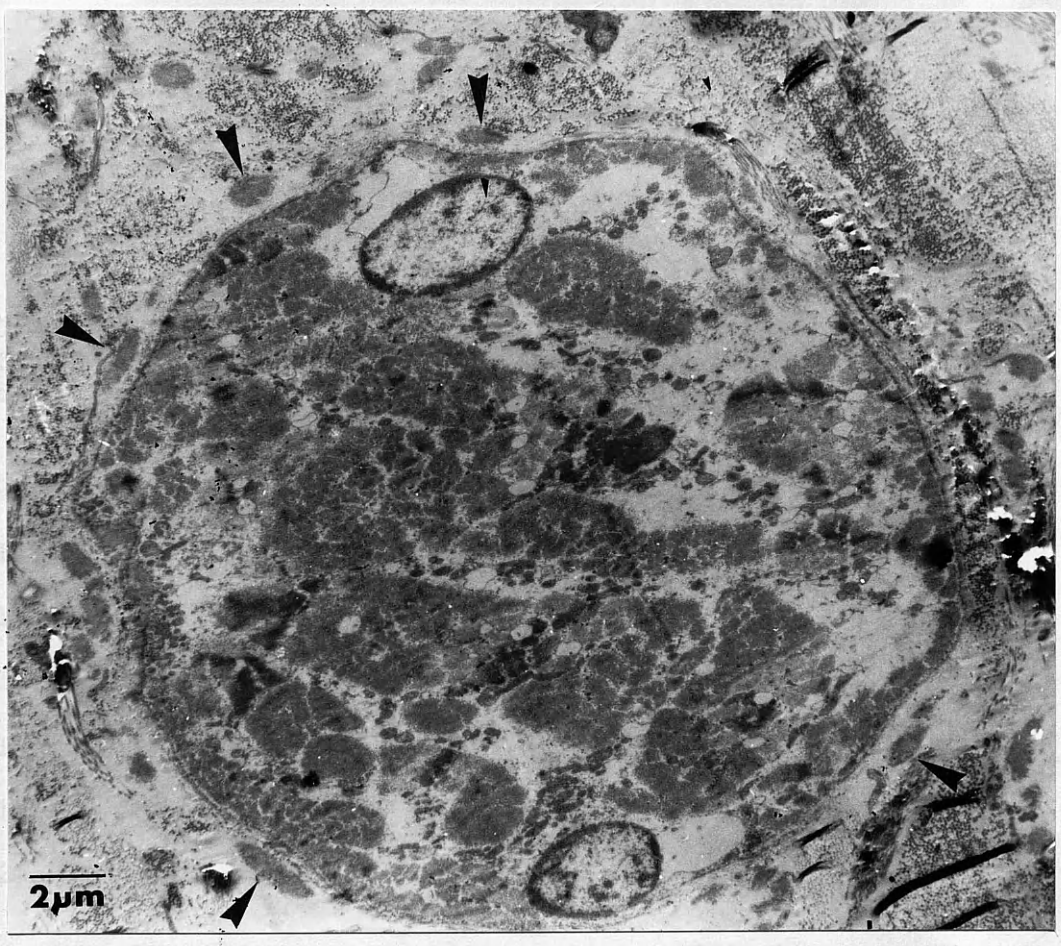
Elastic fibres were assumed to be associated with the muscle fibre if there was no intervening tissue between the elastic and the muscle and the distance between the two was less than 0.5 um.

Table 7 shows the measurements of elastic fibre circumference of the two bag fibres in spindle A and the ratio of elastic fibre to muscle fibre circumference was used as an indicator of the amount of elastic fibre present at each level. The measurement in itself is meaningless but can be used to compare the two fibres with each other and to compare the left and right poles.

Thus at the left pole there was little difference between the two fibres with fibre A having more elastic within the sleeve region but fibre B being longer had elastic associated with it for more of its length.

At the right pole fibre B had much more elastic tissue associated with it than fibre A.

Plate 9



High power electron micrograph of the static bag$_2$ fibre of spindle A sectioned 1000 um extracapsularly. The ultrathin section is stained with palladium chloride and lead citrate. Elastic fibres are arrowed.

TABLE 7

SPINDLE A LEFT POLE

Distance from Equator	NUCLEAR BAG FIBRE A Muscle Fibre Circumf. (M)	Elastic Fibre Circumf. (C)	Ratio (E:M)	NUCLEAR BAG FIBRE B Muscle Fibre Circumf. (M)	Elastic Fibre Circumf. (E)	Ratio (E:M)
938 um	65.6	0	0	58.2	0	0
1140 um	53	34.7	0.65	70.4	8	0.11
1241 um	59.2	37.6	0.63	68	20.8	0.3
1445 um	57.6	51.2	0.88	91.2	20.8	0.22
1547 um	48	32	0.66	91.2	56	0.6
1853 um	24	10.4	0.43	65.6	0	0
2257 um				79.8	28	0.38

RIGHT POLE

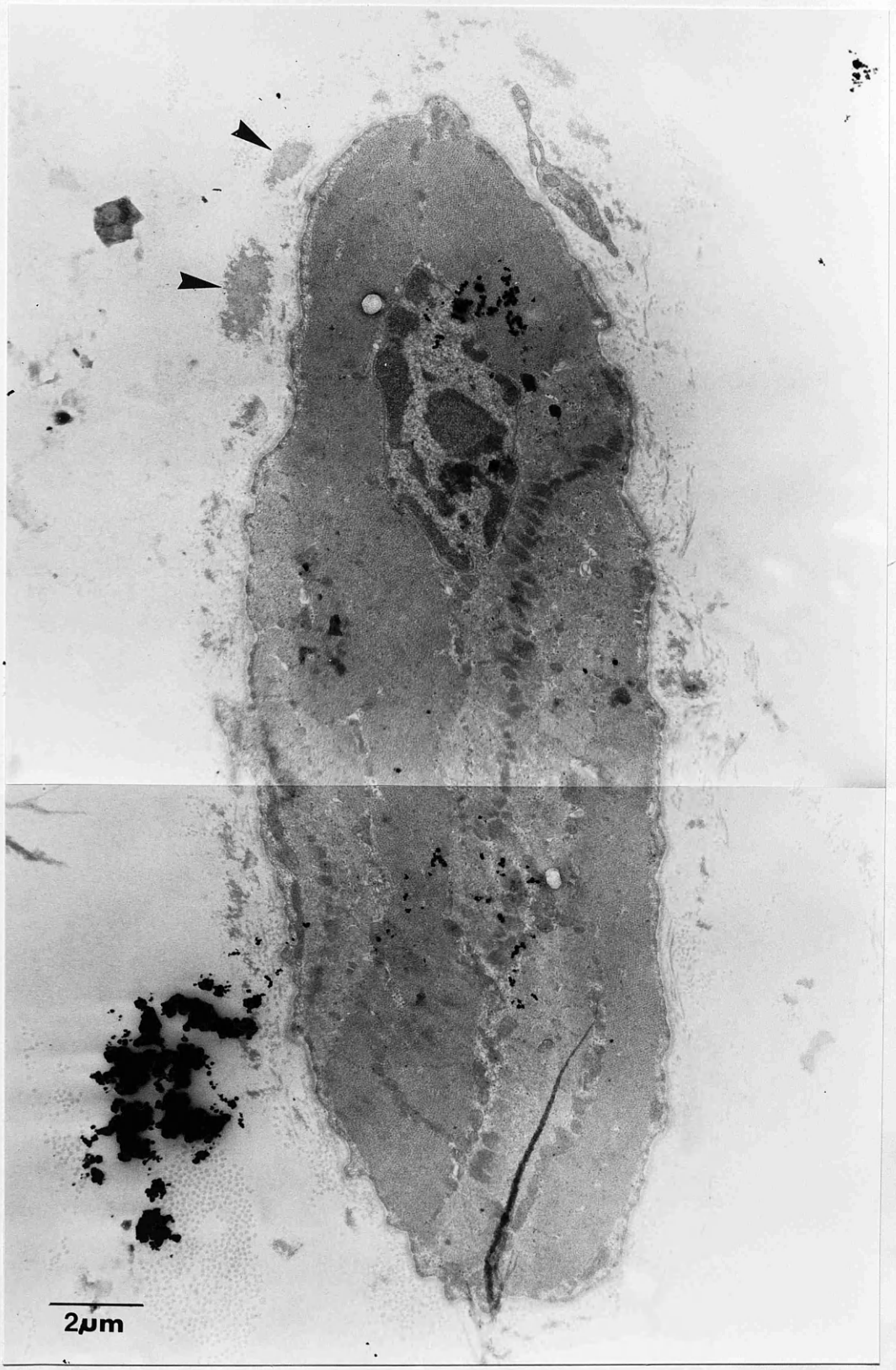
	NUCLEAR BAG FIBRE A			NUCLEAR BAG FIBRE B		
82 um	56	0	0	80	0	0
668 um	48	0	0	56	0	0
1173 um	65.6	0	0	68	0	0
1274 um	58.8	0	0	68	0	0
2304 um	96	0	0	75.2	32	0.42
2607 um	78.4	0	0	64	16	0.25
2809 um	81.6	0	0	66.4	51.2	0.77
3102 um				58.4	25.6	0.44

Table showing ratio of elastic fibre circumference to muscle fibre circumference of nuclear bag fibres A and B in spindle A at both left and right poles.

6.2.2 Spindle B

Fig 24 shows that this spindle had one nuclear bag fibre and ten nuclear chain fibres. Plate 8 shows that there was little elastic tissue to be seen at the end of the sleeve region and 1000 um extracapsularly when compared with plate 7. However what was present can be seen in unstained sections at light microscopy level and is confirmed when ultrathin sections stained with palladium chloride and lead citrate are examined. Plate 10 is an ultrathin section of the nuclear bag fibre of spindle B sectioned 1000 um extracapsularly at the right pole. The elastic fibres are arrowed. A quantitative analysis of the elastic fibres associated with the nuclear bag fibre and the longest nuclear chain fibre was carried out as previously described for spindle A. Table 8 shows the measurements made. At both poles the nuclear bag fibre (fibre A) had marginally more elastic tissue associated with it than the long chain fibre (fibre B). There was more elastic at the right pole than the left pole.

Plate 10



High power electron micrograph of the static bag$_2$ fibre of spindle B sectioned 1000 um extracapsularly. The ultrathin section is stained with palladium chloride and lead citrate. Elastic fibres are arrowed.

