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THE MOTOR INNERVATION OF THE MAMMALIAN MUSCLE SPINDLE

by

M. C. Ip, B.Sc., M.Sc. (H.K.)

being

A thesis presented in candidature for the degree of
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THE MOTOR INNERVATION OF THE

MAMMALIAN MUSCLE SPINDLE

CONTENTS

4. FUSIMOTOR INNERVATION OF MAMMALIAN MUSCLE SPINDLES

1. General Introduction

The muscle spindle has been extensively studied
ever since the detailed histological descriptions by
Ruffini in 1898 which 50 years later were revived and
fortified by Barker in 1948.   Many physiologists as
well as anatomists have devoted themselves enthusias-
tically to various aspects of these complex sense organs
which are said to rank third to the vertebrate eye and
ear in degree of complexity.   It is perhaps because of
the complexity in conjunction with technical difficulties
that there are still many points of disagreement amongst
physiologists and histologists and amongst histologists
themselves.

One of the most important and widely controversial
matters is the mode and nature of the motor innervation
of the spindle, which so far is still not yet fully under-
stood.   It is the hope of the present study to invest-
igate more thoroughly the true picture of the muscle
spindle in this aspect.   However, during the course of
the present study, an interesting phenomenon of 'motor
endplate replacement'(Barker & Ip, 1965a) became evident.
This process, if convincingly proved, should be vitally

important in neurology and neuropathology.   Because of
the probable occurrence of this process, the motor inner-
vation of muscle spindles cannot effectively be described
without constant reference to this possibility.   Thus,
it seems more logical and appropriate to deal with this
subject first before going into details of the fusimotor
innervation.   Following on a description of materials
and methods used, this thesis is therefore divided into
two major sections: 1. Replacement of motoneurons and
their terminals; and   2. Fusimotor innervation of mammalian
muscle spindles.

2.   Materials and Methods

2. 1.   Operations

Normal and de-afferentated hindlimb muscles of cat
and rabbit, as well as some normal rat muscles, were used.
The de-afferentated series of animals consisted of ten
apparently healthy cats and five healthy rabbits of
approximately 2-4 kg body weight.   These young adult
cats, anaesthetized with sodium pentobarbital (Nembutal;

Abbott Laboratories; dosage 36-40 mg/kg) given intra-peritoneally, were laminectomized aseptically and allowed to recover. In the case of similarly healthy rabbits Nembutal anaesthesia was followed by inhalation of ether. In all these cases, the dorsal root ganglia from L6 to S2 were removed as neatly as possible from the right side by a proximal extradural cut followed by a second cut as far distal as root fusion permits, great care being taken to ensure that little or no sensory remnants are left and that no damage is caused to the underlying ventral roots. The corresponding root ganglia of the left side were kept intact to supply the additional normal material as controls. The degeneration period allowed was usually from 20 to 30 days after operation (average 26 days). In another three operations, one cat was de-efferentated, while the other two cats were de-afferentated as well as de-efferentated in order to determine whether mammalian spindles may receive fine sensory or sympathetic innervations as well as motor. Operated animals were killed sometimes with chloroform and sometimes by a blow to the back of the neck at the scheduled time.

2. 2.   Root checks.

The operated roots were fixed in Bouin's fluid, embedded, and sectioned at 10 μ in longitudinal or transverse serial section, and either stained with haematoxylin and eosin or impregnated by Holmes' silver-on-the-slide method (1943) in order to check on the effectiveness of de-afferentation.   The extent of de-afferentation can be further judged by the presence or absence of cell bodies of sensory fibres in the operated root and of surviving afferents, namely Group Ia (Primary), Ib (Tendon-organ), and Group II (Secondary) fibres in the de-afferentated muscle as revealed during the process of teasing.   Damage to ventral roots was searched for in the form of lesions in which regenerating fibres, nuclear proliferation and fibre disorientation were evident, or in the form of profuse collateral sprouting in the operated muscle as described by Edds (1949).   It was found that although damage to ventral root can be largely avoided, de-afferentation can seldom be complete because it is extremely difficult to remove those sensory cell bodies located in the proximal part of the ventral ramus of the mixed nerve or even in the ventral root without causing serious damage (see Text-figure 1;

Text-figure 1.   Photomicrographs of the vertical longitudinal sections at 10 μ of a normal (A) and a de-afferentated (B) spinal root of the cat.   Holmes' silver-on-the-slide method.   Figure A from the first sacral S_1, normal root of the cat.   Figure B from the sixth lumbar L_6, de-afferentated root of cat C159.   In Figure B, the dorsal root ganglion was removed and scar tissue (s.t.) has been formed in its place during the period of 4 weeks' degeneration.   Note in both of the normal and the operated roots taken from two different cats, sensory 'overspills' (o-s.; see text on p. 4) are evident as can be seen by the presence in the ventral root or at the proximal part of the mixed nerve, or outside the dorsal root ganglion beyond the ganglionic cleft, of cell bodies which are obviously imposs-ible to be effectively removed by operation.   1 --1'; 2 -- 2'; and 3 -- 3' represent 3 different levels at which the trans-verse sections in Text-figure 2 a, b, c were taken.

d. r. g. dorsal root ganglion;   g. c. ganglionic cleft; g. c. b. ganglionic cell bodies;   m. n. mixed nerve; o-s. overspill sensory cell bodies;   p. proximal (i.e. nearest to the spinal cord); s. t. scar tissue;   v. r. ventral root.
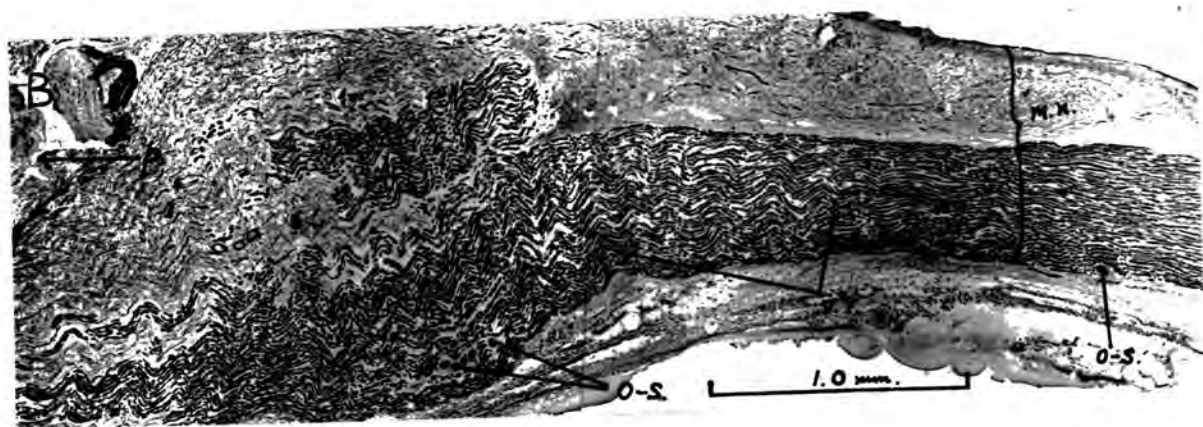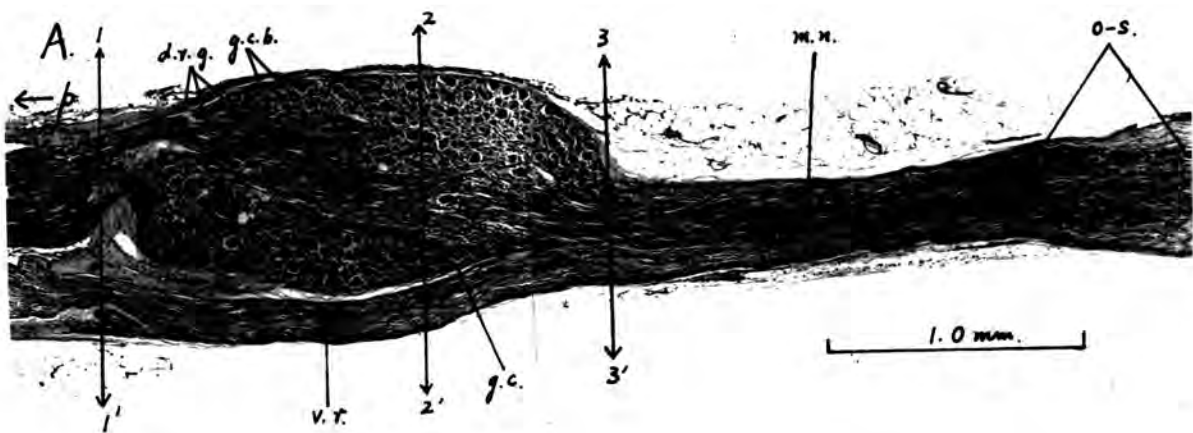
Text-figure 1

see also Sherrington, 1894). In order to ensure that the study is made solely on motor innervation and nothing else, it is important that these measures are taken to check the effectiveness of the de-afferentated operations.

Photomicrographs of three chosen transverse sections at 10 /u impregnated with Holmes' silver method of a de-afferentated 6th lumbar root of cat C167, whose operation is considered as most satisfactory, are given in Text-fig. 2 a, b, & c taken at three different levels from proximal (i.e. nearest to the spinal cord) to distal to illustrate how the checking was achieved. Text-figure 2 a is a section relatively proximal to the former dorsal root ganglion (its corresponding level is indicated in Text-figure 1, 1 -- 1'). It can easily be seen that the en-sheathing perineurium of the ventral root is circular in outline and is thus obviously intact as against the scar tissue formed at the place where the dorsal root ganglion was before operation. In examining section by section the whole transverse series, greatest care was taken to ensure that this ensheathing perineurium was always intact as can be seen in Text-figure 2 b which, being a section taken at a level approximately 1.2 mm more distal to the
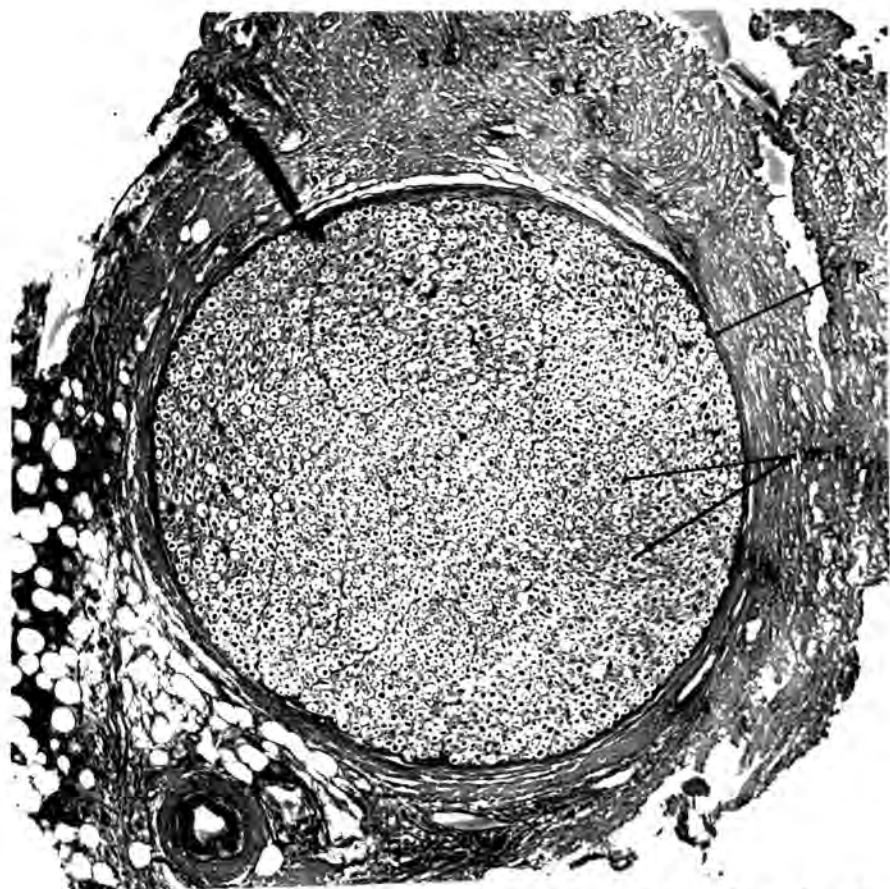
Text-figure 2 a.   Photomicrograph of a transverse
section at 10 μ of the de-afferentated 6th lumbar root of
cat (C167) at proximal end.   It shows the complete
removal of the dorsal ganglion (top part shows scar
tissue of the former dorsal ᵣₒₒₜ ganglion) leaving the ventral
root intact as can be seen from the perfect integuity of
the ensheathing perineurium.   Compare with Text-figure 1,
1 -- 1'.   Holmes' silver-on-the-slide method.

m. a. motor axon;   s. t. scar tissue;   r. p. root
perineurium.

1st (its corresponding level is indicated in Text-figure 1, 2 -- 2') even shows the ganglionic cleft between the dorsal and ventral roots clearly.   In Text-figure 2 c the section taken approximately 0.75 mm more distal to the 2nd (see the corresponding level in Text-figure 1, 3 -- 3') represents the junction where the mixed nerve begins.   It is only when all these conditions are fulfilled for all the spinal roots from L6 to S2 of any one operation that it can be regarded as satisfactory as is the case of cat C167. Because of this, the study of materials from this cat forms the decisive and final judgement on the finding.   But, this ideal situation in de-afferentation can seldom be achieved due to the extreme difficulty or impossibility of removing all sensory cell bodies from all the roots because of the frequent presence of 'overspills' located either in the proximal part of the ventral or dorsal ramus of the mixed nerve (i.e. more distally beyond the level represented in Text-figure 2 c; any attempt to cut beyond this point would naturally involve damage to the ventral root); or even sometimes in the ventral root itself.   Fortunately, this 'overspill' has been found (Barker & Stacey, unpublished observations) not to exceed 0.5 % of the total population of all cell bodies in each dorsal root ganglion, and is more commonly found in sacral than in lumbar roots.   This relative
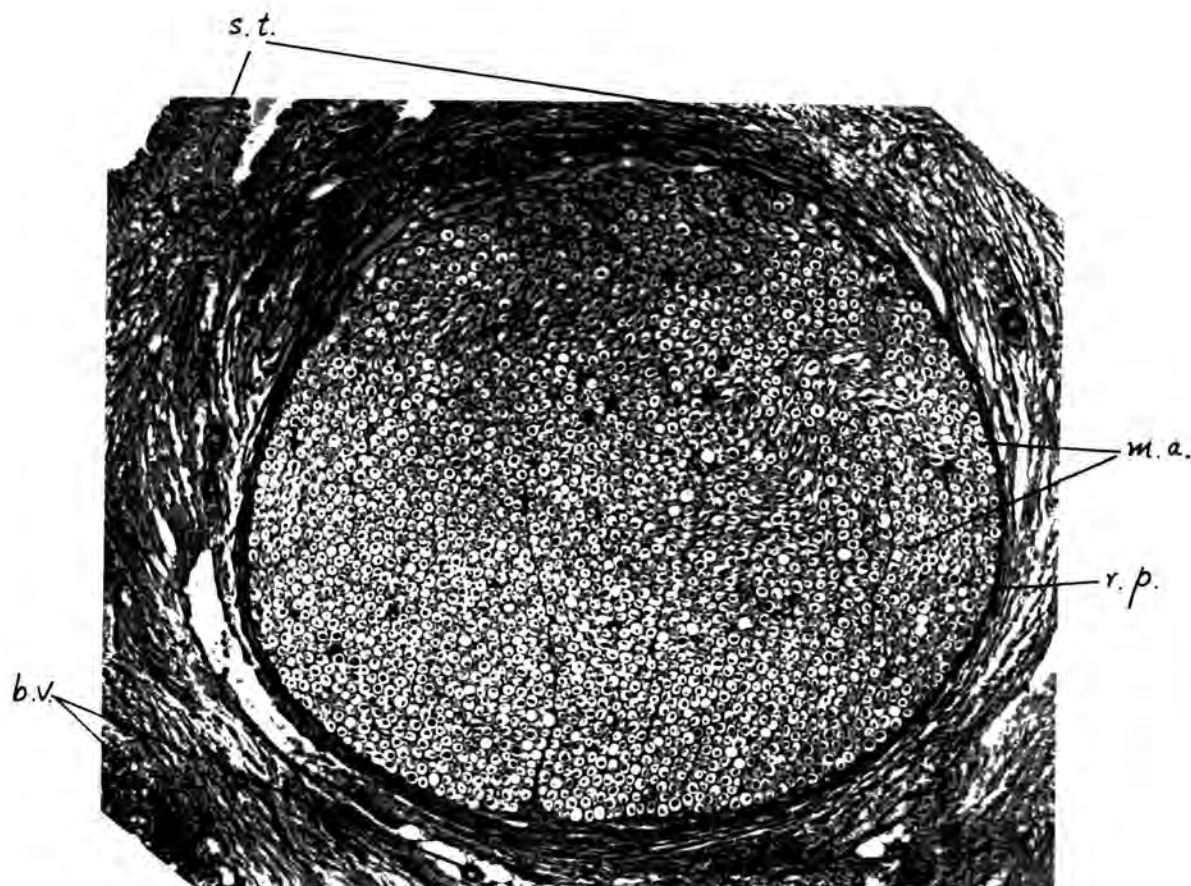
Text-figure 2 <u>b</u>.   Photomicrograph of a transverse
section at 10 μu of the same root.   Again it shows the
complete removal of the dorsal root ganglion and no ventral
root damage.   Note the axons are slanting towards the
dorsal aspect so as to form the mixed nerve slightly more
distally.   This section was taken approximately 1.2 mm
more distal from the previous one.   Compare with Text-
figure 1, 2 -- 2'.   Holmes' silver-on-the-slide method.

b. v.   blood vessel;   m. a.   motor axon;   r. p.   root
perineurium;   s. t.   scar tissue.
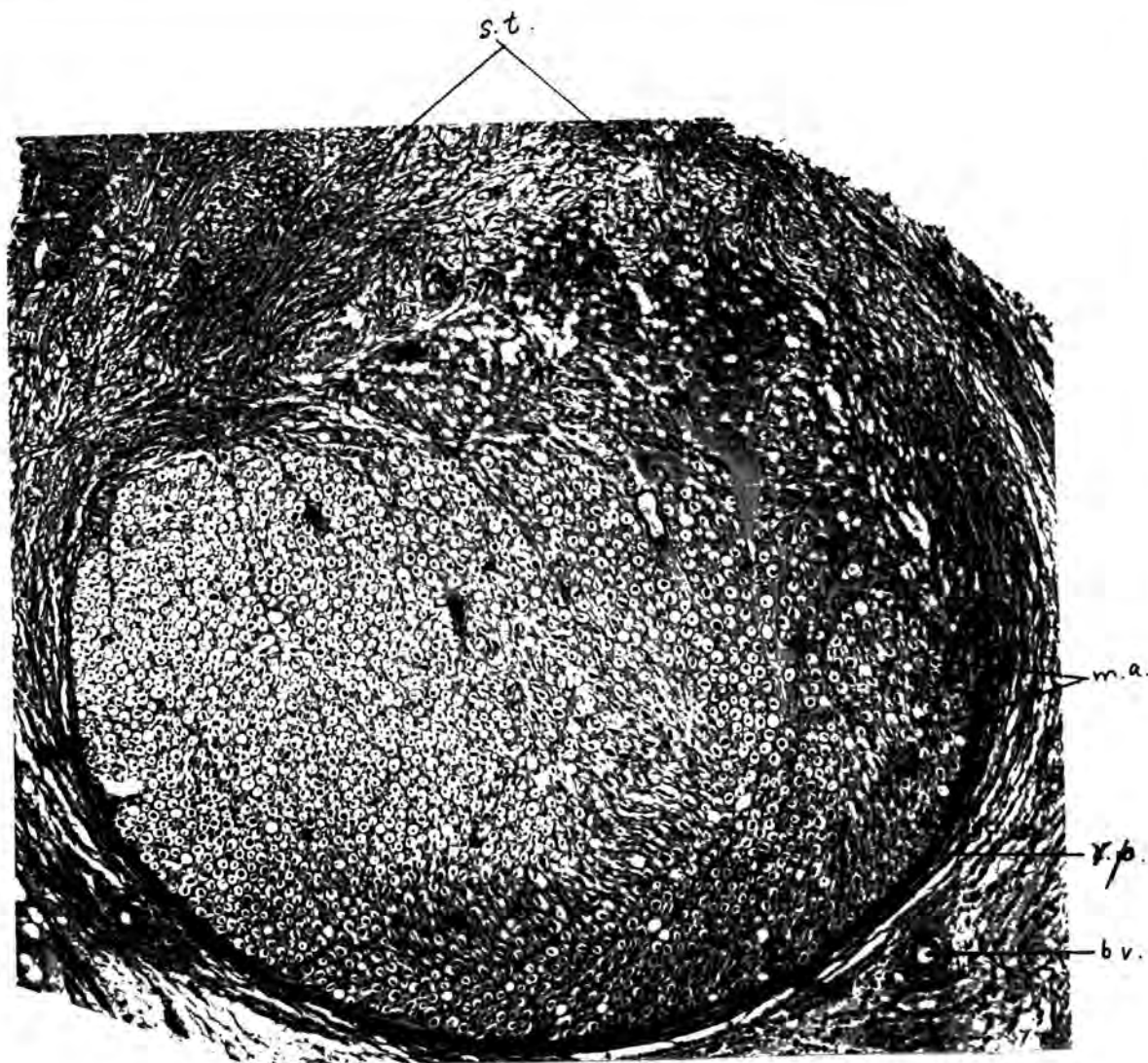
Text-figure 2 <u>c</u>. Photomicrograph of a transverse section at 10 /u of the same root at the extreme distal end approximately at the junction where the mixed nerve begins. Note the slanting of the motor axons is more apparent and extensive. This section was taken approximately 0.75 mm from the second section. Compare with Text-figure 1, 3 -- 3'. Holmes silver-on-the-slide method.

b. v. blood vessel; m. a. motor axon; r. p. root perineurium; s. t. scar tissue.

scarcity of sensory 'overspills' is reflected by the fact
that surviving afferents in the muscles studied have not
been observed in teased preparations, and it is therefore
believed that this factor in no way prejudices the results.

It is conceivable that some damage to the ventral
root fibres during de-afferentation is inevitable (Boyd,
1962a; Gilliatt, 1965), but the important factor is the
extent of damage and whether the consequence of such damage
seriously affects the results. Boyd (1962a) maintains
that the number of fibres in a de-afferentated and a de-
efferentated nerve added together always amounts to close
on 85 % of the number in the corresponding normal nerve.
He attributes 10 % of the loss to the motor and 5 % to the
sensory damage. The experience of our group is that
damage of this order is not inevitable; moreover we question
this method of assessing it. This is because it depends
on an exact numerical bilateral symmetry that does not
obtain, and because it also ignores individual variability.
Just take the soleus muscle of the cat for example, even
the difference of spindle content between bilateral members
of pair can be from 0 to 9, while the range of spindle
content varies from 40 to 70, a difference of 30 (see Chin
et al., 1962) for different soleus muscles. It may be

assumed that every spindle is innervated by one primary
and an average of one secondary fibre (from Barker et al,
1962), and two fusimotor fibres (taking an average of 115
γ-fibres in the soleus nerve from Boyd & Davey, 1962,
divided by the mean of 56 spindles from Chin et al, 1962,
for the soleus muscle).   Expressed in terms of nerve
fibres, this difference would be of the order of 36 for
bilateral pairs and 120 for different soleus muscles.
This variability is illustrated by comparing the average
number of motor fibres in de-afferentated soleus nerves
of 270 given by Boyd & Davey (1962) with the minimum
number of 174 in one de-afferentated soleus given by
Clark (1931), a difference of 96 in number.   Moreover,
if the same calculation is done by adding the number of
fibres in the de-efferentated (A) and de-afferentated (B)
soleus nerves from the values given by various authors,
the total does not amount to 85 % of the corresponding
normal nerves (C) as can be seen in the following data:

A	B	C
No. of sensory fibres in de-eff. soleus n.	No. of motor fibres in de-aff.soleus n.	Total no. of fibres in normal soleus n.

A	B	C
(Barker 193	(Adal,private 247	
et al. 199	communication)	
1962) 237	(Clark, 174	
213	1931) 187	
average 209	192	
	198	
	233	
	average 197	
(Hagbarth & 144		433
Wohlfart,		
1952)		
(Boyd & 180	270	450
Davey,		
1962)		
average 178	average 238	average 442

Thus, if the mean is taken for all these values, then
A + B = 178 + 238 = 416 which is 94 % of 442 in C.   If
the maximum is taken, then A + B = 237 + 270 = 507 which

is higher than 450 by 11 %.   Even if A + B is assumed to

be 85 % of C and therefore 450 should be 100/85 X 450 = 530

for valuing the maximum number of fibres in the normal

nerve in Boyd & Davey's data, 507 would still amount to

96 %.   It is only when the minimum for all values is taken,

then A + B = 144 + 174 = 318 which is 73 % of 433 in C.

Damage to ventral roots at operation would lead to

collateral regeneration of motor fibres in the affected

muscles.   However, judging by the relatively constant

proportion of normal and various sprouting fusimotor term-

inals (see first half of Table 1, page 39 of Results, Section

3.23) occurring in the 12 cat and 10 rabbit spindles from

various de-afferentated animals, it seems very unlikely

that the variable degree of motor damage would affect all

of them to the same extent.   The evidence of all other

histological root-checking methods mentioned elsewhere seems

to suggest that although some motor damage may be inevitable

and de-afferentation is seldom completely satisfactory,

these factors do not affect the results to any significant

extent.

2.3. Staining techniques.

It is a well-known fact that it is fairly difficult to obtain uniformly stained gold chloride preparations, and even if the best staining quality is achieved, individual nerve fibres, especially of motor origin , never offer a clear-cut solid tracing of their courses.   On the other hand, there is no doubt that silver can give a more precise and complete delineation of nervous structure than gold chloride, which gives only coarse granular impregnations, or methylene blue whose application is restricted to thin, superficial muscles.   In the past, the use of silver has been restricted only to whole mounts of thin tissues, or to serial sections from which reconstructions were based. We have therefore devised a new technique (Barker & Ip, 1963) to produce teased, whole-mount, silver-impregnated preparations in which all nerve endings are shown and even the finest nerve fibre is stained solid black enabling it to be traced unmistakenly along its whole course inside as well as outside the spindle (see the silver and gold preparations in Text-figures 3 and 17 for comparison).   By working with teased silver preparations, one has had the advantage of seeing more and with greater precision than

Text-figure 3. Photomicrographs of preparations impregnated by the modified silver technique (Figures a to c) and by the gold chloride technique (Figure d) to show the difference in staining quality exhibited by these two methods. The neurofibrillar framework of the end-plate and its terminal axon is excellently shown by silver which also reveals sole-plate nuclei occasionally as seen in Figure a; but only granular patches are shown in preparations as can be seen in Figure d impregnated by gold which never stains sole-plate nuclei. Note that each individual intrafusal muscle fibre is clearly distinguishable in the silver preparations but very indistinct in the gold chloride preparations.

Figure a, intrafusal motor end-plate from normal rabbit Rb7 vastus intermedius spindle (its tracing is given in Figure 5, Plate 6).

Figures b & c, intrafusal motor end-plates from de-afferentated cat C167 peroneal I spindles (tracing of Figure b is given in Figure 6, Plate 6).

Figure d, intrafusal motor end-plates from a normal cat interosseous spindle.
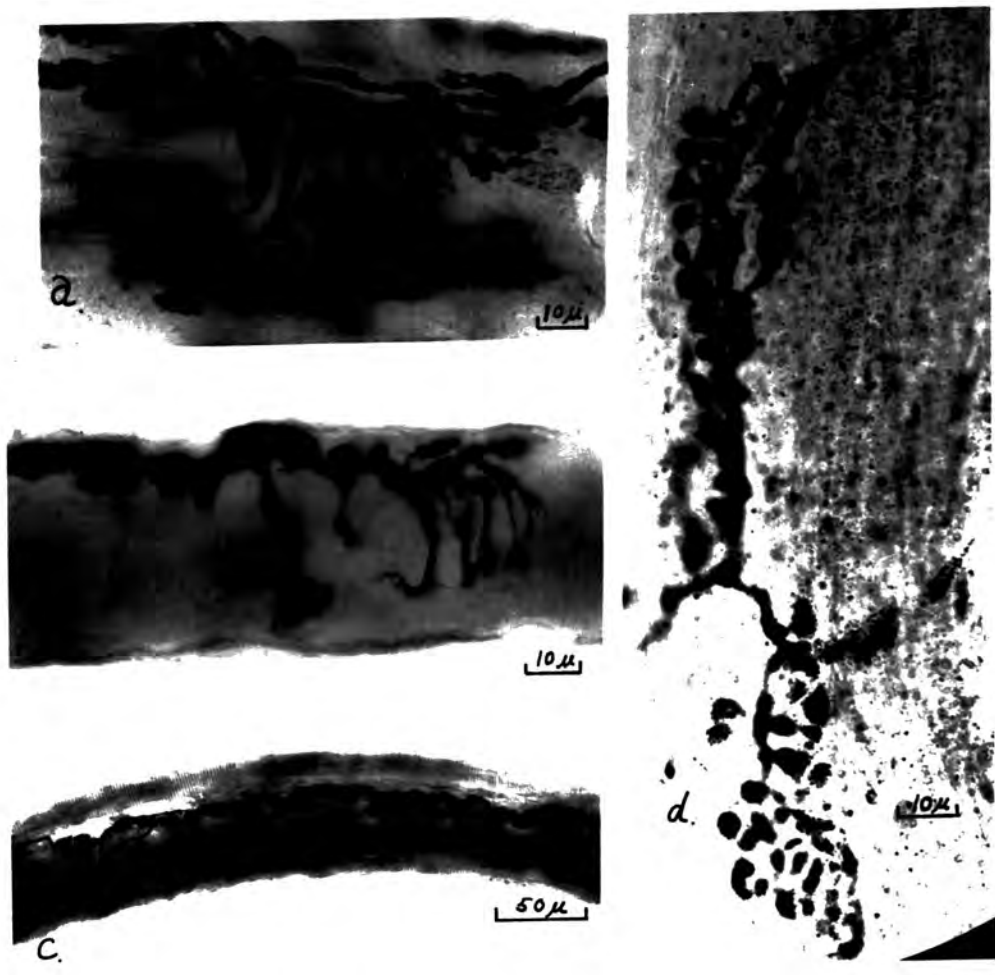
Text-figure 3.

previous investigators who have either hindered by the diff-
iculties of interpretation in reconstructing serial sections
or left guessing by the coarse and granular imperfections of
gold chloride and methylene blue.

Therefore, this modified de Castro's silver block
staining technique, having the merits of silver staining
to exhibit structural details and clarity and at the same
time avoiding the laborious and less convincing reconstruc-
tion from serial sections, is considered to be better than
any other existing methods so far as for the purpose of
studying the motor innervation of mammalian muscle spindle
is concerned. The very well impregnated and overall uni-
form pictures of mammalian muscle spindles, as shown in
Text-figure 4 and many others, cannot be obtained from
gold chloride, methylene blue, or other techniques. The
precise details of the modified de Castro's silver technique
are given as follows (see also Barker & Ip, 1963):

(i) Fix fresh muscle or muscle slices (for better
penetration) for 4 to 6 days in freshly prepared solution
of chloral hydrate, 1g; dissolved in distilled water, 50 ml;
to which 95 % alcohol, 45 ml; and concentrated nitirc acid,
1 ml are added. Longer fixation time is permissible or

Text-figure 4 <u>a</u>.   Photomicrograph of the encapsulated sensory region of a normal muscle spindle of cat teased from the hindfoot lumbrical muscle.   A secondary ending lies on either side of the central primary ending. Note that each individual intrafusal muscle fibre is clearly distinguishable even at this low magnification (150X).

Text-figure 4 <u>b</u>.   Photomicrograph of two normal muscle spindles lying side by side teased from a hindfoot lumbrical muscle of another cat.   Both spindles have a S_1 secondary ending on the right of the primary ending.   Trail endings are present on both poles of them, but end-plates, one of which (e.) is clearly shown, are only present on the right hand pole of these spindles.   Note the difference in size between these two spindles.

Both are teased, whole-mount silver preparations; de Castro technique as modified by Barker & Ip, 1963.

c. w. capsule wall;   e. end-plate;   l. s. lymph space; P. primary ending; S_1 secondary ending;   t. trail ramification.

a.

lymph space   'C' afferent   capsule wall

blood vessel   S₁

b.

t   l.s.   c.w.   P   S₁   l.

t   P   s₁   t

P   t   l.

t

200μ
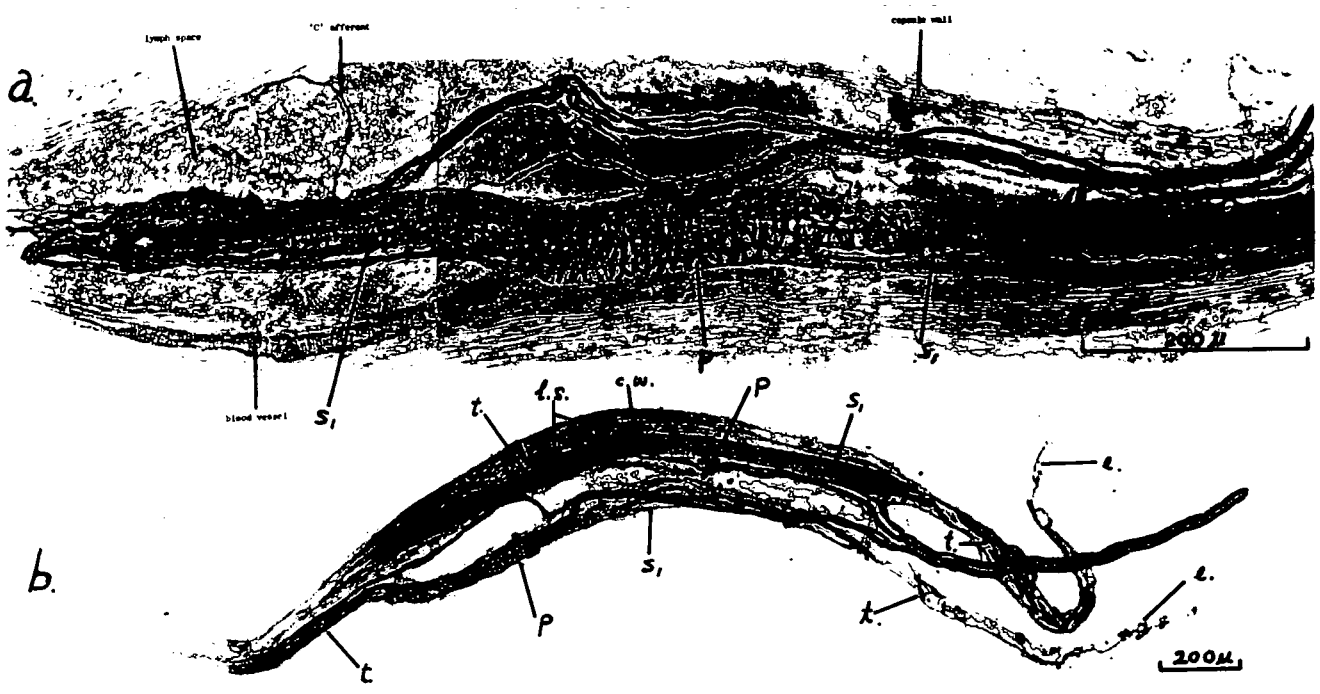
Text-figure 4.

may even be advantageous.

