The innervation of mammalian skeletal muscle by medium and small diameter afferent nerve fibres

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THE INNERVATION OF MAMMALIAN SKELETAL MUSCLE BY MEDIUM AND SMALL DIAMETER AFFERENT NERVE FIBRES

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by

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I. INTRODUCTION

The classical work of Sherrington (1894), which conclusively demonstrated that skeletal muscles and their supplying nerves contained a sensory as well as a motor nerve component, is the basis from which all succeeding neuroanatomical studies of muscle have been developed. In general these studies, like those of Sherrington, have been directed at two separate aspects of muscle innervation, the one being a study of the nerve-fibre composition of the muscle nerve, and the other the study of the nerves at their site of termination in the muscle.

The myelinated sensory fibres in muscle nerves have a total diameter range of between 1.0 μ and 20.0 μ (Sherrington 1894), and can be arranged into three size groups (Lloyd & Chang 1948; Rexed & Therman 1948). The Group I fibres range between 20.0 μ and 12.0 μ in diameter with a peak at 15.0 μ; those of Group II between 12.0 μ and 4.0 μ with a peak at 7.0 μ and Group III between 4.0 μ and 1.0 μ with a peak at 3.0 μ (Lloyd & Chang 1948).

Earlier physiological studies of muscle afferent fibres by Matthews (1933) established the existence of four types of fibre response (each of characteristic frequency discharge) at dorsal root level when the muscle was stimulated in various ways. The A1 and A2 responses were from endings 'in parallel' with the contractile elements of the muscle, since no response was recorded when the muscle was contracting. The B response was initiated during contraction, the ending being 'in series' with the contractile elements. The C response Matthews found to be rare. The endings appeared to lie in the muscle fascia, since when this was stripped from the muscle the response was never recorded. These fibres did not give a response to steady tension, and during contraction their behaviour was variable. 'Often a group of 2-5 impulses is set up during the rise or fall of mechanical activity, sometimes during both' (Matthews 1933 p.31).

That the anatomical grouping of muscle afferent fibres is of physiological significance was demonstrated by Hunt (1954), who calculated the conduction velocities of individual nerve fibres from cat soleus and gastrocnemius muscles by stimulating the muscle nerve and recording from single-fibre preparations at the dorsal root. These conduction velocities exhibited a bimodal distribution equivalent to Groups I and II. Conduction velocities in fibres representative of Group III were seldom detected, 'the principal difficulty being size of the recordable potential' (Hunt 1954 p.121). By more sophisticated methods of stimulation and recording, Paintal (1960) was able to study the responses of Group III fibres to stimuli applied to the muscle. He found that most of them were connected to 'pressure-pain receptors', a few to stretch receptors, and fewer still could not be activated by any mechanical means.

These significant findings led to the conclusion that, generally speaking, the fibres of Group I end in muscle spindles as primary endings and in tendon organs (Matthews' A2 and B responses), while those of Group II end in muscle spindles as secondary endings (Matthews' A1 response). It is generally assumed that Group III fibres probably end in paciniform corpuscles and as free endings (Bessou & Laporte 1961; Barker 1962), and correspond to Matthews' C response.

These physiological procedures have further demonstrated that the sensory-fibre grouping is not strictly delimiting in terms of fibre size and ending response. Certain of the Group I and Group II fibres overlap (Hunt 1954), and some of the pressure-pain receptors have conduction velocities as great as 91m/sec., which in anatomical terms would put their fibres within the Group II and lower Group I ranges (Paintal 1960). Furthermore, some of the slowly conducting (Group III) fibres respond to stretch (Paintal 1960; Bessou & Laporte 1961) that is typical of the fibres of Groups I and II.

Together with the myelinated sensory component, there are in muscle nerves numbers of non-myelinated sensory fibres (C afferents), which, at least in a fascicle of the nerve to vastus medialis of the cat (Ranson & Davenport 1931), were shown to be less than half as abundant as the total (sensory + motor) myelinated fibres.

Activation of these non-myelinated fibres occurs when the sensitive part of the muscle is subjected to pressure with a mechanical threshold between 5 to 10 g.wt. (Iggo 1960). Occlusion of the blood supply combined with prolonged muscle contraction also produces a discharge (Bessou & Laporte 1958; Iggo 1961). Iggo found the discharge to be of low frequency, less than 1/sec., with occasional bursts at 10 to 20/sec. lasting as long as 3 secs. Recording from fine strands of non-myelinated fibres in the muscle nerve, he was further able to record discharges when the mechanically sensitive part of the muscle was warmed above 41°C or cooled below 25°C, there being a number of fibres most sensitive within the temperature range 28°-32°C.

Certain of these findings appear to corroborate many earlier suggestions (Ranson & Billingsley 1916; Hinsey 1927; Denny-Brown 1928; Bishop & Heinbecker 1935; Clark, Gasser & Hughes 1935; Lewis 1942) that the C afferents are 'pain' fibres, although it has been shown that a number of non-myelinated cutaneous afferents respond to light mechanical stimuli and slight temperature changes (Iggo 1959, 1960;

Hensel, Iggo & Witt 1960). These responses to non-noxious stimuli are also a feature of certain C afferents from Skeletal muscle (Iggo 1961).

Free non-myelinated terminal ramifications were first observed by Kolliker (1962) in frog muscle. Since that time, Hinsey (1927), working with cat muscles, has established that at least some of these terminals are derived as a result of myelinated parent-fibre branching close to the site of termination, and that they are undoubtedly sensory in origin. Although it is known that non-myelinated axons of sympathetic origin branch to give extensively ramifying terminals associated with the media of blood vessels (Hinsey 1927; Falck 1962), the relationship between C afferents and their endings in skeletal muscles has never been satisfactorily established. It has been suggested that the non-myelinated axons occasionally associated with muscle spindles, tendon organs and Pacinian corpuscles (Ruffini 1897; Huber & deWitt 1900; Dogiel 1902; Tello 1922; Barker 1948; Goto & Lowenstein 1961; Lowenstein, Goto & Noback 1962) are of C afferent origin, but unequivocal evidence for this is lacking.

In correlating the receptor population of the cat's soleus muscle with the afferent fibre-diameter spectrum of the supplying nerve, Barker, Ip & Adal (1962) found that the Group II and Group III fibres in the muscle nerve were far in excess of the receptor counts.

In terms of unallocated fibres this discrepancy amounted to 35.6% in Group II, and 76.0% in Group III, i.e.29.2% of the total sensory fibre population of the supplying nerve. 'Quantities of Group II and Group III fibres approximately of these proportions'.....(35.6% and 76.0%).....'participate in the innervation of blood vessels and the supply of free endings in the muscle' (Barker, Ip & Adal 1962 p.261). Similarly, Zelena & Hnik (1963) estimated that in rat soleus muscle, which had previously been tenotomized with subsequent repression of encapsulated receptor development, about 30.0% of the sensory nerve fibres supplied free endings in the muscle.

The present investigation was undertaken to obtain an accurate estimate of the non-myelinated sensory component in skeletal muscle nerves, and to provide information about the intramuscular course and mode of termination in the muscle of the small myelinated and non-myelinated sensory nerve fibres.

II. MATERIALS AND METHODS

1. Introduction

In an investigation of the sensory nerve fibres supplying skeletal muscles, it is necessary to remove both the motor and the sympathetic components by differential degeneration. This is done by cutting the ventral roots of the appropriate spinal nerves and removing an equivalent portion of the sympathetic chain. The choice of muscle material for the purposes of this differential denervation is dependent upon a number of factors. To obtain optimum impregnation by the modified de Castro technique (Barker & Ip 1963), small muscles must be used. The smaller the size of the muscle nerve, the easier and more accurate it becomes to count the constituent fibres, and also to cut ultra-thin sections for electron microscopy. There are certain technical limitations of lumbo-sacral sympathectomy which prevent the use of some limb muscles, and together with this there is the possibility that the distribution of sympathetic nerve fibres to limb muscles does not follow the same pattern as the fibres of the spinal nerves.

2. Operating procedures

2.1 The lumbo-sacral sympathetic chain

A preliminary investigation into the nature of the sympathetic chain in the lumbo-sacral region of the cat revealed a number of structural variations that affected the ablation of the chain in this region (Fig.1A-E). In a total of eleven animals it was found that the sympathetic ganglia, including and caudad to S1, gave off a single ramus which joined its equivalent spinal nerve. In four of these animals, the L7 ganglia gave off two rami (Fig.1C) distributed to the L7 and S1 spinal nerves. (In two of these four animals there were no apparent ganglion swellings). In the other seven cases two rami were present only as far as the L6 level, and they were distributed one to the equivalent spinal nerve, and one to the preceding spinal nerve (Fig.1D). In one animal only, the fused S2 ganglion gave off two rami to S2 and S1 spinal nerves. In this particular case there was no apparent S1 ganglion swelling (Fig.1E).

Fusion of the ganglia occurred only at, or below, the S1 level - never above. In six cases it occurred at this level: in three it occurred at the S2 level; in two at the S3 level; and in one instance as far caudad as Ca1. Once fusion occurs, no matter at what level, all the succeeding ganglia are fused. The connecting nerve tracts between ganglia remained paired, except in a single instance, where, after fusion at the S1 level, only one was present (Fig.1A). In one instance the ganglia were not completely fused until the Ca2 level, but connecting strands were present between the paired ganglia at the S1 and the S2 levels (Fig.1B).

The connecting nerve tracts between the L7 and S1 sympathetic ganglia mark the position at which the sympathetic chain passes through the pelvis or over the 'pelvic brim' (Hinsey 1927). From this point caudad the chain becomes more intimately associated with the caudal artery and vein, and consequently during operation it is very difficult to manipulate.

These criteria of ganglionic fusion, number of rami and pelvic brim position were used as a guide during operation to ascertain the level and completeness of sympathetic chain ablation. On killing the animals a thorough dissection was made of the lumbo-sacral region to confirm this.

2.2 Lumbo-sacral sympathectomy

The cat was anaesthetized by an intraperitoneal injection of 40 mg/kg sodium pentabarbitone (Nembutal, Abbott). If after ten minutes it was not sufficiently deep for operative purposes, a supplementary dose of 5 mg/kg was administered. Under these conditions deep anaesthesia was obtained for the duration of the operation, on average one-and-a-half hours.

A retroperitoneal approach to the lumbo-sacral sympathetic chain was taken (Fig.2). Following a primary incision in the skin on the right side, from the angle of the last rib with the backbone to a point overlying the level of the leading edge of the sartorius muscle, and about three inches above the knee, (2.0 kg cat), the underlying fatty deposits are reflected and the superficial dorsal fascia exposed. An incision is then made in the fascia following the line of the primary incision, and on reflection the superficial abdominal muscles are exposed. A further incision is made along the insertion of the superficial abdominal muscle on to the latissimus dorsi muscle. This reveals a layer of deep fat which overlies the deep abdominal muscles. After reflecting the fat, the last incision is made along the line of insertion of the deep abdominal muscle with the latissimus dorsi muscle. The psoas major is then located beneath the deep abdominal muscle. By gentle retraction the connective tissue between these two muscles is separated and the peritoneum located. With slight downward pressure of the peritoneum the vena cava comes into view, and the sympathetic chain at the L5/L6 level is located dorsal to this. The connecting nerve tract between the L5 and L4 sympathetic ganglia is cut, and the distal stump picked up with fine forceps. The connective tissue surrounding the chain is loosened caudad, and as each ganglion is located its rami are cut with fine scissors. In this way the sympathetic chain to the L6 ganglion is loosened. An incision is then made along the line of the insertion of the psoas major with the latissimus dorsi muscle, and the muscles are retracted gently to reveal the L7 sympathetic ganglion. The loosened portion of the chain is again picked up, and the L7 ganglion teased free of its connective tissue, and its rami are cut. The chain between the L7 and S1 ganglia is then loosened from the ventral surface of the pelvis as it passes over the brim, and the caudal artery is gently pressed with a cotton-wool swab while the distal portion of the connecting-nerve tract is loosened and the S1 ganglion located. When this is a fused ganglion, the rami to both left and right spinal nerves are cut, together with the S1 and S2 connecting-nerve tracts. A non-fused S1 ganglion is treated in the same way as the L5, L6 and L7 ganglia. The S1/S2 connecting-nerve tract was always cut and the excised portion of the chain removed and placed in mammalian Ringer's solution. After the operation it was studied under the microscope to check that all of the rami were intact.