TABLE 8

SPINDLE B LEFT POLE

Distance from Zero	FIBRE A Muscle Fibre Circumf. (M)	FIBRE A Elastic Fibre Circumf. (E)	FIBRE A Ratio (E:M)	FIBRE B Muscle Fibre Circumf. (M)	FIBRE B Elastic Fibre Circumf. (E)	FIBRE B Ratio (E:M)
201 um	48	3.2	0.06	66.4	0	0
1601 um	36	0	0	40	0	0
1851 um	40	0	0	48	0	0
2001 um	64.8	0	0	65.6	0	0
2051 um	84	0	0	62.4	0	0
2901 um	84	0	0	84	0	0
3501 um	72	3.2	0.04	68.8	0	0
3801 um				84.8	0	0
		RIGHT POLE				
721 um	49.6	1.6	0.03	58.4	0	0
941 um	43.2	0	0	49.6	4	0.08
1041 um	39.2	4	0.1	42.4	4	0.09
1161 um	48	11.2	0.23	48	6	0.13
1651 um	64	6.4	0.1	54.4	8	0.14
1851 um	56	2.4	0.04	38.4	0	0
2606 um	44	9.6	0.21	44	0	0
2707 um	44	4	0.09	48	0	0
2808 um	44.8	6.4	0.14	52	0	0
2909 um	36	8	0.2	48	0	0

Table showing ratio of elastic fibre circumference to muscle fibre circumference of fibres A and B of spindle B of both left and right poles.

CHAPTER 7

RESULTS : INNERVATION OF BABOON MUSCLE SPINDLES

7.1 ELECTROPHYSIOLOGY

Spindles B and C were studied electrophysiologically.

7.1.1 Spindle C (Fig 25)

Spindle C was isolated and stimulated via an intramuscular nerve branch. Nuclear chain fibre activity was observed in the upper part of the spindle, 1.6 mm from the primary sensory ending and 1.3 mm from the end of the fluid space. The fibres twitched when stimulated at frequencies of 5-100 Hz.

7.1.2 Spindle B (Fig 24)

By stimulating intramuscular nerve branches to spindle B at frequencies varying from 0-100 Hz visible intrafusal fibre activity was observed. Starting at the right hand pole and moving towards the left the following movements were observed.

At 900 um there was a visible contraction site. At this point fibres further out towards the right moved inwards while those to the left moved out. The movement was fast and did not tire easily. It appeared above frequencies of 10 Hz.

Further out there appeared to be a slight movement inwards at lower stimulus strength.

Between the two fluid spaces there was a movement observed which tired easily. The fibres twitched twice at a frequency of 10 Hz but responded mainly between 20 and 100 Hz. The movement was a quick movement typical of chain fibre activity.

Towards the left pole, the largest fibre presumably a nuclear bag fibre gave a fast movement which failed easily at 30 Hz. There was an obvious contraction site at 3,350 um.

The nuclear chain fibres at this pole also twitched but did

not tire as easily as those between the fluid spaces. A contraction site was observed at 2800 um.

Further out at 3800 um a large fibre was observed to move quickly inwards when the nerve was stimulated at 50 Hz. This movement tired easily.

These results are detailed schematically in Fig 27 complementing the montage of spindle B.

7.2 SENSORY INNERVATION

Sensory axons were not studied in any detail at an ultrastructural level but examination of the montage of spindle B shows that (Fig 27) the primary axons in both fluid spaces, identifiable by their large diameter, have less of a spiral configuration than those described in cat spindles.

7.3 AXON DISTRIBUTION TO INDIVIDUAL INTRAFUSAL FIBRES

In spindle B an attempt was made, using both the phase contrast microscope to examine intervening 1 um sections and the electron micrographs taken at 20-100 um intervals, to trace motor axons.

This was a very difficult task as axons can only be identified accurately with the electron microscope. Axons branch profusely at times and can take a very tortuous course appearing more than once in cross section. Only by checking and rechecking drawings and micrographs was it possible to draw a diagram showing the axon distribution to the spindle.

Fig 28 shows the distribution of five motor axons to the spindle together with the location of the 22 motor endings sectioned.

Three axons were selective in distribution in supplying

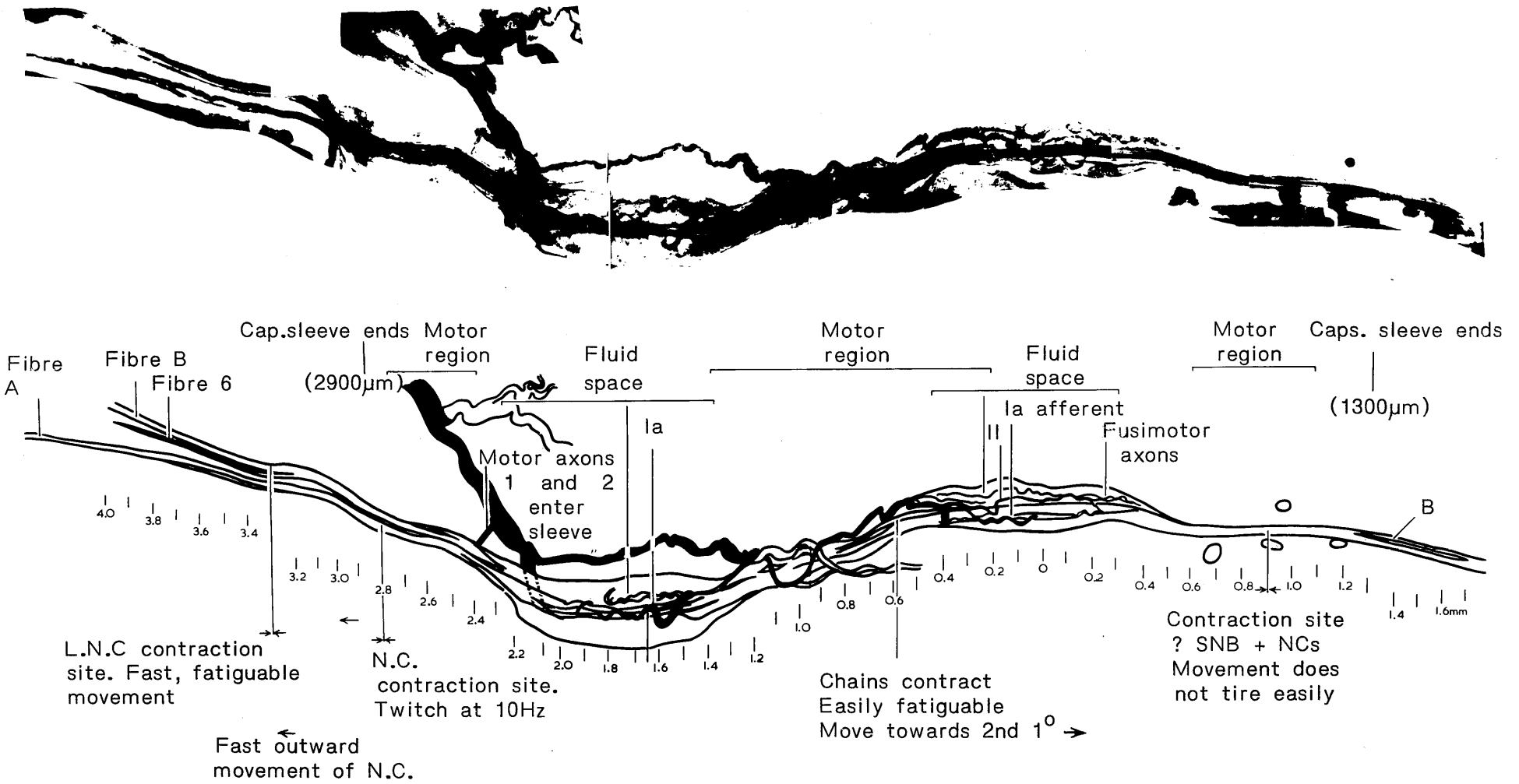


Figure 27 Montage of spindle B with explanatory diagram of electrophysiological findings.