(ii)   Wash for 24 hours in running tap water.

(iii)   Place for 48 hours in 95 % alcohol, 25 ml; concentrated ammonia, 1 drop; or in these proportions.

(iv)   Blot surplus fluid, and incubate for 5 days in 1.5 % silver nitrate at 37° C.

(v)   Reduce for 2 days in freshly prepared solution of hydroquinone, 2g; 25 % formic acid, 100 ml; or in these proportions.

(vi)   Rinse in distilled water; clear and store in glycerine before teasing.   Muscle pieces may be teased after being stored for 1 or 2 days, but glycerine must be changed once or twice if storage period is longer.

(vii)   Mount teased preparation in glycerine and ring coverslip with pitch.

The process of teasing is essentially the same as that in the gold chloride technique employed by Gairns (1930). If the whole muscle was fixed from the beginning, cutting it along the muscle fibres into thin slices prior to teasing may facilitate the process.

This modified de Castro (1925) technique has a high chance of staining nuclei especially those situated in the nuclear-bags (see Text-figure 5 a& c) and nuclear-chains (see Text-figure 5 b & c) of de-afferentated spindles. This is very important in determining whether a traced intrafusal muscle fibre is a nuclear-bag fibre or not. Because, although the relative size of each type of intrafusal muscle fibres can sometimes be used to distinguish whether it is a nuclear-bag or a nuclear-chain fibre, this is not an absolute reliable criterion. The diameter of either nuclear-bag or nuclear-chain fibres always changes along the whole length so that it is not uncommon to find that a nuclear-chain may be as large as or even bigger than a nuclear-bag intrafusal muscle fibre. Eldred et al. (1962) in a study of muscle receptors in the mesial gastroonemius, soleus and extensor digitorum brevis muscles of the cat, remarked that the diameters of intrafusal fibres, measured within the intracapsular space, but distal to the nucleated region, may vary two or threefold. Hence throughout the present study, the presence of nuclear-bags in the equatorial nucleated region is used as the chief and decisive means to determine whether the traced intrafusal fibre is definitely a nuclear-bag fibre.

Text-figure 5.   Photomicrographs **a** & **b** taken at two different foci of the same spot of a de-afferentated spindle from cat peroneal II muscle show distinctively the nuclei of 2 nuclear-bag and 3 nuclear-chain intrafusal muscle fibres in view respectively.   The presence of these nuclei is the chief criterion used to distinguish between nuclear-bag and nuclear-chain intrafusal muscle fibres.   Photomicrograph **c** taken from part of a transverse section at 10 μ stained with haematoxylin and eosin of a normal cat tenuissimus muscle shows the equatorial nucleated region of a spindle on the right and intramuscular blood vessels and nerve trunk on the left.   From this section, two nuclear-bags and four nuclear-chains are clearly distinguishable. Photomicrograph **d** is part of the equatorial region of a de-afferentated cat C159 peroneal II spindle of 30 days degeneration showing the thickening of the ensheathing layer of cross-striations.

b. v. blood vessel;   c. l. capsule lamellae;   e. m. b. extrafusal muscle bundle; i. n. t. intramuscular nerve trunk; l. s. lymph space;   'n-b' nuclear-bag & 'n-c' nuclear-chain intrafusal muscle fibres;   t. c. s. thickened cross-striations.
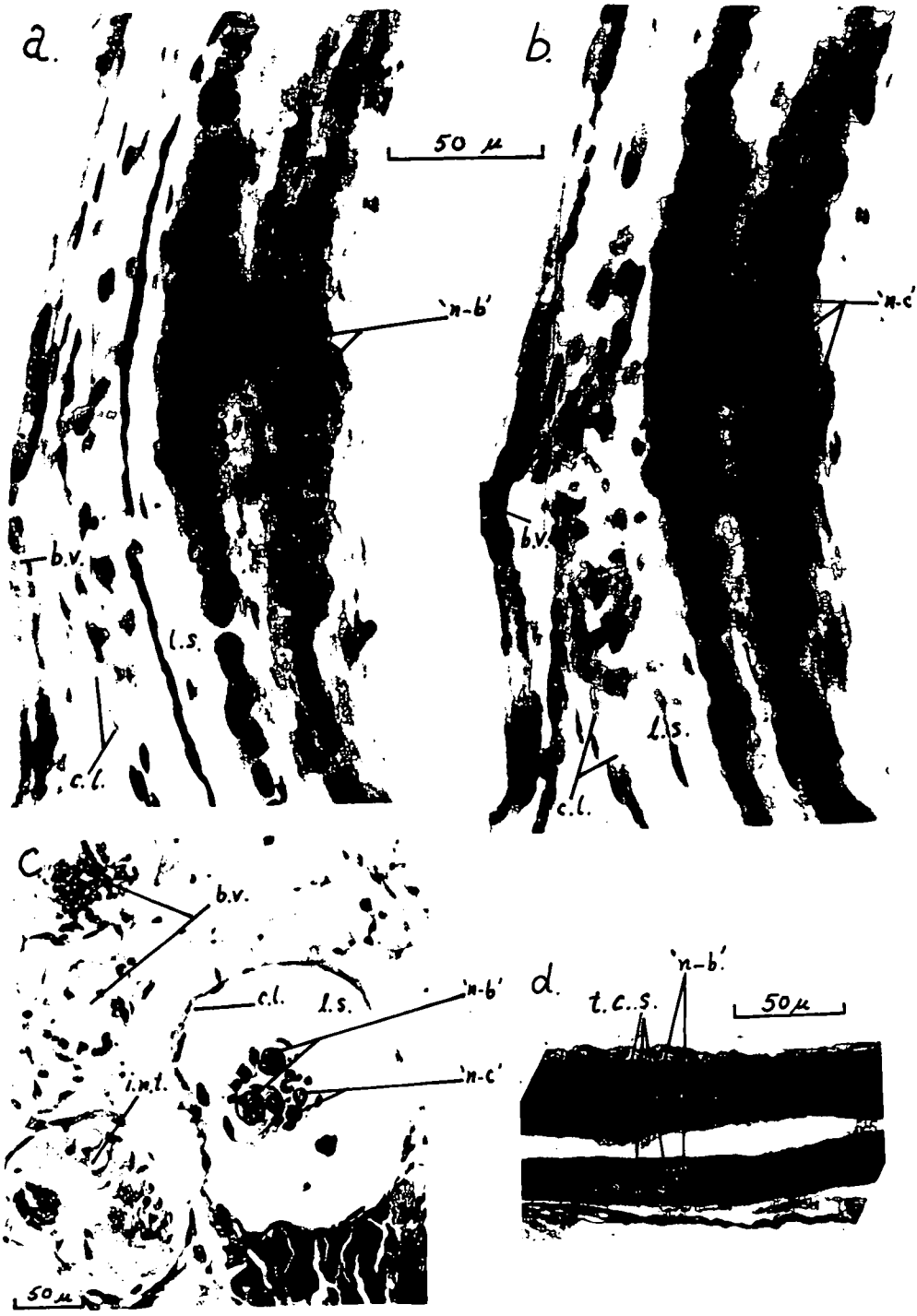
Text-figure 5.

Although nuclei at other sites and the fundamental nuclei of the sole-plate are only capriciously stained (see Text-figure 3 <u>a</u> & 12 <u>b</u> & <u>c</u>), they are often used to help the identification of motor end-plates. Moreover, in preparations impregnated by this silver method, the boundary of every intrafusal muscle fibre is shown much more clearly than in gold chloride preparations (see for example, Text-figures 4 & 6 and compare with Text-figures 3 <u>d</u> & 17 <u>d</u>). As shown in Text-figure 6, the difference in the arrangement and spacing of the cross-striations of the intrafusal muscle fibres also makes the identification of individual intrafusal fibres more easy with greater confidence. It was always used as far as possible to help the separation of the intrafusal bundle into individual muscle fibres for the fusimotor study.

At a later stage of the present investigation, it was found necessary to study the subneural apparatuses of the motor endings whether intrafusal or extrafusal ones. Therefore, other staining methods were also tried and these included Janus Green B (Coupteaux, 1947) which had not much success, and the cholinesterase technique according to Coupland & Holmes (1957) and Silver (1963). A study of

Text-figure 6.   Part of a de-afferentated (d/a)
rabbit spindle pole to show the marked difference in the
arrangement and spacing of the cross-striations in the
intrafusal muscle fibres.   As can be seen easily from
the picture, there are three muscle fibres in view and
possibly a 4th one in the background (out of focus).
This means was often employed in the present study for
the identification and separation of the intrafusal bundle
of spindles into individual intrafusal muscle fibres.
Teased, whole-mount modified de Castro's silver prepar-
ation taken from Rbll peroneal IV muscle (d/a).

the motor subneural apparatuses was made on normal cat
and rabbit muscles as well as de-afferentated material.
Another cat C207 was de-afferentated for this histo-
chemical staining purpose.

2. 4.   Choice and sample of materials

Care was always taken to exclude any muscle in which
the motor supply was suspected to come from the imperfect
root(s), the inclusion of any particular muscle in the de-
afferentation being based on Sprague's chart (1958), so
that the de-afferentated materials from all these operated
animals whose roots were found to contain some degree of
sensory 'overspills' are still useful for motor analysis
to be made with considerable certainty.   The choice of
muscles was based on their distance from the cord, so that
regenerating fibres due to ventral root damage, if any,
would not have had sufficient time to reach them.   The
fastest rate of regeneration, i.e. that after crushing at
4.36 $\pm$ 0.24 mm/day on top of a scar delay of 5.2 days
(see Gutmann et al., 1942), was taken to ensure that even
the fastest regenerating fibres due to accidental crushing
during operation would not have had sufficient time to

reach the muscle. The muscles most frequently used were the peroneal, soleus, and the interosseous muscles because they fit in the above mentioned requirements nicely, but tenuissimus, popliteus, flexor digitorum longus mesial, and lumbrical muscles were also used to include a wider range.

Studies on the sprouting of fusimotor axons were made from both normal and de-afferentated spindles. Most information was obtained from analysing the motor innervation of 12 cat and 10 rabbit spindles in connexion with the fusimotor innervation study (see Section 4). The animals used in studying sprouting and degeneration included 3 cats (C159, C167 and C172) and 2 rabbits (Rb11 and Rb17); and these animals also formed part of the materials used for fusimotor innervation studies. As sprouting became more evident from fusimotor studies, attention was turned to observations on the evidence of sprouting of skeletomotor axons whose endings were thought to be more generalized and less complicated than the fusimotor ones. Observations on the skeletomotor sprouting were all made on muscles taken from normal, apparently healthy hindlimbs of young adult animals. This sample comprised of the following muscles: Cat tenuissimus, peroneals, interossei, and lumbricals; Rabbit soleus and peroneals; rat intertransversarius and gastrocnemius.

Every type of possible sprouting examples was illustrated by a drawing traced from a photomicrograph. The photographic tracings originally made at various convenient magnifications during the course of study are reproduced to a standard scale in the figures for comparison.

Studies on the fusimotor analysis were made from both normal and de-afferentated spindles, most valuable information being obtained from analysing in full detail the motor innervation of a sample of 12 cat and 10 rabbit spindles as just mentioned for fusimotor sprouting analysis (see also Barker & Ip, 1965b). The cat sample contained 4 peroneal, 2 soleus, and 1 popliteus spindles from Cl59; 3 peroneal spindles from Cl67; and 2 peroneal spindles from Cl72. The rabbit sample contained 6 peroneal spindles from Rbll; 1 popliteus and 2 soleus spindles from Rbl7; and 1 vastus intermedius spindle from a normal animal. It was found that very often the motor innervation of only one pole of most cat spindles can be analysed. Since each polar half of a spindle can be considered as separate contractile part (Barker, 1948), motor innervation analysis was often made on polar halves if the whole spindle cannot be studied.

The course of the fusimotor fibres and their termin-
ations whether innervating nuclear-bag or nuclear-chain
intrafusal muscle fibres of each of these spindles was
followed mostly with an oil-immersion lens in full detail
from as near the intramuscular nerve trunk as possible, to
the endings.   The detail of the motor innervation on
either one pole only or the whole spindle as the case may
be was then traced and entered onto a diagrammatic sketch
at approximately X 400 magnification in which the intra-
fusal muscle fibres are drawn out side by side.   Camera
lucida drawing were found not suitable for this purpose.
Tandem spindles, in which 2 or more encapsulated sensory
regions are linked together in series by the continuity of
1, 2, or more intrafusal (exclusively nuclear-bag) muscle
fibres (see Cooper & Daniel, 1956; Barker & Ip, *1961* and Ip, 1961)
were purposely excluded from the present investigation to
avoid unnecessary complexity.   As far as possible, examples
of each characteristic group of the motor termination were
captured with photomicrographs if photogenic, and only those
considered to be worthwhile of showing are given; in other
examples whose characteristics cannot be shown by photomicro-
graphs, tracings alone are given.

The effect of de-afferentation brings about various

structural changes in the spindle. The primary and
secondary or other possible sensory endings gradually
disappear and nothing of a sensory nature remains after
3 weeks' degeneration. There is a gradual loss of nuclei
in the nuclear-bags, but the complete disappearance takes
about a year (see Boyd, 1962b). These nuclei are still
discernible after 4 weeks' de-afferentation as can be seen
from Text-figure 5 a & b. Occasionally, the thickening
of the cross-striations on the circumference of the nuclear-
bags becomes very apparent as in Text-figure 5 d and this
is a great help in distinguishing nuclear-bag from nuclear-
chain intrafusal muscle fibres. According to Boyd (1962b),
there is a slight increase in intrafusal muscle fibre diam-
eter, but no comparative study of this kind is made here.

During the course of the fusimotor analysis, various
methods have been tried in order to isolate individual
intrafusal muscle fibres for studying, but without much
success. For instance, several proteases, including Hyal-
uronidase and trypsin were used before and after the silver
staining in the hope of splitting up the individual muscle
fibres and dissolving the connective tissue around them
and the capsule sheath. But these enzymes either digest
the muscle and nerve fibres, or affect the staining to an

intolerable extent. A micromanupulator was also used in an attempt to break away the capsule and separate the intrafusal muscle fibres. But it was found that even slight handling tends to twist the spindle and make it even worse than without any treatment. Finally therefore, it proved best to study the teased spindle without further treatment. Sometimes, a little pressing may prove helpful (as in the preparation shown in Plate 9). Also a combination of the acetylcholinesterase and the modified silver technique was tried as employed by Wolter(1964). It was thought that if this combination of two techniques works, it would be perfect to reveal the subneural apparatuses with the enzyme activities and the nerve terminals at the same time to decide once for all the exact nature of the trail ramifications. But the brown copper sulphide crystals representing the sites of acetylcholinesterase activities disappeared after the treated tissue was put into the reducer. Further trials should be attempted in the future because it might be very rewarding.

3. Replacement of motoneurons and their terminals

3. 1.   Introduction

It is well known that if a mammalian skeletal muscle is partially denervated, either experimentally or as a result of disease, the affected axons and their terminals undergo retrograde degeneration and the surviving intact axons make new connexions with the denervated end-plates by a process called collateral regeneration (Edds, 1950 & 1953; Hoffman, 1950; Coers & Woolf, 1959). Such reinnervation is generally achieved by outgrowths sprouting either from the subterminal nodes or from the end-plates of the surviving axons. This collateral regeneration is believed to be caused by the influence of a humoral substance released by the transforming Schwann cells of the degenerating axons (Edds, 1953). This neurotropic chemical is called 'neurocletin' by Hoffman (1950), and is thought to be a moderately unsaturated fatty acid probably in the form of glycerides, although the precise nature is uncertain (Hoffman & Springell, 1951). It is suggested that the activities of this neurocletin lie in its ability to penetrate the axolemma and disorganize the axoplasm, and that it may be released

as a water-soluable complex capable of extensive diffusion.

Exactly the same sort of sprouting has been observed in apparently healthy muscles whose motor supply is intact, i.e. in both normal and de-afferentated muscles. In the past, it was assumed that under normal conditions the peripheral terminations of a motoneuron once they have established their morphological identity, would remain unchanged throughout life until the parent motoneuron dies. But in this study, it has become evident that motoneurons have the ability to renew their terminals at intervals under normal conditions by the same growth process that achieves repair under the abnormal conditions of partial denervation. Wilkinson (1929), on the basis of studying differences in growth and development, regarded the morphological variations in forms of motor terminals as representing various developmental stages. He maintained that the 'terminaisons en grappe' could be arranged in a series showing an increasing degree of resemblance to the 'terminaisons en plaque', and called these immature motor endings. He suggested the growing properties of motor terminals in normal muscles, but did not extend this plasticity further so as to raise the question as to whether motor terminals may have only a limited life span and be periodically replaced by

collateral regeneration.

The proof of such a hypothesis is difficult and is beyond the scope of the present study. Therefore, what follows is a description of observations and discussion of such a possibility, sufficient evidence being presented with a hope to render the hypothesis plausible before going into detail about the motor innervation of mammalian muscle spindles.

3. 2.   Results

3. 21.   Suggested probable sequence of the process
         of motor end-plate replacement

In order to facilitate description, the probable sequence of the process of motor end-plate replacement involving both degenerating and sprouting processes has been worked out according to the actual observable examples and is illustrated by a series of diagrams each of which is exemplified as far as possible by photographic evidence plus in some cases, a tracing for simplification.

In the process of degeneration (see Text-figure 7 a),
a normal end-plate undergoes retrograde degeneration by
swelling and then retraction until finally the axon and
ending undergo granular breakdown.   In order to replace
the dying or degenerated end-plate, a motor axon can send
off growing sprouts of axoplasm that do not terminate as
an ending.   For descriptive convenience, a sprout is refer-
red to as nodal if it comes off from the nearest node of the
ending, as preterminal if it originates from just before the
ending, or as ultraterminal if it springs out from one of
the terminal arborizations of the ending (see Text-figure 7 b).
Examples of such fusimotor and skeletomotor sprouts are ill-
ustrated also in Text-figures 8-11 and Plates 1-5.   They
are similar to those formed by intact motor axons in part-
ially denervated muscle (see Coers & Woolf, 1959), and also
to the sprouts formed by the living portion of a cut axon
undergoing regeneration (see Cajal, 1928).   The charact-
eristic feature indicating growth in such axons is the
nature of the tip of the sprout which is either in the form
of a small bulb or ball of axoplasm (see Text-figure 9 c,
d, & j and some in Plates 1 & 2); or of a taper (Text-fig.
9 a, e, & g) in which a neurofibrillar reticulum is often
evident; or sometimes of a small ring (Text-figure 9 b;
see also Cajal's figures 105 & 107 after Tello).

Such a growing sprout may form a new end-plate along-side that borne by the parent axon to form what has pre-viously been described in the literature (Boeke, 1911; Garven, 1925) as 'accessory endings' (see Text-figure 7 c). Alternatively the sprout may form a new ending within the parent plate itself to 'rejuvenate' the old terminal which degenerates (see Text-figure 7 d; also some more in Plate 2). The growing sprouts may also establish new plates on neigh-bouring muscle fibres (Text-figure 7 e), but as with develop-ing and regenerating axons much of the sprouting is spurious and does not necessarily result in forming new endings.   In extreme cases some sprouts can grow out and escape from the spindle (Text-figure 7 f) to make endings on extrafusal muscle fibres or on nearby capillaries (Text-figure 9 j; see also Garven's figure 9 & p. 420, 1925).   Sometimes on their way of growing out and escaping from the spindle, the develop-ing sprout may be trapped in the capsule lamellae (see for example, Text-figure 10 f).   With these postulated sequences of possible replacement hypothesis, one can go into more detail about the various degenerating and sprouting incidences occurring in normal healthy as well as in satisfactorily de-afferentated muscles.

Text-figure 7. Diagrammatic drawings based on observed examples to explain the probable process of motor end-plate replacement.

a. stages of degeneration in normal motor nerve terminals;

b. the terminal branch of a plate-ending motor axon giving off collateral and ultraterminal sprouts at various points of emergence in response to need;

c. how a nodal sprout may develop into a young end-plate on the same muscle fibre adjacent to the old one borne by the same parent terminal branch. Young end-plates of this kind at various stages of development have previously been described as 'accessory endings';

d. how a nodal sprout may also develop a young end-plate situated inside the old one borne by the same parent terminal branch. It is suggested that such configurations may be interpreted as evidence showing 'rejuvenation';

e. the terminal branch of a plate-ending motor axon may also give off collateral or ultraterminal sprouts establishing new plates on neighbouring muscle fibres;

f. sprouting in a fusimotor plate-ending axon, some of the sprouts may escape from the spindle and establish connexion with neighbouring extrafusal muscle fibre or nearby capillary. Situations of this kind are interpreted as accounting for the occurrence of some of the 'β' fibres (see Discussion on p.59).

a
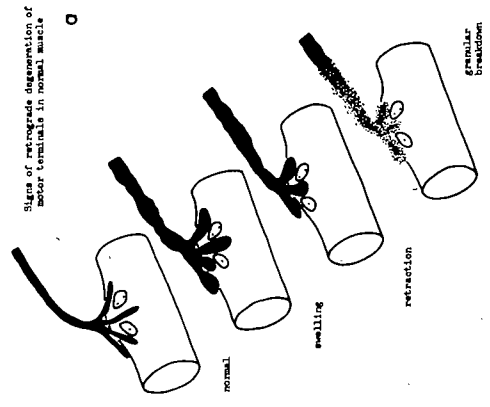
Signs of retrograde degeneration of motor terminals in normal muscle

normal

swelling

retraction

granular breakdown

b
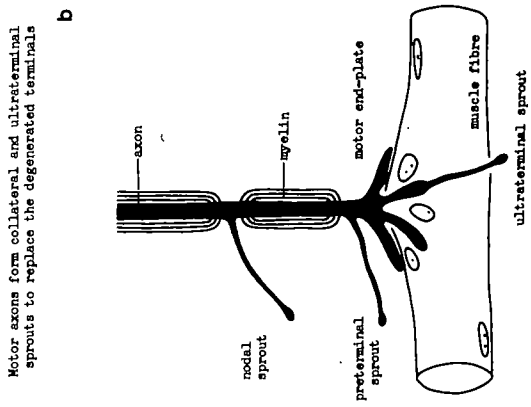
Motor axons form collateral and ultraterminal sprouts to replace the degenerated terminals

axon

myelin

motor end-plate

muscle fibre

nodal sprout

preterminal sprout

ultraterminal sprout

c

A nodal sprout may form a new end-plate alongside that borne by the parent axon

nodal sprout

young end-plate

Young end-plates of this kind have previously been described in the literature as 'accessory endings'

d

Instead of forming an accessory ending near the parent plate a nodal sprout may form one within the parent plate itself. After such 'rejuvenation' the old terminals degenerate.
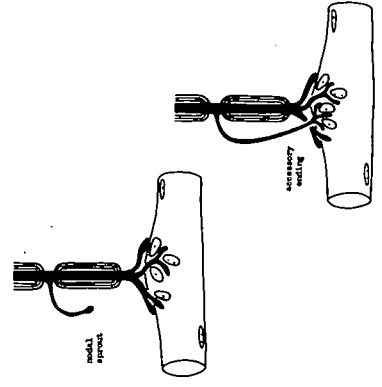
nodal sprout

accessory ending

The collateral origin of such constrictions we once controversial, running to Boeke's claim, on the basis of silver sections, that they constituted a separate innervation by sympathetic fibres.

e

Collateral and ultraterminal sprouts also establish new plates on neighbouring muscle fibres

nodal sprout

ultraterminal sprout
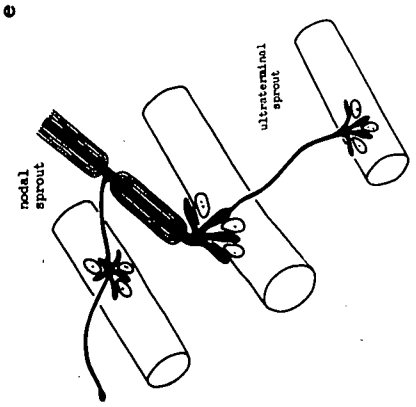
As with developing and regenerating axons, much of the sprouting is spurious and does not result in new endings being formed.

f

extrafusal muscle fibre

spindle nerve trunk

spindle capsule

'escaping' fusimotor sprout

blood vessel

Sprouting and ectopic renewal are more obvious inside than outside muscle spindles owing to the multiple and abnormal nature of fusimotor innervation. Some sprouts actually grow out and escape from its spindle as shown in the diagram below.
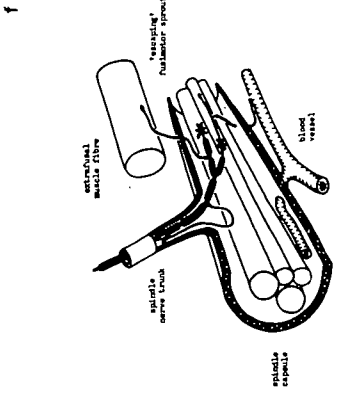
Some of these 'escaping' sprouts may form skeletomotor end-plates and so convert its parent into a spindle-fibre. Such conversions are probably no more than accidental products of the renewal process. They should be placed in a different category from fibres that are more or less equally distributed to spindles and extrafusal muscle fibres.

3. 22.   Terminal motor degeneration in normal muscle

If replacement of motor terminals does occur in
healthy normal muscles as has been suggested (Barker & Ip,
1965a), there should be some evidence of axons and terminals
degenerating as well as growing in these muscles; the
relative abundance observable ~~observable~~ between degenerat-
ing and growing terminals or axons, however, being dependent
on the period during which either of these two processes
takes place.   In other words, if the degenerating process
takes a shorter time than the growing process, there
should be consequently much more chance to meet sprouting
than degenerating axons and terminals and vice versa; and
actually the former one has been found to be more probable.
Degenerating axons and end-plates do occur as swollen,
retracted, or brokendown terminals as illustrated in Text-
figure 8.   All these are examples on extrafusal muscle
fibres.   Degenerating end-plates are also found on intra-
fusal muscle fibres (see Figure 3 in Plate 3).   Replace-
ment seems to be the best reasonable explanation for cases
where one or two degenerating axons and their endings were
sometimes observed among a group of axons and endings whose
staining is of the highest quality (see for example, Figures
7 & 12 in Plate 2).   In Text-figure 8 a which is a tracing

of Figure 7 of Plate 2 for clarity, there are two apparently 'dying back' terminal branches; one of them forms a small retracted plate, while the other appears as a mass of granular axonic debris having evidently degenerated a stage further. Significantly in the same group, there are a lot of terminal branches bearing well-stained normal end-plates and others with nodal sprouts and accessory endings. Nevertheless, degenerating terminal branches and endings do not occur as frequently as sprouting ones, probably because of the greater rapidity of the process of decay than the process of sprouting as mentioned above. Such scarcity is also found in muscles known to be undergoing motor degeneration as a result of disease or experiment. As Coers & Woolf (1959) state:"Even where degeneration is actively progressive, degenerating fibres are not always seen, as in many chronic cases the nerve fibres are affected one by one over a large area, and once degeneration has commenced, disappearance of the affected fibre in most cases speedily ensues..... In acute and severe nerve lesions, for instance in division of nerves or poliomyelitis, the degenerative process is rarely observed in the terminal arborizations, as the motor nerves are quickly destroyed and the axonic debris removed. Occasionally, isolated, deeply staining fragments of the terminal arborizations can be seen in
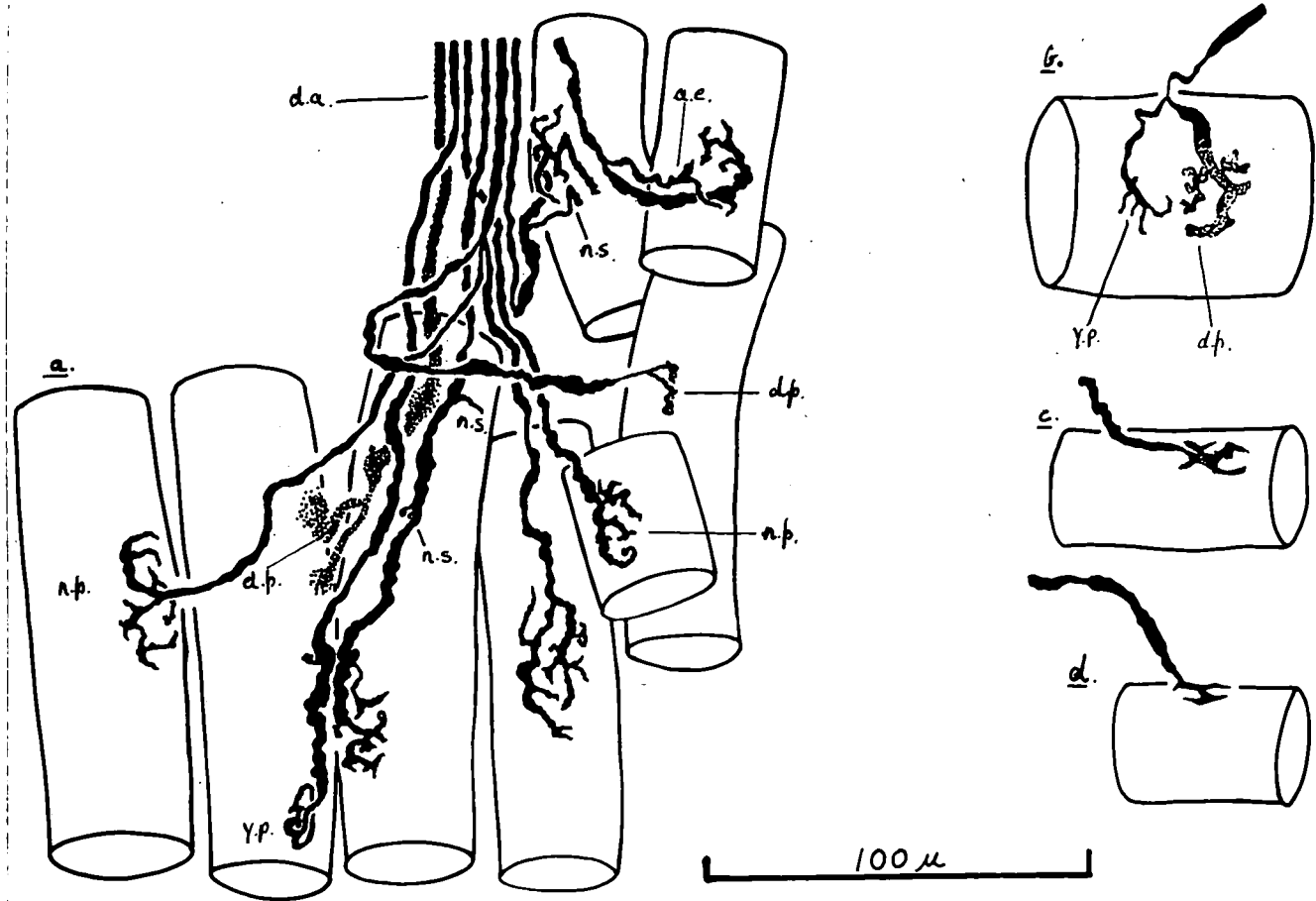
Text-figure 8. Tracings of photomicrographs of teased, whole-mount silver preparations (modified de Castro method) from normal cat tenuissimus muscle to show skeletomotor sprouting and degeneration. Photomicrographs of a, b, and d are given again in Figures 7, 9, and 11, Plate 2.

a. e. accessory ending; d. a. degenerating axon; d. p. degenerating end-plate; n. p. normal end-plate; n. s. nodal sprout; y. p. young end-plate.

methylene blue preparations. This fragmentation is preceded by a transient retraction and swelling of the branches of the terminal arborization. On the other hand, in some cases of 'peripheral neuritis', even in the presence of complete paralysis, there may be very little abnormality observed in the intramuscular nerves or their endings" (p. 46).

On the other hand, there are many examples of 'rejuvenation' of sole-plates. The occurrence of a new small terminal (accessory ending) alongside or upon the existing sole-plate (rejuvenation) can usually be regarded as an indication that the processes which eventually would lead to the complete degeneration and removal of the old terminal have begun. One particular preparation (Text-figure 8 b & Figure 9, Plate 2) illustrates rejuvenation well. The old terminal (marked d.p. on the right side) on the point of 'dying back' is accompanied by a nodal sprout forming a new one alongside, perhaps on the same sole-plate. The granular and swollen appearance of the old terminal, typical of the early stages of end-plate degeneration (Coërs & Woolf, 1959), contrasts sharply with the slender, precise delineation of its newly-formed neighbour. Such configurations bear a striking resemblance to those figured

in hedgehog muscle by Boeke (1911) in which he distinguished between thick and thin branches ramifying within the same sole-plate (see his figures 12, 14, 15, 18). It is suggested, on the basis of scanning all observable evidence and comparing the frequencies, that sole-plates are more frequently 'rejuvenated' than abandoned for even if the decay of axons and terminals and removal of axonic debris are very quick, remains of sole-plates with sole nuclei persist for a considerable time (see also Gutmann & Young, 1944). Abandoned sole-plates are rarely observed although it may be possible that the reinnervation of these supposed abandoned sole-plates takes place quickly so that the existence of them can hardly be easily observed. Generally new replacing terminals appear to be formed just before or just after the old ones have begun to degenerate, or much less frequently, after they have disappeared, so that a sprout may provide an empty sole-plate with a new terminal in the same way that a collateral does after partial denervation, or a regenerating fibre after nerve-injury (see Figure 8 in Plate 2). The preparation in this figure may be an incidence in which the sole-plate was abandoned but has been quickly reinnervated just prior to the fixation.