A binocular operating microscope was used,at a magnification of x 60,from the time that the sympathetic chain was located to the final removal of the ablated portion.

2.3 Ventral root laminectomy

Two weeks after sympathectomy, when the animals had completely recovered, (return to normal eating and weight increase) a lumbo-sacral laminectomy was performed, that entailed cutting the ventral roots of the right L6 to S1 spinal nerves.

It was discovered from previous experience that certain animals developed a resistance to Nembutal. The dosage for this operation was therefore increased to 45 mg/kg and was found to be adequate.

Following the primary mid-dorsal incision at the lumbosacral level, the latissimus dorsi muscles are separated from the backbone to expose the dorsal spines and neural arches of L5 to S2. The upper right quadrate of the L6 to S1 vertebrae are removed to expose the corresponding spinal nerves. By rotating the roots, the separation is located. The dura is cut along the line of separation with a fine scalpel, and the connective tissue between the roots is carefully loosened. The ventral root is then cut and a small portion removed to retard regeneration. The two stumps are picked up with fine forceps to ascertain the completeness of the cut.

The binocular operating microscope is used at x 60 magnification, when the roots are cut.

2.4 Degeneration and regeneration times

Regeneration of sympathetic nerve fibres does not occur after the ablation of the sympathetic ganglia (Hinsey 1927). Ranson (1912) maintained that these fibres are particularly resistant to the normal processes of degeneration, and that some of them remain intact in peripheral nerves three weeks after nerve section. The data of Young (1942) were used to calculate the regeneration rates of nerve fibres of ventral root origin. For animals of about 2 kg weight, the minimum distance from the S1 spinal ganglia to the level of the tibialis-peroneal nerve division is 90mm. Allowing a minimum time of 7.3 days for scar delay and a subsequent growth rate of 3.45 ± 0.16 mm/day, four weeks was considered adequate time for debris clearance of somatic efferents without fear of regeneration occurring. Six weeks was also considered ample time for the degeneration of resistant non-myelinated nerve fibres and subsequent debris removal. The animals were therefore killed six weeks after the first operation.

2.5 Operation checks

On killing the animals, a dissection was performed to check the exact level of sympathetic ablation. This was done by locating the most distal, intact, sympathetic ganglion on the operated side and tracing its connecting ramus to the equivalent spinal nerve. The most distal ganglion removed at operation was that cephalad to the one left intact. Together with the information obtained from normal gross-anatomy dissections, it was possible to establish the exact levels of sympathetic ablation.

To check that the ventral roots had been completely severed without damaging the dorsal-root ganglia, the operated roots were removed, embedded in wax, sectioned at 10 µ, stained by Holmes silver method (Carleton & Drury, 3rd.ed.1957) and studied under the microscope.

The choice of transverse rather than longitudinal sections of the operated roots was adopted because orientation of the roots for perfect vertical longitudinal sectioning is very difficult. Slightly oblique longitudinal sections confuse and often obscure the interpretation of incomplete ventral-root laminectomy. The ventral roots of L6 and L7, 'in vivo', form a gutter in which rests the dorsal root ganglia (Fig.3*a*). If during an operation an incomplete ventral-root cut is made and fibres remain (shaded area in Fig.3*a*) they are not recognizable as ventral-root fibres unless the roots are sectioned through the plane represented by the line A-B. If transverse sections are cut, any intact motor fibres are immediately recognizable because they lie outside the distinct dorsal-root perineurium (Figs.3*a*,*b*) and remain outside until the mixed nerve is reached.

Each series of operated roots was carefully compared with a deliberately damaged series in which a small portion of the right dorsal roots of L7 and S1 had been pinched between fine forceps, left for four weeks, removed, sectioned and impregnated by the Holmes method. These root sections served as a standard for damage (Fig.4A), and if any of the operated roots showed similar damage (Fig.4B) the material from that animal was discarded.

2.6 Overspill of dorsal-root ganglion cells into the mixed nerve and ventral root

On rare occasions in the operated material certain receptors possessed no innervation (Fig.5). At first this was attributed to dorsal-root cell damage incurred during laminectomy, but on re-examination of the sectioned roots the perineurium was found completely intact, and no cell damage was apparent. Sherrington (1894) noted the persistence of 55 myelinated nerve fibres in 10 peripheral nerves of two cats after he had removed the dorsal roots and cut the ventral roots (close to the spinal cord) of the spinal nerves at the lumbo-sacral level, and had allowed between 30 to 39 days for degeneration. He concluded that the persistent fibres 'may be connected with certain of the scattered ganglion cells within the ventral roots' (Sherrington 1894 p.224). In the present investigation the ventral roots of the spinal nerves were not only cut near the cord, but a small portion was actually removed, thus damaging, (or removing), any dorsal-root cells present in the ventral root and therefore causing peripheral degeneration of a certain number of sensory nerve fibres.

To obtain more detailed information of the extent of sensory fibre degeneration likely to occur after ventral root section, a study of 36 spinal nerves (nine each from L6, L7, S1 and S2) from eight cats was made. Serial 10.0 μ longitudinal sections of each root were cut and stained by the Holmes silver method. The afferent-fibre cell bodies in the ventral root were counted together with those situated in the mixed nerve distal to the point of separation of the two roots (Fig.6). The percentage of these overspill cells in relation to the total afferent-fibre cell bodies in each spinal nerve is shown in Table 1 (overleaf). The total numbers of afferent-fibre cell bodies in L7 and S2 are from the data of Duncan & Keyser (1937), and those in S1 are from an estimate of cell bodies in a single dorsal-root ganglion. This was done by counting the nucleoli in each section as representative of individual afferent-fibre cell bodies (Ranson & Billingsley 1918; Duncan & Keyser 1937). The highest percentage overspill is seen in the S1 ganglion which lies within the range of the present investigation, and although it can be assumed that a number of these cells have axons in cutaneous nerves, the rare observations of sensory-fibre degeneration in muscle receptors in the absence of dorsal-root damage is undoubtedly due to the presence of afferent-fibre cell bodies in the ventral root. Kato (personal communication) recently found an afferent fibre of S1 ventral root origin that displayed typical muscle-spindle Group II fibre characteristics. There were spontaneous regular discharges at 30/35 impulses/sec for 3hr at a tension of 10g with an increase to 45/50 impulses/sec at 100g, and a silent period for about 60~~m/sec~~ msec. The conduction velocity was 30m/sec. Dimsdale & Kemp (1966) have recently established both histologically and physiologically the existence of afferent nerve fibres in the L4 ventral root of the rat.

TABLE 1

% 'Overspill' dorsal-root ganglion cells of the total dorsal root ganglion cells in the lower-lumbar and upper-sacral spinal nerves

	L6	L7	S1	S2
C177	-	-	105	21
C82	-	-	14	38
C178	-	8	98	49
C179	-	3	85	19
C176	2	1	34	39
C175	-	1	71	20
C181	-	6	133	45
C184 (right side)	-	-	8	72
C184 (left side)	-	-	22	36
Average overspill	0.2	0.8	63	38
Total number of ganglion cells	-	29,597	13,040	16,934
% overspill	-	0.027	0.4	0.2

2.7 The distribution of sympathetic nerve fibres to hind-limb muscles

To test the assumption that the sympathetic nerve fibres from the lumbo-sacral sympathetic ganglia are distributed to the hind-limb muscles in the same manner as the sensory and motor nerve fibres of the equivalent spinal nerves, the sympathetic chain of one cat (C 204) was removed from the L5 to the L7 ganglia. Fourteen days later the dorsal roots were removed and the ventral roots cut (close to the cord) of the L5 to L7 spinal nerves.

All of the shank and foot muscles were impregnated by the modified de Castro technique. Their supplying nerves were stained by the Holmes method, embedded and sectioned longitudinally at 10 μ intervals. The serial sections of each muscle nerve were studied to ascertain whether or not nerve fibres were present. As a further check, the muscles were teased and the intramuscular nerve trunks, together with areas of blood vessels and connective tissue, were studied. Details of the results are shown in Table 2 (overleaf). It can be seen that the distribution of the sympathetic fibres follows closely that of somatic afferents and efferents as worked out by Sprague (1958).

3. The selection of muscles and their supplying nerves

The smallest muscles of the shank region are the peroneals

TABLE 2

The distribution of sympathetic nerve axons in relation to somatic afferent and efferent nerve fibres (C204. De-afferentated, de-efferentated and sympathectomized L5 - L7)

	Transverse sections of supplying nerve. 10µ (Holmes silver method)	Intramuscular nerve trunks, proprioceptors, blood vessels and connective tissue (de Castro impregnation; teased muscle preparations)
Lat. & mes. plantar	NOT STUDIED	+ (including somatic afferents and efferents)
flexor digitorum longus lat.	+	-
Flexor digitorum longus mes.	-	-
gastrocnem~~e~~ius lat.	+	+
gastrocnem~~e~~ius mes.	+	+
soleus	+	+
tibialis posterior	-	-
popliteus	-	-
extensor digitorum brevis	-	-
peroneus II and III	-	-
extensor digitorum longus	-	-
tibialis anterior	-	-
peroneus 1	-	-

+ indicates the presence of non-myelinated (sympathetic) nerve fibres

- indicates the absence of non-myelinated (sympathetic) nerve fibres which for convenience will be referred to as peroneus I (longus) II (brevis) and III (digiti quinti), and tibialis posterior. Peroneus I and III and tibialis posterior were chosen for the comparative study of the sensory component of muscle nerves made with the light and electron microscopes. For the study of the sensory fibres in intramuscular nerve trunks and the sensory endings in the muscle, extensor digitorum longus, tibialis anterior and extensor digitorum brevis were used in conjunction with the above muscles. The material for an electron microscope study was from two animals (C 234 and C 235) and that for light microscope studies was from a further four animals (C 205, C 213, C 223 and C 228), making six in all.

4. Staining methods

4.1 Muscle nerves

Those muscle nerves studied by light microscopy only were divided into two portions about 1 cm in length. The proximal portion, about 1 cm from nerve entry, was fixed in Bouin solution for 24hr, dehydrated, cleared, embedded in wax and sectioned at 10 μ. The sections were then stained following closely the Holmes silver-on-the-slide technique (Carlton & Drury 1957). The distal portion of the nerve was fixed in a variant of Fleming's fluid (16ml of 1%

chromic acid; 4ml of 2% osmic acid; 1 drop of glacial acetic acid) for 24hr, dehydrated (without washing) in 70%, 90%, 95% and two changes of absolute alcohol, cleared in cedarwood oil for 15hr, embedded and cut transversely at 5.0 µ. After spreading, the sections were hydrated and placed in 3% potassium bichromate at 37°C for 6hr, rinsed and stained in Wolter's haemotoxylin (10-20mls saturated solution of haemotoxylin in 100mls distilled water: 2mls glacial acetic acid) for 15hr. They were then replaced in the 3% potassium bichromate at room temperature for 5mins, differentiated in 0.25% potassium permanganate and flooded with Pal's solution for 5mins, dehydrated, cleared and mounted (Williams & Wendell-Smith 1960).

Those muscle nerves intended for comparative light/electron microscope study were divided into three portions. The proximal (1cm from nerve entry) and distal (4cm from nerve entry) portions were treated as above. The middle portion was fixed for 2hr in 2% osmium tetroxide buffered with Veronal-acetate (Palade 1952), dehydrated and embedded in Araldite. Polymerisation of the Araldite was allowed to take place for two days at 45-60°C, after which the material was blocked and fixed to the microtome chuck. Ultra-thin serial sections were cut with a Huxley ultratome and mounted on 200-mesh copper grids. For maximum contrast at 50kv under the electron microscope the sections were then stained with lead citrate (Reynolds 1963). Drops of the solution were placed in a wax-bottomed petri dish containing a piece of filter paper soaked in lead hydroxide. The grids were floated on to the drops of staining solution, the adhering sections being in contact with the solution. After 15mins they were rinsed in 0.01 N. sodium hydroxide solution and then three times in distilled water.