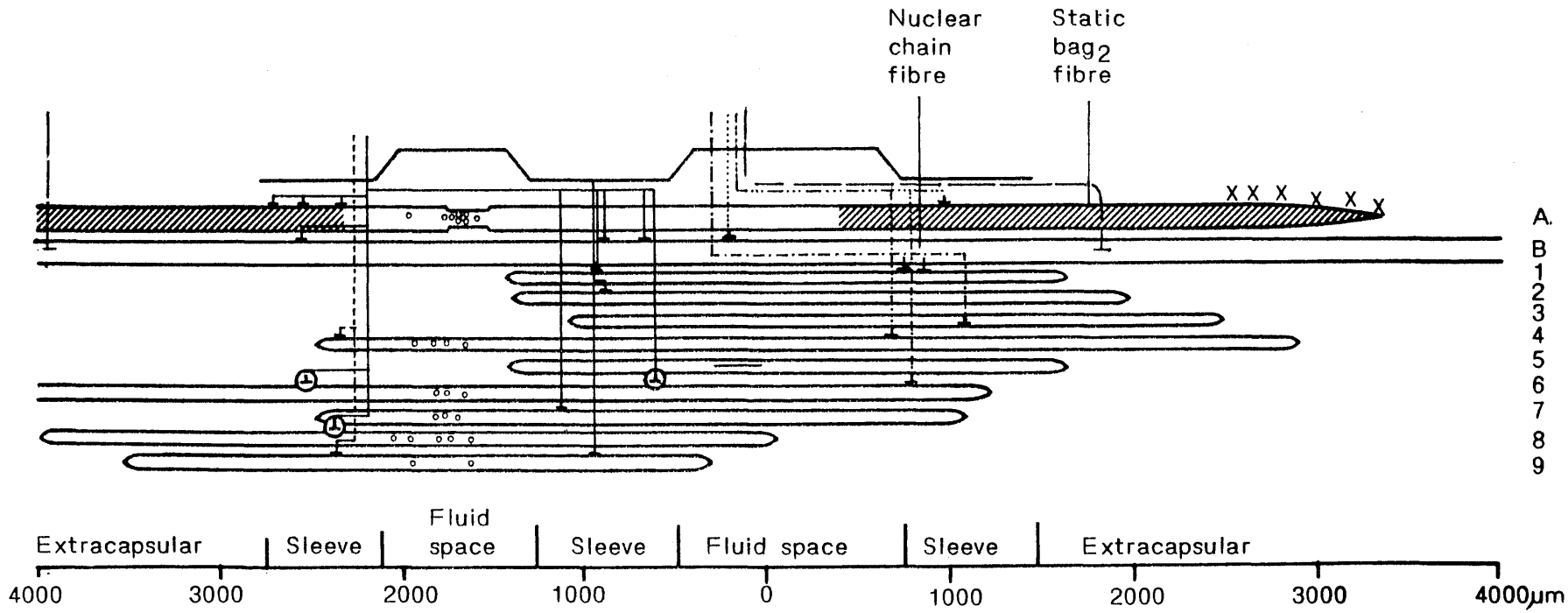
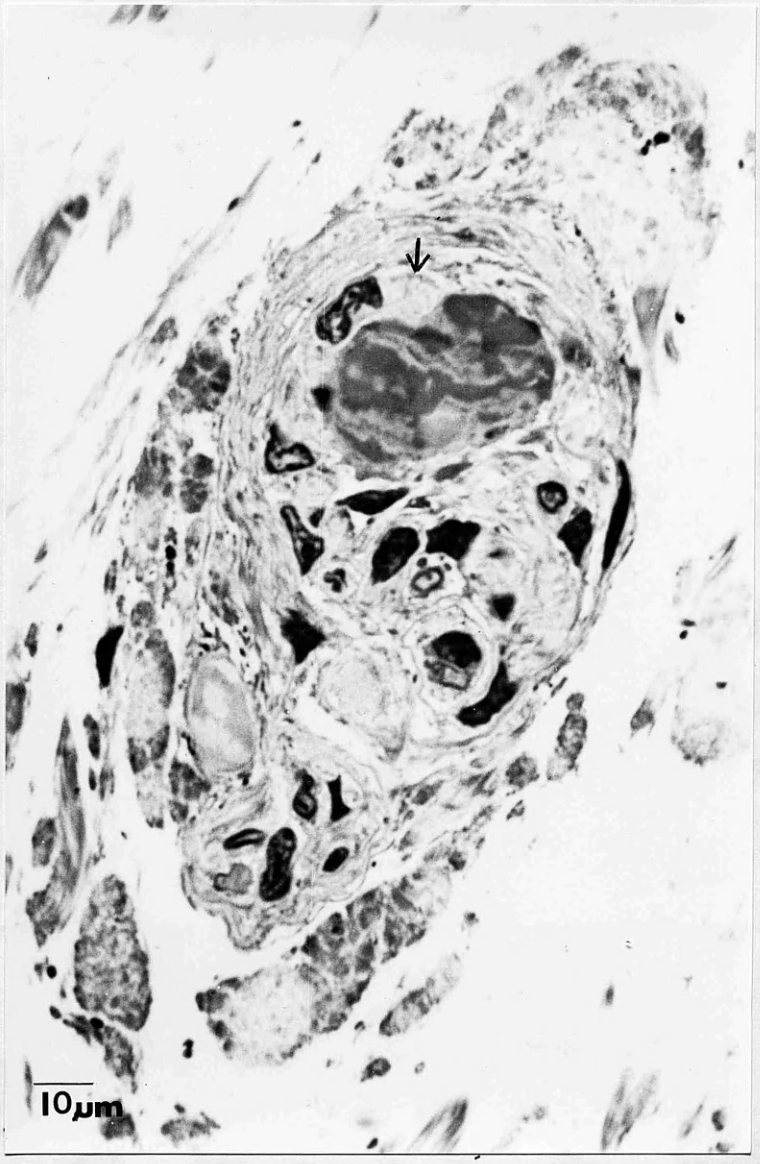


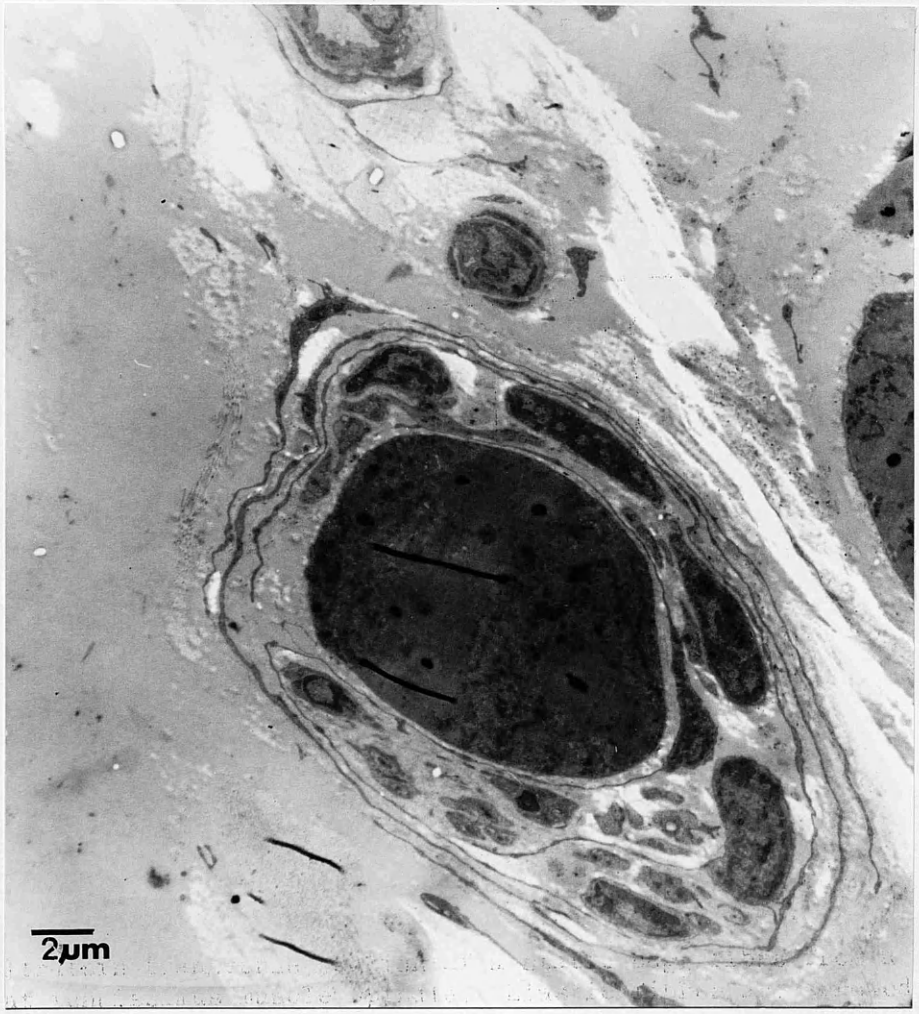
Figure 28 Schematic representation of the distribution of five motor axons to spindle B with the location of the 22 motor endings sectioned. The position of two non myelinated axons are also depicted (—).

Plate 11



High power photograph of a 1 um transverse section of the long chain fibre B in spindle B sectioned 3850 um to the left of the equator. Note the abundance of axon material around the fibre and the probable motor ending (arrowed).

Plate 12



High power electron micrograph of the long chain fibre B in spindle B sectioned 1851 um to the right of the equator. Note the axon material around the fibre but not in synaptic contact with it.

this point and shows clearly the axon material surrounding the fibre.

7.4 MOTOR ENDINGS

The motor endings in spindles A and B were examined ultrastructurally.

Each motor ending was studied through at least one level although some were cut more than once. As the cutting interval was 20 um through the motor region those which were only cut through once were concluded to be less than 40 um long.

Each motor ending was analysed for the following features:

i) protrusion of the muscle fibre underneath the ending.

ii) the degree of indentation of the ending into the muscle fibre. This was done by drawing a line across the ending joining the muscle surfaces at each side and then measuring the depth of the ending below the line at its midpoint, the total height of the ending and then calculating the ratio of depth to total height.

iii) the amount of folding of the post-synaptic membrane. The lengths of the pre and post synaptic membranes were measured and the ratio of pre: post calculated. A value of 1.0 indicated no folding, while a value less than one indicated the presence of folding.

iv) the presence of sole plate nuclei.

v) any expansion of the underlying muscle fibre.

The motor endings could be divided into four types. These have been labelled I, II, III and IV to enhance description.

Type I : These terminals tended to lie superficially on the muscle fibre and did not modify the contour of the muscle fibre. Each ending was less than 20% indented into the muscle. The sole plate was minimal with no nuclei.
Pre: post synaptic membrane ratios were variable but were usually greater than 0.5 indicating that folding was not extensive.

Type II : These terminals lay partially indented into the muscle, usually more than 30%. The muscle fibre contour was affected by the presence of the ending. The sole plate was deep with numerous mitochondria and nuclei. Post junctional folding was absent.

Type III: These terminals were more than 20% indented into the muscle. They were associated with marked muscle protrusion of the underlying fibre and had deep sole plate with nuclei.
Pre: post synatpic membrane ratios were

usually less than 0.5 indicating that folding was extensive.

Type IV : These terminals could not be classified as I, II or III as they possessed mixed features.

7.4.1 Spindle A

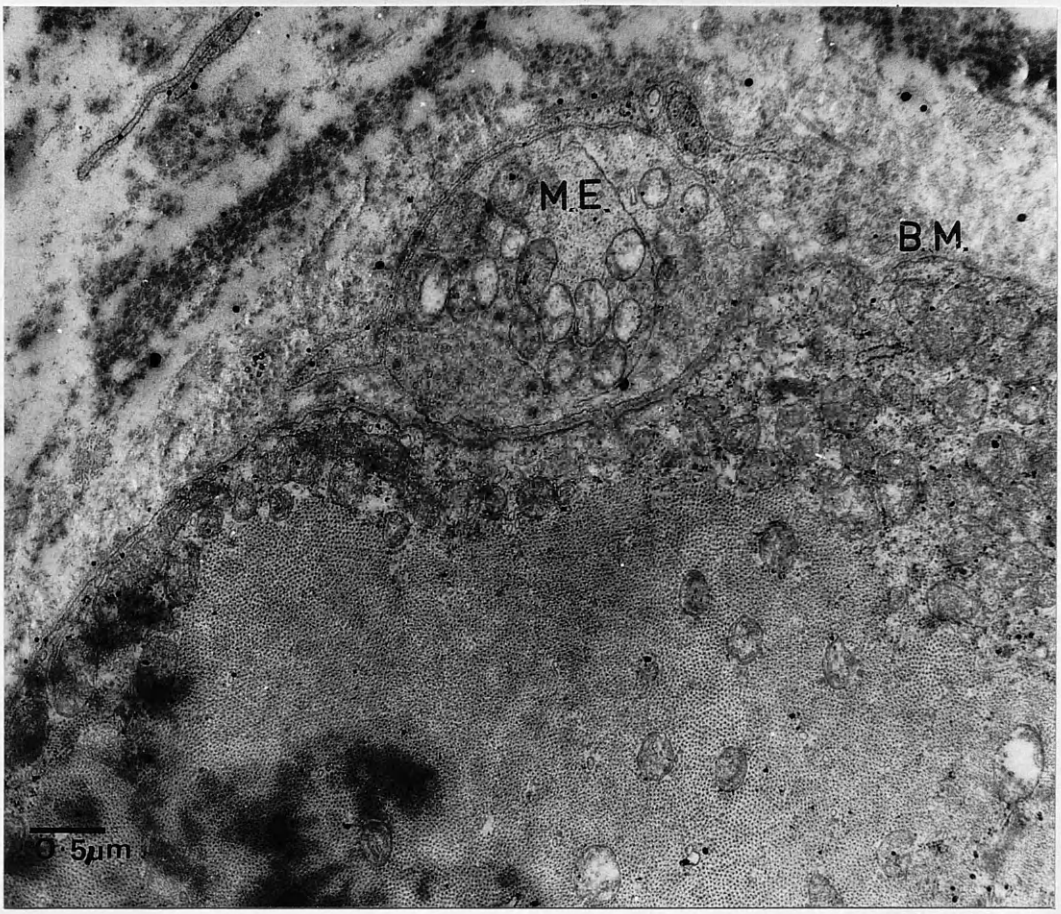
Four motor endings were observed but only one level of each ending was analysed. The endings were found on the two nuclear bag fibres and one nuclear chain fibre (Fig 23).

