A study of the motor supply to mammalian skeletal muscle

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A STUDY OF THE MOTOR SUPPLY TO MAMMALIAN SKELETAL MUSCLE

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by

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I. INTRODUCTION

It has long been known that the motor component in nerves to skeletal muscle shows a bimodal distribution (Eccles & Sherrington, 1930; O'Leary, Heinbecker & Bishop, 1935; Rexed & Therman, 1948; Hagbarth & Wohlfart, 1952). The two modes, designated as alpha (8 - 18 μ, Gasser, 1941) and gamma (2 - 8 μ, Leksell, 1945) groups have been generally accepted to innervate extra- and intrafusal muscle fibres, respectively. Branching of alpha nerve fibres has been shown in a muscle nerve as it approaches and enters a muscle (Eccles & Sherrington, 1930) and around the region of nerve-entry (Adrian, 1925; Cooper, 1929). These fibres are presumed to divide and sub-divide continuously within the muscle to innervate extrafusal muscle fibres, but the nature of branching in the intramuscular region has not been determined beyond von Thiel's (1959) few isolated examples. He traced three efferent nerve fibres of the pyriformis muscle

in the cat, "from the interior of the muscle to the radix anterior"; the study was mainly on fibre diameter and internodal length in consecutive segments of the nerve fibres, although certain kinds of branching were mentioned without quantitative assessments.

The 2 - 8 μ gamma group of Leksell's (1945) has been well established to supply intrafusal muscle fibres. According to Boyd & Davey (1962), a further bimodality occurs within the gamma group in some muscle nerves. The two diameter ranges in the gamma group are said to lie between 1 - 3 μ of thinly myelinated fibres and 4 - 8 μ of thickly myelinated fibres. Boyd & Eccles (1963) later claimed to have demonstrated, by the difference in threshold values and conduction velocities, the existence of two groups of small motor fibres in certain ventral roots and muscle nerves.

The morphology of the mammalian muscle spindle shows the existence of two types of intrafusal muscle fibre having large and small diameters, namely, the nuclear-bag and the nuclear-chain fibres; their proportions vary from animal to animal (Barker, 1948;

Cooper & Daniel, 1956, 1963; Walker, 1958; Swett & Eldred, 1960; Boyd, 1962). An exception has been shown in lagmorph spindles, which have bag fibres only, (Barker & Hunt, 1964), uniquely among mammals so far studied. Boyd (1962) maintains that in the cat, the nuclear-bag fibres which are larger in size, with numerous myofibrils, are innervated by comparatively larger fusimotor fibres (γ_1), ending in discrete motor end-plates; while the nuclear-chain fibres of smaller diameter, with fewer myofibrils, receive much smaller fusimotor fibres (γ_2), ending in some sort of motor 'network'. He postulates that the γ_1 and γ_2 fibres may originate from the two modes in the gamma group and be distributed so as to innervate nuclear-bag and nuclear-chain intrafusal fibres separately (Boyd, 1962). For such a distribution, the small thinly myelinated γ-stem fibres with total diameter of less than 4 μ would have to branch so as to innervate more than twice as many spindles as the large thickly myelinated ones, having total diameters between 4 and 8 μ (Boyd, 1962, calculates the average proportion to be 12 as to 5), since they are from one-half to one-third as numerous in muscle nerves (Boyd & Davey, 1962).

Large fusimotor fibres innervating mammalian muscle spindles have been described by Cilimbaris (1910), Garven, (1925), Barker (1948, 1959, 1962) and Cooper & Daniel (1956). Whether any of these have their origin from alpha fibres has been widely speculated. Branches from alpha fibres innervating both extra- and intrafusal muscle fibres have been observed by Weiss & Dutil (1896), Cilimbaris (1910), Wilkinson (1929), Denny-Brown (1932) and Häggqvist (1960) but have been queried to a certain extent (Barker & Chin, 1961), though it is probably true in amphibia (Gray, 1957) and reptiles (Cipollone, 1898; Perroncito, 1901). From neurophysiological studies, fusimotor fibres responsible for the early-discharging spindles, described in experiments by Granit, Pompeiano & Waltman (1959a, b) and Rutledge & Haase (1961), also suggests such an occurrence, although indirectly. More recently, Bessou, Emonet-Dénand & Laporte (1963a) have succeeded in providing more direct evidence of branches from alpha fibres innervating both extra- and intrafusal muscle fibres. These fibres will henceforth be referred to as β fibres, since the majority of their fibre sizes are smaller than the alpha group, being called slow alphas in accordance with their conduction velocities

by Bessou, Emonet-Dénand & Laporte (1963a). It has also been reported by Kidd (1964) that β motor fibres produce fibre ~~fibre~~ branches to supply both extra- and intrafusal muscle fibres.

Innervation ratios of muscle fibres in various muscles of different animals have been worked out by numerous investigators. The calculation is usually made by counting the number of muscle fibres in the muscle and dividing it by the number of motor nerve fibres in the muscle nerve. However, the number obtained for the motor fibres is not from direct determination, but is usually estimated from the conclusions of Sherrington (1894) and Eccles & Sherrington (1930) that one-half to one-third of nerve fibres in a muscle nerve are afferent. Thus, Porter & Hart (1923), Van Harreveld (1947), Fernand & Young (1951), Krnjević & Miledi (1958), Malmfors & Wersäll (1960a, b), Blevins (1963, 1964), and many others have calculated the motor components for the innervation ratios in different muscles of one animal or another. The innervation ratios presented by Clark (1931) have been based on cats previously de-afferentated to remove the afferent nerve fibres. Hagbarth & Wohlfart (1952) have

estimated the motor component by the difference between a normal muscle nerve and the afferent component. In every previous estimation of innervation ratios all the motor fibres in a muscle nerve have been included in the calculation, and no allowance has been made for the γ and intrafusal components.

Fusimotor innervation ratios have never been attempted although it has been known, from neurophysiological experiments, that a gamma motor fibre in a muscle nerve may innervate more than one spindle (Hunt & Kuffler, 1951; Kuffler, Hunt & Quilliam, 1951; Crowe & Matthews, 1963). Such ratios may only be calculated by having a clear picture of the entire fusimotor component in a muscle nerve and the total number of intrafusal muscle fibres innervated in all the spindles within a muscle.

In previous work, observations have been made mainly on motor fibres in extramuscular nerves and at sites of innervation within muscles. Without the information gained from intramuscular study, any correlation between data at these two levels is by speculation only. In the present investigation, observations have been made

on motor fibres in muscle nerves some distance from entry into muscles which have then been traced through the intramuscular region to the sites of innervation. It was hoped this might throw some light on the following problems:-

a. The nature of branching, in relation to fibre diameter, of gamma-stem fibres, from actual tracings in the intramuscular region in order to link observations between levels at the muscle nerve and the site of innervation into muscle spindles.

b. The distribution of fibre branches from individual gamma nerve fibres to different muscle spindles in the same muscle.

c. The occurrence of branches from motor fibres innervating both extra- and intrafusal muscle fibres, i.e. β fibres, and their nature of distribution.

d. The determination of fusimotor and skeletomotor innervation ratios.

e. The nature of the intramuscular branching of alpha fibres in muscle nerves.

II. MATERIALS

A. The Cat

The present investigation was based on a total of nine adult cats, five of which, cat C158, C167, C175, C188 and C191, had been de-afferentated on the right side from levels Lumbar 6 to Sacral 2. All the materials taken were from cats C158, C167, C175 and C191 in which the de-afferentated ventral roots were intact. Material from C188 was abandoned as some damage to the ventral roots of L6 and L7 was detected (see Methods 2.1). Removal of dorsal root ganglia was mostly successful except in L7 and S2 of cat C175, where a few ganglion cell bodies remained in the distal cut. However, only the first deep lumbrical muscle of this animal was taken and none of the few surviving afferent fibres were observed in the nerve supply. The muscle(s) and/or nerve(s) taken for investigation from the operated cats are shown in Table 1. Three cats, C165, C181 and C182 together with the normal left side of cat C167 were used mainly for the study of intrafusal and extrafusal muscle fibre counts of the first deep lumbrical muscle in the

Table 1. Materials from operated cats.

Reference	Levels operated	Muscle(s) and/or nerve(s) taken	Nature of study
C158	L6 - S2	1st and 2nd DL	A
		1st and 2nd SL	B
		EDB	B
C167	L6 - S2	1st and 2nd DL	A
		1st and 2nd SL	B
		MG, FDL(1), TP	B
		So, MG	C
C175	L6 - S2	1st DL	A
C191	L6 - S2	So, TA, Pop	C

A : Fusimotor and skeletomotor components and their intramuscular distribution.
B : Skeletomotor intramuscular distribution.
C : Motor component in muscle nerve.

DL deep lumbrical, SL superficial lumbrical, EDB extensor digitorum brevis, MG mesial gastrocnemius, TA tibialis anterior, TP tibialis posterior, FDL(1) flexor digitorum longus, lateral, So soleus, Pop popliteus.

normal condition. The extramuscular nature of the nerve to the first deep lumbrical muscle was investigated from a normal cat C174.

The motor component of the muscle nerve, the intramuscular branching of fusimotor and skeletomotor fibres, and the innervation ratios of intrafusal and extrafusal muscle fibres were studied in detail in the first deep lumbrical (DL) muscle of the cat. This muscle was chosen for its small size suitable to the staining technique applied and for the possibility of correlating results with neurophysiological experiments by Bessou, Emonet-Dénand & Laporte (1963a). The other DL muscles of the cat were also examined for fusimotor and skeletomotor fibre branching and for the intramuscular distribution of skeletomotor fibres.

B. The Rabbit

In a total of ten rabbits, two were studied in the normal condition, rabbit Rb 20 for intrafusal muscle fibre morphology and rabbit Rb 62 for motor nerve endings in spindles of lumbrical muscles. The eight

other rabbits, Rb 23, Rb 51, Rb 58, Rb 59, Rb 60, Rb 63, Rb 65 and Rb 68 were all de-afferentated within root levels L6 - S2, for investigation of the motor components. Rabbit Rb 23, however, being the first to be operated, had been de-afferentated in levels S1 - S3 instead of the intended levels from L7 - S2. Routine root checks showed a few remaining ganglion cell bodies in the proximal cut of certain roots, but since these nerve cells were found central to the proximal cut of the roots, the muscle and/or nerves taken for study should not be affected. The possibility of regeneration from these cells could also be discounted (see Methods 1.11). Only one large afferent fibre to the primary ending of a spindle in a lumbrical muscle of rabbit Rb 23 was detected, probably from the neighbouring uncut root L7, but this did not in any way affect on the study of the motor component. Materials taken for study from the operated rabbits are listed in Table 2.

In the rabbit, there is only one set of lumbrical muscles present, corresponding to the deep layer in the cat. The second lumbrical is the largest, the first the smallest, and the third is intermediate in

Table 2. Materials from operated rabbits.

Reference	Levels operated	Muscle(s) and/or nerve(s) taken	Nature of study
Rb 23	S1 - S3	1st, 2nd and 3rd L	A & B
Rb 51	L6 - S2	1st, 2nd and 3rd L	A & B
		So, TA, Pop	C
Rb 58	L7 - S2	1st and 2nd L	B & C
		3rd L	B
Rb 59	L7 - S2	1st, 2nd and 3rd L	B & C
Rb 60	L7 - S2	1st, 2nd and 3rd L	B & C
Rb 63	L7 - S2	1st, 2nd and 3rd L	B & C
Rb 65	L6 - S2	1st and 2nd L	B & C
		3rd L	B
		So, TA, Pop	C
Rb 68	L6 - S2	1st and 2nd L	B & C
		3rd L	B

A : Motor component and intramuscular distribution.
B : Muscle-spindles analysis.
C : Motor component in muscle nerve.

L lumbrical, So soleus, TA tibialis anterior, Pop popliteus

size. These were examined for the nature of fusimotor and skeletomotor branching and for their intramuscular distribution; and also for the presence of different motor nerve endings in the muscle spindles.

C. Cat and Rabbit comparisons

From the shank regions of hindlimbs in both the cat and the rabbit, de-afferentated muscle nerves of soleus, tibialis anterior and popliteus were studied by comparison of motor components, particularly within the gamma group of nerve fibres. Other muscles, namely, the mesial gastrocnemius, tibialis posterior, tibialis anterior, flexor digitorum longus lateral head, popliteus, soleus, extensor digitorum brevis and superficial lumbricals of one animal or another were used for the study of the nature of intramuscular distribution of skeletomotor fibre branches.

III. METHODS

1. Operative procedures

1.1 Laminectomy

In order to study the motor component in muscles and nerves, the relevant spinal roots have to be de-afferentated for the degeneration of sensory nerve fibres. Since nerves and muscles taken for study were mainly from the shank and pes regions of the hindlimb, root levels Lumbar 6 to Sacral 2 were de-afferentated by laminectomy under aseptic condition. There were some differences in the operative procedures between the cat and the rabbit due to variation in the response to anaesthesia and also to the difference in anatomical structure.

1.11 The Cat

In the cat, sodium pentobarbitone (Nembutal, Abbott) was injected intraperitoneally at a dosage of 36 - 40 mgm/kgm (Worden & Lane-Petter, 1957) which was sufficient for the period required for laminectomy. The

dorsal back muscles are opened and the dorsal spines and neural arches of L6 - S2 removed. The plane of cleavage of root separation is easily distinguished, by carefully picking up the dorsal root perineurium and turning it sideways. The tip of a No.11 Swann-Morton scalpel blade may then be inserted into the plane of cleavage so as to separate the roots. Removal of the dorsal root ganglion is performed first by a proximal cut extradurally, followed by a distal cut at the level of root fusion and great care being taken to leave the underlying ventral root intact. The operative procedure was carried out using a binocular microscope at a magnification of x60.

Three to four weeks was allowed for the degeneration of sensory nerve fibres, this period being the optimum one for clearance of degenerated myelin debris but not sufficient for regeneration from any remaining cell bodies of the ganglion central to the excised portion of the dorsal root. The calculation of the rate of regeneration was based on data of a number of previous investigations. Thus, from Young (1942), a latent period of three days being required, from Cajal

(1928), Bentley & Hill (1936) and Gutmann et al. (1941), a regenerating rate of 2.5 mm per day in peripheral nerves, it may be calculated that any regenerating fibres would require at least 80 days before reaching the shank region and an even longer period to reach the pes extremities. As the degeneration period allowed in the present study was between 21 - 28 days, there is no question of any regenerating fibres reaching either the level of the shank or pes regions.

1.12 The Rabbit

Laminectomy in the rabbit was performed under Nembutal and ether anaesthesia. The Nembutal dosage used was 28 mgm/kgm (Croft, 1960), given intravenously through a marginal vein of an ear. This was followed by ether induction from a mask made of a two-inch diameter glass-tube with a few holes in the middle for air regulation and a piece of cotton-wool plugged in the bottom. Ether was administered through one of the holes to the cotton-wool from a drop bottle, the amount given being carefully controlled. The depth of anaesthesia was controlled by observing the limb withdrawal reflex,

induced by pressure on tendons of the limbs and extremities, and by testing for blinking on touching the cornea. Excision of the dorsal root ganglia L6 - S2 was accomplished as in the cat. However, it was found best to approach each root from the posterior side (instead of the anterior side as in cat) because in the rabbit the ventral roots do not lie directly underneath the dorsal root ganglia but are somewhat posterior to them.

2. Histological techniques

2.1 Checks on operations

The general opinion about operations on spinal roots to remove either the sensory or the motor component is that some damage is inevitably caused to the nerve fibres of the remaining root. Such damage is said to vary from animal to animal. Gilliatt maintains (personal communication) that in the baboon, the two components of the spinal roots are so close together that removing one without damage to the other is impossible. Boyd (1962, statement in Hong Kong Symposium on Muscle Receptors) stated that in determining separate afferent and efferent

components of different cats, the total of the two components was always about 15% less than the total number of fibres in the normal nerve. He suggested an allowance of 10% of total nerve fibres counted should be added for the study of the motor component and 5% for the sensory, since damage to motor is usually about twice that of the sensory in his experience. However, our own experience (Barker, Ip & Adal) is that differential denervation can be achieved without significantly damaging the surviving root in a high proportion of animals, and that it is not necessary to allow for 15% damage. For example, if the number of motor fibres, 247, counted from the de-afferentated soleus nerve of cat C191 (see Results, 3.4) is added onto 209, which is the average number of sensory fibres from four soleus de-efferented nerves (Barker, Ip & Adal, 1962), the total, 456, compares well with the average value of 450 in the normal nerve given by Boyd & Davey (1962). A more extensive discussion on this topic is being reported by Ip (1965). It is common knowledge that there are individual variations in the total number of fibres present in nerves and roots; even between contralateral limbs of the same cat, as pointed out by the difference in spindle counts between the two

sides (Barker & Chin, 1960). Thus, adjustment by allowing for a constant percentage of fibres damaged during operation is of little significance to the true situation.

These factors have been borne in mind during the course of the present study. The morphology of the spinal roots in the cat and rabbit, however, is such that separation into the motor and sensory components during operation is feasible and the removal of one virtually without damage to the other is possible. Certain routine root check procedures have been followed (see below) to ascertain the success of each operation. No adjustment has been made on data from studies of motor components since there is no substantial basis for an appropriate allowance to be determined. Moreover, even if an adjustment of the order of 10% is made it does not produce any significant effect on the small number of motor nerve fibres supplying the small lumbrical muscles studied in this investigation.

In order to ascertain the completeness of ganglion removal and to detect any possible damage to the ventral root, the following checks have been carried

out for all the de-afferentated animals:-

(i) The operated roots were fixed in Bouin's fluid and serial transverse or longitudinal sections at 10 μ were examined after applying either Holmes's (1943) silver-on-the-slide method or haematoxylin and eosin staining. There is no difficulty in examining for the absence of nerve cell bodies from an operated root to ascertain complete dorsal root ganglion removal, but the examination of a ventral root for possible damage requires more careful study. The typical picture of a ventral motor root after a successful de-afferentation is shown in Plate 1. It is from a transverse section of root Lumbar 6 of cat C167. Note that the whole of the ventral root is intact with normal motor axons distributed throughout the area. In Plate 2, a section of root Lumbar 6 of cat C188 is shown with areas of damage among normal motor axons. Damage to the ventral motor root is exhibited by proliferation of nuclei and disorientation of nerve axons in the area affected. Ventral root damage in the area directly beneath the dorsal root ganglion was probably inflicted during operative procedures. Damage at the peripheral area of the root, however, was possibly

Plate 1. Successfully de-afferentated root.

The whole of the ventral root intact and encirculated within the root perineurium (rt.peri.); motor axons (m.ax.) are distributed uniformly throughout the root. From Lumbar 6, cat C167, T.S. 10/u, Holmes's silver-on-the-slide method. cap.-capillaries.

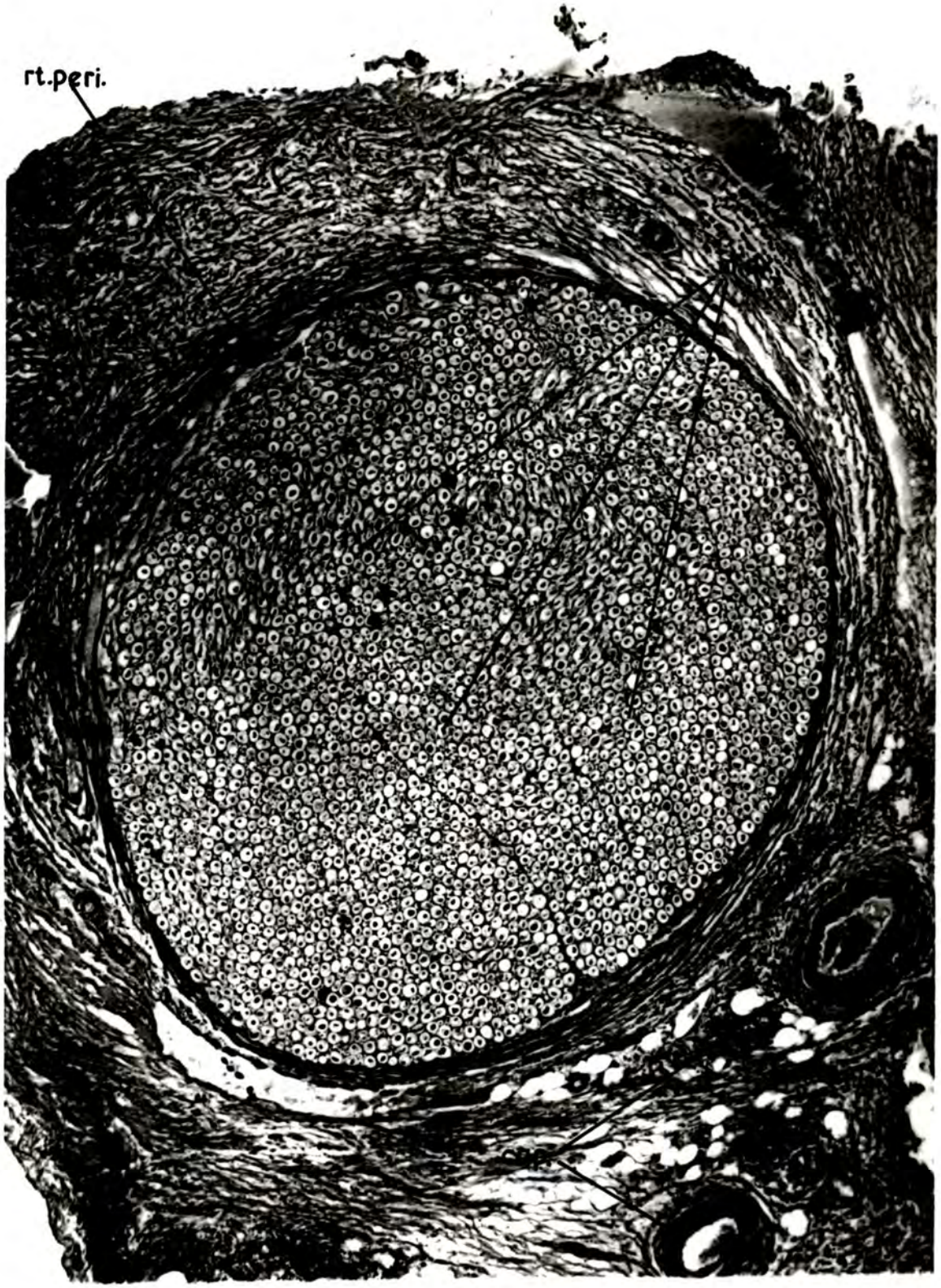
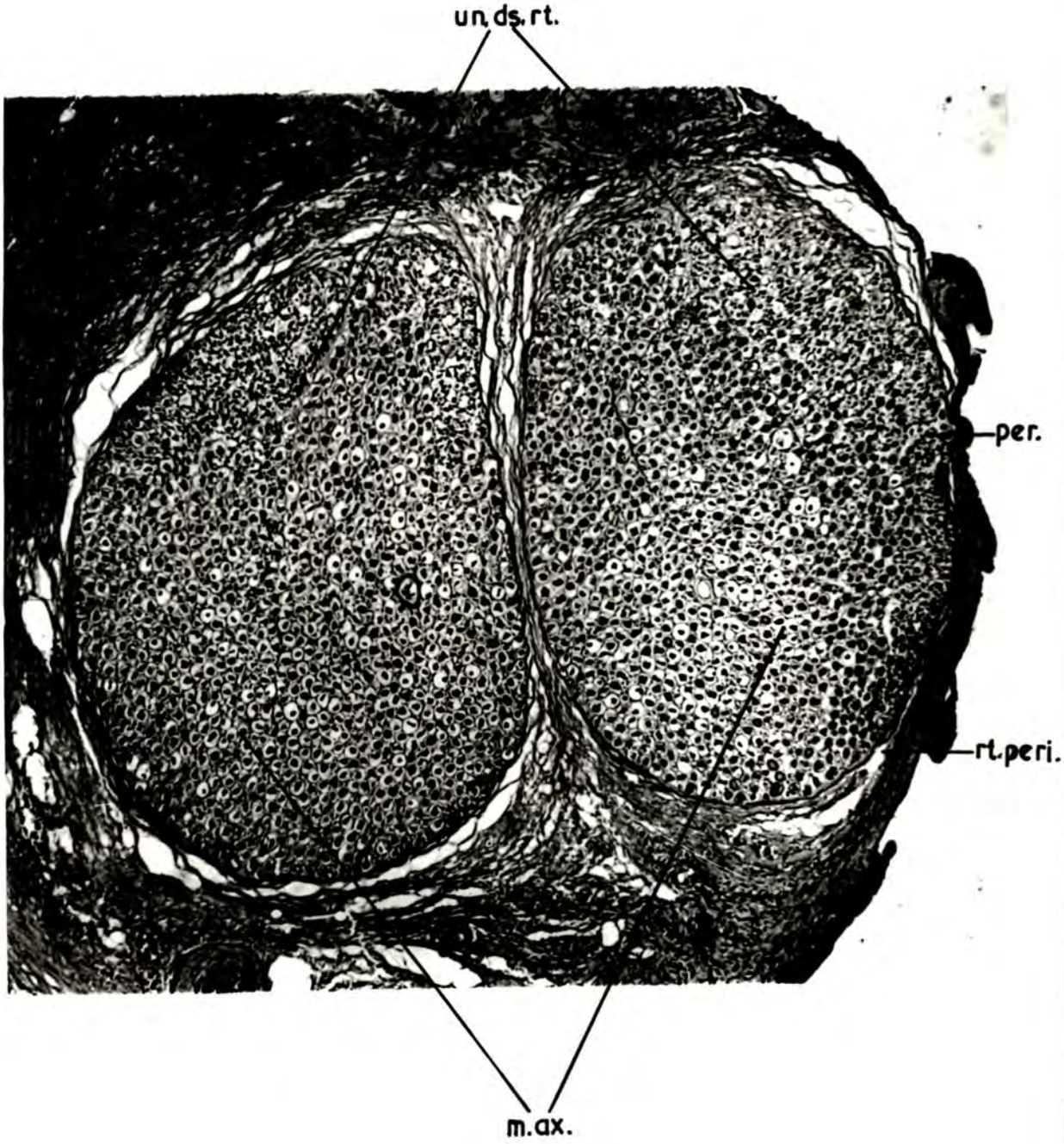

rt.peri.

Plate 2. Damaged de-afferentated root.

Note the proliferation of nuclei and disorientation of nerve axons in damaged areas of the ventral root at (i) underneath the dorsal root (un.ds.rt.) probably due to handling during operation, and (ii) at the periphery (per.) due to ischaemia from capillary damaged. Motor axons (m.ax.) uniformly distributed in other areas. Two fasciculi of ventral root surrounded by the root perineurium (rt.peri.). From Lumbar 6, cat C188, T.S. 10/u, Holmes's silver-on-the-slide method.


un. ds. rt.
per.
rt. peri.
m. ax.

due to ischaemia from damaged capillaries supplying the affected area. As damage to the ventral roots of L6 and L7 was detected in cat C188, all materials taken from this cat were abandoned (see Materials, A).

(ii) Observations were made, during teasing of operated muscles, for any remaining sensory fibres supplying proprioceptors in the muscles by the 'osmic/glycerine' or 'teased silver' preparations (see techniques below).

(iii) The occurrence of extensive collateral regeneration (see e.g. review by Edds, 1953) in the efferent nerves to extrafusal muscle fibres in teased silver preparations would also indicate damage to ventral root fibres during operation. No such collateral regeneration was observed in the material upon which the results are based.

2.2 The study of muscle nerves

2.21 By teasing

The nature of branching of motor nerve fibres

in the extramuscular region of a muscle nerve was investigated by teasing the motor fibres previously stained with osmium tetroxide and cleared in glycerine. From a freshly-killed, de-afferentated cat, a muscle nerve was traced as far proximal as possible to its source of origin from a large nerve trunk. For example, the nerve to the 1st DL muscle was followed to a common nerve trunk where it emerged together with the nerve supply to the first interosseous muscle; the nerves to the soleus and mesial gastrocnemius muscles were traced to their origins from the large tibial nerve in the femoral region (see Text-figure 1). The muscle nerve was then cut at the level of nerve entry into the muscle and at a little distance proximal to the point of its origin from the nerve trunk, the distance between being measured. A piece of library card frame was used to mount the nerve which was lightly stretched to prevent coiling during fixation, and the tissue was placed in 1% osmic acid overnight. Clearing in glycerine was followed the next day through 30% and 50% stages. Teasing of nerve fibres was carried out under 50% glycerine with a binocular microscope at a magnification of x80.

2.22 By sectioning

The study of muscle nerves by sectioning was accomplished by fixing the nerve in a variant of Flemming's fluid (16 ml of 1% chromic acid, 4 ml of 2% osmic acid and 1 drop of glacial acetic acid) and stained by a modified Weigert-Pal technique (Fernand & Young, 1951). Muscle nerves dissected from freshly-killed animals were taken as far proximal from nerve entry as possible to avoid extramuscular fibre branching (Eccles & Sherrington, 1930). They were lightly stretched and adhered to small library card frames before immersing into the fixative. Processing by dehydration with different graded alcohols were carried through and the tissue embedded, using cedarwood oil as a clearing medium. Transverse sections were cut at 5 μ from paraffin blocks and mounted. Staining solutions of the methods, namely, 3% potassium dichromate; haematoxylin in absolute alcohol with glacial acetic acid; and differentiation with 0.25% potassium permanganate and Pal's solution were used in the usual manner. A standard time for each stage of the staining procedure was observed for uniform staining in all nerve sections. From the prepared slides, the best

sections were selected for photography which was done by direct projection onto bromide paper at a constantly checked magnification of x1000. The photographs were then joined together to form a montage of the whole section. Measurements of nerve fibres were made by matching from a piece of perspex with round scales of diameter 1mm, 2mm, ..., corresponding with fibre diameter of 1 μ, 2 μ, etc. (see e.g. Fernand & Young, 1951). Measured fibres were pricked with a needle and recorded at the same time to avoid re-counting. Individual fibres were constantly checked against the section under the high power of a microscope (x400) and any doubts, especially regarding fibres of small calibre, were always carefully scrutinized. Counted fibres were then grouped according to their sizes and histograms drawn in relation to the percentage in individual muscles.

The sources of possible errors in this technique have been fully discussed (Gutmann & Sanders, 1943; Aitken, Sharman & Young, 1947; Evens & Vizoso, 1951; Quilliam, 1956; Williams & Wendell-Smith, 1960) and most of which have been discounted to show any significant effect in the study of nerve fibre population, with

relation to the external diameter.

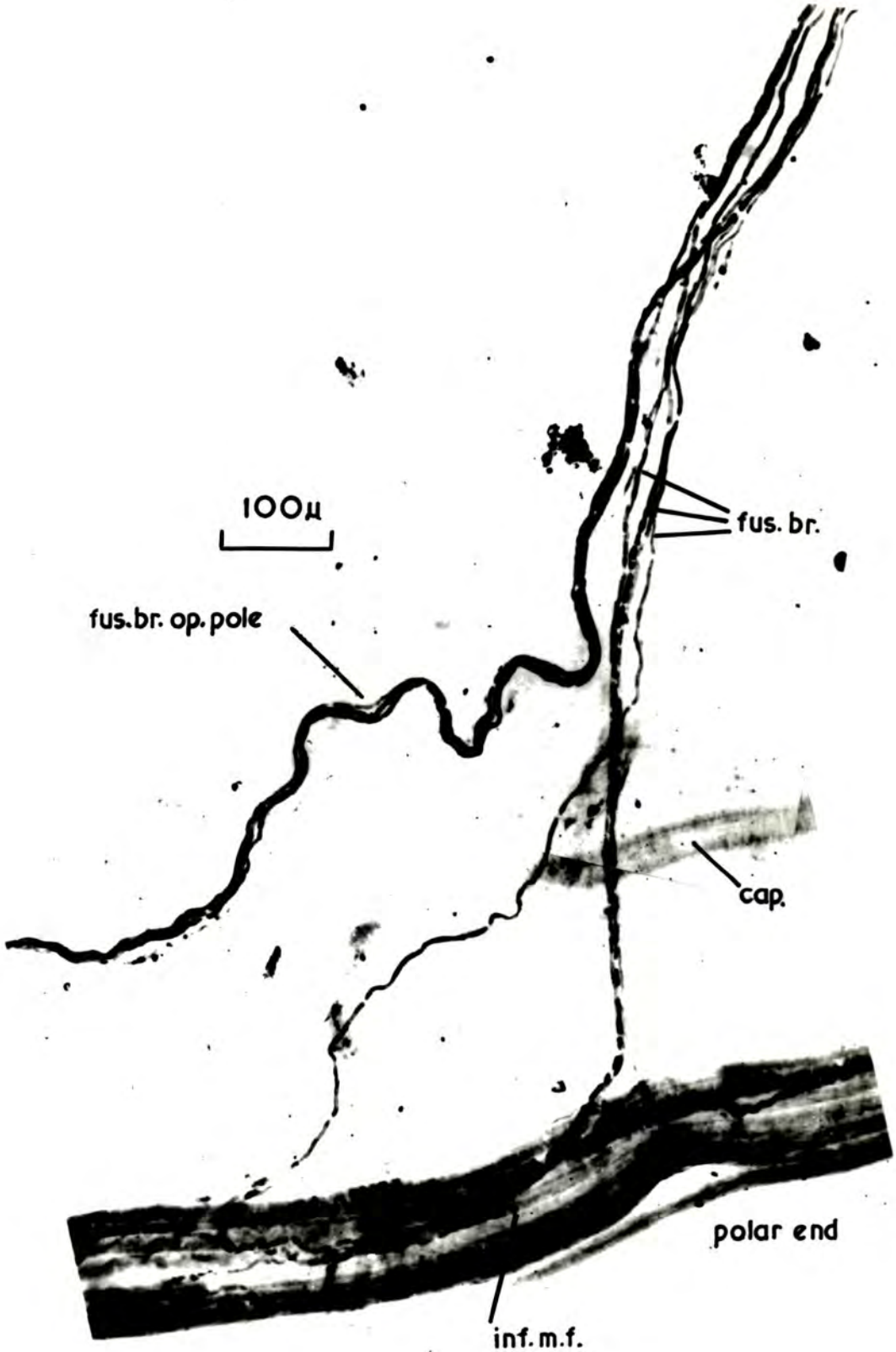
2.3 Intramuscular study of nerve fibres

After a proximal portion of the nerve supply was taken for study in transverse section by the Weigert-Pal technique, the muscle intended for intramuscular study together with the remaining portion of nerve intact, was stained with 1% osmic acid. Before staining, the muscle was carefully teased in mammalian Ringer's solution under a binocular microscope at a magnification of x80; extrafusal muscle fibres were separated as much as possible to ensure a good osmic acid penetration for the staining of myelinated nerve fibres. After leaving in osmic acid for about three hours, the tissue was transferred to 30% glycerine with 1% potassium dichromate overnight. Final clearing was completed by two changes of pure glycerine. Further teasing was done by removing the extrafusal muscle fibres (showing a good yellowish-brown background from the dichromate colour against the darkly stained myelinated nerve fibres) and cutting the nerve terminals as close to the muscle fibres as possible. Muscle spindles and their motor nerve supply

(fusimotor, Hunt & Paintal, 1958) were left intact to distinguish them from the cut nerves to extrafusal muscle fibres (skeletomotor). The perineurium of the main nerve trunk was opened and nerve fibres within spread out as much as possible. The preparation was finally mounted in glycerine and careful compression applied as necessary in order to facilitate the spreading of nerve fibres. This 'osmic/glycerine' preparation, as it is referred to throughout this study, was then examined and tracings of all nerve fibres and branches in the intramuscular course to the levels of innervation were carried out. Measurements of external diameters of stem fibres and their branches were taken by an average of a number of recordings wherever possible. Measurements were also made of the length in the total intramuscular course of all fibres and branches from the point of entry into the muscle to the point(s) of entry into the spindle(s), together with measurements of the distances between successive sites of branching. This technique has been initially reported by Adal & Barker (1965a). Plate 3 demonstrates the nature of the staining by the technique described, and shows the fusimotor fibre branches in the intramuscular region and their entry into a spindle pole.

Plate 3. Osmic stained nerve fibres teased from the intramuscular region.

Fusimotor fibre branches (fus.br.) innervating intrafusal muscle fibres (inf.m.f.) at the polar region of a spindle. (cap.- capillary; fus.br.op.pole-fusimotor fibre branches to opposite pole).


100μ
fus. br.
fus.br. op. pole
cap.
polar end
inf. m.f.

That thinly myelinated nerve fibres of small calibres (3 μ or less) in de-afferentated muscles could possibly be of sympathetic origin has also been taken into consideration. It would have to be a considerably large sympathetic nerve fibre for any degree of myelination to occur, and the area of its distribution in a muscle would certainly be extensive. However, individual small myelinated nerve fibres observed intramuscularly in all the de-afferentated preparations from the lumbrical muscles innervated one muscle spindle only, without prior branching and retained their myelin (see Results). Since there is still no histological evidence of a sympathetic innervation of muscle spindles and in view of the fact that the small, thinly myelinated nerve fibres innervated individual muscle spindles only, it is most unlikely that they are sympathetic in origin.

The intramuscular branching of the skeletomotor supply was fully examined in order to ascertain whether any of the 2 - 4 μ terminal branches had been involved in fusimotor innervation. There is no doubt in the present study that whenever such collateral innervations occurred, they had been preserved intact with the other

fusimotor fibres during the teasing process, provided the collateral branch was myelinated. The occurrence of unmyelinated fusimotor axons innervating muscle spindles would have gone unobserved in these osmic stained preparations but a study of the motor supply to ten spindles from three DL muscles by the teased silver method (see Methods 2.4) indicated that such a contribution was negligible. The range of fusimotor axons or axon branches were from 3 - 10 giving an average of 5.8. This compares with an average of 5.7 myelinated fusimotor fibres or fibre branches with a range of 2 - 11, supplying the eighteen DL spindles studied by the osmic/glycerine technique (Tables 3, 4, 5 & 6 and Text-figure 3).

The nature of the intramuscular distribution of skeletomotor nerve fibres was studied by carefully mapping out certain areas of muscle. This was achieved by the service of a contour projector (Shadowgraph), throwing an image at a magnification of x50. Camera lucida drawings from a microscope image projector ('Britex') were also made. The nerve fibres from different side branches of nerve trunks in the area observed were traced through their courses under the high-power

magnification (x400) of a microscope. The directions at which the nerve fibres run in relation to the proximal (direction of origin from muscle nerve) and distal (direction of innervation at the ending) points were noted and indicated appropriately by different arrows in the drawing. The final product resulted in a sketch of a certain area of a muscle, showing the distribution of nerve fibres in the intramuscular region with arrows indicating their travelling directions within branching nerve trunks (see Text-figure 13).

2.4 Silver impregnation for spindle innervation

Teased, silver impregnated preparations have been used when it was necessary to acquire information about the intrafusal motor endings of the muscles studied. The advantage of the technique is that all fusimotor fibres to the spindles are stained, whether myelinated or unmyelinated, and observations can be carried further inside the spindles to the terminal nerve endings, in relation to the innervating nerve fibres. The technique is based on a modification of de Castro's (1925) version of one of the block silver impregnation method of Cajal

(1903) and extended further by Barker & Ip (1963) in such a way that the muscle tissues may be teased.