4.2 Muscles

The technique used to demonstrate nerve fibres in skeletal muscle was one based on Cajal's (1903) block silver-impregnation method, modified by de Castro (1925) and further extended by Barker & Ip (1963), to allow for whole, teased preparations.

This type of preparation in the study of fine-nerve fibres is of particular importance since the silver is taken up evenly by the smallest of non-myelinated axons resolvable with the light microscope. Gold chloride and methylene blue show only patchy distribution in these fine-calibre axons. Moreover, the necessity of working on a single cutting plane per muscle (Hinsey 1927; Denny-Brown 1928) introduces a certain error into the identification of free nerve endings. Mechanical distortion, which is minimal when sections are cut, is a possible disadvantage in the present study. However, the actual relationship of nerve fibres in whole, teased preparations is little altered during the teasing process, since 'areas' of tissue are carefully removed from the muscle debris and mounted. This introduces the added advantage that there is no limiting plane of study, no interference from darkly-stained extrafusal muscle fibres, and no discontinuity of nerve fibres.

The muscles were fixed for 4-6 days in a freshly prepared solution of chloral hydrate 1gm; 95% alcohol 45ml; distilled water 50ml; concentrated nitric acid 1ml. They were washed for 24hr in running tap water and then transferred to a solution of 95% alcohol 25ml; 1 drop ammonia for 48hr. Incubation was for 5 days at 37°C in a 1.5% solution of silver nitrate. Reduction was allowed to take place for two days in a freshly prepared solution of 2gm hydroquinone in 100ml of 25% formic acid. After reduction, the muscles were rinsed in distilled water and cleared (and stored) in glycerin before teasing.

5. Nerve-fibre diameter measurements

To obtain an overall picture of the sensory fibre outflow from skeletal muscle, it is necessary to observe different sections of the same nerve, one stained to demonstrate the myelin sheath and the other the axon. These procedures were employed so that firstly direct comparison could be made between the myelinated fibre distribution of the muscle nerves under investigation and those of previous studies (Lloyd & Chang 1948; Rexed & Therman 1948; Boyd 1962; Barker 1962), and secondly so that the proportional distribution of myelinated and non-myelinated fibres could be determined on the same muscle nerve.

The best-stained sections of the Weigert-Pal and Holmes methods were photographed. In all cases the sections for comparative studies were never more than 50 μ apart, except in the studies with the electron microscope material when they were separated by about 1cm. Photography of the Weigert-Pal sections was done by direct projection on to bromide paper. The Holmes sections were photographed by normal procedures. At all times a constant check was made that the magnification was 1,000 times. At this magnification the sections were photographed in portions, and the resulting prints stuck together to produce a montage of the whole section. Measurement of the nerve-fibre diameters in the Weigert-Pal stained sections was done by placing standard circles (1.0mm; 2.0mm; 3.0mm;.......20.0mm), which had been cut in a sheet of perspex, over each nerve fibre of the montage and ascertaining the circle of best fit. The diameters were noted and the fibre images pin-pricked. During this procedure continuous reference was made to the actual section by observation at 1,000 times under the microscope.

Although it is possible to see a 'halo-like' shadow of the myelin sheath around Holmes silver-impregnated axons, this 'halo' becomes increasingly ~~more~~ difficult to identify in the smaller fibres, and there comes a point when it is impossible to say whether certain axons are invested with myelin or not. For this reason the first estimate of the number of non-myelinated fibres in the muscle nerves was made by subtracting the total of myelinated fibres (Weigert-Pal technique) from the total of axons counted in a section of the same nerve impregnated by the Holmes silver method.

The histograms of the nerve-fibre diameter distribution of the total sensory outflow from the muscles are composite in nature, based on data obtained from the same nerve by the two histological techniques.

By dividing the conduction velocity of a myelinated nerve fibre by a factor of 6 (Hursh 1939) an estimate of the total diameter of that fibre in the fresh state may be obtained. So that comparisons can be made between previous physiological and histological data, and fibre diameters of silver-impregnated nerve fibres (de Castro technique, Barker & Ip 1963) an additional factor is necessary to convert silver diameters into fresh-state diameters.

It is known that little or no shrinkage occurs when nerve fibres are stained by the modified Weigert-Pal method that employs osmic acid as a fixing agent (Williams & Wendell-Smith 1960). External diameters of nerve fibres fixed in osmic acid can therefore be considered as fresh-state total diameters. Comparisons were made between the diameters of osmic-fixed nerves and silver-impregnated nerves in equivalent muscles of the cat's foot. In this way a conversion factor was obtained for the purposes of converting the diameters of all silver-impregnated nerve fibres into equivalent fresh-state diameters.

Samples of primary, secondary and fusimotor nerve fibres, to spindles of osmic-teased and silver-impregnated lumbrical muscles of the cat, were taken as representative of the total nerve-fibre diameter distribution in these muscles. Each fibre measurement was an average of between six and ten internodal, total diameter measurements made between the point at which the nerve fibre left the intramuscular nerve trunk and the penultimate internode (Table 3 overleaf). The histograms in Fig.7 were constructed from these data. The differences between the peak-diameter values obtained by the two histological techniques is 2.0 μ for the primaries, 4.0 μ for the secondaries, and 1.0 μ for the fusimotor nerve fibres. In all cases the lower of the two peak-diameter values was obtained for the silver-impregnated nerve fibres.

Each fibre-size group (primary, secondary and fusimotor) of both histological techniques was divided into equal portions, and the average fibre diameter for each portion was calculated (Table 3). The equivalent average diameter measurements of the silver-impregnated and osmic-stained nerve fibres were plotted against one another (Fig.8). A correlation coefficient of 0.95 was obtained, and the regression line of the average osmic-acid diameter measurements (x) on the average silver diameter measurements (y) was inserted. This line was represented by the formula $x = 1.52y - 0.11$. Therefore the equivalent fresh diameter of a silver-impregnated nerve fibre is almost one and a half times its total diameter.

TABLE 3

Internode diameter measurements of nerve fibres in cat lumbrical muscles

	fusimotor			secondary			primary		
	diam.(μ)	No.	% total	diam.(μ)	No.	% total	diam.(μ)	No.	% total
SILVER (de Castro)	1	4	8	3	4	16	5	4	14
	2	20	40	4	12	48	6	6	20
	3	19	38	5	9	36	9	6	20
	4	6	12				8	9	29
	5	1	2				9	2	7
							10	1	3
							11	2	7
	Average diam. bottom 50% = 1.9 μ			Average diam. bottom 50% = 3.7 μ			Average diam. bottom 50% = 6.1 μ		
	Average diam. top 50% = 3.3 μ			Average diam. top 50% = 4.7 μ			Average diam. top 50% = 8.1 μ		
	diam.(μ)	No.	% total	diam.(μ)	No.	% total	diam.(μ)	No.	% total
OSMIC	2	35	40	5	1	5	7	1	4
	3	41	47	6	4	10	8	1	4
	4	12	14	7	6	30	9	3	12
				8	7	35	10	9	36
				9	2	10	11	7	28
							12	2	8
							13	2	8
	Average diam. bottom 50% = 2.6 μ			Average diam. bottom 50% = 6.4 μ			Average diam. bottom 50% = 10.2 μ		
	Average diam. top 50% = 3.3 μ			Average diam. top 50% = 8.1 μ			Average diam. top 50% = 12.0 μ		

NOTE The OSMIC data for secondary and primary fibres are from Adal (unpublished)

The OSMIC data for fusimotor fibres are from Adal & Barker (1965)

The SILVER data for fusimotor fibres are from Ip (1965 Ph.D. thesis)

The extension of the regression line much below the lower-diameter point on the graph is of little value since nerve fibres below this range do not possess a myelin sheath stainable by either of the techniques. Extension of the line above the highest inserted point is of value because in muscles more proximal to the spinal cord than are the lumbricals, nerve fibres of greater diameter are found.

To ascertain whether 1.41 still holds as a silver/fresh conversion factor in the upper range of the nerve-fibre spectrum, a fibre-diameter spectrum of a large fascicle of a de-efferentated and sympathectomized soleus muscle-nerve impregnated by the de Castro technique was constructed from measurements made approximately 1cm prior to nerve entry. Each measurement is an average of five measurements made along two to three internodal lengths (Fig.9). The peak value of each group, when extrapolated into an equivalent osmic diameter corresponds closely with those obtained by Lloyd & Chang (1948) and Barker, Ip & Adal (1962) for soleus muscle, using Weigert-Pal technique for myelinated nerve fibres.

Extension of the regression line above the highest inserted point on the graph is therefore justified, and it follows that 1.41 (approx. 1.5) can be used as a silver/osmic (fresh) conversion factor throughout the fibre-diameter spectrum of myelinated nerve fibres.

In the results, the diameters of myelinated nerve fibres are adjusted diameters. That is, they have been measured in silver but presented in the text as if they had been measured in the fresh state. So that the scale of the diagrams and figures does not appear inconsistent, the diameters of the nerve fibres, as measured in silver-impregnated preparation have been placed in brackets after the adjusted diameter e.g. a 2.5 μ fibre as measured in silver will be indicated as:- 3.5 μ (2.5 μ) [(2.5 x 1.4 = 3.5)].

III. RESULTS

1. Muscle nerves

1.1 The myelinated component

A trimodal distribution of myelinated sensory fibres can be seen in all of the nerves studied (Fig.10A-C). The fibre-diameter range in tibialis posterior was between 1.0 μ and 22.0 μ; in peroneus I between 2.0 μ and 21.0 μ; and in peroneus III between 2.0 μ and 16.0 μ. The reason for this variation in maximum diameters is difficult to explain in terms of any single morphological or functional factor. Extensor muscles usually receive larger sensory fibres than their flexor antagonists (Lloyd & Chang 1948). Peroneus I and peroneus III partake in the flexion of the foot, but only peroneus III has a significantly lower maximum-size distribution than the antagonist extensor foot muscles, of which tibialis posterior is one. The presence of large sensory fibres in peroneus I cannot therefore be explained on this basis. The only other possible explanation is that of tapering (Fernand & Young 1951), which can lead to a reduction in diameter of as much as 8.0 μ in Group I fibres between spinal ganglia and the primary ending in the muscle spindle (Adal & Barker 1962). The muscle nerves of peroneus I and tibialis posterior lie at approximately the same distance from the spinal ganglia. Peroneus III is, however, about 20 cm more distal, which may account for the restricted diameter distribution as a result of peripheral tapering in the large diameter fibres.

The peak values of the individual fibre Groups also varied. In peroneus III the Group III peak occurred at 2.0 µ, whereas in peroneus I and tibialis posterior it was 3.0 µ. The Group II peak of peroneus III was between 6.0 µ and 9.0 µ; in peroneus I it was at 8.0 µ, and in tibialis posterior at the usual 7.0 µ. The Group I peak of peroneus III was found to be at the usual 15.0 µ, although the upper limit of the group was only 16.0 µ. Peroneus I and tibialis posterior Group I peaks were present at 16.0 µ and 19.0 µ, respectively.

In both tibialis posterior and peroneus I Group I always contained the greatest number of fibres (48.7% and 38.1%, respectively); Group II contained 38.1% and 35.3%, respectively, and Group III 13.0% and 16.7%, respectively. The percentage distribution of the fibres in the nerve to peroneus III was:- Group I: 28.3%, Group II: 50.7%, Group III: 20.8%. As can be seen, the medium and small size sensory fibres constitute just over 70% of the total, whereas in the other two nerves they account for approximately 50%. The significance of these variations is not clear. It may be that the muscle-spindle and tendon-organ population of peroneus III is small in relation to the number of paciniform corpuscles and free endings, but there is no data in the literature to confirm this.