The motor ending on nuclear bag A was located 905 um from the equator towards the right pole and was within the sleeve region. Two terminals were located at this level. The post synaptic membrane beneath each terminal was smooth (plate 13) and the two were indented 30% into the muscle. The muscle contour itself was visibly altered by the presence of the ending. The underlying sole plate was deep containing numerous mitochondria and nuclei. The underlying muscle fibre was not expanded. This ending was labelled type II.

Two motor endings were found on nuclear bag B, at both the right and left poles within the sleeve regions as shown in Fig 24. The post synaptic membrane beneath both endings was smooth and they did not indent the muscle fibre or visibly alter its contour. The sole plate was minimal in amount and the underlying muscle was not expanded (plate 14). These endings were labelled type I.

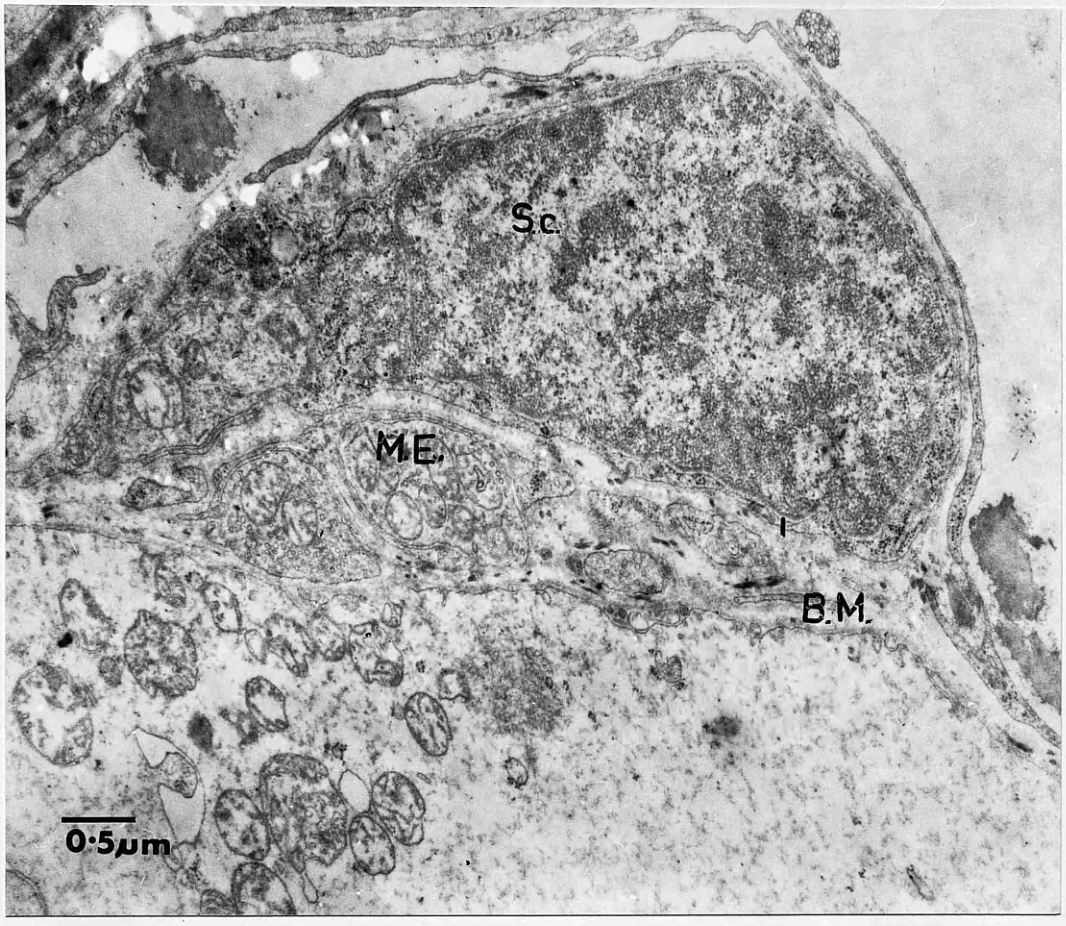
The motor ending on the nuclear chain fibres was located 605 um from the equator towards the right pole at the beginning of

Plate 13



High power electron micrograph of a motor ending on nuclear bag A, spindle A, sectioned 905 um towards the right of the equator. M.E.,: motor ending, B.M.,: basement membrane.

Plate 14



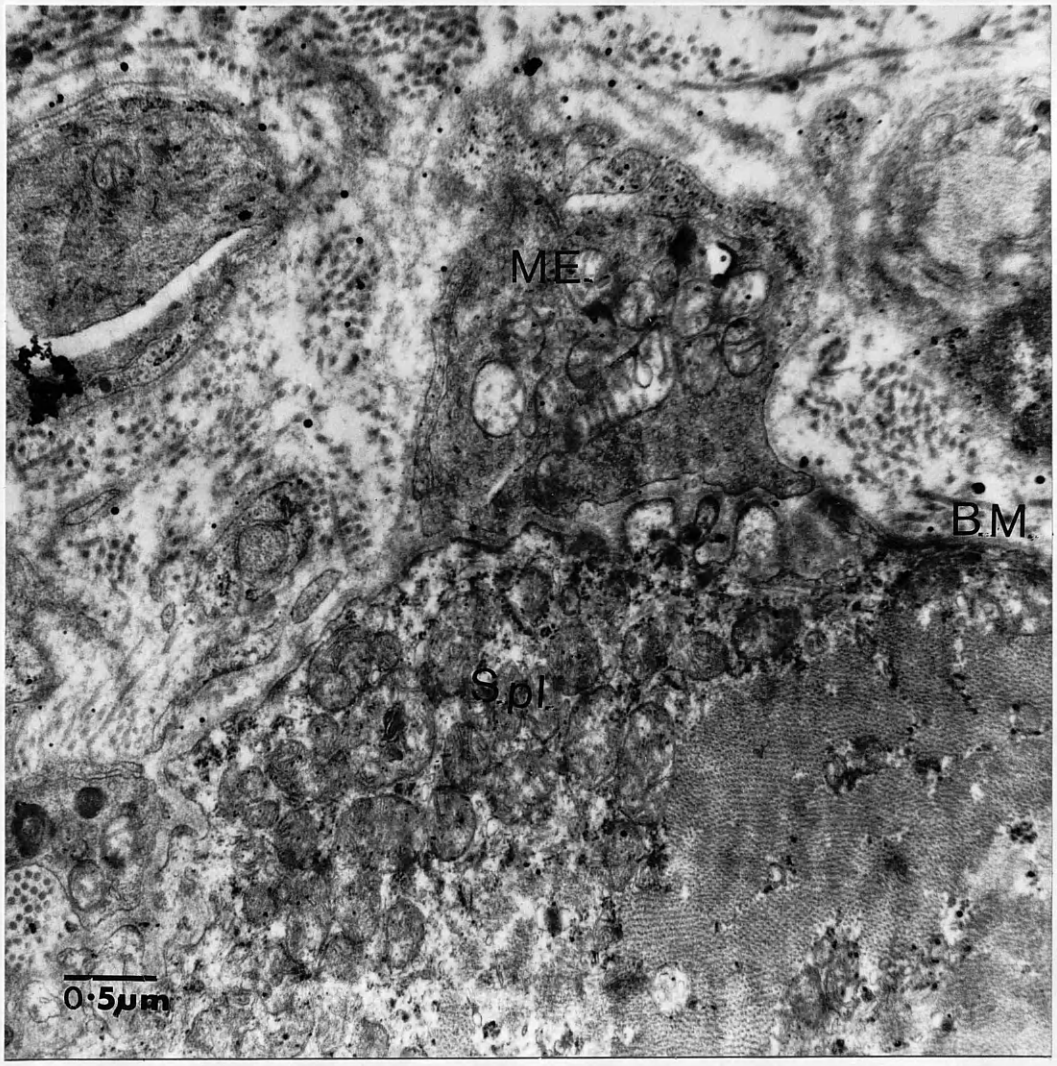
High power electron micrograph of a motor ending on nuclear bag B, spindle A, sectioned 1186 um towards the left of the equator. M.E.,: motor ending, B.M.,: basement membrane, S.c.,: Schwann cell.

the sleeve region (Fig 23).

Two terminals were observed under which there was obvious subjunctional folding. The terminals did not indent the fibre but its contour was affected by the presence of the ending. The underlying sole plate was deep with numerous mitochondria but no nuclei (Plate 15). This ending was labelled type IV.

Table 9 summarises the findings of the motor endings examined in spindle A.

Plate 15



High power electron micrograph of a motor terminal of the ending found on a nuclear chain fibre in spindle A. The ending was sectioned 605 um towards the right of the equator. M.E.,: motor ending, S.pl.,: sole plate, B.M.,: basement membrane.