Muscles from freshly-killed animals were fixed for four days in a freshly made up solution containing chloral hydrate, 1gm; 95% alcohol 45 ml; distilled water, 50 ml; and concentrated nitric acid, 1 ml; the size of the muscle had no critical effect on the staining. After fixing, the tissues were washed for twenty-four hours in running tap water followed by placing for forty-eight hours in ammoniacal alcohol made up with 95% alcohol, 25 ml and concentrated ammonia solution, 1 drop. The next stage was followed by first blotting off surplus fluid then incubating for five days in a 1.5% silver nitrate solution at 37°C. At the end of the incubation period, the tissues were reduced for two days in a freshly made up solution of hydroquinone, 2 gm and 25% formic acid, 100 ml. The tissues after reduction were rinsed with distilled water then cleared and stored in glycerine. Spindles with as much as possible of their fusimotor fibres intact were teased out from muscles and mounted in glycerine for study of fibre innervation and the related nerve endings.

2.5 Extrafusal and intrafusal muscle fibre counts

The determination of extrafusal and intrafusal muscle fibre content was ascertained by examining serial transverse sections of the 1st DL of the cat. The muscle dissected from a freshly-killed animal was laid on a piece of library card before immersion in Bouin's fixative . Paraffin embedding, serial transverse sections at 10 μ, staining with haematoxylin and counter-staining by eosin were carried out in the usual way.

For the study of spindle morphology, muscle spindles were carefully examined from end to end in every section. Data on the number of intrafusal muscle fibres for each spindle and the nuclear-bag and nuclear-chain fibre ratios were accummulated. From an aggregate figure, the average number of intrafusal muscle fibres for a spindle was calculated. The average figure was used for all spindles in a muscle and together with the fusimotor component, the innervation ratio of intrafusal muscle fibres was then estimated.

For the determination of the number of extrafusal

muscle fibres, a section through the belly of the lumbrical muscle was chosen for photography at a magnification of x200. Such a section included all the fibres which run in parallel from origin to insertion in the muscle. From the montage of the whole section the muscle fibres were counted and then pricked by a needle to avoid recounting. The section was referred to under a microscope as often as necessary whenever in doubt. This count, together with the skeletomotor component of the muscle nerve, enabled the innervation ratio of the extrafusal fibres in the muscle to be determined.

3. Correlation of different techniques

As the present investigation involved the study of motor nerve fibres both extra- and intramuscularly, and also in relation to their nerve endings, the result from a single technique cannot, due to its limitations, provide a full solution of the problems involved. Each technique is only indicative of a certain specific study, but usually, results of two suitable techniques are combined in order to produce answers to certain problems.

Thus, the following correlations between data of different techniques were made for specific problems:-

(i) The nature of nerve fibres, whether thickly or thinly myelinated, as observed in sections from a proximal portion of a muscle nerve by the Weigert-Pal technique, was correlated with observation on the same muscle nerve as teased both extra- and intramuscularly using the osmic/glycerine technique.

(ii) The study of fusimotor innervation of muscle spindles in teased, silver preparations was compared with the osmic/glycerine findings of the same nerve prior to nerve entry. Comparisons of fusimotor supply to individual muscle spindles were also made between the teased silver and the osmic/glycerine preparations to account for any difference in number, if any, between myelinated and unmyelinated fusimotor fibres innervation.

(iii) In the evaluation of innervation ratios, whether for extrafusal or intrafusal muscle fibres, the counts of each category from haematoxylin and eosin stained sections of the muscle were correlated with separate

skeletomotor and fusimotor components of the muscle nerves, determined by the intramuscular study in osmic/glycerine preparations.

IV. RESULTS

A. The Cat

1. Extramuscular study of motor nerve fibres

The extramuscular nature of the motor component of a muscle nerve was studied in the nerve supply to the 1st DL, soleus and mesial gastrocnemius muscles of the cat. Each muscle nerve was traced as far proximal as possible to their source of origin from a larger nerve trunk, for example, the 1st DL nerve being traced to its common nerve trunk with the nerve to the first interosseous muscle; the soleus and mesial gastrocnemius nerves being followed to their origins from the tibial nerve. The muscle nerves were stained with osmium tetroxide and carefully teased to search for fibre divisions in relation to the distance from nerve entry into the muscle.

1.1 Branching of muscle nerves

The nerve supply to the 1st DL muscle in the

hindlimb of a normal cat C174 was traced to a distance of 55 mm back to its emergence from a common nerve trunk with the nerve to the first interosseous muscle (Text-figure 1, a). Careful examination at this point within the common nerve trunk showed that nerve fibres ran confluent and parallel with one another without any axon branching. Continuous teasing of the 1st DL nerve towards the muscle showed that branching into two smaller nerve trunks occurred at 1 mm prior to nerve-entry, and at this level axon branching also began. The nerves to the soleus and mesial gastrocnemius muscles of the de-afferentated cat C167 were traced to their origins from the tibial nerve in the femoral region, covering a distance of approximately 95 mm and 80 mm, respectively, from the muscle (Text-figure 1, b & c). Extensive teasing around the junction of the tibial nerve where the two muscle nerves originated did not reveal any axon branching and the nerve fibres were confluent with one another. The two nerves ran side by side from their origins at the tibial nerve to the level of the gastrocnemius muscle, from whence the soleus nerve continued further on its own. In tracing the soleus nerve more distally, branching into two smaller nerve trunks

Text-figure 1. Schematic representation of motor fibres in muscle nerves showing the extramuscular nature.

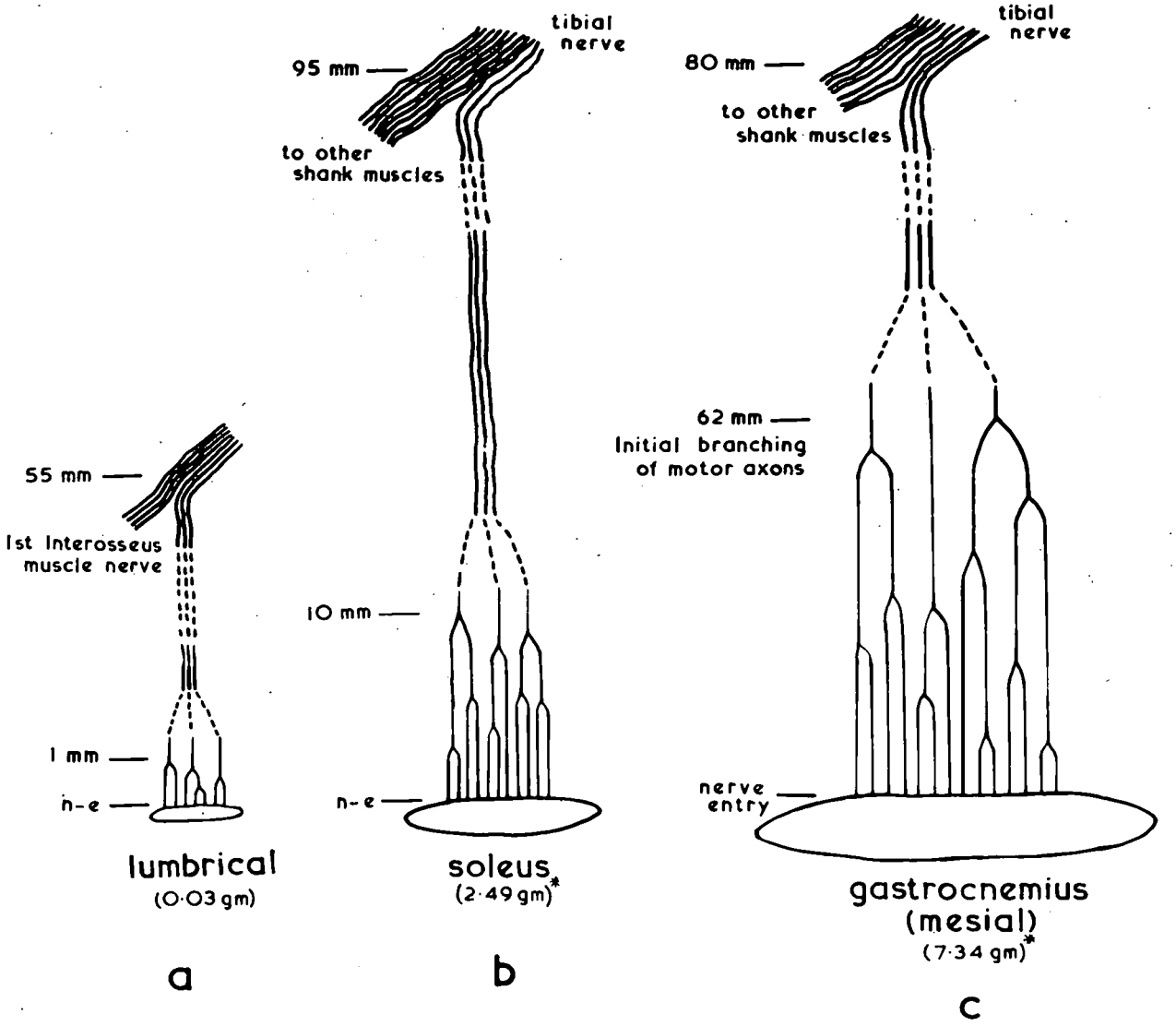
Note that (i) motor nerve fibres run in confluent with one another in common nerve trunk and segregate off to individual muscle nerves without branching, (ii) initial motor axon branching occurs at a distance from a muscle depending on the muscle size. Only three motor fibres are drawn for each muscle nerve.

Figure a. The first deep lumbrical muscle nerve from cat C174 traced 55 mm back to its emergence from a common nerve trunk.

Figure b. The de-afferentated soleus nerve from cat C167 traced approximately 95 mm back to its origin from the tibial nerve.

Figure c. The de-afferentated mesial gastrocnemius muscle nerve from cat C167 traced approximately 80 mm back to its origin from the tibial nerve.

(* Chin, Cope & Pang, 1962).


tibial nerve
95 mm
to other shank muscles
10 mm
n-e
soleus
(2·49 gm)*
b
tibial nerve
80 mm
to other shank muscles
62 mm
Initial branching of motor axons
nerve entry
gastrocnemius (mesial)
(7·34 gm)*
c
55 mm
1st Interosseus muscle nerve
1 mm
n-e
lumbrical
(0·03 gm)
a

occurred at a distance of 10 mm from nerve-entry; it was at this level that axon branching began also. The nerve to the mesial gastrocnemius muscle gave branches of smaller nerve trunks at a distance of 62 mm from the muscle where initial axon branching was also observed.

1.2 Branching of motor axons

In the nerve to the 1st DL muscle of the unoperated cat C174, initial axon branching was observed, as stated above, at a distance of 1 mm from nerve entry into the muscle. Branching of nerve fibres at this level was found only in those with calibre sizes above 10 μ, and they were probably of the motor component since branching of large afferent fibres has not been demonstrated (Eccles & Sherrington, 1930). Initial branching of motor nerve fibres was observed as distal as the nerve-entry or sub-entry levels of this muscle in de-afferentated cats C158 and C167. The nature of intramuscular branching and distribution of motor fibres in this muscle will be dealt with later in detail. In the soleus muscle nerve, initial axon branching was shown at a distance of 10 mm from nerve-entry and branching of nerve fibres was

encountered more frequently towards the muscle. In the large mesial gastrocnemius muscle nerve, initial axon branching occurred at a considerably longer distance of 62 mm from the muscle and branching of nerve fibres occurred abundantly as the nerve supply approached the muscle. The frequency of axon branching was not studied quantitatively in the large shank muscles. The results are represented diagrammatically in Text-figure 1. In order to simplify the figure, only three motor nerve axons were drawn as an illustration within each muscle nerve.

1.3 The nature of motor fibres in the extramuscular region

These results indicate that motor nerve fibres in the cat run parallel with one another in large nerve trunks without branching. A muscle nerve originates from a large nerve trunk (e.g. soleus from tibial nerve), with emergence of motor nerve fibres by segregation only. As the nerve supply approaches a muscle, axon branching usually begins to occur at a point some distance away. The distance between this point and nerve entry varies

from muscle to muscle, and is somewhat proportional to the size of the muscle. Thus, in this study, the distances are 1 mm for the small lumbrical muscle (mean weight, 0.03 gm); 10 mm for the soleus (mean weight, 2.49 gm, Chin, Cope & Pang, 1962); and 62 mm for the mesial gastrocnemius (mean weight, 7.34 gm, Chin et al., 1962).

2. Intramuscular study of fusimotor fibres

2.1 The number of lumbrical muscles studied

The results of intramuscular branching of fusimotor fibres are based on observations in three 1st and two 2nd deep lumbrical muscles of three de-afferentated cats, namely, the 1stDL of cat C158, C167 and C175 and the 2nd DL of cat C158 and C167 (see Materials, Table 1). Except for the 2nd DL of cat C158, where only one spindle was analysed, all the muscles and their nerve supply were studied by a correlation of two techniques. This was achieved by sectioning a proximal portion of the muscle nerve (see Methods 2.22) and staining the remaining muscle and nerve supply by the osmic/glycerine method (see Methods 2.3). A section of the

muscle nerve provides information about the number, diameter, and degree of myelination of the nerve fibres, and the intramuscular study by osmic staining reveals their fate in branching and distribution within the muscle. The results obtained are therefore based on direct observation by actual tracings in the intramuscular region, between the nerve fibres at the level of the muscle nerve supply and the level of entry into muscle spindles.

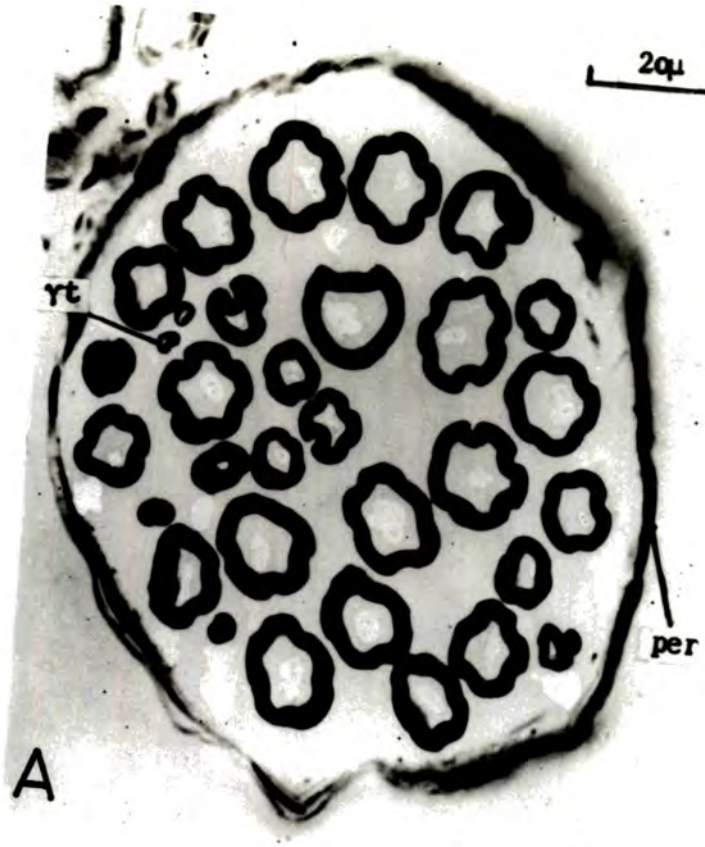
2.2 The nature of the first deep lumbrical muscle

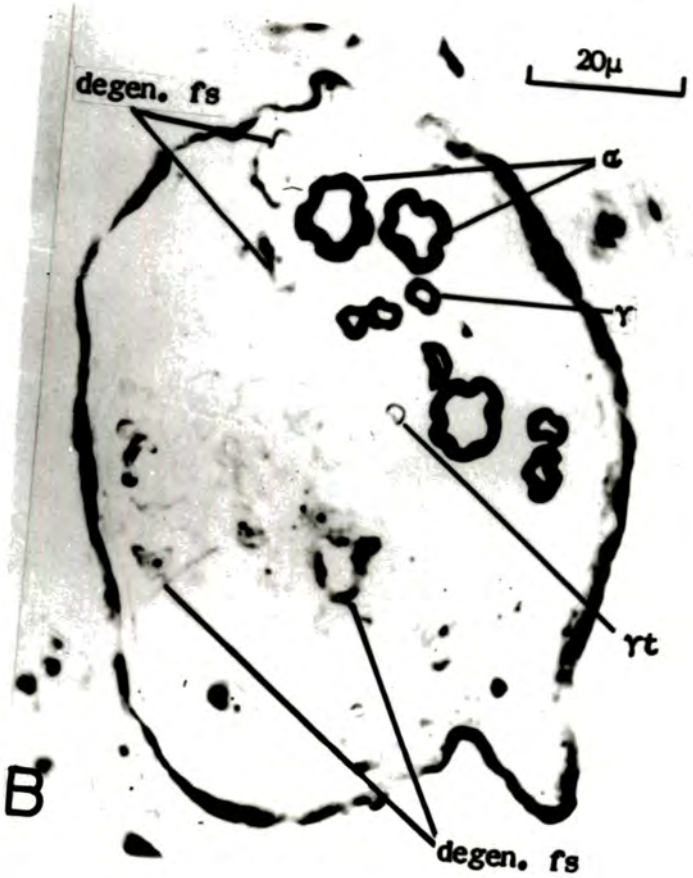
The 1st DL muscle of the cat contains four to six spindles, and is supplied by approximately twenty to thirty myelinated fibres of which about half are motor; the 2nd DL is similar. Plate 4, figures A & B show a section of a normal 1st DL muscle nerve compared with one which has been de-afferentated. There are two thinly-myelinated small fibres in the normal nerve; one such fibre is seen in the de-afferentated nerve. The remaining fibres are all thickly myelinated.

An osmic/glycerine preparation showing the

Plate 4. Normal (Figure A) and de-afferentated (Figure B) nerves to the first deep lumbrical muscle of the cat.

Osmic sections at 5 μ. (α-alpha fibre, γ-gamma fibre, γ_t - thinly myelinated gamma fibre, per. - perineurium, degen.fs.- residue from degenerated fibres).


20μ
rt
per
A


20μ
degen. fs
α
γ
rt
B
degen. fs

intramuscular distribution of nerve fibres to muscle spindles is exhibited in Plate 5, which is from the 1st DL muscle of cat C167. In the cat, the distribution of intramuscular nerve trunks is such that there are independent small nerve branches containing groups of fusimotor fibres going towards individual muscle spindles. Each spindle receives one or more such fusimotor nerve trunks. As will be seen later, this pattern of supply is not found in the rabbit (cf. Plate 19).

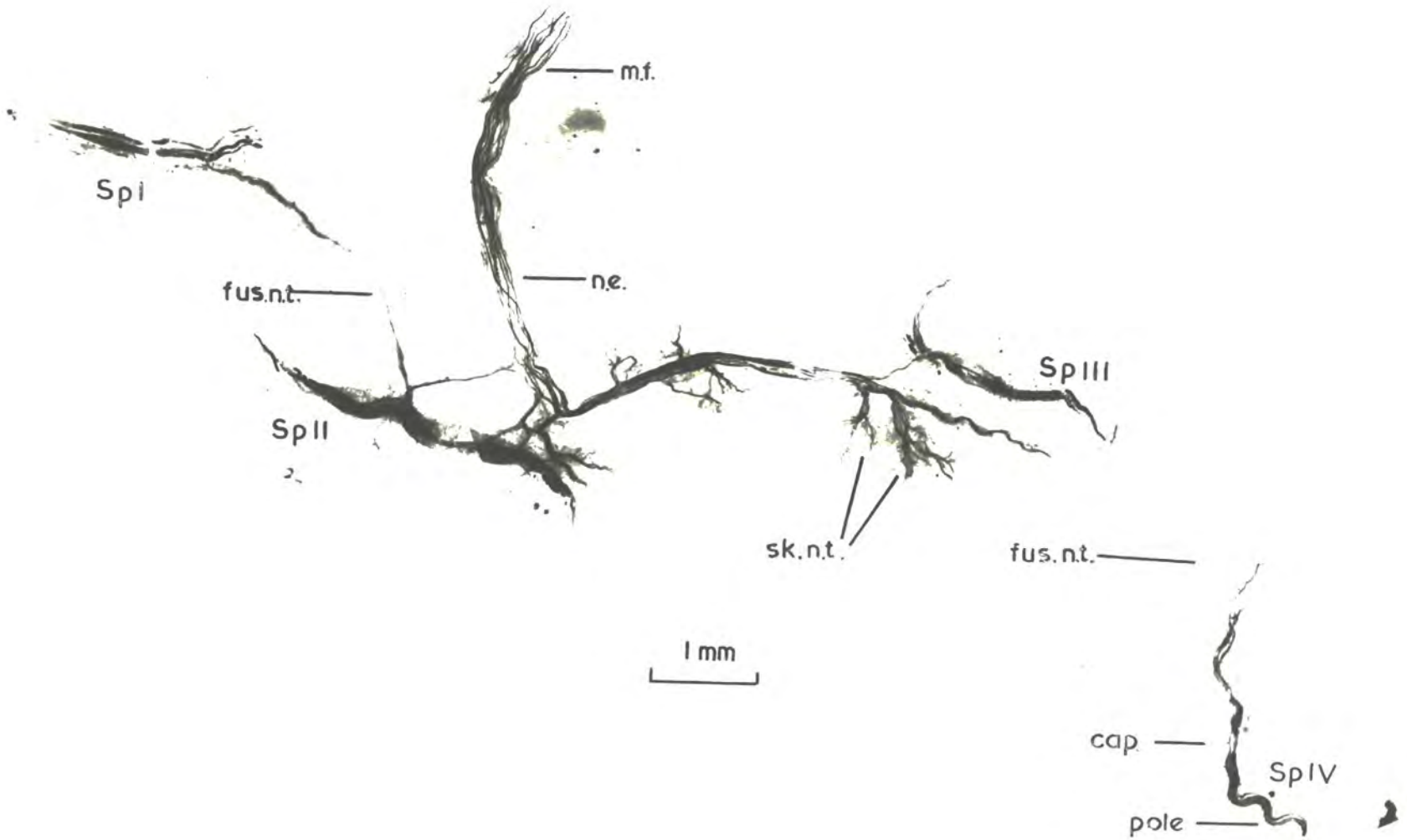
2.3 Total fusimotor fibres investigated

The intramuscular course of twenty-seven out of twenty-eight stem motor fibres supplying eighteen muscle spindles were traced with certainty to the level of spindle entry (Tables 3, 4, 5 & 6 and Text-figure 3). Further branching of fusimotor fibres upon entering a spindle could not be traced satisfactorily with osmic staining, but this is of no consequence to the present study since the difference in diameter between Boyd's γ_1 and γ_2 fibres is already established at spindle entry.

In tracing the origin of individual fusimotor

Plate 5. An osmic/glycerine teased preparation from the first deep lumbrical muscle of cat C167.

Note the independent fusimotor nerve-trunk (fus.n.t.) to individual spindles which is lacking in the rabbit (cf. Plate 19).
n.e. - nerve entry; m.f. - motor fibres in de-afferentated muscle nerve, cf. Plate 8; sk.n.t. - small skeletomotor nerve-trunks; cap. - spindle capsule; pole - spindle pole; Sp I - IV, spindles one to four.


m.f.
Sp I
fus.n.t.
n.e.
Sp III
Sp II
sk.n.t.
fus.n.t.
1 mm
cap
Sp IV
pole

fibres entering a muscle spindle, it was possible to determine whether any of them were derived from β-stem fibres. Of the twenty-eight stem fibres referred above, five were of the β type, and their intramuscular courses were traced as far as possible (Text-figure 3).

2.4 The fusimotor supply to individual lumbrical muscles

An osmic section of the de-afferentated nerve to the 1st DL muscle of cat C158 cut approximately 12 mm from its entry into the muscle showed a total of ten myelinated fibres, three large and seven small, the smallest fibre being thinly myelinated (Plate 6, figure A). These fibres were also observed in the osmic/glycerine preparation at a level nearer the muscle (Plate 6, figure B). The osmic stained preparation of this muscle and nerve is shown in Text-figure 2 which was drawn from an image projector (Shadowgraph). The final positions assumed by the muscle spindles was dependent on the process of mounting the preparation, but their relative distances to the point of nerve-entry could be measured. There were six spindles present in this muscle, five lying

Plate 6. Supply of skeletomotor (α) and fusimotor (γ) to first deep lumbrical muscle of cat C158.

Figure A. Osmic section of de-afferentated nerve cut approximately 12 mm from its entry into muscle. γ_t: thinly myelinated γ fibre.

Figure B. Teased osmic preparation of same nerve approximately 1 mm from its entry into the muscle.

Figure C. Schematic representation of the intramuscular branching and distribution of γ fibres a - g to the six spindles (Sp I - VI) in the muscle. (cf. Table 3) Scale indicates intramuscular length (mm) of each fibre and levels at which branching and spindle entry occur. Average total diameters (μ) of the fibres and their branches are indicated by the numbers placed in relation to them. Fibres and fibre branches entering spindles as 'γ_1 fibres' shown in black; 'γ_2 fibres' in white. Asterisks denote the fibre and fibre branches photographed at this level in Plate 7, figure A.

(Adal & Barker, 1965a).

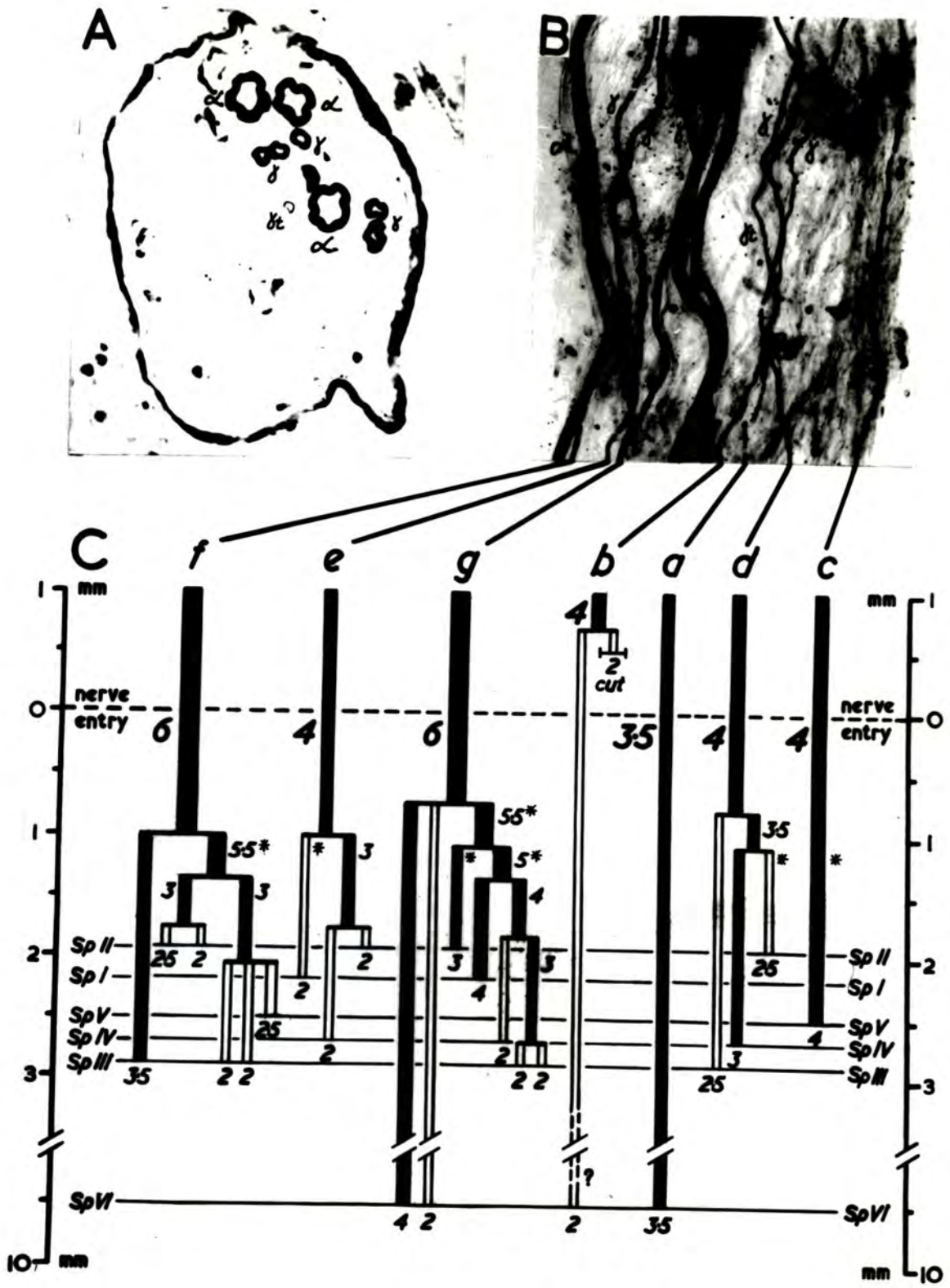
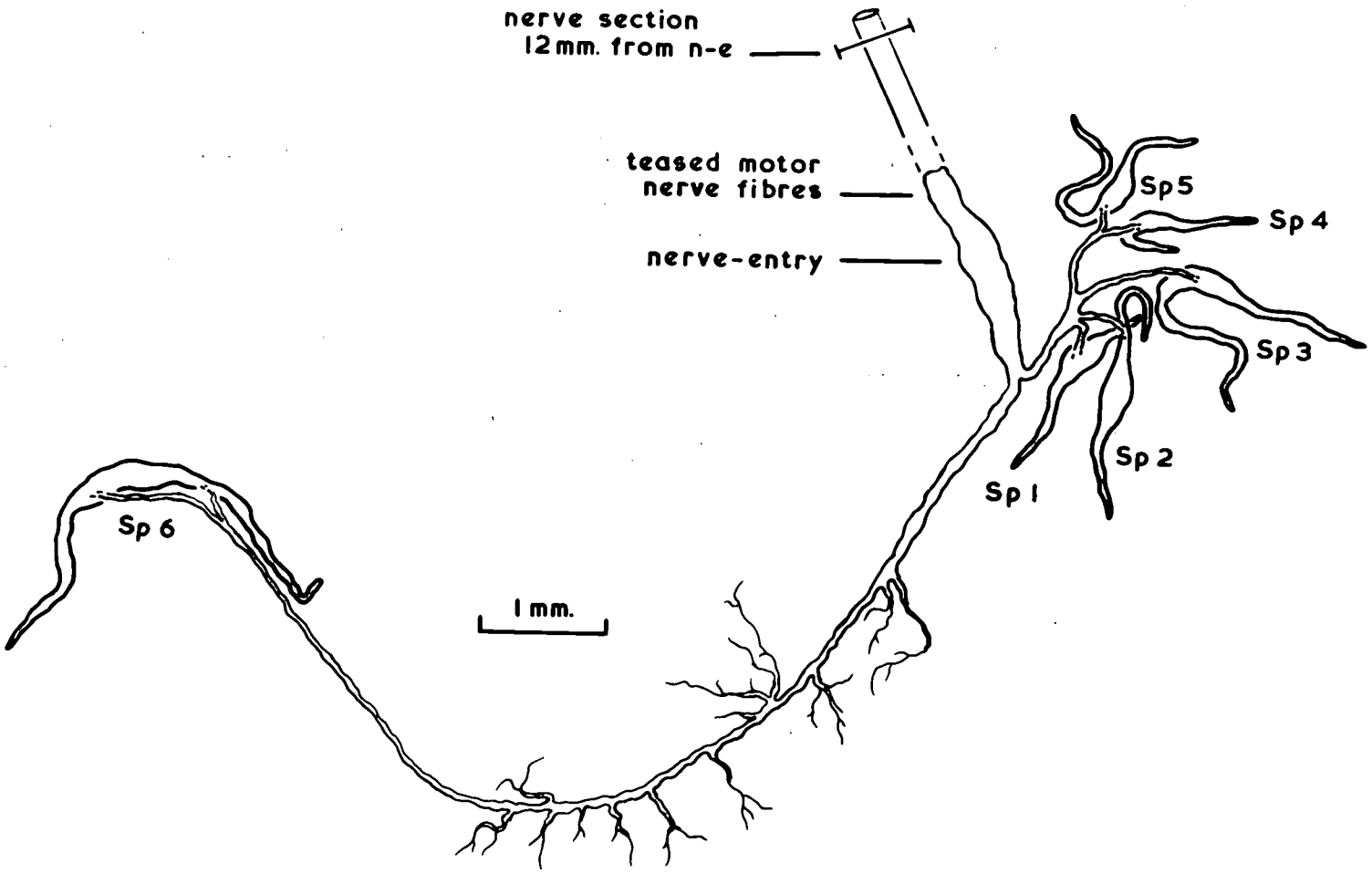

A
B
C
f
e
g
b
a
d
c
mm
nerve entry
cut
Sp II
Sp I
Sp V
Sp IV
Sp III
Sp VI
1
0
1
2
3
10

Table 3. Intramuscular branching of fusimotor fibres innervating the first deep lumbrical muscle of cat C158.

C 158 1st deep lumbrical			FUSIMOTOR BRANCHES diameter (μ) at spindle entry					
			Sp I	Sp II	Sp III	Sp IV	Sp V	Sp VI
STEM MOTOR FIBRES diameter (μ) in muscle nerve	γ	a : 3.5						3.5
		b : 4.0	Gives rise to two 2μ branches: one cut in teasing; the other doubtfully innervates Sp VI					
		c : 4.0					4.0	
		d : 4.0		2.5	2.5	3.0		
		e : 4.0	?.0	2.0		2.0		
		f : 6.0		2.0, 2.5	2.0, 2.0 3.5		2.5	
		g : 6.0	4.0	3.0	2.0, 2.0	2.0		2.0, 4.0
	α	A : 12.5	B : 13.5 C : 13.5					

Text-figure 2. Cameralucida drawing of an osmic/glycerine preparation of the first deep lumbrical muscle of cat C158.

The section and the teased nerve supply of the muscle are shown in Plate 6, figures A & B. Sp - spindle. Small skeletomotor nerve trunks are given off from the main intramuscular nerve-trunk.


nerve section
12mm. from n-e
teased motor
nerve fibres
nerve-entry
Sp 5
Sp 4
Sp 3
Sp 2
Sp 1
Sp 6
1 mm.

2 - 3 mm from the point of nerve-entry and the sixth at a distance of 9.5 mm away from the same point (Text-figure 2). It is a characteristic in the 1st DL that the spindles are distributed in this manner, i.e. all but one lie within certain levels from nerve-entry and an odd one out at some distance away.

An intramuscular study of the preparation showed that the three large fibres in the muscle nerve with stem diameters of 12.5 μ (fibre A), 13.5 μ (fibre B) and 13.5 μ (fibre C) were purely skeletomotor (α, Table 3). The seven small fibres, two with stem diameters of 6.0 μ (fibres g & f), four 4.0 μ (fibres b, c, d & e) and one 3.5 μ (fibre a) constituted the fusimotor supply to the six spindles (Sp I - VI) in the muscle. The nature of the intramuscular branching and distribution of the seven γ fibres supplying these spindles is shown diagrammatically in Plate 6, figure C.

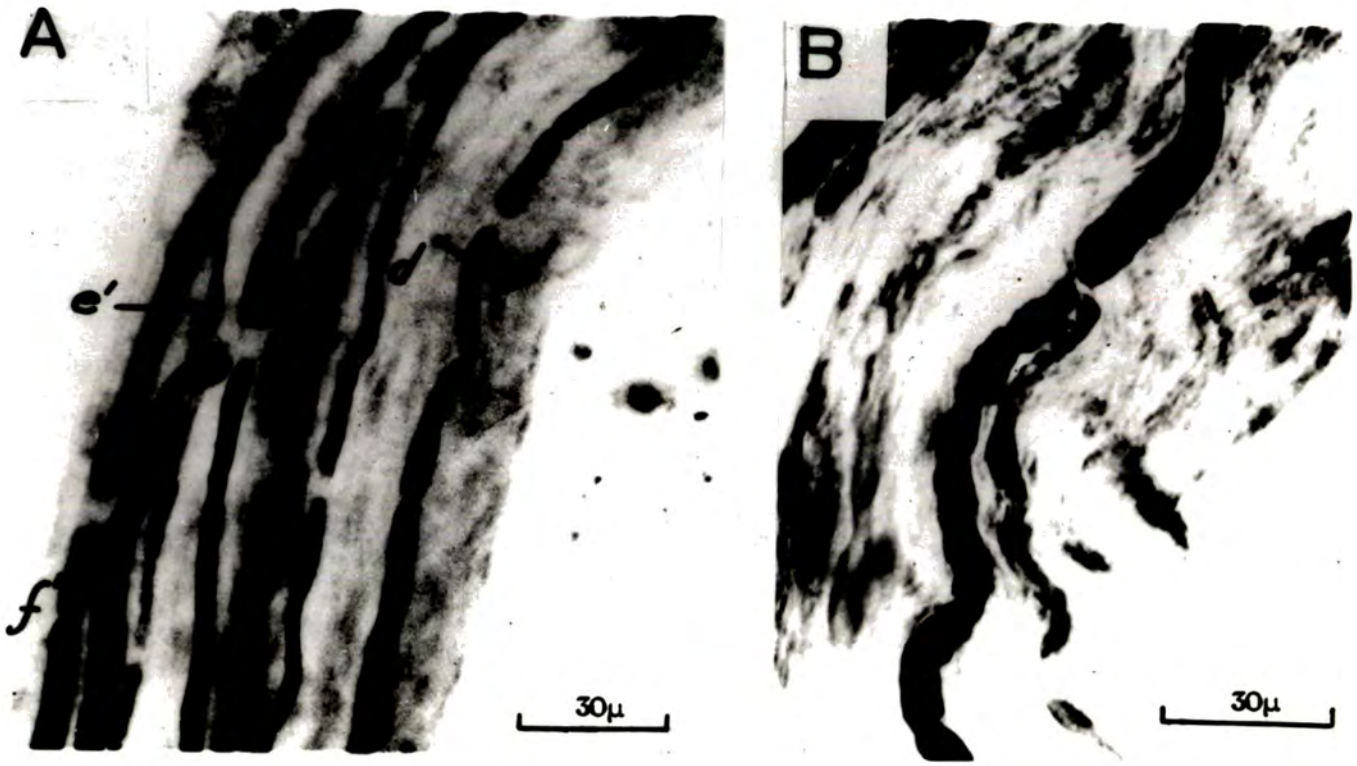
Plate 7, figure A shows an intramuscular division of a large fusimotor fibre (5.5 μ, fibre g'), being a branch of fibre g (stem diameter 6.0 μ) into branches of 5.0 μ and 3.0 μ. Another fusimotor fibre

Plate 7. Intramuscular fusimotor fibre branching producing daughter fibres of large and small calibre.

Figure A. A 5.5μ intramuscular branch (g') of the γ fibre g supplying the 1st DL muscle of cat C158 is shown dividing into 5.0μ (left) and 3.0μ (right) branches. The fibre c and branches of fibres d, e, and f are also seen. The fibre and fibre branches present in this photograph correspond with those asterisked in Plate 6, figure C.