3. 23. Fusimotor sprouting

Most spindles in the cat and rabbit receive a diffuse
type of motor terminal, besides the normal well-known dis-
crete end-plates, that has been called 'trail ending' (for
more details see Barker & Ip, 1965b; and Section 4).
Recognition of trail endings, however, is often obscured
by unmyelinated outgrowths from plate-ending fibres that
do not terminate in sole-plates.   These unmyelinated out-
growths are interpreted as growing sprouts of axoplasm
which, as mentioned before, can be of either nodal, pre-
terminal, or ultraterminal nature, according to their point
of origin from the axon or end-plate (see p. 25).   Examples
of these sprouts from both normal and de-afferentated
spindles are illustrated in Text-figures 9, 10 and in Plate 1.
In form they are exactly similar to those formed from
intact motor axons in partially denervated muscles as des-
cribed by Coërs & Woolf (1959) and also to various shapes
of regenerating sprouts depicted by Cajal (1928).   The
characteristic shapes of the tips of these sprouts have been
described on p. 25.   Measurements of sprout diameter give
a range from 0.5 μ to 2.5 μ; the largest ball of axoplasm
measured 11.0 μ across being borne at the end of a 2.0 μ
thick sprout (Text-figure 9 c and Figure 4, Plate 1).

Another one nearly as large can be seen in Figure 7, Plate 1, also coming off from an ultraterminal sprout. A typical ball of axoplasm is also shown in Figure 5, Plate 5. Terminal bulbs or ball of axoplasm were also demonstrated by Coërs & Woolf (1959) using methylene blue on rat and human intrafusal muscle fibres and were called by them as unbranched small motor nerve endings (see their figs. 69 & 70).

It is true that there are many more sprouts which cannot be revealed by the light microscope as used in the present investigation, for with the electron microscope, Causey & Hoffman (1955) and Coërs & Woolf (1959) have seen nodal sprouts as thin as 0.1 μ growing from collaterally regenerating motor axons which are certainly beyond the highest resolving power of the light microscope. Nodal sprouts were found to occur most frequently, nearly twice as many as either preterminal or ultraterminal ones (see totals for fusimotor sprouts in columns 5, 6, and 7 in Table 1, p. 39). Nodal sprouts are usually unbranched and restricted to one sprout per node, and it is always the nearest node next to the ending that most frequently gives rise to sprouting. Perhaps this may be partly due to the short diffusion distance from degenerating sites and partly due to the unintentional bias taken to look for such

Text-figure 9.   Tracings based on photomicrographs of teased, whole-mount silver preparations (modified de Castro method) to show collateral and ultraterminal sprouting of plate-ending fusimotor axons in both normal (n) and de-afferentated (d/a) cat and rabbit spindles from the following muscles : a, rabbit peroneal I (n); b, cat tenuissimus (d/a); c, cat peroneal I (d/a); d, cat tenuissimus (n); e, cat popliteus (d/a); f, rabbit peroneal IV (d/a); g, cat interosseus (d/a); and i, cat peroneal II (d/a); a fusimotor end-plate without evidence of sprouting ('normal ending') from a cat interosseous spindle (d/a) is shown in h for comparison.   Photomicrographs of a, c, and d are reproduced in Figures 3, 4, and 9, Plate 1. Photomicrograph of g is reproduced in Figure 1, Plate 4; that of i in Figure 2, Plate 3; and that of h in Text-figure 12 b respectively.

b. v. blood vessel;   n. s.   nodal sprout;   p. s.   preterminal sprout; u.s.   ultraterminal sprout.
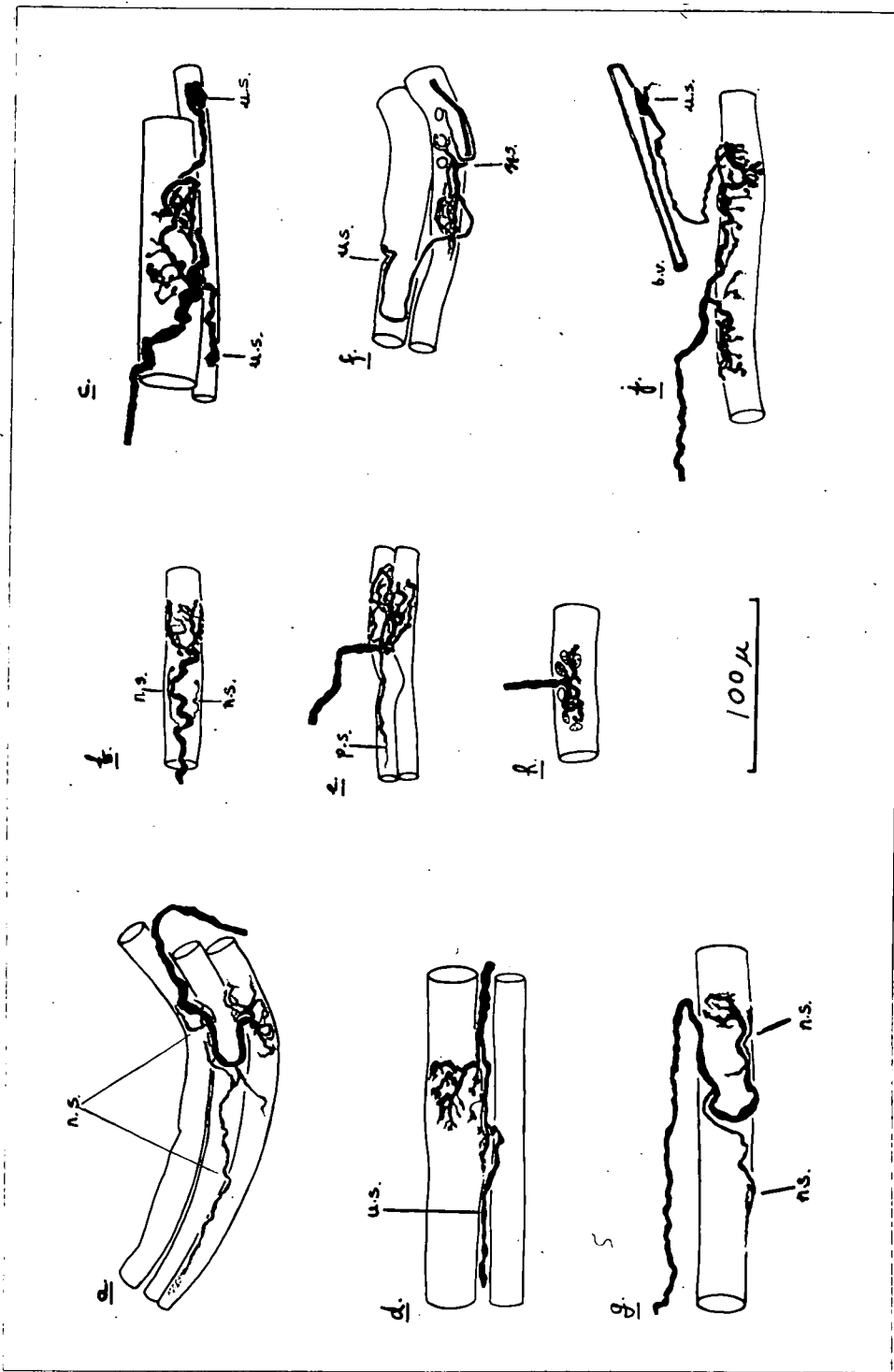
Text-figure 9.

examples around the terminals.   All sprouts in Text-figure 9 are considered as young ones, and these are just the beginning of the story, i.e. they had not developed far away from the axons or end-plates bearing them when they were arrested by fixation.   Some more examples of fusi-motor sprouting at various stages are given as photomicro-graphs in Plate 4.

There are other examples thought to be later stages in sprouting (see Text-figure 10) and in these cases they can be interpreted to show replacement evidence more clearly. The simplest condition is achieved when a nodal sprout grows just a short distance to form a small end-plate adja-cent to the old one borne by the same parent axon, both being situated on the same muscle fibre (Text-figure 10 a, b, and Figure 5 in Plate 1).   Sometimes 2 or 3 subterm-inal nodes may all give rise to sprouts contributing to the formation of accessory endings (see Text-figure 10 e and Figure 7, Plate 1).   The next step would be the further growth of the accessory ending, together with the myelin-ation of the nodal sprout that formed it, leading to the condition in which two fully developed plates lying adjacent to each other are given off by a terminal motor axon, resembling the extrafusal 'double' motor endings described

by Cole (1955) and others. An ultraterminal sprout may also form a new end-plate near the parent ending, though not on the same muscle fibre, such as that in Text-figure 10 $\underline{d}$ in which the ultraterminal has developed into a configuration that could be regarded as a young end-plate in the process of formation (compare with Perroncito's figure 12, plate 2, 1902). Text-figure 10 $\underline{h}$ and Figure 2 in Plate 1 show a nodal sprout of an extensive end-plate forming a new ending 140 μ away from it on an adjacent intrafusal muscle fibre, and then growing 280 μ further on to terminate in the form of a typical bulb of axoplasm. A similar example is shown in Figure 1, Plate 1.

However, much of the sprouting encountered in the present study is not so obviously purposive. For example, the nodal sprouts shown in Text-figure 10 g have each grown more than 200 μ away from the parent axon and appear to be still growing. In some cases sprouts have been observed to escape from the spindle, and some have been trapped in the capsule lamellae as mentioned above. Text-figure 9 $\underline{j}$ and Figure 2 in Plate 3 illustrate such an escaping sprout belonging to an end-plate situated near the polar end where the ensheathing capsule has become thinned out by decreasing the number of lamellae. The

Text-figure 10.   Tracings of photomicrographs of teased, whole-mount modified de Castro's silver preparations to show the motor terminals with advanced sprouting of plate-ending fusimotor axons in both normal (n) and de-afferentated (d/a) cat spindles from various hindlimb muscles.   Motor terminals with accessory endings (a.e.) are shown in a from superficial lumbrical (n), b from peroneal III (n) and e from peroneal I (d/a).   A motor terminal showing double motor ending is shown in g from superficial lumbrical (n) spindle.   The remaining tracings illustrate examples of sprouts well advanced in growth in spindles from the following muscles (all d/a):   d, 1st deep lumbrical; f, flexor digitorum longus, mesial head; g, peroneal II; and h, popliteus.   Photomicrographs of a and h are reproduced in Figures 5 and 2, Plate 1; those of b, d, e in Figures 5, 6, 7, Plate 4; and that of f in Figure 1, Plate 3 respectively.

a. e.   accessory ending;   o. p.   old end-plate;   u. s.   ultraterminal sprout;   y. p.   young end-plate.
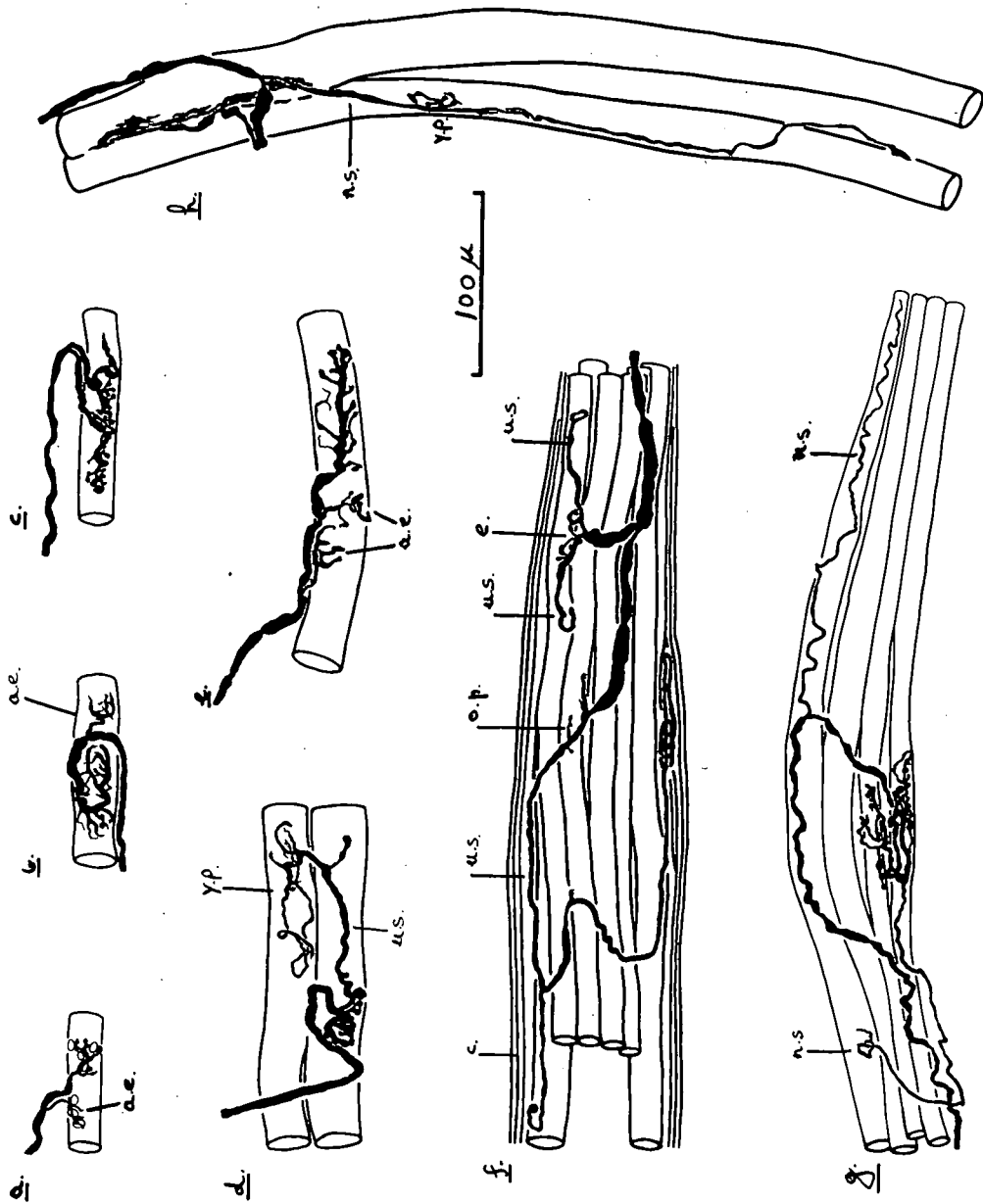
Text-figure 10.

sprout is apparently growing away and making a connexion
on meeting a nearby capillary.   A similar case is shown
in Figure 10, Plate 1.   In Text-figure 10 _f_, an active
replacing fusimotor axon innervating the polar region of
a de-afferentated cat spindle as seen coming from the right
side of the picture was interpreted as originally giving
rise to two end-plates on two adjacent nuclear-chain muscle
fibres.   The plate on the right still looks normal with
two ultraterminal sprouts on either side of the parent
plate, while the one on the left has degenerated (marked
o.p.) and has sent off a long ultraterminal sprout.   One
branch of this terminates as a bulb on top left corner of
the picture whereas the other branch, well advanced in
growth, has penetrated between the lamellae of the invest-
ing capsule and then doubled back and forth upon itself
as if trying to escape.   In another case, a sprout was
observed to leave a de-afferentated rabbit spindle by
growing up into the small nerve twig conveying the fusi-
motor supply so that both the sprout and the parent axon
came to lie side by side in the nerve twig travelling in
opposite directions.   Some more similar cases were found
to occur in trail-ending axons of de-afferentated cat
spindles to be mentioned later in Section 4.

Peculiar arborizations quite different from end-plates or trail ramifications were sometimes found in the tendinous substance of the intrafusal muscle fibres especially of those short and tendinous spindles which are usually situated near the tendon or aponeurosis (Barker & Ip, 1961; Ip, 1961). On following carefully the origin of these arborizations it is possible to show that in many of these cases they are actually branches from a plate fusimotor fibre (see Figures 1 & 3 in Plate 5) or perhaps from a trail fibre (see fu' b in Figure 1, Plate 5); or in other words, they are motor in origin. Figure 1, Plate 5 shows clearly 2 definite arborizations on the tendinous substance of a de-afferentated cat spindle. One of these (t.a.1) is supplied by a branch from a plate fibre (fu' a) of 3.0 μ axon diameter which sends a definite typical end-plate with an ultraterminal sprout (u.s.) to innervate the very end of the intrafusal muscle fibre as can be seen on the left side of the figure. The other arborization (t.a.2) is supplied by another fibre (fu' b) of 1 μ axon diameter. This is probably a trail fibre because there are trail fibres and endings innervating this pole and this fibre is lost in the midst of these. Figure 2, Plate 5 shows some wavering terminal branches from a fusimotor fibre terminating on both muscular and

tendinous substance of the intrafusal muscle fibres of a normal rabbit spindle; while Figure 4 is a similar case with a ball of axoplasm which is so characteristic of the sprouting axon. In Figure 3, only the very end of the tendinous pole of a de-afferentated cat spindle is shown at a higher magnification to show the terminals of these arborizations more clearly. A very fine axonic outgrowth can be seen coming out from one of these arborizations (t.a.1). It is not certain whether this axonic outgrowth actually terminates somewhere outside the spindle pole or explores on its way of growing, but that it is a sprouting of some sort or other there is no doubt at all. In fact, this arborization with its axonic outgrowth is found to come from an ultraterminal of an end-plate supplied by a fusimotor fibre of 3.6 μ axon diameter. The other arborization (t.a.2) is also a branch (probably a nodal sprout) arising from the nearest node of the same end-plate. Thus, all circumstantial evidence revealed by the teased, whole-mount silver preparations seems to point to the fact that they are probably growing sprouts that go astray and terminates accidentally on tendons. Whether such terminals are functional or may be only vestigial must await further investigations to decide.

In order to determine the frequency of sprouting
exhibited by plate-ending fusimotor axons in normal and
de-afferentated muscles, the nature of the terminal
branches of such axons innervating 12 cat and 10 rabbit
spindles was studied. The number of those terminal
branches showing collateral or ultraterminal sprouting
was ascertained as against the number of those ending in
'normal' plates, i.e. bearing single, discrete end-plates
without sprouts issuing either from the ending or the
subterminal region of the axon. The number of terminal
branches that formed double motor endings, and those that
formed an accessory ending in addition to a normal plate
was also counted. The results are given in Table 1.
In the sample of 12 cat spindles, there was a total of
115 plate-ending terminal branches of which 68 (or 59.1 %)
terminated as 'normal' plates while 35 (or 30.4 %) exhibited
nodal, preterminal, or ultraterminal sprouting. If
accessory endings are regarded as young plates newly formed
by nodal sprouts, and their number is added to the total
of other sprouting terminal branches, the frequency of
sprouting is increased to 33.9 %. The sample of 10
rabbit spindles yielded a similar analysis: 54 (or 57.4 %)
of a total of 94 terminal branches terminated as 'normal'
plates; and 32 (or 34.0 %) exhibited definite sprouting,

TABLE 1. TERMINATION AND SPROUTING OF FUSIMOTOR AND SKELETOMOTOR TERMINAL AXON BRANCHES

Figures in brackets indicate the percentage that each count is of the relevant total in column I

	I	II			III			% of sprouts in (1)	% of sprouts + accessories in (1)
	total no. terminal branches examined	motor endings			sprouts				
		normal	accessory	double	nodal	preterminal	ultraterminal		
fusimotor									
12 cat spindles	115	68 (59.1)	4 (3.5)	8 (7.0)	15 (13.0)	11 (9.6)	9 (7.8)	30.4	33.9
10 rabbit spindles	94	54 (57.4)	4 (4.3)	4 (4.3)	16 (17.0)	7 (7.4)	9 (9.6)	34.0	38.3
totals	209	122 (58.4)	8 (3.8)	12 (5.7)	31 (14.8)	18 (8.6)	18 (8.6)	32.0	35.8
skeletomotor									
cat tenuissimus	312	227 (72.8)	30 (9.6)	27 (8.6)	18 (5.8)	10 (3.2)	-	9.0	18.6
cat interosseus	127	94 (74.0)	22 (17.3)	7 (5.5)	4 (3.2)	-	-	3.2	20.5
rabbit soleus	91	73 (80.2)	8 (8.8)	2 (2.2)	6 (6.6)	2 (2.2)	-	8.8	17.6
rat intertrans-versarius	37	27 (73.0)	4 (10.8)	-	2 (5.4)	1 (2.7)	3 (8.1)	16.2	27.0
totals	567	421 (74.3)	64 (11.3)	36 (6.3)	30 (5.3)	13 (2.3)	3 (0.5)	8.1	19.4

a proportion increased to 40 (or 38.3 %) if accessory
endings are included. Double motor endings are excluded
from these assessments of sprouting since their formation
is more conjectural than in the case of accessory endings.

The diffuse nature of the trail ending makes the
detection of its sprouting, if any, much more difficult
to determine. Moreover, since the extent of the unmyel-
inated portion of the trail ending and the precise location
of its synaptic contact has not yet been established with
certainty, sprouting of trail terminals cannot be satis-
factorily searched for in collateral and ultraterminal
situations. Cholinesterase preparations give only a very
crude and darkly-stained picture of the enzyme activities
along most part of the juxta-equatorial regions and thus
offer no clear-cut solution to this determination (see
Text-figure 15 and Hess's figure 8). Nevertheless, 2
observations seem to suggest that sprouting does occur.
First, a filament of axoplasm from some of the trail term-
inals may sometimes be seen to terminate in the form of a
small bulb at the tip (see Figure 8 in Plate 1); such
filaments are probably sprouts. Second, in two satisfact-
orily de-afferentated cat spindles, an unmyelinated branch
of a trail ending was observed to leave the area of term-

ination and depart from the spindle by turning back into the nerve twig supplying the fusimotor innervation (see Text-figures 33 & 34 in Section 4). Unfortunately, in neither case was it able to locate the end of the branch, and it seems possible that each was destined to innervate another spindle. A more probable interpretation, however, is that these stray branches were growing sprouts, for a similar example has already been noted (p. 35) where a sprout from a plate-ending axon was escaping from a de-afferentated rabbit spindle in this fashion.

3. 24. Skeletomotor sprouting

All examples observed for sprouting from motor axons innervating extrafusal muscle fibres were taken from various normal apparently healthy muscles of the cat, rabbit and rat. Groups of well-impregnated skeletomotor terminal branches were selected at random. These groups comprised of a total of 312 terminal branches from cat tenuissimus, 127 from cat interosseus, 91 from rabbit soleus, and 37 from rat intertransversarius, making a total sample of 567 terminal branches. As with the determination of plate-ending fusimotor axon sprouting, the number of those exhibiting either collateral or ultraterminal sprouts was counted

as against the number terminating as 'normal' plates.
The number of those bearing accessory endings and doubles
was also determined. The results are summarized in the
second part of Table 1 on page 39 for comparison with those
of fusimotor sprouting. Exactly the same number of classes
was observed and thirty well-stained groups of terminal
branches were classified in a similar way. The average
number of branches per group was 19 in a range of 7 to 50.
Terminal branches exhibiting collateral or ultraterminal
sprouting were observed in 17 groups; those bearing access-
ory endings occurred in 26 groups; and those terminating in
double motor endings were encountered in 15 groups. Only
in 2 groups both being teased from a rabbit soleus muscle
did all the terminal branches (a total of 18) terminate as
'normal' end-plates. Of a total of 567 terminal branches
examined, 421 (or 74.3 %) terminated as 'normal' end-plates;
46 (or 8.1 %) exhibited collateral or ultraterminal sprouting,
a proportion increased to 110 (or 19.4 %) if those branches
bearing collaterals to form accessory endings are included.
As with the case of fusimotor axon sproutings, nodal sprouts
occurred twice as many times as preterminal ones, but the
occurrence of ultraterminal sprouts was much less frequent
than that found in spindles (only 0.5 % as against 8.6 %).
The frequency of branches forming accessory endings was

much higher (11.3 % as against 3.8 % in fusimotor sproutings),
that of branches forming double motor endings about the
same (6.3 % as against 5.7 %).   In the only other known
quantitative assessment of this kind, Wilkinson (1929)
gave a proportion of over 90 % of a total of 500 end-plates
taken from the interosseous muscle of a cat as 'typical
ramifications'; judging from his figure 39, the rest appear
to be accessory and double motor endings.   It seems likely
that he relied either on gold or on methylene blue prepar-
ations or a mixture of both for this assessment.   But
neither technique would permit such classification to be
made with the same certainty and confidence as the precise,
clear-cut modified silver method does (compare some of the
preparations by modified silver technique with those by
gold chloride method as demonstrated in Text-figures 3 & 17).

        Some traced examples of skeletomotor sprouting and
accessory and double motor endings are illustrated in Text-
figure 11 and some of them plus other photogenic ones are
reproduced as photomicrographs in Plate 2.   Most of these
are similar to their fusimotor homologues, but others
deserve some comment.   In Text-figure 11 $\underline{f}$, the terminal
branch of a skeletomotor axon divides into a large and a
small branch at a point 60 μ from the end-plate to which

both branches contribute ramifications; and a similar case

is photographed in Figure 3 of Plate 2 where a nodal sprout

forms an accessory ending alongside the old terminal of

the same parent axon.   In Text-figure 11 e, the pre-

terminal sprout (marked p.s.) of a normal extrafusal end-

plate has grown for some distance in the process of end-

plate formation (marked y.p.) onto another extrafusal

muscle fibre.   What is interpreted as a later stage of

this type of sprouting where a mature plate is formed on

an adjacent extrafusal muscle fibre by the advanced growth

of a rather short preterminal sprout is shown in Text-fig.

11 j.   In fact, the original plate and its younger off-

shoot constitute a double motor ending because both are

borne by the same terminal branch, but since they are

situated on different muscle fibres this would be using

the term in a different way than applied by Cole (1955)

and others.   It is suggested that the smaller and slender

branch in these configurations is a nodal sprout which

forms an accessory ending within the parent plate itself

instead of forming one next to the parent plate.   Conse-

quently, a time may come when the accessory ending grows

bigger and equals the size of the old one to form double

motor endings.   The collateral origin of such configur-

ations was once controversial owing to Boeke's (1911) claim,

Text-figure 11. Tracings of photomicrographs of teased, whole-mount, modified de Castro's silver preparations to show motor terminals with sprouting of skeletomotor axons innervating extrafusal muscle fibres of various normal muscles of the cat, rabbit and rat.

a & b, from rat intertransversarius; c, rat gastrocnemius; d, cat tenuissimus; e, rat peroneal I; f, rabbit peroneal I; an extrafusal motor end-plate without evidence of showing sprouting ('normal ending') from rabbit soleus is shown in g for comparison; h, cat interosseus, double motor ending; and i, rabbit soleus. Photomicrographs of a, b, and f are again reproduced in Figures 1, 2, and 5, Plate 2.

a. e. accessory ending; n. s. nodal sprout; p. s. preterminal sprout; u. s. ultraterminal sprout; y. p. young end-plate.
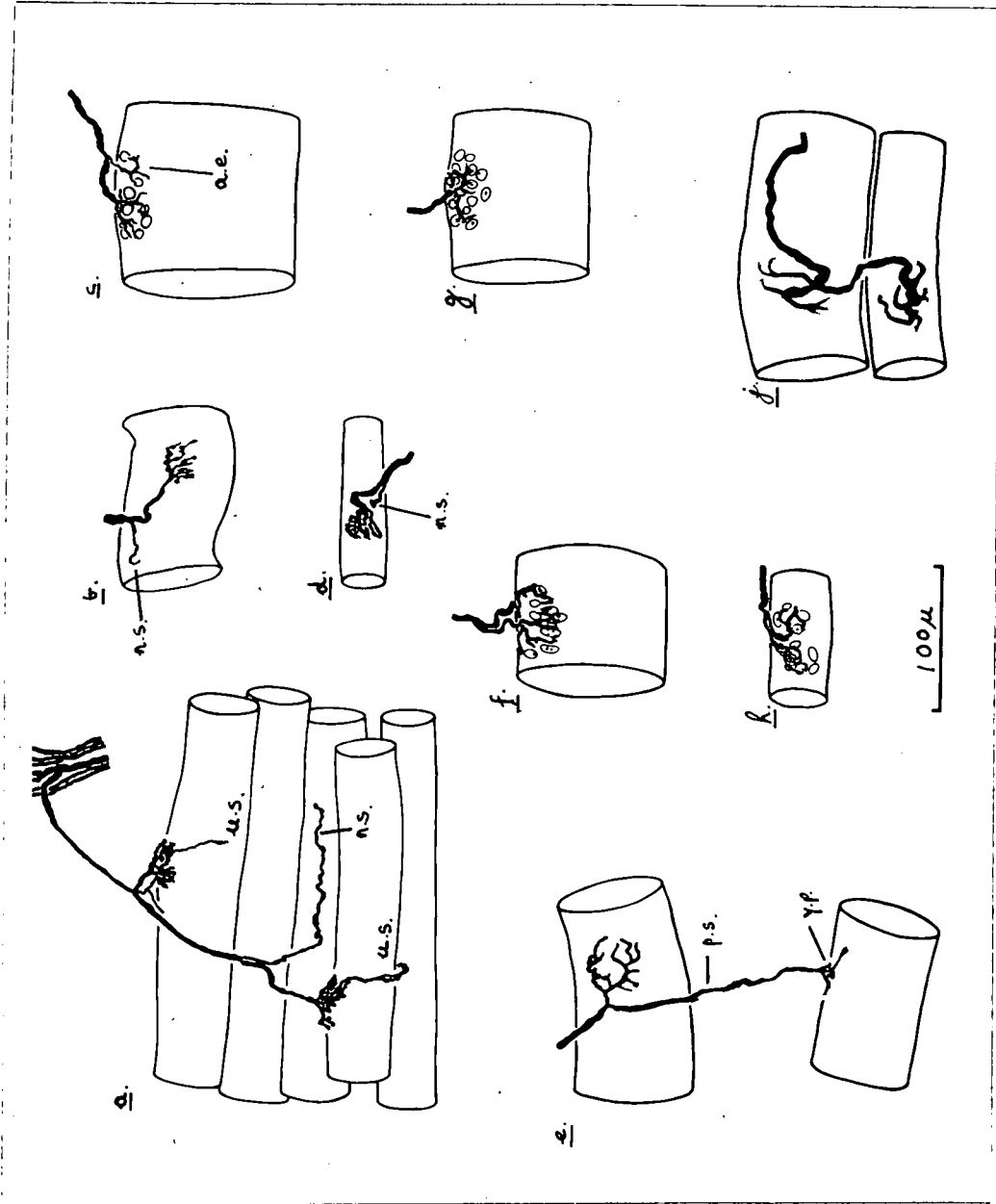
Text-figure 11.

on the basis of studying silver serial sections, that they
constituted a separate innervation by sympathetic fibres,
but this was substantially denied by Wilkinson (1929) and
others.

3. 3.   Discussion

The motor terminals have been described as 'eminence
of Doyere', 'terminal nerve plate of Rouget', and 'sole-
plate of Kühne' (Hinsey, 1934).   In the 324 figures of his
classical paper, Kühne (1887) was the first to show a
thorough account of a great variety of forms in the motor
end-plates found in various vertebrates, and this compre-
hensive study still remains one of the chief sources of
information on this subject.   Since then, there have been
various investigators , such as, to name only a few, Tello
(1917), Boeke (1911), Garven (1925), Wilkinson (1929),
Gutmann & Young (1944), and Coers & Woolf (1959) who were
interested in and have contributed to much of the knowledge
in this field.   Therefore, the morphology of a typical
end-plate is not discussed here.   In the present study,
a wide range of muscles to include both slow (or red) and
rapid (or pale) extensors or flexors is used and according

to my impression, differences of motor end-plates between one and the other muscle or between muscle fibres of various sizes do not occur, or at least are not great enough to be noticeable.

The repeated and unfailing finding of collateral or ultraterminal sprouting and accessory endings of motor axons in normal as well as in de-afferentated muscle removes much of the suspicion that might otherwise cast on the validity of this finding had muscles from only operated animals been studied. Nevertheless, it is important to discuss the possible grounds for such suspicion. It could be argued that partial motor denervation might have been effected in the de-afferentated animals by (1) damage to the underlying ventral roots at operation; (2) severance of motor axons that supposedly either pass through the dorsal roots or have their cell bodies located within the ganglia; or (3) transneuronal degeneration of those motor axons monosynaptically connected with primary afferents. If the muscles examined had their motor supply partially denervated for one or more of the above reasons, the sprouting could be accounted for by the collateral regeneration of the remaining intact axons. The first possibility has been catered for and can thus be ignored (see Methods). The existence

of motor axons issuing from dorsal roots is ruled out by
Sherrington (1894) and Nevin (1930).   Hughes (1965) re-
ported that transneuronal degeneration, after severance
of the central processes between spinal ganglia and cord
in developing Anura, affects 30 % to 50 % of the motor
axons in muscle nerves, but this has no effect in the adult.
Mammalian motoneurons can survive intact after de-afferen-
tation even if this is accompanied by transection of the
cord on either side of the operated roots (Tower, 1937;
Cook, Walker & Barr, 1951).

Then, there only remain two other factors which might
account for sprouting, applicable to normal as well as
operated muscles, namely age and incidental injury.   The
number of myelinated fibres in human spinal roots is known
to decrease with age; e.g. there is a gradual decrease in
the average number of myelinated fibres in the human spinal
roots after the 2nd and 3rd decade, the highest decade
average being in the 10-19 year-old group (Corbin & Gardner,
1937).   The average fibre content of the 8th and 9th
thoracic roots from 70-79 year old cadavers was found to
be 27 % less than in 10-19 year old cadavers and a similar
decline was found in the number of dorsal root ganglion
cells (Gardner, 1940).   Duncan (1934) found decreases of

up to 10 % in the ventral root fibres of 800 day-old rats.
Presumably the neurons that survive compensate peripherally
for such losses by collateral regeneration, although the
ability to do so probably decreases with age as judged by
loss of mobility in old persons and animals.   But this
factor can be discounted since the study was made exclusively
on young adult animals.   In any event it is unlikely that
a gradual loss of this kind could have accounted for such
a high degree of sprouting observed.   Incidental injury
to superficial muscles, resulting in neural damage followed
by collateral regeneration, could no doubt account for some
of the sprouts seen in tail and foot muscles.   Indeed,
Fullerton & Gilliatt (1965) have reported neural damage to
occur in the feet of ^guinea pigs kept in wire-mesh cages.   Miledi
(1962a) established, at least in the frog, that motor nerve
fibres can be induced to form functional nerve-muscle junc-
tions at sites other than the original neuromuscular junction
provided that the muscle has been subjected to injury.   But
it seems very unlikely that the observed sproutings are of
this nature, because the range of sampling has been such,
both among animals and muscles, that incidental injury should
have no serious interference.

It is, therefore, regarded as established that the

motor axons of mammals do undergo collateral and ultraterminal sprouting under normal conditions. Indeed the finding of the presence of ultraterminal fibres and accessory endings is not new and has been reported in a wide variety of vertebrates under normal as well as various experimental conditions (see reviews by Hinsey, 1934; Tiegs, 1953). It has been suggested that this is a general property of the vertebrate motoneuron. It seems plausible to interprete an accessory ending as a young motor ending formed by a nodal sprout, whether the ending is located within the end-plate or alongside it. No other satisfactory explanation has been advanced to account for their presence. The claims of Boeke (1911), Kulchitsky (1924a), Hunter (1924, 1925) that they represent a separate innervation of sympathetic origin have long since been refuted; and in the context of the present findings Tello's (1922) suggestion that they represent vestiges of a foetal condition, and Cajal's (1925) that they should be regarded as errors of development, carry little weight.