1.2 The non-myelinated component

1.21 Light microscopy - the proportion of myelinated to non-myelinated fibres

In transverse sections of silver-impregnated muscle nerves, the non-myelinated axons can be seen in bundles throughout the sections (Fig.11 & Fig.12a). They are most abundant near the periphery and are easily distinguishable from degenerated fibre debris, as they maintain a regular outline when the fine focus of the objective is moved through the section.

With the light microscope the arrangement of non-myelinated axons in such a bundle is seen more clearly in teased preparations of intramuscular nerve trunks impregnated by the modified de Castro method (Barker & Ip 1963). At high magnification the non-myelinated axons can be seen contained within a lightly stained sheath. This arrangement was first noticed by Remak (1838) and studied more fully by Nageotte (1922), but it was not until Gasser's (1955) electron-microscope studies that the intimate relationship between the non-myelinated axons was fully understood. They were found to be contained within the cytoplasm of Schwann cells and subsequently the whole structure containing the axons has been called a Schwann-cell bundle. In Fig.12b there are 6 non-myelinated axons that appear to intertwine throughout their course in the Schwann-cell bundle. At a point of division the axons re-group to produce two separate bundles that contain 2 and 4 axons, respectively.

In this part of the study, the individual axons were counted wherever possible. However, some of the very small bundles appear in the silver sections as 'single axons', as noted by Gasser (1955) in his study of non-myelinated dorsal-root fibres, and so they were counted as single axons.

Details of the data obtained from each muscle nerve are shown in Table 4 (overleaf). It can be seen that the number and proportion of myelinated fibres and non-myelinated axons varies in the individual nerves, but that the average proportion of 2·2:1 myelinated to non-myelinated is a constant feature of all three nerves. If the non-myelinated fibres are aggregated within the 0.0 μ to 1.0 μ diameter range, they form a large fourth Group at the left-hand end of the sensory fibre spectrum (Fig.13).

TABLE 4

Nerve fibre data obtained by light microscopy

		Myelinated fibres(W-P)	Total axons (Holmes)	Non-myelinated axons	Proportion myel: n/myel.	Average proportion myel:n/myel.
tibialis posterior	C223	182	237	55	3.3:1	2.2:1
	C228	211	335	124	1.7:1	
	C234	127	221	84	1.5:1	
	C235	130	185	55	2.4:1	
peroneus I	C223	83	118	35	2.4:1	2.2:1
	C228	128	177	49	2.6:1	
	C234	87	137	50	1.7:1	
peroneus III	C234	67	97	30	2.2:1	2.2:1

and f. each appear to communicate with the exterior by two mesaxons, and d. appears to do so by a branched mesaxon. These apparent anomalies can be explained in terms of the branching of Schwann-cell cytoplasm that produces a system of interdigitating, cytoplasmic filaments. The whole Schwann-cell complex in Figure 17 is in fact three separate filaments of cytoplasm enclosed within a single basement membrane, and the axons d, e, and f. are in process of being enclosed by their appropriate cytoplasmic filaments. Their mesaxons are not yet developed, and the previously assumed mesaxons are dividing spaces between the three cytoplasmic filaments. The presence of an extremely fine cytoplasmic filament closely juxtaposed to a large mass of cytoplasm in obvious association with a nucleus (Fig.14) suggests this interdigiting arrangement. The fine filament is interpreted as a strand of cytoplasm from a Schwann cell other than the one to which the nucleus belongs. The axons, which are found between two regions of cytoplasm in transverse sections (Figs. 14, 16, 17, 18), are interpreted as being in process of leaving one Schwann-cell bundle and entering another.

Some of the non-myelinated axons are seen as very elongated structures (Fig.16.Ax) compared with the relatively circular or oval appearance of most axons in the Schwann-cell cytoplasm. It seems certain that they are in process of changing their course in the Schwann-cell bundle and have consequently been cut in oblique section. In support of this observation, the non-myelinated axons in teased intramuscular nerve trunks can be seen to do just this at intervals along their course. Individual axons can also be seen in the intramuscular nerve trunks to leave one bundle entirely and enter another some distance away (Fig.52c). This behaviour of non-myelinated axons in the muscle nerve is illustrated in Fig. 16, where three single axons (ax_1, ax_2, n.ax.) surrounded by a layer of Schwann-cell cytoplasm are seen to be detached from the main bundle and surrounded by their own basement membranes.

1.22(b) The proportion of non-myelinated to myelinated fibres

To establish the number of non-myelinated axons in a muscle nerve, the following procedure was adopted to avoid the production of an electron micrograph montage of a whole nerve section, large areas of which do not contain Schwann-cell bundles. A section was found that was only partly obscured by the grid bars. Those areas of the section that were visible were scanned, working along horizontal and then vertical strips. When Schwann-cell bundles were found, they were photographed, and their relative position to certain conspicuous myelinated fibres was noted on a previously prepared rough sketch of the section. Having photographed all of the visible bundles, further successive sections were scanned in the same way, and Schwann-cell bundles obscured by the grid bars in the first section were photographed.

The data obtained from two nerves by this procedure are seen in Table 5 (overleaf). The proportions of 1:2 and 1:1.9 myelinated to non-myelinated represents almost a four-fold increase in the non-myelinated component over the observed result obtained by the Holmes method, and an increase of about one third of that obtained when the de Castro technique was employed (Fig.19).

1.3 The total sensory component in muscle nerves

The diameters were measured of those non-myelinated axons that were rounded or slightly oval in shape, and those that had obviously been cut in longitudinal section, having altered their course in the Schwann-cell bundle. The diameter range was found to be between 0.15 μ and 1.20 μ. The diameter distribution of 357 non-myelinated sensory axons is shown in Fig. 20A in terms of a histogram. A single large peak occurs at 0.35 μ and 90% of the fibres have diameters within the 0.15 μ to 0.55 μ range.

TABLE 5

Non-myelinated (C afferent) axon counts obtained with the electron microscope from two tibialis posterior nerves

C235 Plate reference	No. of n/my.axons	C234 Plate reference	No. of n/my.axons
A208	10	A90	16
198	19	95	11
199	5	92	10
200	15	93	12
201	9	71	11
202	5	84	18
203	6	91	20
204	18	94	9
205	10	86	7
206	20	98	3
208	15	100	10
209	9	85	4
211	10	74	5
213	16	72	12
188	3	89	10
189	9	75	9
190	5	79	11
191	5	76	15
194	10	80	7
220	21	77	20
215	22	78	19
227	19		
total non-myelinated axons	261		239
myelinated fibres (from Table 4)	130		127
proportion myelinated: non-myelinated	1:2.0		1:1.9

These figures are similar to those obtained by Gasser (1955) for the non-myelinated sensory nerve fibre distribution in cat dorsal roots (L7 and S1). The histogram in Fig. 20B is a reconstruction of Gasser's data, and it can be seen that axon diameters range from 0.10 μ to 0.80 μ with a large peak at 0.30 μ. Ninety-six per cent of the fibres are present within the 0.15 μ to 0.60 μ range.

The complete diameter distribution of sensory axons and fibres in the nerve of tibialis posterior of the cat is seen in Fig.21 (cf. Fig.14). Those non-myelinated axons with diameters greater than 1.0 μ have been added to the small myelinated fibres with a diameter less than 2.0 μ, so that the 1.0 μ diameter range includes both myelinated fibres and non-myelinated axons. For convenience, the non-myelinated axons are shown to extend from 0.0 μ to 1.0 μ but, as can be seen from Fig.20A, the smallest axons in this range are 0.15 μ in diameter.

2. Muscles

2.1 The constituent elements of muscles

The individual muscles of the cat consist of a number of tissue elements, the most obvious being the extrafusal muscle fibres. The individual muscle fibres, which are surrounded by an endomysial connective-tissue sheath, are arranged into fascicles. Each fascicle is separated from its neighbour by a sheath of connective tissue, the ~~ep~~perimysium. All of these connective-tissue membranes are contunuous throughout the muscle, and at the junction of the muscle fibres with the tendon they extend among the tendon elements, and ensheath the whole tendon. According to Ham & Leeson (1965), these membranes constitute a harness between the tendon and the muscle fibres, allowing contraction of the muscle fibres to be effective through the non-contractile tendon elements without rupture occurring at their junction. Apart from this important harnessing function, the connective-tissue skeleton affords a pathway within the muscle for the innervating nerve trunks and blood vessels, and a site for the deposition of fat. Fat cells are often found in close association with the large nerve trunks and blood vessels (Fig.22).

Muscle spindles occur throughout the muscle and lie in parallel with the extrafusal muscle fibres. Tendon organs lie in series with the extrafusal muscle fibres, and are situated at musculo-tendinous junctions. Paciniform corpuscles are scattered throughout the connective-tissue network, but occur most frequently in the tissue close to the surface of the muscle and at musculo-tendinous junctions.

2.2 The vascular and nerve supply to muscles

The supplying artery and vein, which enter the muscle as branches of the main limb vessels, divide continually and in parallel with one another throughout the body of the muscle to produce successively smaller vessels until the capillaries are formed around the individual extrafusal muscle fibres. Occasionally a very small arteriole and venule join to form a pre-capillary anastomosis, or A-V bridge. All of the branching vessels up to the capillary level are surrounded by a distinct adventitia. That of the arterioles is always thicker than that of the venules at any one level of branching. The capillaries are devoid of an adventitial coat.

The muscle nerve usually enters the muscle together with the supplying blood vessels and divides continuously throughout the muscle tissue to produce successively smaller branches. These branches, which contain the innervating sensory nerve fibres, are of two distinct types, one large and the other small. The larger and consequently the most obvious are those which form the intramuscular nerve 'tree'(Fig.23). The branches divide as do the branches of a tree and course throughout the muscle. They contain the large and medium-sized myelinated sensory fibres that innervate the encapsulated receptors, as well as small myelinated sensory fibres and non-myelinated sensory axons. Occasionally two branches of the intramuscular nerve tree re-join in a nerve-trunk anastomosis. This is not a typical feature of their anatomy.

The small type of nerve trunk is that which forms the vascular nerve plexus. Small side branches of the intramuscular nerve trunks anastomose with other similar branches to form a network of vascular nerve trunks that tend to follow the course of the innervating artery and vein and their smaller branches (Fig.24). This network is not unlike the subcutaneous nerve plexus described by Woollard, Weddell & Harpmann (1939), although it is much looser in appearance. The constituent nerves of the vascular nerve trunks are of two types only, namely small-myelinated fibres and non-myelinated axons. They innervate the blood vessels, the connective tissue network and musculo-tendinous junctions, and occasionally the encapsulated receptors.

In normal muscle material the intramuscular nerve trunks are seen to contain, in addition to the sensory-fibre component, both myelinated fibres of ventral-root origin and non-myelinated axons of sympathetic origin. The vascular nerve trunks contain only a sympathetic contribution in addition to their sensory nerve component. Evidence of these degenerated nerves is seen in de-efferentated and sympathectomized muscle material. In normal muscle tissue there is a third type of nerve trunk that contains exclusively the motor nerves to extrafusal muscle fibres. These nerve trunks are of course totally absent in de-efferentated and sympathectomized muscle.

Figure 25 is a cut-away diagram of de-efferentated and sympathectomized skeletal muscle illustrating the derivation and distribution of the intramuscular and vascular nerve trunks, and the course and distribution of the vascular system.

2.3 Sensory endings in the muscle

Three types of encapsulated receptor were present in all the muscles studied, namely the muscle spindle, the tendon organ and the paciniform corpuscle. They are supplied with four types of sensory (encapsulated) ending derived from fibres of Groups I, II and III. A fifth type of sensory ending is present throughout the muscle tissue, which is termed a non-encapsulated or free ending. (Regand & Favre 1904).