Figure B, A 6.0μ intramuscular branch of the γ fibre r (stem diameter 6.5μ) supplying the 2nd DL muscle of cat C167 (see Table 5) is shown dividing 2.2 mm from nerve entry into 5.5μ (left) and 2.0μ (right) branches. The smaller branch enters a spindle as a 'γ_2 fibre' 0.7 mm further on; the larger branch undergoes further division to supply another spindle 3.7 mm away with three 'γ_1' and two 'γ_2' fibres.

(Adal & Barker, 1965a).


A
e'
f
30μ
B
30μ

(fibre c, 4.0 μ) and fibre branches (fibre d'', e' & f') are also shown. The fibre and fibre branches correspond with those asterisked in Plate 6, figure C.

Consistent size relationship between diameters of stem fibres and their branches at spindle entry is not evident. For example, the large thickly-myelinated 6.0 μ γ fibres g and f (Plate 6, figure C) gave rise to thirteen branches; four of these enter spindles as large fibres (3.0 - 4.0 μ), and nine as small fibres (2.0 - 2.5 μ). If a difference of 0.5 μ is allowed for adjustment as between total and axon diameters, the fibres may be regarded as a supply of four γ_1 axons and nine γ_2 axons. According to Boyd's hypothesis, these nine γ_2 axons should all be branches of the small thinly-myelinated 3.5 μ fibre a, and this fibre should also supply axons to all six spindles present. But fibre a showed no branching at all in its intramuscular course. It supplied only one spindle which it entered with its total diameter at 3.5 μ, the same as the value of its stem fibre, or in other words, as a γ_1 axon of about 3 μ.

A similar study of an osmic section of the

nerve to the de-afferentated 1st DL of C167 taken at about 12 mm from its nerve entry showed the presence of eleven nerve fibres, four large and seven small; the smallest again being thinly myelinated (Plate 8, figure A). The nerve fibres shown in the section were examined in the osmic/glycerine preparation of the muscle nerve (Plate 8, figure B). The branches and distribution of these nerve fibres as determined from intramuscular tracing are shown in Table 4. Two large fibres with stem diameters of 13.0 μ (fibre D) and 13.5 μ (fibre E) showed a pure skeletomotor distribution. The two other large fibres with diameters of 9.0 μ (fibre m^1) and 12.5 μ (fibre m^2) gave rise to branches supplying both extra- and intrafusal muscle fibres, i.e. were β. The remaining seven γ fibres, composed of two fibres with a stem diameter of 7.5 μ (fibre n & o), two of 5.5 μ (fibres l & m) and one each of 5.0 μ (fibre k), 4.0 μ (fibre j) and 2.5 μ (fibre h), provided the main fusimotor supply to four spindles in this muscle. As in the previous muscle, there was no evidence of size relationship between the diameters of the γ-stem fibres and their intramuscular fusimotor branches; for example, fibre o (stem diameter 7.5 μ) and k (5.0 μ) produced intramuscular

Plate 8. The motor supply to the first deep lumbrical muscle of cat C167.

Figure A. Osmic section of the de-afferentated nerve cut approximately 12 mm from its entry into the muscle.

Figure B. Teased osmic preparation of same nerve approximately 1 mm from its entry into the muscle. h - o: γ fibres (h is thinly myelinated). D,E: α fibres. m^1, m^2 : β ('mixed') fibres (cf. Table 4).

(Adal & Barker, 1965a)

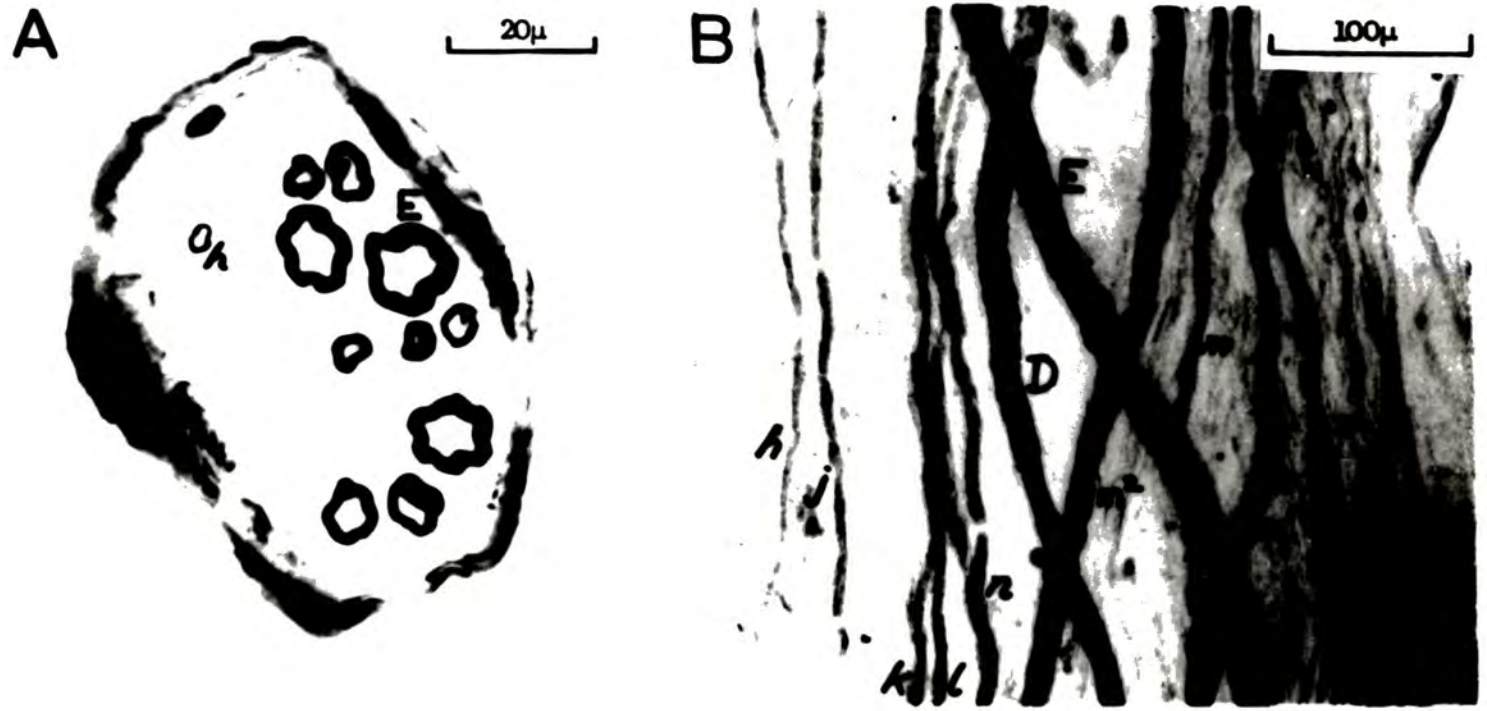

A
20µ
E
B
100µ
E
D

Table 4. Intramuscular branching of fusimotor fibres innervating the first deep lumbrical muscle of cat C167.

C 167 1st deep lumbrical			FUSIMOTOR BRANCHES diameter (μ) at spindle entry				SKELETOMOTOR BRANCHES no. & diameter (μ)	
			Sp I	Sp II	Sp III	Sp IV	terminal	others
STEM MOTOR FIBRES diameter (μ) in muscle nerve	γ	h : 2.5	2.5					
		i : 4.0		4.0				
		k : 5.0	3.0, 3.5	2.5				
		l : 5.5	4.0	3.5				
		m : 5.5			3.0, 3.5, 3.5			
		n : 7.5				3.5, 4.0, 4.5		
		o : 7.5		3.5, 4.0		2.5, 3.0, 3.0 3.0 3.5		
	β	m^1 : 9.0	3.0, 3.5	4.0	2.5	3.0	3.0, 3.0	6.5
		m^2 : 12.5			3.0		7 @ 2.5–4.0	9 @ 4.5–7.5
	α	D : 13.0 E : 13.5						

branches of both γ_1 and γ_2 calibre. The thinly-myelinated γ-stem fibre h (2.5 μ) also supplied only a single spindle without previous intramuscular branching.

Another study in the 2nd DL of cat C167 showed much the same sort of findings. An osmic section of the muscle nerve revealed the presence of eleven fibres, all thickly myelinated except for the smallest one which was thinly myelinated. Intramuscular tracing showed that seven fibres, fibre F (stem diameter 7.5 μ), G (11.5 μ), H (12.5 μ), J, K, L, (13.0 μ each) and M (13.5 μ) were purely skeletomotor in nature; three (fibres p, q and r, stem diameter 3.0 μ, 5.5 μ and 6.5 μ, respectively) were purely fusimotor; and one (fibre m3, 7.5 μ) was β (Table 5). The fusimotor branches supplied the three spindles present in the muscle. The occurrence of large γ-stem fibres dividing into large (γ_1) and small (γ_2) branches is demonstrated here by fibre r (stem diameter 6.5 μ). Its initial division, just within nerve-entry, gave rise to a large (6.0 μ) and a small (2.0 μ) branch. The smaller branch innervated a spindle (Sp III, Table 5) 7.5 mm away from the point of nerve-entry as a γ_2 fibre. The larger intramuscular branch divided once more at a

Table 5. Intramuscular branching of fusimotor fibres innervating the second deep lumbrical of cat C167.

C 167 2nd deep lumbrical			FUSIMOTOR BRANCHES diameter (μ) at spindle entry			SKELETOMOTOR BRANCHES no & diameter (μ)	
			Sp I	Sp II	Sp III	terminal	others
STEM MOTOR FIBRES diam. (μ) in muscle nerve	γ	p : 3.0		3.0			
		q : 5.5	2.5	3.0, 3.5			
		r : 6.5	2.0, 2.0, 3.0 3.5, 3.5	2.0	2.5		
	β	m^{s} : 7.5	3.0, 3.5	3.0, 3.0, 4.0	2.5, 2.5, 3.5 3.5	2.5, 3.5	
	α	F : 7.5 G : 11.5 H : 12.5 J : 13.0 K : 13.0 L : 13.0 M : 13.5					

distance of 2.2 mm from the point of nerve-entry into branches of 5.5 μ and 2.0 μ (Plate 7, figure B). The smaller branch innervated a spindle (Sp II, Table 5) 0.7 mm further on as a γ_2 fibre. The larger fibre gave rise to three γ_1 and two γ_2 fibres innervating another spindle (Sp I, Table 5) 3.7 mm further away. The consistency of size relationship between diameters of γ-stem fibres and their branches is again not exhibited as fibres q (stem diameter 5.5 μ) and r (6.5 μ) both gave branches of γ_1 and γ_2 calibre. The smallest thinly-myelinated γ fibre of stem diameter 3.0 μ (fibre p) innervated a single spindle entering it as a γ_1 fibre.

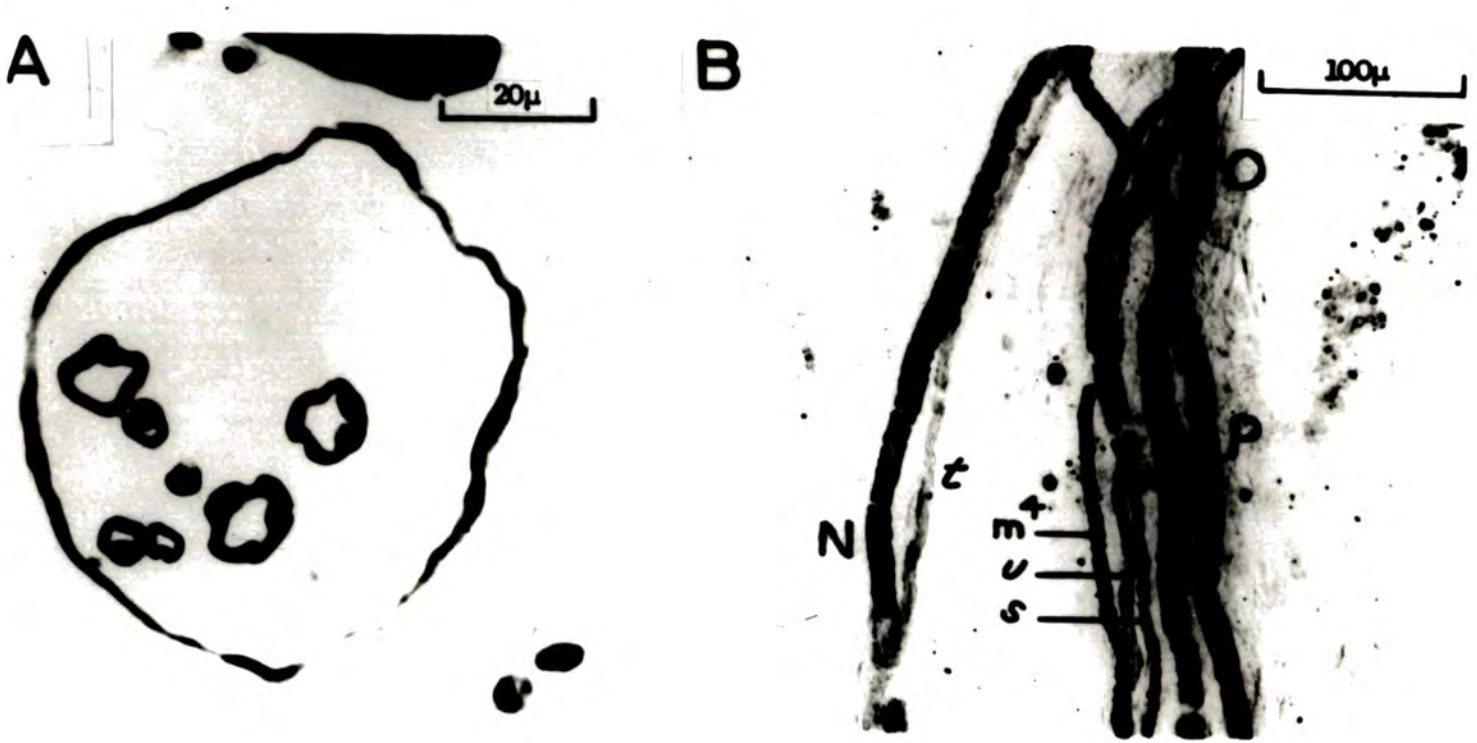
In the 1st DL of cat C175, a situation occurred where no thinly-myelinated γ-stem fibre was present. An osmic section of the muscle nerve taken approximately 10 mm from nerve-entry showed the presence of seven fibres, three large and four small, all thickly myelinated (Plate 9, figure A). These fibres may be seen in the teased osmic/glycerine preparation shown in Plate 9, figure B. Intramuscular tracing showed that the three large fibres, fibre N (stem diameter 12.5 μ), O (13.0 μ) and P (13.0 μ) were purely skeletomotor in nature (α); three small fibres,

Plate 9. The motor supply to the first deep lumbrical muscle of cat C175.

Figure A. Osmic section of the de-afferentated nerve cut approximately 10 mm from its entry into the muscle.

Figure B. Teased osmic preparation of same nerve approximately 2 mm from its entry into the muscle. s, t, u: γ fibres (all thickly myelinated). N, O, P: α fibres. m^4: β ('mixed') fibre (cf. Table 6).

(Adal & Barker, 1965a).


A
20μ
B
100μ
O
P
N
ES

fibre s, t and u of stem diameters 4.5 μ, 5.0 μ and 5.5 μ, respectively, were purely fusimotor (γ); and the remaining small fibre m^4 , 6.0 μ, was β (Table 6). The fusimotor supply to the four spindle present in the muscle was provided entirely by thickly-myelinated fibres, the smallest of which had a total diameter of 4.5 μ (fibre s, Table 6 and Plate 9, figures A & B). These branched so as to produce a mixture of γ_1 and γ_2 fibres, thus demonstrating that γ_2 fibres can be present in a muscle in the absence of thinly-myelinated γ-stem fibres in the motor supply.

In the only spindle analysed from the 2nd DL of cat C158, the fusimotor supply consisted of three γ-stem fibres, v, w and x (stem diameters 2.5 μ, 4.5 μ and 3.5 μ, respectively) and a larger fibre m^5 (8.5 μ), the last being a β fibre (Text-figure 3). The smallest of the γ fibres, v and x, also proceeded to enter the spindle without division in the intramuscular region. Fusimotor branches derived from the β fibre m^5 were of both 'γ_1' and 'γ_2' calibre.

Table 6. Intramuscular branching of fusimotor fibres innervating the first deep lumbrical of cat C175.

C 175 1st deep lumbrical			FUSIMOTOR BRANCHES diameter (μ) at spindle entry				SKELETOMOTOR BRANCHES no. & diameter (μ)	
			Sp I	Sp II	Sp III	Sp IV	terminal	others
STEM MOTOR FIBRES diam. (μ) in muscle nerve	γ	s : 4.5	2.0	2.0, 3.0	2.5	3.0		
		t : 5.0		3.0, 3.5	3.5	3.5		
		u : 5.5	3.5, 3.5	2.0, 3.0 3.0		4.0		
	β	m : 6.0		2.5, 2.5 2.5	2.0, 2.5 3.0, 3.0	4.0	2.0	
	α	N : 12.5 O : 13.0 P : 13.0						

2.5 The nature of branching and distribution of fusimotor fibres

A survey of the results shows that the γ-stem fibres in the lumbrical muscles of the cat have a stem diameter range of 2.5 - 7.5 μ and each stem fibre may contribute from one (without intramuscular division) to seven (e.g. fibre g, o & r) fusimotor branches (Tables 3, 4, 5 & 6 and Text-figure 3). Fusimotor branches from a single γ-stem fibre may innervate from one to five muscle spindles, usually from two to three. On the other hand, a single spindle may receive fusimotor fibre branches arising from as many as four different γ-stem fibres (e.g. Sp II, 1st DL, C158; Sp II, 2nd DL, C167). Generally speaking, the larger the diameter of the γ-stem fibre the greater is its frequency of intramuscular branching. From the tracing of a total of eighteen thickly-myelinated γ-stem fibres (4.0 - 7.5 μ), seven (fibres c, j, l, m, n, t and w) give branches of γ_1 calibre only (total diameters 3.0 - 4.5 μ); two (fibres b & e) produce γ_2 fibres only (total diameters 2.0 - 2.5 μ); and nine (fibres d, f, g, k, o, q, r, s and u) produce a mixture of both γ_1 and γ_2 calibre. All the five thinly-

myelinated γ-stem fibres traced (stem diameters 2.5 - 3.5 μ) show a similar nature in that each travels to a spindle and enters it without branching in the intramuscular region, three as γ_1 (fibres a, p & x) and two as γ_2 (fibres h & v) axons. These results show that the intramuscular branching of γ fibres is such that they do not supply γ_1 and γ_2 fibres to spindles in accordance with their stem diameters and degree of myelination as proposed by Boyd (1962).

2.6 The occurrence of β fibres

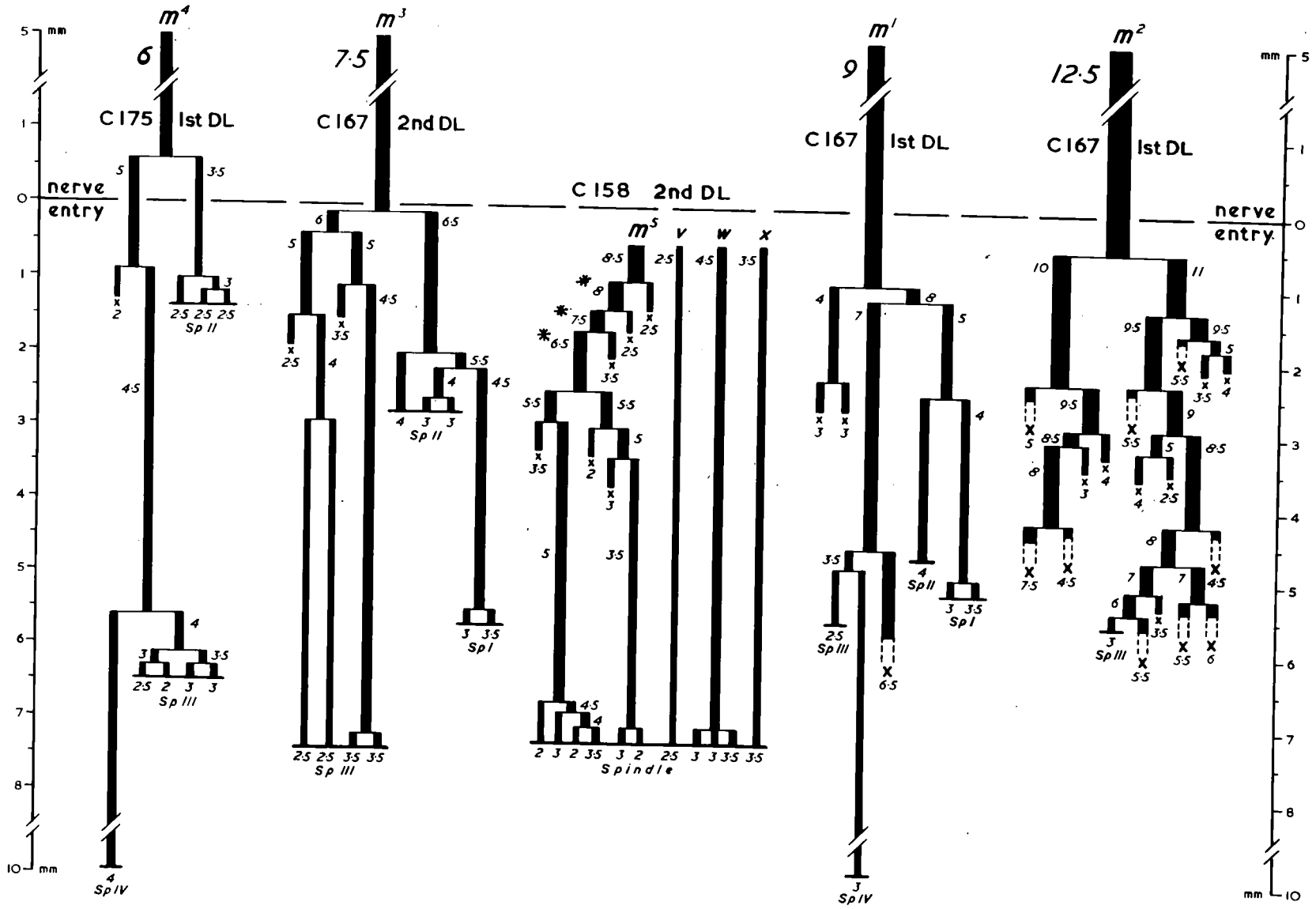
In the present study of five lumbrical muscles, the 1st DL of cat C158 was the only muscle that did not receive a β fibre in its motor supply. A total of five such fibres were found in the other four lumbrical muscles. They were all thickly myelinated; one produced a single fusimotor branch of γ_1 calibre and the rest gave fusimotor branches of both γ_1 and γ_2 calibres. Their distribution is shown diagrammatically in Text-figure 3; four of these fibres are also listed in Tables 4, 5 & 6 (fibres m^1 - m^4).

Text-figure 3. Schematic representation of the intramuscular branching and distribution of five β ('mixed') fibres, m^1 - m^5.

These fibres contribute to the innervation of four out of five cat deep lumbrical (DL) muscles studied. Scale indicates intramuscular length (mm) of each fibre and the levels at which spindle (Sp) entry or extrafusal (X) innervation occurs. Average total diameters (μ) of the fibres and their branches are indicated by the numbers placed in relation to them.

* Divisions and branches of fibre m^5 photographed at this level in Plate 15.

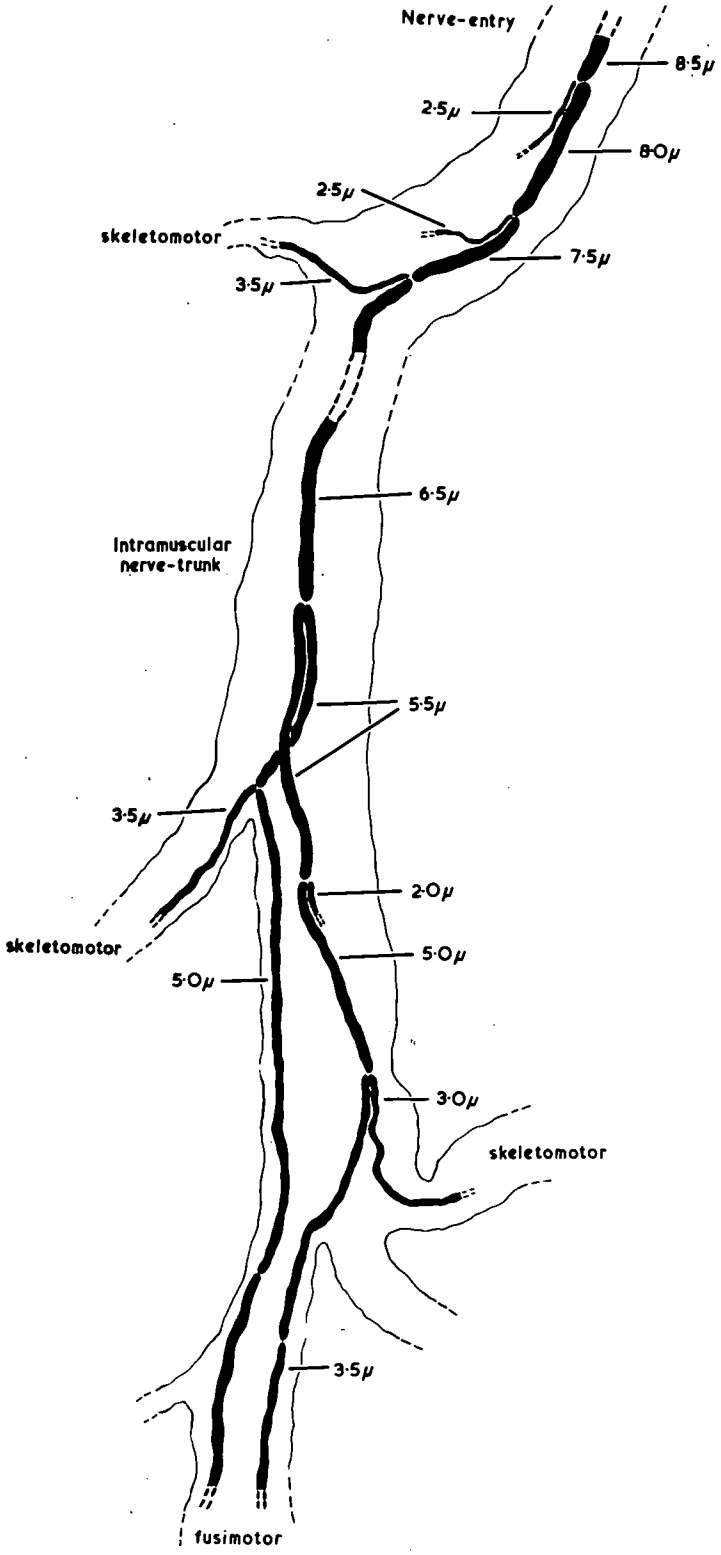
(Adal & Barker, 1965a).


m4
6
C175 1st DL
m3
7·5
C167 2nd DL
C158 2nd DL
m5
V
W
X
m1
9
C167 1st DL
m2
12·5
C167 1st DL
nerve entry
nerve entry
mm
mm
mm
mm
Sp II
Sp III
Sp I
Sp IV
Spindle

The β fibre $\underline{m}^4$ (stem diameter 6.0 μ), of the 1st DL in cat C175 gave fusimotor branches of γ_1 and γ_2 calibre to supply three spindles. A single skeletomotor fibre branch of 2.0 μ innervated an extrafusal muscle fibre. In the 2nd DL of cat C167, β fibre $\underline{m}^3$ (stem diameter 7.5 μ) was involved in supplying fusimotor branches also of both γ_1 and γ_2 calibre to three spindles. There were two fibre branches of 2.5 μ and 3.5 μ that were skeletomotor in nature. In the only spindle analysed in the 2nd DL of cat C158, a β fibre $\underline{m}^5$ (diameter 8.5 μ) was found to be involved in the fusimotor supply. It gave rise to as many skeletomotor fibre branches as fusimotor branches (six branches of each kind). The fusimotor fibre branches were of both γ_1 and γ_2 calibre. Text-figure 4 shows a camera lucida tracing of β fibre $\underline{m}^5$ in its course through the intramuscular nerve-trunk, giving off branches as shown diagrammatically in Text-figure 3. Plate 15 shows two divisions within the intramuscular nerve-trunk of the same nerve fibre; these divisions are in relation to those asterisked in Text-figure 3. The β fibre $\underline{m}^1$ (stem diameter 9.0 μ) of the 1st DL of cat C167, gave fusimotor branches to supply four spindles, and skeletomotor branches of a larger proportion, in having two 3.0 μ

Text-figure 4. Camera lucida drawing of β fibre m^5, showing its nature of branching in the intramuscular region.

Tracing from an osmic/glycerine preparation of the second deep lumbrical muscle of cat C158. (cf. Text-figure 3).


Nerve-entry
8·5μ
2·5μ
8·0μ
2·5μ
skeletomotor
7·5μ
3·5μ
6·5μ
Intramuscular
nerve-trunk
5·5μ
3·5μ
2·0μ
skeletomotor
5·0μ
5·0μ
3·0μ
skeletomotor
3·5μ
fusimotor

branches and one 6.0 μ in size. The fusimotor fibre branches were again of both γ_1 and γ_2 calibre. In the 1st DL of cat C167, the β fibre $\underline{m}^2$ (stem diameter 12.5 μ) showed only a single fusimotor branch of γ_1 calibre contributing to a spindle, and the majority of the fibre branches were skeletomotor in nature.

In the sample of β fibres studied, the range of the total diameter is from 6.0 μ (fibre $\underline{m}^4$) to 12.5 μ (fibre $\underline{m}^2$) and between these two extremes, the nature of distribution varies from being predominantly fusimotor to predominantly skeletomotor, respectively. Fibre $\underline{m}^5$, with a total diameter of 8.5 μ, lies in midway between the two extremes. In this group of β fibres, two (fibres $\underline{m}^1$ & $\underline{m}^3$) contribute to the innervation of all spindles in a muscle; one (fibre $\underline{m}^4$) supplies branches to three out of four spindles and two (fibres $\underline{m}^2$ & $\underline{m}^5$) supply a single spindle only.

Only by tracing the full course of these fibres has it been possible to reveal the mixed nature of their distribution. The fact that they have eluded convincing demonstration by previous histologists (see Barker & Chin,

1961, for references) may be attributed to the restriction of observations to the site of the spindle and its immediate surroundings; and to the nature of the techniques employed, which usually omitted the essential preliminary of de-afferentation.

3. A study of innervation ratios

The innervation ratios (i.e. the average number of extrafusal muscle fibres innervated by branches of a single motor nerve) given by the majority of previous investigators (for references, see Introduction) have been calculated by dividing the estimated or counted number of extrafusal muscle fibres within a muscle by the estimated or counted number of motor fibres supplying it. In such calculations, the whole of the motor component is taken to supply extrafusal muscle fibres only, while the fusimotor supply to the intrafusal muscle fibres of the spindles in the muscle is neglected. In view of the considerable proportion of fusimotor fibres in the motor component of a muscle nerve, such innervation ratios are therefore comparatively low; each skeletomotor nerve fibre should innervate more extrafusal muscle

fibres than those indicated by the innervation ratios from previous investigators. Innervation ratios of fusimotor fibres in relation to intrafusal muscle fibres have not, as yet, been determined. An estimation for such a ratio requires the combined data of the pure fusimotor component in the muscle nerve and the total number of intrafusal muscle fibres within a muscle.

In the present study, the nature of intramuscular branching of fusimotor fibres and the fusimotor component of the muscle nerve have been thoroughly investigated in the 1st DL muscle of the cat (Tables 3, 4, 5 & 6). Combining this data with those of the intrafusal muscle fibre counted in all the spindles within the muscle, it should be possible to calculate the innervation ratio of the intrafusal muscle fibres of this muscle. It is more indicative to refer to this ratio as the 'fusimotor innervation ratio'.

The skeletomotor nature of the muscle nerve has also been investigated separately from the fusimotor component. With the data of the extrafusal muscle fibre count, the innervated ratio between the skeletomotor

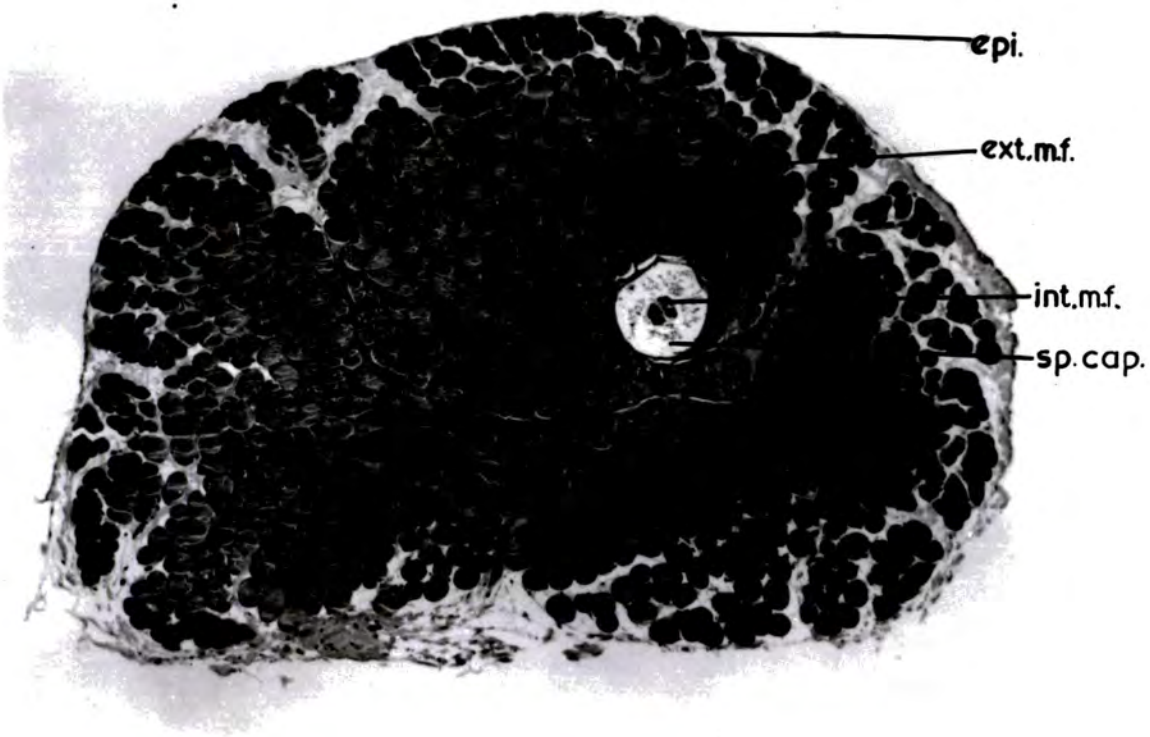
nerves and the extrafusal muscle fibres could be calculated. As this innervation ratio is determined between the pure skeletomotor component of the muscle nerve and the extrafusal muscle fibres, it is proposed to refer to it as the 'skeletomotor innervation ratio'.

3.1 Skeletomotor innervation ratios in lumbrical muscles

The extrafusal muscle fibre counts of the 1st DL were determined in two cats (see Methods 2.5). The number of extrafusal muscle fibres from the 1st DL of cat C165 was 1020 (Plate 10). Another count in the 1st DL of cat C181 was 800, giving an average of 910. The total number of motor fibres present in the de-afferentated muscle nerves of the 1st DL of cats C158, C167 and C175 was 10, 11 and 7, respectively, giving an average of 9.3. According to the usual method of calculation, i.e. dividing 910 extrafusal muscle fibres by 9.3 motor nerve fibres, the innervation ratio is therefore 1 : 98. This calculation, however, does not account for the distribution of the fusimotor and the mixed nerve fibres to the intrafusal muscle fibres. For example, in the

Plate 10. A section of the first deep lumbrical muscle taken for extrafusal muscle fibre count from cat C165.

T.S. 10μ, haematoxylin & eosin. ext.m.f. - extrafusal muscle fibre; int.m.f. - intrafusal muscle fibre; sp.cap. - spindle capsule, epi. - epimysium.


epi.
ext.m.f.
int.m.f.
sp.cap.

nerve to the 1st DL of cat C158, only three of the ten fibres in the motor component were purely skeletomotor in nature (Table 3, fibres A, B and C). With the extrafusal muscle fibre count of 910, the actual skeletomotor innervation ratio should be 1 : 303, i.e. 910 extrafusal muscle fibres divided by 3 purely skeletomotor nerve fibres. In cat C175, the same skeletomotor innervation ratio of 1 : 303 would be obtained since there were also three purely skeletomotor nerve fibres in the muscle nerve (Table 6, fibres N, O and P). In cat C167, however, the β fibre m^1 gave a more or less equal number of fusimotor and skeletomotor branches (Table 4 & Text-figure 3), and the number of skeletomotor fibres should therefore be 3.5 (Table 4, fibres D, E, m^2 & m^1). The skeletomotor innervation ratio here is therefore 1 : 260, i.e. 910 muscle fibres divided by 3.5 skeletomotor nerve fibres. Taken as a whole, the data indicates a skeletomotor unit in this muscle of approximately 1 : 300. The complete data are summarized in Table 7.

Table 7. Innervation ratios of skeletomotor fibres in the first deep lumbrical muscle of the cat.

Reference	No.fibres in motor nerve (a)	No.skeletomotor nerve fibres (b)	No.extrafusal muscle fibres (c)*	Usual ratio calculated (c/a)	Actual innervation ratio (c/b)
Cat C158	10	3	910	1 : 91	1 : 303
Cat C167	11	3.5	"	1 : 83	1 : 260
Cat C175	7	3	"	1 : 130	1 : 303
Average	9.3	3.1	"	1 : 98	1 : 300

*Average of two 1st DL muscles from cat C165 and C181.

3.2 Fusimotor innervation ratios in lumbrical muscles

3.21 Intrafusal muscle fibre counts

The intrafusal muscle fibre counts of the 1st DL of the cat were determined by serial transverse sections of the muscle (see Methods 2.5) in four cats, C165, C167, C181 and C182. In the 1st DL of cat C165, there were six muscle spindles provided with a total of 13 nuclear-bag fibres (range 1 - 3) and 24 nuclear-chain fibres (4 in each spindle); a total of 37 intrafusal muscle fibres. In cat C167, the 1st DL contained seven spindles composing of 18 bag fibres (range 1 - 4) and 24 chain fibres (range 3 - 4, except one spindle with one chain fibre only), giving a total of 42 intrafusal muscle fibres. In the 1st DL of C181, five spindles consisted of 11 bag fibres (range 2 - 3) and 25 chain fibres (range 4 - 6), resulting an aggregate of 36 intrafusal muscle fibres. In cat C182, the 1st DL showed the presence of five spindles having 13 bag fibres (range 1 - 3) and 19 chain fibres (range 3 - 5) giving a total of 32 intrafusal muscle fibres.

From the four 1st DL muscles studied, 23 muscle

spindles composed of 55 nuclear-bag fibres and 92 nuclear-chain fibres, yield a total of 147 intrafusal muscle fibres. The average number of intrafusal muscle fibres in each spindle was 2.4 bag and 4.0 chain fibres giving a total of 6.4 muscle fibres per spindle. The data are summarized in Table 8.