It has been suggested (Barker & Ip, 1965a) that the purpose of such sprouts is to replace old terminals which are degenerating, or have degenerated, having fulfilled a limited life span. In our paper (Barker & Ip, 1965c),

we have suggested that (1) a sole-plate may receive a new ending from a nodal sprout derived from the same terminal branch as the old plate, which degenerates; (2) a sprout may establish a new plate alongside the old plate both coming from the same terminal branch, the old plate may degenerate quickly, or persist for a time so that a double motor ending is formed (though probably permanent double endings also exist); (3) less frequently, a sole-plate site is abandoned and a new one established by a sprout elsewhere on the muscle fibre; and finally (4) as with developing and degenerating axons, much of the sprouting is spurious and does not necessarily result in new endings being formed.

It may be argued that the motor terminals, in the living muscles, may be actually in a constant state of 'amoeboid' movement so that the various forms of motor end-plates seen after fixation represent the configurations each exhibited by an end-plate at a particular moment caught at the time of fixation. But judging from the elaborate way of forming the myoneural synapse (see Gutmann & Young, 1944), and the complicated systems of 'furrows' in the synaptic gutters as revealed by the electron microscope (Robertson, 1956b), this possibility is very unlikely.

Perhaps it is because of such a complicated and elaborate system of synaptic contact that motor end-plate sites are more often re-juvenated than abandoned. Newly emptied motor end-plates, whose nerve terminals have degenerated either because of nerve-injury or experimental denervation, have been observed to offer easier sites for reinnervation by approaching regenerating axons. They are also able to persist for long periods if arrival of reinnervating axons is prevented (see Gutmann & Young, 1944).

Cycles of replacement are commonly found in other tissues, such as blood corpuscles, epithelium, etc., and indeed, have been established to occur among cutaneous sensory axons. Tello (1932) observed both normal and degenerating bulbous (paciniform) corpuscles in human external genitalia, and concluded that the receptors were continually being renewed. Such corpuscles, as well as freely ending nerves, have been described by FitzGerald (1961, 1962) as undergoing 'cyclic growth and decay' in the epidermis of the pig's snout. It has also been established that taste receptors are constantly renewed by ordinary epithelial cells that pass into the taste bud and become innervated (Beidler, 1961), and it is known that cutaneous sensory axons react by sprouting to partial

experimental denervation (Weddell, Guttman & Gutmann, 1941).
Therefore, it is highly probable that both sensory and
motor axons exhibit this dynamic activity of sprouting in
maintaining their populations of peripheral terminals in
a state of equilibrium in order to keep the muscles working
efficiently and smoothly.   Perhaps the same is true of
their central processes; the sprouts described by Nageotte
(1906), Cajal (1928) and Illis (1964b) growing out from
atypical cells in spinal ganglia, either from the cell body
or proximal part of the axon, might be engaged in such
renewal.

On the other hand, it is conceivable that replacement
of nerve endings is inevitable in surface epithelia exposed
to constant wear and tear, and that no such renewal is
required by muscle afferents.   Maybe axonic growth and
degeneration in muscle is restricted to motoneurons and
is in some way linked with a similar turnover of muscle
fibres.   The longitudinal division of both intrafusal
(Barker & Gidumal, 1961) and extrafusal (Susheela, 1964)
muscle fibres in the adult could be connected with such
a process, but althougher it seems a remote possibility.

All evidence seems to point to the fact that as a

rule the nervous system is capable of renewing its terminals wherever the most urgent need is required and much work has to be done, and so it is absolutely unlikely that the myoneural junction is an exception. The renewal process is supposed to take place only in the pre-synaptic part because evidence has shown that re-innervated old end-plates are functional (Gutmann & Young, 1944). Then, it seems highly probable that the vesicles, which are found especially abundant in the axoplasm just inside the terminal arborization, may be responsible for such a renewal. This is because, as Katz (1961) suggested, that the working of motor terminals depends on the amount of acetylcholine released across the synaptic gap, and that these vesicles are supposed to be the seat of manufacturing this chemical. It would be entirely probable that at intervals, these tiny vesicles may become exhausted after a continual production for a certain period of time and need to be renewed or replaced if the working of the myoneural junction is still required. These vesicles aan be renewed by those formed in the central axoplasm and transported distally to the endings, or they can be abandoned and the endings get their recruitment of vesicles by forming replacing sprouts with a complete set of fresh vesicles. It may be equally possible that both types of renewal of vesicles take place.

End-plates poisoned by botulinum toxin are no longer func-
tional because the neuromuscular transmission across the
synaptic gap is blocked, although no structural changes or
abnormalities were found in the end-plate itself (see Thes-
leff, 1960).   Unfortunately, it is beyond the scope of the
present study to investigate such poisoned muscles histo-
logically so as to see whether replacement configurations
are as common as in experimentally denervated or normal
muscles.   It would not be surprising to find that replace-
ment configurations may be fewer in number because of the
fact that either there are no degenerating axons and myelin
in releasing 'neurocletin', or the functional mechanism is
apparently unaffected by the toxin so that no renewal need
is required for these otherwise normal end-plates of the
poisoned muscles.   But this has to remain conjectural and
speculative before a full study or thorough understanding
of the mechanism of the toxin is achieved.

The finding by Morris (1953) that the growth of new
fibres occurs only in areas where degenerated and intact
fibres are closely intermingled does favour the suggestion
of collateral regeneration being caused by the influence
of a humoral substance released by transforming Schwann
cells of the degenerating axons (Edds, 1953).   Morris further

showed that new fibres appeared as early as 4 days after
incomplete denervation, and by the 4th week only a few were
still present, but none were seen later than 6 weeks.
Surely new fibres should occur all the time if replacement
does occur in normal muscles.   Edds (1949, 1950), on the
other hand, stated that the process of collateral sprouting
was at a maximum during the 1st month and continuing up to
the 4th.   Although the time of emergence of new fibres and
their absence after 6 week in Morris'es account should be
regarded as relative scarcity due to the limitation of the
technique of sectioning he employed, it may be used as a
rough estimate of the time for sprouting to start to make
its appearance and the period of maturation of sprouts.

The degree of sprouting exhibited by fusimotor plate-
ending axons is shown to be greater than that by skeleto-
motor ones as can be seen from Table 1.   This was suggested
(Barker & Ip, 1965c) to be due to the fact that intrafusal
muscle fibres receive both a multiple (Barker, 1948) and a
polyneuronal (Hunt & Kuffler, 1951) innervation from motor
axons.   Cases were found in the present study where some
nuclear-bag intrafusal muscle fibres received up to 6 end-
plates in cat as well as rabbit spindles, with as many as
three connected to the same axon terminal branch.   If this

interpretation is correct, then the degree of sprouting in skeletomotor terminals should be much greater in extraocular muscles and human vocal muscles for these both receive a multiple motor innervation (Feindel, Hinshaw & Weddell, 1952; Rossi & Cortesina, 1965). Preliminary observations on the extraocular muscles of the cat suggest that this is so, and it seems probable that the atypical motor endings of Retzius (1892), the small sites of cholinesterase activity near main end-plates (Kupfer, 1960), and the 'thin nerves with simple endings' seen in extraocular muscles (Wolter, 1964) are all expressions of the replacement process.

It has been suggested (Thesleff, 1960) that acetyl-choline sensitivity is restricted to sole-plate loci, and that the acetylcholine-sensitive locus spreads centrifugally after denervation, becoming restricted to the sole-plate regions again on reinnervation. If this is so, then every functional motor end-plate should be accompanied by an acetylcholine-sensitive locus which can easily be shown by the cholinesterase technique. In view of this it is sur-prising to note the findings of Wolter (1964) who states that the 'simple endings of thin nerve fibres' can be seen without cholinesterase next to motor end-plates with exten-sive cholinesterase accumulation, while other thin nerve

fibres which form isolated simple ring- or button-shaped endings always exhibit extensive deposition of cholinesterase. He also remarks that the large accumulation of cholinesterase around the motor end-plates makes it difficult to determine whether the simple ring or button endings contain cholinesterase. Unfortunately it cannot be decided whether all these thin nerve fibres of Wolter are skeletomotor axons. Nevertheless, it seems probable that the simple endings are nodal sprouts at a very early stage in forming accessory endings so young that no cholinesterase has had time to develop yet, while the ring or button endings with heavy accumulation of cholinesterase represent replacing sprouts at later stages.

Another factor which could affect the degree of sprouting might be the size of the innervation ratio, i.e. the size of the motor unit. Fulton (1926) calculated this ratio to be 1 to 140 for the tenuissimus muscle of the cat. Clark (1931) by counting the number of motor fibres in the de-afferentated nerves and the number of muscle fibres in cross sections of the respective muscles gave an average value of 1 to 120 in the soleus and 1 to 165 in the extensor digitorum longus of the cat. Of course in all these studies, both fusimotor and skeletomotor fibres were treated together.

Only recently, Adal & Barker (1965a) separated the fusimotor from the skeletomotor innervation, and were thus able to calculate the skeletomotor innervation ratio for the cat's 1st deep lumbrical muscle to be 1 to 300 as against a fusimotor one of only 1 to 9.   If there is some significant relationship between the degree of sprouting and the size of the motor unit, a high degree of skeletomotor sprouting should be expected to occur in such muscles as the tensor tympani and stapedius which were shown to have an innervation ratio of about 1 to 3 in the cat (Blevins, 1963; 1964); or in the extraocular muscles which were found by Bors (1926) to have an innervation ratio of 1 to 6 in man. Indeed, the extraocular muscles of the cat studied in the present investigation do show an extensive degree of sprouting compared with ordinary hindlimb muscles.

One other interesting factor may be a species difference in the degree of sprouting.   Edds & Small (1951) have shown that in the monkey there was only a limited degree of collateral regeneration after slight denervation, but no collateral regeneration was observed after extensive denervation.   If this is true, then it would seem that the lability of the nervous tissue to regenerate is diminished in higher mammals.   A comparative study of the degree

of sprouting in different species of animals is needed to prove this point.

The occurrence of motoneurons innervating both extra-fusal and intrafusal muscle fibres (Bessou, Emonet-Denand & Laporte, 1963a, 1965; and Adal & Barker, 1965a) raises a query relevant to the replacement hypothesis.   Adal & Barker (1965a) found that some of these motor fibres branch so as to innervate both extrafusal and intrafusal muscle fibres almost equally, while in other cases only one term-inal branch makes a cross connexion to create a skeleto-fusimotor distribution.   It seems probable that those sprouts seen growing away from spindle poles (see p. 34-35) may eventually establish such trivial connexions as a result of either fusimotor or skeletomotor sprouting in the immed-iate vicinity of the spindle.   Otherwise some mechanism must be postulated whereby a skeletomotor plate cannot be renewed by a fusimotor sprout, and vice versa, and this seems unlikely.

One major difficulty concerning the proof of motor end-plate replacement, or the quantitative assessment of the rate of replacement, is the fact that one parent motor fibre may innervate muscle fibres in widely different parts

of the muscle so that the motor unit is not confined to single groups of neighbouring muscle fibres (Cooper, 1929; Feindel, Hinshaw & Weddell, 1952; Morris, 1953). This means that any given patch of motor end-plates in skeletal muscle is derived from different parent axons and so will not exhibit a synchronous rate of turnover.

It remains for future work to establish the existence of motor end-plate replacement beyond doubt. If such a process does occur in normal muscles, certain considerations of the replacement possibility will be necessary in the assessments of neuromuscular diseases and their diagnosis. To Coërs & Woolf (1959) sprouting of motor axons in human biopsy specimens is exclusively an expression of pathological reaction to various diseases of the motoneuron, and the collateral reinnervation is supposed to remain as a permanent alteration of the normal pattern. For example, they consider that the terminal innervation pattern remains the same in the muscle biopsy of a patient who has long since been recovered from an attack of poliomyelitis for thirty-six years so that the collateral reinnervation remains as a permanent alteration of the normal pattern, leaving an 'indelible evidence' in the terminal innervation pattern (see their figure 125). New interpretation of the

reinnervation study of nerve endings after nerve injury
will also be necessary.   The formation of various patterns
of sprouts in the process of replacement has to be con-
sidered if the regenerating fibres are returning to the
site of myoneural junction.   In the past it has been
assumed that such regeneration is only concerned with the
restoration of a permanent population of static, normal
end-plates that injury had destroyed and the structural
form of the end-plate is assumed to remain unchanged through-
out life under normal conditions (see e.g. Gutmann & Young,
1944).   Finally, it could be of great interest to know
the normal rate of turnover in different species of animals
and the way this is affected by such factors as age, hyper-
trophy, tenotomy, and disease.

4. Fusimotor innervation of mammalian muscle spindles

4. 1.   Introduction

Over a hundred years have elapsed since the first
discovery of the muscle spindle as an anatomical unit by
Kölliker (1862) in frogs and by Kühne (1863) in mammals,
but agreement is still lacking about many points of the
finer details of its structure, especially the nature and
mode of the motor innervation of the mammalian muscle
spindle.   As early as 1893, Kerschner had already claimed
an efferent innervation in spindles, but Ruffini (1898)
who had done so much on the sensory side was inclined to
deny their efferent innervation and depicted beautifully
various forms of intrafusal end-plates which he regarded
as sensory in nature.   By selective degeneration of the
sensory and sympathetic components to muscles of the cat,
Hinsey (1927), and Hines & Tower (1928) demonstrated that
when the somatic motor innervation from the ventral roots
was the only innervation left intact, the plate-like
endings were still present on intrafusal muscle fibres.
They described fibres supplying these plates as 'small
myelinated ones which lost their myelin near their term-

ination'. Nevertheless, it was not until in 1945 that
the motor nature of these 'gamma efferents' on the spindle
poles was first decisively established by the electrophy-
siological study of Leksell on the 'gamma efferent' fibres
by recording the action potentials and conduction rate in
the small ventral root fibres from cat. It was further
convincingly confirmed by Hunt & Kuffler (1951.) and
Kuffler, Hunt & Quilliam (1951) working on single fibre
stimulation.

But recently, Boyd (1961; 1962b) postulates that there
is a dual motor innervation of mammalian muscle spindles by
γ_1 fibres terminating in the form of end-plates on nuclear-
bag muscle fibres and γ_2 fibres terminating in the form of
'network' endings on nuclear-chain muscle fibres. He
believes that γ_1 & γ_2 fibres encountered at spindle sites
are derived from large and small γ-stem fibres in the muscle
nerve, respectively. By reconstructing serial transverse
sections impregnated with silver, Barker & Cope (1962) have
already doubted this hypothesis. The finding of Steg
(1962) that frequently there is only one γ fibre innervating
the tail muscles of the rat which usually have more than
one spindle consisting of both nuclear-bag and nuclear-chain
intrafusal muscle fibres (Ip, 1962, unpublished observation)

again raises a query about this clear-cut duality and seg-
regation of fusimotor fibres.   Furthermore, Adal & Barker
(1965a; 1965b) have shown that the diameter of a fusimotor
fibre or fibre branch near the spindle site has no particular
relevance to the diameter of its stem-fibre.   Therefore, it
is the hope of the present study to determine whether there
is any truth in Boyd's duality and segregation hypothesis,
and to establish beyond doubt the nature and mode of the
motor innervation of the mammalian muscle spindle.

The work of Adal & Barker (1965a) has further shown
that the great majority of intramuscular motor axons en-
countered near spindle sites were fibre branches (see their
Table 1); only rarely were unbranched γ-stem fibres found
entering spindles.   Therefore, in the present study, made
only on the vicinity of spindles, the chances are much
greater in dealing with fibre branches than with fibres.
Thus, since it is cumbersome in description repeatedly to
refer to the 'fibres or fibre branches' entering a spindle,
the term 'fibre branches' only will be used throughout.

Since the anatomical structure of the mammalian
muscle spindle is well-known, it will not be described in
detail here.   But in order to describe the results of

the fusimotor study in terms of intrafusal muscle fibres
and the respective regions in the spindle, it is necessary
to make a few points briefly about spindle morphology.
It is well known that most mammalian spindles are composed
of two kinds of intrafusal muscle fibres.   One is the
nuclear-bag fibre with numerous large spherical nuclei
closely packed to form a 'bag' at the equatorial region
and a 'myotube' region on either side (Barker, 1948; see
also Text-figure 5 a).   The other muscle fibre, usually
of smaller diameter, with a chain of nuclei in the central
core at the equatorial region is the nuclear-chain fibre
(Boyd, 1960; see also Text-figure 5 b).   Although each
type of intrafusal fibre has a distinct range of diameter,
this may change considerably along their entire length
(Swett & Eldred, 1960b).   On top of this, each spindle
usually consists of several fibres of both types wrapped
together in the form of a bundle.   On the sensory side,
it is generally accepted that the sensory innervation of
the mammalian muscle spindle typically consists of a primary
or 'annulo-spiral' ending supplied by a primary or Group Ia
fibre of 12 to 20 μ in diameter; or with mostly one or
sometimes two secondary or 'flower-spray' endings supplied
by secondary or Group II fibre(s) of 4 to 12 μ.   Spindles
with one primary and one secondary occur most frequently in

the rabbit (Barker, 1948) and in the cat (Barker & Ip, 1961;

Boyd, 1962b); but in the cat's Vth pes interosseus, simple

spindles with one primary ending only are most common.

A useful nomenclature for describing in a simple way

the number and position of the sensory endings was recently

advocated by Boyd (1962b) and will be employed in the present

investigation. Each primary sensory ending usually stretches

for a length of approximately 300 μ of the nuclear-bag

region and each secondary sensory ending generally occupies

a length of the intrafusal bundle from 300 to 500 μ with

an average of 400 μ on either side of the primary ending.

According to this descriptive classification, any spindle

can be divided into a P (primary) region about 300 μ long

and a series of S (secondary) regions, namely, S_1 (Nearest

to P), S_2, S_3 ... etc. each about 400 μ long on either side

of the P region. For example, both P S_1 and P S_2 represent

spindles with one primary ending and one secondary ending

only, but the latter is further denoted by the symbols to

have the secondary ending situated farther away from the P

region than the first by approximately 400 μ and so on.

Since there is a considerable overlap in position between

the secondary sensory innervation and the fusimotor inner-

vation, the latter will sometimes be referred to as occupying

the S_1, S_2, or, S_3 positions, as convenient, in the des-
criptions that follow.

4. 2. Results

4. 21. Plate endings

In its general structure, the intrafusal motor end-
plate is like an extrafusal one as has been pointed out
before (Barker, 1948). Essentially, each motor end-plate
(see Text-figure 12) consists of several (an average of
5.8 given by Gutmann & Young, 1944) twigs arranged in a
random manner terminating either as fine tapers, neuro-
fibrillar brushes, bulbs, or rings so as to constitute a
plate-like structure on a mass of granular sarcoplasm, the
sole-plate. Within the sole-plate protoplasm lie the
large, oval nuclei each containing one to three nucleoli.
As mentioned before (see p. 15), although the sole-plate
nuclei are stained only occasionally by this silver method,
the typical characteristics and shapes of the sole-plates
are sufficient to enable them to be identified even in the
absence of the nuclei. In fact, the failure of staining
the nuclei is sometimes advantageous in revealing more

clearly the details of the terminal twigs which are so often obscured by the darkly stained sole-plate nuclei (for example compare Text-figures 3 _b_ with 3 _a_ & 12 _c_). Two motor end-plates from different muscles or even from different animals such as the rabbit and the cat may have very similar appearance and arrangement in their terminal twigs as can easily be seen in Text-figure 3 _a_ & _b_. In other words, a means of identifying motor end-plates according to muscles or mammalian species has not yet been achieved so that the mammalian motor end-plate can be regarded as a pretty uniform structure throughout the series. Some examples of extrafusal and intrafusal motor end-plates of cat and rabbit are given as tracings in Plate 6.

The terminal axon of plate-ending fibre branches may terminate on the intrafusal muscle fibres either perpendicularly (as in Text-figure 12 _b_) or from the side (as in Text-figure 12 _a_ & _c_). Nevertheless, in either case, intrafusal motor end-plates exclusively have longer dimension along the axis than across the width of the muscle fibre. But extrafusal motor end-plates do not follow this pattern. Therefore, it is more convenient and perhaps more significant to take the longest dimension which is thereby arbitrarily termed 'length' as the size
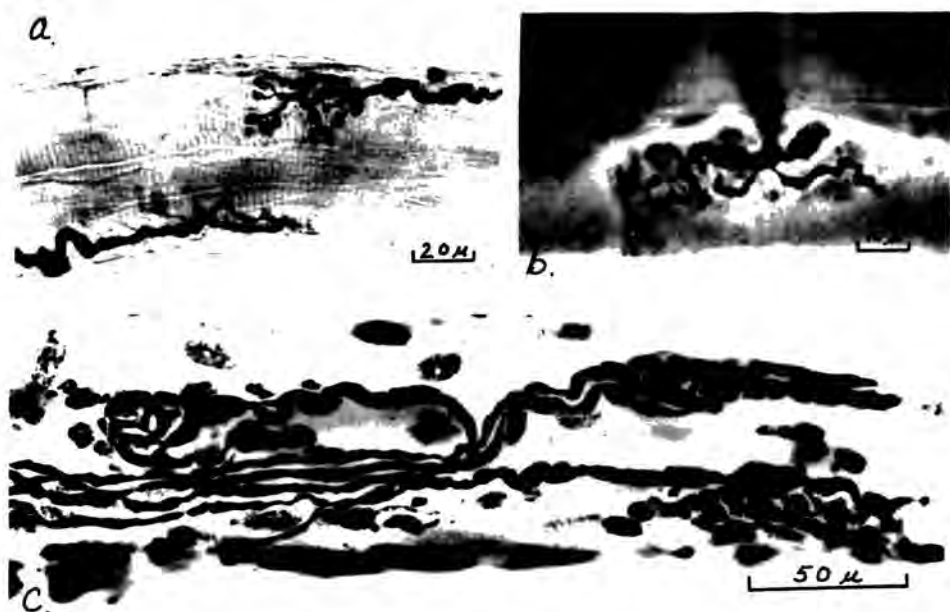
Text-figure 12.   Photomicrographs of intrafusal motor
end-plates ('normal', without sign of sprouting) found in
de-afferentated (d/a) spindles of cat and rabbit.   They all
show the general structure of a typical motor end-plate.
Teased, whole-mount, silver preparations (de Castro's method
as modified by Barker & Ip, 1963).   Note that sole-plate
nuclei are sometimes shown by this method as in b & c.

    a.   cat C159 interosseus (d/a), its tracing is repro-
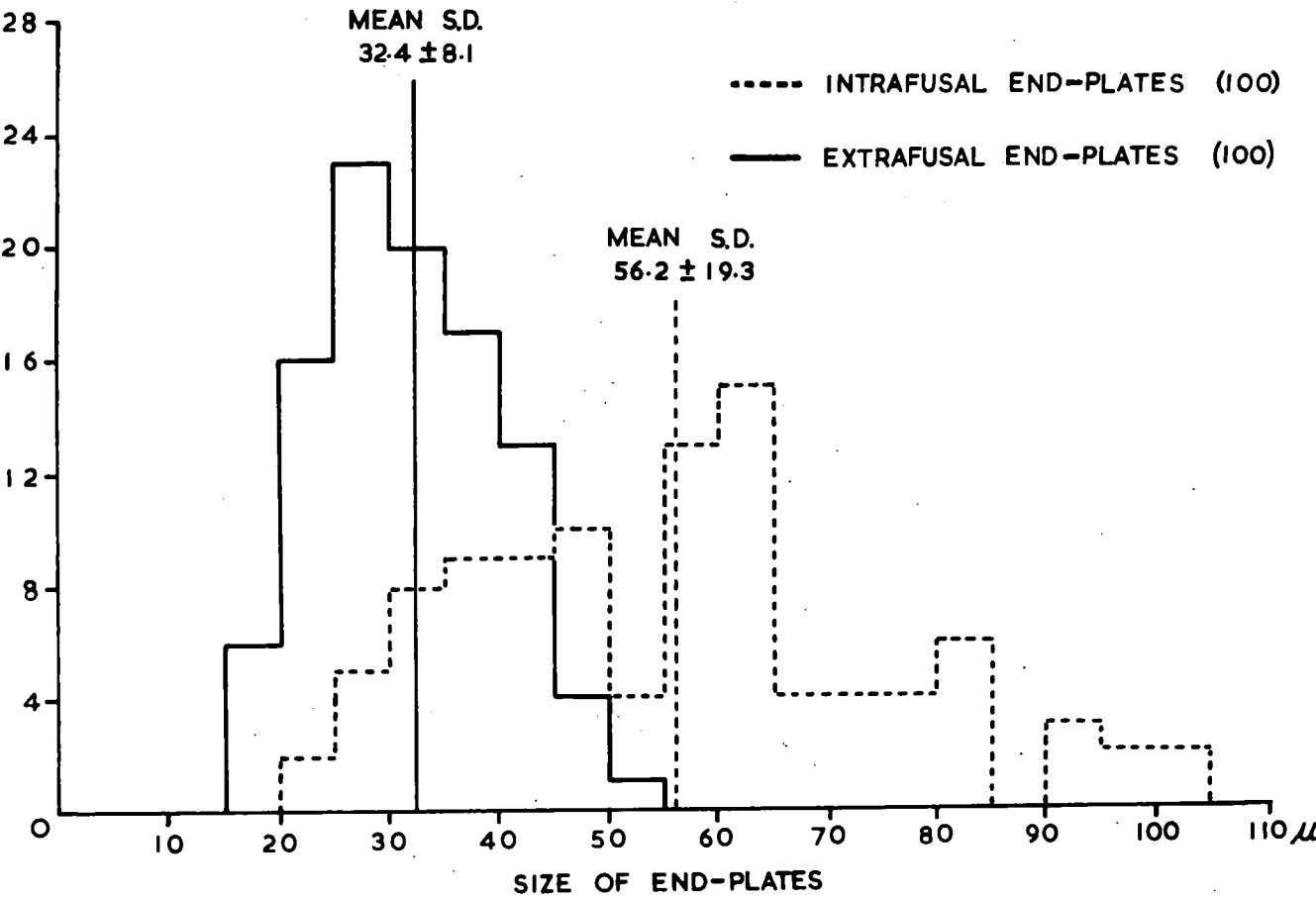duced in Figure 7, Plate 6;
    b.   cat C159 interosseus (d/a), its tracing is repro-
duced in Figure 3, Plate 6;
    c.   rabbit Rb.7 vastus intermedius (v).

of both intra- and extrafusal motor end-plates.   There
is an appreciable difference in length between extrafusal
and intrafusal motor end-plates.   The average length of
extrafusal motor end-plates is smaller than that of intra-
fusal ones, and also extrafusal motor end-plates have a
narrower range compared with intrafusal ones; while extra-
fusal muscle fibres are usually four times or more larger
than intrafusal nuclear-bag fibres in volume.   Consequent-
ly, extrafusal end-plates only occupy comparatively a tiny
area of the muscle fibre whereas the intrafusal ones occupy
nearly the whole width of the muscle fibre (compare the
tracings in Figures 1 & 2 with those in the rest of Plate 6).

In Text-figure 13, a hundred of extrafusal motor end-
plates taken at random from the tenuissimus and interosseous
muscles of the cat and a equal number of intrafusal motor
end-plates taken similarly from the peroneal I & II, tenui-
ssimus, and soleus muscles of the cat were measured, the
longest dimension in each case being taken.   The length of
the extrafusal motor end-plates varies from 15 to 55 μ
with a mean at 32.4 μ ± 8.1 μ S.D. while that of the
intrafusal ones varies from 20 to 105 μ with a mean at
56.2 μ ± 19.3 μ S.D.   The value obtained in the present
study is in good agreement with that given by Barker (1948)

CAT

MEAN S.D.
32·4 ± 8·1

MEAN S.D.
56·2 ± 19.3

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••••• INTRAFUSAL END-PLATES (100)