TABLE 9

FIBRE	DISTANCE FROM EQUATOR	PRE:POST SYNAPTIC MEMBRANE	INDENTATION: DEPTH OF MOTOR ENDING	MUSCLE FIBRE PROTRUSION	SOLE PLATE
A	905 um (R)	1 1 0.625	0.05 0.33 0.375	+++	Deep +mitochondria +nucleus
B	1186 um (L)	1	0.1	-	Little
C	605 um (R)	0.45 0.7	0.025 0.15	+	Deep + mitochondria no nucleus

Table showing the features of the motor endings located in Spindle A on nuclear bag fibres A and B and nuclear chain fibre C.

7.4.2 Spindle B

A total of 22 motor endings were examined (Fig 24,28).

Nuclear bag fibre A had four endings all of which lay superficially being less than 20% indented into the muscle fibre. There was little subjunctional folding and minimal sole plate. These endings thus came into the type I division (Plate 16 is an electron micrograph of one of the endings).

The long chain fibre B also had four endings which were more than 35% indented, had marked subjunctional folding and had deep sole plates. They were also associated with marked muscle protrusion. These endings came into the type III division. (Plate 17 is an electron micrograph of one of these endings).

The other chain fibres, apart from chain fibre 5 which was not innervated, had motor endings which varied in complexity. They varied from those which lay superficially and had wide, shallow subjunctional folds to those which had long finger-like projections of muscle extending round the terminals (Plate 18 shows three motor endings on three different chain fibres highlighting these features).

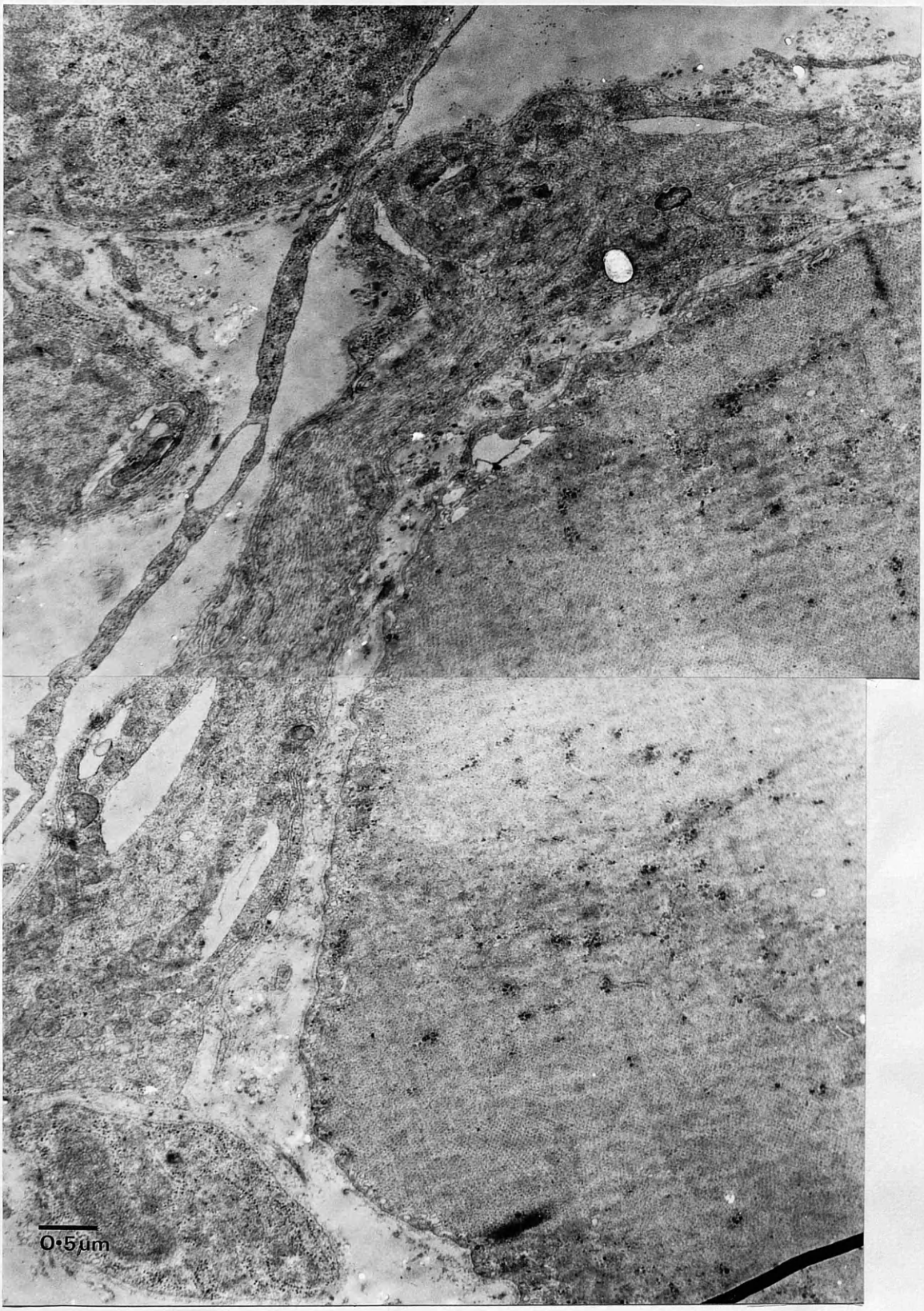
Chain fibres 1 and 6 were the only fibres which possessed both type I and type III endings.

7.4.3 Lengths of Intrafusal Motor Plates

The lengths of the four types of ending are shown in Fig 29.

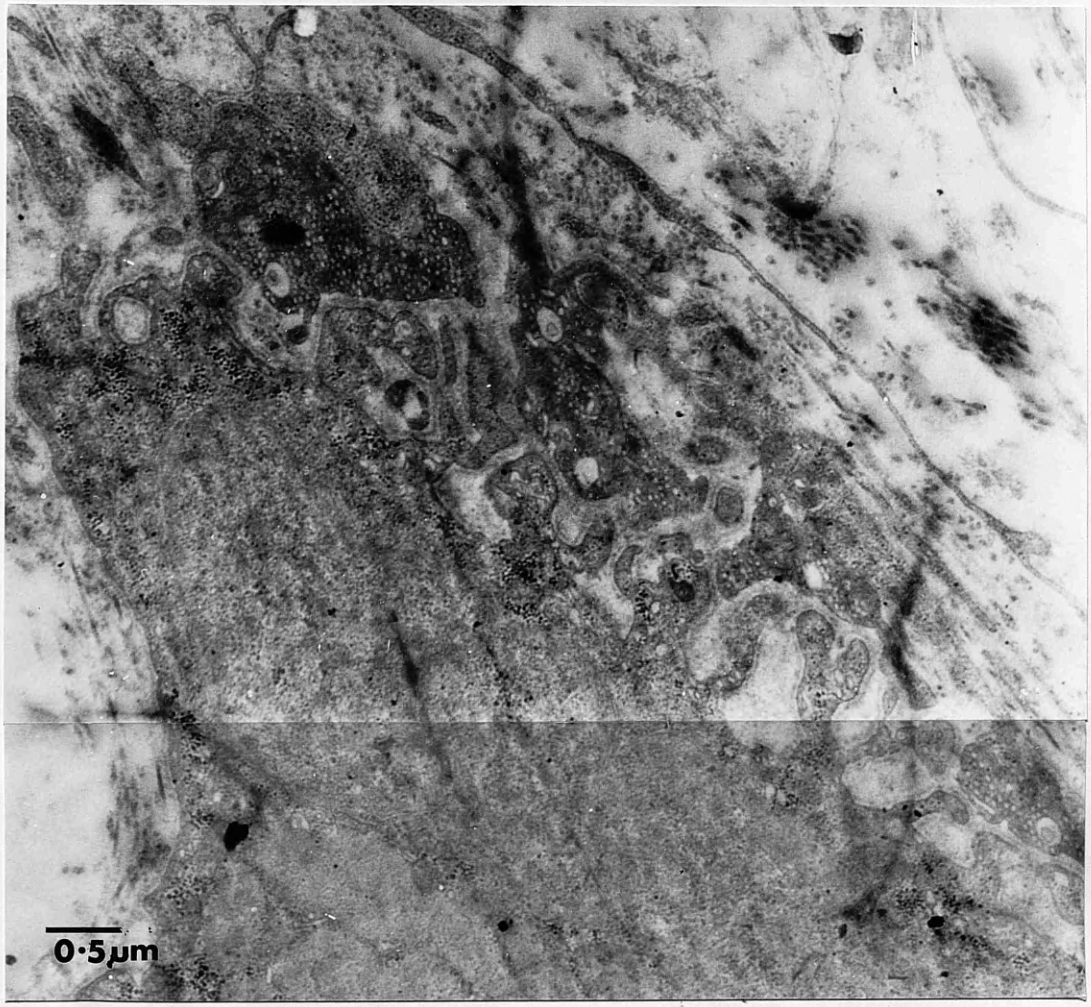
All the plates are less than 140 um in length with those on nuclear chain fibres being smaller than those on bag fibres. The mean length of end-plates on chain fibres was 53.3 um while those on bag fibres was 76 um.

Plate 16



High power electron micrograph of motor ending on nuclear bag fibre B, spindle A, sectioned 2800 um towards the left pole.

Plate 17



High power electron micrograph of motor ending on long nuclear chain fibre B, spindle B, sectioned 2581 um towards the left pole.