The total number of intrafusal muscle fibres in a spindle from the 1st DL shows a range of 4 - 8; the number of nuclear-bag fibres may range from 1 - 4 and the nuclear-chain fibres from 1 - 6. Examples of spindles having bag and chain fibres outside the average are spindles Sp 3, C165 & Sp 3, C167 (1 bag fibre); Sp 4, C167 (4 bag fibres); Sp 5, C167 (3 bag, 1 chain); Sp 4, C181 (6 chain fibres); and Sp 2, C182 (1 bag, 5 chain). Taken as a whole in each individial cat, the muscle spindles in cat C182 have more bag fibres while those in cat C181 have more chain fibres than the average. Plate 11 & 12 show some examples of muscle spindles with different ratios of nuclear-bag and nuclear-chain fibres.

3.22 Direct calculation of fusimotor innervation ratios

The figure of 6.4 muscle fibres per spindle must

Table 8. Intrafusal muscle fibre counts of the first deep lumbrical muscle from four cats, C165, C167, C181 and C182.

Reference	Number of spindles	Number of bag fibres	Number of chain fibres	Total number of intrafusal fibres
Cat C165	6	13	24	37
Cat C167	7	18	24	42
Cat C181	5	11	25	36
Cat C182	5	13	19	32
Total of four muscles	23	55	92	147
Average number of intrafusal muscle fibres per spindle		2.4	4.0	6.4

Plate 11. Variety of nuclear-bag/nuclear chain ratios in spindles of the first deep lumbrical muscle of the cat.

Figure A. A spindle most commonly found in this muscle, consisting of 2 bags and 4 chain fibres.

Figure B. A spindle with 2 bags and 5 chain fibres.

Figure C. A spindle with 2 bags and 6 chain fibres.

T.S. 10 μ, haematoxylin & eosin. b - bag fibre, c - chain fibre, cap. - capsule, n. - nerve supply, p.sp. - polar region of a neighbouring spindle, ext.m.f. - extrafusal muscle fibre.

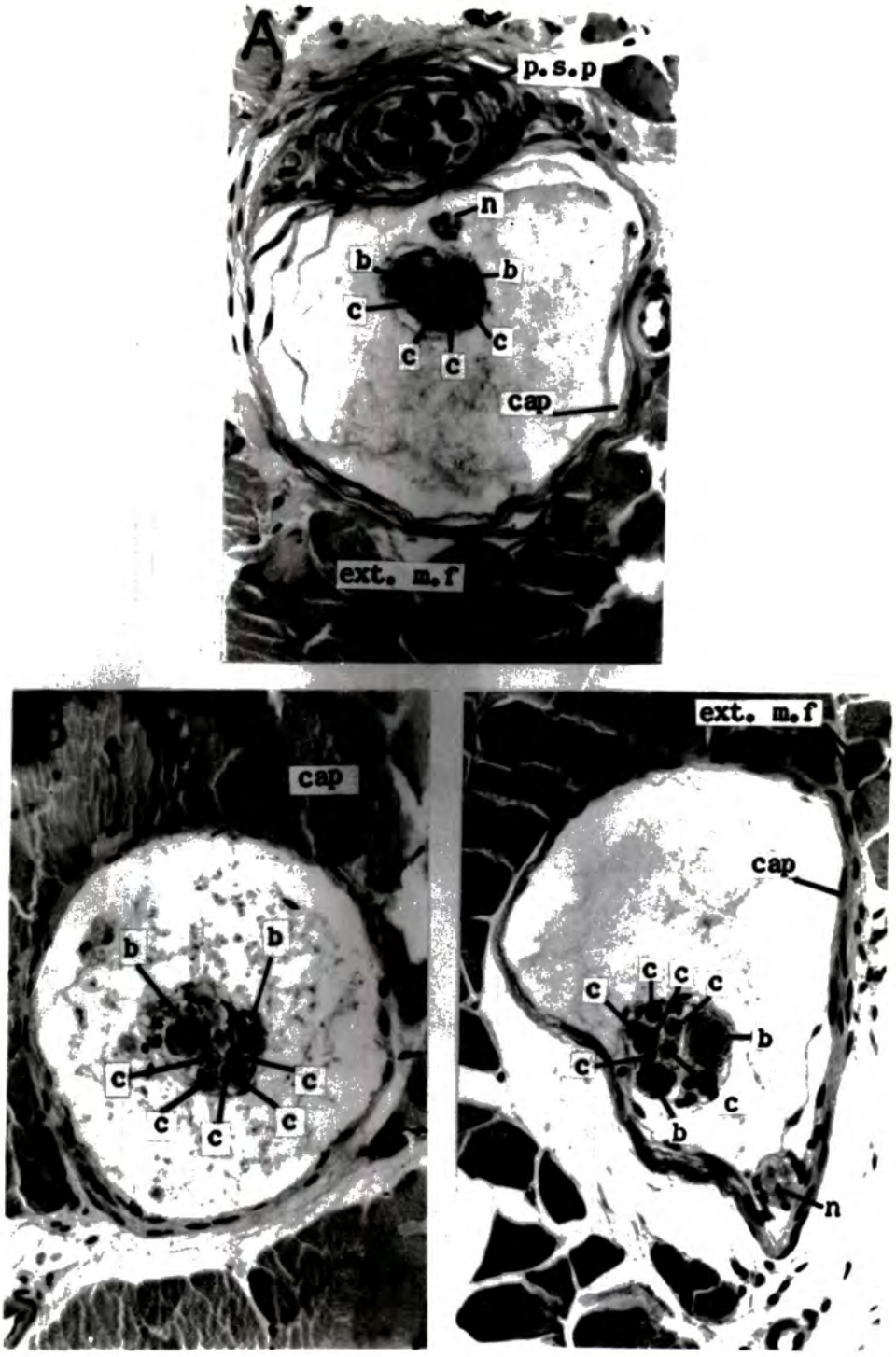

A
p.s.p
n
b
b
c
c
c
c
c
cap
ext. m.f
cap
b
b
c
c
c
c
c
ext. m.f
cap
c
c
c
c
b
c
c
b
n

Plate 12. Variety of nuclear-bag/nuclear-chain ratios in spindles of the first deep lumbrical muscle of the cat.

Figure A. A spindle with 1 bag and 4 chain fibres.

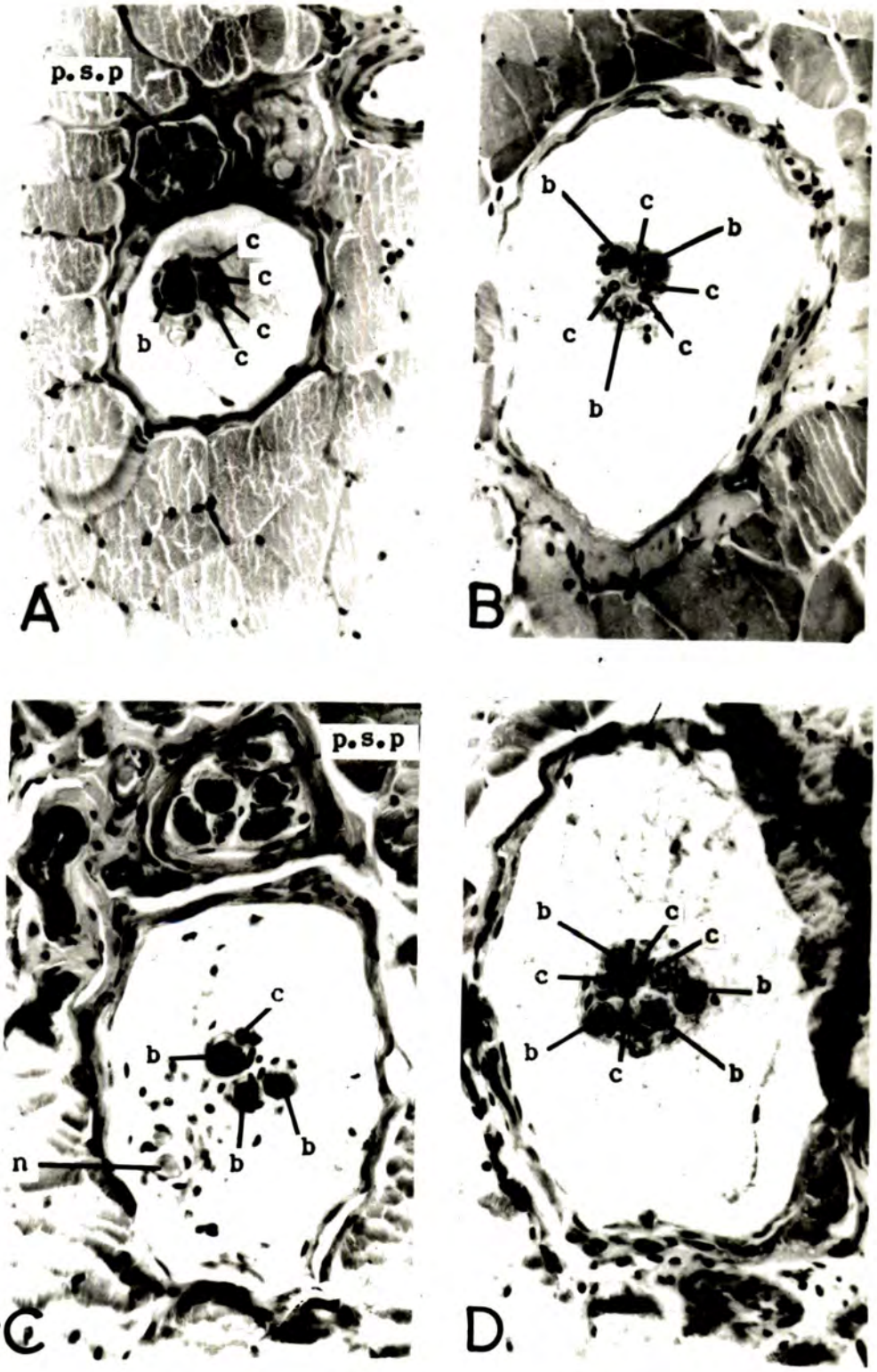
Figure B. A spindle with 3 bags and 4 chain fibres.

Figure C. A spindle with 3 bags and 1 chain fibre.

Figure D. A spindle with 4 bags and 4 chain fibres.

T.S. 10 μ, haematoxylin & eosin.

Abbreviations as Plate 11.


p.s.p
c
c
c
b
c
A
b
c
b
c
c
c
b
B
p.s.p
c
b
b
b
n
C
b
c
c
c
b
b
c
b
D

be doubled for the calculation of innervation ratio since each intrafusal muscle fibre consists of two contractile polar regions (muscle-fibre poles) separated by a relatively non-contractile area containing a bag or chain of nuclei. If this value is then divided by the number of purely fusimotor fibres in the muscle nerve, the innervation ratio of fusimotor fibres may be obtained. For example, there were six spindles in the 1st DL of cat C158 (Table 3, Sp I - VI) giving a total of 76.8 (i.e. 6 x 6.4 x 2) intrafusal muscle-fibre poles. The number of purely fusimotor fibres in its nerve supply was seven (Table 3, fibres a - g). The fusimotor innervation ratio is therefore 1 : 11 (i.e. 76.8 divided by 7) or one γ fibre supplying eleven muscle-fibre poles. Similarly, in the 1st DL of cat C167, four muscle spindles (Table 4, Sp I - IV) gave a total of 51.2 (i.e. 4 x 6.4 x 2) muscle-fibre poles. There were seven purely fusimotor fibres (Table 4, fibres h - o), and half of the β fibre m^1 should also be included in the fusimotor supply, giving a total of 7.5 fusimotor fibres. The fusimotor innervation in this muscle is therefore 1 : 6.8 (i.e. 51.2 divided by 7.5). Again, in the 1st DL of cat C175, four spindles (Table 6, Sp I - IV) with a total of 51.2 muscle-fibre

poles (i.e. 4 x 6.4 x 2) were supplied by four fusimotor fibres (Table 6, fibres s, t, u & m^4) giving a fusimotor innervation ratio of 1 : 12.8 (i.e. 51.2 divided by 4). The three determinations together produce an average ratio of 1 : 10. The results are summarized in Table 9.

3.23 Adjusted calculation of fusimotor innervation ratios

This method of calculation, however, does not allow for the fact that each pole of an intrafusal muscle fibre receives numerous motor endings indicating some degree of overlap in fusimotor fibre distribution. The following calculation by another approach would take the overlapping distribution into account.

In studying six polar regions from spindles in peroneal muscles of the cat, by the teased silver method, Barker & Ip (unpublished observations) have shown that the fusimotor fibres or fibre branches innervate an an average of 2.5 muscle-fibre poles (range 1 - 8). Assuming that each fusimotor fibre or fibre branch on entering a muscle spindle in the 1st DL muscles of cats

Table 9. Innervation ratios of fusimotor fibres in the first deep lumbrical muscle of the cat.

Reference	Number of spindles (Sp)	Number of muscle-fibres poles (Sp x 6.4 x 2)	Number of fusimotor fibres	Innervation ratio
Cat C158	6	76.8	7	1 : 11
Cat C167	4	51.2	7.5*	1 : 6.8
Cat C175	4	51.2	4*	1 : 12.8
			Average	1 : 10

*Occurrence of β fibres included.

C158, C167 and C175 (Tables 3, 4 & 6) also innervated 2.5 muscle-fibre poles, a total of 20 fusimotor fibres (γ and β included) would be involved in supplying 180 muscle-fibre poles giving a fusimotor innervation ratio of 1 : 9 (i.e. 180 muscle-fibre poles divided by 20 fusimotor fibres). If the β fibres were excluded, the ratio is 1 : 8.5.

Thus, from the data obtained for the fusimotor innervation ratio, the average fusimotor unit in the 1st DL muscle of the cat may be envisaged as comprising one γ or β fibre distributed to nine intrafusal muscle-fibre poles, and from the nature of intramuscular branching, it may be concluded that these are located in from one to five spindles, usually in two or three (Tables 3, 4 & 6).

3.24 Fusimotor innervation ratios calculated according to a dual motor innervation of muscle spindles

Using the same data of intrafusal muscle fibre counts and the fusimotor component of the nerve supply

to the 1st DL of the cat, the fusimotor innervation ratio calculated by Boyd's (1962) hypothesis would be quite different (see Table 10). Boyd's postulation is that in the spindle there is a segregated motor innervation of nuclear-bag and nuclear-chain fibres by the larger γ_1 and smaller γ_2 nerve branches derived from thickly and thinly myelinated γ-stem fibres, respectively, in the muscle nerve. Taking this into account, it should be possible, then to calculate the fusimotor innervation ratios of nuclear-bag and nuclear-chain muscle fibres separately; i.e. by dividing the total number of bag or chain fibres in all the spindles in a muscle by the number of thickly or thinly myelinated fusimotor fibres in the muscle nerve. For example, in the 1st DL of cat C158, six spindles with 14.4 bag fibres (i.e. 6 x 2.4 bag fibres per spindle, Table 8) or 28.8 bag-fibre poles (i.e. 14.4 x 2, Table 10) innervated by six thickly myelinated γ-stem fibres (Table 3 & Plate 6, figure A) would give a fusimotor innervation ratio of 1 : 4.8 for nuclear-bag fibres. On the other hand, six spindles produce 24 chain fibres (i.e. 6 x 4.0 chain fibres per spindle, Table 8) or 48 chain-fibre poles (Table 10) innervated by one thinly myelinated γ-stem fibre (Table 3 & Plate 6, figure A) and the fusimotor

Table 10. Innervation ratios of thickly and thinly myelinated γ-stem fibres in the first deep lumbrical muscle of the cat, calculated according to Boyd's (1962) hypothesis.

Ref.	Number of spindles	Aver. no. chain-fibre poles (Sp x 4 x 2)	No. thinly myel. γ-stem fibres	Inner. ratio chain fibres	Aver. no. bag-fibre poles (Spx2.4x2)	No. thickly myel. γ-stem fibres	Inner. ratio bag fibres
C158	6	48	1	1 : 48	28.8	6	1 : 4.8
C167	4	32	1	1 : 32	19.2	6.5*	1 : 3.0
C175	4	32	0	?	19.2	4*	?

* Occurrence of β fibres included. ? No basis for calculation.

innervation ratio of chain fibres is therefore 1 : 48. Similarly, for the 1st DL of cat C167, the fusimotor innervation ratio of nuclear-bag fibres is 1 : 3.0 and chain fibre is 1 : 32. In the 1st DL of cat C175, however, the absence of thinly myelinated γ-stem fibre renders such calculations impossible.

The two estimations from the 1st DL of cats C158 and C167 produce an average fusimotor innervation ratio of approximately 1 : 4 for bag fibres and 1 : 40 for chain fibres. In other words, a thickly myelinated γ-stem fibre would branch to innervate four bag-fibre poles and a thinly myelinated γ-stem fibre would innervate forty chain-fibre poles. According to this calculation, the smaller, thinly myelinated γ-stem fibre would branch and innervate intrafusal muscle fibres ten times more than a larger, thickly myelinated one; i.e. smaller thinly myelinated γ-stem fibres have much larger fusimotor innervation ratios than larger, thickly myelinated γ-stem fibres.

3.3 Estimated innervation ratios of some other muscles

It is possible to re-assess the data on skeletomotor innervation ratios of some muscles previously investigated by other workers, and to estimate their fusimotor innervation ratios. As the purely skeletomotor or fusimotor components in these muscle nerves have not been ascertained, nor intrafusal muscle fibre counts made, the following assumptions have to be made in the re-assessments.

Firstly, fibres in the motor component of muscle nerves having a total diameter less than 8 μ (Leksell's 2 - 8 μ gamma group, 1945) are regarded as fusimotor in nature, and those larger than 8 μ as skeletomotor. This, however, would not include the occurrence of any β fibres, which for this exercise shall be ignored. Secondly, as intrafusal muscle fibre counts for other hindlimb muscles have not been made, the average number of 6.4 intrafusal muscle fibres per spindle, determined in the 1st DL muscle, will be used throughout for the calculation of fusimotor innervation ratios. This figure

of 6.4 intrafusal muscle fibres per spindle is probably a good average for all spindles in cat hindlimb muscles. For example, Barker & Gidumal (1961) gave an average of two large and four or five small intrafusal muscle fibres in spindles from the rectus femoris muscle. Boyd's (1962) average figures for intrafusal muscle fibres in spindles studied from cat tenuissimus, soleus and interosseous muscles were 2.2 bag fibres and 4.1 chain fibres. The value of 6.4 intrafusal muscle fibres per spindle is thus very close to these findings.

3.4 Re-assessment of some cat innervation ratios determined by previous workers

In one of Clark's (1931) determinations, the extrafusal muscle fibre count of the soleus muscle is 27,261 and the number of nerve fibres in the motor supply is 233. The innervation ratio obtained in the usual way gives a value of 1 : 117, and the average ratio from a number of his determinations is 1 : 120. In the present study, a count in the number of motor fibres in the de-afferentated nerve to the soleus muscle of cat C191 was 247 of which 130 (52.6%) were larger than 8 μ. We

may therefore assume that 52.6% or 123 motor fibres in Clark's soleus count were skeletomotor in nature. The re-assessed innervation ratio is obtained from dividing the number of extrafusal muscle fibres, 27,261 by 123 skeletomotor nerve fibres giving a value of 1 : 222 (Table 11).

In the determination by Hagbarth & Wohlfart (1952), the number of extrafusal muscle fibres in the tibialis anterior is 37,000 with a motor supply of 337 nerve fibres. The innervation ratio from the usual calculation gives a value of 1 : 110. In the present study of the motor supply to the same muscle in cat C191, 172 (58.5%) of the total 294 nerve fibres has been shown to be larger than 8 μ. This percentage would give 197 out of the 337 motor nerve fibres of Hagbarth & Wohlfart's (1952) to be skeletomotor in nature. The re-assessed innervation ratio is thus 1 : 188

The adjusted calculations presented here give an approximate innervation ratio of 1 : 220 for soleus as compared with 1 : 120 of Clark's (1931), and 1 : 190 for tibialis anterior as compared with a ratio of 1 : 110

Table 11. Re-assessed innervation ratios for the soleus and tibialis anterior muscles of the cat.

Muscle	No. of motor nerve fibres	Fibres > 8 μ %	Fibres > 8 μ no.	No. Muscle fibres	Inner. ratio usual calc.	Inner. ratio adjusted calc.	Discrepancy
Soleus	233*	52.6	123*	27,261* (Average 1 : 120)*	1 : 117*	1 : 222	83%
Tibialis anterior	337+	58.5	197+	37,000+	1 : 110+	1 : 188	73%

*Clark (1931); +Hagbarth & Wohlfart (1952).

calculated by Hagbarth & Wohlfart (1952) for this muscle. From these two determinations, the percentage difference in the values of innervation ratios calculated by the usual and the adjusted methods is between 73 - 83%.

3.5 Further cat fusimotor innervation ratios estimated

As mentioned above, the average number of 6.4 intrafusal muscle fibres per spindle is used for calculation of the total number of intrafusal muscle fibres in all the spindles within a muscle. In taking the mean spindle count of 56 for the soleus muscle (Chin, Cope & Pang, 1962), the number of intrafusal muscle-fibre poles would be 56 x 6.4 x 2 or 716.8. In the de-afferentated soleus nerve of cat C191, there were 117 fibres less than 8 μ out of a total of 247. The fusimotor innervation ratio in this case is 1 : 6.1 (i.e. 716.8 divided by 117, Table 12). Similarly, in the tibialis anterior muscle, a mean of 71 spindles (Barker & Chin, 1960) would give 71 x 6.4 x 2 or 908.8 intrafusal muscle-fibre poles. There were 122 fibres less than 8 μ out of a total of 294 in the de-afferentated nerve to the tibialis anterior

Table 12. **Fusimotor innervation ratios of soleus, tibialis anterior and mesial gastrocnemius muscles of the cat.**

Muscle	No. of motor nerve fibres	No. fibres < 8 μ(a)	Spindle count (Sp)	Total muscle-fibre poles(b) (Sp x 6.4 x 2)	Fusimotor inner.ratio (b/a)
Soleus	247	117	56+	716.8	1 : 6.1
Tibialis anterior	294	122	71#	908.8	1 : 7.5
Gastroc. mesial	431*	152*	62+	793.6	1 : 5.2

*Eccles & Sherrington (1930); #Barker & Chin (1960); +Chin, Cope & Pang (1962).

muscle of cat C191. The fusimotor innervation ratio in this muscle is thus 1 : 7.5 (i.e. 980.8 divided by 122, Table 12). In the mesial gastrocnemius muscle, 62 spindles (Chin et al., 1962) would produce 62 x 6.4 x 2 or 793.6 intrafusal muscle-fibre poles. In the de-afferentated nerve to the same muscle, Eccles & Sherrington (1930) found 152 fibres less than 8 μ in a total of 431. This would give a fusimotor innervation ratio of 1 : 5.2 (793.6 divided by 152) in this muscle (Table 12).

3.6 Possible relationship between fusimotor innervation ratios and muscle size

A comparison of fusimotor innervation ratios estimated so far shows a value of 1 : 10 in the small deep lumbrical muscle compared with 1 : 5.2 in the large gastrocnemius muscle in the cat. The tibialis anterior and soleus muscles, with sizes between the two above, have innervation ratios also between the two extremes. This seems to be an indication of a possible relationship between the size of a muscle and its fusimotor innervation ratio. In order to find out the extent of this possible relationship, further fusimotor innervation ratios have

been estimated for as many other muscles as possible, using the histological data of Barker & Chin (1960); Chin, Cope & Pang (1962) and Boyd (1962). Similar assumptions have been taken for the method of calculation as described above (cf. Results 3.3). Results from the estimations are shown in Table 13.

A superficial study of the muscles listed in accordance with their difference in weight and the innervation ratios (Table 13) does not show very convincing evidence for a relationship. However, if the muscles are grouped into three categories, with the small and large muscles at the extremes and the intermediate sizes in between, then the corresponding innervation ratios would show a relationship with the three different muscle-size groups. For example, the small muscle group including tibialis posterior and deep lumbrical muscles would have fusimotor branches from a γ-stem fibre innervating about 8 - 11 intrafusal muscle-fibre poles (fusimotor innervation ratios of 1 : 7.9 and 1 : 11, respectively, Table 13); similarly, for the intermediate muscle size group the approximate number of intrafusal muscle-fibre poles innervated by a γ fibre would be

Table 13. Estimated fusimotor innervation ratios of a number of hindlimb muscles in the cat.

Muscle	Mean wt.(gm)[+]	Total no.[*] motor fibres	No. γ[*] fibres (a)	Spindle number[+] (Sp)	Total muscle-fibre poles(b) (Sp x 6.4 x 2)	Fusimotor inner.ratio
1st DL	0.03	10	7	6	76.8	1 : 11
TP	0.78	90	50	31	396.8	1 : 7.9
FDL,m	1.06	300	110	48	614.4	1 : 5.6
Soleus	2.49	270	115	56	716.8	1 : 6.2
FDL, l	3.25	420	155	75	960.0	1 : 6.2
TA	4.57	350	165	71[#]	908.8	1 : 5.5
ST	6.41	700	380	114	1459.2	1 : 3.8
MG	7.34	480	180	62	793.6	1 : 4.4

DL deep lumbrical (present study, cat C158); TP tibialis posterior; FDL,m (l) flexor digitorum longus, mesial (lateral); TA tibialis anterior; ST semitendinosus; MG mesial gastrocnemius

[#] Barker & Chin (1960); [+] Chin, Cope & Pang (1962); [*] Boyd (1962).

(The fusimotor innervation ratios for TA and MG muscles being lower in these calculations than those in Table 12 is due to Boyd's,1962, figures for motor nerve fibres to these muscles are considerably high).

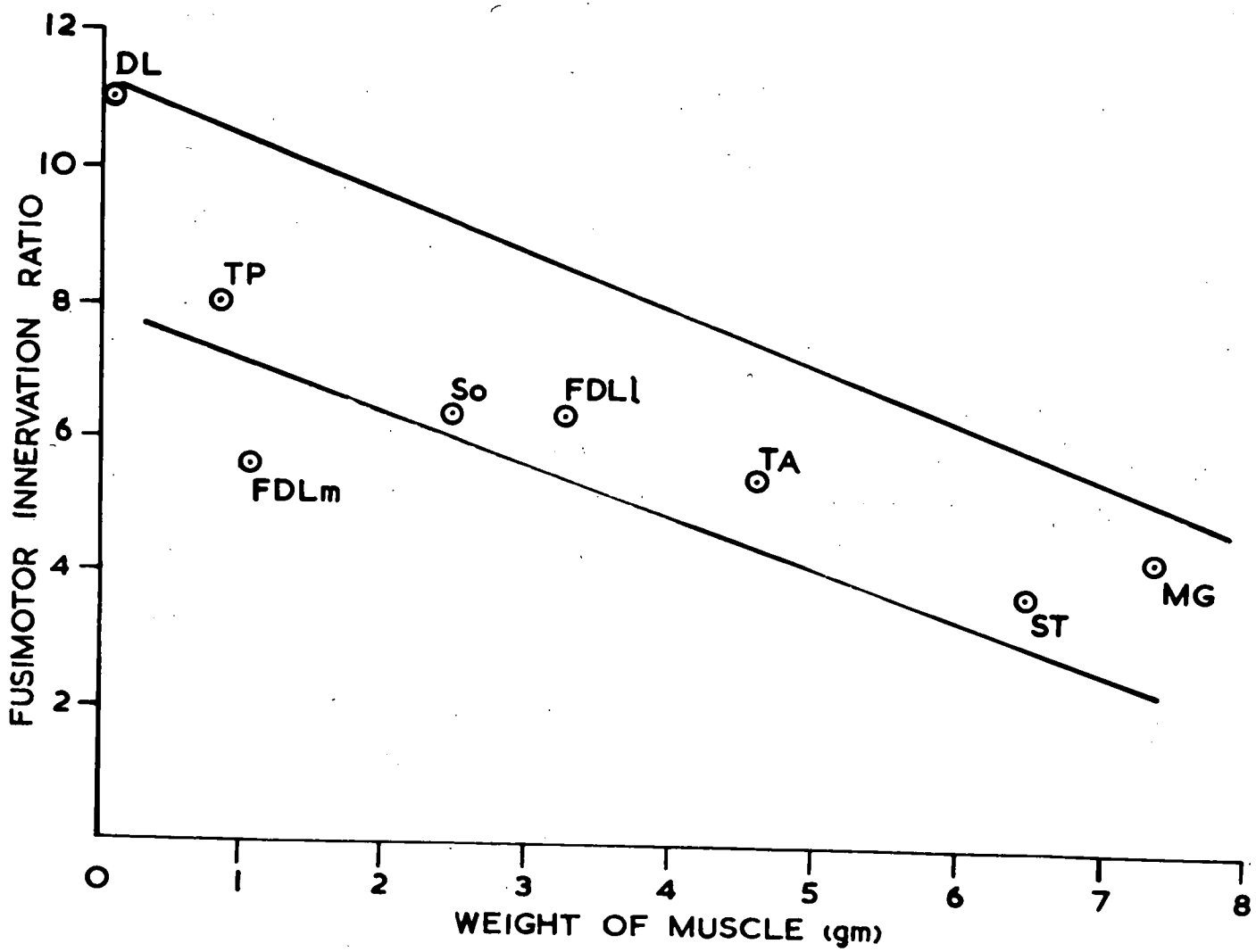
between about 5.5 - 6.5; and for the large muscle group it would lie approximately between 3.5 - 4.5. The general trend from the graph plotted with the estimated results shows a similar indication of the relationship between the fusimotor innervation ratio and the size of a muscle (Text-figure 5). The results indicate that the small muscles containing higher spindle densities and fewer γ-stem fibres in the motor supply, have higher fusimotor innervation ratios, while the large muscles having lower spindle densities and larger proportion of γ-stem fibres have lower fusimotor innervation ratios. By and large, muscles of intermediate sizes between the two extremes show a similar relationship.

4. Intramuscular study of skeletomotor fibres

4.1 Total skeletomotor fibres investigated

Intramuscular branching of skeletomotor fibres was investigated by the osmic/glycerine technique from the 1st and 2nd DL muscles of two cats, C158 and C167. The 1st superficial lumbrical muscle of cat C158 was examined for intramuscular skeletomotor distribution.

Text-figure 5. Estimated fusimotor innervation ratios of some hindlimb muscles of the cat plotted against the mean weight of the muscle. (cf. Table 13).


12
10
8
6
4
2
FUSIMOTOR INNERVATION RATIO
DL
TP
So
FDLl
FDLm
TA
MG
ST
0
1
2
3
4
5
6
7
8
WEIGHT OF MUSCLE (gm)

All the fibres in the muscles studied were analysed except in the 2nd DL of cat C158 where only one fibre was examined. There were three fibres examined from the 1st DL of both cats C158 and C167 (fibres A, B and C, Table 3; fibres D, E and m^2, Table 4), seven from 2nd DL of cat C167 (fibres F - M, Table 5), and one from 2nd DL of cat C158 (fibre m^5, Text-figure 3), giving a total of fourteen fibres analysed. The range in total diameter of these motor fibres was from 7.5 - 13.5 μ; there were four fibres of each with diameter of 13.5 μ and 13.0 μ, three of 12.5 μ, and one each of 11.5 μ, 8.5 μ and 7.5 μ. These fibres were examined in the proximal part of the muscle nerves by transverse sections stained by the Weigert-Pal technique (see e.g. Plate 6, figure A, 1st DL C158; Plate 8, figure A, 1st DL C167).

4.2 Equal and unequal branching of motor nerve fibres

The nerve fibres supplying a DL muscle usually lie in the main intramuscular nerve-trunk giving off small side-branches. A parent fibre may split up at a node into two, three, four, or even five daughter axons.

Of the 199 nerve fibre divisions observed from the fourteen motor fibres (12α, 2β), 172 were of the dichotomy type, 19 trichotomy, 6 quadri- and 2 penta-chotomy, representing approximately 86%, 10%, 3% and 1%, respectively. The calibre of daughter axons from a division of a parent fibre may be quite equal in size, or may differ quite markedly. If the difference in the total diameter between daughter axons is greater than 2μ, branching of the parent nerve fibre is regarded as unequal. If the difference in size of the daughter axons is 2μ or less, the branching of the parent nerve fibre is taken as equal. Using these criteria, the sample contained 110 equal branchings and 89 unequal, a percentage proportion of approximately 55 : 45. Various examples of branching are shown in Plate 13, figures A & B. The occurrence of unequal branching predominates in the main intramuscular nerve-trunk, the percentage proportion of unequal to equal branching was approximately 60 : 40 (3 : 2). Deeper into the muscle and the side nerve branches, equal branching was more frequent, the percentage ratio here was about 25 : 75 (1 : 4). Divisions into four or five daughter fibres occurred mainly in the deepest intramuscular regions of the nerve bundles, at the pre-terminal

Plate 13.

Intramuscular branching of skeletomotor fibres.

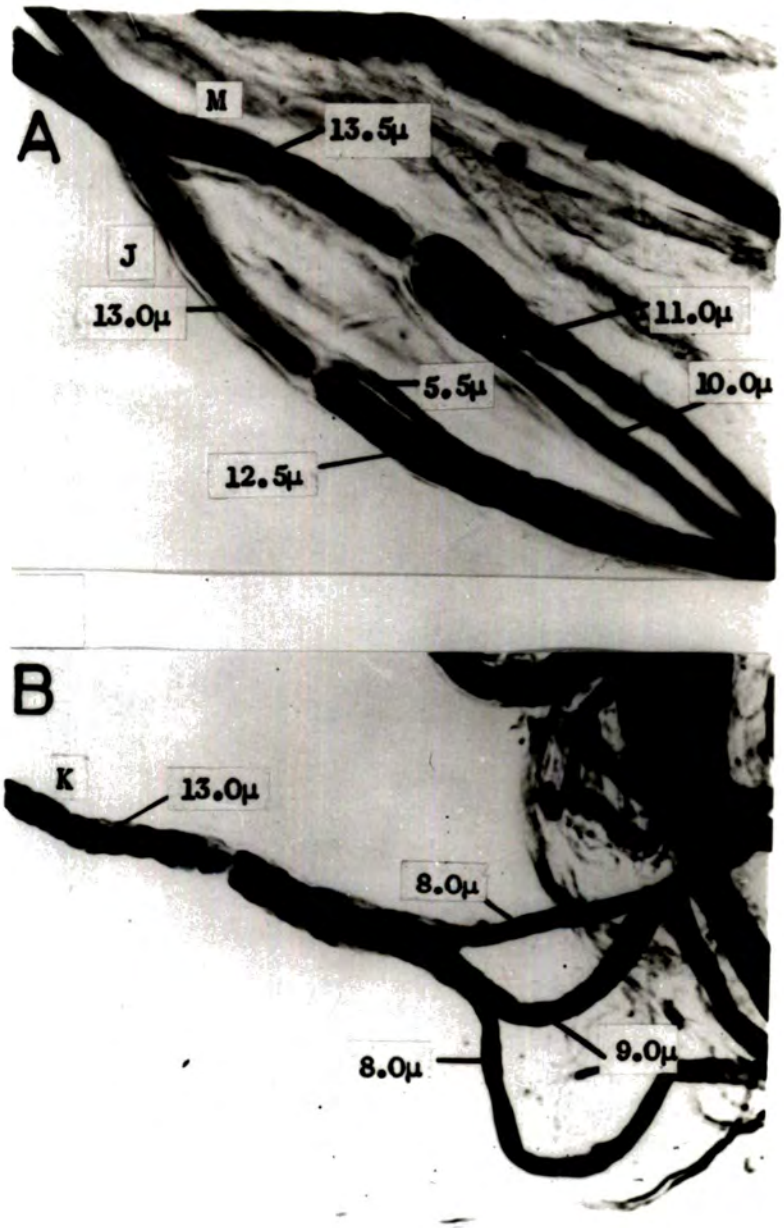
Figure A. Examples of nerve fibre branching by dichotomy:-

(i) equal branching where daughter fibres are very similar in size (10.0 μ & 11.0 μ from 13.5 μ, α fibre M, Table 5).

(ii) unequal branching where daughter fibres differ markedly in size (12.5 μ & 5.5 μ from 13.0 μ, α fibre J, Table 5).

Figure B. An example of nerve fibre division by trichotomy. Three daughter fibres of very similar sizes (8.0 μ, 8.0 μ & 9.0 μ) produced from an equal branching of a parent fibre (13.0 μ, α fibre K, Table 5).

Osmic stained, teased preparations from the 2nd DL muscle of cat C167.


A
M
13.5μ
J
13.0μ
11.0μ
10.0μ
5.5μ
12.5μ
B
K
13.0μ
8.0μ
8.0μ
9.0μ

or terminal levels.

4.3 The course of axons to small nerve trunks

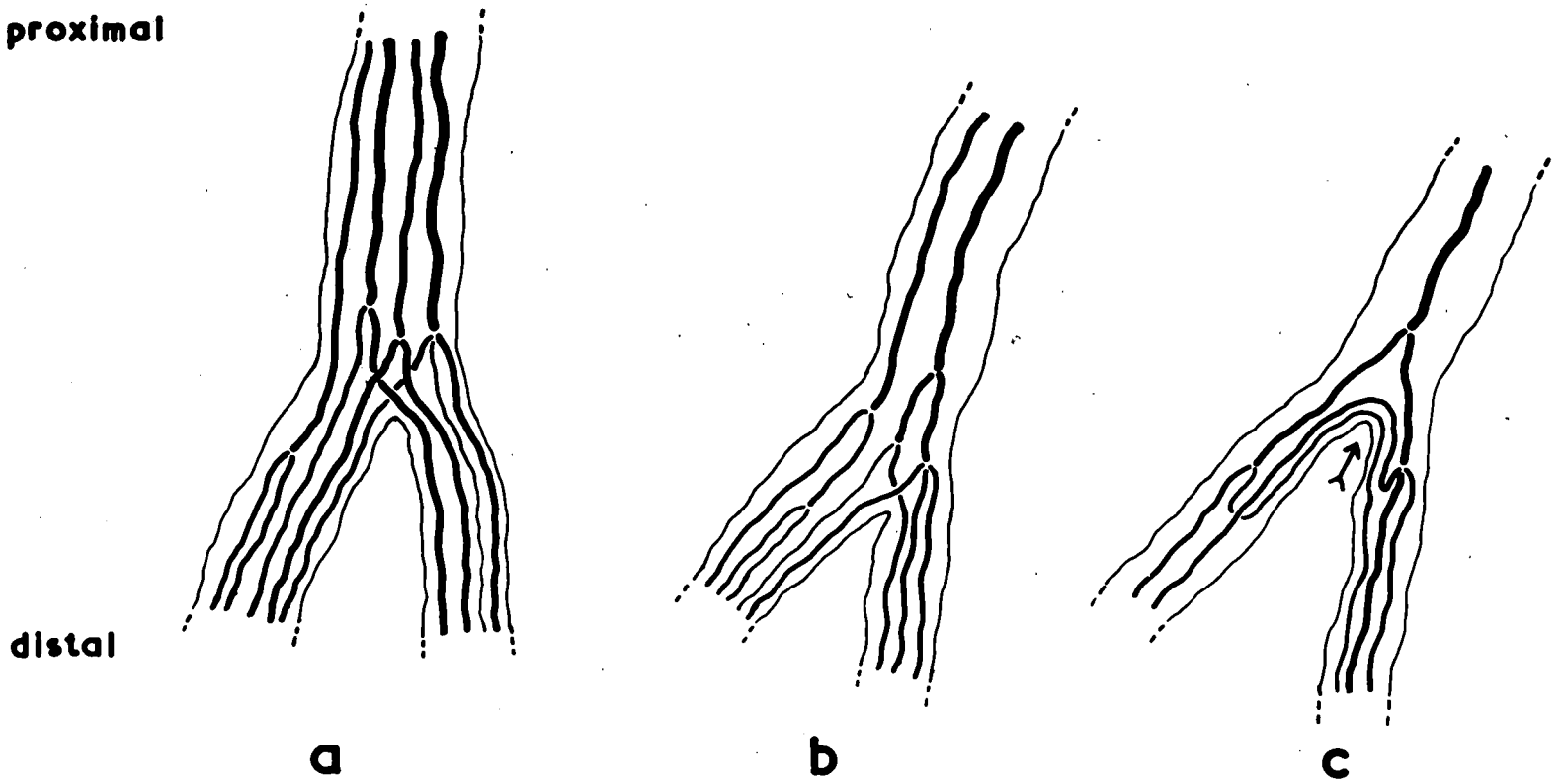
Initial divisions of axons occurred in the main nerve trunk at the junction of dividing into side branches; the region involved may be slightly above or below this level (Text-figure 6, a, b and c). Many axon divisions were encountered in the region of the main intramuscular nerve-trunk where two side branches were close together. Sub-branching of daughter axons might take place some distance away from the parent division, either at the next nerve-trunk bifurcation or might occur almost immediately, in some after only a very short internode of less than one millimetre (Text-figure 6, b). The shortest internode recorded was only 0.11 mm long with an external diameter of 10.5 μ, observed from the branching of fibre M in the 2nd DL of cat C167. However, no quantitative study of short internodes in relation to external diameter was attempted. Short internodal type of axon divisions were especially frequent in a particular type of axon branching (see 4.43 below). Daughter axons usually pursued separate routes, one

Text-figure 6. Illustrations of initial branching of skeletomotor fibres in lumbrical muscle nerves.