——— EXTRAFUSAL END-PLATES (100)
```

SIZE OF END-PLATES

Text-figure 13. Comparative end-plate length histograms of random samples of extrafusal (from tenuissimus and interosseous muscles of cat) and intrafusal (from peroneal I & II, tenuissimus and soleus muscles of cat) motor end-plates of a hundred each as measured under a X40 lens from teased, modified silver preparations. The extrafusal motor end-plates vary from 15 to 55 /u with a mean at 32.4 /u ± 8.1 /u S.D.; while the intrafusal motor end-plates vary from 20 to 105 /u with a mean at 56.2 /u ± 19.3 /u S.D.

who gave the measurement of intrafusal motor end-plates
in the hindlimb muscles of the rabbit as 50 to 70 μ long.
Wilkinson (1929) on the measurements of 24 normal motor
plates obviously taken from the extremity muscles of the
cat gave a range from 35 to 45 μ in length with an
average of 36 μ which also evidently coincides with the
mean of 32.4 μ for the extrafusal motor end-plates in the
present investigation.   Working with an entirely different
technique, Coërs & Woolf (1959) from measurements of a
series of human limb muscles gave a mean value for the
diameter of the subneural apparatus of 32.2 μ ± 10.5 μ
S.D. which is again in perfect agreement with the present
value.   Throughout the present series, no significant
correlation was found between the size of the motor end-
plate and the size of the muscle fibre it terminates on.
In other words, a small muscle fibre may have a compara-
tively larger end-plate than a large muscle fibre, and
vice versa.

The intrafusal end-plates are generally situated
farther away from the equatorial region more towards the
middle and ends of the polar region.   A typical example
of the distribution of the intrafusal end-plates can be
seen from a de-afferentated cat C167 peroneal spindle,

the motor innervation in one pole of which was accurately traced and drawn onto a diagrammatic drawing given in Text-figure 14.   In part B of this figure is shown a plate-ending fibre branch innervating only one nuclear-bag intra-fusal muscle fibre with two end-plates occupying a length of about 340 μ between the S_3 & S_4 region.   Therefore as usual, these end-plates are located extracapsularly.   A similar example found in a normal cat spindle prepared by the cholinesterase technique (after Coupland & Holmes, 1957; 18 hours incubation at pH 4.0) is given in Text-figure 15. As can be seen from this picture, the region innervated by discrete end-plates is towards the middle and end of the spindle pole.

Cases where the same end-plate has been supplied by more than one fusimotor fibre branch have been observed. Each of these 'complex' end-plates has dimensions either larger than or towards the largest limit of the normal intrafusal end-plates but is supplied by fusimotor fibre branches usually of different diameters.   Such cases were also observed by Barker (1948) and Barker & Cope (1962). In Text-figure 16 a, the intrafusal end-plate extends for about 120 μ and is supplied by three terminal axons of 2.8, 1.3 and below 1.0 μ which unfortunately cannot be
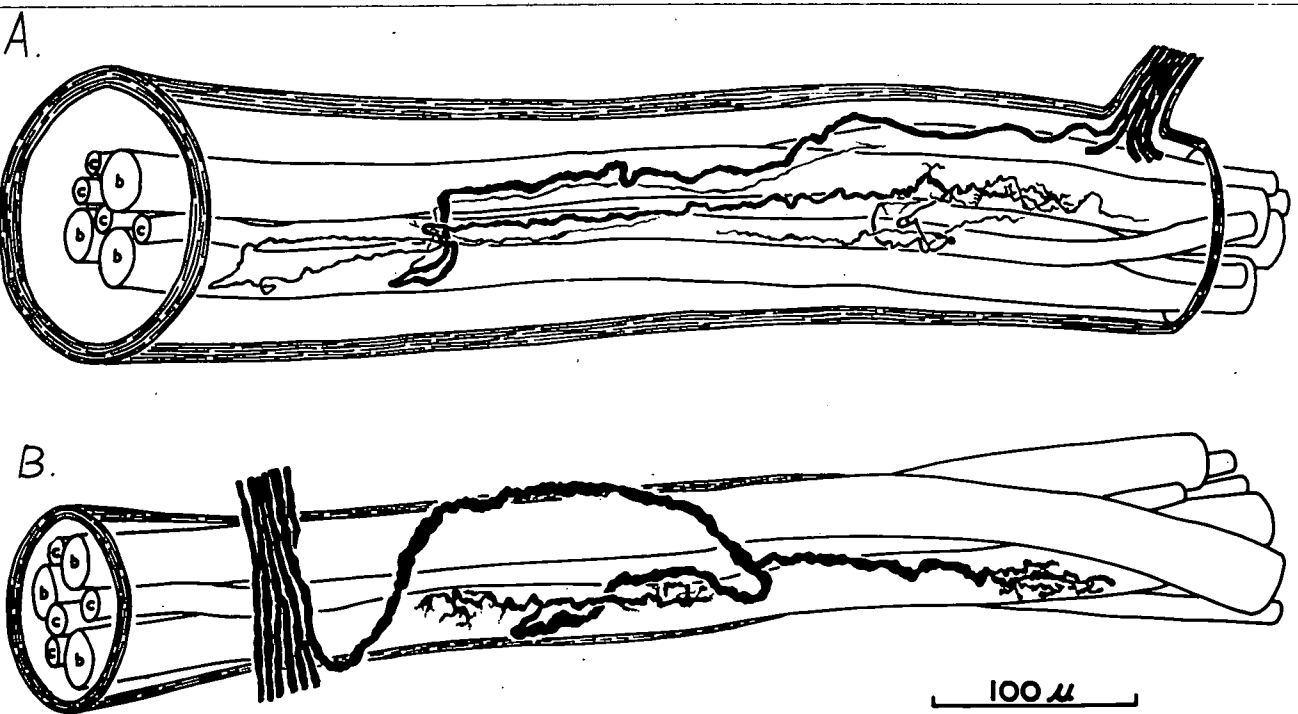
Text-figure 14. Accurate tracings of a trail ending (A) and motor end-plates (B) on a diagrammatic drawing of one pole of a de-afferentated cat C167 peroneal I spindle consisting of 3 nuclear-bag (b) and 4 nuclear-chain (c) intrafusal muscle fibres. All other motor innervations are omitted. B is continuous with A and is cut with 150 μ in between removed to suit the space. The right hand side is the polar end while the left hand side is the equatorial region, the centre of which is approximately 500 μ from the cutting surface of A. The intrafusal bundle is encapsulated in a lymph-filled capsule completely in A, but partly in B. Therefore, as is usually the case, the trail ending is regarded as intracapsular and the plate ending extracapsular.

Text-figure 15.   Photomicrograph of a normal cat spindle prepared by the cholinesterase technique (after Coupland & Holmes, 1957; 18 hours incubation at pH 4.0) to show two distinctly different kinds of motor terminations on the intrafusal muscle fibres of the mammalian muscle spindle.   On the left is the discrete motor end-plates situated towards the extremity of the pole and on the right is the diffuse multiterminal or trail endings situated towards the equatorial region.   It is conceivable that some end-plates are located among this diffuse type of motor endings in the region of overlap marked x.   eq. reg.   equatorial region;   e. p.   discrete end-plate;

t.   diffuse multiterminal or trail ending.

traced very far back because they are lost in the nerve trunk but I am in the impression that they belong to separate fibres. What appears in Text-figure 16 b is an end-plate of more definitely 'complex' in nature found in rabbit peroneal I spindle, occupying approximately a length of 70 μ of the intrafusal fibre. It is supplied by 3 fusimotor fibre branches of 2.0, 1.9 and below 1.0 μ in diameter which can be traced back for a distance over 3 mm from the spindle and still remain separate and different in size.

4. 22. Trail endings

In addition to the usual, typical motor end-plates, there is a second fundamentally different type of fusimotor ending called trail ending (Barker & Ip, 1965b). The term 'trail ending' will be used to denote the whole innervation supplied by a fusimotor trail fibre branch and thus it is usually made up of a number of trail ramifications. Trail endings are present in a majority of rabbit as well as cat spindles. In structure, as the name applies, trail ramifications exist as single tapered fine axonic threads, a number of fine trails running in any direction, or brush-like terminations (see examples in Text-figure 17, and
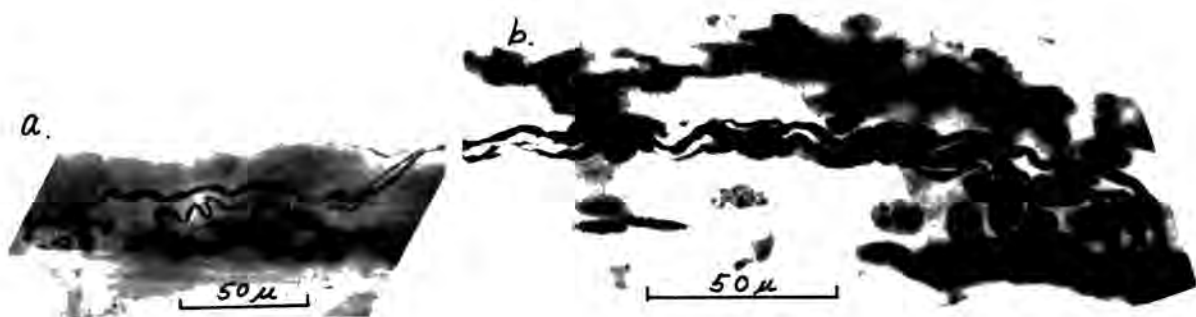
Text-figure 16.   Photomicrographs of teased, whole-
mount modified de Castro's silver preparations of normal
(n) cat in a and de-afferentated (d/a) rabbit in b spindles
to show intrafusal end-plates supplied by several plate
fusimotor terminal branches.

a, a complex intrafusal motor end-plate supplied by three
    fusimotor terminal axons in a cat C165 tenuissimus (n)
    spindle;

b, a complex intrafusal motor end-plate of a rabbit Rb11
    peroneal I (d/a) spindle innervated by three fusimotor
    terminal axons which on being traced back to the incoming
    nerve trunk, remain separate and different in size for
    over 3 mm.

Text-figure 17. Photomicrographs of teased, whole-mount preparations impregnated by the modified silver method (a, b, c, & e) de-afferentated (d/a) cat and rabbit spindles for showing examples of trail ramifications. That of a preparation impregnated by the gold chloride method from a normal (n) cat spindle is given in d for comparing the staining capacity between these two methods. The neurofibrils are excellently shown as solid black lines by silver as can be seen in a, b, c & e; but only appear as coarse, granular lines in gold preparations as can be seen in d. Only trail ramifications are shown in a, b, c & e; but in d a few end-plates are shown as well towards the left, and part of the primary spirals is visible on the right. What lying across and underneath the thick sensory nerve fibres is interpreted as trail ramifications same as those revealed by silver except in a less satisfactory appearance due to the coarse, granular staining capacity of gold. They are taken from:
a, cat C159 peroneal I (d/a) spindle (see its tracing in Fig. 11, Plate 7); b, cat C159 interosseous (d/a) spindle (see its tracing in Figure 12, Plate 7); c, rabbit Rb17 peroneal I (d/a) spindle; d, part of a spindle pole of a cat C41 rectus femoris (n) spindle; e, a tendinous pole of a cat C159 peroneal II (d/a) spindle consisting of trail ramifications only. Note cross-striations and individual intrafusal muscles are distinctly distinguishable.
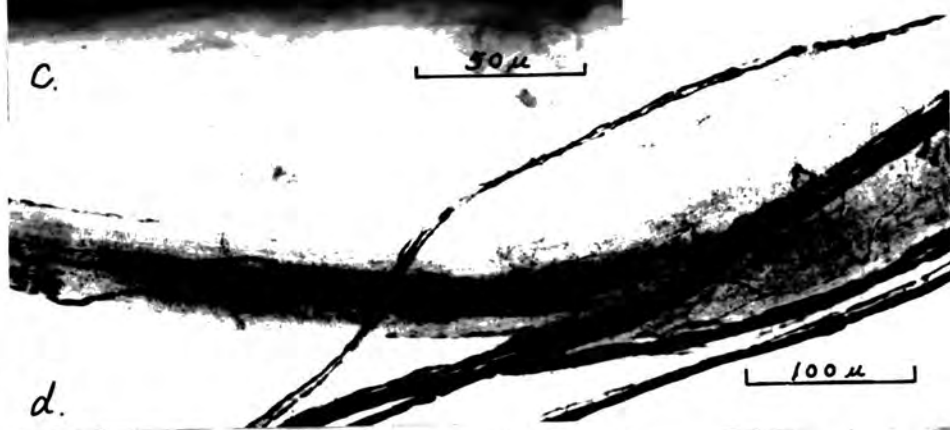
Text-figure 17.

Plates 7 & 8). In short, they exist in quite a different form from the typical motor end-plates, and appear as diffuse trailing terminations usually unaccompanied by nuclei whcih even though if present, offer no definite characteristics to be distinguished from the other nuclei in the muscle fibre. In determining the type of endings, great care must be taken to distinguish between the trail ramifications and the replacement configurations as pointed out by Barker & Ip (1965a). On following the trailing terminations of replacement, there is always a connexion with a definite end-plate somewhere (see for example, Plates 1 & 4). Also it must be borne in mind that very often there are some trail ramifications appearing very plate-like. In such cases, the best solution is perhaps to follow every possible branch of the parent fibre to see whether any particular plate-like termination is really an end-plate or a plate-like trail ramification (see for example, Figures 4 & 5 of Plate 8).

A portion of a de-afferentated cat soleus spindle given in Text-figure 18 illustrates the difference in form between trail ramifications and replacement configurations very well. This picture shows clearly that the replacement configuration (r.s.) is very much like the trail

ramification (t.) in appearance and can only be identified as not a trail ramification by following its course back to x, the point of branching of its parent axon, where another branch by the same axon terminates as definite end-plates.

Trail endings were found either in one or both poles in 252 out of a total of 274 (i.e. 92 %) cat spindles and in 23 out of a total of 33 (i.e. 69.7 %) rabbit spindles (see Table 2). They exist either as the only motor termin- ations or as a combination with plate endings in the whole pole of a spindle. They can be found even in spindles teased from the most satisfactorily de-afferentated materials with 4 week degeneration (for example, Figures 1 & 2 in Plate 8). Moreover, by careful study, they can be observed in normal spindles (for example, Figures 5-8 in Plate 7) in such an abundance that they can hardly be attributed to sensory-pain or sympathetic origin. Trail endings are also demonstrable in short-term degenerated spindles with only one week degeneration (see Methods) so that they are very unlikely abnormal endings arising as a result of degenera- tion induced perhaps by a chemical substance released from the de-afferentated spindle. Hinsey (1927), and Hines & Tower (1928) found no innervation in spindles after both

Text-figure 18.   Part of a de-afferentated (d/a) cat spindle pole.
It shows two motor end-plates (el) by a fusimotor fibre branch (p.f.1) on
a nuclear-chain (n-c) intrafusal muscle fibre with a ball of axoplasm (b.a.)
on the extreme top right corner.   Two end-plates (e2) situated on a nuclear-
bag (n-b 1) muscle fibre are supplied by another fusimotor fibre branch
(p.f.2).   On another nuclear-bag (n-b 2) muscle fibre, a trail ramification
(t.) and a replacement configuration (r.s.) are also clearly shown.   Notice
that the replacement configuration is given off by the same terminal axon
that gives rise to the nuclear-chain end-plates (el) and the point of
branching is seen at x.   Teased, whole-mount silver preparation from cat
C159 soleus (d/a); de Castro's method as modified by Barker & Ip, 1963.

b. a. ball of axoplasm;   b. v.   blood vessel;   el, end-plates on nuclear-
chain muscle fibre;   e2, end-plates on nuclear-bag muscle fibre;
n-b. 1 & 2, nuclear-chain intrafusal muscle fibres;   n-c, nuclear-chain
intrafusal muscle fibre;   p.f. 1 & 2,   plate fusimotor fibre branches;
r. s. replacement configuration;   t.   trail ramification;   t. f. trail
fusimotor fibre branch;   x. point of branching of p.f. 1.
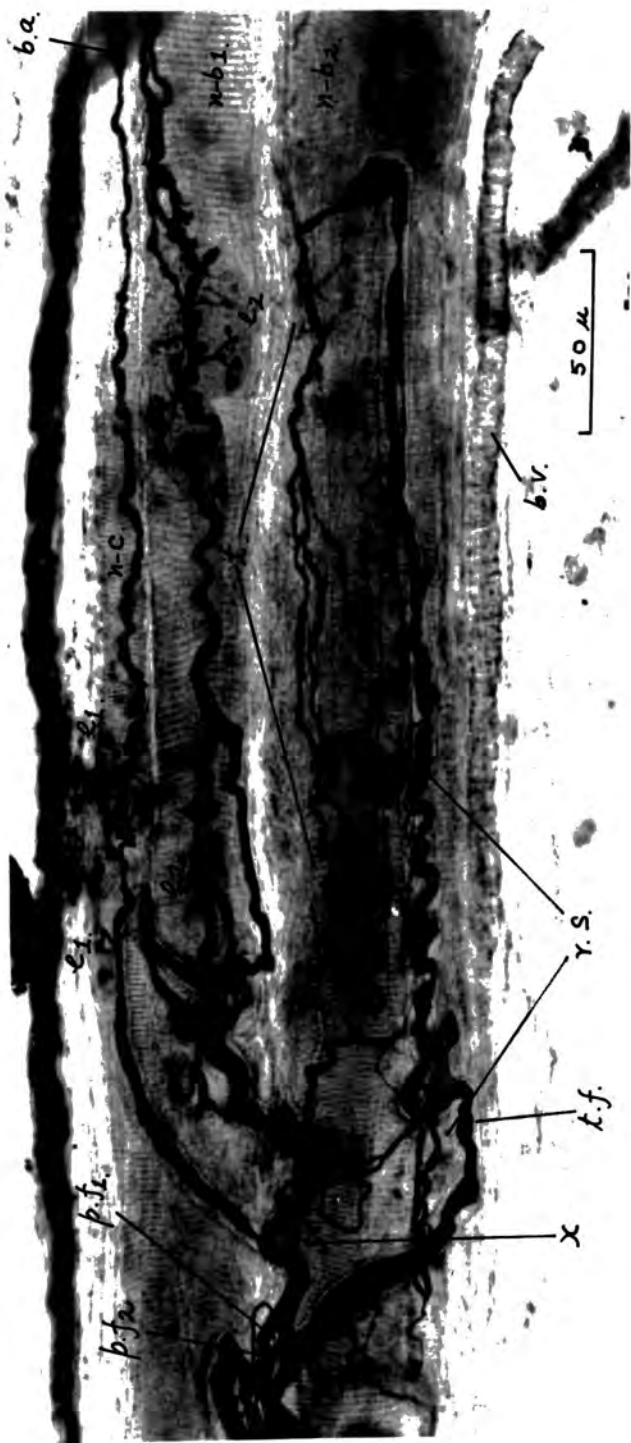
Text-figure 18.

sensory and motor roots were out. In my own de-afferen-
tated and de-efferentated materials same negative result
was obtained although nicely stained fine nerve fibres of
1 to 2 μ or less were found in nearby intramuscular nerve
trunks and blood vessels. Thus, trail endings are very
unlikely sympathetic in origin.

Usually, trail endings are situated near or at the
juxta-equatorial regions, and if expressed in Boyd's
terminology, they are usually situated in the S_1 and S_2
positions although sometimes they can be found to extend
to the S_3 position. A typical example of the distribution
of the trail ramifications can be seen in part A of Text-
figure 14. It illustrates the innervation supplied by
a trail-ending fusimotor fibre branch, innervations by
other motor fibre branches being omitted to give a clearer
picture. The trail ending, extending for a length of
approximately 350 μ, innervates one nuclear-bag and three
nuclear-chain intrafusal muscle fibres in a region of the
intrafusal bundle consisting of three nuclear-bag and four
nuclear-chain muscle fibres, about 600 to 1000 μ from the
centre of the equatorial region, i.e. almost the entire
length of the S_2 region. As can be seen from the drawing,
the ending is regarded as intracapsular because the region

of the intrafusal bundle where the trail ending terminates
is completely within the lymph-filled capsule. The posi-
tions of the trail ramifications in relation to those of
the end-plates can also be seen in a normal cat spindle
prepared by the cholinesterase technique in Text-figure 15.
As can be seen from the picture, there are two distinct
types of motor innervation on this spindle pole. The
diffuse multiterminal type (compare with the figures of
Hess, 1961) is located towards the equatorial region on
the right while a discrete end-plate type is situated to-
wards the extremity of the pole on the left. Although
the capsule seems a little collapsed, it can be seen that
most part of the diffuse multiterminal type of motor inner-
vation is within the capsule sheath while the discrete end-
plate type is outside. A region of overlap where some
end-plates can be detected among the diffuse multiterminal *ending*
can be seen somewhere in the middle of the spindle pole.
Thus, both Text-figures 14 & 15 illustrate the consistency
in the distribution of the plate and trail endings along
the polar region of the spindle very well.

4. 23. <u>Motor innervation of intrafusal muscle fibres;</u>
<u>whether types of motor termination depend on</u>
<u>types of muscle fibre</u>

Twelve cat and ten rabbit spindles were analysed in order to determine the complete fusimotor innervation of every individual intrafusal muscle fibre by examining each muscle fibre with the highest optical magnification (10 X or 25 X eyepieces and oil immersion objective). It was ascertained whether the spindle motor innervation consists of plates only, trails only, or plates and trails together on either one or both polar halves of individual intrafusal fibres; and whether in cat spindles there is any segregation of the 2 types of motor endings on the 2 types of intrafusal fibres.

It is consistently found that plate endings are present on nuclear-bag (see Figures 3-8 in Plate 6) as well as on nuclear-chain muscle fibres (see Figures 9-11 in Plate 6 & Plate 9). Similarly, trail endings may terminate on either nuclear-bag (see Text-figure 17 a) or nuclear-chain muscle fibres (see Figures 1 & 2 in Plate 8). Alternatively, each type of ending may terminate on a mixture of nuclear-bag and nuclear-chain muscle fibres, or may exclusively lie on one or the other. In order to illustrate the manner in which a fusimotor fibre branch terminates on the intrafusal muscle fibres of the mammalian spindle, the detailed terminations of 3 fusimotor fibre branches (2 plate-fibres and

1 trail-fibre) from 3 different de-afferentated cat spindles were carefully drawn to scale and given in Text-figure 19. These 3 fusimotor fibre branches had been unravelled (in places it was necessary to break the continuity of the axon which was represented by dotted lines) and the whole innervation of each of them was isolated out free from muscle fibre for the sake of clarity and simplicity. These 3 fusimotor fibre branches all terminate on a mixture of nuclear-bag and nuclear-chain muscle fibres. A is a plate-ending fusimotor fibre branch of 3.0 μ axon diameter innervating both poles of a de-afferentated cat C172 peroneal II spindle. It sends off 8 end-plates without any sign of sprouting to terminate on 2 nuclear-bag and 3 nuclear-chain muscle fibres of this spindle. B is again a plate-ending fusimotor fibre branch of average axon diameter of 2.7 μ innervating one pole of another de-afferentated cat C159 peroneal II spindle. It sends off 5 end-plates to innervate all the 3 nuclear-bag muscle fibres and 7 smaller end-plates to innervate 5 out of 6 nuclear-chain muscle fibres of this spindle. In addition, there are a total of 10 possible sprouts coming off either from a terminal axon or from an end-plate. C is a trail-ending fusimotor fibre branch of average axon diameter of 3.8 μ innervating one pole of a de-afferentated

cat Cl67 peroneal I spindle. It supplies as many as 20 trail ramifications to 1 out of 2 nuclear-bag and 4 out of 5 nuclear-chain muscle fibres of this spindle pole.

Frequently both types of endings occur on the same muscle fibre, though the distance between them may be far apart. Sometimes an end-plate and a trail ramification may be found on different sides of the same site (see Text-figure 20). Text-figure 20a shows three photomicrographs taken at different foci of the same region of a normal rabbit spindle pole to illustrate this point. In a, one of the intrafusal muscle fibres is shown to receive an end-plate and a trail ramification only about 125 μ apart located on the surface of the same muscle fibre as can be seen from the first picture. The next focus brings out the muscle fibre more clearly into view. The last focus reveals that in addition, another trail ramification at the same spot but on the other side of the muscle fibre where the end-plate terminates. In b, a similar condition is found in a de-afferentated rabbit spindle. Both an end-plate and a trail ramification can be found terminating on the different sides of the same spot of an intrafusal muscle fibre as can be seen from

Text-figure 19.   Accurate tracings of three separate fusimotor fibre branches innervating three de-afferentated (d/a) spindles from hindlimb muscles of the cat.   Tracings were based on teased, whole-mount modified de Castro's silver preparations.   Innervations by other fusimotor fibre branches were omitted.   In places, the continuity of the axons was broken for unravelling or shortening and was represented by double dotted lines. These three fusimotor fibre branches all terminate onto a mixture of nuclear-bag and nuclear-chain intrafusal muscle fibres.

A, A plate-ending fusimotor fibre branch of average axon diameter of 3.0/u innervates both poles of a cat C172 peroneal II (d/a) spindle.

B, A plate-ending fusimotor fibre branch of average axon diameter of 2.7/u innervating one pole of a cat C159 peroneal II (d/a) spindle.

C, A trail-ending fusimotor fibre branch of average axon diameter of 3.8/u innervating one pole of a cat C167 peroneal I (d/a) spindle.

b. nuclear-bag intrafusal muscle fibre;   o.   nuclear-chain intrafusal muscle fibre;   E. R.   equatorial region;   s.   sprouting.
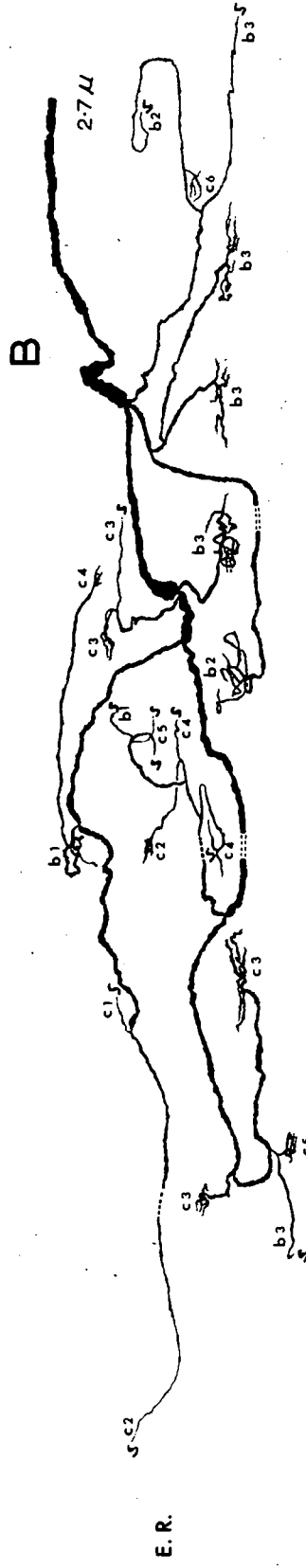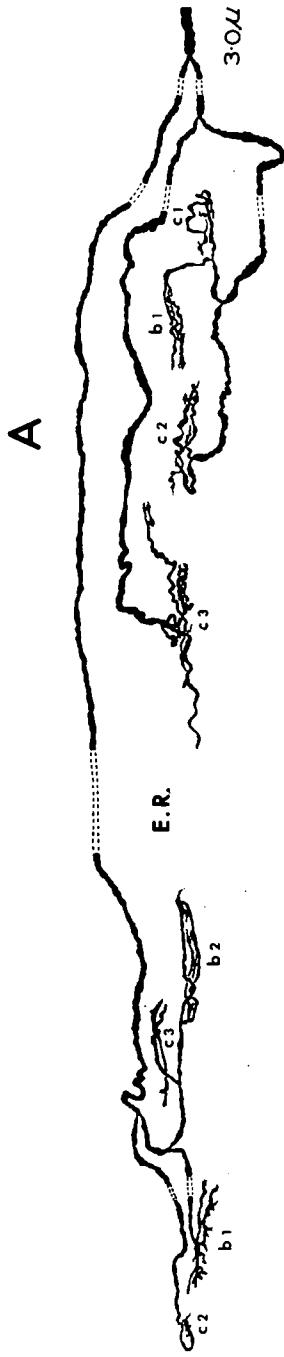
Text-figure 19.

the two photomicrographs taken at different foci. Very
often, two or more end-plates supplied by the same plate
fibre branch can be found terminating on the same intra-
fusal muscle fibre (for example, Text-figure 14 B) or on
different ones (for example, Text-figure 3 c & d).
Similarly, two or more trail ramifications supplied by
the same trail fibre branch may terminate either on the
same or on different intrafusal muscle fibres (see for
example, Text-figure 17 a & b). Moreover, two or more
end-plates or trail ramifications terminating on the same
intrafusal muscle fibre may also be supplied by two or more
different fusimotor fibre branches. For instance, the two
trail ramifications in both Text-figure 17 b and Figure 1,
Plate 8 are supplied by two trail fibre branches.

Because of their mode of termination, it is possible
to classify fusimotor fibre branches into 3 different types,
i.e. those innervating nuclear-bag fibres only; those inner-
vating nuclear-chain fibres only; and those innervating a
mixture of both, sometimes in equal proportions or sometimes
mostly on one type or the other. For descriptive conven-
ience, a system of symbols is used to represent the motor
innervation of spindles or their polar halves. If P is
used for designating plate endings, T for trail ramifications

Text-figure 20 a.   Three photomicrographs of part of a normal (n) rabbit spindle taken at the same region but at different foci to show that both plate and trail ramifications terminating on the same intrafusal muscle fibre.   In the first picture, a trail ramification and an end-plate are located approximately 125 μ apart on the surface of the same intrafusal muscle fibre (the lowest one).   The next focus brings the intrafusal muscle fibre more clearly into view with the two terminals just mentioned are still vaguely visible.   As can be seen from the arrangement and spacing of the cross-striations, three muscle fibres can be distinguished.   The final focus in the third picture reveals another trail ramification on the other side of the same spot where the end-plate terminates.   Taken from rabbit Rb7 vastus intermedius (n).

Text-figure 20 b.   Two photomicrographs of part of a de-afferentated (d/a) rabbit spindle taken at two foci on the same spot to show a similar situation.   In the left picture, a plate ending can be detected near the edge of the top muscle fibre.   The next focus on the right brings out a trail ramification at the same spot but on the other side of the muscle fibre.   Taken from rabbit Rb11 peroneal IV (d/a).

e. p. end-plate;   t.   trail ramification.
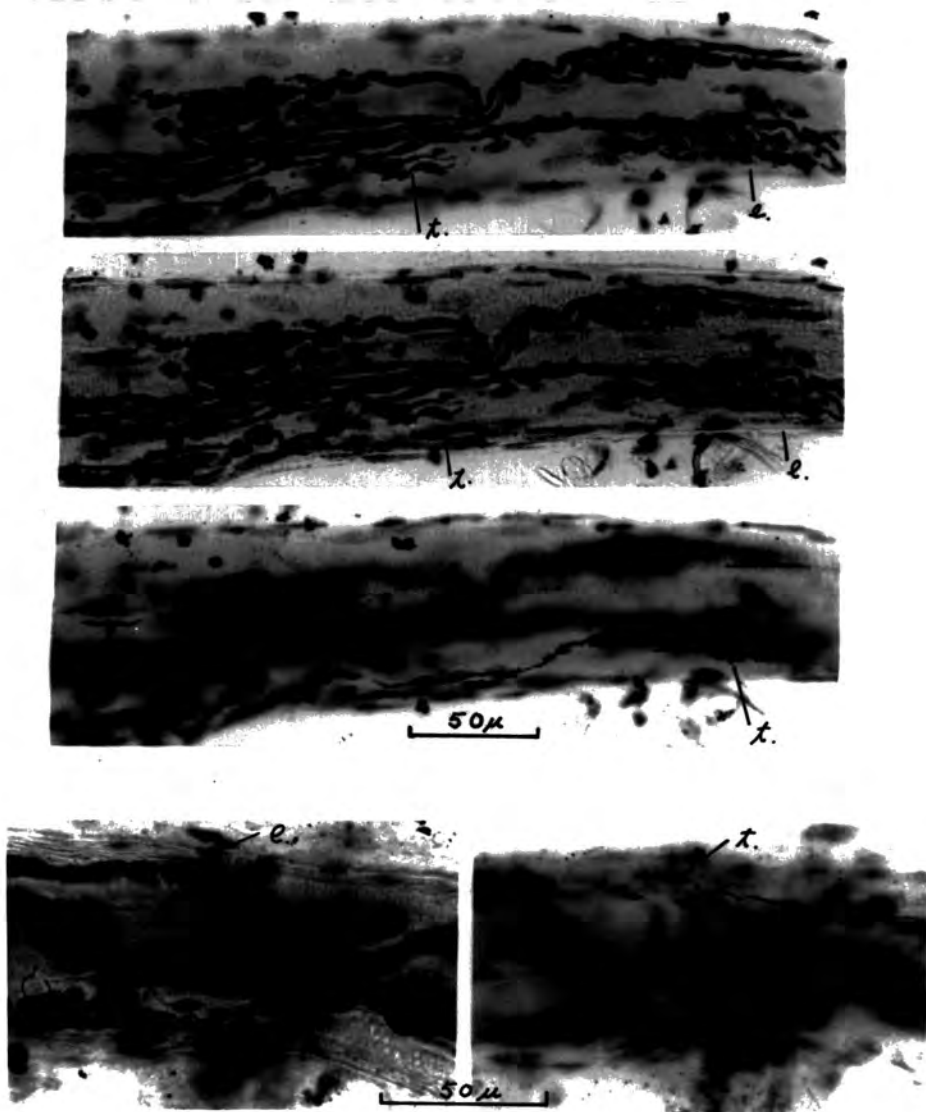
Text-figure 20.

on nuclear-bag muscle fibres; and p for denoting plate
endings and t for trail ramifications on nuclear-chain
muscle fibres; with corresponding italics to denote endings
supplied by the same fusimotor fibre branch innervating
both types of muscle fibres; the motor innervation of all
analysed de-afferentated spindles can be diagrammatically
represented in a symbolized form.   In Text-figure 21, the
complete motor innervation of one polar half of a de-affer-
entated rabbit spindle was analysed and then individual
polar intrafusal fibres with their endings were drawn
separately in a semi-diagrammatic form.   One of these
intrafusal polar halves (shaded) was isolated out to
illustrate how its motor innervation was represented.
This polar half is supplied with 2 end-plates and 2 trail
ramifications at sites as indicated.   If this polar half
is arbitrarily divided into 3 approximately equal lengths,
then its motor innervation is written as

$$\text{P} \qquad \text{P T} \qquad \text{T} \quad :$$
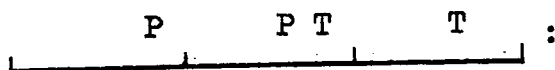
and so on (the colon represents the region of equatorial
nucleation).   The other polar half of the same intrafusal
fibre was also similarly analysed and found to contain only
one end-plate at the middle of the polar half so that the

motor innervation of this entire intrafusal muscle fibre
is represented by

$$\underline{\qquad \underset{P}{\qquad} | \underset{P\ T}{\qquad} | \underset{T}{\qquad} |} \quad : \quad \underline{\qquad | \underset{P}{\qquad} | \qquad |}$$

The sample of 12 cat and 10 rabbit spindles were all thus
symbolized but only two of each of them are given in Text-
figures 22 & 23.

It can be seen that the nuclear-bag or nuclear-chain
muscle fibres may have just one single or more motor rami-
fications per fibre, spaced close together or very far
apart and yet may have no motor innervation over much of
their length (see also Boyd, 1962b). Either type of muscle
fibres may have only one type of motor ending, or a mixture
of both plate and trail ramifications. Roughly speaking,
however, the distribution of motor terminations can be
grouped approximately into three equal regions the extent
of which depends on the length of each individual spindle
pole concerned. It can be seen that the motor innervation
of a spindle pole may consist of either P (plates) only,
a mixture of PT (plates and trails) together, or T (trails)
only so that six possible combinations of motor innervation
of spindles as a whole can be sorted out in this respect,

Text-figure 21.   Diagrammatic drawing of one pole of a de-afferentated rabbit spindle (Rbll peroneal IV) with all its motor innervation completely analysed.   One of the four nuclear-bag intrafusal polar halves is isolated out (shaded) to illustrate how its motor inner-vation can be represented in a symbolized form in the lower part of the figure.   The drawing was based on a tracing from a teased, silver pre-paration (modified de Castro method).   In the symbolized form, each polar half is represented by a line arbitrarily divided into three app-roximately equal parts with the position of the appropriate ramifications entered accordingly into these three regions.   The equatorial region is indicated by a colon (:).   The complete motor innervation of this spindle is given as the 1st spindle in Text-figure 22 and again as Sp. 4 in Text-figure 25.

P -- plate ending;   T -- trail ramification.

RABBIT

200μ

P   PT   T

P   PT   T:
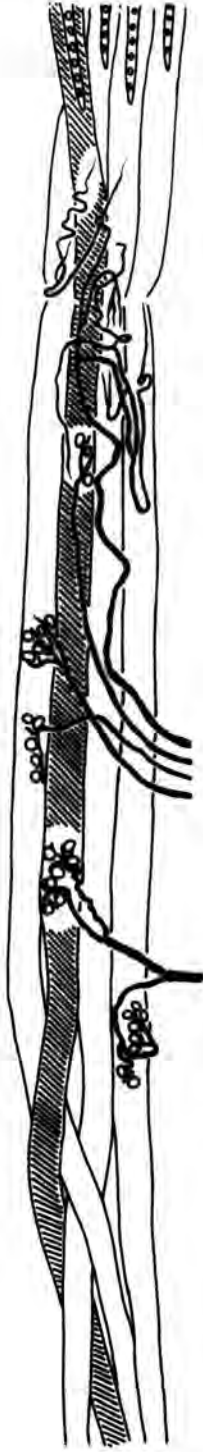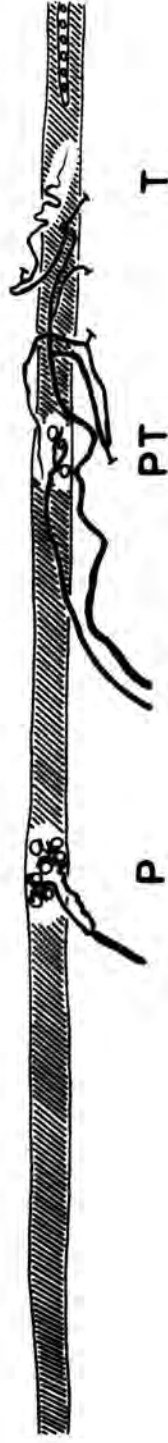
P   PT   T: P

Text-figure 21.

Text-figure 22. Symbolized representations of two de-afferentated rabbit spindles in a similar way as out-lined in Text-figure 21 or as described in the text on page 81. Each intrafusal muscle fibre is divided into two polar halves by the equatorial region represented by a colon (:) at about the middle. The ratio on top refers to the number of plate fibre branches to that of trail fibre branches innervating the whole spindle; while ratios below apply to the proportions of these two types of fusi-motor fibre branches going to innervate each polar half only. Drawing based on tracings of the modified de Castro's silver preparations. The complete motor innervation of these two spindles is represented graphically as Sp 4 and Sp 1 respectively in Text-figure 25.

P -- end-plate; P -- end-plates supplied by one and the same plate fusimotor fibre branch that innervates both polar halves of the spindle; T -- trail ramification.

RABBIT

9/1

```
_____,P P, T_,  : _____,    P ,  P ,
_, P ,  PT,   T_,  : _____,    P    ,
_____,    TTT,  : _____,      P ,
_, P ,      T_,  : _, P, P ,P ,
        4/1        5/0
```

6/0