2.31 Fibres and endings associated with the muscle spindle

Typical muscle spindles receive both primary and secondary endings, the parent fibres of which are usually derived from Groups Ia and II respectively. A total of twenty-four Group Ia fibres were traced from the muscle spindle to nerve entry, a distance that varied between approximately 10.0mm and 0.8mm, and they remained unbranched throughout their intramuscular course. Forty Group II secondary fibres were similarly traced back to nerve entry, a distance that varied between 9.5mm and 0.8mm. Again branching did not occur in the intramuscular nerve trunks. The only branching that was obvious in the Group II fibres was that which occurred close to the muscle spindle and produced between two and three secondary endings IN THE SAME SPINDLE. Only eight such examples were found in a total of 198 spindles with intact portions of intramuscular nerve trunks. These observations are in full agreement with those of von Thiel (1959) and Barker, Ip & Adal (1962) concerning the 1:1 relationship of Group Ia and Group II fibres and their respective endings in the muscle.

Some secondary endings are situated well towards the polar ends of the spindle, at sites Boyd (1962) designates as the 'S4', 'S5' positions, and their parent fibres are never greater in diameter than 4.0 μ. This compares with 'S1', 'S2', and 'S3' secondary fibres which have a diameter range of between 5.0 μ and 12.5 μ. Out of a total of 318 teased spindles, five 'S4' and one 'S5' secondary endings were supplied by fibres measuring between

2.5 µ and 4.0 µ in diameter. These diameters are within the Group III range and their secondary endings are much less complex than 'S1' secondary endings. The terminal axons of the 'S4' and 'S5' endings are always in contact with intrafusal muscle fibres around which they are very loosely coiled. They are never associated with the capsule or connective tissue of the spindle. They represent one end of a structural series that has the complex, tightly-coiled primary ending at the opposite extremity (Fig.26a-f).

Certain spindles possess a form of sensory ending that is often extremely diffuse with extensive ramifying terminals contained largely in the receptor. Figure 27 is an illustration of such a spindle. The primary and secondary endings are virtually obscured by the mass of ramifying terminals produced by two Group III fibres that enter the spindle at the left hand pole. They branch profusely within the spindle and shower terminals not only throughout the spindle but also into the connective tissue that surrounds it. In Fig.28a and Fig.28b the individual fibres of the left-hand pole have been separately traced to their terminations. They are both myelinated fibres of Group III which divide profusely to give fine terminals throughout the spindle located in the connective tissue between the intrafusal muscle fibres, and within the capsule. The 3.0 µ parent fibre (Fig.28b) produces a branch, before it actually enters the spindle, that divides to give terminals in the connective-tissue sheath of the polar end of another spindle, and a second in connective tissue close to this spindle. The 4.0 µ fibre (Fig.28b) produces endings in the studied area, largely in the capsule of the spindle. However, a branch (1.5 µ) is seen to cross the equitorial region and divide continually to give further endings in the opposite pole that were too complex to trace out. A branch from the 3.0 µ parent fibre (Fig.28a) also crosses the equitorial region and produces similar complex terminal ramifications in the opposite pole.

Only six examples of such sensory endings were found out of a total of 318 spindles examined. Some produced complex terminal ramifications, other consisted of only a few branched terminals (Fig.29a), and the simplest type consisted of a single non-myelinated 0.5 µ fibre that entered the spindle with the other sensory fibres and terminated without branching in the connective tissue between two intrafusal muscle fibres (Fig.29b). Whether or not this simple terminal was the result of myelinated fibre branching in the intramuscular nerve trunk could not be established. All of these terminals are interpreted as free endings. Their mode of termination is not typical of nerve fibres that usually end in muscle spindles in that they are not closely or specifically associated with the intrafusal muscle fibres. Their ramifications are not coiled as are primary and secondary ramifications, but end parallel with the intrafusal muscle fibres. The actual terminal ramification, although it produces neurofibrillary expansions, appears as a tapering structure. Secondary ending ramifications produce far more neurofibritary expansions, which are connected by very fine strands of axoplasm, giving a totally different appearance to the ending. The terminal ramifications of the muscle-spindle free ending occur in connective tissue within the capsule wall and associated with intrafusal muscle fibres. They also occur, as branches of the parent fibre, outside of the spindle. Secondary endings are exclusively associated with the intrafusal muscle fibres.

2.32 Fibres and endings associated with the tendon organ

Tendon organs are innervated by a single Group Ib fibre which can usually be traced back to nerve entry and found to be unbranched. Occasionally branching may occur, and such a fibre is seen to supply two and sometimes three tendon organs. Out of a total of 120 Group Ib fibres, 88 (73.3%) supplied one tendon organ, 26 (21.7%) supplied two tendon organs, and 6 (5.0%) supplied three tendon organs. This is in agreement with results obtained by Pang (see Barker 1967). Those Ib fibres that branch do so only in the distal reaches of the intramuscular nerve trunks, never in the large branches in the belly of the muscle.

As in the muscle spindle, free ramifying terminals have been observed associated with the tendon organ. They occur among the tendon elements both inside and outside of the capsule.

Figure 30 shows a 3.0 μ fibre (f_1) that divides to give a 2.5 μ fibre which, after losing its myelin sheath at the terminal node (t.n.), branches to form a number of terminals between the tendon elements. One of the terminals of this ending courses towards a small arteriole in the tendon tissue and ends in the adventitia. The resultant terminals of the other small myelinated fibre (f_2) also end in this region in the adventitia of the arteriole and among fat cells. Such innervation is sometimes restricted to the capsule (Fig.31a). None of the fibres actually enters the tendon organ, although some are associated with the surrounding connective tissue and blood vessels. Of 126 teased tendon organs, only 5 received this type of innervation. In teased preparations of normal cat and rabbit material a further four examples of free ramifying terminals associated with tendon organs and musculo-tendinous junctions were found (Fig.31b,c).

2.33 The paciniform corpuscle

Forty-two out of sixty-five teased paciniform corpuscles (approximately 65%) were present either within a tendon-organ capsule wall or within the organ itself (Figs.32a,b: 33a,b). The remainder were found in the musculo-tendinous tissue close to tendon organs (Fig.32c) or in connective tissue close to small blood vessels and vascular nerve trunks (Fig.32d).

Twenty-four of one hundred and fifty eight (15%) teased preparations of tendon organs had paciniform corpuscles closely associated with them. Any number between one and five have been found in this situation, and their innervation is derived as a result of branching of a single myelinated nerve fibre.

Figure 33a shows the division of a 7.0 μ fibre as it crosses a tendon organ. The resultant branches (4.0 μ and 3.5 μ in diameter) course for some distance before the paciniform corpuscles are reached. They then lose their myelin sheaths and enter the corpuscles, one of which lies outside the capsule and the other inside.

A larger group of paciniform corpuscles derived from a 10.5 μ parent fibre is seen in Fig.33b. At the point of division four small fibres are produced. These ultimately end in five corpuscles, all of which adhere to the outside of the tendon-organ capsule.

In Fig.34 a small region of the musculo-tendinous junction is seen to contain two tendon organs and the polar end of a muscle spindle. A 12.0 μ parent fibre in the small intramuscular nerve trunk divides to give eight paciniform corpuscle endings. Five of them are closely associated with the two tendon organs, and the other three are present close to the nerve trunk.

Branching of sensory nerve fibres destined to produce paciniform corpuscle endings occurs in those small, distal, intramuscular nerve trunks that course in the direction of the proprioceptors; in this instance the tendon organs. It has never been observed to occur in the large intramuscular nerve trunks, in those instances when it was possible to trace the corpuscle parent-fibres back to nerve entry.

Most of the nerve fibres that innervate paciniform corpuscles are of Group III origin (Fig.32a). However, some of them are seen to be well within the Group II range, even in the region of fibre branching (Fig.33). The parent fibre in Fig.34 is undoubtedly of lower Group I origin.

2.34 The free ending

2.34(a) The form of the free ending ramification

The naked axonic ramification seen in Fig.35a is approximately 0.7 μ in diameter. At intervals neuro-fibrillary expansions of the axoplasm are visible; these become more frequent towards the tip. Immediately proximal to the terminal expansion the axon has a diameter of about 0.4 μ. Free ramifications smaller in diameter than this are not uncommon (Fig.35b). In almost all such terminals, the actual ending tapers abruptly from a neuro-fibrillary expansion to a fine point in a very short distance (0.5 μ). Schwann-cell nuclei invest the terminal ramifications, and they appear to be particularly abundant in this region of the terminal.

The majority of free endings are present within the perimysium, which contains the large and small arterioles and venules as well as a certain amount of fatty tissue. Termination occurs in the adventitia of the arterioles and venules, in the connective tissue itself, in the endomysium at musculo-tendinous junctions, and occasionally endings are associated with fat cells. Free endings have never been found in the capillary network, although a few instances have been observed of terminals between two extrafusal muscle fibres (which possess this network) at the musculo-tendinous junction. In sectioned muscle material (Hinsey 1927), they are occasionally seen in the endomysium in the belly of the muscle.

The free endings associated with intrafusal muscle fibres, the capsules of spindles and tendon organs, and with tendon tissue, have already been described (Figs. 27; 28a,b; 29a,b; 30; 31a,b,c). This form of innervation is seen infrequently compared with the regular presence of free endings in the perimysium and its associated structures.

2.34(b) Pre-terminal branching and the free ending

Pre-terminal branching, that is, branching usually outside the limits of the intramuscular and vascular nerve trunks, produces a great variety of free endings from the simple through to the comples (Fig.36a-d).

The criterion for the classification of those free-ending fibres that possess a myelin sheath is the identification of the terminal node. Any subsequent branching and terminals are considered as the ending (Fig.37A). The free ending of a non-myelinated axon (C afferent) is considered as the branching and ramifications produced by the axon after it has left the Schwann-cell bundle (Fig.37B).

As can be seen in Fig.37A, two types of ending are produced from myelinated parent fibres; the nodal type and the terminal type. In general the nodal type is less complex than the terminal type and consists of only a few ramifications produced as a result of axon branching (Figs.36*a*,*b*; 38*a*,*b*), although more extensive axon branching does occur (Fig.38*c*,*d*). The ramifications of the terminal type of ending are usually more widely distributed and innervate a greater area of tissue (Fig.39*a*-*d*), although they have been observed to be restricted in their distribution (Fig.39*e*).

The ramifications of the simple ending, whether it is nodal or terminal in nature, tend to be associated with only one kind of tissue element; connective tissue (Figs.38*a*; 39*e*) or blood-vessel adventitia (Figs. 36*a*; 38*b*). The more complex the ending, the more ubiquitous are its ramifications. They may be distributed to a number of tissue elements. Those of the endings in Fig.38*d* end in connective tissue, in blood-vessel adventita, in association with fat cells and in the peri~~m~~neurium of a small nerve trunk that contains the parent fibre. Similarly in Fig.38*c* ramifications are seen in the blood-vessel adventitia and in connective tissue between the vascular nerve trunk and the blood vessel. The ramifications of the terminal ending in Fig.39*c* are in connective tissue and associated with fat cells. Those in Fig.39*d* end in the adventitia of a small arteriole as well as between the extrafusal muscle fibres at the musculo-tendinous junction. In Fig.39*a* ramifications occur in blood vessel adventitia and close by in connective tissue.

Occasionally complex terminal endings are restricted in their distribution, as are the simple nodal endings. In Figs. 36d; 39b, the ramifications are restricted to blood-vessel adventitia and connective tissue respectively.

Although free endings are fairly restricted in that their ramifications innervate only a small area of tissue, the myelinated parent fibres from which they are derived have a comparatively large sensory field, since one such fibre may produce a number of endings at different and widely-spaced sites in the muscle. The ending illustrated in Fig.38d is one of at least two that are both nodal endings of the same myelinated parent fibre (Fig.40). The distance between them is approximately 3.2mm and it is probable that the continuing parent fibre, which was broken during preparation, produced at least a terminal ending at a more distal site. The endings in Fig.36d and 39c are both from the same parent fibre, and their relationship to one another is shown in Fig.41.

The extreme ramifications of the endings illustrated in Fig.42a,b are separated by a distance of approximately 0.8mm. Their disposition in relation to one another is seen in Fig.43, together with further terminal ramifications of endings derived from the same parent fibre. It can be seen from this illustration that the terminal ramifications of free endings of a single myelinated parent fibre innervate an area of the muscle that is at least 1.5mm^2.