Figure a. Branching of skeletomotor fibres occur most frequently around the region of the muscle nerve dividing into smaller trunks.

Figure b. Consecutive division of nerve fibres may occur almost immediately in some cases, giving rise to very short internodes between two fibre divisions.

Figure c. Some daughter fibres from a nerve branching after the junction of the nerve-trunk division may travel recurrently (indicated by arrow) from one side branch to another. (cf. Plate 14, figures A & B).


proximal
distal
a
b
c

going off in a side branch while the other carried on in the main nerve trunk. There was also wide variation in the nature of the courses pursued by daughter axons depending on the relation between the point of division from the parent axon and the level of the side nerve branch. The point of axon division might be before or at about the level of a side branch, in which case the daughter axon might course off immediately after division (Text-figure 7, a), or it might travel for a small distance backwards in a recurrent nature before reaching the side branch (Text-figure 7, b). A daughter fibre might even exhibit a double recurrent nature by travelling further on immediately after fibre division, doubled back for some distance, then reverse its direction of travel once more before going off a side branch (Text-figure 7, c). In cases where the point of fibre division was after the level of the side branch, the daughter fibres also exhibited similar recurrent and double recurrent nature (Text-figure 7, d & e). Their recurrent routes at the side branch bifurcations made it difficult to deduce the direction of travel of the fibres without knowing their origin (Plate 14, figures A & B; Text-figure 6, c). Thus, an axon from a parent nerve division in

Text-figure 7. Illustrated varieties of nerve fibre branches travelling to side nerve trunks after fibre divisions.

Figure a. Fibre division occurring before a side nerve-trunk, fibre branch travels directly to side nerve-trunk.

Figure b. Fibre branch shows a recurrent route before travelling to side nerve-trunk.

Figure c. Fibre branch shows double recurrent routes before travelling to side nerve-trunk.

Figure d. Fibre division occurring after side nerve-trunk, fibre branch travels back immediately after division to the side nerve-trunk, giving a recurrent nature at the junction (indicated by arrow). (cf. Plate 14, figures A & B).

Figure e. Fibre branch shows double recurrent routes before travelling to side nerve-trunk, also giving recurrent nature at the junction.

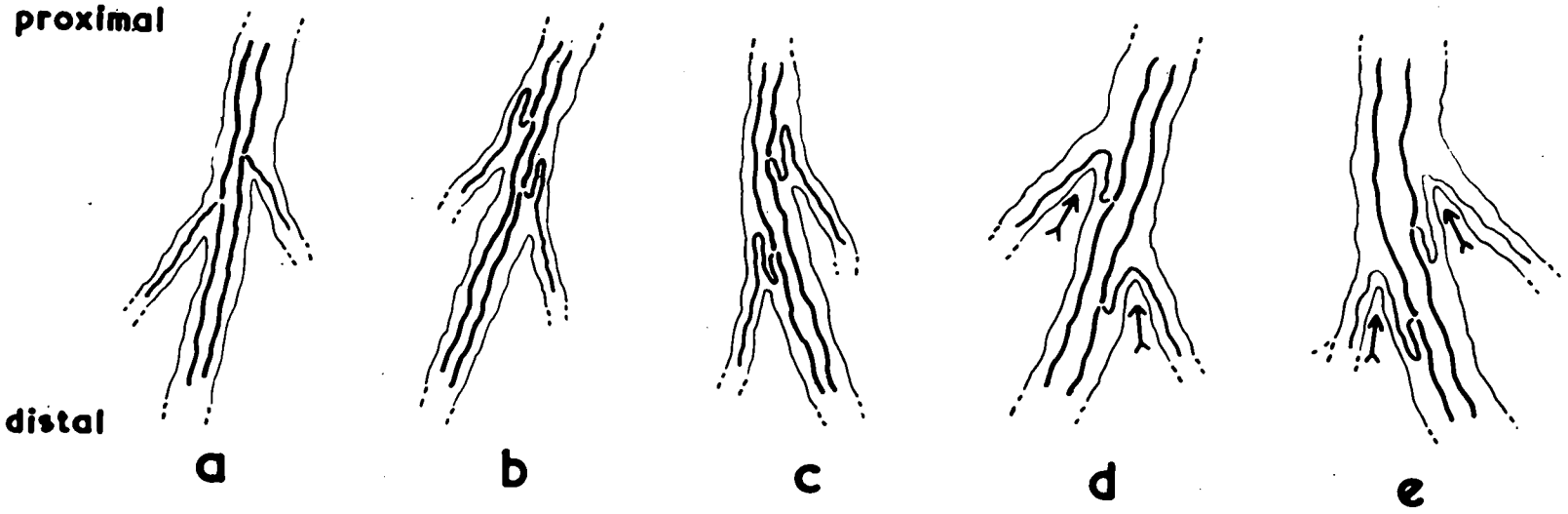
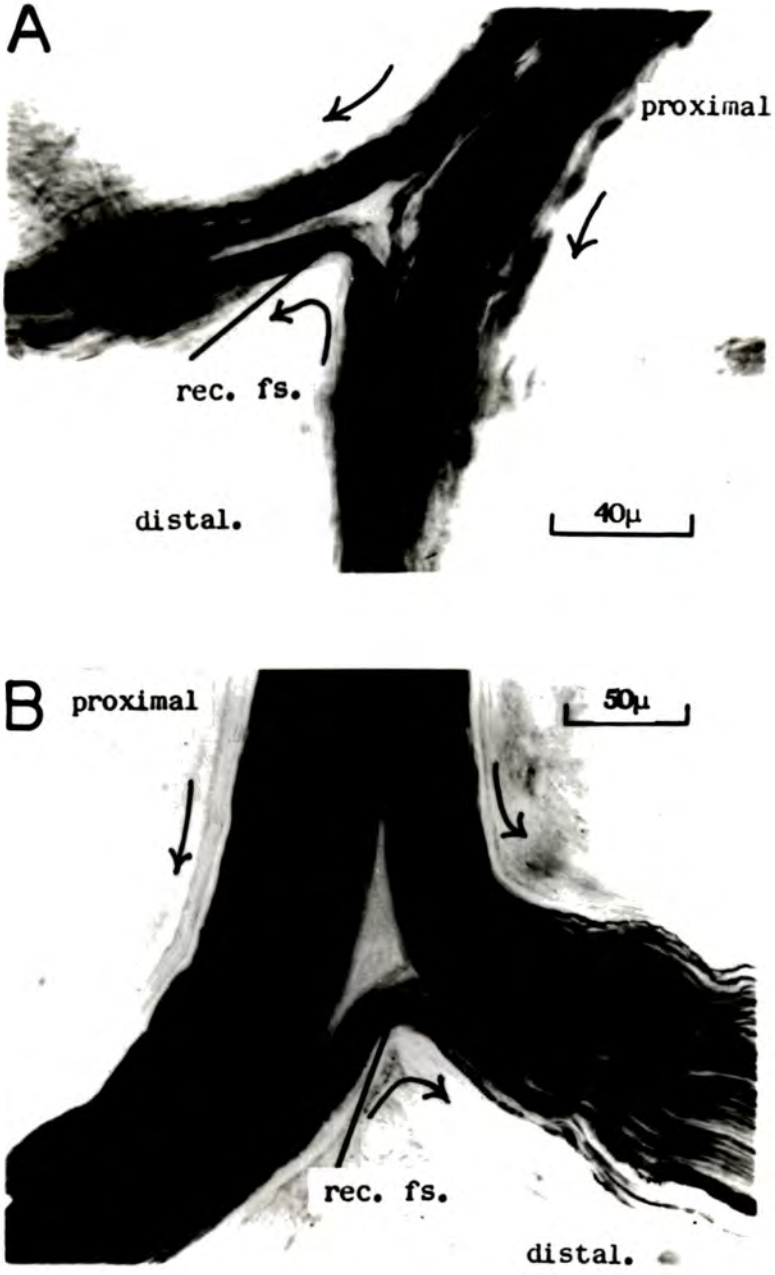

proximal
distal
a
b
c
d
e

Plate 14. Recurrent nerve fibres at the junction of nerve-trunk division.

Figure A. Intramuscular division of a nerve into two smaller nerve-trunks from the first deep lumbrical muscle of cat C158. Several motor nerve fibres can be seen at the junction to travel recurrently from one small nerve-trunk to the other. Osmic stained, teased preparation. Arrows indicate direction of travel of nerve fibres. rec.fs. - recurrent nerve fibres.

Figure B. Similar recurrent nature of nerve fibres in an intramuscular nerve-trunk division from the flexor digitorum longus, lateral head, of a normal cat. Teased silver preparation.


A
proximal
rec. fs.
distal.
40μ
B
proximal
50μ
rec. fs.
distal.

the main nerve trunk does not necessarily proceed by the shortest possible route to a side nerve trunk.

4.4 Types of skeletomotor fibre branching

It has long been known and accepted that motor nerve fibres in a muscle nerve increase to a large number within a muscle by numerous branching and sub-branching, but the exact nature of intramuscular branching has not been clarified. The manner in which skeletomotor fibres branch so as to supply their motor-units was found to occur in three main types, grouped according to their distribution into the form of a spatial configuration, and not according to their actual anatomical distribution within the muscle.

4.41 Pyramidal type (Text-figures 8, a & 9)

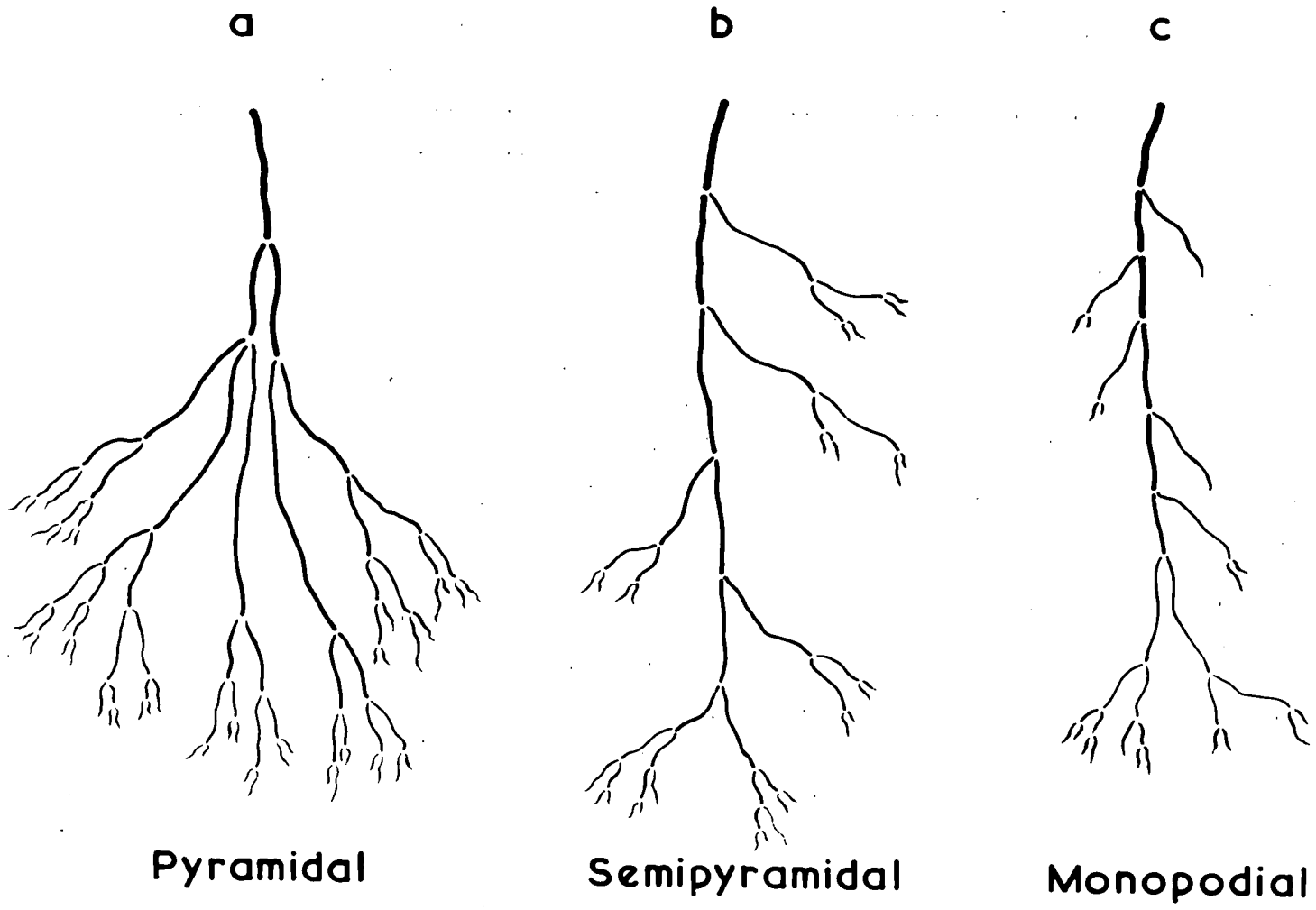
Daughter axons from an α parent may divide by dichotomy or trichotomy and the newly formed axons rarely differ very much in size in this type of branching. The initial few divisions are usually in the main intramuscular nerve-trunk. Deeper within the muscle,

Text-figure 8. Illustrations of the three main types of skeletomotor fibre branching.

Figure a. 'Pyramidal' type. Dichotomy or trichotomy of fibres, usually by equal branching (daughter fibres similar in size to within 2μ, see text). Fibre branches build up a pyramidal configuration in spatial distribution.

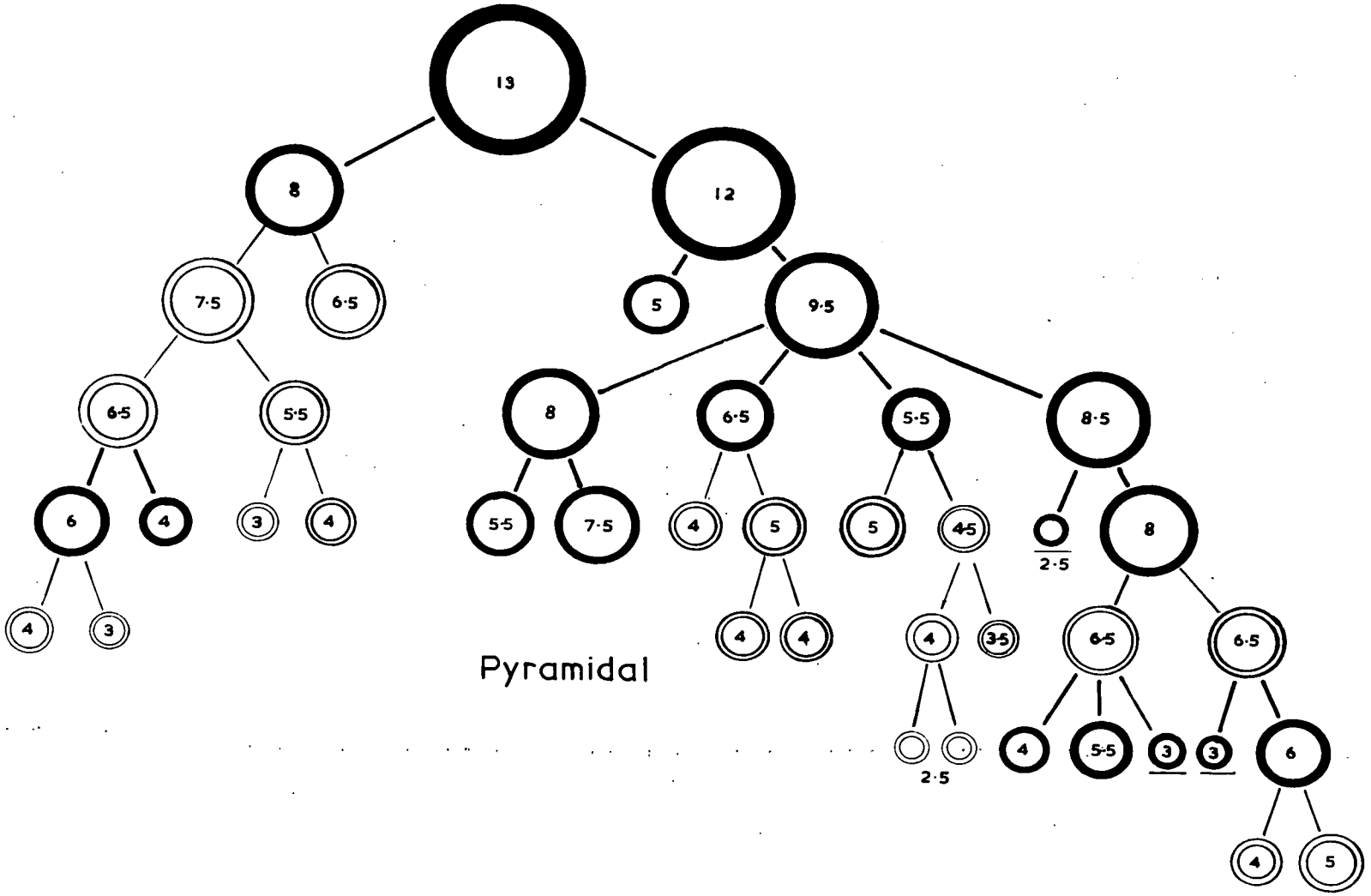
Figure b. 'Semipyramidal' type. Dichotomy of fibres mainly by unequal branching. Large daughter fibre persists in main intramuscular nerve-trunk, smaller daughter fibre travels to side nerve-trunk. Fibre branches build up into a semipyramidal configuration.

Figure c. 'Monopodial' type. Dichotomy of fibres but by very unequal branching producing marked difference in size of daughter fibres. Large daughter fibre persists in main nerve-trunk, small daughter fibre travels to side nerve-trunk.


a
b
c
Pyramidal
Semipyramidal
Monopodial

Text-figure 9. Schematic representation of 'Pyramidal' type of motor fibre branching.

Fibre D from the first deep lumbrical muscle of cat C167 (cf. Table 4), stem diameter 13.0μ shown at top of figure. A total of 19 fibre divisions traced, 11 by equal and 8 by unequal branching; 17 of the fibre divisions by dichotomy and one each by tri- and quadri- chotomy. (black circles, thick lines - unequal branching; white circles, thin lines - equal branching; terminal fibre branches underlined).


13
8
12
7·5
6·5
5
9·5
6·5
5·5
8
6·5
5·5
8·5
6
4
3
4
5·5
7·5
4
5
5
4·5
2·5
8
4
3
4
4
4
3·5
6·5
6·5
2·5
4
5·5
3
3
6
4
5

Pyramidal

daughter axons course off to side branches where divisions of a similar nature continue until a large number of fibres is formed at terminal levels in various depths of the muscle. An example is illustrated from fibre D in the 1st DL muscle of cat C167 (Text-figure 9). The α stem motor fibre measuring 13 μ divided and sub-divided to give a number of daughter axons. Daughter axons from divisions further into the muscle did not differ markedly, e.g. 6 μ and 4 μ from 6.5 μ; 4.5 μ and 5 μ from 5.5 μ; and 7.5 μ and 5.5 μ from 8 μ and so on. Continued branching and sub-branching of these axons built up the axon number into a typical pyramidal configuration.

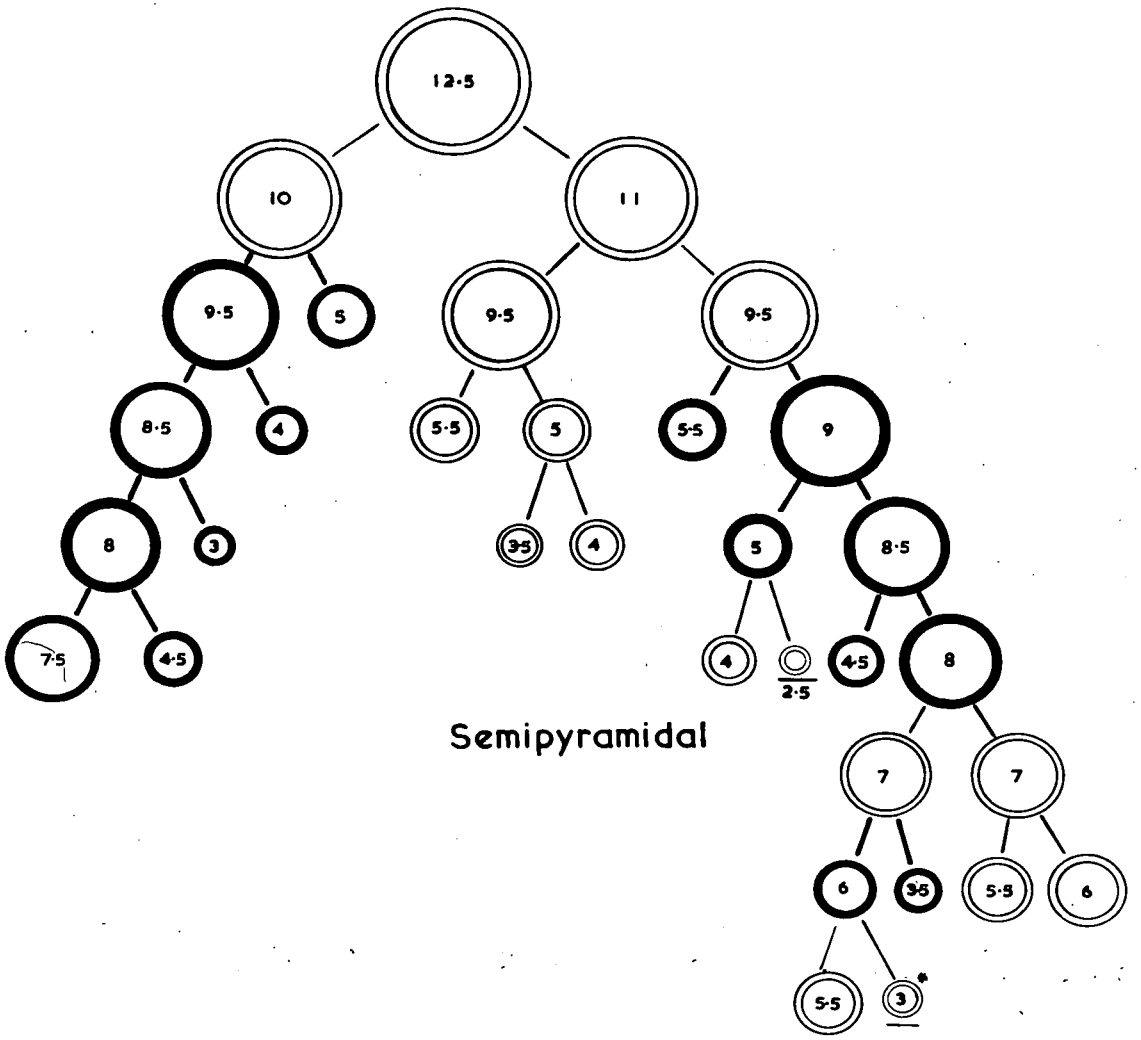
4.42 Semipyramidal type (Text-figures 8, b & 10)

Branching in this type is mainly by dichotomy and usually occurs in the main intramuscular nerve-trunk. There is greater difference between diameters of daughter axons, the smaller axon usually goes to a side nerve branch while the larger axon, not much less in size than the parent, persists in the main nerve trunk. Further dichotomy of the same nature occurs from this main daughter axon at the next side-branch. The smaller

Text-figure 10. Schematic representation of 'Semipyramidal' type of motor fibre branching.

Fibre $\underline{m}^2$ from the first deep lumbrical muscle of cat C167 (cf. Table 4), stem diameter 12.5μ. A total of 16 fibre divisions traced, equal and unequal branching being half and half. Usually, unequal branching predominates in this type of motor fibre branching but the more successful tracings of some terminal fibre divisions increased the number of equal branching. All fibre divisions by dichotomy. Representation of fibre branching as in Text-figure 9.

*fusimotor fibre branch.



Semipyramidal

daughter axons going to side branches may then divide once or twice before innervating muscle fibres, in a miniature pyramidal type of arrangement. The continuing larger daughter axon in the main nerve trunk finally terminates deeper in the muscle, presumably in a similar manner as those in the side branches. This type is shown as in Text-figure 8, b and exhibited by fibre $\underline{m}^2$ in the 1st DL muscle of cat C167 (Text-figure 10). The β parent motor fibre of 12.5 μ divided to give branches of 10 μ and 11 μ. Further divisions of the 10 μ branch gave the daughter pairs of 9.5 μ and 5 μ; 8.5 μ and 4 μ and so forth. The larger daughter axon decreased only a little in diameter in relation to the precedding parent fibre and persisted in the main nerve trunk. The smaller daughter axon might presumably terminate with a few more divisions down the side nerve branches, although further tracings in these cases were unsuccessful. On the other side, the 11 μ fibre gave rise to two 9.5 μ branches. Further divisions from one of these showed the same semipyramidal nature. Note that the parent β fibre is predominantly skeletomotor in nature, giving only one fibre branch at the terminal level to innervate a muscle spindle. The 9.5 μ branch, however, showed a typical

pyramidal type of branching, intermingled with other nerve fibres.

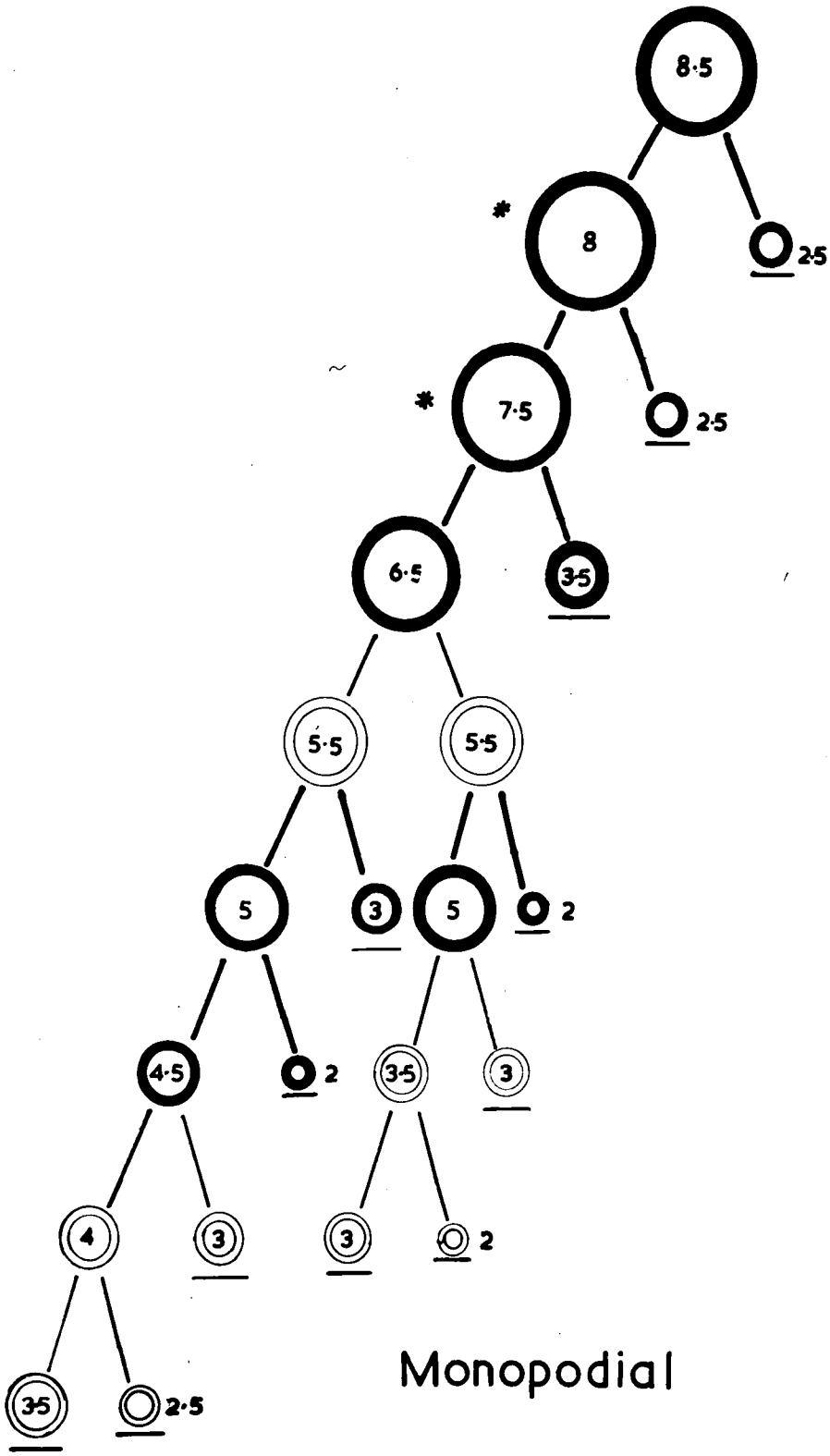
4.43 Monopodial type (Text-figures 8, c & 11)

This type of branching is shown when the growth of the main axon is accompanied by small branches being given off at intervals. Botanists use the term 'monopodial' to describe such branching in plants and it is therefore adopted here. Branching is mainly by dichotomy but the daughter axons differ greatly in size. These tiny off-shoots tend to occur at consecutive short internodes and they course off to the nearest side trunk where they terminate in muscle fibres, or at most undergo one more division before innervation. The main axon courses on down the main nerve trunk and finally terminates by further divisions prior to innervation (Text-figure 8, c). Fibre $\underline{m}^5$, from the 2nd DL of cat C158 demonstrated this type of branching (Text-figure 11). The β parent motor fibre of 8.5 μ divided just after nerve-entry to give two branches of 8 μ and 2.5 μ. Further divisions from the persisting larger branch gave consecutive daughter pairs of 7.5 μ and 2.5 μ; 6.5 μ and 3.5 μ, respectively

Text-figure 11. Schematic representation of 'Monopodial' type of motor fibre branching.

Fibre $\underline{m}^5$ from the second deep lumbrical muscle of cat C158 (cf. Text-figures 3 & 4 and Plate 15). Fibre diameter 8.5 μ after nerve-entry. A total of 11 fibre divisions, 6 by unequal and 5 by equal branching. Most of the equal branching were from divisions of terminal fibre branches. All fibre divisions by dichotomy. Representation of fibre branching as in Text-figure 9.

*axon divisions photographed in Plate 15.

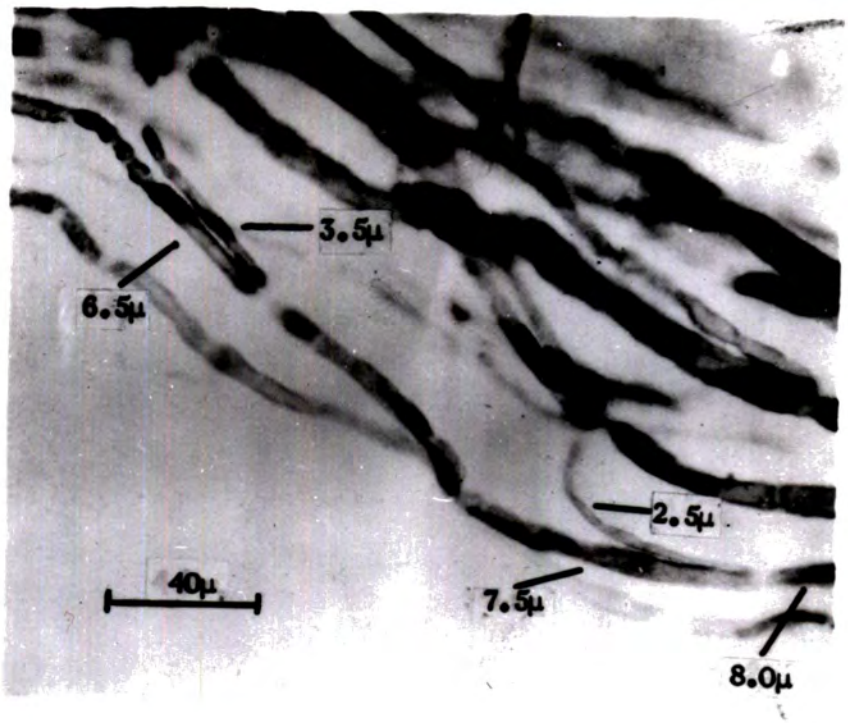


Monopodial

Plate 15.

'Monopodial' type of motor fibre branching.

Dichotomy from an 8.0 μ motor fibre into two daughter branches, one at 7.5 μ which is only slightly smaller than the parent, but differs markedly with the other branch at 2.5 μ. A similar nature is shown in the consecutive unequal fibre branching, giving branches of 6.5 μ and 3.5 μ (cf. Text-figures 4 & 11). Note the very short internodal length of 0.16 mm between the two fibre divisions. Osmic stained, teased preparation of fibre $\underline{m}^5$ from the second deep lumbrical muscle of cat C158.


3.5μ
6.5μ
2.5μ
40μ
7.5μ
8.0μ

(Plate 15 & Text-figure 11). Note the tiny axon branch, the very short internode of only 0.16 mm in length (down to about 0.10 mm in some cases) between the two divisions and the persisting main axon in the main intramuscular nerve trunk. The two smaller axon branches in this case travelled in the same side nerve trunk and terminated as skeletomotor fibres without any division. The distribution of this β fibre shows half its terminal branches being fusimotor and half skeletomotor.

von Thiel (1959), in a study of a few isolated motor nerve fibres in the pyriformis muscle of the cat, showed examples of nerve fibre divisions which he called 'collateral branching'. One branch from a fibre division was very thin compared to the other which did not differ very much in size from the parent nerve. This may probably be the initial few nerve fibre divisions of the 'monopodial' type, but no further quantitative analysis was given in his investigation, although there were indications that some other types of fibre branching occurred.

4.5 The frequency of occurrence of different types of branching

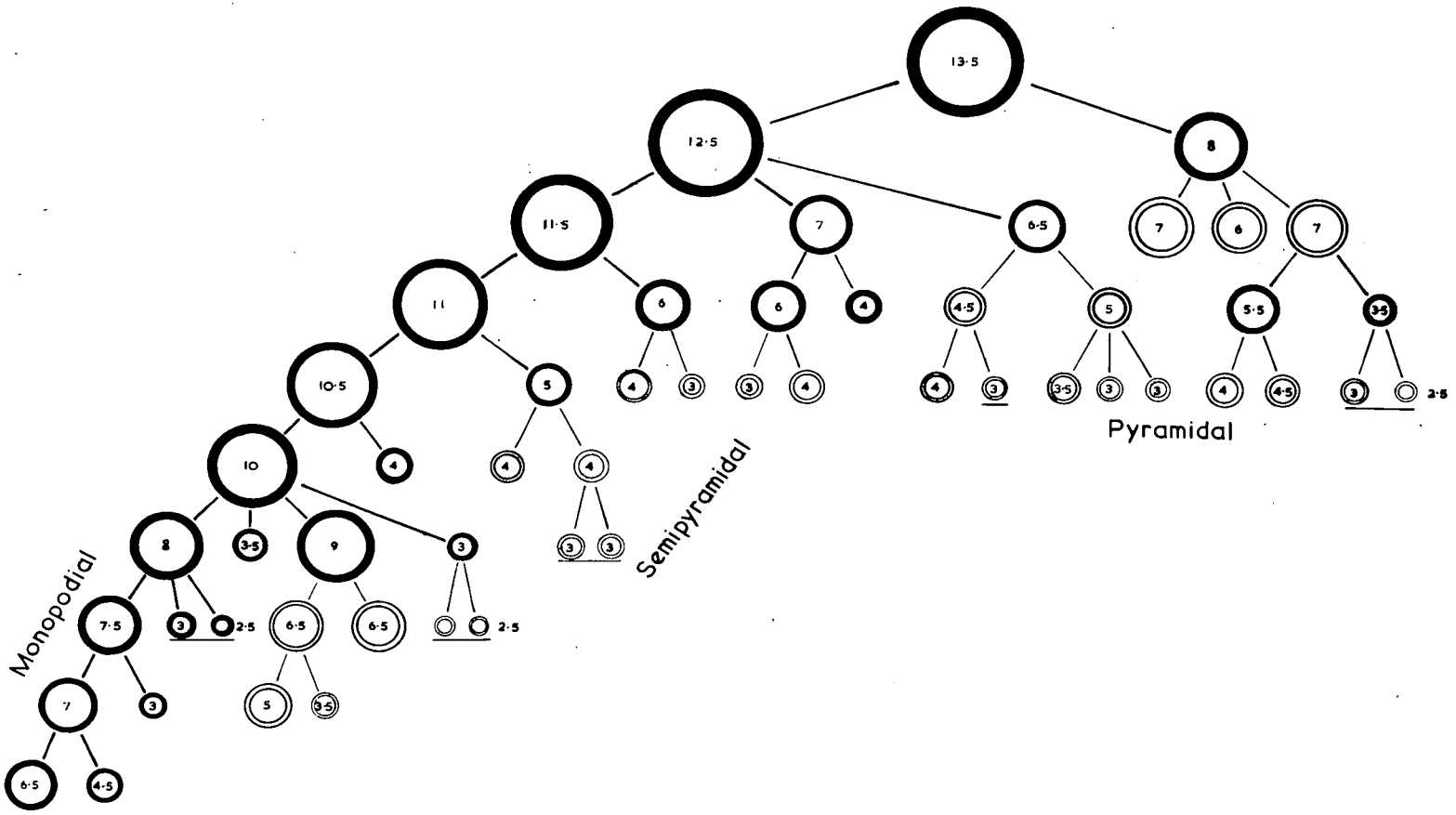
By far the most common is the pyramidal type of branching. It not only occurs frequently in intramuscular nerve trunks and side branches but is typical of most of the pre-terminal and terminal branching. At these levels, the large number of fibres is the result mainly of this type of branching. The semipyramidal type occurs to a lesser extent and the monopodial type is the least common. Daughter fibres from an α motor fibre may have different combinations of the three types of branching in various proportions. In fibre E, from the 1st DL muscle of cat C167 (Text-figure 12), the same α motor fibre has all the three types of branching in different proportions.