```
_____, P ,P_,  : _____,    P    ,
_, P ,  P_,  : _____,    P    ,
_____,    P_,  : _____,      P ,
_____, P ,  : _____,    P P,
        4/0        3/0
```

Text-figure 22.

Text-figure 23.   Similarly symbolized representa-
tions of two de-afferentated cat spindles.   In this case
shorter lines are used to represent nuclear-chain muscle
fibres and italics to denote ramifications supplied by
fusimotor fibre branches that innervate both types of
muscle fibres, otherwise everything is the same as in
Text-figure 22.   Drawing again based on tracings of the
modified de Castro's silver preparations.   The complete
motor innervation of these two spindles is reproduced in
Sp 10 and Sp 7 respectively of Text-figure 24.

   ⚲ represents end-plates supplied by one and the
same plate fibre branch that innervates both nuclear-bag
and nuclear-chain muscle fibres on both poles of the
spindle.

Text-figure 23.

namely:

      PT:TP - plates and trails on both poles;

      PT:T   - plates and trails on one pole,

                    trails only on the other;

      PT: P - plates and trails on one pole,

                    plates only on the other;

      P :T   - plates only on one pole,

                    trails only on the other;

      P : P - plates only on both poles;

      T:T   - trails only on both poles.

In order to determine which is the most common type of spindles according to this kind of classification, a larger sample from a wider range of muscles was added to the original sample of 22 completely analysed spindles. For this purpose, it is sufficient to know only what type of motor endings is present on each pole without the necessity of following each individual intrafusal muscle fibre. Therefore, a greater number of spindles from different muscles can be included so long as the staining renders the ramifications suitable to be distinguished as plates or trails. It may be added that sometimes even normal spindles can be used and in this case the sensory innervation can be identified as well. Thus a total of

274 cat spindles (180 d/a & 94 normal) taken from 9 hind-
limb muscles, namely soleus, peroneal I, II & III, popli-
teus, flexor digitorum longus mesial head, tenuissimus,
superficial and deep lumbricals; plus 33 rabbit spindles
(27 d/a & 6 normal) from 5 hindlimb muscles, viz. vastus
intermedius, peroneal I, III & IV, and soleus, all being
treated together, was analysed and the results are given
in Table 2 (the 22 completely analysed cat and rabbit
spindles being included).

Thus, it can be seen that the PT:TP type, occurring
fairly constantly among all the muscles used with an aver-
age of 61.3 %, is the most common type of motor innerva-
tion in cat spindles; while in rabbit spindles, PT:TP,
PT:P and P:P types are more or less equally common because
all these 3 types occur in 33.3, 36.4 and 30.3 % respec-
tively.   The occurrence of spindles without trail endings
on either pole, i.e. P:P type, is relatively high in rabbit
(30.3 %) as compared with that in cat (8.0 %) whereas the
T:T type of spindle has not been observed.   It is inter-
esting to note that the occurrence of the PT:T type is
exceptionally high in the lumbrical muscles with an average
of 37.8 % as against a mean of 5.0 % in all the other muscles;
and that of PT:P is highest in soleus (33.3 %) but lowest in

Table 2 :   Motor Innervation Types of Cat & Rabbit Spindles in %.

Motor Innerv. Types	Cat Spindles											Rabbit Spindles*
Muscles	Soleus	P I	P II	P III	Popl.	FDL	Tenu.	S.lumb.	D.lumb.	Total No.	%	
PT : TP	55.6	54.8	68.8	78.4	64.0	63.6	52.4	59.1	61.5	168	61.3	33.3
PT : T	0	6.5	3.1	13.5	4.0	9.1	1.6	40.9	34.6	29	10.6	0
PT : P	33.3	22.6	15.6	5.4	28.0	18.2	30.2	0	3.9	52	19.0	36.4
P : P	11.1	9.7	9.4	2.7	4.0	9.1	15.8	0	0	22	8.0	30.3
P : T	0	6.5	3.1	0	0	0	0	0	0	3	1.1	0
T : T	0	0	0	0	0	0	0	0	0	0	0	0
Total No. of Spindles	27	31	32	37	25	11	63	22	26	274	100.0	100.0

* D/S as well as normal spindles in these samples

peroneal III (5.4 %); although all other types occur in proportions fairly constant for all the muscles used.

In order to see whether there is any correlation between the motor and the sensory types of innervation in cat and rabbit spindles, a similar analysis was made on 100 normal cat and rabbit spindles in which the sensory endings could be identified. This sample comprised of 38 tenuissimus, 43 superficial and deep lumbricals, and 13 peroneal spindles of the cat and 6 vastus intermedius spindles of the rabbit. The results of this analysis are given in Table 3. From this it is clear that both end-plates or trail ramifications may be found next to a primary or to a secondary ending, and that there appears to be no significant correlation between the sensory and the motor innervation. In this sample, PT:TP is again the most common type of motor innervation.

4. 24. Motor innervation of individual intrafusal fibres as a whole one or as two separate polar halves

Occasionally, one polar half but never both halves of an intrafusal fibre of a spindle may be found to receive

Table 3: Motor Innervation Types of Cat and Rabbit Spindles with different Types of Sensory Innervations in %.

Motor Innervation Types \ Sensory Innervation Types	Pr	Pr S	P& > 2S	Mean % Total No.
PT : TP	51.6	54.5	64.0	56.0
PT : T	29.0	22.7	4.0	20.0
PT : P	6.5	20.5	24.0	17.0
P : P	12.9	2.3	8.0	7.0
P : T	-	-	-	-
T : T	-	-	-	-
Total Number of Spindles	31	44	25	100

no motor innervation at all (see the 3rd nuclear-bag
intrafusal muscle fibre in the 1st spindle of Text-figure
23). In spindles with one pole short and tendinous, it
was very often found that only trail ramifications but no
definite end-plates were present (as shown in Text-figure
17 e). Thus, it can be worked out that there are ten
possible types of individual entire intrafusal muscle
fibres from one polar end to the other. That is:

> P : P = plates only on both polar halves of the
> muscle fibre;

> P : - = plates only on one and no motor innerva-
> tion on the other half;

> PT: P = plates and trails on one and plates only
> on the other half;

> PT: - = plates and trails on one and no motor
> innervation on the other half;

> T: P = trails only on one and plates only on
> the other half;

> T: - = trails only on one and no motor innerva-
> tion on the other half;

> T:T = trails only on both polar halves of the
> muscle fibre;

> PT:TP = plates and trails on both polar halves
> of the muscle fibre;

PT:T — plates and trails on one and trails

only on the other half;

: - — no motor innervation on both polar

halves of the muscle fibre.

The tenth one, however, has never been observed as men-
tioned above.

Data of such an analysis must necessarily be col-
lected from completely analysed spindles. The results
of analysing 41 individual intrafusal muscle fibres of
the 10 rabbit spindles, plus 10 nuclear-bag and 15 nuclear-
chain individual intrafusal muscle fibres of the 4 com-
pletely analysed cat spindles are given in Table 4. It
is clear that the P : P type occurs most frequently (41.4 %)
in the intrafusal muscle fibres of rabbit spindles. In
the case of nuclear-bag muscle fibres of cat spindles, the
PT: P type is most common; but both PT: P and T: P types
are equally common in nuclear-chain muscle fibres. Thus,
the six possible kinds of motor innervation of spindles
mentioned in the previous section can be derived from
various combinations of nine of these ten types (because
: - type has never been observed) of intrafusal muscle
fibres.

Table 4 : Motor Innervation Types of Individual Intrafusal
fibres from pole to pole.

| Motor Innervation Types | Individual Intrafusals from Pole to Pole | | | | | |
| | Rabbit (bags only) | | Bags of Cat | | Chains of Cat | |
	Number	%	No.	%	No.	%
P : P	17	41.4	3	30	2	13.3
P : -	6	14.6	0	0	2	13.3
PT : P	11	26.8	5	50	3	20.0
PT : -	3	7.3	0	0	1	6.7
T : P	2	4.9	1	10	3	20.0
T : -	0	0	1	10	1	6.7
T : T	0	0	0	0	2	13.3
PT : TP	1	2.5	0	0	0	0
PT : T	1	2.5	0	0	1	6.7
- : -	0	0	0	0	0	0
Total Number of Intra-fusals taken	41		10		15	

• 10 rabbit and 4 cat D/S spindles completely analysed are used
to give these data.

- means that pole without any motor innervation

Throughout the present study, the pattern of the motor innervation in both cat and rabbit spindles is treated in more detail in terms of polar halves rather than of whole intrafusal muscle fibres. The chief reasons for this preference are firstly, the spindle is known to be separated at the middle by a relatively non-contractile equatorial region of sensory innervation into two contractile units---the poles, which are therefore, better regarded as separate functional units physiologically and anatomically; and secondly, due to the technical difficulty as mentioned before (see Methods). Therefore, very often only one pole of a spindle especially cat spindles which are more complicated by having a larger number of intrafusal fibres consisting of both nuclear-bag and nuclear-chain fibres, is analysable so far as the detailed motor innervation is concerned. Hence, if only polar halves are considered, it was observed that there are four types of motor innervation in them. That is, those with plates only (P); those with a mixture of plates and trails (PT); those with trails only (T); or those without any motor innervation (-). In this case, data can be collected from completely analysed spindle poles as well. The results of analysing a total of 78 rabbit intrafusal polar halves, 38 nuclear-bag and 68 nuclear-chain intra-

fusal polar halves of cat from the 12 completely analysed cat and 10 rabbit spindles are given in Table 5. It can be seen from this table that polar halves of nuclear-bag muscle fibres of both rabbit and cat spindles are predominantly innervated by plate endings only (62.8 % in rabbit and 52.6 % in cat); but those of nuclear-chain muscle fibres of cat spindles are innervated most commonly by either plate endings only (36.8 %) or trail ramifications only (35.3 %) in about equal proportions.

4. 25. The distribution and number of motor ramifications in individual intrafusal muscle fibres of the completely analysed spindles

In order to determine the distribution of motor ramifications in cat and rabbit spindles in terms of their distance from the centre of the equatorial region and their numbers, it is necessary to standardize the length of the polar halves of the muscle fibres. This is because spindles vary in length so that the most distant polar terminal of a very short spindle may be equidistant from or even closer to the equatorial region than most of the proximal terminals of a very long spindle. Therefore, the significant correlation, if any, between the distance of plate

Table 5 :   Motor innervation types of polar halves of individual
intrafusal muscle fibres.

Motor innervation types	Rabbit (N-bags only)		N-bags of Cat		N-chains of Cat	
	Number	%	Number	%	Number	%
P	49	62.8	20	52.6	25	36.8
PT	17	21.8	12	31.5	11	16.1
T	3	3.9	4	10.6	24	35.3
-	9	11.5	2.	5.3	8	11.8
Total	78	100.0	38	100.0	68	100.0

Data from the 12 completely analysed cat spindles (4 with 2 poles
and 8 with only 1 pole analysed) and 10 rabbit spindles.

- means polar halves without any motor innervation.

and trail ramifications and their relative numbers would
be difficult to be realized if all spindles remain, as
they are, variable in length. By measuring the approx-
imate lengths (because often the extreme end of the spin-
dle is cut during preparation) of all polar halves of the
12 cat spindles, the mean value of nuclear-bag or nuclear-
chain polar halves was found to be 3.4 mm or 2.9 mm
respectively. Then the length of every individual polar
half was accordingly converted to the appropriate mean
value with its ramifications in each case likewise pro-
portionately corrected, all measurements being taken from
the centre of the equatorial nucleation. Take a simple
case for example, suppose a nuclear-bag polar half measures
1.7 mm with a trail and a plate 0.5 mm and 1 mm from the
centre of the equatorial region, then this polar half is
converted to the standard length of 3.4 mm with the trail
and the plate correspondingly 1 mm and 2 mm from the cen-
tre and so on. Similarly, the length of each polar half
of all intrafusal muscle fibres in the 10 rabbit spindles
was measured and converted in the same way and the mean
value of 2.1 mm was obtained. The positions of the motor
ramifications were corrected accordingly. After such
conversions, all end-plates (black circles) and trail
ramifications (black triangles) were entered into the

corresponding1y standardized polar halves (vertical lines) according to their calculated distance from the centre of the equatorial region.   Text-figure 24 A & B illustrates this information for the 12 cat spindles analysed, and Text-figure 25 similarly illustrates the 10 rabbit spindles.   The mean for the position of all the end-plates and the trail ramifications was found to be 1.4 mm & 1.03 mm for nuclear-bag polar halves; and 1.25 mm & 0.83 mm for nuclear-chain polar halves of the cat; while the corresponding value for the rabbit polar halves being 0.9 mm & 0.65 mm respectively (the mean is represented by a dotted line in each case).   The difference between the positions of plate and trail ramifications is not great, but this is understood because of the exceptions encountered.   If these exceptions are discarded, the difference should be much more obvious.

Generally speaking, the distribution of the motor ramifications in intrafusal polar halves is bimodal, i.e. trail ramifications usually occupy the juxta-equatorial region while plate endings occupy the middle and extreme end of the polar halves.   There is a region of overlap (between the dotted lines) where plate and trail ramifications intermingle one another.   In order to avoid confusion
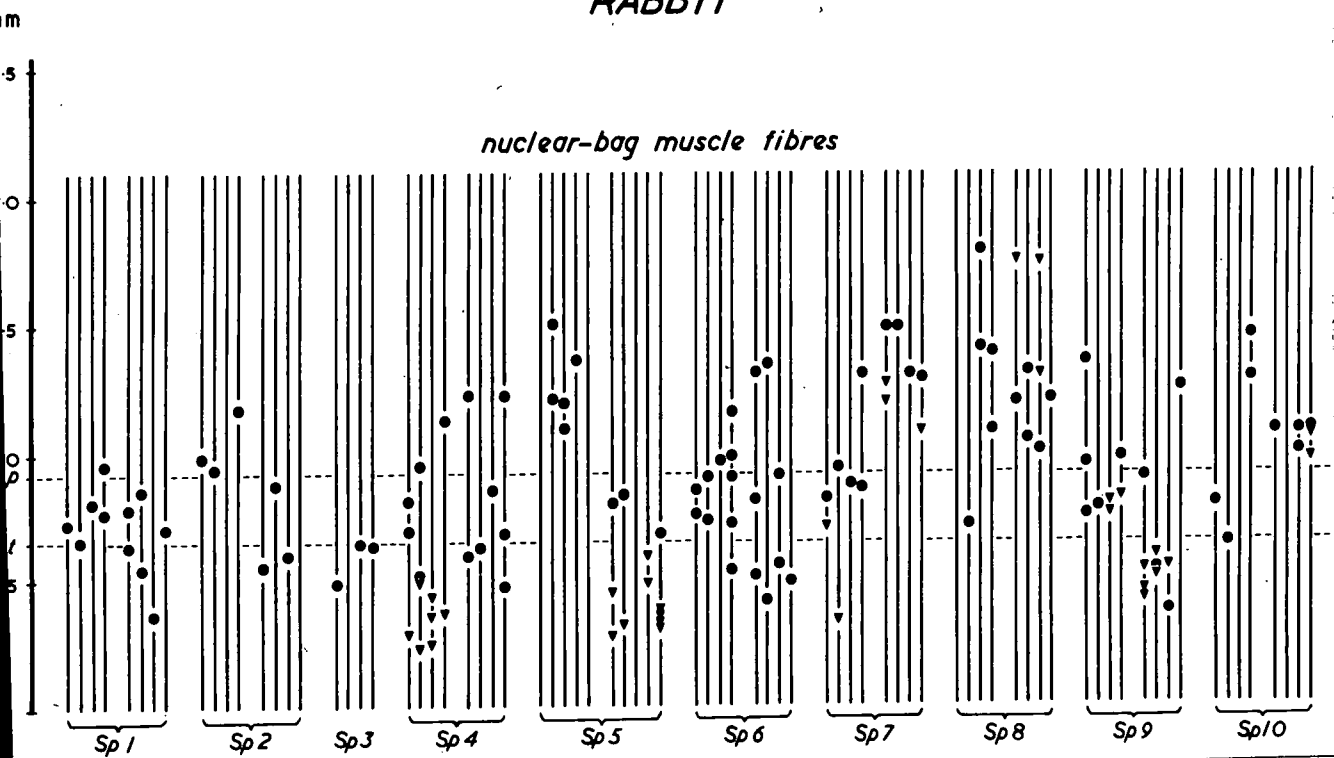
Text-figure 24. Graphic representation of the 12 completely analysed de-afferentated cat spindles. Each nuclear-bag (A) and nuclear-chain (B) polar half is represnted by a line. In case of whole spindles, the intrafusal muscle fibres are cut at the middle and put side by side separated by a wider gap to represent the two poles of the spindle in such a way that the 1st polar half of the 2nd pole is the counterpart of the 1st one of the 1st pole. All polar halves are standardized to the mean polar length, measurements being taken from the centre of nucleation in the equatorial region (0 mark) with the positions of the ramifications proportionately converted (see text on p. 94-95). It would be understood that the difference between the means of the plate and trail positions should be greater than it is now if, for example, the very exceptional 10 most distant trail ramifications in Sp 12 are excluded from Figure B. Then the mean of the positions of the trail ramifications should be lying around 0.7 mm. The key shows how plates are represented by filled circles and trails by filled triangles.

Sps 1, 2, & 3 from C159 peroneal II; Sps 4 & 5 from C172 peroneal II; Sp 6 from C159 peroneal III; Sp 7 from C159 popliteus; Sps 8 & 9 from C159 soleus; Sps 10-12 from C167 peroneal I. All are d/a.
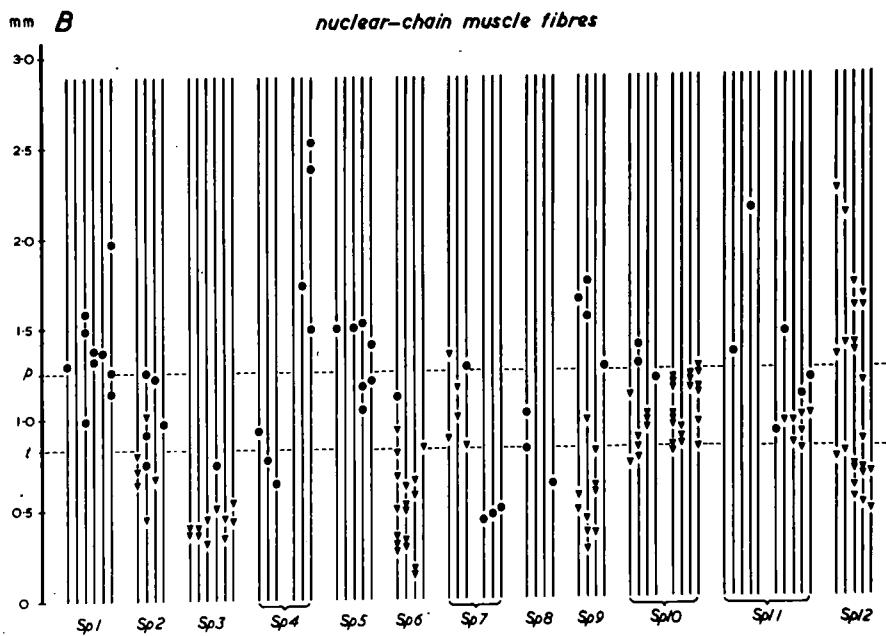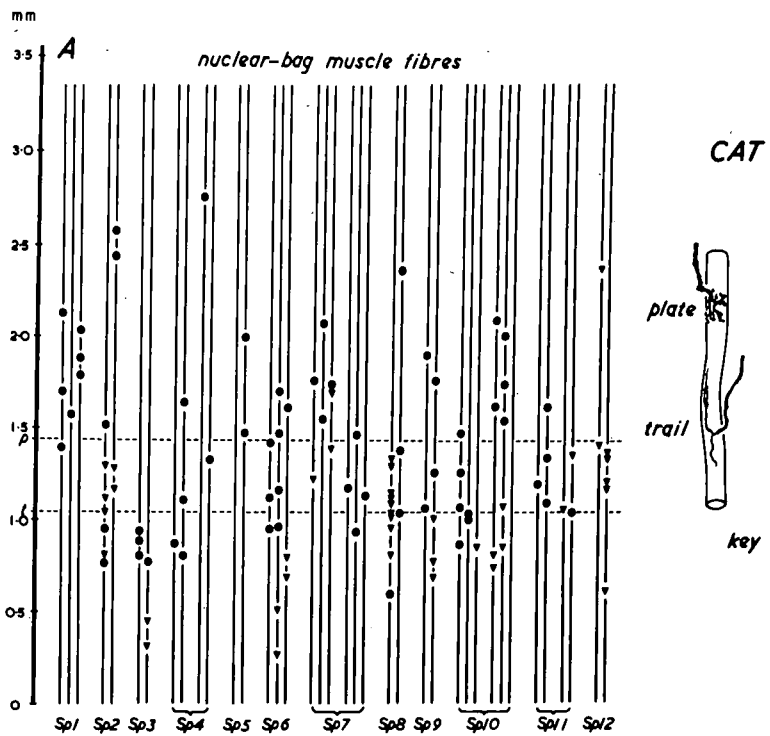
Text-figure 24.

Text-figure 25. Graphical representation of the 10 completely analysed rabbit spindles whose intrafusal muscle fibres (entirely of nuclear-bag fibres) are represented by lines. As in Text-figure 24, the two polar halves of all spindles are cut at the middle and similarly arranged into two groups. All polar halves are similarly standardized as before (see also text on p. 94-95). Again if those six exceptional trail ramifications in the right hand pole of both Sp 7 & Sp 8 are excluded, the mean for the trail ramifications would become lying around 0.5 mm. The same key as in Text-figure 24 was applied here. Sps 1, 2, & 3 from Rb11 peroneal I muscle (d/a); Sps 4 & 6 from Rb11 peroneal IV (d/a); Sp 5 from Rb11 peroneal III (d/a); Sps 7 & 8 from Rb17 soleus (d/a); Sp 9 from Rb17 peroneal I (d/a); Sp 10 from Rb7 vastus intermedius (n).

and complication, the extent of the lymph space or capsule length was omitted in both figures. But generally, trail ramifications are intracapsular and plate endings are extracapsular. It is also clear from these two figures that polar halves may receive a variable number of ramifications.

It is found that there are 64 end-plates and 41 trail ramifications terminating on the 38 nuclear-bag polar halves and 51 end-plates and 106 trail ramifications on the 68 nuclear-chain polar halves of the sample of 12 cat spindles. Hence, the average number of end-plates would be 1.7 (64/38) per each nuclear-bag polar half and 0.75 (51/68) per each nuclear-chain polar half. Similarly the average number of trail ramifications would be 1.1 (41/38) per each nuclear-bag and 1.56 (106/68) per each nuclear-chain polar half. If both plate and trail ramifications are treated together by adding their average numbers, then each nuclear-bag polar half would receive a total of 2.8 (1.7 + 1.1) and each nuclear-chain polar half 2.3 (0.75 + 1.56) motor ramifications (plates and trails). If the average number of motor ramifications for the entire intrafusal muscle fibre can be calculated by doubling that for the polar halves, then the average

number of end-plates is 3.4 per nuclear-bag and 1.5 per
nuclear-chain intrafusal muscle fibre.   Similarly, the
average number of trail ramifications is 2.2 per nuclear-
bag and 3.0 per nuclear-chain intrafusal muscle fibre.
It follows that if both plates and trails are treated
together, the average number of all the motor ramifica-
tions present would be 5.6 per each entire nuclear-bag
and 4.5 per each entire nuclear-chain intrafusal muscle
fibre.   While Boyd (1962b) in an assessment of the similar
kind, gives 4 γ_1 end-plates per nuclear-bag and 4 γ_2 end-
ings per nuclear-chain muscle fibre.   However, from the
assessment of the present study, it is certain that nuclear-
bag muscle fibres receive more end-plates while nuclear-
chain muscle fibres receive more trail ramifications.

In the case of rabbit spindles, there are altogether
94 end-plates and 35 trail ramifications in a total of 78
nuclear-bag polar halves.   Therefore in a similar way,
the average number of end-plates and trail ramifications
can be calculated to be 1.2 (94/78) and 0.45 (35/78) per
each nuclear-bag polar half respectively.   Again if the
entire intrafusal muscle fibre is taken into account, then
there would be 2.4 end-plates and 0.9 trail ramifications
making a total of 3.3 (2.4 + 0.9) motor endings per each

intrafusal muscle fibre in rabbit spindles. Barker
(1948) gives an estimate of 2 end-plates per each rabbit
intrafusal muscle fibre, and it is thus quite near the
figure obtained for the end-plates alone in the present
study. The difference between the average number of end-
plates and that of trail ramifications in either nuclear-
bag or nuclear-chain muscle fibres of cat spindles is
relatively small (3.4 against 2.2 for nuclear-bag and
1.5 against 3.0 for nuclear-chain muscle fibres) as com-
pared with the difference in rabbit spindles in which
end-plates occur nearly three times as often as trail
ramifications (2.4 against 0.9). This relatively higher
ratio of plate to trail ramifications in rabbit spindles
follows from the fact that rabbits have higher proportion
of plate-only spindles (see Table 2 on p. 85). Finally,
rabbit intrafusal muscle fibres can be seen to receive
fewer motor ramifications than cat ones although both
animals have more or less the same number of end-plates
per intrafusal muscle fibre.

4. 26 Distribution of plate and trail fusimotor fibre branches in terms of nuclear-bag and nuclear-chain muscle fibres

In a total of 51 plate fusimotor fibre branches found near or at spindle sites, innervating the 12 completely analysed cat spindles, 25 (or 49 %, nearly half) have their endings terminating on nuclear-bag intrafusal muscle fibres only; 17 (or 33 %) on nuclear-chain muscle fibres alone; and 9 (or 18 %) on both nuclear-bag and nuclear-chain muscle fibres together (see Table 6). In a similar total of 25 trail fusimotor fibre branches, 4 (or 16 %) have their endings on nuclear-bag muscle fibres only; 7 (or 28 %) on nuclear-chain muscle fibres alone; and 14 (or 56 %, more than half) on both nuclear-bag and nuclear-chain muscle fibres together. For plate fibre branches, those inner-vating nuclear-bag muscle fibres only are most common; while for trail fibre branches, those innervating both nuclear-bag and nuclear-chain muscle fibres together are much more numerous. The approximately comparative ratios of plate to trail fibre branches are 3:1 for those having terminations on nuclear-bag muscle fibres only; 1:1 for those innervating nuclear-chain muscle fibres alone; but 1:4 for those terminating on both types of intrafusal

Table 6 : Motor innervation on nuclear-bag fibres only, nuclear-bag and nuclear-chain fibres. and nuclear-chain fibres only.

Types of Fusimotor fibres	CAT Innervation of Fusimotor fibres on							RABBIT
	N.-bags only		N.-bag & -chain		N.-chains only		Total	Total
	Number	%	Number	%	Number	%		
Plate fibres	25	49	9	18	17	33	51	57
Trail fibres	4	16	14	56	7	28	25	12
Appr. Plate fibres	3		1		2			
ratio Trail fibres	1		4		2			

Data from the 12 completely analysed cat spindles (4 with 2 poles and 8 with only 1 pole analysed) and 10 rabbit spindles.

muscle fibres.   Also it can be seen that plate fusimotor
fibre branches occur twice as often as trail fusimotor
fibre branches (as far as near or at spindle sites is
concerned).   In the case of the 10 completely analysed
rabbit spindles, there are altogether 57 plate and 12
trail fibre branches found near or at spindle sites.
Hence, the ratio of plate to trail fibre branches in the
rabbit spindles is nearly 5:1 (compare with the ratio of
2:1 in cat spindles).

4. 27. Number of polar halves innervated by single fusimotor fibre branches

Fusimotor fibre branches may contribute terminals
to either one, some, or to all the intrafusal muscle fibres
in a spindle pole, and sometimes to a variable number of
muscle fibres in both poles.   The number of intrafusal
muscle fibres involved in the motor innervation by single
fusimotor fibre branches varies from one to eight (3 bag:
5 chain) in the cat, and from one to five in the rabbit.
It is complicated if nuclear-bag and nuclear-chain muscle
fibres are treated separately.   Because, for instance,
the involvement of 3 muscle fibres in the motor innerva-
tion of a single fusimotor fibre branch may be of either
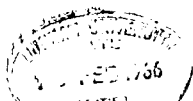
1 bag:2 chain; 2 bag : 1 chain; 3 bag; or 3 chain and so
on.   Therefore, for the sake of simplicity, both types
of intrafusal muscle fibres are treated together.   Since
the number of muscle fibres involved by a single fusimotor
fibre branch may be variable in both poles, two polar halves
of the same muscle fibre innervated by a fusimotor fibre
branch are treated as one muscle fibre while two polar
halves of different muscle fibres whether in the same or
different spindle poles innervated by a fusimotor fibre
branch are treated as two being affected by that fusimotor
fibre branch.   Thus in this way, all plate and trail fusi-
motor fibre branches of both cat and rabbit spindles from
the sample analysed in the previous section are classified
according to the number of intrafusal fibres involved in
the motor innervation by each of them.   The results are
given in Table 7.

It is difficult to determine whether single fusimotor
fibres are in reality fibre branches of the same fusimotor
fibre higher up in the nerve trunk, especially if they are
cut short during teasing or cannot be traced further back.
It is conceivable that many of those fibres traceable for
only up to 1.0 mm back to the nerve trunk could be the
terminal fibre branches of a fewer number of fusimotor

Table 7.   Classification of fusimotor fibres according to the number of intrafusal muscle fibres each of them innervates. Both in cat and rabbit are treated together. Each * means one fusimotor plate fibre innervating both poles.

Types of fusimotor fibres	Traceable distance of indivi. fusimotor fibres	Number of intrafusal muscle fibres each individual fusimotor fibre innervates								Total Number
		1	2	3	4	5	6	7	8	
Plate	0.1 – 1.0 mm	55	14*	4	1	1*				75
	1.0 – 1.5 mm	6	2	2						10
	1.5 – 2.0 mm	2	1	1		1*			1	6
	> 2.0 mm	6	5	4*	1	1*				17
Total number of fibres > 1.0mm		14	8	7	1	2			1	33
Trail	0.1 – 1.0 mm	11	8	3	6	1	1			30
	1.0 – 1.5 mm		1				1			2
	1.5 – 2.0 mm				1					1
	> 2.0 mm		1		1	1		1		4
Total number of fibres > 1.0 mm			2		2	1	1	1		7

fibres. Naturally, the further back the fusimotor fibre is traced, the more chance there is that it is a fibre and not a fibre branch. For the present purpose, it seems reasonable to take into account fusimotor fibres with traceable distance greater than 1.0 mm. Then, as can be seen from Table 7 there are 33 plate and 7 trail fusimotor fibres that can be traced back for more than 1 mm as single and separate fibres. For the plate fibres, a majority (14/33 or 42.4 %) terminate on one intrafusal polar half only. Approximately equal numbers of them terminate on 2 or 3 intrafusal polar halves. Those terminating on more than 4 intrafusal polar halves are comparative rare. For the trail fibres, they usually innervate more than one intrafusal polar half, and none innervates one polar half only. In this sample of plate fibres, there are altogether 5 fibres innervating both poles of the spindle and each is indicated by an asterisk in Table 7. It is clear that there are 3 out of 33 plate fibres (or 1 in 11) innervating both poles of the spindle. If, however, only those fibres terminating on 3 or more polar halves are taken into con-sideration, then the proportion becomes 3 out of 11 (or approximately 1 in 4). This last ratio seems in good agreement with the proportion of static fibres that give the 'driving' effect (see Discussion). It is hoped that

in the light of these findings, a significant correlation
between the two anatomical distinct groups of plate and
trail fusimotor fibres and the two functional different
groups of static and dynamic fibres may be interpreted in
a more satisfactory manner and will be discussed in more
detail in the last part of the Discussion.

4. 28. <u>Significant correlation of axon diameter
between plate and trail fusimotor fibre
branches and β or 'mixed' motor fibres</u>

Although there is no significant correlation between
the axon diameter of fusimotor fibre branches and the type
of motor endings they supply as claimed by Boyd, there is
a tendency for the largest fibre branches to supply plate
endings and the smallest ones to supply trail endings.
The axon diameter of 50 plate and 50 trail fibre branches
was measured around spindle sites at about 1.0-1.5 mm from
spindle entry with an oil immersion lens (same method as
employed by Boyd, 1962b) and the measurements were con-
structed into a histogram (Text-figure 26).   Although
there is a considerable overlap between plate and trail
fibre branches of 1.5 to 3.0/u axon diameter, fibre branches
measuring below 1.5/u or above 3.0/u at spindle sites are

almost exclusively trail and plate fibre branches, res-
pectively.

Around spindle sites branching of fusimotor fibre
branches before entering into spindles was frequently
observed (see Text-figure 27). In this preparation, a
fusimotor plate fibre branch (fu' a) of $3.0\,\mu$ branches
into two fibres approximately $300\,\mu$ away from the spindle
it innervates. The right branch terminates as an end-