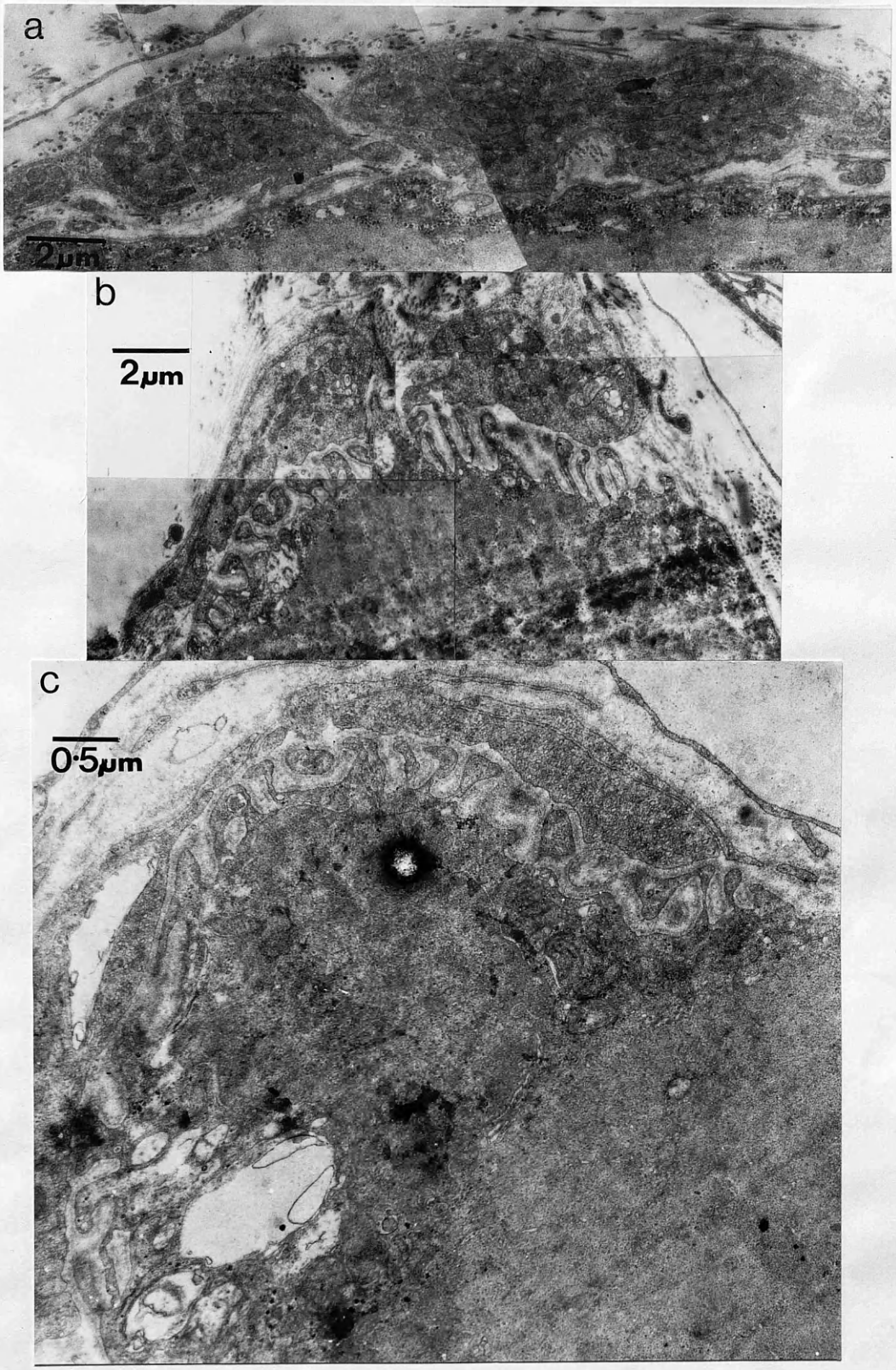


Plate 18

High power electron micrographs of motor endings found on nuclear chain fibres in spindle B showing the variation in complexity from those which lay superficially with wide shallow subjunctional folds (a) through those which lay superficially but had numerous subjunctional folds (b) to those which had long finger-like processes extending round the terminals (C).

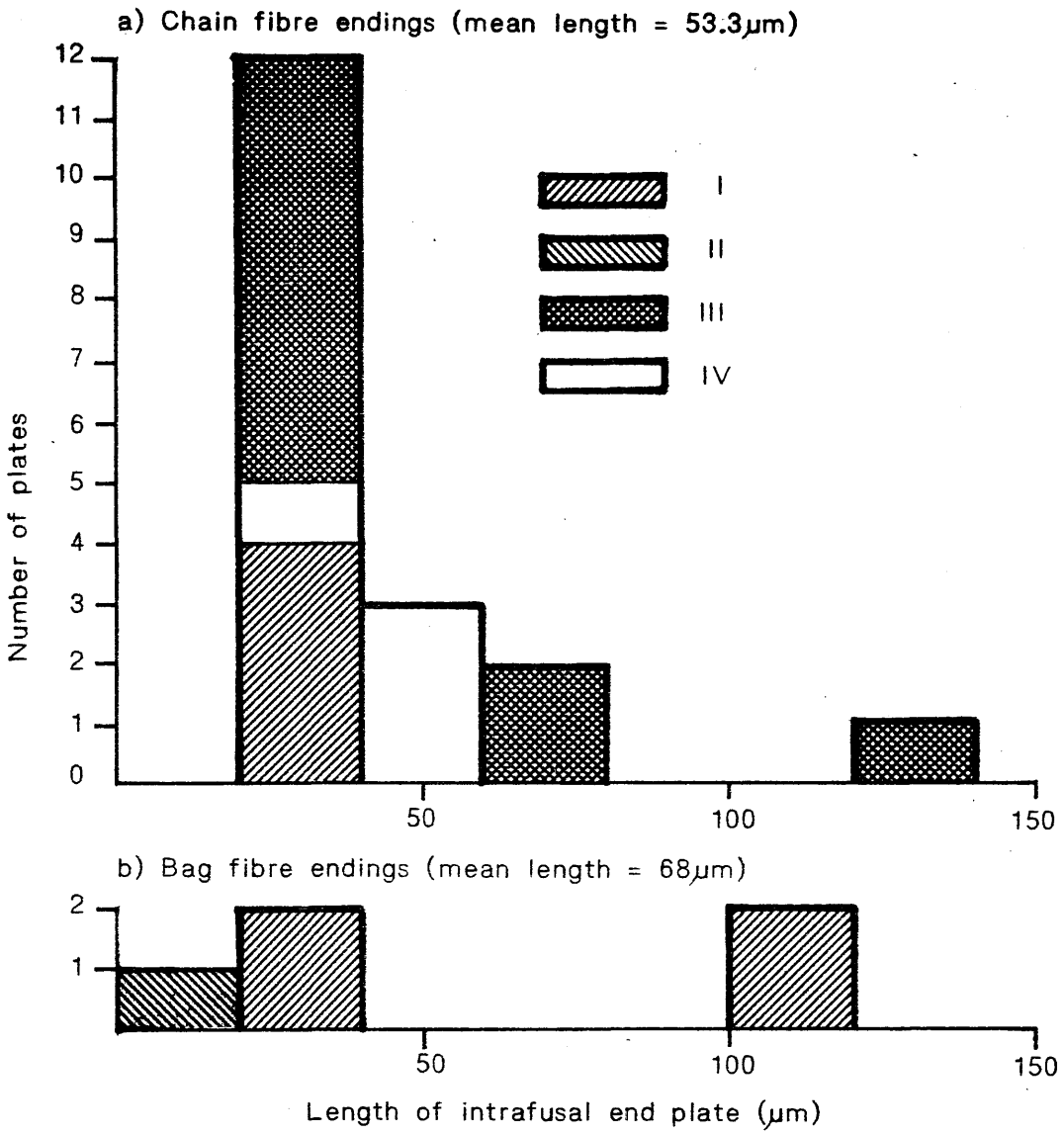


Figure 29 Showing the lengths of the intrafusal fibre endings of the types I-IV described in the text.

7.4.4 Preterminal Unmyelinated Axons

The length of the pre-terminal unmyelinated axon supplying each ending varied from less than 20 um to 100 um. All the type I endings had short lengths of axon supplying them, less than 40 um.

The type IV endings were innervated by unmyelinated axons less than 50 um while the type III endings had longer lengths of unmyelinated axon (Table 10).

7.4.5 Intrafusal Fibre Thickening in Motor Region

The muscle fibre underlying each ending was examined to see if there was any fusiform swelling.

Bag fibre A did not expand at all in the region of the motor endings.

The long chain fibre B did not expand appreciably although each ending was associated with protrusion of the muscle fibre.

The chain fibres all expanded beneath the motor endings except for chain fibre 1 which was expanded under its type III terminal but not under either of its type I terminals.

TABLE 10

Distance from Equator of Ending on each Fibre (um)	Length of Unmyelinated Axon (um)	Pre:Post Synaptic Membrane Length	Indentation	Muscle Fibre Protrusion	Sole Plate
I					
A (821-921)	20	0.505	0.2	--	+
(-2481)	20	0.64	0.14	--	++
(-2601)	20	0.35	0	--	+
(-2721-2821)	20	0.68	0.1	--	+
1 (601)	20	0.83	0.2	--	+
(-1021)	20	0.31	0	--	+
2 (-1001)	20	0.45	0	--	+
6 (-2641)	40	0.66	0.06	--	+
IV					
4 (581-621)	40	0.6	0.48	--	++
(-2401)	50	0.49	0.5	+++	+
7 (-1141-1261)	20	0.46	0.34	+	+
8 (-2541-2561)	20	0.46	0.17	+	+
III					
B (-201)	20	0.38	0.97	+++	+++
(-751-871)	20	0.52	0.51	+++	+++
(-1001-1061)	20	0.41	0.35	+++	+++
(-2581-2641)	40	0.44	0.42	+++	+++
1 (761)	40	0.41	0.22	+++	+++
3 (1001)	20	0.44	0.52	+++	+++
6 (681)	100	0.37	0	+++	+++
(-701)	50	0.37	0.48	+++	+++
9 (-1041)	20	0.36	0.31	+++	+++
(-2421)	50	0.28	0.41	+++	+++

Table summarising the features of the motor endings located in spindle B.

CHAPTER 8

DISCUSSION

Certain features of baboon spindles have been examined in some detail. These are:

i) the general morphology

ii) the M-line distribution in the intrafusal fibre

iii) the elastic fibre distribution

and

iv) the fusimotor innervation

These findings will now be discussed in turn and compared with those in human and cat spindles.

8.1 GENERAL MORPHOLOGY OF BABOON MUSCLE SPINDLES

The number of spindles in different lumbrical muscles was variable, but the arrangement within the muscles was similar to that in the cat (Boyd 1962). A typical baboon muscle spindle is reconstructed in Fig 20 and when compared with Fig 3 which is a typical cat spindle the two are very similar. Two types of intrafusal fibre, distinguishable by their nuclear arrangement and their diameter, were found in every spindle in agreement with what is found in both cat (Boyd 1962) and human (Cooper and Daniel 1963) spindles.

8.1.1 Number of Intrafusal Fibres Per Spindle

Baboon spindles were found to contain 4-15 intrafusal fibres per spindle similar to the range (2-14) found in human spindles (Cooper and Daniel 1963) but more than the range (6-8) found by Boyd 1962, in cat spindles.

The number of nuclear bag fibres in baboon spindles was 1-5

(mean 2.29) and the number of nuclear chain fibres was 0-10 (mean 4.9). This was again similar to the ranges found by Cooper and Daniel (1963) in human spindles but slightly more than that found by both Kucera and Dorovini-zis (1979) in human spindles and by Boyd (1962) in cat spindles.