4.6 Pattern and extent of distribution of motor fibre branches

As motor nerve fibres are followed deeper into the muscle, their pattern of distribution and direction of 'flow' becomes more confused at the pre-terminal and

Text-figure 12. Schematic representation of three types of fibre branching exhibited by the same motor fibre.

Fibre E from the first deep lumbrical muscle of cat C167 (cf. Table 4), stem diameter 13.5μ. A total of 24 divisions traced, 11 by unequal and 14 by equal branching; 19 of the divisions by dichotomy, 4 by trichotomy and 1 by quadrichotomy. Representation of fibre branching as in Text-figure 9.

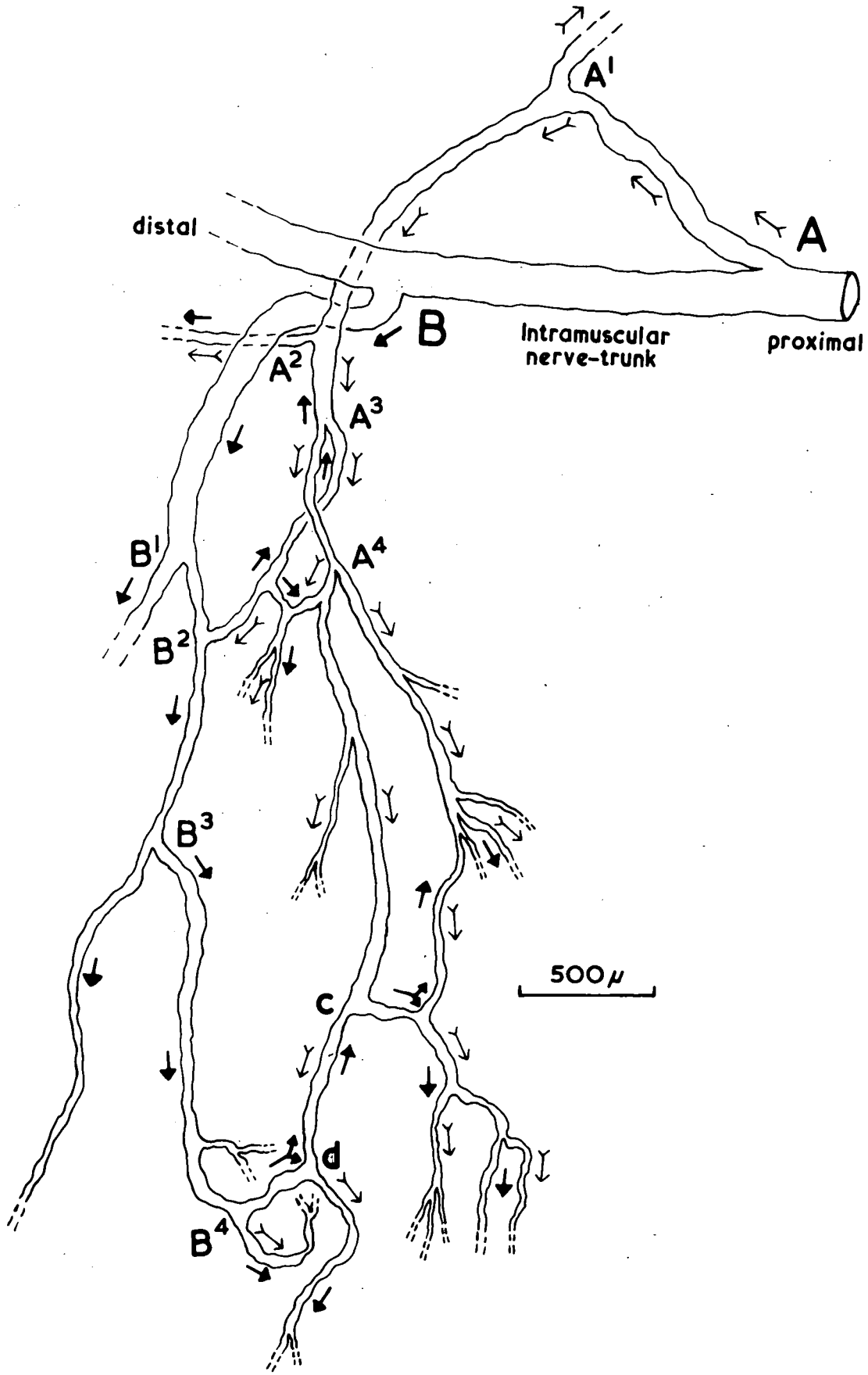

13·5
12·5
8
11·5
7
6·5
7
6
7
11
6
6
4
4·5
5
5·5
3·5
10·5
5
4
3
3
4
4
3
3·5
3
3
4
4·5
3
2·5
10
4
4
4
3
3
8
3·5
9
3
7·5
3
2·5
6·5
6·5
2·5
7
3
5
3·5
6·5
4·5
Monopodial
Semipyramidal
Pyramidal

terminal levels. This is due to the fact that not all nerve fibres branching from a nerve-trunk bifurcation proceed to innervate adjacent muscle areas. Some of these travel for variable distances then divide and subdivide into smaller nerve groups forming parts of a neural pattern. A proportion of these branches may continue in their original direction but others may double back in a recurrent manner. A similar pattern of distribution is contributed by nerve fibres from neighbouring side branches and this results in the formation of a complex neural pattern. These patterns cover muscle areas of different magnitudes.

One of these neural patterns in the 1st superficial muscle of cat C158 was chosen for study in detail (Text-figure 13). The side branch A from the main intramuscular nerve-trunk bifurcated a number of times at points A^1, A^2, A^3 and A^4. Branches from these bifurcations travelled to different areas, taking part in a number of neural configurations. Another side branch B, with bifurcations at B^1, B^2, B^3 and B^4 also took part in the neural pattern. The direction of 'proximal-distal' flow in the axon branches from both nerve branches A and B

Text-figure 13. Camera lucida tracing of a part of the neural pattern formation by motor nerve bundles within the intramuscular region.

From the first superficial lumbrical muscle of cat C158, osmic/glycerine preparation. Side nerve branches A and B from main intramuscular nerve-trunk. Further divisions of nerve bundles occur at junctions A^1, A^2, A^3 & A^4; B^1, B^2, B^3 & B^4, respectively. Different arrows designate the origin of motor fibre branches from either A or B and also indicate the direction of travel. For example, between junctions c and d, the nerve bundle consists of fibre branches originating from both A and B side nerve branches and travelling in opposite directions. Area of tracing approximately 8 sq.mm.


A¹
distal
A
B
Intramuscular
nerve-trunk
proximal
A²
A³
B¹
A⁴
B²
B³
500 μ
C
d
B⁴

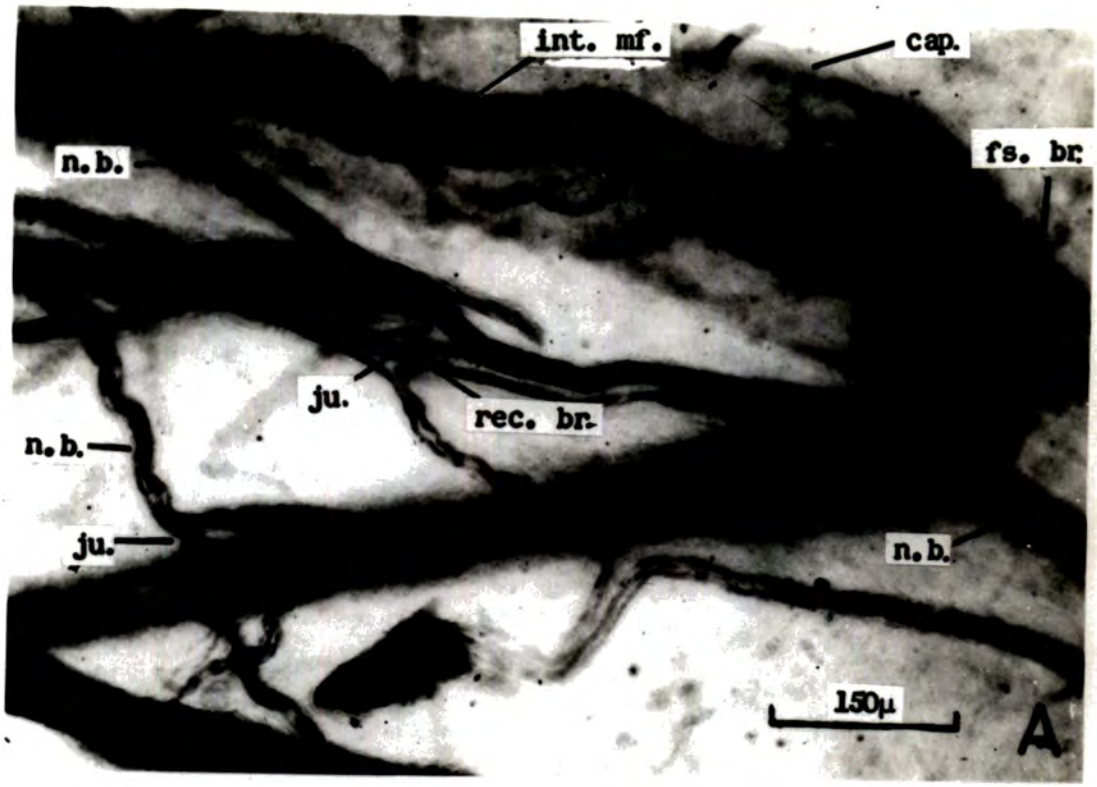
is illustrated by different arrows. Thus, observation of the axon branches between points c and d showed that they originated from side branches of both A and B. Between these points, axon branches originating from A travelled in the opposite direction to those from B. The occurrence of axon divisions in every junction of the neural pattern, together with the recurrent nature of their courses results in a large area of distribution. The area studied was approximately 8 mm^2. Plate 16, figure A shows part of a neural pattern, and figure B demonstrates the chaotic flow and recurrent nature of some axon branches at a junction of the neural pattern. Such patterns have been reported in certain muscles of the rabbit and macaque monkey by Feindel et al. (1952), who described them as being of 'plexiform nature' at the pre-terminal and terminal levels, and 'anastomoses' in relation to nerve-trunk and nerve bundles. In the present study, neural patterns were found not only in the superficial and deep lumbrical muscles but also in the extensor digitorum brevis, flexor digitorum longus, lateral head, popliteus, soleus, tibialis posterior, tibialis anterior and gastrocnemius muscles of the cat.

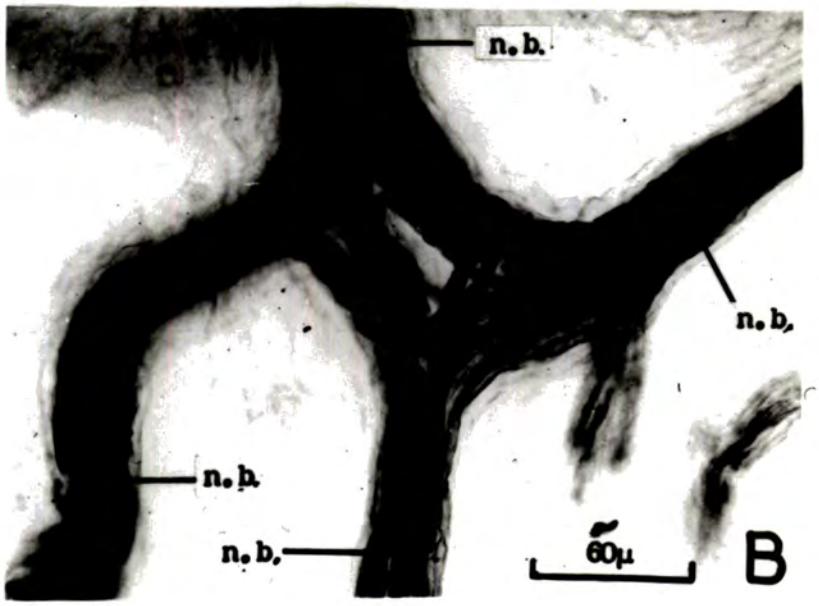
Upon entry into the intramuscular region, an

Plate 16. Intramuscular neural patterns of motor fibre branches.

Figure A. Some intramuscular neural patterns of motor fibre branches are shown from the first superficial lumbrical muscle of cat C158. Osmic/glycerine, teased preparation. n.b. - nerve bundles, rec.br. - recurrent fibre branches at junction (ju.) of nerve bundle division, fs.br. - fusimotor fibre branches, inf.m.f. - intrafusal muscle fibre of a spindle, cap. - spindle capsule.

Figure B. A junction from part of a neural pattern where fibre branches from a few nerve bundles converge/depart from/to different directions, giving a complicated distribution of fibre branches. Same preparation as in Figure A.


int. mf.
cap.
n. b.
fs. br.
ju.
rec. br.
n. b.
ju.
n. b.
150μ
A


n. b.
n. b.
n. b.
n. b.
60μ
B

∝motor fibre divides at nearly every bifurcation of the intramuscular nerve trunk. Daughter fibres course towards different side branches and innervate separate areas of muscle fibres in different depths of the muscle. Moreover, the occurrence of recurrent and double recurrent courses of nerve fibres in the nerve trunk and formation of neural patterns at the pre-terminal and terminal levels contributes to a most complex distribution. It is therefore impossible to assess the extent of a single motor-unit within a muscle by histological method. Indeed, all the evidence suggests that a certain degree of overlapping in motor-units is inevitable (see e.g. Adrian, 1925; Cooper, 1929; Van Harreveld, 1946).

B. The Rabbit

1. The motor supply of lumbrical muscles

1.1 Extramuscular study of the motor component

The nature of the motor supply to the three lumbrical muscles in the rabbit's hind-foot has been examined from eight rabbits (rabbit Rb 23, Rb 51, Rb 58, Rb 59, Rb 60, Rb 63, Rb 65 & Rb 68; see Materials B and Table 2) from which twenty-one de-afferentated lumbrical nerves were investigated by the teased osmic/glycerine preparations. The muscles are smaller than those in the cat, particularly the 1st lumbrical, and the motor supply consists of no more than a dozen nerve fibres at most, even in the largest 2nd lumbrical. The spindle content of each muscle has been analysed by either the osmic/glycerine technique or by teased silver preparations. The results are shown in Tables 14, 15 & 16. A preliminary communication has been reported by Adal & Barker (1965b).

1.11 The motor component of individual lumbrical muscles

From eight de-afferentated 1st lumbrical muscles, the number of motor fibres supplying individual muscles was from 1 to 5, the common number being 3 and 4 (Table 14). Only three of the muscle nerves showed the presence of motor fibres with total diameters less than 8 µ, and there were only four such fibres out of a total of twenty-five motor nerve fibres studied in this sample. The smallest fibre encountered was at 5 µ (Rb 60) and the largest did not exceed 11 µ (Rb 63 & Rb 65). The number of spindles in the muscle was between three and four with two exceptions each having only a single spindle. Plate 17, figure C shows the motor supply to the 1st lumbrical muscle of rabbit Rb 60 taken approximately 5 mm prior nerve-entry from an osmic stained preparation. The total diameters of the three fibres were 5µ, 8 µ and 9 µ, respectively.

In a study of eight de-afferentated 2nd lumbrical muscle nerves, the number of fibres in the motor supply of individual nerves observed was from 3 to 12

Table 14. The number and sizes of motor fibres in the nerve supply and the number of spindles in the first lumbrical muscle of the rabbit. (Fibres less than 8 μ are underlined.)

Reference	Number of motor fibres	Total diameter (nearest μ)	Number of spindles
Rb 23	1	@ 8 μ.	1
Rb 51	3	1 @ 8, 1 @ 9, 1 @ 10.	3
Rb 58	4	1 @ 6, 1 @ 7, 1 @ 8, 1 @ 9.	4
Rb 59	3	1 @ 8, 2 @ 10.	4
Rb 60	3	1 @ 5, 1 @ 8, 1 @ 9.	1
Rb 63	5	1 @ 8, 1 @ 9, 2 @ 10, 1 @ 11.	3
Rb 65	4	1 @ 7, 1 @ 9, 1 @ 10, 1 @ 11.	3
Rb 68	2	1 @ 8, 1 @ 9.	3

Rb 59 - cf. Text-figure 15; Rb 60 - cf. Plate 17, figure C.

Plate 17. De-afferentated nerves to lumbrical muscles of the rabbit.

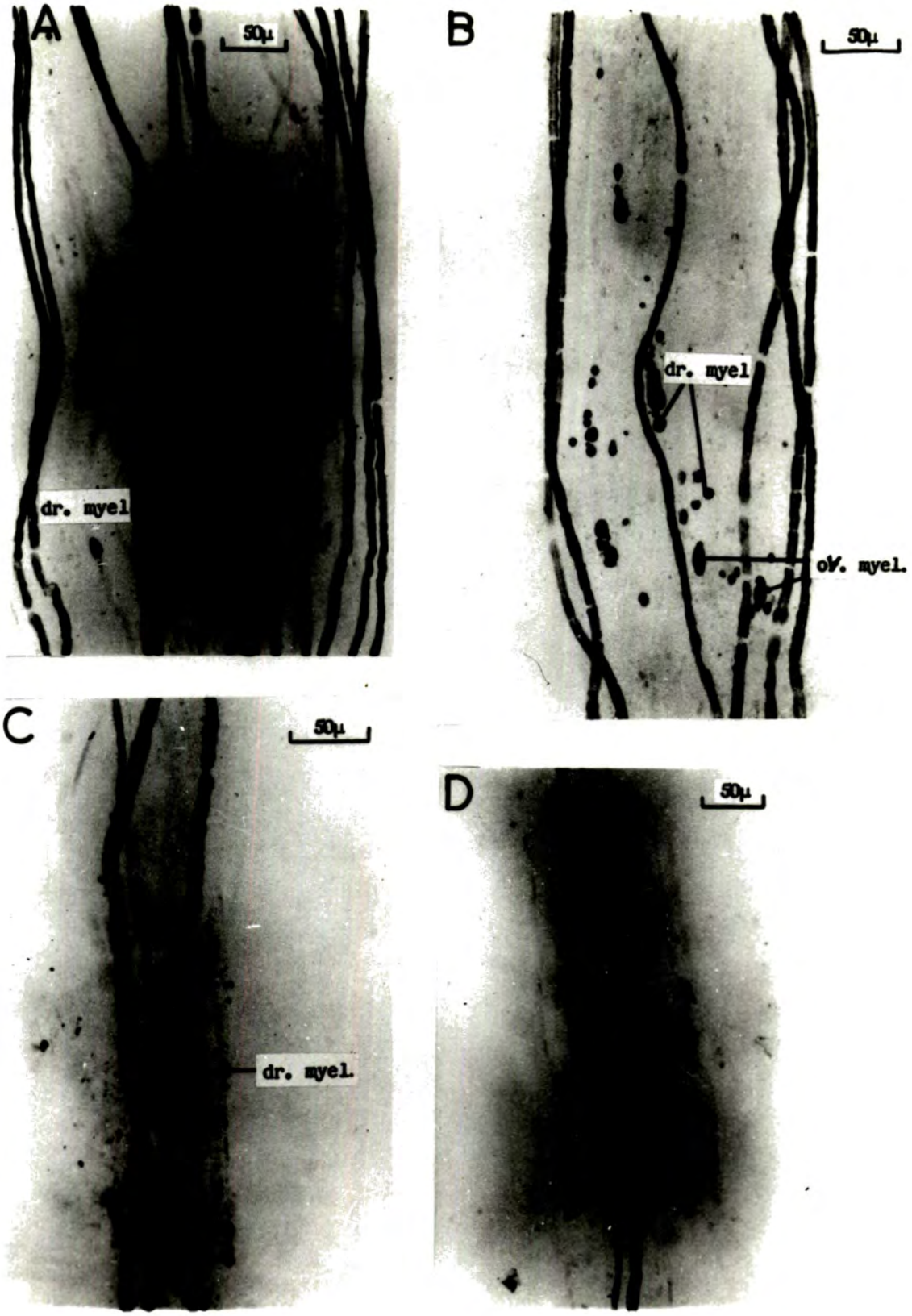
Figure A. De-afferentated nerve supply to the 2nd lumbrical muscle of rabbit Rb 58 showing eleven motor fibres. Two fibres below 8 μ measured at 7 μ (cf. Table 15).

Figure B. Six motor fibres in the de-afferentated nerve to the 2nd lumbrical muscle of rabbit Rb 60. Two fibres below 8 μ measured at 6 μ and 7 μ (cf. Table 15).

Figure C. Three motor fibres in the de-afferentated nerve supply to the 1st lumbrical muscle of rabbit Rb 60. Fibre diameters at 5 μ, 8 μ and 9 μ (cf. Table 14).

Figure D. The de-afferentated nerve supply to the 3rd lumbrical muscle of rabbit Rb 60 showing only two motor fibres, one at 4 μ and one at 9 μ (cf. Table 16).

ov./dr. myel. - ovoids/ droplets of degenerated myelin.


A
50µ
dr. myel
B
50µ
dr. myel
ov. myel.
C
50µ
dr. myel.
D
50µ

(Table 15). Thirteen of the total of sixty-two fibres measured were less than 8 μ; two (Rb 59 & Rb 68) out of the eight muscle nerves examined were without motor fibres of this calibre. The smallest fibre had an external diameter of 6 μ (Rb 65); the largest did not exceed 11 μ. The spindle content of the muscle was between 3 and 5. The de-afferentated nerve supply to the 2nd lumbrical muscle of rabbit Rb 58 showed eleven fibres with total diameters measuring between 7 and 10 μ (Plate 17, figure A & Table 15). In rabbit Rb 60, the de-afferentated nerve to the 2nd lumbrical showed six nerve fibres with diameter range of 6 - 9 μ (Table 15); the teased preparation is shown in Plate 17, figure B, at a distance of about 8 mm from nerve-entry. The nerve to the 2nd lumbrical of rabbit Rb 51 consisted of twelve fibres with a diameter range of 7 to 10 μ (Table 15); a section taken approximately 9 mm from nerve-entry showed all the fibres to be thickly myelinated (Plate 18, figure A & B).

A total of eighteen motor fibres were examined from five de-afferentated 3rd lumbrical muscle nerves. The number of fibres in the motor supply to this muscle was from 2 to 5 (Table 16). Two (Rb 51 & Rb 59) of the

Table 15. The number and sizes of motor fibres in the nerve supply and the number of spindles in the second lumbrical muscle of the rabbit. (Fibres less than 8 μ are underlined).

Reference	Number of motor fibres	Total diameter (nearest μ)	Number of spindles
Rb 23	8	1 @ 7, 3 @ 8, 4 @ 9.	4
Rb 51	12	4 @ 7, 3 @ 8, 2 @ 9, 3 @ 10.	5
Rb 58	11	2 @ 7, 4 @ 8, 3 @ 9, 2 @ 10.	5
Rb 59	3	1 @ 9, 1 @ 10, 1 @ 11	6
Rb 60	6	1 @ 6, 1 @ 7, 2 @ 8, 2 @ 9.	3
Rb 63	7	2 @ 7, 3 @ 8, 2 @ 9.	3
Rb 65	10	1 @ 6, 1 @ 7, 4 @ 8, 2 @ 9, 2 @ 10.	4
Rb 68	5	1 @ 8, 2 @ 9, 1 @ 10, 1 @ 11.	5

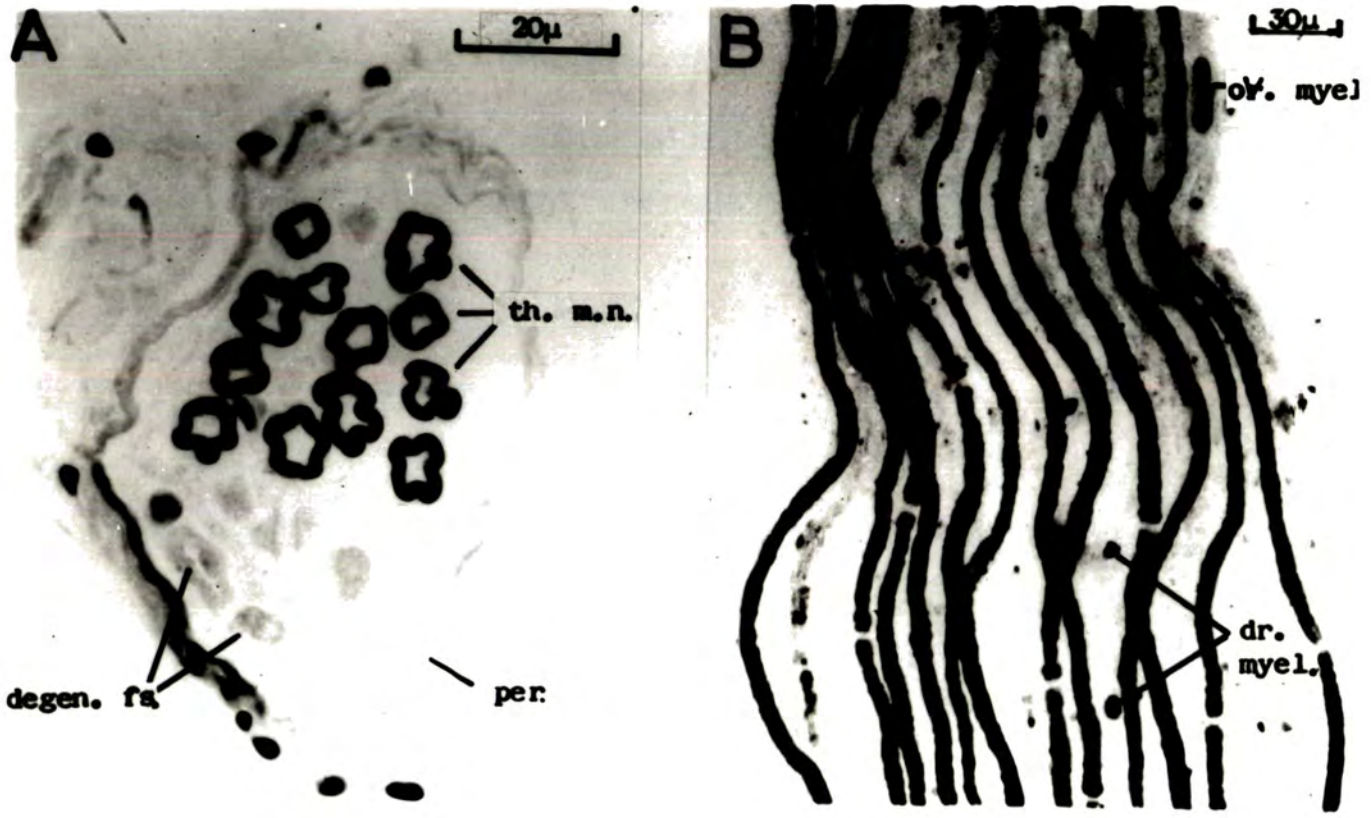
Rb 51 - cf. Plate 18, figures A & B.
Rb 58 & Rb 60 - cf. Plate 17, figures A & B.

Plate 18. De-afferentated nerve supply to the second lumbrical muscle of rabbit Rb 51.

Figure A. Osmic section at 5μ of the nerve approximately 9 mm from its entry into the muscle showing twelve motor fibres all thickly myelinated. Four fibres below 8μ all have diameters at 7μ (cf. Table 15).

Figure B. Osmic stained, teased preparation of the same nerve 4 mm from its entry into the muscle.

per. - perineurium, th.m.n. - thickly myelinated motor fibres, degen.fs. - residue from degenerated nerve fibres, ov./dr. myel. - ovoids/droplets of degenerated myelin.


A
20µ
th. m.n.
degen. fs
per.
B
30µ
ov. myel
dr.
myel.

five muscle nerves examined were without motor fibres less than 8 μ; only three fibres in the sample of eighteen were within this calibre. The smallest fibre was 4 μ in diameter; the upper limit of fibre size was 10 μ. The number of spindles in this muscle was between 1 and 3. The motor supply to the 3rd lumbrical muscle of rabbit Rb 60 is shown in Plate 17, figure D. It was taken 12 mm from nerve-entry and consisted of only two fibres measuring 4 μ and 9 μ.

Results from the twenty-one lumbrical muscle nerves examined showed a total of 105 motor fibres in which 19 measured less than 8 μ. Only twelve of these nerves consisted of motor fibre of γ sizes below 8 μ, the remaining nine nerves were without a γ component.

1.2 Intramuscular study of the motor fibre distribution

1.21 Distribution of fusimotor fibre branches

Investigation into the intramuscular region of the lumbrical muscles in the rabbit for the nature of fusimotor branching has been carried out in six muscles

Table 16. The number and sizes of motor fibres in the nerve supply and the number of spindles in the third lumbrical muscle of the rabbit. (Fibres less than 8 μ are underlined).

Reference	Number of motor fibres	Total diameter (nearest μ)	Number of spindles
Rb 23	4	1 @ 7, 1 @ 8, 2 @ 9.	2
Rb 51	3	1 @ 9, 2 @ 10.	1
Rb 59	5	1 @ 8, 2 @ 9, 2 @ 10.	3
Rb 60	2	1 @ 4, 1 @ 9.	2
Rb 63	4	1 @ 7, 1 @ 8, 1 @ 9, 1 @ 10.	1

Rb 51 - cf. Text-figure 14; Rb 60 - cf. Plate 17, figure D.

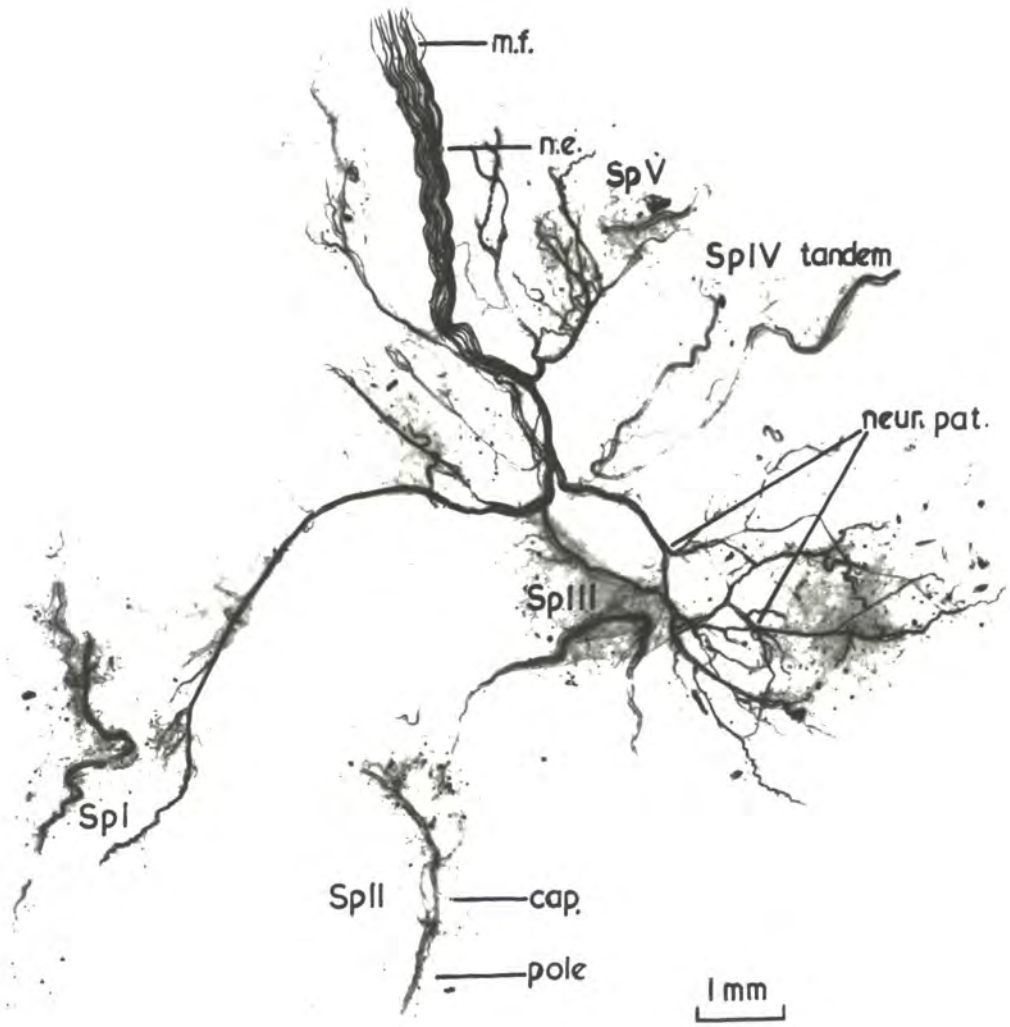
from two rabbits, Rb 23 & Rb 51. Due to the difference in the intramuscular distribution of the nerve fibres, a parallel study to that accomplished in the cat is practically impossible. The difference is best illustrated by a comparison between an osmic/glycerine preparation of a rabbit lumbrical muscle (rabbit Rb 51, 2nd lumbrical, Plate 19) and one of a cat (cat C167, 1st DL, Plate 5).

In the rabbit, the intramuscular distribution of nerve fibres exhibits an even more complicated system of neural pattern than those in the cat, covering large areas in such a way that it is quite impossible to trace individual nerve fibre branches. The most notable absence in the rabbit's preparation is the lack of independent fusimotor fibre branches. In addition to these difficulties, the muscle spindles in the rabbit lumbrical muscles derive their fusimotor fibre branches from β fibres only in many cases (see below).

Nevertheless, the nature of intramuscular branching of fusimotor fibres was ascertained to a certain extent from two muscles. One of these was the 3rd lumbri-

Plate 19. An osmic/glycerine teased preparation from the second lumbrical muscle of rabbit Rb 51.

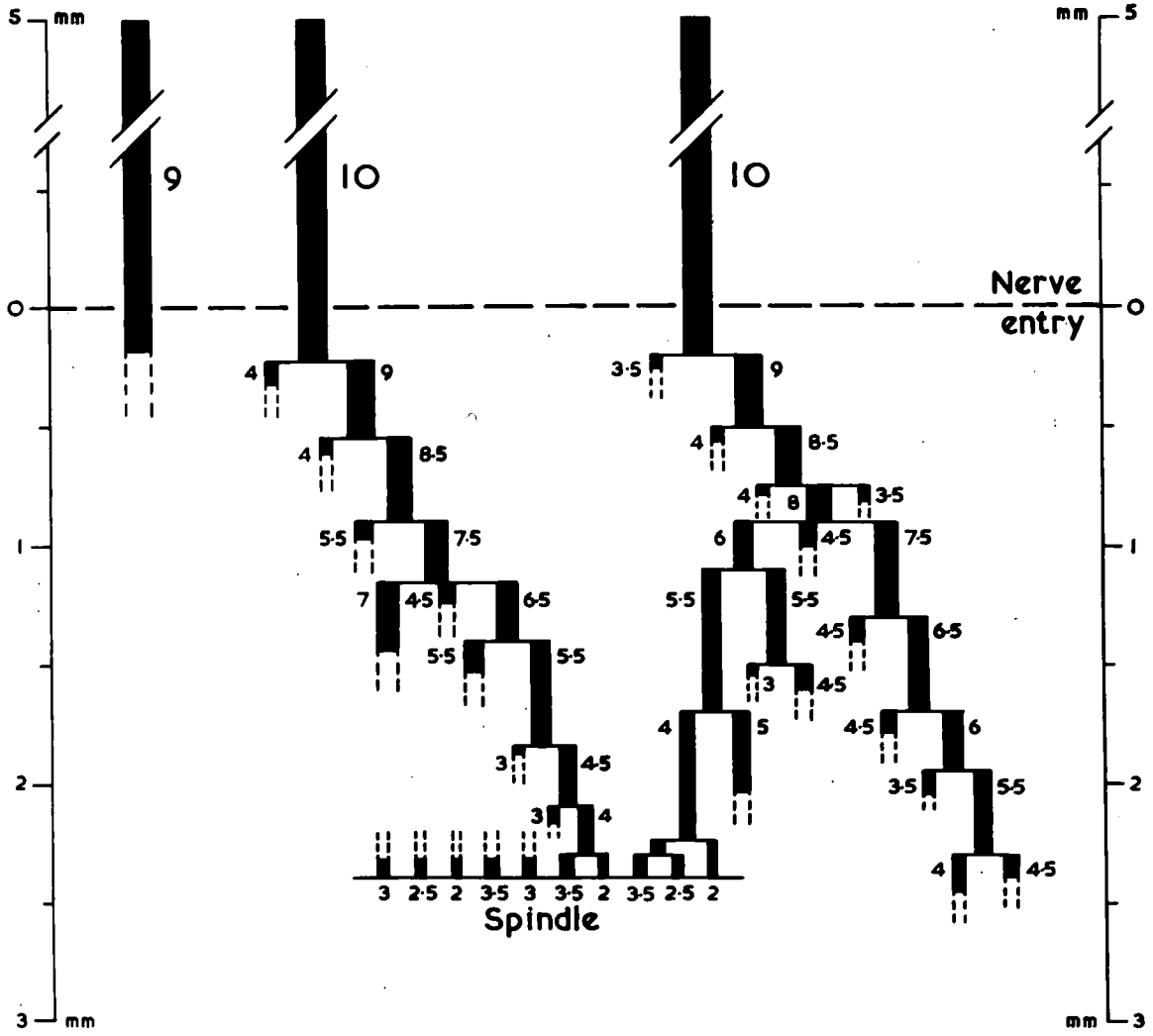
Note the extensive neural patterns (neur.pat.) from connections of nerve bundles and the absence of the independent fusimotor nerve-trunks found in the cat (cf. Plate 5). n.e. - nerve entry , m.f. - motor fibres in muscle nerve , cap. - spindle capsule, pole - spindle pole, Sp I - V, spindles one to five.


m.f.
n.e.
SpV
SpIV tandem
neur. pat.
SpIII
SpI
SpII
cap.
pole
1 mm

cal muscle of rabbit Rb 51. There were three motor fibres in the nerve supply, one 9 μ and two 10 μ, all of which branched intramuscularly to innervate extrafusal muscle fibres. Five of the ten axons supplying the only spindle present were traced to originate from the two 10 μ, fibre; the derivation of the remaining fibres could not be traced with certainty (Text-figure 14 & Table 16). The other muscle studied was from the first lumbrical muscle of rabbit Rb 59. There were three fibres in the nerve supply, one measuring 8 μ diameter and two 10 μ. From teased silver preparations, the muscle showed the presence of four spindles, two of which were tandem. In tracing the origin of the fusimotor fibre supply to one of the tandem spindles, four of these fibres, three of 2 μ and one of 3 μ, proved to be derived from an 8 μ fibre (Text-figure 15). As this was very near to the region of nerve-entry, this 8 μ fibre might be the same one as observed in the muscle nerve, or might originate from any one of the 10 μ fibres. Since a γ component in the muscle nerve was lacking, the fusimotor supply in this muscle must have originated from β fibres. A further example, which was rather unique, was shown in the nerve to the 1st lumbrical muscle of rabbit Rb 23, where the motor supply

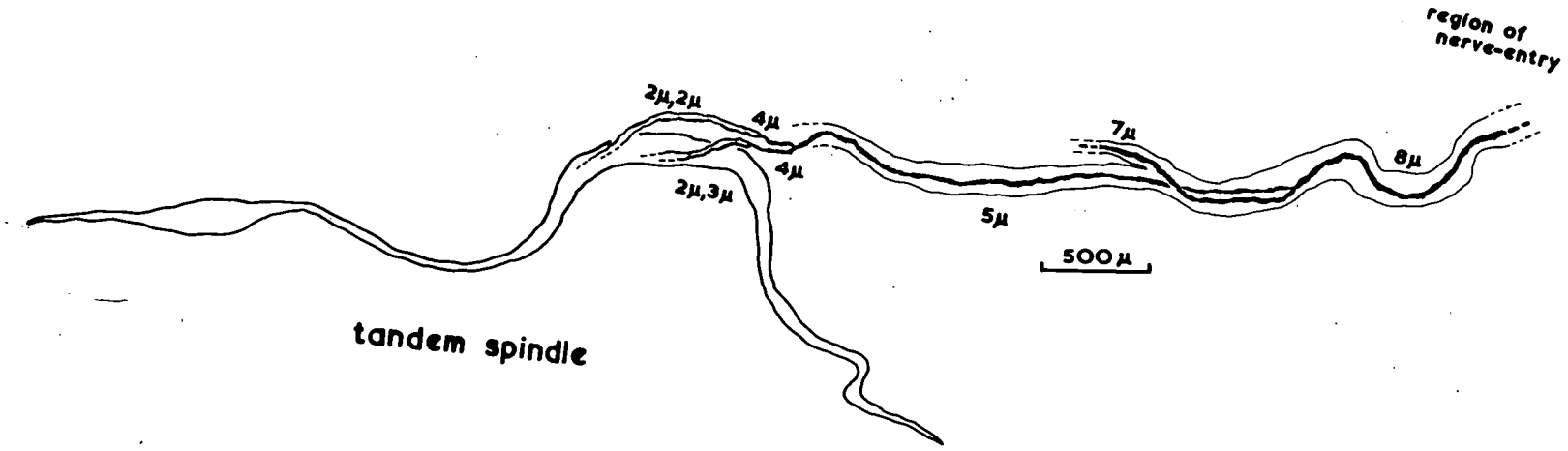
Text-figure 14. Schematic representation of fusimotor fibre branches from β stem fibres innervating a muscle spindle in the lumbrical muscle of the rabbit.