plate on the pole of the spindle not shown in the figure.
The left one terminates as an extensive end-plate on a
nuclear-bag intrafusal muscle fibre and three small plates
plus a sprout on three nuclear-chain muscle fibres and also
another sprout can be seen in the middle between the end-
plate and the three small ones. The branching may be very
uneven, e.g. in Text-figure 28, one fusimotor trail fibre
branch (fu' a) of $3.1\,\mu$ axon diameter divides unequally to
give off branches of 1.4 and $2.9\,\mu$. A plate fibre branch
(fu' b) of $1.9\,\mu$ similarly divides but less unevenly, into
branches of 1.3 and $1.6\,\mu$ to innervate the two poles of the
spindle approximately 0.5 mm away. In another preparation
(not shown), one fusimotor plate fibre branch of $5.0\,\mu$
divides unevenly to give rise to two branches of 3.0 and
$4.0\,\mu$ to innervate two spindles. Searching extensively

Text-figure 26.   Comparative histograms of the axon diameter of
plate and trail fusimotor fibre branches of fifty each as measured in
teased, whole-mount de Castro's silver preparations as modified by
Barker & Ip, 1963.   Each reading is an average of six measurements made
at distances approximately 1 to 1.5 mm from spindle entry.   The sample
was taken at random and consisted of 5 peroneal I, 5 peroneal II, 2
peroneal III, 5 popliteus and 4 soleus de-afferentated cat spindles.
Notice that the majority of overlap occurs between fibre branches of
axon diameter from 1.5 to 3.0 μ.
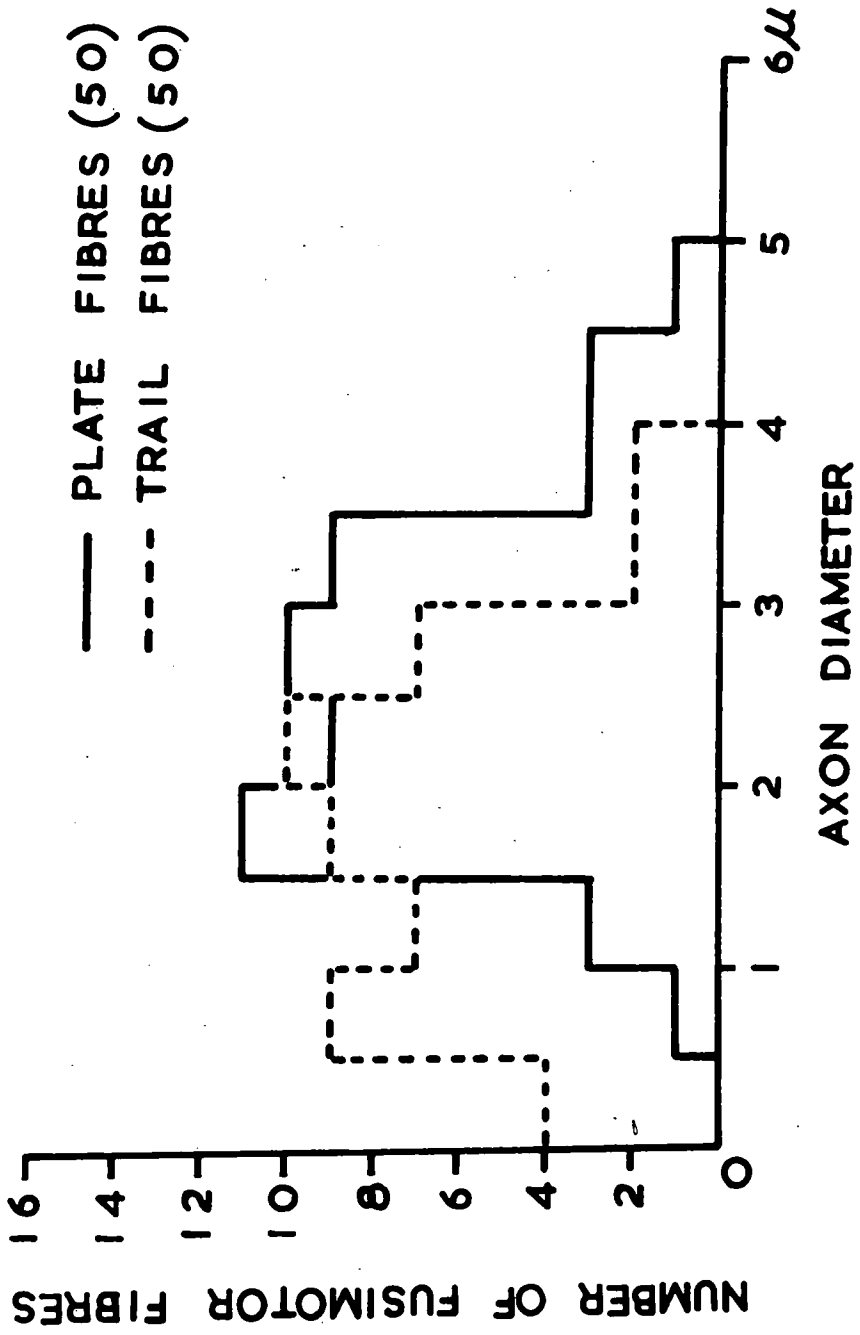
Text-figure 26.

Text-figure 27.   The entire fusimotor innervation
of one pole of a de-afferentated cat C159 popliteus spindle.
A 3.0 µu fusimotor fibre branch (fu' a) coming down from the
top left hand corner gives off two branches (a & b) at X.
The right hand branch (a) terminates as an end-plate on a
nuclear-bag intrafusal muscle fibre of the other pole not
shown in the picture.   The left hand branch (b) terminates
as an end-plate (e1) on a nuclear-bag intrafusal muscle
fibre and 3 small plates (e2) plus one sprout (s1) on three
nuclear-chain muscle fibres on extreme right and another
sprout (s2).   Another 2.0 µu fusimotor fibre branch (fu' b)
coming from the left side gives off an extensive end-plate
(e3, seen in edge-on view) terminating on a nuclear-bag
muscle fibre and a young plate (y.p.) plus a terminal bulb
(b.a.) on extreme right on another nuclear-bag muscle fibre.
Two more end-plates (e) can be seen given off by another
fusimotor fibre branch (fu' c)   Teased, whole-mount modified
de Castro's silver preparation.

b. a. ball of axoplasm;   e 1, 2, 3 intrafusal motor end-
plates;   fu' a, b, c fusimotor fibre branches;   s. sprout;
y. p. young end-plate.
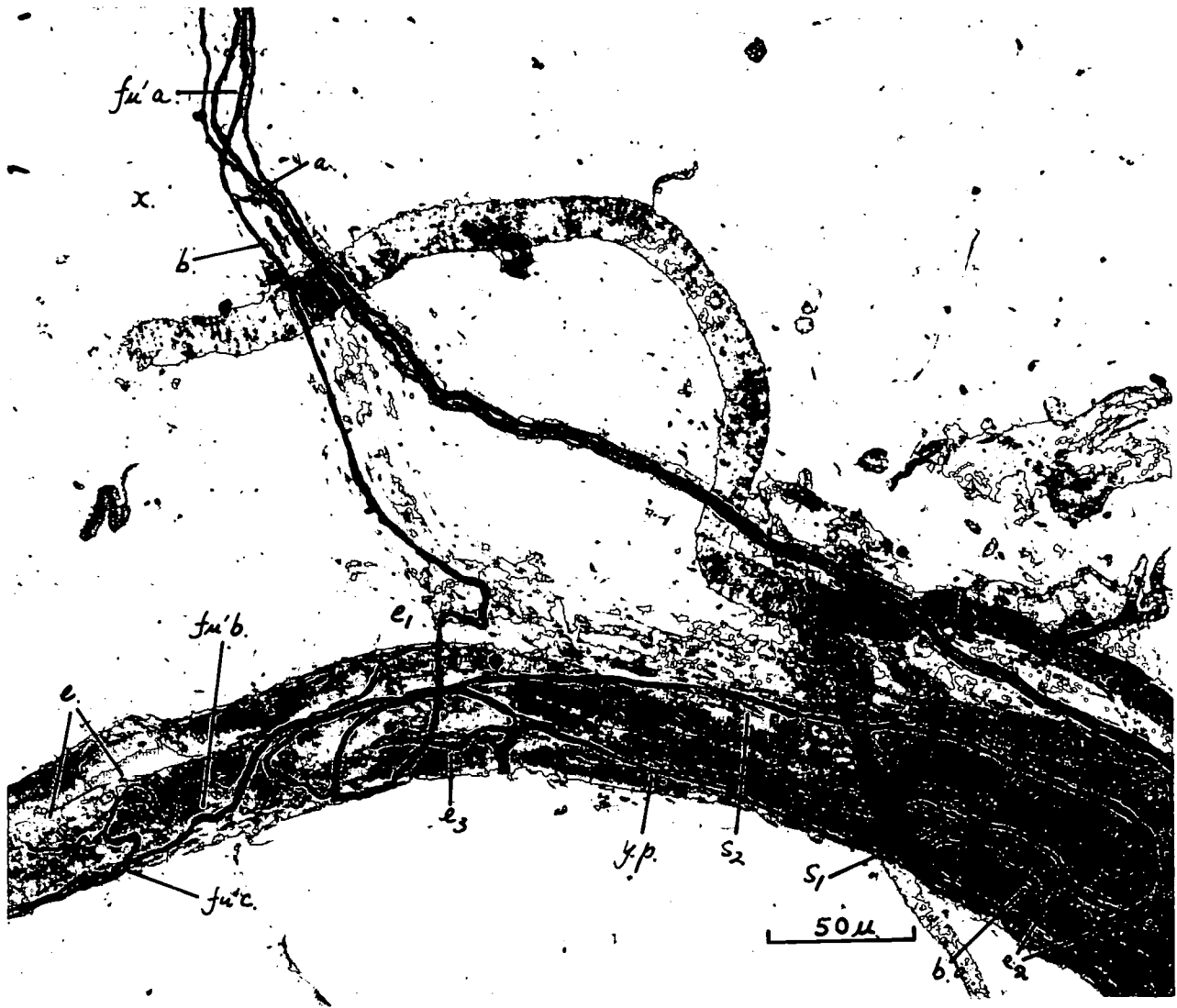
Text-figure 27.
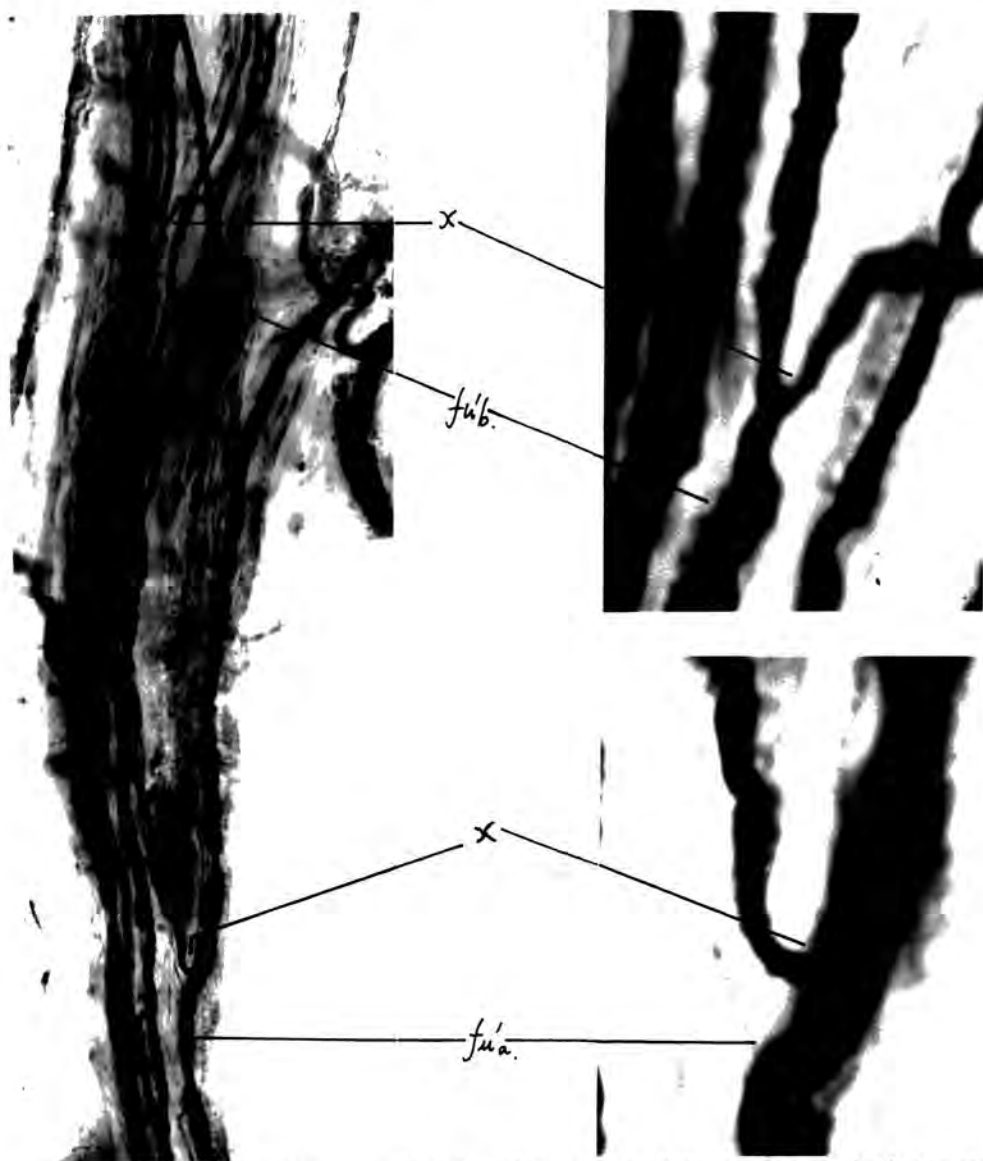
Text-figure 28.   Photomicrographs of the intramuscular
bundle of motor nerves approximatelt 0.5 mm from a spindle to
show the uneven branching of motor nerve fibres.   Teased,
whole-mount modified de Castro's silver preparation taken from
a de-afferentated cat C159 soleus muscle.   The right hand side
pictures show the points of branching more clearly at a higher
magnification.   See text on page 106.

fu' a & b, fusimotor fibre branches;   x,   point of branching.

for these cases is beyond the scope of the present study. Moreover, a detailed account of this uneven motor fibre branching intramuscular or around spindle sites is given by Adal & Barker (1965a).

In eight cases of cat and two cases of rabbit, a motor fibre was observed to supply end-plates to both intrafusal and extrafusal muscle fibres (see Text-figures 29 & 30). But motor fibres supplying trail ramifications to intra- and extrafusal muscle fibres have not been observed, nor have trail ramifications ever been observed terminating on extrafusal muscle fibres only.

4. 29. <u>Fusimotor fibre branches innervating two or more spindles and sudden drop in diameter of fibre branches after entering the spindle</u>

Six cases in cat and one case in rabbit where branches of the same fusimotor fibre innervate two or three spindles have been found. Only some of these cases are given in Text-figures 31 & 32. Text-figure 31 shows three such cases in which a fusimotor plate-ending fibre branch innervates two spindles close together terminating as end-plates. These fusimotor fibre branches that innervate more than one

Text-figure 29.   Part of a de-afferentated (d/a)
spindle of rabbit (a) and cat (b).   In each of them a single
motor fibre branch innervates both the extrafusal (on the
left) and the intrafusal (on the right) muscle fibres.
Whole-mount modified de Castro's silver preparations teased
from the following muscles:
a, rabbit Rb17 interosseus (d/a);   b, cat C159 interosseus
(d/a).
e. e. extrafusal end-plate;   i. e. intrafusal end-plate.

Text-figure 30. The low power photomicrograph on the
left shows that a motor fibre branch (m.f.) innervates both
intrafusal and extrafusal muscle fibres. One branch (b.a.)
of it terminates as an extrafusal end-plate (the middle one
of the three). The other branch (b.b.) innervates a muscle
spindle nearby on the top right corner across a group of
extrafusal muscle fibres. The inset is at a higher magnifica-
tion to show more clearly the point of branching (x) of this
motor fibre. Modified silver preparation teased from cat C159
interosseus (d/a). E. e. extrafusal end-plates;
i. e. intrafusal end-plates.

spindle may terminate either on a mixture of nuclear-bag and nuclear-chain muscle fibres of one spindle (for example, spindle a & spindle c in Text-figure 32) and on nuclear-bag fibre alone in another spindle (spindle b in Text-figure 32) or more often on only nuclear-bag muscle fibres of all spindles concerned.   Other combinations of nuclear-bag and nuclear-chain muscle fibres in all the spindles innervated by such fusimotor fibre branches have not yet been observed, but I would rather reserve to say that there is no such combined terminations by these fibre branches. So far, motor fibre branches terminating as trail ramifications in two or more spindles have not been seen.

Sometimes fusimotor trail fibre branches exhibit a sudden drop in axon diameter especially after entering the spindles.   Both Text-figures 33 & 34 illustrate these cases. In Text-figure 33, a fusimotor trail fibre branch of 2.9/u axon diameter exhibits a marked sudden drop in diameter to less than 1/u and remains thin for the whole course of its innervation for a distance of 500/u in this de-afferentated cat spindle.   A similar sudden drop in diameter is also shown in Text-figure 34.   Incidentally in both cases, one of their branches exhibits a 'recurrent' phenomenon by turning back into the incoming nerve trunk (see also p. 40).
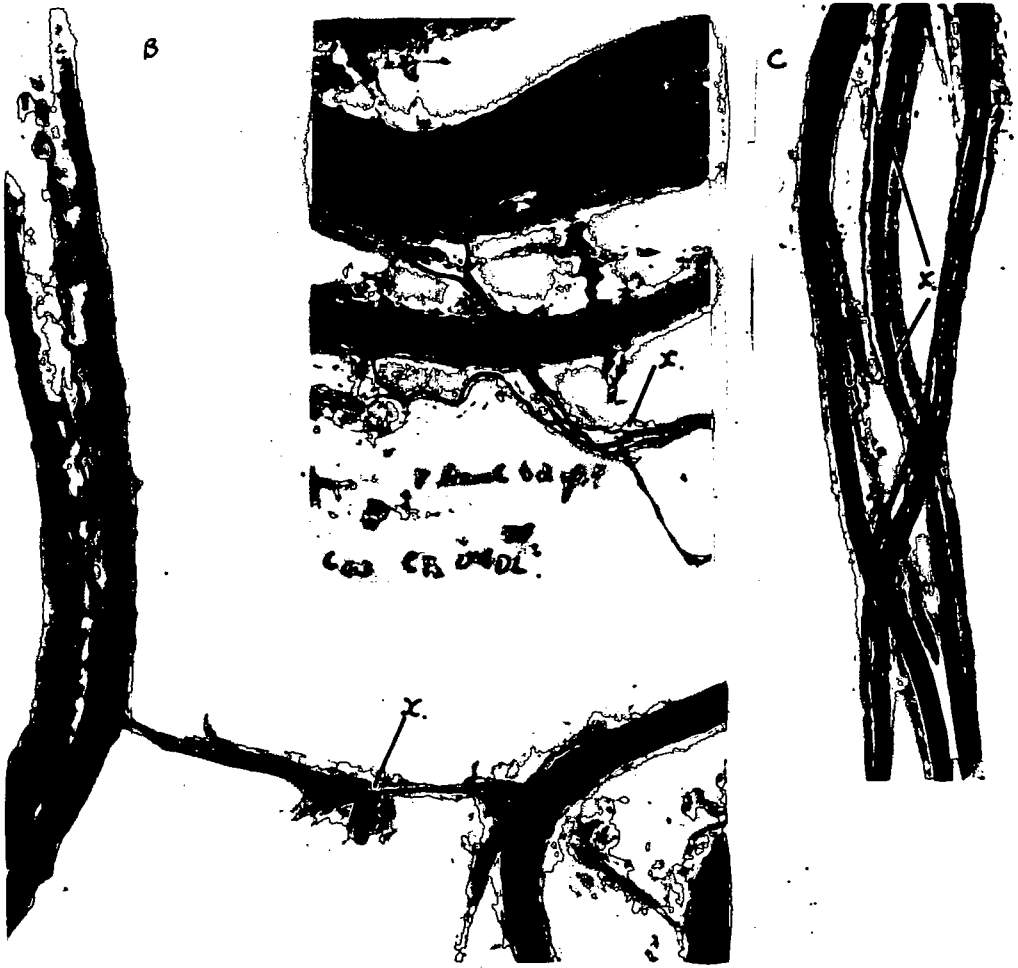
Text-figure 31.　Photomicrographs of normal (n) and
partial de-afferentated (p. d/a) cat spindles showing in
all of them a fusimotor fibre branch innervating more than
one spindle.　Whole-mount modified de Castro's silver pre-
parations of spindles teased from the following muscles:

a, cat CT2 2nd deep lumbrical (p. d/a);　b, cat C160 1st
deep lumbrical (n);　c, cat C169 superficial lumbrical (n).
x,　point of branching of the fusimotor fibre branches.

Text-figure 32.   Accurate tracings of the polar halves of three normal muscle spindles from the 2nd deep lumbrical muscle of cat C172 to show the motor innervation of more than one spindle by single fusimotor plate-ending fibre branches.   The spindles are slightly re-orientated to suit space.   Part of the periaxial lymph space with the investing capsule lamellae is shown in each case. There are two fusimotor plate fibre branches innervating these three spindles, all other innervations being omitted for simplicity.   Both fibres divide into two branches; one branch from both fibres innervates the nuclear-bag muscle fibre of the middle spindle (Sp. b), while the other branch of a fusimotor fibre (fu' a) and that of the other (fu' b) innervate a mixture of nuclear-bag and nuclear-chain muscle fibres of the top (Sp. a) and bottom spindle (Sp. c) respectively.

b, nuclear-bag intrafusal muscle fibre;

c, nuclear-chain intrafusal muscle fibre.

*sp. a.*

fu′a.

fu′b

sp. b.

100μ

sp. c.
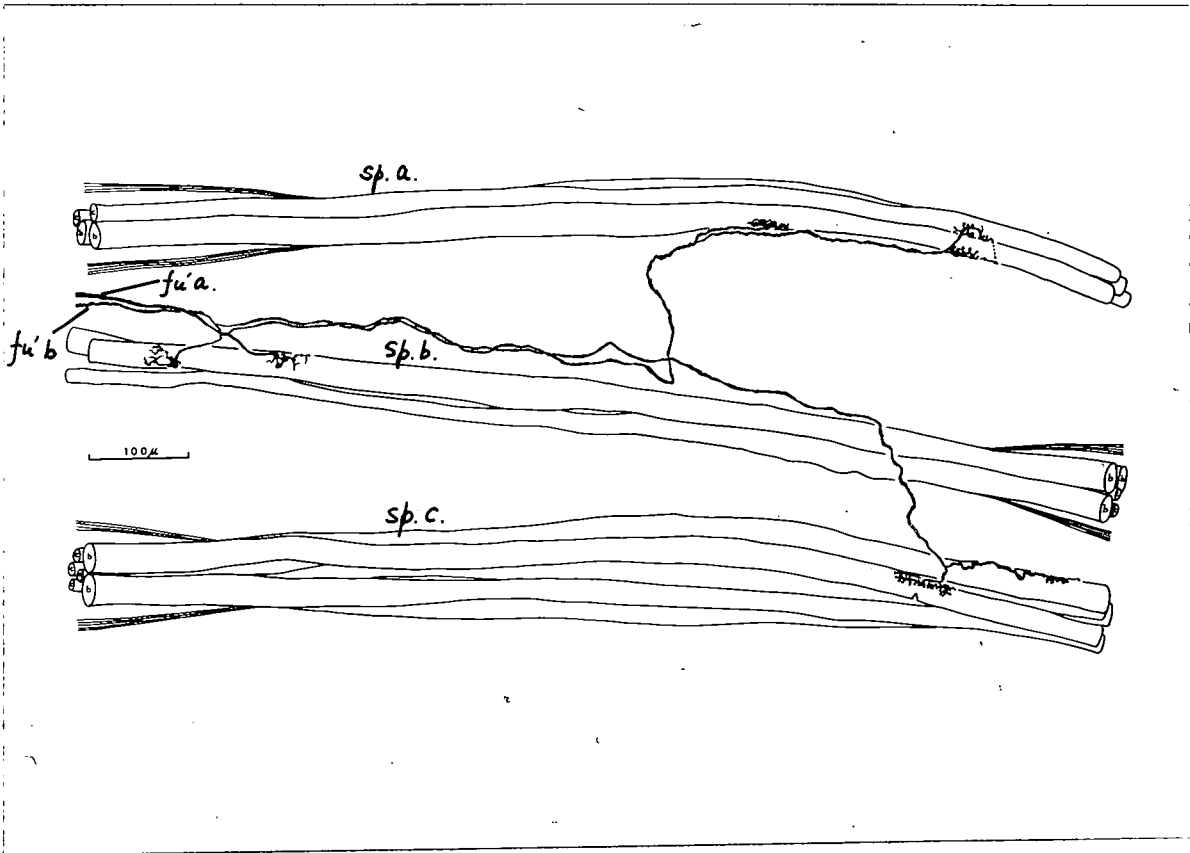
Text-figure 32.

Text-figure 33.   Photomicrograph of part of a cat C167 peroneal I (d/a)
spindle showing the sudden drop in axon diameter of a fusimotor trail fibre
branch (fu' a).   This trail fibre branch of 2.9 μ axon diameter drops suddenly
in diameter to less than 1 μ after entering the spindle.   Then it divides into
two branches.   The top branch after going towards the equatorial region for a
distance of 500 μ turns back into the incoming nerve trunk.   It gives off two
trail ramifications (out of focus) one before and one after it turns back.
The other branch (not shown) gives off one trail ramification each to a
nuclear-bag and a nuclear-chain muscle fibre.   The course of all these branches
and ramifications remains thin at about 1 μ.   Teased, whole-mount modified de
Castro's silver preparation.   fu' a fusimotor trail fibre branch;
r. f. 'recurrent' axon;   x. point of branching.

Text-figure 34.   Photomicrograph of part of the equatorial region of a de-afferentated (d/a) cat C167 peroneal I spindle.   A fusimotor trail fibre branch with average axon diameter of $2.5\,\mu$ is seen to divide at 'x' giving rise to three branches one of which (lowest in the picture) again divides into two branches innervating the two poles of the spindle.   The second branch innervates the right pole while the third one (r.f.) turns back and goes along the same path of its parent fibre and is lost in the bigger nerve trunk approximately $1600\,\mu$ from the spindle.   This trail fibre branch exhibits a sudden drop in diameter after entering the spindle.   Incidentally, two nuclear-bags (n-b.) occurring at different levels are clearly visible as indicated.   Teased, whole-mount modified de Castro's silver preparation.

c. l.   capsule lamellae;   fu' a   fusimotor trail fibre branch;   l.s.   lymph space;   n-b.   nuclear-bag; r. f.   'recurrent' fibre branch;   x.   point of branching.
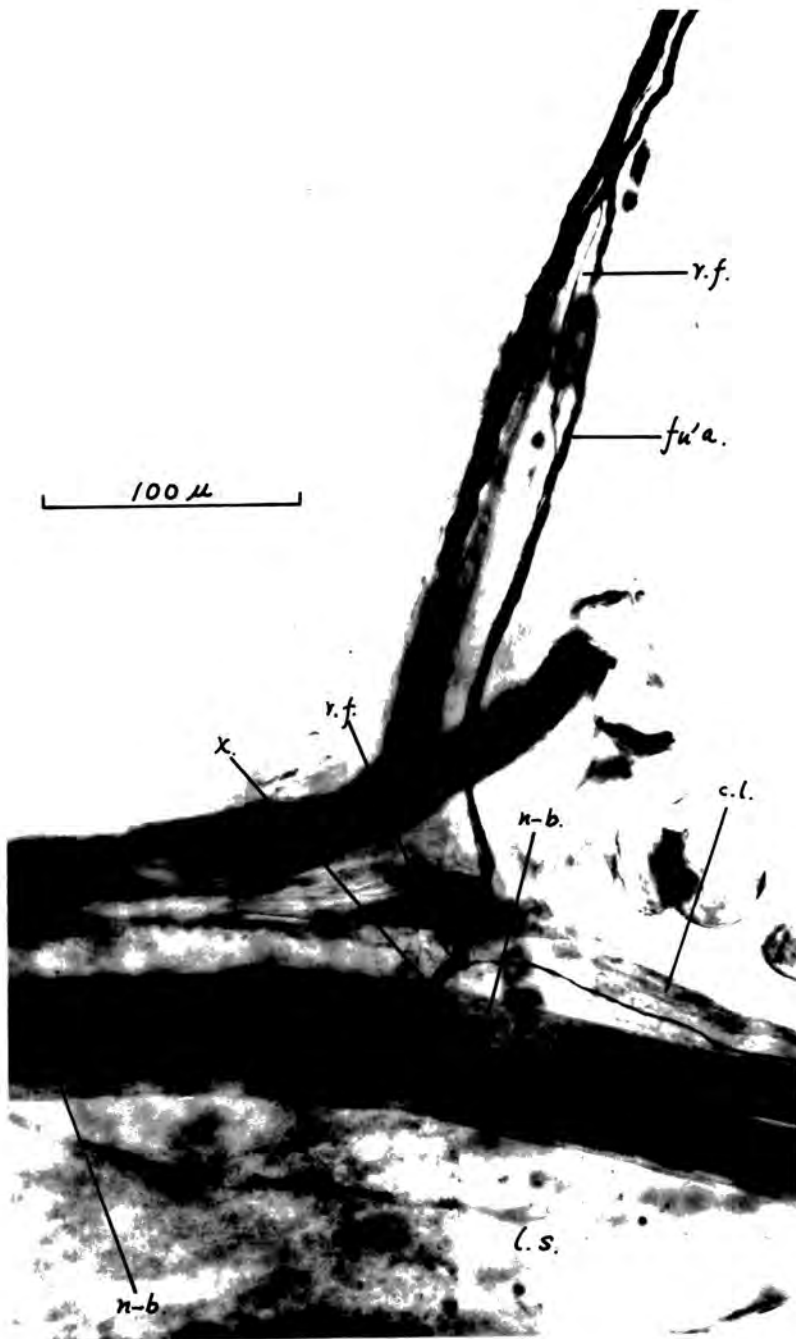
Text-figure 34.

5. Discussion

The existence of trail endings in the majority (92 %
in the cat, and 69.7 % in the rabbit) of normal as well as
de-afferentated spindles shows that they are a true
structural type of motor ending and definitely not of sym-
pathetic nor sensory origin. Although Threadgill (1947)
has put forward possible evidence of afferent conduction
via sympathetic ganglia, such a possibility does not merit
serious consideration. This is because firstly, his claim
by itself needs further supporting evidence histologically.
Secondly, such afferent conductions were demonstrated by
him to subserve pain sensation only and besides, usually
a consecutive series of several dorsal root ganglia was
removed during de-afferentation thus greatly reducing the
possibility of such an unusual pathway for afferent fibres.
Moreover, the absence of any visible innervation in spindles
after both sensory and motor roots are cut as found by
Hinsey (1927), Hines & Tower (1928), and in the present
investigation demonstrates that the trail ending is not
sympathetic in origin.

Even allowing for the maximum occurrence of 0.5 % of

sensory overspill would not account for such a large number
of trail ramifications as found in all de-afferentated
spindles.   In fact, the absence of any primary (Group Ia),
tendon-organ (Group Ib), and secondary (Group II) fibres or
endings in so many extensively teased muscles from de-
afferentated animals whose roots were found to contain
sensory overspill, suggests that these aberrantly placed
cells may probably take part in the innervation of the skin.
The presence of trail endings in equally abundant propor-
tions in spindles from the most satisfactorily de-afferentated
cat C167, which was found to have no sensory overspill,
naturally forms another conclusive clue to show that they
are definitely motor in origin.

The simple collateral sprouting demonstrated by Edds
(1950; 1953) takes more than one week to make its appearance.
If the trail ramifications are in reality sprouting of
similar kind as a result of or enhanced by the effect of de-
afferentation, then their presence in short-term de-
afferentated spindles of one week degeneration period should
be scarce and negligible.   But it was found that this was
not so because the trail ramifications in these spindles
were already as well established and extensive as in any
other de-afferentated spindles of longer degeneration period.

The repeated and unfailing finding of trail endings in high proportions in both normal and de-afferentated spindles removes the suspicion that they may be collateral sprouts of plate-ending fibre branches undergoing replacement. The fact that trail endings are characteristically juxta-equatorial renders it unlikely that they are replacement configurations, for these may occur anywhere. Moreover, they can further be confirmed as motor by the cholinesterase demonstration of Hess (1961) who demonstrates that at the juxta-equatorial regions of rabbit and rat spindles there is a multiterminal type of motor endings which were found also to be the case in the cholinesterase preparations in the present study and have thus been confidently interpreted as the equivalent of trail endings in silver preparations. Kupfer (1960), on the basis of the cholinesterase localization in muscle spindles of the human extraocular muscles, also states that there appear to be two types of motor nerve endings.

It is also quite probable that Boyd's γ_2 net-works demonstrated by a less satisfactory and more granular staining method using gold which is undoubtedly unable to stain the fine axon tips distinctly, correspond in reality to a mixture of trail ramifications plus possibly some small end-

plates on the nuclear-chain intrafusal muscle fibres.
Even Boyd (1962b) admits that --"in a few spindles one of
the nuclear-bag fibres may have a γ_2 ending in the S_2
region in addition to the usual γ_1 end-plates in the S_3
region ...(p. 127)", and considers such as perhaps an
anatomical peculiarity. But it has been shown in the
present study that very often nuclear-bag muscle fibres
may receive both plate and trail endings each occurring
at the same region described by him. It seems more likely
that the gold method would offer much less chance of
detecting such γ_1 and γ_2 endings on the same intrafusal
muscle fibre and finding those fusimotor fibres innervating
both nuclear-bag and nuclear-chain muscle fibres as can be
observed in the more delineated silver impregnated prepara-
tions. Nevertheless, no matter whether it is the multi-
terminal endings of Hess, γ_2 net-works of Boyd, or trail
endings of ours, these endings are usually shown to occur
near or at the juxta-equatorial regions, and thus, in
position alone, apart from any possible structural identity,
they correspond to one another so well that they could be
nothing but the same type of motor endings and represent a
second true structural form of mammalian intrafusal motor
endings in addition to the usual well-known plate endings
which are generally situated more distally from the equatorial

region as has been shown in many photographic examples.
The sudden drop in diameter exhibited exclusively by trail-
ending fusimotor fibre branches found in the present study
and reported in the γ_2 fusimotor axons by Boyd further
suggests that both trail and γ_2 endings are the same thing.

Grouping of fusimotor fibres into functionally and
morphologically distinct classes according to their diameter
or conduction velocity measurements alone is evidently far
too simple and arbitrary as can be seen from the work of
Bessou, Emonet-Denand & Laporte (1963a; 1965), Crowe &
Matthews (1964b), Brown, Crowe & Matthews (1965), and Adal
& Barker (1965 a, b). Both thickly-myelinated large axons
of diameter between 2.5μ and 4.0μ (Boyd's γ_1) and thinly-
myelinated small axons of diameter between 1μ or less, and
2.0μ (Boyd's γ_2) at spindle sites can be derived from large
or small γ-stem fibres of total diameter between 4.0 - 7.5μ
or 2.5 - 3.5μ or even from mixed (β) fibres of 6.0 - 12.5μ
in the muscle nerve as shown by Adal & Barker (1965a).
Even at spindle sites, unequal branching and sudden drop in
diameter (exhibited by trail-ending fibres) of fusimotor
fibres to considerable extent are often encountered in the
present study. Naturally, both these phenomena can occur
higher up in the intramuscular nerve trunk or muscle nerve

and can easily lead to confusion and erroneous interpretation
of fusimotor grouping. Thus, it was agreed in the Stock-
holm symposium that the γ_1 and γ_2 terminology of Boyd
should be abandoned and instead, plate and trail fusimotor
fibres should be used in its place.

With regard to the α-fibre participation in the inner-
vation of the mammalian muscle spindles, practically all
motor fibres of total diameter above 6.0μ may contribute
terminations to both extrafusal and intrafusal muscle fibres
according to Adal & Barker's finding (1965a). However, it
is shown that only those motor fibres of medium size are the
real 'mixed' motor fibres in the sense that they contribute
almost equally to both types of muscle fibres whereas motor
fibres of the α group tend to have much greater contribution
to extrafusal muscle fibres and motor fibres towards the γ
range tend to innervate intrafusal muscle fibres predomin-
antly. It is for this reason that it has been suggested
(see Section 3; also Barker & Ip, 1965b) that the trivial
cross-connexion of such predominantly skeletomotor or pre-
dominantly fusimotor fibres may be the result of skeleto-
motor or fusimotor sprouting respectively. Therefore,
such a cross-connexion which is usually found only in the
vicinity of spindles by ordinary histological methods cannot

be determined with certainty whether it comes from predominantly skeletomotor, predominantly fusimotor, or true 'mixed' motor fibres.

Although cases of the skeleto-fusimotor connexions are only rarely found in the vicinity of spindles in the present study, the actual percentage of occurrence of these cases is much higher as indicated by the electrophysiological findings of Granit, Pompeiano & Waltman (1959a; b), Rutledge & Haase (1961), and Bessou, Emonet-Dénand & Laporte (1963a; 1965). This discrepancy is not surprising if one considers the extreme difficulty of finding by ordinary histological methods all such possible skeleto-fusimotor collateral branchings (see Barker & Chin, 1961), unbroken during the course of preparation from start to finish and not obscured from observation by the presence of other tissues. Moreover, even if this is achieved, only those occurring relatively close to spindle sites in the most favourable conditions may have a chance of being observed. In fact, the intramuscular distance between the point of branching into skeleto-fusimotor and the point of the cross-connexion in some cases is shown to be longer than 8 mm even in a tiny muscle such as the 1st deep lumbrical muscle of the cat (see m_4 in text-figure 1 of Adal & Barker, 1965a). Such a

connexion, especially in much larger shank muscles, could never be preserved intact in ordinary routine teasing and consequently is extremely difficult to be detected. The same sort of technical difficulty would naturally be expected for those cases in which one fusimotor fibre branch innervates more than one spindle. In a similar way, the probable occurrence of fusimotor fibre branches innervating two, three, or more spindles with trail terminations cannot be dismissed because of the same reason. In fact, it seems most probable that fusimotor fibres of this kind do exist because physiologically single static or dynamic fusimotor fibres have been found to have effect on more than one primary ending by Crowe & Matthews (1964b), and Brown, Crowe & Matthews (1965).

The fairly constant and comparatively high percentage of PT:TP motor innervation type in spindles throughout various hindlimb muscles of the cat may possibly indicate that this is the most favourable type of spindle to provide the fullest and most effective control of the afferent discharge. In such spindles, two kinds of contraction effected by two types of morphologically distinct motor endings produce two different types of afferent discharge probably for compromising the change in length and the rate of lengthening and shortening over a longer span of

the muscle in which they are embedded, since they contain both types of motor endings on both poles. If this is so, it would not be difficult to realize why spindles with purely plate terminations on both poles occurred in about 8 % of the cat spindles while no spindle was found to have only trail terminations on both poles, although approximately 12 % of the cat spindles were innervated by only trail endings on one of their poles (see also Boyd, 1962b, p. 116 "one or two tenuissimus spindles have γ_1 end-plates at one end only but this is rare"). Thus it seems to suggest that spindles with purely one type of motor control over both poles are present while spindles with purely another type of motor control on both poles are absent. Either the actual situation is much more complicated than the straight-forward correlation of plate/ static and trail/dynamic (or vice versa), or perhaps some other mechanisms are involved such as the relative number and position of the motor endings, or even perhaps only one type of fusimotor control is essential while the other type is optional for the normal working of the mammalian spindle. The occurrence of trail endings on only one of the poles especially that one which is short and tendinous of spindles frequently found in most of the tiny muscles of the foot is perhaps another significant phenomenon.

Finally, it must be pointed out that there may be some
significance in the percentage of different types of
spindles according to their types of motor innervation
in the slow (represented by soleus) and the fast (repre-
sented by FDL) muscles as can be seen from Table 2,
columns 2 & 7, on page 85.   Then it would be of the utmost
interest to find out among other significant structural
changes whether there is any change in the percentage
composition of various spindle types in the reinnervated
spindles of the slow (soleus) and the fast (FDL) muscles
which have been subjected to experimental cross-reinner-
vation (Buller, 1965).

It is difficult to understand why sometimes two motor
endings of different types occur so close together as to
occupy the different sides of the same site.   Whether they
work in harmony, or separately so that one modifies the
action of the other, is hard to tell.   However, innerva-
tions on widely spaced regions of the intrafusal muscle
fibres either by the same or different types of motor
endings could be understood in the sense that for the best
working efficiency of the spindle, it should be able to
have different controls by a variety of motoneurons in
order to give a wider control of either differential or

summation in nature on some, even if not on all, of its
intrafusal muscle fibres.   On the other hand, it would
be difficult to explain those innervations in some of the
intrafusal muscle fibres by several motor endings of the
same motoneuron.   As has been shown, such intrafusal
muscle fibres may receive several motor terminations some-
times very close together, supplied by the same fusimotor
fibre branch.   It may be argued that the purpose of such
a multiple innervation is for one motor terminal to reinforce
the action of the other and vice versa so that by having
more motor terminals from the same motoneuron, an intra-
fusal muscle fibre can be sure to receive message from this
motoneuron amplified manifold at the ending level compared
with having only one motor terminal.   Alternatively, it
may be supposed that motor terminals cannot function
continuously and this seems to be the case because Luco &
Rosenblueth (1939) showed that after prolonged stimulation
of a curarized muscle until the curarization was over, the
muscle still remained unresponsive and thus concluded that
the end-plate fatigue had occurred probably due to exhaustion
of the transmitter, acetylcholine.   Thus, it seems probable
that the arrival of nerve impulses at the motor terminal
triggers off (but not initiates, see Katz, 1961) a sudden
increased liberation of the transmitter and this can only

happen possibly for a certain number of times after which
the transmitter becomes temporarily exhausted so that the
motor terminal remains quiescent for the accumulation of
a new supply of the transmitter.   During this quiescent
period, which may be very brief, the terminal is no longer
responsive.   If there is any truth in this, then it would
seem to be a great advantage for motoneurons to have
several, instead of just one, motor terminals near one
another on the same muscle fibre acting as a safety reserve
to make sure that muscle fibre they innervate to function
uninterruptedly.   This is perhaps because it may be possible
that the exhaustion takes place one by one in succession,
although very briefly, between the terminals of the same
fusimotor fibre terminating on the same muscle fibre.

The motor unit of the skeletomotoneurons is generally
so large that the failing of some of their terminals whose
transmitter has become temporily exhausted would be im-
material and undetected so long as the overall working
efficiency is concerned compared with the relatively
smaller fusimotor unit.   The low innervation ratio (small
motor units), and multiple or polyneuronal innervations in
the eye and some tiny muscles such as the human vocalis
muscle (see Rossi & Cortesina, 1965) may all point to the

same direction to ensure that these small muscles can
perform delicate functions of extreme precision and
constant steadiness.   Thus, it seems highly probable
that a short-term continually repeated stimulation of the
motor terminal may lead to its temporary exhaustion or
fatigue to response normally whereas a long-term continual
working of the motor terminal may necessitate its repair
or replacement in part or altogether.

Despite of the enormous difference in length and in
diameter between extrafusal and intrafussl muscle fibres ,
the difference in length of their end-plates are compara-
tively much less marked.   There is perhaps a definite
optimal junctional area for each type of muscle fibre in
the normal functioning of the motor endings.   The fact
that the mean length of intrafusal motor end-plates is
greater than that of extrafusal ones does suggest that
there is an optimum junctional area.   The width of the
intrafusal muscle fibre is much smaller and is near the
limit of the width of most of its end-plates so that any
expansion in area of intrafusal end-plates in approximation
to the size of extrafusal ones naturally goes lengthwise.
What seems peculiar is that although theoretically the
motor end-plates could increase their size by wrapping

round the muscle fibres they innervate, this has not been
observed in this study. Instead of forming giant-sized
end-plates, motoneurons are able to increase their terminals
junctional area by folding to form what is called 'synaptic
gutters' as demonstrated by Robertson (1956b). By so
doing, the actual area of junctional contact of the end-
plate would be increased manifold. It is probable that