The ramifications of Group IV fibre endings innervate almost exclusively the adventitia of blood vessels (Figs.44*b*; 45), although a few instances have been observed of connective-tissue innervation (Fig.44*a*). They have never been observed in association with fat cells. Branching of the axon, after it has left the Schwann-cell bundle, is always extensive, and the extreme ramifications are widely separated. Those illustrated in Fig.45 are 0.8mm apart.

If the free ending of the non-myelinated C afferent is considered to be the whole of the branching complex after the parent fibre has left the Schwann-cell bundle, then it covers a much greater sensory field than does even the most complex ending of myelinated fibre. On the other hand, if it is compared with the sum total of endings from individual myelinated parent fibres, its distribution is less extensive. These comparisons do not, however, take into account the extent of myelinated and non-myelinated parent-fibre branching within the large vascular and intramuscular nerve trunks.

2.34(d) Free endings and their parent fibres

In a few preparations it is possible to observe a single ending or ramification, trace its parent fibre back to nerve entry, and so establish the sensory-fibre Group from which it is derived. In all such preparations it is only possible to link one or two free endings or ramifications with the parent fibre, although the extent of intramuscular branching of this fibre suggests that there are further endings in the muscle derived from it.

Figure 46 is a composite illustration of the intramuscular branching of a Group II sensory nerve fibre that produces two free endings in the muscle. The parent fibre, which is 7.0 μ in diameter at nerve entry, branches for the first time 6.5mm inside of the muscle. One of the resultant fibres (3.5 μ) can be followed into a small intramuscular nerve trunk. This fibre divides to give two fibres, one of which is 2.0 μ in diameter. This is the parent fibre of two terminal-type free endings, one of which ramifies in connective tissue, and the other among fat cells. The fat cell ending is illustrated in greater detail in Fig.35b).

Figure 47 is a similar composite illustration, but of the intramuscular branching of a Group III fibre. The parent fibre (3.5 μ) branches 4.0mm inside of the muscle to give two fibres, one of which is 3.0 μ in diameter. This fibre enters an intramuscular nerve trunk containing a Ib fibre and branches after coursing along this nerve trunk for 0.2mm. One fibre continues along the nerve trunk, but the other (2.0 μ) leaves it and produces free ramifying terminals in connective tissue close by.

Although only three free endings can be demonstrated as a result of branching of these two sensory myelinated nerve fibres, that intramuscular branching occurs to produce other broken fibres, is an indication of further free endings in other regions of the muscle. It follows that the sensory field of a single myelinated nerve fibre is, therefore, much greater in extent than that covered by a single free ending.

The single free-ending Group IV axon illustrated in Fig.45 is seen in Fig.48 to be one of many contained in a Schwann-cell bundle. This bundle can be traced from the region of the free-ending terminal ramifications for approximately 5.0mm back to nerve entry. None of the axons is derived from a myelinated parent fibre, as the bundle maintains its identity throughout its intramuscular course.

2.34(d) Branching of sensory nerve fibres and axons in intramuscular and vascular nerve trunks

Although it is extremely difficult to obtain preparations of free endings in muscle tissue as well as their parent fibres at nerve entry, examination of whole, teased preparations of large and small intramuscular nerve trunks only, reveals much the same picture of intramuscular branching Group III and Group II free-ending fibres as well as an occasional example of Group I free-ending fibre branching.

Figure 49 is a composite diagram of such branching derived from Group I and III fibres. The first division of the Group I (14.0 μ) fibre occurs approximately 1.5mm after nerve entry and produces two fibres, one 11.0 μ and the other 7.0 μ. The resultant branching of the 7.0 μ fibre produces at least one axon, less than 1.0 μ in diameter. The 11.0 μ fibre continues in the nerve trunk for approximately 1.5mm and then divides to give three fibres, 5.5 μ, 3.5 μ, and 3.5 μ. The two 3.5 μ fibres produce a 1.0 μ axon and a 1.5 μ fibre in a small nerve trunk. The Group III fibre seen in this preparation divides approximately 4.0mm distal of nerve entry producing two fibres (3.0 μ), one of which branches again to give two 1.5 μ fibres.

Similarly in Fig.50 a Group II fibre can be traced from nerve entry into the muscle. Persistent branching of the fibre produces a number of non-myelinated axons in the small intramuscular and vascular nerve trunks.

The initial division of these Group I, Group II and Group III nerve fibres occurs most often in the large intramuscular nerve trunks that contain fibres representative of the whole sensory component. Their resultant branches eventually enter the small vascular nerve trunks, which in itself suggests eventual free-ending termination.

The approximate dimensions of the sensory field in the muscle covered by the extreme endings of the Group I fibre (Fig.49) is at least 2.0mm^2. This approximation is based on the distance between the 5.5 µ fibre, on the left of the figure, and the 1.0 µ axon at the bottom right of the figure. The 5.5 µ fibre obviously branches much more before it produces free endings in other regions of the muscle.

The same principle of estimating the sensory field of the Group III fibre (Fig.49) cannot be applied because both of the fibres run parallel in the same intramuscular nerve trunk.

The endings of the Group II fibre in Fig.50 have a sensory field of at least 3.5mm^2 since the 7.0 µ branch of the first division can be traced along the intramuscular nerve trunk for a further 3.0mm.

The small nerve trunks of the vascular network contain only small myelinated fibres and non-myelinated axons. (Fig.51<u>a</u>). The small myelinated fibres are derived either as a result of larger myelinated fibre branching (Fig.51<u>b</u>) or by direct entry without branching of Group III fibres (Fig.51<u>c</u>). The non-myelinated axons are derived either by branching of the myelinated fibres (Fig.51<u>d</u>), or by branching of the non-myelinated (Group IV) axons, or by direct entry of these axons from the intramuscular nerve trunks (Fig.51<u>e</u>). Those non-myelinated axons of myelinated parent-fibre origin can usually be distinguished from axons of Group IV origin in that they tend to remain unassociated with other non-myelinated axons. In other words they remain as individuals and are not grouped in Schwann-cell bundles. On the whole they are slightly larger in diameter (about 1.0 μ). However, occasional observations have been made of non-myelinated axons of unequivocal myelinated parent-fibre origin entering the Schwann-cell complex of non-myelinated Group IV axons (Fig.52<u>a</u>).

The behaviour of Group IV axons in the intramuscular nerve trunks is similar to the rest of the fibres of the sensory component, in that they tend to take a single directional course, distal to proximal.

In the vascular network direction seems to be of little consequence. The myelinated fibres run in opposite directions to one another in the same vascular nerve trunk, and it is often difficult to trace fibres to their likely point of leaving the trunks, unless actual division can be observed and the branches of such a division followed in their various courses (Fig.52b). Some of the non-myelinated (Group IV) axons are extremely difficult to follow in silver preparations because of their small size. Also, occasional axons have been seen to be running in the opposite direction to the main flow of axons in the same Schwann-cell bundle (Fig.52c).

2.4 The sensory innervation of skeletal muscle

Figure 53 is a schematic representation of the innervation of skeletal muscle by sensory myelinated nerve fibres and non-myelinated axons. It is based exclusively on observations made during the present investigation on de-efferentated and sympathectomized cat hindlimb muscles impregnated by the modified de Castro silver technique (Barker & Ip 1963).

The parent fibres that innervate encapsulated receptors are arranged down the left side of the figure. Any branching that occurs in these fibres does so only in the distal reaches of the intramuscular nerve trunks. Branching of the Group Ib fibre indicates innervation of only two tendon organs, although as many as three have been observed with their innervation derived from a single Ib fibre.

The Group I/II fibre is indicated as innervating more than two paciniform corpuscles. As many as five have been observed to receive their innervation from a single Group I fibre.

Group Ia, II and III fibres to muscle spindles have never been seen to branch in the intramuscular nerve trunks. The only branching that does occur is immediately pre-terminal in nature with resultant fibres supplying endings in the same spindle.

The parent fibres of free endings are arranged down the right side of the figure. The Group III free-ending fibres that enter the encapsulated receptors are indicated as branching in the intramuscular nerve trunks. This is based on actual observation of such branching.

The non-myelinated axon (IV) which ends in the spindle could not be traced back as far as nerve entry, but it was seen to retain its identity for some distance along the intramuscular nerve trunk that supplied the spindle. For this reason it was considered to be of Group IV origin. Unequivocal Group IV axons have been observed to branch in their course in the Schwann-cell bundles and to produce free endings in both blood-vessel adventitia and in connective tissue.

The Group III, II and I parent fibres, which produce terminals in blood-vessel adventitia, connective tissue and in fat, branch extensively in both the intramuscular and vascular nerve trunks as do the Group III fibres that produce free terminals in tendon, at musculo-tendinous junctions and in the capsules of tendon organs.

IV. DISCUSSION

The present investigation is concerned largely with a study of the innervation of skeletal muscles by nerve fibres that end either in paciniform corpuscles or as free endings. It has involved a study of (1) sensory fibres in muscle nerves and (2) the endings and their parent fibres in the muscle.

Sensory fibres in muscle nerves

Light microscope studies have shown that myelinated sensory nerve fibres of dorsal root origin are present in muscle nerves (Sherrington 1894), and that they fall into three diameter groups (Lloyd & Chang 1948). Also present in the dorsal roots are small sensory-fibre cell bodies that produce non-myelinated peripheral axons (Ranson 1912). These axons are distributed largely to cutaneous nerves where, according to Ranson & Davenport (1931), they outnumber the myelinated fibres by a proportion of 1 : 3.7; myelinated to non-myelinated. These authors also estimated that in muscle nerves the non-myelinated axons are less than half as abundant as the myelinated fibres by a proportion of 1 : 0.4 myelinated to non-myelinated.

It must be remembered that the two studies of Ranson &

Davenport (1931) are not strictly comparable in terms of sensory innervation of cutaneous and muscle nerves since the myelinated fibres of the muscle nerve (vastus medialis) are of both motor and sensory origin. Sherrington (1894) has shown, by differential degeneration, that there are approximately equal numbers of myelinated motor and sensory nerve fibres in the nerve to vastus medialis and therefore any interpretation of Ranson & Davenport's findings must take this into account. Since the present study is concerned exclusively with the sensory component in muscle nerves, Ranson & Davenport's data have been re-calculated in the light of Sherrington's findings and they show that the myelinated and non-myelinated fibres in vastus medialis are present in approximately equal proportions.

According to Hinsey, Hare & Phillips (1939) the sensory nerve fibre component of the phrenic nerve of the cat consists of more non-myelinated axons than myelinated fibres by a proportion of 1 : 3, myelinated to non-myelinated. This nerve supplies the innervation not only to skeletal muscle of the diaphragm but also to peritoneum and pleura.

An investigation with the electron microscope in the present study has shown that in skeletal-muscle nerves non-myelinated axons are twice as abundant as myelinated fibres

(1 : 2 myelinated to non-myelinated). It now seems likely that the proportion of myelinated to non-myelinated axons in the saphenous (Ranson & Davenport 1934) and phrenic (Hinsey, Hare & Phillips 1939) nerves is probably of the order of 1 : 16 and 1 : 12 respectively.

As a result of their study of the determination of the numbers of fibres and cells in the dorsal roots and ganglia of the cat, Duncan & Keyser (1937) came to the conclusion that there is a 1 : 1 relationship between afferent nerve fibre cell bodies in the ganglia and the sensory nerve fibres (both myelinated and non-myelinated) in the roots. Their study was made with the light microscope and from their data it is possible to calculate the proportions of sensory myelinated fibres to non-myelinated axons in cervical, thoracic, lumbar, sacral and caudal dorsal roots. The average proportion of myelinated fibres to non-myelinated axons in all of the roots that Duncan & Keyser studied is 1 : 1.9. If this figure is also considered as an under-estimate of non-myelinated axons and is increased to 1 : 8 myelinated to non-myelinated, then the sensory fibres and axons that flow into the spinal cord are greatly in excess of the number of afferent cell bodies in the dorsal root.