From an osmic/glycerine preparation of the third lumbrical muscle of rabbit Rb 51. The nerve supply consists of three β motor fibres measuring 9μ. 10μ and 10μ (cf. Table 16). Five of the ten fusimotor fibre branches innervating the only spindle in the muscle originate from the two 10μ β-stem motor fibre in the muscle nerve. The remaining fusimotor fibre branches cound not be traced with certainty.


5 mm
mm 5
9
10
10
Nerve entry
0
0
1
1
2
2
3 mm
mm 3
4 9
4 8·5
5·5 7·5
7 4·5 6·5
5·5 5·5
3 4·5
3 4
3 2·5 2 3·5 3 3·5 2 3·5 2·5 2
Spindle
3·5 9
4 8·5
4 8 3·5
6 4·5 7·5
5·5 5·5
4·5 6·5
3 4·5
4 5
4·5 6
3·5 5·5
4 4·5

Text-figure 15. Camera lucida drawing of fusimotor fibre branches originating from a large motor fibre to innervate a tandem spindle in a lumbrical muscle of the rabbit.

Teased silver preparation from the first lumbrical muscle of rabbit Rb 59. There were three β -stem motor fibres, 8μ, 10μ and 10μ in the nerve supply (cf. Table 14) to the muscle with four spindles (two tandems). Fusimotor fibre branches, three of 2μ and one of 3μ, traced to their origin from an 8μ motor fibre around the region of nerve entry. Irrespective of their source from any of the stem motor fibres, the fusimotor fibre branches are of β fibre origin since there is no γ component in the muscle nerve supply.


region of
nerve-entry
2μ,2μ
4μ
7μ
8μ
4μ
2μ,3μ
5μ
500μ
tandem spindle

consisted of one 8 μ fibre only (Table 14). This single fibre provided the skeletomotor supply to the whole muscle as well as the fusimotor innervation of the only spindle present.

These results, taken in conjunction with those of the preceeding section indicate a higher degree of β innervation of spindles such as also appear to occur in rat tail muscles (see Kidd, 1964).

1.22 Motor endings in lumbrical muscle spindles

It has been shown by Barker & Ip (1965b) that there are two kinds of motor endings in spindles of the cat and the rabbit. One is the usual end-plate type, which is normally found in the polar region some distance from the sensory endings; another is a diffuse type, the 'trail-ending', located at the juxta-equatorial region. However, there is a difference in the morphology of the intrafusal muscle fibres in the spindles of the two animals. In the spindles from the vastus intermedius, tenuissimus and soleus muscles of the rabbit, the intrafusal muscle fibres consist of nuclear-bag fibres only

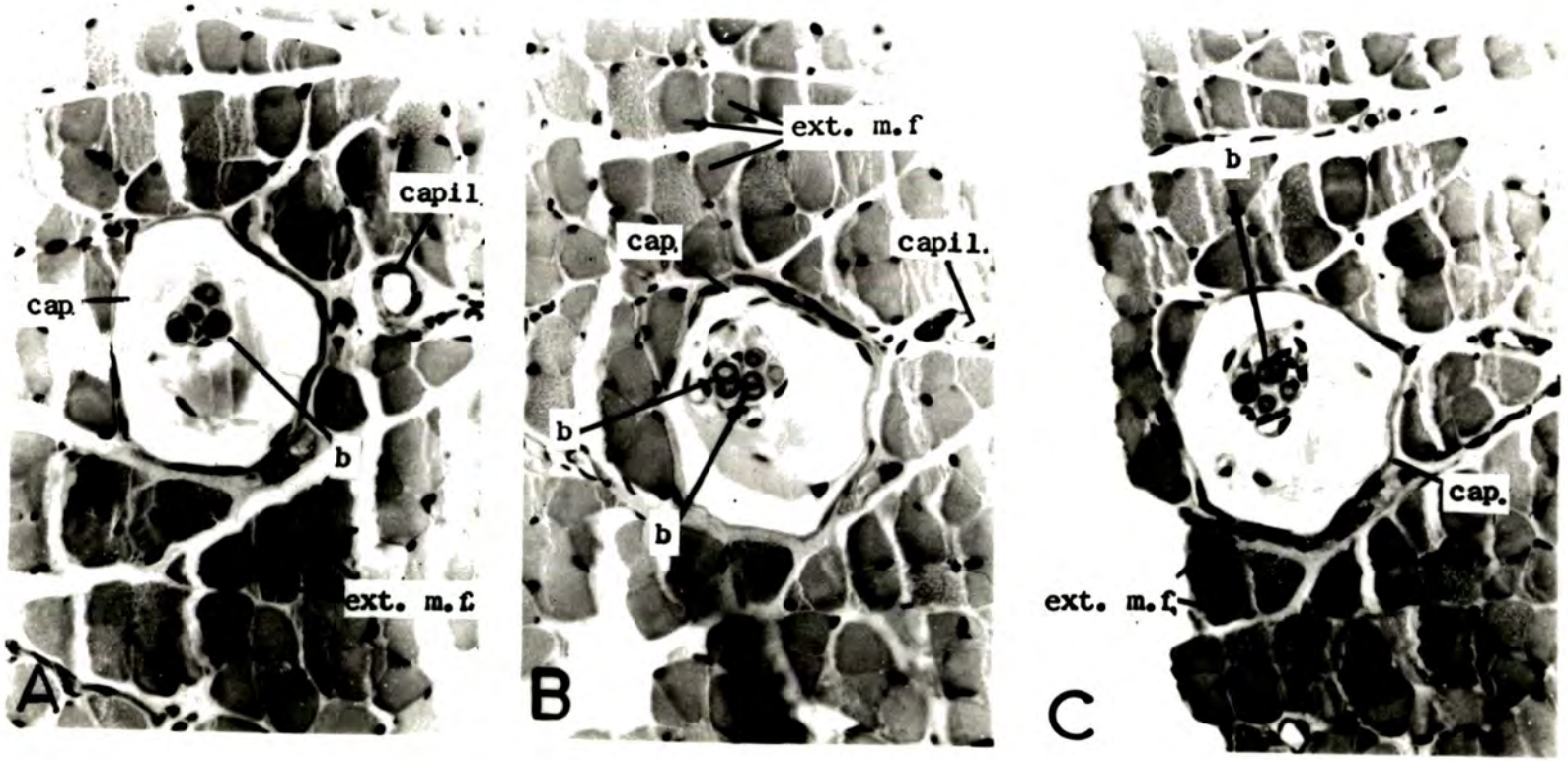
(Barker & Hunt, 1964); the nuclear-chain fibres, commonly found in cat spindles, are lacking.

In the present investigation, the intrafusal muscle fibres of the spindles in the lumbrical muscles have been examined to ascertain whether they also consist of nuclear-bag fibres only. This was determined by studying serial transverse sections of the 2nd lumbrical muscle of rabbit Rb 20. There were five spindles in the muscle, each of which consisted of four nuclear-bag fibres. Only one fibre was doubtful as to whether it might not be a chain fibre. Plate 20 shows sections from the capsular region of one of the spindles, with four nuclear-bag fibres, lying side by side at slightly different levels, exhibited by different sections.

A total of 92 spindles from 21 lumbrical muscles, stained by the teased silver method, were examined from seven rabbits, one normal (rabbit Rb 62) and six being de-afferentated previously (rabbit Rb 58, Rb 59, Rb 60, Rb 63, Rb 65 & Rb 68) . Motor endings in the majority of the spindles were of the end-plate type. The occurrence of 'trail ending' was encountered only in two

Plate 20. A spindle showing nuclear-bag fibres only from the second lumbrical muscle of rabbit Rb 20.

Serial transverse sections at 10μ, haematoxylin & eosin. The four nuclear-bags lie at slightly different levels as shown by consecutive sections. b - bag fibre, cap - spindle capsule, ext.m.f. - extrafusal muscle fibre, capil. - capillary.


capil.
cap.
b
ext. m.f.
A
ext. m.f
cap.
capil.
b
b
B
b
cap.
ext. m.f.
C

spindles, both were from the second lumbrical muscle, one in rabbit Rb 63 and the other in rabbit Rb 65. A reference to the nerve supply (Table 15) showed that fibres below 8 μ were present in the motor component of the two muscle nerves.

These results indicate that both types of plate and trail motor endings occur in spindles of rabbit lumbrical muscle, and that the plate ending is by far more common than the trail ending. Plate endings were found in spindles of muscles having a motor supply of nerve fibres with or without a γ group; whereas the trail endings encountered on the two occasions were in spindles of muscles supplied by fibres among which were those less than 8 μ and are therefore presumably γ. No trail endings occur extrafusally, so it may therefore be concluded that they are exclusively of γ origins unlike plate endings which may be supplied to spindles by either γ or β fibres.

1.23 Motor fibre branches to extrafusal muscle fibres

Investigation of the intramuscular branching of motor nerve fibres to extrafusal muscle fibres in the

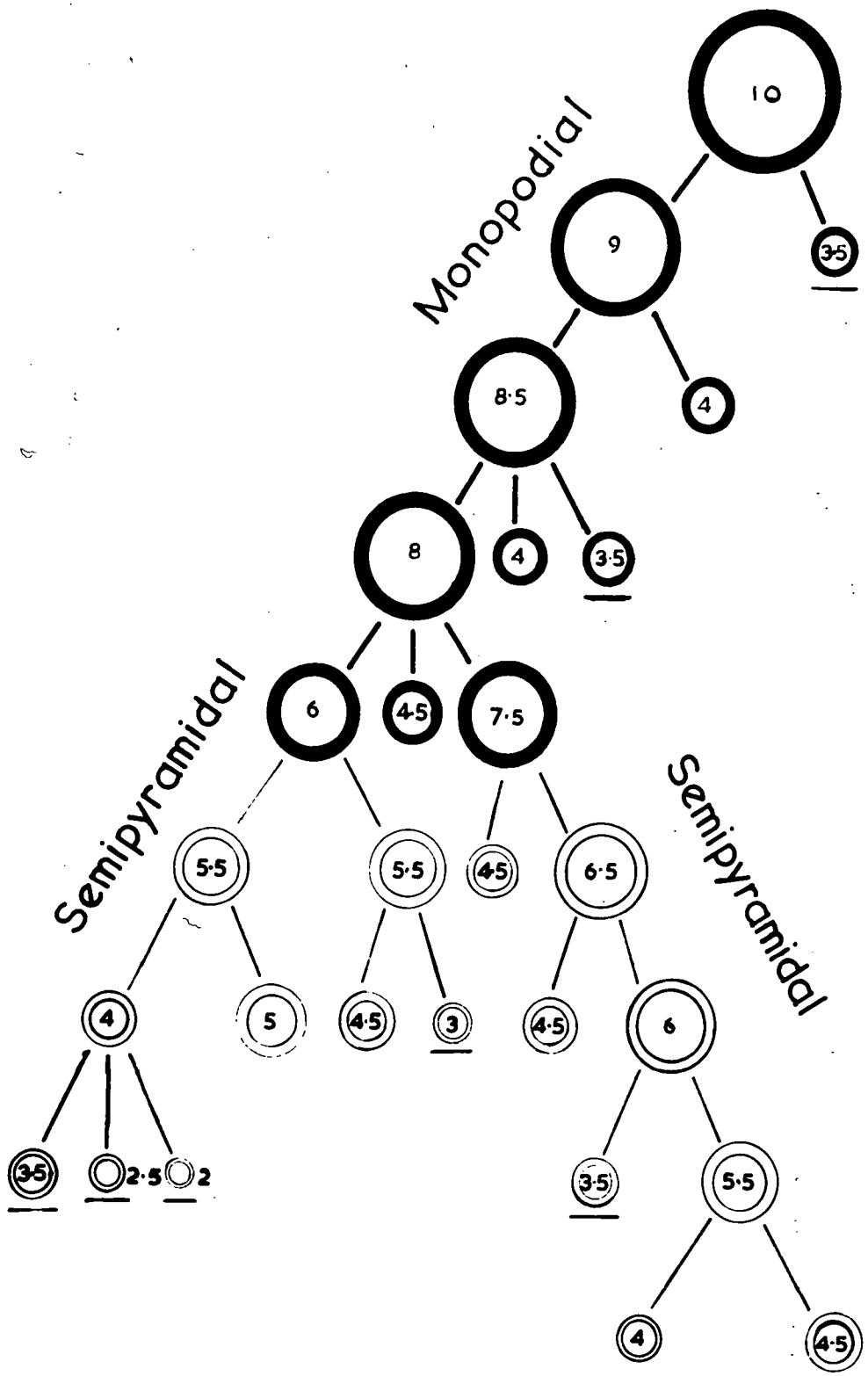
rabbit has been carried out only to a limited extent. This is due to the fact that a large amount of the motor fibres supplying the lumbrical muscles are β fibres, as a result of which the intramuscular tracing of fibre branches is made more difficult. In fact, the two motor fibres traced from the muscle nerve to the intramuscular region both showed a mixed nature. The two fibres from the third lumbrical muscle of rabbit Rb 51 exhibited the same external diameter of 10 μ (Text-figure 14). The nature of branching of these two fibres, one of which is shown in Text-figure 16, seems to show a mixture of 'semipyramidal and 'monopodial' types. From the study of the fibre branches and their neural pattern of distribution within the muscle, there is every reason to suppose that all three types of skeletomotor intramuscular branching found in the cat also occur in the rabbit.

C. Some motor comparisons between cat and rabbit

The postulation that γ-stem fibres less than 4 μ in diameter specifically innervate the nuclear-chain muscle fibres in cat spindles (Boyd & Davey, 1962; Boyd, 1962) leads to the question as to whether such small

Text-figure 16. Schematic representation of a motor fibre branching intramuscularly from the lumbrical muscle of the rabbit.

Osmic/glycerine preparation from the third lumbrical muscle of rabbit Rb 51. Both 'Semipyramidal' and 'Monopodial' types of branching are exhibited. A total of 12 divisions traced, 9 by dichotomy and 3 by trichotomy; also 8 by equal and 4 by unequal branching. Representation of fibre branching as in Text-figure 9.


Monopodial
10
9
3·5
8·5
4
8
4
3·5
Semipyramidal
6
4·5
7·5
5·5
5·5
4·5
6·5
Semipyramidal
4
5
4·5
3
4·5
6
3·5
2·5
2
3·5
5·5
4
4·5

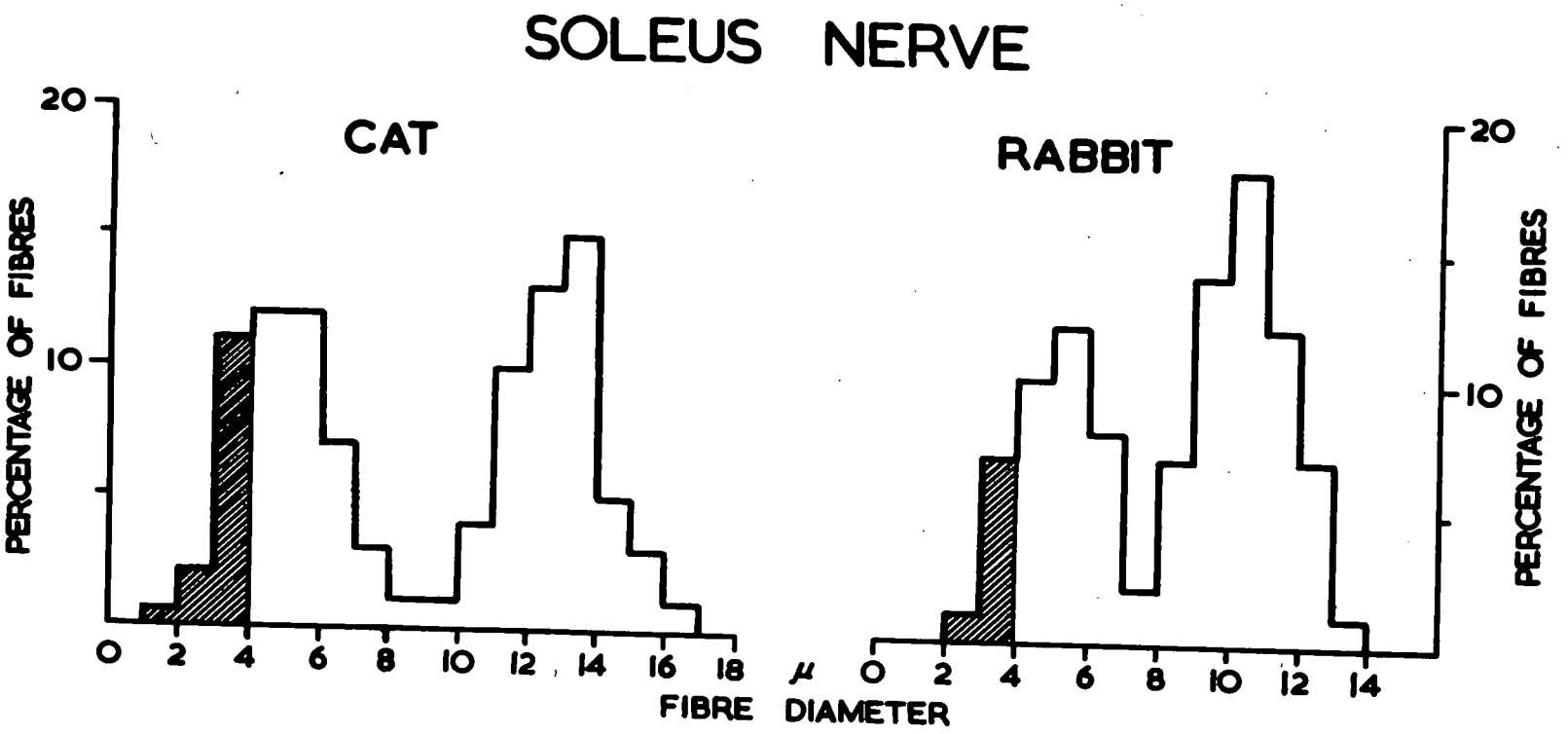
γ fibres are absent in rabbit muscle nerves that supply spindles without nuclear-chain muscle fibres (Barker & Hunt, 1964). Comparisons were made in the present study from de-afferentated fibre-diameter spectra of nerves supplying the soleus, tibialis anterior and popliteus muscles of the cat and rabbit. The usual bimodality from α and γ groups of motor fibres was exhibited in all muscle nerves. However, small γ-stem fibres were present in all rabbit nerves though in smaller proportions than in the cat. The results were based on de-afferentated muscle nerves from one cat, C191, and two rabbits, Rb 51 & Rb 65.

1. Comparisons of the soleus, tibialis anterior and popliteus muscle nerves

A comparison between fibre-diameter spectra of the nerves to the soleus muscle of the cat and the rabbit has shown that the percentage of small γ-stem fibres less than 4 μ in the 2 - 8 μ fibre group is 29% in the cat and 19% in the rabbit (Text-figure 17). The small γ fibre percentage in the same muscle nerve of another rabbit (Rb 65) is slightly lower, at 13%. A similar comparison of fibre-diameter spectra of the nerves to

Text-figure 17. Comparison of fibre-diameter spectra of de-afferentated soleus muscle nerves of a cat (C191) and a rabbit (Rb 51).

Gamma fibres of small calibre are present in the rabbit nerve though in smaller proportion than in the cat, in spite of rabbit spindles having nuclear-bag fibres only. Percentage of gamma fibres less than 4μ within the 2 - 8 μ gamma group (striped area) occurs at 19% in the soleus nerve of the rabbit (13% in rabbit Rb 65) and 29% in the cat.


SOLEUS NERVE
CAT
RABBIT
PERCENTAGE OF FIBRES
20
10
0 2 4 6 8 10 12 14 16 18
μ
FIBRE DIAMETER
0 2 4 6 8 10 12 14
20
10
PERCENTAGE OF FIBRES

tibialis anterior muscle shows the percentage of small γ fibres in the cat being 43% as to 28% in the rabbit (Text-figure 18). Again, in the nerves to the popliteus muscle, the percentage of small γ fibres in the cat is 27% as to 5% in the rabbit (Text-figure 19), though only 2% from the determination of another rabbit (Rb 51).

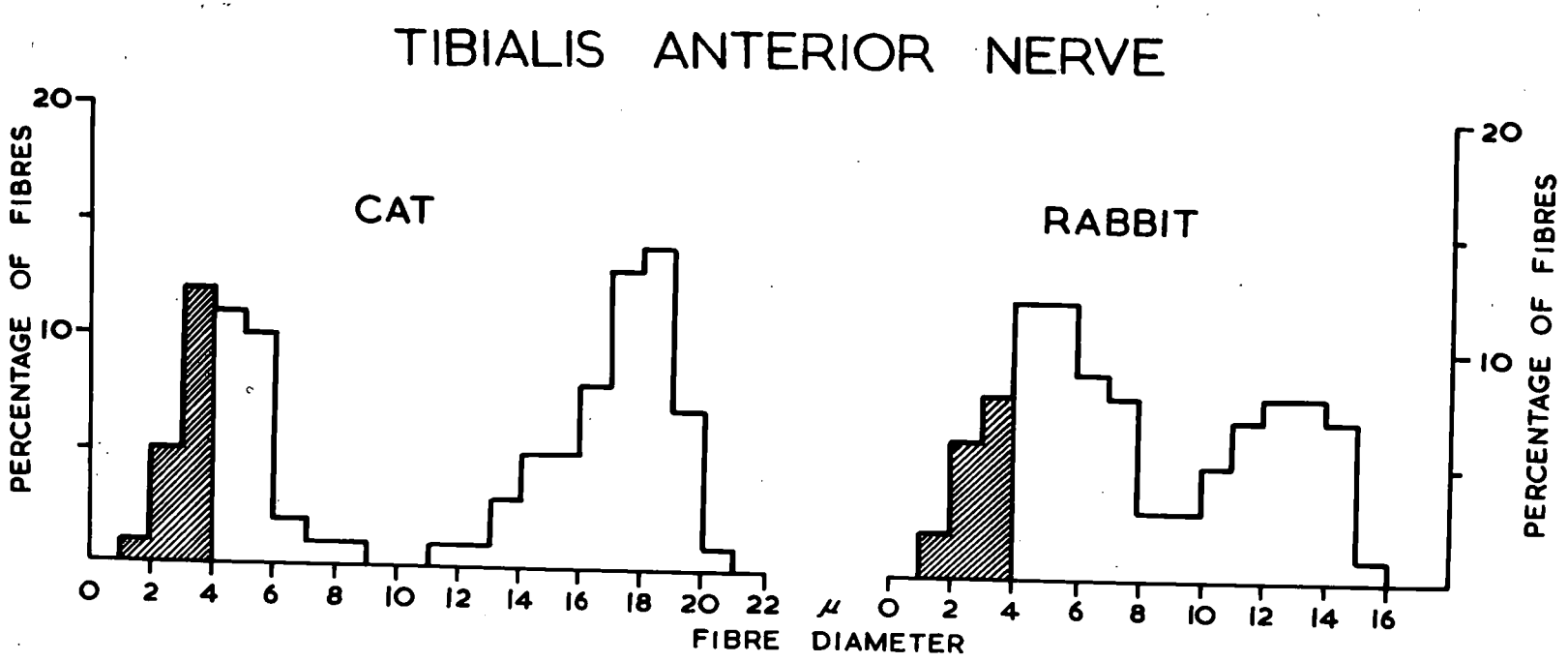
2. Comparisons of lumbrical muscle nerves

Results from de-afferentated lumbrical muscle nerves of the cat and the rabbit give an even more interesting comparison in the fibre-diameter spectra. Data from the cat lumbrical nerves give a total of 39 fibres from four muscles of three cats (Tables 3, 4, 5 & 6). A total of 105 nerve fibres have been examined in twenty-one lumbrical muscle nerves from eight rabbits (Tables 14, 15 & 16). The fibre diameters in the cat lumbrical nerves are spread over a wide range throughout the α and γ groups (Text-figure 20). In the rabbit, however, the range in fibre diameter is much more limited and the distribution of nerve fibres show only a unimodal peak.

The unimodal distribution of nerve fibres in

Text-figure 18. Comparison of fibre-diameter spectra of de-afferentated tibialis anterior muscle nerves of a cat (C191) and a rabbit (Rb 65).

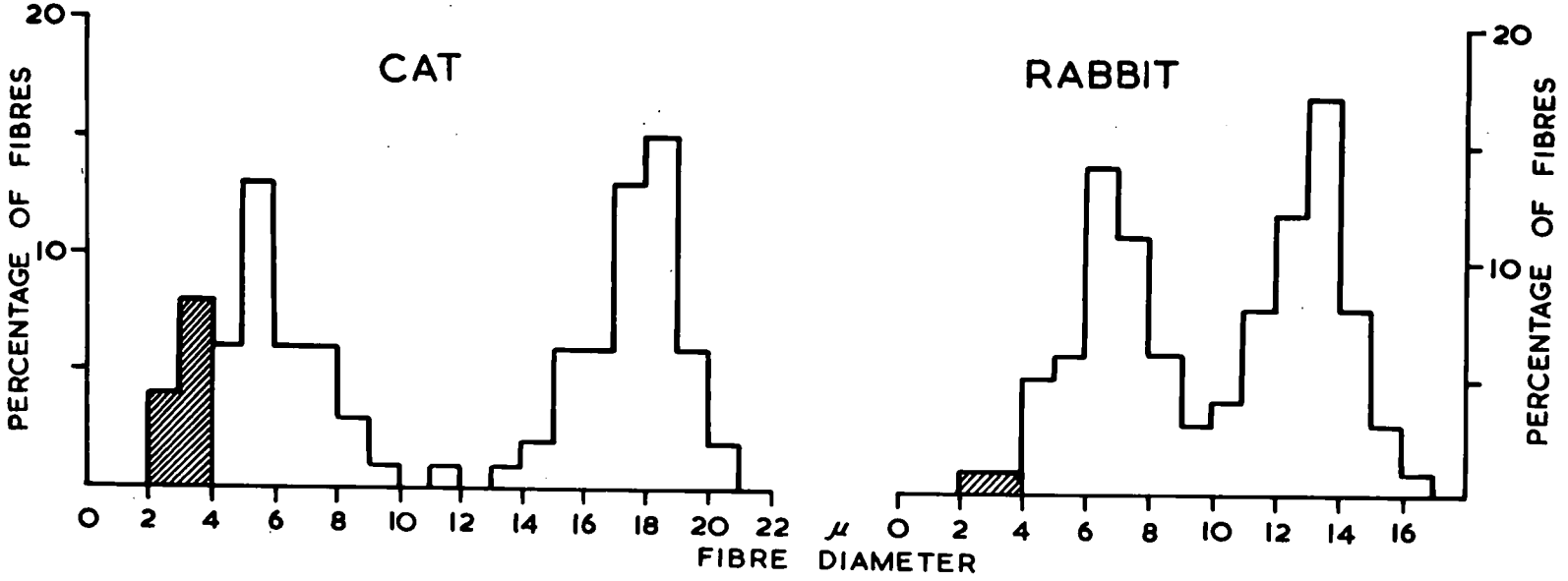
Percentage of gamma fibres less than 4μ within the gamma group occurs at 28% in the rabbit and 43% in the cat.


TIBIALIS ANTERIOR NERVE
CAT
RABBIT
PERCENTAGE OF FIBRES
20
10
0 2 4 6 8 10 12 14 16 18 20 22
μ 0 2 4 6 8 10 12 14 16
FIBRE DIAMETER

Text-figure 19. Comparison of fibre-diameter spectra of de-afferentated popliteus nerves of a cat (C191) and a rabbit (Rb 65).

Percentage of gamma fibres less than 4μ within the gamma group occurs at 5% in the rabbit (2% in rabbit Rb 51) and 27% in the cat.

POPLITEUS NERVE

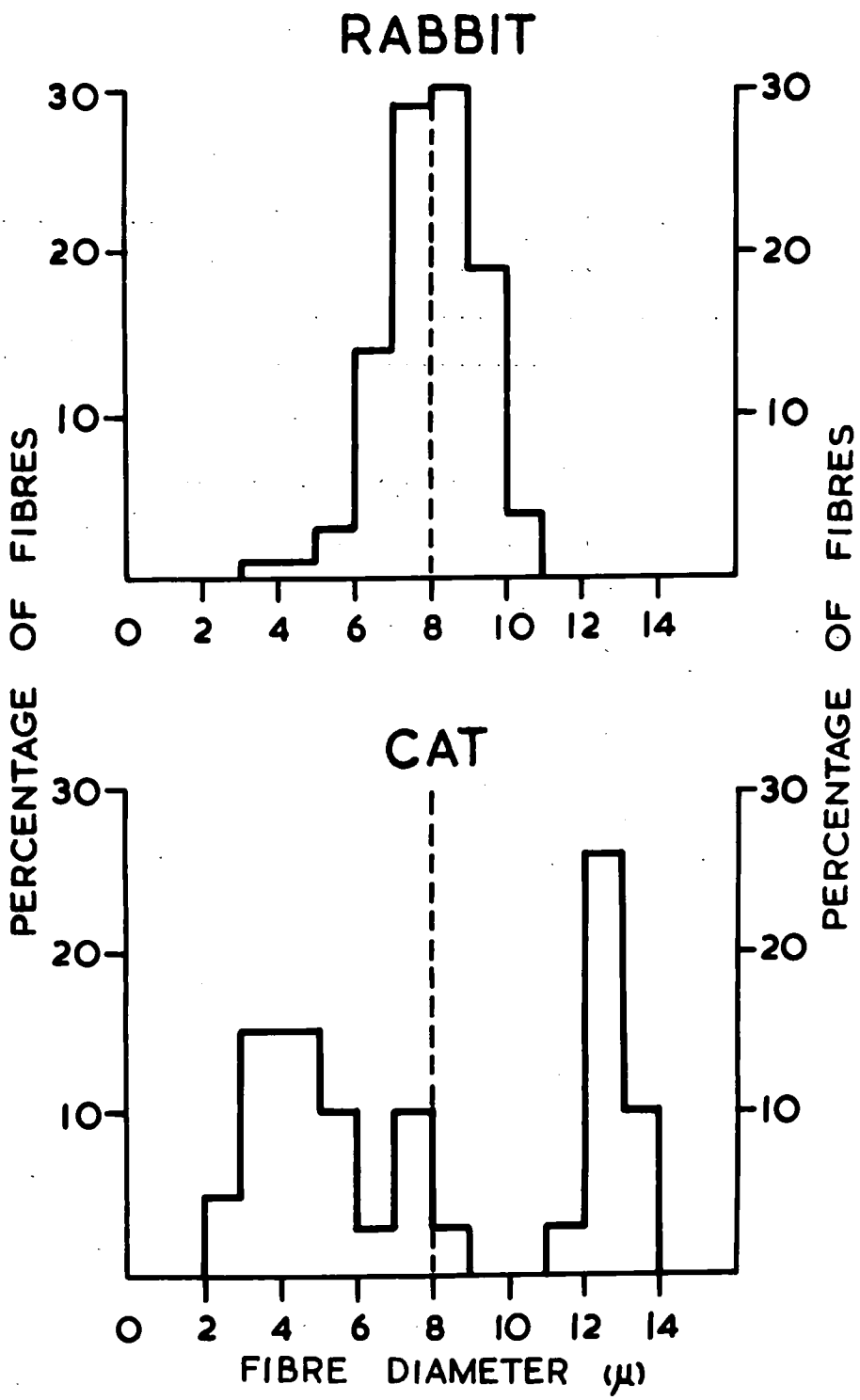


the efferent fibre-diameter spectra of small muscle nerves at the extremity is due not to the lack of muscle spindles and therefore devoid of a γ group (Fernand & Young, 1951), nor to peripheral tapering and branching of motor nerve fibres only (Adal, 1961) but is due mainly to the common occurrence of β fibres.

Text-figure 20. Comparison of fibre-diameter spectra of motor fibres, being a total of all de-afferentated lumbrical muscle nerves studied from cats and rabbits.

A total of 39 motor fibres from four deep lumbrical muscle nerves of three cats (cf. Tables 3, 4, 5 & 6) and 105 motor fibres from twenty-one lumbrical muscle nerves of eight rabbits (cf. Tables 14, 15 & 16). A bimodal distribution of fibre diameters into α and γ groups is shown in the histogram of the cat lumbrical nerves; a unimodal distribution mainly of β fibres is shown in the diameter spectrum of the rabbit lumbrical nerves.

LUMBRICAL NERVES



V. DISCUSSION

Extramuscular branching of muscle nerves

It has long been known that nerve fibres supplying muscle undergo branching, but there is relatively little information about the nature and degree of their branching. The usual indirect approach by most investigators is to examine two transverse sections of a muscle nerve, taken some distance apart, ensuring no side trunks to have been given off in between. The increase in number of nerve fibres and the absence or reduction in number of those of large calibre in the distal section, as compared to the proximal, indicates the occurrence and degree of branching.

In the present study, it has been demonstrated that initial branching of motor fibres occurs at a certain distance from the muscle, in close relation to the level at which the muscle nerve begins to divide into smaller nerve-trunks. This level is considerably below the origin of the nerve from a larger common nerve trunk

where motor fibres do not undergo branching but are simply segregated into two streams. Usually, motor fibres begin to divide further away from a larger muscle than from a small one, though there is not a direct linear relationship for all muscles. In the small deep lumbrical muscles of the cat's hindlimb, initial branching of motor fibres in the muscle nerves has been observed to occur only one millimetre away from the muscle; in some cases, division of nerve fibres does not occur until after nerve entry.

Eccles & Sherrington (1930) demonstrated in the mesial gastrocnemius nerve of the cat that between 62 and 9 mm from entry into the muscle, there was an increase of 153 nerve fibres, and the distance of the nerve dividing into two smaller nerve-trunks was 56 mm from nerve-entry. They pointed out that a section taken from a muscle nerve for the study of its fibre population should be as far proximal as possible to avoid the occurrence of branching. This is certainly true for large muscles, but less important when dealing with small ones. It is interesting to note that Gilliatt (1965), in a study by stimulation and recording from the human fore-arm,

has concluded that motor axon branching in a nerve to muscles in the hand begins in the region of the elbow; it seems very doubtful that branching occurs so far away from cat muscles.

Intramuscular skeletomotor component

It is generally known that motor fibres of a muscle nerve undergo profuse branching and increase in number upon entry into a muscle. In this study, the nature of such branching has been classified into three main types, namely, the 'pyramidal', 'semipyramidal' and 'monopodial' types, with the frequency of occurrence in that order. It might be possible that the common occurrence of the 'pyramidal' and 'semipyramidal' types of motor fibre branching represent the normal division of nerve fibres within a muscle. The 'monopodial' type of branching might be interpreted in relation to the process of motor end-plate replacement (Barker & Ip, 1965a). The thin fibre branches in this type of branching might be the product of the replacement process as re-innervating fibre branches to end-plates.

In the present study, it has been shown that unequal axon branching predominates over equal branching in the main intramuscular nerve-trunk but in the side nerve branches at pre-terminal and terminal level, the situation is reverse. From the data of Cooper (1929), seven out of the listed ten fibre divisions from the sartorius muscle nerve of the cat showed unequal branching and one example of trichotomy was also demonstrated. Examples of dichotomy were also shown by Eccles & Sherrington (1930) from the semitendinosus, soleus and mesial gastrocnemius muscle nerves of the cat.

It has been consistently found in the present study that intramuscular branching of axons occurs most frequently around division of nerve trunks, either a little before or at the junctions. Daughter axons usually travel in different directions, normally, one towards a side nerve, the other continuing along the main nerve-trunk. This provides the histological picture to the physiological work of Adrian (1925) who recorded action potentials at both ends of the tenuissimus muscle which was cut at the middle with the nerve branches intact and stimulated at one end only. The same explanation applied

to the work of Kuffler, Hunt & Quilliam (1951) when potentials were recorded in both nerve branches of this muscle when a single nerve fibre in the related ventral root was stimulated.

In the intramuscular region of a number of cat hindlimb muscles, the motor nerve axons show neural configurations from which smaller bundles of axons are given off to innervate groups of motor end-plates. Such neural configurations have been observed in human muscles (Frohse, 1898), and in muscles of rabbit and macaque monkey (Feindel et al., 1952). It has yet to be investigated whether such neural configurations of axons occur in other lower groups of animals. Tiegs (1953) gave a personal observation in his review that ".... in intercostal muscle of python, the thin nerve twigs can be seen traversing the muscle tissue for considerable distances, delivering motor endings to adjacent fibres, but without any interweaving".