In general therefore baboon spindles are more like human spindles in having larger complements of intrafusal fibres within them than cat spindles.

8.1.2 Intrafusal Fibre Length

Nuclear bag fibres in baboon spindles ranged in length from 3600 um to 9500 um (mean 5935 um). Boyd (1962) did not find nuclear bag fibres in cat spindles which were less than 4000 um and the mean length was never less than 6830 um.

Nuclear chain fibres in baboon spindles ranged in length from 1000 um to 7400 um (mean 3400 um). In cat spindles, nuclear chain fibres never exceeded 6000 um, (Boyd 1962).

In baboon spindles therefore the difference in lengths of nuclear bag fibres and nuclear chain fibres is not as great as in the cat.

8.1.3 Long Chain Fibres

The incidence of long chain fibres in single baboon lumbrical spindles was 30%. The incidence per spindle pole was 22%. In the tenuissimus muscle of the cat Barker (1976b) found an incidence of 29.4% while Kucera (1980) found an incidence of 11.66%. Kucera also found that 1.2% of tenuissmus spindles had a long chain fibre at both poles. In this study the incidence was 4%. Thus it would appear that, as in cat, the long chain fibre is an important subtype of baboon nuclear chain fibre.

Spindle B in this study had one long chain fibre which was a long chain at both poles. This was labelled fibre B (Fig 25). This spindle also had another two long chain fibres, fibres 4 + 6, which were long chains at one pole only. Fibre B was much greater in diameter than the other chain fibres throughout its length. It was also larger than the nuclear bag fibre especially extracapsularly. This was also the case for chain fibre 6. This feature has been noted by Kucera (1982) with respect to long chain fibres in the cat.

8.1.4 Intrafusal Fibre Diameter

As in cat spindles, the nuclear bag fibres in baboon spindles tended to be larger than the nuclear chain fibres, however this was not a reliable criterion by which to identify them especially as nuclear chain fibres tended to be larger at the poles than at the equator.

8.1.5 Tandem Spindles and Single Bag Spindles

The incidence of tandem spindles in baboon lumbrical muscles was 13.3%. This compares with 11% in cat soleus muscle, 24% in cat medial gastroanemius. (Swett and Eldred 1960a) and 16-20% in cat rectus femoris (Barker and IP 1961).

In all the tandem spindles examined one fluid space contained far more fibres than the other, usually an extra bag fibre plus some chain fibres. This is also the case in cat spindles (Kucera 1982b).

Spindle B however appeared to have only one bag fibre and a long chain fibre linking both fluid spaces. Kucera (1982b) studied nineteen complete tandem spindles in the cat tenuissimus muscle and found that each tandem had two bag fibres in at least

one fluid space. He found one spindle which contained a long chain fibre passing through both equators along with a bag2 fibre. He also found that every two bag capsule contained a long chain fibre while the single bag capsules never contained a long chain fibre. Single bag spindles were found in 13% of baboon lumbrical muscles.

Bakker and Richmond (1981) found single bag spindles in one third of spindles studied in cat neck muscles, 50% of these could be traced into tandem linkages. In this study one spindle out of four was traced into a tandem linkage ie 25%. Bakker and Richmond (1981) noted that the single bag spindles always contained a static bag2 fibre. Kucera (1982b) found only 1.3% of cat tenuissimus muscle spindles were single bag fibre spindles and the single bag could be either a static bag2 fibre or a dynamic bag1 fibre. In this study the single bag fibre in spindle B was presumed to be a static bag fibre on the basis of its M-line distribution, elastic fibre distribution and motor innervation.

The functional significance of a single bag fibre spindle is unclear. If may be modified because the bag1 fibre is usually absent, presumably lowering the dynamic sensitivity. The high incidence of single bag fibre spindles in tandem linkages must be physiologically significant. It the spindle response pattern is predominantly influenced by events in its immediate muscle environment, a single spindle in a tandem complex might be expected to signal information that is repeated in the spindle to which it is linked. This may provide the CNS with comparative information regarding the same muscle event.

Different complements of chain fibres in both spindles

linked in a tandem would also be expected to signal comparative information to the CNS about the same muscle event.

It would appear therefore that tandem spindles and single bag spindles are physiologically important and from this small study it looks as though baboon spindles are in general similar to those found in the cat.

8.1.6 Capsules of Muscle Spindles

The capsule length varied from 850 um to 4100 um (mean 1977 um). This was shorter than that found by Cooper and Daniel (1963) in human spindles where it ranged from 1000 um to 5000 um (mean 3500 um).

8.2 M-LINE DISTRIBUTION

In the three spindles studied ultrastructurally two contained two nuclear bag fibres and one contained only one nuclear bag fibre. The striking feature which emerged was that one bag fibre per spindle had obvious M-lines in transverse section within 361 um of the equator, two having M-lines within 125 um.

In the two spindles which had two bag fibres per spindle the other bag fibre in one, had no M-lines at all as far as the spindle was sectioned (Spindle C), while the second bag in Spindle A developed M-lines at a distance of 2141 um from the equator.

All the nuclear chain fibres had M-lines demonstrated at some point along their length except for and chain fibre 8 spindle B. For the purpose of this study it was the larger fibres which were of more interest. Since the findings are so similar to those found by other authors, the bag fibre which had

obvious M-lines near the equator can be labelled the static bag2 fibre. Barker et al (1976b) & Banks et al (1977) found the static bag2 fibre to have M-lines in the sleeve region in cats, rabbits and rats. Kucera and Dorovini-zis (1979) in their work on human muscle found that the static bag2 fibre had M-lines at the end of the sleeve region.

The dynamic bag1 fibre in all these studies aquired M-lines further out than the static bag2 fibre. In cats and rabbits, M-lines became distinct extracapsularly, while in rats the M-line was never distinct. In humans the M-line became distinct extracapsularly.

In this study one bag fibre in one spindle acquired M-lines 500 um extracapsularly. The other spindle was incompletely sectioned. Presumably therefore in baboon spindles the dynamic bag1 fibre possessed M-lines extracapsularly.

The size of the M-line which occurs in the middle of the H zone in the sarcomere is only 70 nm as compared to the size of the sarcomere which is 2.2 um. The likelihood of cutting through the M-line in transvere section is quite small but assuming that the muscle is not aligned perfectly horizontal an M-line in at least one sarcomere could be expected to be sectioned. This means that it may be only a very small patch of M-line that is visible in transverse section with the electron microscope.

When present the M-line is easily seen as a hexagonal arrangement of myosin filaments connected by cross bridges.

The absence of the M-line is less easily determined but if it is never seen in cross section at any level, it can be quite confidently assumed to be absent.

Other factors which can reduce the clarity of the M-line

appearance are the degree of shortening or stretching of the muscle but it should still be visible (Knappeiss & Carlsen 1965).

The variation in M-line structure must have a functional basis.

The precise function of the M-line is still obscure. It does seem to have a mechanical role to play in the maintenance of normal actin-myosin relationships within the separate fibres during muscle contraction. If this is so, well developed M-lines may be a more necessary requirement of muscle fibres undergoing rapid or vigorous twitch contractions, while slower contracting fibres may not need them.

Smith (1966) found that small diameter intrafusal fibres twitched vigorously to direct stimulation whereas large fibres gave a small movement in response to a single stimulus.

Boyd (1966) observed similar contractions to indirect stimulation via fusimotor axons.

Bessou and Pagès (1972) recorded action potentials in chain fibres and junctional potentials in nuclear bag fibres. This is similar to what is found in twitch and slow extrafusal fibres. In the former single shock stimuli to large motor axons results in propagated action potentials and twitch like contractions. These extrafusal muscle fibres are found to possess well developed M-lines, numerous mitochondria and a well developed sarcoplasmic reticulum. Slow extrafusal fibres on the other hand respond with local non propagated action potentials and slow contractions to repetitive stimulation of small motor nerves. These fibres do not possess M-lines, have less mitochondria and a poorly developed sarcoplasmic reticulum.

It is interesting to compare the electrophysiological findings of spindle B with the ultrastructural. At the right pole a fast fatigue resistant movement was observed which was thought to be due to contraction at the static bag2 fibre and two chain fibres. These were all shown to have M-lines at this pole. The movement which occurred between the fluid spaces was quick but tired easily. This was due to contraction of fibres B, 1, 2, 6, 7 and 9. By far the largest ending was found on fibre B which was observed to have only faint M-lines at the right pole. This might account for the greater fatiguability of this movement.

Towards the left pole two movements were observed one tiring less easily than the movement between the fluid spaces. This was thought to be due to movement of fibres 4 and 9 both of which had M-lines, fibre 9 having M-lines in the region of the motor endings. The other movement at this pole which did tire more easily was thought to be due to fibres A, B, 6 and 8. Fibres B and 8 did not have M-lines at all while fibre 6 had M-lines visible at the right pole only.

A quick movement which tired easily was also seen further out towards the left pole and was thought again to be due to contraction of the long chain fibre which was not observed to have M-lines at this pole.

Thus the presence of M-lines may enable a fibre to respond more quickly to nerve stimulation with a movement more resistant to fatigue.

In converse it has been shown that the opposite effects can occur when the fibres are immersed in succinyl choline.

Fehr (1965) found that intrafusal fibres underwent

contraction after intravenous injections of succinyl choline, nuclear bag fibres contracting and relaxing more rapidly than nuclear chain fibres. Smith (1966) also noted similar findings when he immersed intrafusal fibres in succinyl choline.

Gladden (1976) noticed that the dynamic bag1 fibre responded more quickly than the static bag2 fibre to acetyl choline in the perfusate around isolated spindles.

Thus it is possible that in fibres with less rigid and poorly defined M-lines the actin filaments may be more inclined to pass each other in the H-zone when exposed to acetyl choline.

In summary therefore it appears that baboon spindles are similar to other mammalian spindles in terms of M-line distribution. The difference between static and dynamic bag fibre distribution is more obvious than in the human and is a useful distinguishing marker. More work needs to be done on a quantitative basis to support this conclusively.

8.3 ELASTIC FIBRE DISTRIBUTION

As in other mammals, elastic fibres in baboon muscle were found to be more prevalent in association with intrafusal fibres than with extrafusal fibres.

Nuclear bag fibres were observed to have much more elastic associated with them than nuclear chain fibres. This was noted in cat spindles in 1972 by Gladden.

Also more elastic tissue was found at the end of the capsule sleeve region than elsewhere as noted by Cooper and Daniel (1967) in human and rat spindles.