Gasser (1954) has suggested, on similar evidence, that the central projections of non-myelinated afferent cell-bodies branch in the dorsal-root ganglion before they enter the root. He points out a difference in appearance between Schwann-cell bundles in the root from those present in cutaneous nerves. He describes the non-myelinated axons in cutaneous nerves as being suspended in the Schwann-cell cytoplasm by a single mesaxon, one axon to one mesaxon. In the roots a number of axons are suspended in the cytoplasm of the Schwann cell by a single mesaxon.

I have found both types of axon arrangement within the Schwann-cell cytoplasm. I have also found that the size distribution of non-myelinated axons in muscle nerves differs only slightly from that found by Gasser (1954) for non-myelinated axons in spinal roots. This is in contrast to the difference between the size distribution of spinal-root non-myelinated axons and cutaneous non-myelinated axons that Gasser (1954) found himself.

The existence of a fourth Group of sensory nerve fibres has now been conclusively established in muscle nerves which range in diameter from between 0.15 μ and 1.20 μ with a peak diameter value at 0.35 μ. There is no distinct delineation in terms of diameter between the smallest Group III myelinated fibres and the largest Group IV non-myelinated axons, and only when the electron microscope is used is it possible to establish the difference between them on morphological grounds.

Endings and parent fibres in the muscle

Apart from the Group Ia, Group II secondary and Group Ib tendon-organ endings, there are three other types of endings in skeletal muscles. Two are encapsulated and the other is unencapsulated or free.

In this study only one type of encapsulated ending was found, namely the paciniform corpuscle ending. The other type, the Pacinian corpuscle ending, is only occasionally encountered within muscles, particularly the flexor digitorum longus (Barker 1962). Pacinian corpuscles in this muscle derive their innervation not from the muscle nerve but from the interrosseous nerve that innervates about 60 Pacinian corpuscles, approximately half of which occur in a cluster at the lower end of the tibia between the lateral head of the flexor digitorum longus muscle and the tibial periosteum (Barker 1962).

Paciniform corpuscles and their parent fibres

Paciniform corpuscles occur largely at musculo-tendinous junctions in association with tendon organs, i.e. 65% of all paciniform corpuscles observed in this study. The remainder are present either at musculo-tendinous junctions unassociated with tendon organs, or in the perimysium close to blood vessels and small vascular nerve trunks. Those associated with tendon organs usually occur in groups on the capsule wall or inside the tendon organ itself (Ruffini 1897), and their innervation is derived from a single parent nerve fibre. The more corpuscles that derive their innervation from a single fibre, the greater in diameter this fibre is. As many as eight corpuscles variously distributed in an area of musculo-tendinous junctions have been observed, with a Group I parent fibre (12.0 μ). Parent fibres of 10.5 μ, 7.0 μ (GRII) and 3.5μ(GRIII) have also been observed to innervate groups of corpuscles (GRII) and single corpuscles (GRIII). Paciniform corpuscles occur in human skin (Winkelmann 1960), in the foot pads of the cat (Malinovsky 1966) and in joint capsules (Stilwell 1957; Hromada & Polacek 1958) of human and macaque hand and foot, and cat hindlimb. Fitzgerald (1962) has made a study of paciniform corpuscles ("corpuscula bulboidea") in the snout and oral mucous membrane of the pig.

All of the above authors, except Fitzgerald (1962) have found much variation and branching of the terminal axon within the paciniform corpuscle. Hromada & Polacek (1958) and Malinovsky (1966) maintain that these variations are of evolutionary significance in terms of lamillated-corpuscle development from a free-type ending to an organized encapsulated-type ending. Hromada & Palacek (1958) classify their observed endings in an evolutionary progression. They maintain that the hedgehog, low in the evolutionary scale, has proportionally more branching terminal-axon corpuscles in its joint capsules than has the cat (high up the evolutionary scale). Moreover Malinovsky (1966), working in the same laboratory, dismisses Fitzgerald's (1962) view, that branching of the terminal axons and 'ghost' axons may be a form of corpuscle replacement, on the grounds of deficient histological technique and the observation of artefacts due to the thinness of his sections (15-30 μ). Malinovsky (1966) agrees with Hromada & Polacek (1958) and also maintains that 'the variability of the receptors of cats' foot pads does not correspond to the picture of the receptors in the joint capsules of the same animal, but more to the joint receptors of the dog' (Malinovsky 1966, p.98). In terms of the recent evolutionary development of mammals, this presents bizarre possibilities!

In skeletal muscles I have found only 4 paciniform corpuscles with terminal axon branching actually within the corpuscle; this represents 6.1% of the total corpuscles studied. This branching occurred only once in each corpuscle and produced two terminal axons. It was never as extensive as seen by Malinovsky (1966) in the foot pad of the cat. Also in the present study only 1 example (1.53%) was found of two corpuscles fused at their bases with innervation from the same axon; 4.45% of Malinovsky's (1966) preparations were of this type. Fitzgerald (1962) also observed them but does not give any data of their occurrence in the pig's snout.

The degree of variability of the axon terminals in paciniform corpuscles seems to be related to their position in the body. Any receptors in joint capsules, foot pads, or snout, are subject to almost continuous stimulation and the possibility of their wearing out and being replaced seems to have been overlooked or disregarded by most workers in the field, with the exception of Fitzgerald (1962); Barker & Ip (1966) have conclusively demonstrated that changes occur, involving sprouting and degeneration of motor nerve fibres, related with the motor end plate replacement in normal skeletal muscles. There seems no reason why sensory nerve fibres and endings should not exhibit similar replacement changes, and it is almost certainly this process that Fitzgerald (1962) has observed in paciniform corpuscles of the pig's snout. It also seems more feasible that the confusion of axon branching in the paciniform corpuscles that Hromada & Polacek & Malinovsky observed and the variability of the corpuscle itself may be phases in either the replacement of old axons by new, or the complete replacement of whole corpuscles for new. The scarcity of any profound variability in paciniform corpuscles in muscle may possibly be related to their being rather less affected by mechanical deformation and less quickly worn out because of only occasional, as opposed to almost continual, stimulation.

Free endings

Free endings are ubiquitous in their distribution in skeletal muscles. Their axon ramifications are associated with representative parts of virtually every tissue element of skeletal muscles; intra- and extrafusal muscle fibres, the perimysial connective tissue, the connective tissue of muscle spindle and tendon organ capsule walls, the vascular system (excluding the capillary network), tendon tissue and fat cells. Moreoever ramifying axons of the same free ending innervate different tissues and free endings of the same parent fibre innervate different regions of the same muscle. Extensive branching of Group I, II and III free ending parent fibres has been categorically demonstrated, in this study, to occur particularly in small intramuscular and vascular nerve trunks. This observation dispels any doubts that Hinsey (1927) had when he stated 'Our observations do not permit us to conclude that these fibres and endings are not derived eventually from large myelinated fibres' (p.134). However it is in disagreement with his statement (Hinsey 1927 p.126) ...... 'branching of an unmyelinated fibre from a myelinated one.......... if it occurs at all, is very rare'. Moreover it goes some way towards providing a possible ending in the muscle for the discrepant GR I, II and III fibres that Barker, Ip & Adal (1962) found when they correlated the receptor population of soleus muscle of the cat with myelinated nerve fibres in the muscle nerve. The terminal ramifications of free endings of Group IV non-myelinated axons are mainly restricted to the adventitia of arterioles and venules, although they have been observed in perimysial connective tissue and between the intrafusal muscle fibres of a single muscle spindle. Those endings, which in the literature have been referred to as accessory (Tello 1922) or concommittant (Ruffini 1897) are undoubtedly only one form of free-ending termination associated with encapsulated receptors, and are in no way distinct from any other form of free ending. The free ending, like the universal aunt, is everyone's relation. It occurs in virtually every part of the body, with the possible exception of bone matrix. It is the only type of nerve ending that is so universally distributed.

Free nerve endings have been described in the cornea, the tooth pulp, tympanic membrane, mucous and serous membranes (see Woollard, Weddell & Harpmann 1940); in tendon, aponeuroses, periosteum, fascia, ligaments, joint synovial membranes (Weddell & Harpmann 1940; Stilwell 1957; Ralston III, Miller & Kasahara 1960; Polacek 1965; Becton, Winkelmann & Lipscomb 1966), in the viscera (Lim 1960), and in skin overlying various part of the body of humans and other mammals (Woollard, Weddell & Harpmann 1940; Weddell, Palmer & Pallie 1955; Miller, Ralston III & Kasahara 1958; Miller & Kasahara 1959; Winkelmann 1960). It is in observations of skin that most of the information concerning free endings has accumulated.

The terminal ramifications of free endings in skin do not differ structurally from those observed in skeletal muscles. They occur in both the superficial and deep layers of the dermis and are associated with connective tissue, blood vessel adventitia, and subcutaneous fat as well as with encapsulated receptors (Krause end-bulbs, Meisner and Pacinian corpuscles).

In the dermal layer of the ear of an appropriately sympathectomized rabbit there is a plexus of nerve trunks that contains all sizes of sensory nerve fibres. The nerve trunks of this plexus in turn give rise to a more superficial plexus that contains only small myelinated fibres and non-myelinated axons. It is from this superficial plexus that the majority of free-ending fibres arise (Woollard, Weddell & Harpmann (1940).

The only difference that could be observed between free-ending innervation in skin and muscle is the variation in density of the pre-terminal nerve plexuses; that is, between the superficial skin plexus and vascular nerve plexus in muscle. The superficial plexus in skin, which contains the free-ending parent fibres, is very dense in appearance. In muscle it is extremely loose. In general, the farther from the surface of the body, the less dense this plexus becomes. An intermediate type is seen in fascia, and a type more dense than in fascia, but less dense than in skin, is seen in periosteum (Feindel, Weddell & Sinclair 1940).

The relative density of the pre-terminal nerve plexus in the variety of tissue just cited is undoubtedly a reflection upon the relative abundance of small myelinated fibres and non-myelinated axons in the plexus and consequently upon the abundance of free endings derived from these fibres and axons in this variety of tissues.

It is not known whether any free endings in the skin are derived from large diameter parent fibres, but it is known from the observations of Brashear (1936) that a number of free endings in the tooth pulp are derived from parent fibres 9 μ in diameter. This is within the Group II range.

Physiological considerations

From about the beginning of the present century there has been much controversy concerning the functional significance of specific groups of nerve fibres and their endings. This began largely as a result of observations of cutaneous C afferents and their specific mediation of painful sensation. More recently specificity of function was applied to the individual groups of sensory fibres demonstrable in muscle nerves. Observations made during the present investigation show conclusively that no one Group of nerve fibres is specific in terms of endings in the muscle. Also on the basis of this study, and observations of various other authors, the idea of exclusive C afferent participation in the mediation of painful sensation is rejected. It is believed that in muscles, as in skin, the C afferents, together with a large proportion of myelinated sensory fibres, take part in a proposed gate control mechanism (Melzack & Wall 1965) of painful sensation.

Specificity of Fibre Groups

That the diameter Grouping of myelinated sensory fibres in muscle nerves was a reflection of their function was first proposed by Hunt (1954) on the basis of previous observations that only Group I fibres end in muscle spindles as primary endings and in tendon organs, and only Group II fibres end in muscle spindles as secondary endings. Together with his own electrophysiological recordings and measurements of conduction velocities, he demonstrated that the results obtained by the conversion of conduction velocity into diameter of a sample of fibres from muscle spindles and tendon organs fitted very closely with the diameter distribution and peak values of Group I and II fibres obtained with histological techniques by Lloyd & Chang (1948). However, Hunt & McIntyre (1960) later demonstrated that certain receptors in the flexor digitorum longus muscle with Group I parent fibres, responded, under natural conditions, to vibration and tap stimuli. This is not a typical muscle-spindle or tendon-organ fibre response. Paintal (1960) has shown that although the majority of 'pressure-pain' receptors in muscle are of Group III parent fibre origin, four of them had conduction velocities of 55, 66, 76, and 91m/sec. When the conduction velocities of these fibres are converted (Hursh 1939) their diameters are, respectively, 9.1 μ, 11.0 μ, 12.6 μ, and 15.1 μ that puts them within the Group II and I ranges. Moreover Paintal (1960) has shown that 2 of a sample of 31 Group III fibres showed discharges characteristic of muscle stretch receptors.