Motor-units

It is practically impossible to explore the

exact territory covered by a single motor-unit from histological studies even in a muscle as small as the deep lumbrical of the cat. An exception is found in the odd rabbit lumbrical muscle which is supplied by only one motor nerve fibre, in which case, the whole muscle constitutes one unique motor-unit. Instead of proceeding straight off to side nerve trunks, the recurrent and double recurrent nature of axon branches is an initial indication that motor axons do not pursue the shortest possible routes to the site of motor innervation. Moreover, the neural configurations involving numerous other axons tend to add further difficulties in the tracing of the muscle area innervated by individual parent nerve fibres. It is evident that the areas covered by neighbouring motor-units overlap considerably and the muscle fibres innervated by axon branches from a parent nerve are scattered throughout an area covered by numerous axon branches of different parent nerve origins.

The nature of overlapping of motor-units within a muscle stems from different contributions of motor nerve fibres deriving from a number of spinal roots to various muscle nerves. The small muscles at the extrem-

ities of the macaque monkey receive a different segmental motor supply (Sherrington, 1892). Overlapping of the distribution of spinal nerves contributing to various muscle nerves of the hindlimb in the rat has been shown by Browne (1950). The motor cell columns of the lumbo-sacral spinal cord with relations to spinal roots and muscle nerves of the hindlimb in the cat, determined by Romanes (1951) has been further extended by Sprague (1958). It has also been shown by Wohlfart (1949) that in certain diseases of motor neurones resulting in some denervation of human muscles, the unit of muscular atrophy does not correspond to the muscle fasciculus.

That muscle fibres innervated by nerve fibre branches of the same motor-unit are scattered over a certain area of a muscle has been shown by recordings of action potentials in separate parts of a muscle produced by electrical stimulation of single axons. This has been demonstrated by Adrian (1925) and Kuffler, Hunt & Quilliam (1951) and by Cooper (1929). Van Harreveld (1946) has shown that in cutting one of the spinal nerves innervating the sartorius muscle of the rabbit, histological examinations of the muscle fibres two weeks later

have shown intermingling of normal and atrophic muscle fibres.

Some indications of the extent of a single motor-unit in the deep lumbrical muscles can be seen in the present study from the intramuscular branching of skeletomotor fibres. Within the muscle, branches from an α motor fibre have been traced to side nerve trunks at a distance of nearly 7 mm from nerve-entry and to continue one or two millimetres further down to the level of muscle-fibre innervation. Buchthal, Ermino & Rosenfalck (1959) recorded the spread of action potentials from motor-units of different human muscles by means of multi-electrodes and found that the fibres of a motor-unit were detected in a circular area of 5 - 7 mm diameter for the muscles of the upper extremities and of 7 - 10 mm in the lower extremities.

Innervation ratios

In the past, numerous innervation ratios estimated for different muscles of one animal or another have not taken either the γ component of the motor supply

or the intrafusal muscle fibres into account. In re-estimating the innervation ratios for two cat muscles after the required adjustments, the values obtained are higher than those previously determined by about 70 - 80%.

Fusimotor innervation ratios, however, have not been previously determined. This is due mainly to the lack of detailed knowledge of the fusimotor component of the nerve supply and the related intrafusal muscle fibre analysis for individual muscles, without which calculations for such ratios are not possible. Even in electrophysiological studies, stimulation of motor fibres of low conduction velocity and presumably of the gamma group failed to develop any measurable tension. Kuffler, Hunt & Quilliam (1951) were unable to record any contraction from the soleus and tenuissimus muscles of the cat on stimulation of small motor fibres conducting at 15 - 55 m/sec in the ventral roots. McPhedran, Wuerker & Henneman (1965) also failed to record any measurable tension on stimulating motor nerve fibres conducting under 40 m/sec to the same muscle; they were unsuccessful with the gastrocnemius muscle as well, working on nerve fibres

with conduction velocities between 10 - 50 m/sec (Wuerker, McPhedran & Henneman, 1965).

The separate estimations of innervation ratios for both extra- and intrafusal muscle fibres in the deep lumbrical muscle determined in this investigation is made possible only after the skeletomotor and fusimotor components in the muscle nerve were analysed independently in detail within the intramuscular region. The value of 1 : 300 given for skeletomotor innervation ratio and 1 : 9 given for fusimotor innervation in the first deep lumbrical muscle of the cat should be genuine figures representing the average proportion of motor nerve fibres to muscle fibres innervated, since separate entities of extra- and intrafusal muscle and nerve fibres have been taken into account.

Gamma component

Since the well-established work of Leksell (1945) that gamma efferent fibres are responsible for the innervation of muscle spindles, individual gamma fibres have further been demonstrated to supply several

spindles within a muscle by Hunt & Kuffler (1951), Kuffler, Hunt & Quilliam (1951), and more recently by Crowe & Matthews (1963, 1964b) and Brown, Crowe & Matthews (1965). The nature of the fusimotor branching and distribution within a muscle has been observed in the present study by actual tracings of fusimotor fibres through their intramuscular course, from γ-stem fibres in the muscle nerve to the site of innervation in the muscle spindles. Crowe & Matthews (1964b) demonstrated different frequencies of response produced by three primary endings from stimulation of a single fusimotor fibre. This is probably attributed to the variation in the number of fusimotor fibre branches distributed to different spindles in the muscle from a single γ-stem fibre. It has also been observed that individual muscle spindles receive fusimotor innervation deriving from different γ-stem fibres, as shown by neurophysiological experiments (Kuffler, Hunt & Quilliam, 1951).

From direct observation it has been possible to track the course of γ-stem fibres down to their terminal branches. In the case of the γ component, Boyd (1962) postulates the occurrence of a size relationship

in such a way that the large, thickly myelinated and the small, thinly myelinated γ-stem fibres give rise to large γ_1 fibres and small γ_2 fibres, respectively, at the level of spindle entry. In relation to the diameter measurements of Boyd (1962) and Boyd & Davey (1962), the thickly myelinated γ-stem fibres with total diameters between about 4.0 and 8.0 μ would branch intramuscularly to give γ_1 fibres, reaching the spindles with axon diameters between 2.5 and 4.0 μ; and the thinly myelinated γ-stem fibres, having total diameters less than 4.0 μ, would branch to give γ_2 fibres with axon diameters between 1.0 μ or less and 2.0 μ at spindle level. To obtain such a distribution, the small thinly myelinated γ fibres would have to branch more frequently than the thickly myelinated ones (average proportion 12 : 5, Boyd's calculation, 1962), since they are from a half to a third as numerous in muscle nerves (Boyd & Davey, 1962).

However, results from intramuscular tracings show that the diameter at which a fusimotor fibre or fibre branch enters a spindle has no direct relationship to the diameter of its stem fibre in the muscle nerve.

In addition, thinly myelinated γ fibres in muscle nerves do not branch as frequently as the thickly myelinated ones. In general, the frequency of intramuscular branching is greater in the larger, thickly myelinated γ-stem fibres, not the reverse. There is also no significance in the suggestion of a functional duality within the γ component in such a way that each spindle receives an innervation from fast, thickly myelinated 4.0 - 8.0 μ γ-stem fibres of lower threshold, and slow thinly myelinated, higher threshold γ-stem fibres with diameters of less than 4.0 μ. Only four out of the eighteen spindles studied received fibre branches from both large and small γ-stem fibres. In fact, small γ_2 fibre branches are shown to innervate spindles in one muscle that received a γ component of only thickly myelinated nerve fibres. The work of Steg (1962, 1964), showing a single γ fibre innervating an average of three spindles present in the lateral segmental tail muscles of the rat is indicative of a similar nature. The same conclusion is indicated even if the thinly myelinated γ group is taken as fibres measuring less than 3.0 μ (Boyd, 1962) instead of less than 4.0 μ (Boyd & Davey, 1962).

Boyd (Boyd & Davey, 1965) has recently alleged that Adal & Barker (1965a) misunderstood his classification of the relative diameters of the γ_1 and γ_2 axons supplying the two types of motor endings in muscle spindles, which he emphasized to be based on a number of criteria. Although the occurrence of a dual motor system suggested by Boyd (1962) is based on different classified observations, namely, two kinds of intrafusal muscle fibres, two kinds of motor ending, γ_1 and γ_2 fibre branches etc., a closer examination into any one of the criteria is legitimate. Since no direct relationship is shown in the present study between γ_1 and γ_2 fibre branches and the thickly and thinly myelinated γ-stem fibres, it must be concluded that Boyd's speculations insofar as they are based on fibre diameter are unjustified. In addition, the discrepancy in the measurements of nerve fibres between Boyd's (1962) axon diameter (gold chloride stained) and the external diameter (osmic acid stained, Adal & Barker, 1965a) has already been adjusted by allowing a difference of 0.5 μ between these two measurements. This sdjustment should be adequate as Boyd (1962) stated that "The values of diameter given ..... are those of axon diameter, but it may be assumed that the total

diameter was only slightly greater." Therefore, any fibre with external diameter of 2.5 μ or less in Adal & Barker's (1965a) measurements should be equivalent to 2.0 μ or less in axon diameter measurements of γ_2 fibres, since the axon diameter near muscle spindles was given by Boyd (1962) as 4.0 - 2.5 μ for γ_1 and 2.0 - 1.5 μ for γ_2 fibres. There is no question that any small fibre branches have been missed since all the fusimotor fibre branches entering muscle spindles have been examined. Moreover, a comparison of the average number of fusimotor fibre branches per spindle in the lumbrical muscle between the osmic acid and the teased silver techniques has shown the difference to be negligible (5.7 as compares with 5.8), indicating that all fusimotor fibre or fibre branches innervating the spindles studied have been examined.

Fusimotor fibres and fibre branches exhibiting a duality in fibre diameters, as presented by Boyd (1962), have not been consistent from the level of a muscle nerve to the site of innervating muscle spindles. At the level prior to entry into a muscle, two groups of γ-stem fibres with thick and thin myelination have been demonstrated

(Boyd & Davey, 1962), while within the muscle, it has been found that "In the intramuscular nerve branches two groups of fusimotor axons of different diameter are not obvious.. ..... in the spindle themselves the difference in diameter of the two types of axon is often very striking ...." (Boyd, 1962). Thus, it has been shown for the three different levels that the fibre diameters of motor nerves exhibit a dual nature at the muscle nerve (thickly and thinly myelinated γ-stem fibres), a rather normal distribution as a group in fibre calibre in the intramuscular region, and a clear-cut segregation again near to and within muscle spindles (γ_1 / γ_2 fibres). In order to attempt at an explanation for such a distribution of motor nerve fibres, certain conclusions could be drawn in terms of the nature of branching of these fibres in the intramuscular region. Firstly, if no branching has occurred, then the motor nerve fibre sizes should remain segregated at all levels. This, of course, is a postulation at the extreme but it does illustrate the outcome of the situation. Secondly, if the frequency of branching is the same for all γ fibres whether thickly or thinly myelinated, then since the γ-stem fibres exhibit a duality in sizes, the increase in number of fibre branches

from the same frequency of fibre divisions of both types should also produce a segregation of fibre sizes at all levels. Thirdly, if the frequency of branching is greater within the thinly myelinated γ group (as Boyd, 1962, calculates in the proportion of 12 : 5 in comparison with the thickly myelinated ones), then this should also produce a segregation of motor fibre branches in the intramuscular region since the γ_2 fibre branches from thinly myelinated γ-stem fibres would remain to be small in sizes from more divisions while the γ_1 fibre branches from thickly myelinated γ-stem fibres would remain larger in calibre from fewer divisions. Finally, if the frequency of branching is greater in the thickly myelinated γ group (as shown in the present study), then fibre branches from the thickly myelinated γ fibres together with those from fewer divisions of the thinly myelinated ones would produce a more normal distribution as a group of fibre diameters throughout the range of fibre sizes in the intramuscular region, which is precisely the situation existing within this region.

In the study of fusimotor fibre branches innervating intrafusal muscle fibres, Barker & Ip (1965b) have

shown two kinds of motor ending, namely, the plate and trail ending, lying in different locations in muscle spindles of the cat and rabbit. These endings are found to innervate both the nuclear-bag and nuclear-chain muscle fibres of cat spindles, and are not segregated between them. As maintained previously by Barker & Cope (1962) and Barker, Cope & Ip (1962), no correlation is shown between the size of a motor fibre or fibre branch entering a spindle and the type of intrafusal muscle fibre it innervates. Thus, the diameters of γ fibres, whether stem or branch, are of no great significance and their nature and location of termination as motor endings in the spindle is more relevant.

A dual motor system suggested to occur in muscle spindles by Boyd (1962) is incompatible with some recent neurophysiological conclusions. Henneman, Somjen & Carpenter (1965a) recently showed that during a slowly increasing stretch of an extensor muscle in a decerebrate cat, the smallest alpha motoneurones of the muscle were the first to be reflexly discharged and the larger nerve cells were incorporated into action in the order of increasing sizes. They later (1965b) demonstrated that

the excitability of motoneurones is an inverse function of their cell sizes and the inhibitibility is a direct function of cell sizes. In other words, larger motoneurones are less excitable and more susceptible to inhibition; small motoneurones are more excitable and less susceptible to inhibition. In addition, a relationship was shown between the diameter of a motor fibre and the size of the motor-unit it supplies (McPhedran, Wuerker & Henneman, 1965; Wuerker, McPhedran & Henneman, 1965). Henneman & Olson (1965) finally incorporated all the current findings in a study on the principle in the design of muscles. They concluded that ".... the functional properties of motor-units .... depend upon the size of the motoneurons which innervate them: the size of the cell dictates its excitability, its excitability determines the degree of use of the motor-unit,.... It is suggested that this size principle also governs the properties of gamma motor-units." In applying the size principle of these conclusions on the γ component of motor nerves, the larger γ fibres should innervate 'fusimotor-unit' of larger sizes than the smaller ones. It has been demonstrated in the intramuscular region that large γ-stem fibres do branch more extensively than

the small ones which is as expected for their distribution to 'fusimotor-units' of larger sizes. The reverse nature postulated by Boyd (1962) that small γ-stem fibres undergo more branching and presumably innervate 'fusimotor-units' of larger sizes than large γ-stem fibres is exactly the opposite of all these findings.

The suggestion that γ-stem fibres of less than 4.0 μ in total diameter and of thin myelination specifically innervate nuclear-chain muscle fibres in cat spindles has led to an extension in the present investigation into the study of muscle nerves that supply rabbit spindles lacking nuclear-chain muscle fibres (Barker & Hunt, 1964). The existence of small γ-stem fibres in all the rabbit muscle nerves studied, though in smaller proportions than in the cat, shows that there is no substance in the postulated dual relationship between γ_1 and γ_2 fibre branches from the γ-stem fibres innervating separately the two types of intrafusal muscle fibres in spindles.

The functional significance of fusimotor fibres and their related endings in spindles within muscles have

been investigated by various neurophysiological experiments. Matthews (1962) isolated two functionally distinct kinds of γ motor fibres to the soleus muscle of the cat and named them dynamic and static fusimotor fibres, identified by the respective frequencies of response from the primary ending produced by repetitive stimulation of the fibres at constant muscle length and during the dynamic phase of muscle stretching. Matthews and his co-workers (Jansen & Matthews, 1962; Matthews, 1962, 1964; Crowe & Matthews, 1964a, b) presented arguments that the dynamic fusimotor fibres may correspond to the γ_1 fibres of Boyd's (1962) supplying the nuclear-bag intrafusal muscle fibres and the static fusimotor fibres may correspond to the γ_2 fibres supplying the nuclear-chain intrafusal muscle fibres, though they pointed out that such a correlation was based on the histological structure of the spindle by Boyd (1962) only without any support from direct experimental evidence.

There is certainly no doubt as to the existence of two functionally significant types of γ fibres, namely the dynamic and static fusimotor fibres, from experimental evidence. However, equating these fibres directly with

two independent motor system within the muscle spindle in accordance with Boyd's γ_1 and γ_2 motor fibres would be against certain findings as yet unexplained by the arguments.

Firstly, there is considerable overlap in the range of conduction velocities without any characteristic specific range for dynamic and static fusimotor fibres in all investigations. For example, Matthews (1962) showed a range in conduction velocity of 24 - 38 m/sec for dynamic fusimotor fibres and 23 - 45 m/sec for static fusimotor fibres. No marked difference in conduction velocity between these fibres was found by Crowe & Matthews (1964b) or Appelberg, Bessou & Laporte (1965). In addition, Brown, Crowe & Matthews (1965) showed that individual spindles might be supplied by a dynamic and a static fusimotor fibre of the same conduction velocity. Assuming the conduction velocity of medullated fibres varies with their diameter (Hursh, 1939), the diameters of the dynamic and static fusimotor fibres would overlap considerably. Secondly, the functional difference in the two types of fusimotor stimulation was demonstrated from dynamic and static fusimotor fibres which were

selected specifically in preference for their strong contrasting types of effect, although the existence of intermediate or other types of fusimotor fibres could not be excluded (Matthews, 1962). This would have to be borne in mind when these results are equated into the motor innervation of the spindles in general. Finally, if the functional difference between dynamic and static fusimotor fibres in the cat spindles were equated in terms of their distribution to the two types of intrafusal muscle fibres, the same interpretation would not apply to rabbit spindles in which only one type of intrafusal muscle fibre, the nuclear-bag fibre, occurs (Barker & Hunt, 1964), and also consists of both dynamic and static fibres in their fusimotor innervation (Emonet-Denand, Laporte & Pagès, 1964).

The suggestion by Boyd (Boyd & Davey, 1962; Boyd, 1964) that the small thinly myelinated γ-stem fibres differ in motor function from the large thickly myelinated ones has not been supported by physiological experiments. Brown, Crowe & Matthews (1965), with this question in mind, have specifically chosen static fusimotor fibres of slow conduction velocities (21.9 - 16.4

m/sec) and therefore, presumably to be small γ-stem fibres, to compare with those of fast conduction velocities, but no consistent functional difference has been observed between them. Boyd (1965; Boyd & Davey, 1965), however, put forth a further suggestion recently that both the dynamic and static fusimotor fibres are contained within the thickly myelinated γ-stem fibre group, and that the thinly myelinated γ fibres may have a function of their own which has yet to be investigated. In view of Henneman & Olson's (1965) findings, however, it seems more probable that the varying proportion of thickly and thinly myelinated γ fibres in various muscle nerves is associated with different degree of γ excitability.

In a study of the functional relationship between fusimotor fibres and their motor endings, Bessou & Laporte (1965) investigated the effects of fusimotor stimulation by determining the intrafusal muscle fibre potentials produced from stimulation of the dynamic and static fusimotor fibres. It was found that stimulation of static fusimotor fibres produced diphasic or triphasic potentials, considered as 'propagated potentials' similar to those described by

Eyzaguirre (1960). These potentials were normally observed over only one-half of the spindle, at some distance away from the equatorial region. In constrast, stimulation of dynamic fusimotor fibres produced monophasic potentials which were generally recorded near the equatorial region of the spindle. These results provide a new interpretation in the correlation of the distribution of fusimotor fibres within the muscle spindles as suggested by Bessou & Laporte (1965). With their production of different potentials at various locations when stimulated, the dynamic and static fusimotor fibres correlate well with the histological findings of trail-endings and plate-endings (Barker & Ip, 1965b), respectively, within muscle spindles.

β fibres

The occurrence of motor fibres in mammalian muscle nerves giving branches to supply both extra- and intrafusal muscle fibres (β fibres) have long been suspected and various conclusions have been made either by direct histological observations or by indirect physiological studies. Their existence as suggested

from indirect evidence by different interpretations of of various physiological experiments have been fully discussed by P.B.C.Matthews's (1964) review with an open mind for other possible interpretations. It is only from the more recent work of Bessou, Emonet-Dénand & Laporte (1963a, b, c; 1965) that direct evidence of the existence of β fibres in the innervation of muscle spindles has been demonstrated convincingly by neurophysiological methods.

The β fibres examined in the lumbrical muscles of the cat give a diameter range of 6.0 - 12.5 μ, in comparison with one of 2.5 - 7.5 μ for the γ fibres traced and one of 7.5 - 13.5 μ for the α fibres. These histological findings are closely related to measurements of conduction velocities of β and α fibres in the first deep lumbrical muscle nerve of the cat shown by Laporte and his co-workers, namely, 31 - 61 m/sec for β (Bessou, Emonet-Dénand & Laporte, 1965) and 42 - 78 m/sec for α fibres (Bessou, Emonet-Dénand & Laporte, 1963b). The distribution of β fibres are found to vary from being predominantly fusimotor to predominantly skeletomotor in nature. A β fibre may provide

fusimotor branches to innervate only one spindle within a lumbrical muscle or may innervate all the spindles present. On the other hand, the fusimotor supply of a muscle spindle does not necessarily contain fibre branches from a β fibre; in fact, β fibres are not present in all lumbrical muscle nerves, a point in agreement with Bessou, Emonet-Dénand & Laporte (1965).

β fibres have also been demonstrated in the work of Brown, Crowe & Matthews (1965) in the tibialis posterior muscle nerve of the cat; the conduction velocities fall within the range of 59 - 84 m/sec and their detection is said to be 'sporadic'. The higher rate of conduction velocities and therefore relatively larger fibre diameters in the β fibres of this muscle is due to the muscle size and the related fibre-diameter spectrum being larger than the lumbricals.

The situation found in the lumbrical muscles of the rabbit is that β fibres occur more frequently, since in many cases, both fusimotor and skeletomotor supply are derived from motor fibres without a γ component. It is not surprising that, in some cases, β fibres

contribute to the fusimotor supply to spindles within a muscle in spite of the presence of a γ component in the muscle nerve; in others, the fusimotor supply to spindles is replaced entirely by β fibres in the absence of a γ component. This situation has been suspected in the caudal muscles of the rat by Kidd (1964) who found the motor supply to derive from β fibres.

It is difficult to assess the occurrence and functional significance of β fibres. They might be a special group of fibres which, from reflex action, produce additional tension to the muscle together with some increase in activity of spindle endings. However, their absence in some muscles and sporadic detection in others make this interpretation rather doubtful. Moreover, the tension from some of the units innervated by these fibres is negligible. Another interpretation is that they might be a kind of evolutionary vestige. In the lower animals, the intrafusal muscle fibres are closely related to the extrafusal muscle fibres in having a common motor innervation and functional combination. In the higher animals, the condition might be that a more delicate control of muscular activity is progress-

ively developed through the evolutionary process to produce a separate and independent group of motor fibres for a specialized function. In this way, the β fibres, having an intermediate distribution and action between skeletomotor and fusimotor fibres, could then be considered as mere vestiges of such a process. A further possibility in the production of β fibres should not to be excluded. Barker & Ip (1965a) have recently demonstrated that motor nerve endings do not maintain a fixed morphological entity but degenerate periodically followed by renewal. β fibres with only one or two terminal branches of fusimotor nature could well be accidental products from the process of regeneration in the renewal of degenerated motor nerve endings in the spindles. β fibres if produced by this means should not, however, be confused with those which show an approximately equal distribution to both extra- and intrafusal muscle fibres.

A schematic presentation of the motor innervation of cat skeletal muscle

The principal issues in the difference between the past and the present knowledge of the intramuscular branching of fusimotor fibres and their distribution and termination within cat muscle spindles are now presented for comparison. The dual motor innervation of the spindle in the form of two separate efferent systems according to Boyd (1962) is shown schematically in Text-figure 21. The nuclear-bag and nuclear-chain intrafusal muscle fibres are said to have different types of motor endings independently, namely, the motor end-plate and motor 'network', respectively, and these in turn receive separate large γ_1 and small γ_2 fusimotor branches from thickly and thinly myelinated γ-stem fibres within the γ group in the muscle nerve. The smaller proportion of the thinly myelinated γ-stem fibres in the muscle nerve would have to branch more extensively in the intramuscular region to produce such a distribution in the innervation of spindles. Results from this study were based on observations in muscle nerves and at surrounding sites of innervation into muscle spindles; the nature

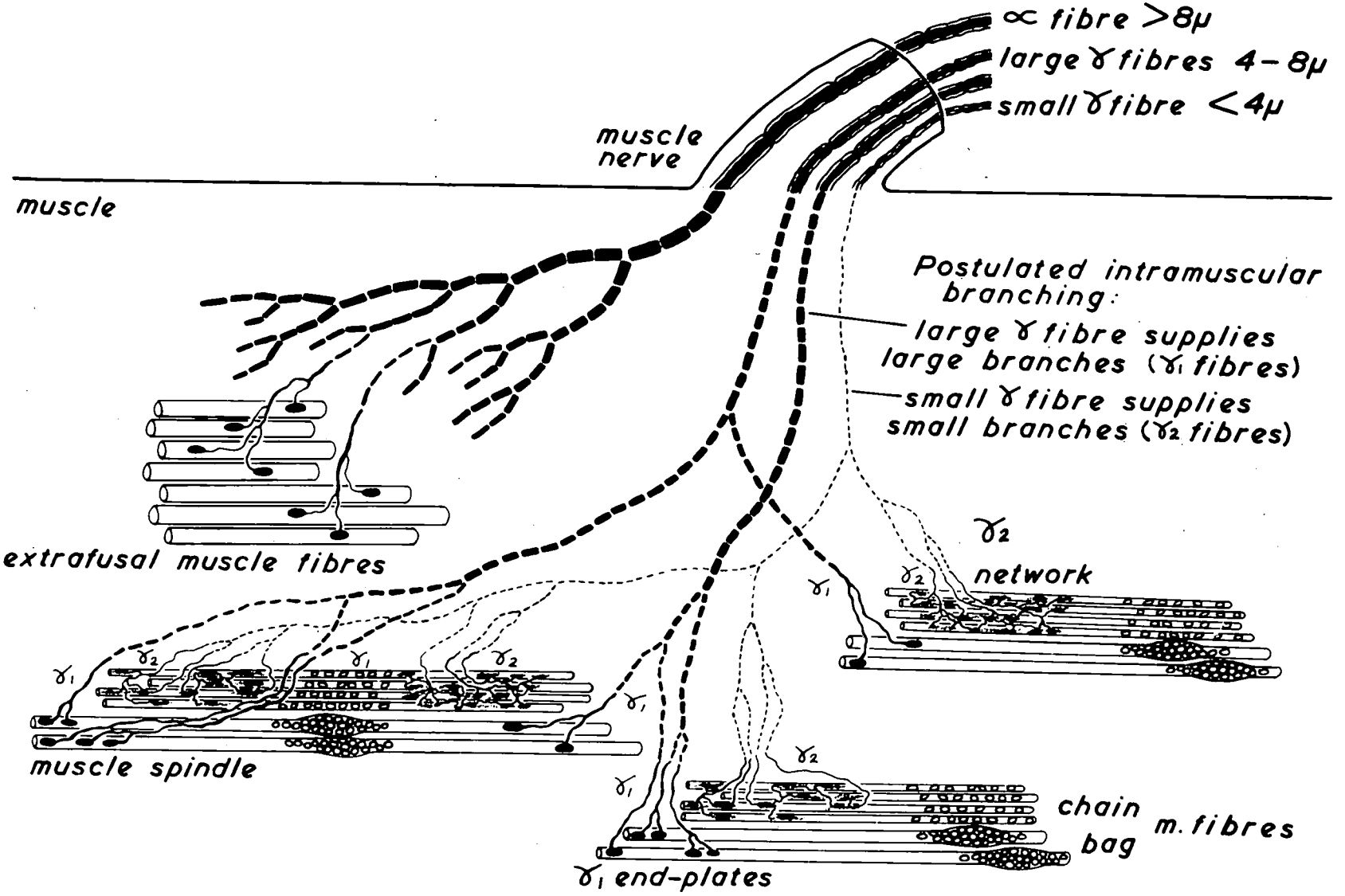
Text-figure 21. Schema of intramuscular branching and distribution of motor nerve fibres in a skeletal muscle according to Boyd's (1962) hypothesis.

(i) Large, thickly myelinated γ-stem fibres of 4 - 8μ show little branching and their fusimotor fibre branches, γ_1, innervate only nuclear-bag muscle fibres of spindles, ending as end-plates.

(Ii) Small, thinly myelinated γ-stem fibres of less than 4μ branch more extensively and their fusimotor fibre branches, γ_2, innervate only nuclear-chain muscle fibres of spindles, ending as 'network'.

(iii) The proportion of intramuscular branching between thinly and thickly myelinated γ-stem fibres is given as 12 : 5 (Boyd, 1962).

(iv) Motor fibres with branches innervating both extrafusal and intrafusal muscle fibres have not been detected.


∝ fibre >8μ
large γ fibres 4–8μ
small γ fibre <4μ
muscle nerve
muscle
Postulated intramuscular branching:
large γ fibre supplies large branches (γ1 fibres)
small γ fibre supplies small branches (γ2 fibres)
extrafusal muscle fibres
γ2 network
muscle spindle
γ1 end-plates
chain bag m. fibres

of nerve fibres in the intramuscular region were derived by postulation only. Fibre branches of β origin innervating muscle spindles have not been demonstrated.

The new picture of the situation, based on the results of the present study as well as those currently accomplished (Barker & Ip, 1965b; Barker, 1965) is shown in Text-figure 22. Branching of nerve fibres in the intramuscular region have been actually traced and this provides in detail, the nature and distribution of nerve fibres within the muscle, from the level of the muscle nerve to the site of innervation. The source of origin and reference of each nerve fibre represented in the muscle nerve is shown in the following table:-

Nerve fibre in muscle nerve	Origin	Reference to data
13.5 μ	C158, 1st DL	Fibre C, Table 3
12.5 μ	C167, 1st DL	Fibre m?, Table 4
5.5 μ	C167, 1st DL	Fibre l, Table 4
6.5 μ	C167, 2nd DL	Fibre r, Table 5
3.0 μ	C167, 2nd DL	Fibre p, Table 5

DL - deep lumbrical muscle

Text-figure 22. Schematic representation of the nature of intramuscular branching and distribution of motor nerve fibres from actual teasing in the intramuscular region of skeletal muscles.

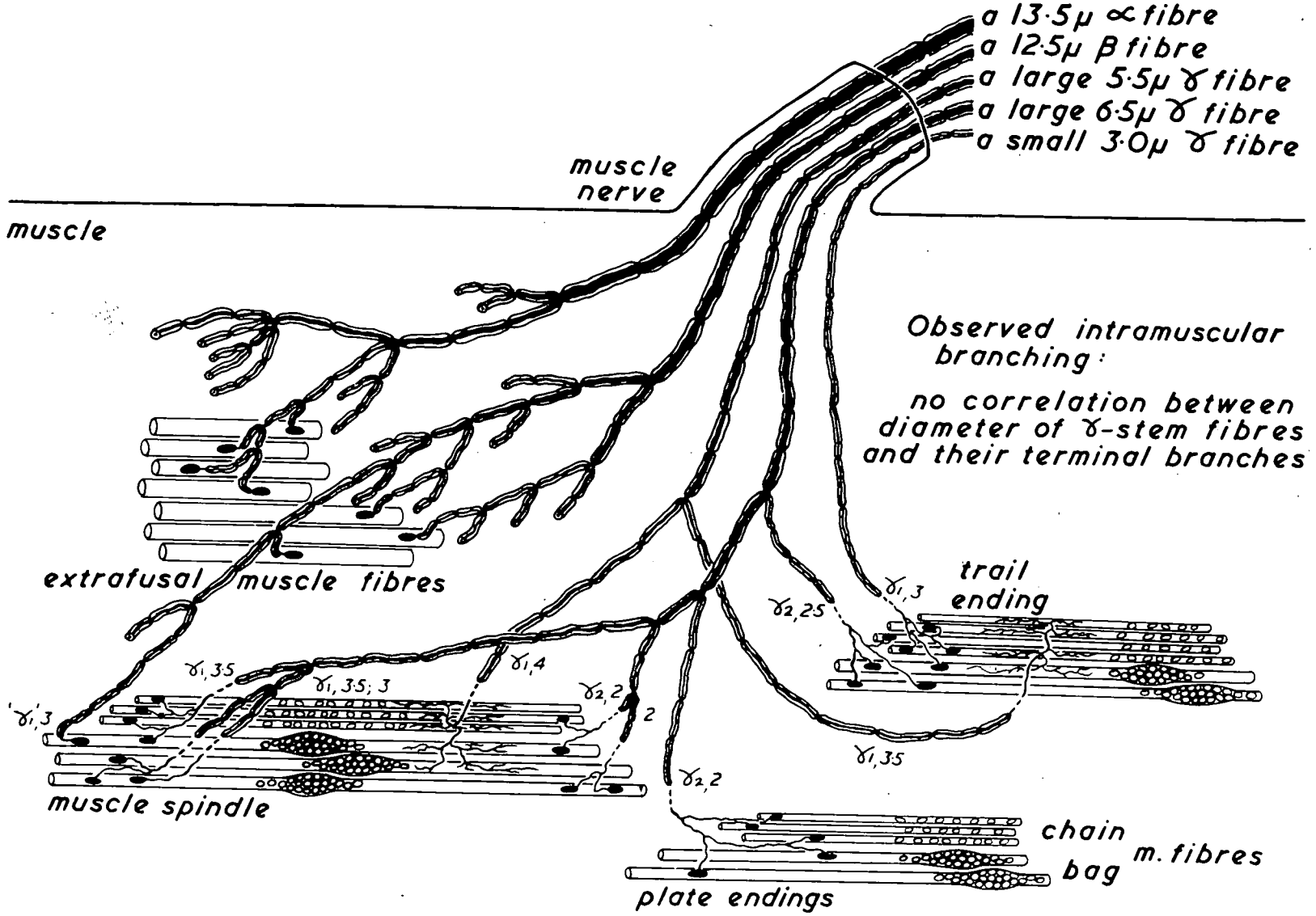
(based on the work of Adal & Barker 1965a and Barker & Ip, 1965b).

(i) There is no direct relationship between diameters of fusimotor fibre branches in the intramuscular region prior to spindle entry and the diameter and degree of myelination of γ-stem fibres.

(ii) Fusimotor fibre branches innervate both nuclear-bag and nuclear-chain muscle fibres of spindles, irrespective of their diameters.

(iii) Fusimotor fibre branches may innervate motor endings of the 'plate' and 'trail' types and these endings lie in both the nuclear-bag and nuclear-chain muscle fibres of spindles. The only difference is the location of these motor endings within the spindle.

(iv) Some motor fibres (β fibres) may give fibre branches to supply both extra- and intrafusal muscle fibres.


a 13·5μ ∝ fibre
a 12·5μ β fibre
a large 5·5μ γ fibre
a large 6·5μ γ fibre
a small 3·0μ γ fibre
muscle nerve
muscle
Observed intramuscular branching:
no correlation between diameter of γ-stem fibres and their terminal branches
extrafusal muscle fibres
trail ending
γ1,3
γ2,25
γ1,3·5
γ1,3·5; 3
γ1,4
γ2,2
γ1,3
2
γ1,3·5
γ2,2
muscle spindle
chain
bag
m. fibres
plate endings

No consistent size relationship is shown between the diameters and degree of myelination of γ-stem fibres in the motor supply and the fibre branches entering muscle spindles. The recent knowledge of two types of motor endings in the spindles, the end-plates and the trail-endings (Barker & Ip, 1965b), are distributed to both nuclear-bag and nuclear-chain intrafusal muscle fibres, definitely not segregated between them. The calibre of fusimotor fibre branches innervating the two kinds of motor endings are also without segregation. The locations of the two types of endings lie in different positions within the spindle, the end-plates normally nearer the polar regions and the trail-endings around the juxta-equatorial regions. A fibre branch from β origin innervating a muscle spindle is also included.

In view of the fact that no significance is shown between γ_1/γ_2 fusimotor fibre branches and their origin from different γ-stem fibres, and in order to avoid further confusion, it has been generally agreed at the recent Nobel Symposium (1965) in Stockholm that these terms, γ_1/γ_2 , should be abandoned.

VI. SUMMARY

1. In osmium tetroxide preparations, the extramuscular course of the 1st deep lumbrical, soleus and mesial gastrocnemius muscle nerves of the cat were traced back to their origin from larger nerve trunks where no branching of nerve axons occurred. Initial branching of motor axons above 10/u begins at a certain distance from the muscle; this distance being greater in larger muscles than in smaller ones.

2. In teased, osmium tetroxide preparations the intramuscular course of twenty-seven out of twenty-eight stem motor fibres supplying eighteen spindles was traced to the level of spindle entry in three 1st and two 2nd deep lumbrical muscles removed from the de-afferentated hindlimbs of three cats.

3. The nature of the intramuscular branching of the γ fibres was such that they did not supply γ_1 and γ_2 fibres to the spindles in accordance with their stem diameters and degree of myelination as proposed by Boyd(1962). Of eighteen thickly myelinated γ-stem fibres traced (4.0 - 7.5/u), seven branched so as to produce γ_1 fibres only (3.0 - 4.5/u), two produced γ_2

fibres only (2.0 - 2.5 μ) and nine produced a mixture of both (all measurements refer to total diameters.

4. In general, the larger the diameter of the γ-stem fibre, the greater is its frequency of intramuscular branching. Five thinly myelinated γ-stem fibres traced (2.5 - 3.5 μ) did not branch at all in their intramuscular course; each supplied one spindle only, three entering as γ_1 fibres, two as γ_2 fibres.

5. Five of the motor fibres traced innervated both extra- and intrafusal muscle fibres. The stem diameter of these β fibres ranged from 6.0 to 12.5 μ, and at these two extremes their distribution varied from being predominantly fusimotor to predominantly skeletomotor, respectively.

6. The skeletomotor innervation ratio for the cat 1st deep lumbrical muscle is calculated to be 1 : 300. The fusimotor innervation ratio is calculated to be one γ or β fibre to nine intrafusal muscle-fibre poles. Re-assessed innervation ratios for the cat soleus and tibialis anterior muscles are higher than those previously determined by about 70 - 80%.

7. In teased, osmium tetroxide preparations twelve skeletomotor and two β fibres in two 1st and

one 2nd deep lumbrical muscles from the de-afferentated hindlimbs of two cats were examined for the nature of intramuscular branching and distribution. Their intramuscular branching was shown to be of three main types, namely, 'pyramidal', 'semipyramidal' and 'monopodial' types.

8. A daughter axon from a parent nerve fibre division within the main intramuscular nerve trunk may not proceed direct to a side nerve trunk but may travel by a recurrent route. This depends on the point of axon division in relation to the level of the side nerve trunk. The distribution of skeletomotor axon branches in the intramuscular region is in the form of neural configurations with numerous axon branches travelling in different directions. It is impossible under these conditions to trace the extent of individual motor-units histologically.

9. In teased, osmium tetroxide preparations the motor component of twenty-one de-afferentated nerves to the lumbrical muscles of eight rabbits was examined. 105 motor nerve fibres were measured in which 19 were less than 8μ. Only twelve of the nerves consisted of a γ component, the remaining nine were having β fibres only.

10. In teased, silver preparations of spindles in these muscles, both types of motor ending, namely, the 'plate' and 'trail' ending were observed although the former is by far the commoner of the two. In a total of 92 spindles from 21 muscles, trail endings were found to occur in only two spindles from separate muscles which received γ fibres in the motor supply. In other muscles, there were no trail endings in the spindles although γ fibres were present in the nerve supply.

11. Small γ fibres with total diameter less than 4 μ were found to occur in the soleus, tibialis anterior and popliteus muscle nerves that supply rabbit spindles lacking nuclear-chain fibres, although in smaller proportions than in the cat.

12. The nature of intramuscular branching and distribution of motor nerve fibres in cat skeletal muscle is shown by a schematic presentation.

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