Assuming that M-line indentification of the dynamic bag1 fibre and the static bag2 fibre was correct the following points can be made:

1. In baboon spindles the static bag2 fibre can have elastic at both poles (Spindle A) or at one pole only (Spindle B). This is unlike what is found in cat spindles in which the static bag2 fibre has elastic at both poles.

2. The dynamic bag1 fibre can have elastic at one pole only (Spindle A). Elastic is not associated with motor endings particularly since the pole of the dynamic bag1 fibre which had the motor ending had very little elastic. It is possible that the pole which had more elastic had no innervation and that the presence of elastic may be due to the greater stress imposed on this pole from the pull by the contracting pole

3. In baboon spindles the static bag2 fibre cannot be distinguished from the dynamic bag1 fibre on the basis of elastic fibre distribution as in the cat.

4. Elastic fibre distribution in baboon spindles is also not like that in human spindles. In at least some of these one nuclear bag fibre had a cuff of elastic close to

the equator. (We do not know if this is found in all human spindles as a systematic study of a large number of spindles has not been done, but we know it is found in both young and aged individuals). Despite the large number of baboon spindles examined this cuff was not present.

Elastic fibres must play some functional role in spindle mechanics.

At the equator, elastic fibres were found within the inner capsule as in human spindles but did not appear to be associated with any particular fibre. They may therefore strengthen the equatorial region so that it can withstand contraction of the intrafusal fibres on both sides.

It is possible that as the fibres are stretched either passively or due to contraction outwith the equator, the spirals of the primary sensory endings are compressed by the elastic fibres, the pressure being related to the strength of contraction and the tension within the muscle (Gladden 1974).

At the end of the capsular sleeve region it seems likely that the elastic fibres perform some form of mechanical anchorage for the nuclear chain fibres and nuclear bag fibres to the capsule.

The distribution of elastic fibres along the intrafusal fibres is not continuous in the baboon.

It would appear that extracapsularly elastic fibes perform more of a structural role. The more vigorous the contraction ability of the fibre the more elastic that is required to anchor it. Thus the nuclear chain fibres are anchored at each end to the

capsule whereas the nuclear bag fibres and long chain fibres end within the extrafusal muscle fibres. The static muclear bag contracts more vigorously than the dynamic nuclear bag so it requires more elastic to support it.

In summary therefore, although differences in elastic fibre distribution to intrafusal fibres in baboon spindles were found, these were not significantly helpful in distinguishing the static nuclear bag fibre from the dynamic nuclear bag fibre as in the cat. The pattern was more like that found in human spindles than cat spindles.

8.4 INNERVATION OF BABOON MUSCLE SPINDLES

8.4.1 Sensory Innervation

Primary axons were found in every spindle studied ultrastructurally, the tandem spindle having one primary axon per fluid space. Both bag fibres and chain fibres were supplied by the primary axon as is the case in cat spindles (Boyd 1962) and human spindles (Cooper and Daniel 1963, Kennedy 1970).

The spiral form of the primary axon seen in cats was perhaps not so striking in spindle B of the baboon (Fig 27). This was a feature of human spindles noted by Cooper and Daniel 1963, Kennedy 1970 and Swash and Fox, 1972.

8.4.2 Position of Motor Plates Relative to Intrafusal Fibre Behaviour in Spindle B

900 um towards the right pole (Fig 24, Fig 27) a visible contraction site was seen in spindle B when studied electrophysiologically. The movement was fast and did not tire easily. Further out another slight movement inwards was seen.

Ultrastructural studies revealed a motor ending on the nuclear bag fibre between 821 um and 921 um. The axon innervating this fibre also innervated chain fibres 4 and 6 at 580 um and 680 um respectively. Presumably stimulation at this axon resulted in contraction of all three fibres with the movement of the bag fibre being the one most visible.

Further out at 1001 um another ending was found on chain fibre 3 which was innervated by a second axon. This axon also supplied an ending on chain fibre 1 at 761 um. The slight movement observed was probably due to stimulation of this axon.

In between the fluid spaces a quick movement was observed which tired easily. Motor endings were found on fibres B, 1, 2, 6, 7 and 9 between the fluid spaces and all were supplied by the same axon. The movement was therefore due to stimulation of this axon.

At 2800 um towards the left pole, chain fibres were seen to contract, which did not tire as easily as those between the fluid spaces. At 3300 um a fast movement which tired easily was observed. This latter movement included contraction of a large fibre, thought to be a nuclear bag fibre.

Ultrastructurally several motor endings were found between 2400 and 2800 um. Two axons supplied these endings. One innervated chain fibres 4 and 9 at 2401 um and 2421 um respectively, the other innervated nuclear bag fibre A at 2487-2821 um, long chain fibre B at 2581-2641 um and chain fibres 6 and 8 at 2641 um and 2541 um respectively.

It is possible that the fast movement which did not tire easily was due to chain fibres 4 and 9 contractions and that the other movement was due to fibres A, B, 6 and 8 contracting. The

axon supplying the latter fibres also innervated the endings between the fluid spaces.

The discrepancy between the distances measured in the living spindle and the fixed spindle are greater the further from zero the spindle was cut. This is probably due to a combination of fixation shrinkage and observer error. However it has been observed by Arbuthnott et al (1982) that chain type behaviour in cat spindles was of two types, a more pronounced twitching associated with their Mc plates at the point of contraction, and a weaker contraction associated with Ma plates which could be a distance of 300 um from the contraction site. It is interesting to note that more endings which were similar to Arbuthnott et als type Ma plates occurred at the left pole of this spindle so this could also explain the discrepancy in distances measured.

At 3800 um towards the left pole a large fibre was observed to move inwards. It was again a quick movement which tired easily and at 3740 um a motor ending on fibre B was thought to have been missed on sectioning (Plate 11). This ending was probably innervated by an extracapsular axon.

8.4.3 Motor Endings on Baboon Intrafusal Fibres

Four types of motor ending on baboon intrafusal fibres have been described. These were labelled types I, II, III and IV. A total of 26 endings were examined.

The criteria used in defining the types I, II and III were the same as those used by Arbuthnott et al in defining their Ma, Mb and Mc endings respectively. The type IV ending in this study was defined as an ending with features which could not be classified into one of the other three groups.

The static bag2 fibre of both spindles A and B were innervated by type I endings which corresponds to the Ma endings of Arbuthnott et al. This finding supports their conclusion that static bag2 fibres in cat spindles are innervated by Ma type endings. However folding, although less extensive than in type II or type III endings, was more often present than in the Ma endings of Arbuthnott et al.

The dynamic bag1 fibre of spindle A was innervated by type II endings which corresponds to the Mb endings of Arbuthnott et al. This is again in agreement with what they found in cat spindles.

The long chain fibre B, of spindle B, was innervated by type III endings which corresponds to the Mc endings of Arbuthnott et al and again supports their finding that Mc endings are found only on nuclear chain fibres. However Arbuthnott et al describe a fourth type of ending called Md which resembles an extrafusal fibre ending and which is usually found on long chain fibres extracapsularly. It is possible that the ending missed on sectioning the long chain fibre extracapsularly was an Md ending but if so this would oppose what Arbuthnott et al found, which was that only one type of ending innervates a particular fibre pole.

In this study however chain fibre 1 of spindle B had two endings labelled type I and type III or Ma and Mc if the classification of Arbuthnott et al is used. This was a finding never reported by the latter authors. It is possible that the type I ending was actually a type III ending cut at its outer edge. Each of these endings was cut through only once.

The eight chain fibres of spindle B and the one chain fibre

of spindle A were innervated by type I, III or IV endings. It is also possible that the type IV endings were part of a type III ending which had not been sectioned through its more complex part.

A type III ending could not of course be mistaken for any other ending.

Thus it is not possible to be certain from this study whether there are three types of motor ending on chain fibres or two types (type I and type III) or whether in fact there is a gradation in complexity of one type of ending. Further work needs to be done to clarify this, in particular each ending needs to be sectioned through more than one level to be certain of its type.

Axons which are non-selective in distribution to both nuclear bag fibres and nuclear chain fibres (Fig 28, Table 10) can terminate in both type I and type III endings. This was found by Arbuthnott et al in the cat, in which non-selective axons can end in both Ma and Mc plates. There is no doubt that the endings on the nuclear bag fibre of spindle D are type I or Ma endings as they were cut through more than one level.

It is not possible from this study to say whether selective axons terminate in only one type of ending for the reasons already quoted.

8.4.4 Lengths of Intrafusal Motor Plates

The lengths of the nuclear chain fibre endings was smaller than the nuclear bag fibre endings which is in agreement with Boyd (1962) who found the $\gamma 2$ network which primarily supplied chain fibres to have smaller end plates than the endings on nuclear bag fibres. Arbuthnott et al (1984) found that the mean

length of static nuclear bag endings from five tenuissimus spindles to be 52 um, the dynamic nuclear bag fibre endings to be 54 um and the nuclear chain fibre endings to be 41 um. For baboon spindles the values were 70 um for bag fibres and 53.3 um for chain fibres. Thus the ending lengths are not dissimilar for both species.

8.4.5 Preterminal Unmyelinated Axons

It is interesting to note that the endings on bag fibres had shorter lengths of preterminal unmyelinated axons than those on nuclear chain fibres. This was largely what Swash and Fox (1972) found in human spindles. In cat spindles the length of the preterminal unmyelinated axons was not helpful in distinguishing the motor endings.

8.4.6 Intrafusal Fibre Thickening

Swash and Fox (1972) noted that the muscle fibre underlying p2 plates, which could occur on both nuclear bag fibres and nuclear chain fibres, was swollen. In this study the muscle fibre underlying nuclear chain fibres was swollen except for chain fibre 1 under its type I endings and the long chain fibre. Thus swelling of the underlying muscle would not appear to be related to the motor ending type or intrafusal fibre type.

In conclusion therefore the motor innervation of the baboon spindle when compared to the cat using the classification of Arbuthnott et al (1982) is similar in some respects. These are:-

i) the dynamic bag1 fibre is innervated by IIb type endings.

ii) the static bag2 fibre is innervated by IIa type

endings.

iii) the nuclear chain fibre is innervated by Ma and/or Mc endings. However a fourth type of ending has also been described which may be a variation of of the Mc ending.

iv) long chain fibres are innervated by Mc endings intracapsularly.

v) non-selective axons can end in Ma endings on static bag2 fibres and Mc endings on chain fibres.

vi) the lengths of the motor endings are similar.

vii) the degree of swelling of the underlying intrafusal fibres was not helpful in distinguising different motor endings.

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