I have found paciniform corpuscles and free ending parent fibre diameters within the Group II and I as well as Group III ranges. I have also demonstrated 'S4'and 'S5' secondary endings with parent fibre diameters of less than 4.0 μ (GRIII). These observations support Paintal's (1960) conclusion that 'The Group II band of afferent fibres can no longer be regarded as a homogenous group of stretch receptors, owing to a fair number of fibres connected to pressure receptors' (p.268). Even though Paintal observed a 15.1 µ parent fibre of a pressure-pain receptor, he seems reluctant to consider the Group I band of fibres as heterogenous in nature. I believe, on the evidence of the present study, that the Group I band is also heterogenous. This is supported not only by Hunt & McIntyre's (1960) inclusion of tap and vibration fibres in this Group, but also by the fact that Barker, Ip & Adal (1962) observed a discrepancy between myelinated afferent fibres in the soleus muscle nerve of the cat and the encapsulated receptor population in the muscle that amounted to approximately 30% of the total sensory fibres present in the nerve. The majority (76%) of Group III fibres, 35.6% of Group II fibres, and 10.6% of Group I fibres, could not be allocated to receptors. Hnik & Payne (1966), working with rat gastrocnemeus muscles, have shown that an increase in spontaneously discharging nerve fibres occurs following chronic de-efferentation of muscle. This increase occurs in 'non-proprioceptor' nerve fibres (probably free-ending fibres) and represents 29.0% of a sample of de-efferentated muscle nerve fibres. These fibres have a mean conduction velocity of 56.7 m/sec and a range between approximately 30 and 90 m/sec. They are therefore exclusively of Group I and Group II origin. For technical reasons no recordings were made from spontaneously active Group III fibres, but Hnik & Payne (1966) maintain that if this were possible it would demonstrate a proportion of 'non-proprioceptor' nerve fibres in muscle nerve far in excess of 29.0%.

Stimulation of C afferents produces reflex activity in anaesthetized experimental animals in the form of vasodilation, pupillary dilation and struggling. Bishop & Heinbecker (1928) showed that the threshold of response to electrical stimulation of nerve fibres varies inversely with fibre diameter and in man weak stimuli activated rapidly-conducting low-threshold fibres so that the high-threshold C fibres were assumed to be specific pain fibres. Ranson & Billingsley (1916) drew similar conclusions based on observations that the central projections of non-myelinated cutaneous afferent axons run into Lissaur's tract and are accompanied by only small numbers of myelinated afferent fibres. The bulk of the myelinated afferent fibres run over the substantia gelatinosa into the cuneate fasciculus. After cutting the fibres of one or other of these 'systems' in the cord and observing the reflex effects produced in the animal, it was concluded that the non-myelinated fibres mediate the protopathic sensations of pain and probably heat (Head 1920). Similar conclusions have been drawn by White & Sweet (1955) from clinical observations of patients with spinal cord lesions, in which the appropriate small axon system had been accidentally severed with total loss of painful sensation but retention of touch and two point discrimination. Unfortunately, clinical severance of the non-myelinated fibre system in the cord is rarely successful for the relief of morbid pain sensation (see White & Sweet 1955); in fact, it often intensifies the condition. Further experimental evidence for the participation of C afferents in the mediation of pain was supplied by Clark, Gasser & Hughs (1935) who used compression and asphyxia to block activity in A and B fibres and so produced activity only in C fibres by electrical or mechanical stimulation of the whole (cutaneous) nerve.

Heinbecker, Bishop & O'Leary (1933) have shown that at least in a human subject $A\delta$ fibres are also responsible for the mediation of pain. Direct histological and physiological evidence for this comes from Brookhart, Livingston & Hangen (1953) who showed that it is myelinated parent fibres in man and the cat that produce free ramifying terminals in the tooth pulp and only painful sensation can be elicited from this region. These fibres have a diameter range between 1.5 μ and 9.0 μ in man (Brashear 1936). The findings of Paintal (1960) and Bessou & Laporte (1961) are also significant in this respect. They found that Group III fibres in the cat gastrocnemius/soleus muscle-nerve were activated when the muscle was squeezed or injected with 6% NaCl; stimuli that produce a painful sensation in man. The present investigation has shown that Group III fibres (1 - 4 μ in diameter), among others, produce free and paciniform corpuscle endings in cat skeletal muscles.

The above results demonstrate that nerve endings with parent-fibre diameters of less than 9 µ can be activated by noxious stimuli. They are not conclusive evidence of the exclusive mediation of noxious stimuli by these fibres, nor are they conclusive evidence that only small-diameter nerve fibres and axons mediate these stimuli.

Muscle C afferents of the cat not only respond under conditions of ischaemia,which is man is very painful (Besson & Laporte 1958; Iggo 1961),but also when heavy localized pressure is applied either to the muscle or its tendon (Iggo 1961). Some of the same fibres that respond to heavy localized pressure also respond under high and low temperature stimuli and a few axons respond to thermal stimuli that are non-noxious in quality (Iggo 1961). Cutaneous C afferents have been far more extensively studied than those in muscle nerves (see Douglas & Ritchie 1962) and it appears that not only do they respond under noxious conditions of stimulation, but also under a wide variety of non-noxious conditions.

There are two basic, and conflicting, views concerning the mechanisms of so called 'painful sensation'. The observations cited above fall into two categories that either lend support or challenge the specific or pattern theories of pain.

Ranson & Billingsley (1916), cited above, are categoric in their belief that pain is a specific modality transmitted in specific fibres to the cord, and in the cord along specific tracts to the brain. On the other hand, Weddell, Palmer & Pallie (1955) are equally categoric when they conclude that 'there is not and never really has been convincing histological evidence for the commonly accepted statement that morphologically specific nerve endings subserve each of the primary modalities of cutaneous sensibility' (Weddell, Palmer & Pallie p.188), namely touch, heat, cold and pain. Noordenbos (1959) maintains that it is only as a result of the modifying effect of a rapidly-conducting large-fibre system upon a slowly-conducting, multisynaptic small-fibre system in the cord that pain is appreciated. Melzack & Wall (1965) are of the opinion 'that no single theory so far proposed is capable of integrating the diverse theoretical mechanisms' and 'that these mechanisms have not received any substantial experimental verification' (Melzack & Wall 1965 p.974). They propose a theory of pain mechanisms whereby impulses from peripheral receptors are modulated in the spinal cord by the interaction of the central projections of peripheral nerve fibres and special nerve cells contained in the substantia gelatinosa and the dorsal horn (Fig.54).

Positive and negative feedback mechanisms associated with pre-synaptic facilitation and inhibition of volleys of impulses arriving in the spinal cord have been shown to be mediated by the cells of the substantia gelatinosa (Wall 1958; Mendell & Wall 1965). The effect of impulses arriving in large and small peripheral nerve fibres on the first transmission cells of the dorsal horn is being continually adjusted. This adjustment constitutes a gate control mechanism. This mechanism is, in turn, influenced by a central control mechanism (Melzack & Wall 1965).

Impulses in the larger-diameter fibres, which arrive first in the cord, are immediately effective at both cortical and spinal levels since the larger fibres produce an ascending branch which travels in the dorsal column-lemniscus system, as well as a branch to the substantia gelatinosa. Because the larger fibres adapt quickly, their effect is short-lived, but they have, by their excitatory effect upon the substantia cells, reduced the threshold of the first transmission-cell synapse to subsequent impulse vollies. In other words they have 'closed the gate'. Subsequent arrival of impulses in small-diameter fibres, whose central projections end only in the substantia gelatinosa, are in effect blocked. However, these fibres are slowly adapting with longer effectiveness on the substantia cells. They inhibit the substantia cells, thus lowering the threshold of the first transmission-cell synapse. They 'open the gate' to subsequent volleys of impulses. Once the first transmission cells are activated, painful sensations are appreciated at cortical levels and persist until the activity in small fibres is reduced below that in the larger fibres and the gate is closed. Scratching or rubbing of an affected area can initiate activity in the larger-diameter fibres with subsequent reduction in the sensation of pain. A central control mechanism, triggered by activity in the larger-diameter fibres, also has an effect on the output (or threshold) of the first transmission cells. The effectiveness of the central control is probably best understood in terms of the general condition of the body. Someone in poor health will have a lower threshold to pain than someone in good health.

Melzack & Wall (1965) based their theory on physiological data obtained from large and small afferent nerve fibres from the skin and the terminal relationships of these fibres with other nerve cells in the spinal cord. Figure 55 is a modified version of their original figure. It illustrates the proposed cord connections of myelinated fibres and non-myelinated axons from muscle (and from skin) that are concerned with the gate control mechanism of painful sensation.

It seems unlikely that the myelinated nerve fibres from muscle spindles and tendon organs are part of the pain gate-control mechanisms, since their central projections by-pass the dorsal horn and synapse in the posterior funiculus. McIntyre (1962) has shown that massive hindlimb muscle stretch is not appreciated at cortical level and'in all probability the stretch receptors of muscle play no part in conscious proprioception' (McIntyre 1962 p.28). Moreoever, he has shown that stimulation of a few Group I and II fibres is appreciated at cortical level and the central projections of these fibres travel in the dorsal horn lemniscal system, one of two pathways that Melzack & Wall suggest may be taken by fibres of the central control trigger. On the basis of the evidence found in the present study it seems likely that those Group I and II fibres in muscle nerves not concerned with muscle spindles and tendon organ innervation may be in some way associated with the gate control mechanism.

It seems clear that a large part of the sensory innervation of the body consists of free nerve endings derived not only from non-myelinated axons and small myelinated fibres, but also from medium and large myelinated fibres. Judging from present evidence it seems probable that they take part in a central gate-control mechanism of 'painful' sensation. However, the exact functioning of this mechanism is not fully understood, and the manner in which the variety of synaptic connections influence one another is yet to be elucidated.

I believe that an understanding of the true functional significance of free endings is only just beginning, and that they probably play a much greater part in general sensory perception than was ever previously imagined.

V. SUMMARY

1. The non-myelinated sensory (C afferent) axons in muscle nerves, impregnated by the Holmes-silver method and studied with the light microscope, are shown to be approximately half as abundant as the myelinated sensory nerve fibres; 2.2:1 myelinated to non-myelinated. In muscle nerves (just after entry into the muscle) impregnated by the de Castro technique and studied with the light microscope, the myelinated fibres are less abundant than the non-myelinated axons; 1:1.6 myelinated to non-myelinated.

2. A study with the electron microscope of the morphology of non-myelinated sensory nerve axons (C afferents) in muscle nerves is described.

3. With the electron microscope a more detailed count of non-myelinated axons in muscle nerves is possible. They are twice as abundant as the myelinated fibres; 1:2 myelinated to non-myelinated.

4. The non-myelinated sensory axons in muscle nerves have diameters between 0.15 μ and 1.20 μ with a peak value at 0.35 μ.

5. A histogram of the fibre distribution of the total sensory component of tibialis posterior is illustrated. The fibres fall into 4 Groups according to their diameters.

6. The nerve trunks in skeletal muscles are of two distinct types. A large intramuscular type that contains nerve fibres representative of the whole sensory component, and a small vascular type that contains only small sensory myelinated fibres and non-myelinated axons.

7. Paciniform corpuscles are often associated with tendon organs. As many as 8 have been observed that receive their innervation from a 12.0 µ parent fibre. Group III II and I parent fibres innervate paciniform corpuscles.

8. Free-ending ramifications occur in fat, connective tissue, blood vessel adventitia and tendon as well as inside muscle spindles and tendon organs and in their capsule walls. Ramifications in different sites may be derived from the same parent fibre.

9. Free endings are derived from Group I II III & IV parent fibres that are seen to branch extensively in vascular and intramuscular nerve trunks.

10. The innervation of skeletal muscle by sensory myelinated nerve fibres and non-myelinated axons is illustrated schematically.

11. The role of these endings and their parent fibres is discussed in the light of Melzack & Wall's (1965) theory of a central pain gate-control mechanism.

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