a propagated contraction of the muscle fibre occurs only
when a certain minimum amount of the chemical transmitter,
acetylcholine, released by the nerve terminal across the
synaptic gap in response to the nerve impulses is reached.
Maybe the effective area needed for the passage of this
minimum amount of transmitter across the gap to cause pro-
pagated contractions approaches the total area of the
gutters in the end-plate. Then the amount of acetylcholine
able to cross the synaptic gap of the axon tip of trail
ramifications would be hundreds or thousands times smaller
owing to their obviously much smaller junctional area even
if a large amount of transmitter is caused to released pre-
synaptically in response to the same or stronger stimulus.
Consequently, the trail ramifications can just cause a
small and localized contraction due to the sub-threshold
amount of transmitter passing across the junction which
fails to reach the minimum level required for large and

propagated contractions.   But the electron microscope
photographs of spindles shown recently by Landon at the
Dundee symposium (1965) do not seem to show any sign of
synaptic gutters in the intrafusal motor plate endings.
Whether the discrepancy is due to the application of two
different techniques employed by Robertson and Landon, or
more likely due to the actual significant difference between
the structure of the extrafusal and intrafusal motor end-
plates is uncertain.

It has been shown that not only there is a difference
in behaviour of the primary and secondary endings under
various mechanical stimuli (Cooper, 1959; Lundberg &
Winsbury, 1960; Harvey & Matthews, 1961b; Appelberg, 1962;
Bessou & Laporte, 1962; Bianconi & van der Meulen, 1963);
but there is also a difference in the frequency of afferent
discharge when the muscle is passively stretched dynamically
or at constant lengths (Harvey & Matthews, 1961b; Bessou
Laporte, 1962).   Furthermore, two functionally distinct
kinds of fusimotor fibres were discovered to have different
effects on the response of the primary ending (Jansen &
Matthews, 1961, 1962b; Matthews, 1962; Crowe & Matthews,
1964a).   Therefore, functionally speaking, fusimotor fibres
can be classified either as dynamic fibres if they increase

the 'dynamic index' or as static fibres if they do not alter or even decrease this index. The dynamic index, which is really an arbitrary measure of the response of the sensory ending to the dynamic component of the stimulus (stretching), is defined by Crowe & Matthews (1964 a, b), on the suggestion of Laporte, as the slowing of the discharge frequency of the ending occurring on completion of the dynamic phase of stretching as measured over the first 0.5 second. Or in other words, the dynamic index is just the measure of the difference between the frequency discharge of the sensory ending before the end of the dynamic phase of stretching and that occurring at final maintained stretch 0.5 sec. after stretching is ceased. It has been demonstrated, as mentioned before, in the spindles of the cat and rabbit there are two morphologically distinct types of motor endings; one is the discrete end-plate and the other is the diffuse or multiterminal trail ending (Hess, 1961; Boyd, 1962; Barker & Ip, 1965b) suggesting possibly two means of activating the intrafusal muscle fibres. Naturally these two different ways of classifying two distinct types of fusimotor fibres and motor endings seem to be in good agreement and suggestion has been proposed that the dynamic fibres correspond to Boyd's γ_1 fibres with plate endings and static fibres correspond to Boyd's γ_2 fibres with net-

work endings (Matthews, 1962; Crowe & Matthews, 1964 a, b;
Brown, Crowe & Matthews, 1965; Appelberg, Bessou & Laporte,
1965).   In trying to explain the possible mechanism of the
action of fusimotor fibres in altering the behaviour of the
primary ending, Jansen & Matthews (1962a), Crowe & Matthews
(1964a), and Matthews (1964) suggested that Boyd's γ_1 fibres
terminating as end-plates on nuclear-bag fibres, and his γ_2
fibres terminating as the γ_2 network on nuclear-chain fibres
should correspond well with dynamic and static fibres,
respectively.   At the first instance, this correlation
seemed all right for the cat spindles.   But the convincing
evidence established by Emonet-Dénand, Laporte & Pagès (1964)
who found both static and dynamic fibres present in the
spindles of the rabbit's hindlimb muscles which are known
to contain spindles with nuclear-bag fibres only (Barker &
Hunt, 1964) renders this interpretation less convincing.
Since the rabbit spindle also has both plate and trail
endings on the same intrafusal fibre, as established by the
present study, it seems much better to correlate the mechanism
of static and dynamic actions with these two structurally
distinct forms of motor endings rather than with two
segregated intrafusal fibre systems.

Moreover, to be able to perform two types of different

contractions by the same muscle fibre, the mammalian intra-
fusal muscle fibres would not be the only one in the animal
kingdom. It has been postulated that the muscle fibres of
some insects have multiple as well as polyneuronal innerva-
tions, and that they have the ability to response to
different axons, fast or slow (Pringle, 1939; Hoyle, 1957b).
Then it seems quite possible that a similar mechanism can
also be applied to the mammalian intrafusal muscle fibres
which have also multiple and polyneuronal innervations.
Bessou & Laporte (1965) have recently produced evidence to
establish beyond doubt that the cat's tenuissimus spindle
can perform both localized and propagated contractions. By
shifting two electrodes over the cat's tenuissimus muscle
the equatorial region where the primary ending lies of whose
spindles can be located with precision (Bessou & Laporte,
1962) and whose spindles are in series each being separated
by several millimeters, Bessou & Laporte (1965) could obtain
both localized, non-propagated post-synaptic and propagated
diphasic action potentials from single spindles of these
muscles. They obtained localized, non-propagated post-
synaptic action potentials from the intrafusal bundles of
single spindles if the electrodes were located at about
1.3 mm from the equatorial region on stimulating single
fusimotor fibres having a dynamic effect (see their figure 1).

They also found that on shifting the bipolar electrode along the axis of the spindle away from the equatorial zone, the form of potentials of the intrafusal fibres changed and they could get propagated diphasic potentials if the electrodes are situated at about 2.5 mm from the equatorial zone on stimulating fusimotor fibres with a static effect on the primary ending (see their figure 2). There was also a transitional zone where both types of potentials may overlap and superimpose. Allowing for shrinkage or contraction due to fixation, these two zones correspond exceedingly well to the mean positions of the trail and plate endings respectively. Therefore, it seems probable that dynamic fusimotor fibres terminate near the equatorial region as trail endings which can bring about localized, non-propagated contractions, whereas static fusimotor fibres terminate towards the middle or end of the spindle pole as plate endings which can bring about propagated contractions of the intrafusal fibres.

If this finding is accepted, then the mechanism for giving rise to static and dynamic responses of the primary ending by static/plate and dynamic/trail fusimotor fibres can be explained with the help of the recordings of Crowe & Matthews (1964a, figure 1). In Text-figure 35, an

extension of the spindle as a result of passive stretching of the muscle in the absence of fusimotor stimulation causes the nuclear-bag fibre to narrow with the spirals of the primary ending correspondingly extended apart; thus stimulating the primary to fire (see B). During the dynamic phase of extension, the frequency discharge increases progressively probably due to the continuous deformation of the spirals. When stretching is stopped, the frequency discharge drops a little bit, as the tension caused by the extension at the final length is maintained. With the stimulation of a plate-ending static fibre, the strong propagated contraction (represented by thick arrows in C) tends to draw the spirals apart even in the absence of extension, thus causing deformation, and the primary ending fires as can be seen from the first part (initial length) of record C. During the dynamic phase of stretching, the continuous extension pulls the spirals wider apart thus increasing the frequency discharge of the primary ending. But since the static stimulation has already caused a considerable amount of deformation of the spirals, the dynamic extension cannot cause much more to increase the frequency discharge to a great extent. As should be expected, the frequency discharge should remain almost the same as stretching is stopped so that the dynamic index (see p. 124)
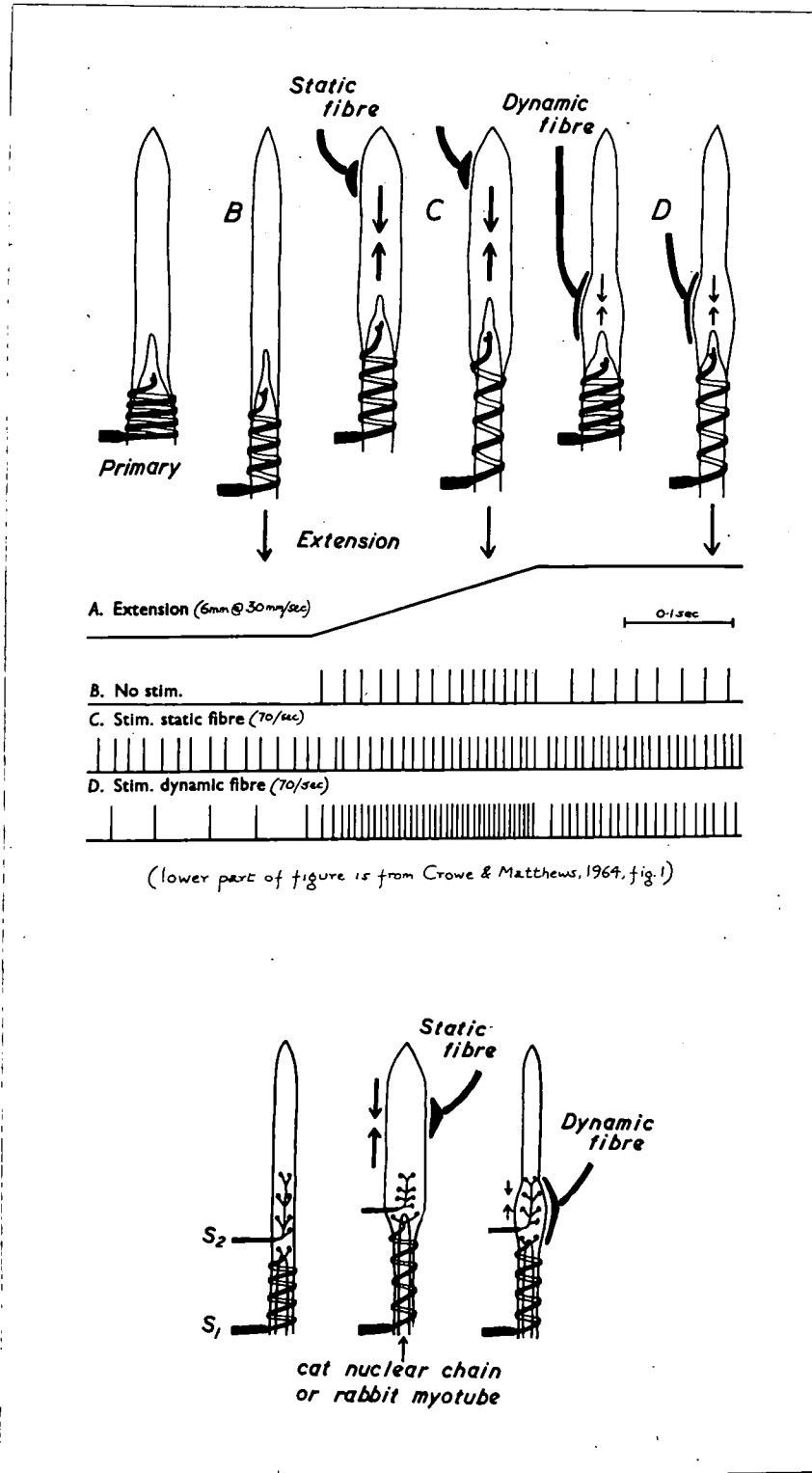
is not or just slightly altered, since the total deforma-
tion exerted by these two forces acting together is nearly
the same at the final length as during the final stages of
the dynamic phase of stretching. Thus it can be inferred
that the degree of deformation and consequently the rate
of frequency discharge of the primary ending produced by
the stimulation of this particular static fibre is greater
than that produced by the maintained extension of 6 mm as
can be seen from the first and the last parts of records
C & B respectively. Stimulation of the static fibre at
constant muscle length increases the degree of deformation
or rate of frequency discharge of the primary ending more
than similar stimulation of the dynamic fibre does, as can
be seen by comparing the number of spikes in the first part
of both records C & D. But during the dynamic phase of
stretching with the stimulation of the trail-ending dynamic
fibre, the frequency discharge of the primary ending increases
enormously because the rate of deformation on the sensory
ending is much greater in this case. At the final length
when stretching has stopped the rate of deformation slows
down but in this case, the rate should be expected to be
greater than the similar situation of the static fibre so
that the frequency discharge drops to a greater extent
causing an increase of the dynamic index (see D).

The behaviour of secondary endings in response to the stimulation of static and dynamic fusimotor fibres is less understood. This is probably due to the fact that the situation in secondaries is more complicated than that in primaries because different forms of secondary endings are known to exist. For instance, there are S_1, S_2, S_3 etc. secondary endings each being slightly different in structure. The S_1 secondary ending, situated next to the primary ending, is quite different from the others by having its terminals with spirals and coils wrapping round the cat's nuclear-chain fibres or the rabbit's myotubes; while the other secondary endings are much more diffuse and 'flower-spray' like scattered in the polar region of the intrafusal muscle fibres (see Barker & Ip, 1960). Nevertheless, it is suggested that the various patterns of the behaviour of secondary ending so far reported from physiological findings could also be explained by this idea (see bottom part of Text-figure 35). A localized, small contraction caused by the stimulation of a dynamic fibre terminating very near or actually on the site of the S_2 secondary innerva-tion naturally deforms the S_2 ("flower-spray") ending more than a contraction caused by the stimulation of a static fibre occurring farther away from the S_2 secondary ending. On the other hand, a stimulation of a static fibre causing

a greater and propagated contraction must have a stronger pulling force to deform the S_1 secondary ending usually in the form of spirals and coils just like the primary endings, to a much greater extent.   This interpretation seems to explain well those static fibres found by Crowe & Matthews (1964b) to excite powerfully the secondary ending the behaviour of which was more or less similar to that of primary endings during static fusimotor stimulation.

However, the study of the effect of stimulating static or dynamic fusimotor fibres on the sensory endings has so far been made only under such mechanical stimuli as linear extension and releasing, vibration, and sinusoidal stretching etc.; and it is a surprise to find that nothing much has been reported about their effects produced under changes of pressure.   The experiment of Bridgman & Eldred (1964) shows convincingly that the mammalian muscle spindle is an extremely sensitive pressure-detecting organ (see also the technique of locating spindles employed by Bessou & Laporte, 1962).   A very light pressure exerted by the extremity of a vibrissa, touching the exact spot of the equatorial region, is sufficient to cause an immediate firing of the sensory endings of the spindle, both primary and secondary endings being equally sensitive to this stimulus.

Text-figure 35.   Diagrammatic drawing (not to scale)
to show how the static and dynamic effects are brought
about by the stimulation of the respective fusimotor
fibres when the muscle is subjected to extension by
stretching on the primary afferent discharges in the
first part of the figure and on the S_1 and S_2 secondary
afferent discharges below.   The middle part is reproduced
from Crowe & Matthews, 1964a, figure 1 for illustration
(see text in Discussion on p. 127).

Static
fibre

Dynamic
fibre

B

C

D

Primary

Extension

A. Extension (6mm @ 30mm/sec)   0.1 sec

B. No stim.

C. Stim. static fibre (70/sec)

D. Stim. dynamic fibre (70/sec)

(lower part of figure is from Crowe & Matthews, 1964, fig. 1)

Static
fibre

Dynamic
fibre

S_2

S_1

cat nuclear chain
or rabbit myotube

Text-figure 35.

Spindles are provided with a bag-shaped capsule with several
layers of lamellae, characteristic of most pressure-sensitive
structures such as the Pacinian and paciniform corpuscles.
No doubt when a muscle is passively stretched, an increase
in pressure would be created and exerted on the bulging
capsule to compress it in towards the intrafusal bundle
thus causing the sensory endings to fire off. The extension
of the capsule as a result of tension caused by the passive
stretching would also cause a compression or flattening of
the capsule to increase this pressure further still. This
pressure increase would be expected to be greater or more
drastic the quicker the rate of stretching or compression.
When stretching is stopped, then the pressure inside the
capsule would be released by reducing the volume of fluid
by leaking out through the ends of the capsule. This may
perhaps explain partly or even wholly the adaptation of the
primary ending because it seems unlikely to ascribe this
adaptation to tension alone since the tension caused by the
stretching still exists as the final length remains longer
than the initial length. This may also possibly explain
the difference between the results of stimulating a dynamic
fibre in soleus and tibialis posterior (Brown, Crowe &
Matthews, 1965) arises from the difference in pressure
change caused by the different patterns in the arrangement

of the muscle fibres in these two muscles in relation to the direction of stretching.

So far it has generally been assumed that the static and dynamic fusimotor fibres may be directly correlated with the two morphologically distinct types of fusimotor fibres. Is this justified? It is known (Crowe & Matthews, 1964b) that there are various different or intermediate types of fusimotor fibres that are admittedly difficult to classify as either static or dynamic because they have only a weak action on the sensory endings. As has been described, a fusimotor fibre branch may have several endings on the same or several intrafusal muscle fibres either in one or both poles of the spindle or may have just one single ending in the whole spindle. Undoubtedly, those fusimotor fibres with several endings on most or all of the intrafusal muscle fibres of a spindle would certainly have, on being stimulated, stronger effect than those with only one; while those with several endings on both poles of a spindle would be expected to have an effect still even stronger. Hence, it would not be surprising to find some fusimotor fibres have stronger but others have weaker effects under the same conditions of stimulation. Then, it may be possible that those fusimotor fibres with a clear-cut static or dynamic

effect would presumably be plate or trail fibres with heavy
innervations on a larger proportion of the intrafusal
muscle fibres of the spindle on either one or even possibly
both poles of the spindle.   While those intermediate fibres
with weak action on the sensory ending may just represent
plate or trail fibres having a single or just a very few
terminals on a minority of the intrafusal polar halves at
different positions of the spindle so that the effects
they produce are not always identical to one another.   The
strength of the effect produced may depend on the summation
in proportion to the number of intrafusal muscle fibres
involved and the number of ramifications supplied by each
of them in that particular spindle and possibly plus the
pressure change factor.   It could also be possible that
plate-ending static fibres with very low polar involvement
in their innervation can simulate the dynamic effect of
trail-ending dynamic fibres, but altogether it must remain
as a speculation at present.

Since generally a static fibre is able to produce a
strong propagated contraction on being stimulated by every
single stimulus, the frequency of such propagated contrac-
tions would depend on the number of single stimuli put in.
This in turn would deform the primary ending which it

affects at the same frequency as that of the single stimuli. Then the above interpretation may be significantly applied to explain why only one out of five (in soleus) or one out of three (in tibialis posterior) static fibres can give the'driving'effect. The results have shown (Table 7) that there are three out of eleven plate fibres (or 1 in 4) innervating both poles of spindles with reasonably heavy termination on relatively high proportion of all the intra-fusal muscle fibres of the spindle. Since this ratio is in good agreement with that of the 'driving' static fibres, it seems highly probable that only those plate fibres with the strongest static effect possibly by having multiple terminals on both poles involving larger number of intra-fusal muscle fibres can produce a 'driving' on the sensory ending of the spindle.

6. Summary

1.   The motor innervation of de-afferentated as well as normal cat and rabbit spindles was investigated in teased silver preparations, much information being obtained from a sample of 12 cat and 10 rabbit spindles.

2.   A process of growth and decay of motor terminals was evident in both de-afferentated and normal skeletal muscles.   In the sample of 12 cat and 10 rabbit spindles the proportions of plate-ending fusimotor axons showing sprouting were 30.4 % and 34.0 %, respectively.   If 'accessory endings' are regarded as young plates newly-formed by collateral sprouts, the degree of sprouting is increased to 33.9 % and 38.3 %, respectively.

3.   In a sample of 567 skeletomotor terminal branches examined in normal muscle taken from cat, rabbit, and rat, 8.1 % showed sprouting, or 19.4 % if the collaterals forming accessory endings are included as sprouts.

4.   Some evidence of retrograde degeneration among the terminal branches of skeleto- and fusimotor axons is

described.   It is suggested that sprouting effects the replacement of old end-plates which degenerate after a limited life-span; and that it is a general property of the vertebrate motoneuron for its peripheral terminals to undergo cyclic renewal in this way.   Some of the implications of the replacement hypothesis, e.g. with regard to neuromuscular pathology, are discussed.

5.   In a spindle sample taken from 10 cat and 5 rabbit de-afferentated spindles, two morphologically distinct fusimotor endings were found.   In addition to the usual well-known end-plates, there is a second type of fusimotor ending, the trail ending, found in the majority of normal as well as de-afferentated mammalian spindles (92 % in the cat; 69.7 % in the rabbit).   There is also a distinct regional distribution of these two endings in the spindle: end-plates are usually located towards the middle and end of the spindle poles, while trail endings are usually situated near the equatorial region.   There is also usually a region of overlap.

6.   Different forms of trail ramifications and end-plates are described, and a distinction is made between a trail ramification and sprouts growing out from plate-ending

fusimotor fibres.

7. Length measurements give a mean of $32.4\,\mu \pm 8.1\,\mu$ S. D. (range from 15 to $55\,\mu$) for extrafusal motor end-plates, and a mean of $56.2\,\mu \pm 19.3\,\mu$ S. D. (range from 20 to $105\,\mu$) for the intrafusal ones.

8. In cat spindles plate and trail endings were found either on nuclear-bag fibres only; or on nuclear-chain fibres only; or on a mixture of both. Each intra-fusal muscle fibre may receive a variable number of motor endings either consisting of plates only, trails only, or most frequently, both plates and trails.

9. Spindles with both plates and trails on both poles (PT:TP type) are the most common, occurring in 61.3 % of the cat spindles studied, while in rabbit spindles, PT:TP, PT:P & P:P types are about equally common occurring in 33.3 %, 36.4 % and 30.3 %, respectively. Spindles with trails only have not been found. The occurrence of spindles without any trails on either pole, i.e. P:P type is relatively high in rabbit (30.3 %) as compared with cat (8.0 %). There appears to be no significant correlation between the type of sensory and the type of motor innervation.

10.   For individual intrafusal muscle fibres, the
P:P type occurs most frequently (41.4 %) in rabbit spin-
dles; PT:P type (50 %) in cat nuclear-bag fibres; and PT:P
& P:T types (both 20 %) in cat nuclear-chain fibres.   If
only polar halves of intrafusal muscle fibres are considered,
those with plates only are most common for the nuclear-bag
polar halves of both cat and rabbit, but those with plates
only, or trails only, are equally common for nuclear-chain
polar halves of cat.

11.   The number of plates or trails per each polar
half of intrafusal muscle fibres was determined.   In cat
spindles it was found on average to be 1.7 end-plates and
1.1 trails making a total of 2.8 motor endings (plates and
trails) per nuclear-bag polar halves; and 0.75 end-plates
and 1.56 trails making a total of 2.3 motor endings per
nuclear-chain polar half .   In rabbit spindles, the average
distribution was found to be 1.2 end-plates and 0.45 trails,
or 1.65 motor endings per each polar half.

12.   In a sample of 51 plate and 25 trail fusimotor
fibre branches found near or at sites of the 12 completely
analysed cat spindles, nearly half (25 or 49 %) of the plate
fibre branches terminate on nuclear-bag fibres only; while

more than half (14 or 56 %) of the trail ones terminate on both nuclear-bag and nuclear-chain muscle fibres.   In the 10 completely analysed rabbit spindles, the proportion of plate to trail fibre branches is 57 to 12.

13.   A fusimotor fibre branch may terminate on from 1 to 8 (3 bag: 5chain) polar halves in the cat, and on from 1 to 5 in the rabbit.   A classification of fusimotor fibre branches with traceable distance greater than 1.0 mm shows that the majority of plate fibres terminate on just one intrafusal muscle fibre, but trail fibres tend to innervate 4 intrafusal muscle fibres or more.

14.   The correlation between the two morphologically distinct groups of fusimotor endings with the two functionally different fusimotor fibres is discussed.   It is suggested that the significant correlation of plate to static and trail to dynamic may not be a straight-forward and simple one.   The possibility is discussed of the influence of the relative number of motor endings involved in a spindle resulting in different degrees of modifying the afferent discharge.

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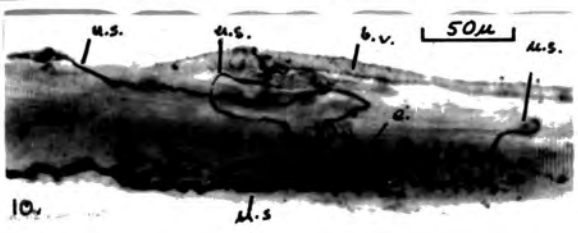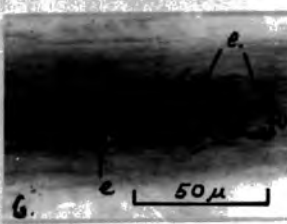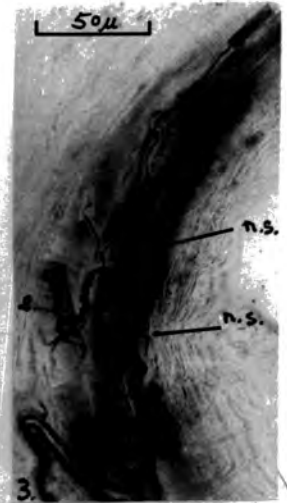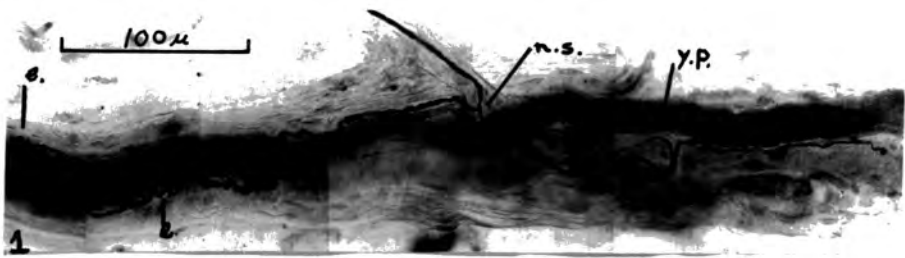containing cholinesterase in striated human eye
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Plate 1.

Photomicrographs taken from teased, whole-mount silver preparations
(modified de Castro's method) of fusimotor terminals exhibiting sprouting
in both normal (n) and de-afferentated (d/a) cat and rabbit muscle spindles.

a. e. accessory ending;   b. v. blood vessel;   d. e. double ending;
e. end-plate;   n. s. nodal sprout;   s. sprout;   u. s. ultra-
terminal sprout;   y. p. young end-plate.

Figure 1.   A fusimotor axon enters the polar region of a de-afferentated
rabbit soleus spindle to form 2 apparently normal end-plates on the left.
A nodal sprout given off to the right has formed a young plate and then
grown further on to terminate as a small bulb at tip of sprout.

Figure 2.   A similar situation to that in Figure 1, from a d/a cat
popliteus spindle.   Note terminal bulb at tip of the nodal sprout and
the plate the sprout has begun to form (its tracing is given in Text-
figure 10 h, see also text on p. 34).

Figure 3.   A fusimotor terminal axon bearing nodal sprouts from two
nearest successive nodes, terminates as a 'normal' end-plate on a normal
rabbit peroneal I spindle (its tracing is given in Text-figure 9 a).

Figure 4.   An intrafusal motor end-plate bearing two ultraterminal
sprouts in a d/a cat peroneal I spindle.   The sprout on the right has a
large ball of axoplasm measured approximately 11 μ in diameter at its tip
(tracing given in Text-figure 9 c, see also text on p. 31).

Figure 5.   Part of the fusimotor innervation of a nuclear-bag muscle
fibre in a cat lumbrical spindle (n).   The terminal branch of the axon
on the left forms a nodal sprout and a double motor ending one of which

Plate 1 (cont.).

is interpreted to be 'dying'. The branch on the right bears an accessory

ending (a.e.) adjacent to an apparently 'normal' end-plate borne by the

same parent branch (see also Text-figure 10 a).

Figure 6. Double motor ending on the intrafusal muscle fibre of a

normal cat tenuissimus spindle.

Figure 7. The terminal branch of a fusimotor axon forms a 'normal'

plate at its end and bears two nodal sprouts that are establishing young

plates or accessory endings (a.e.) from two nearest consecutive nodes.

An ultraterminal sprout from another axon is seen across the top of the

picture terminating in a ball of axoplasm on the left.

Figure 8. A suspected sprout of a similar nature to those found in

plate-ending axons with terminal bulb at tip located among the terminals

of a trail ending in a cat peroneal I spindle (d/a).

Figure 9. An intrafusal motor end-plate with an ultraterminal sprout

in a cat tenuissimus spindle (n). Its tracing is given in Text-fig. 9 d.

Figure 10. A fusimotor axon innervating the polar region of a cat

soleus spindle (d/a) shows both nodal and ultraterminal sprouting in an

extensive manner. Two of the ultraterminal sprouts have grown outside

the spindle, and one of them started to ramify on a nearby capillary.

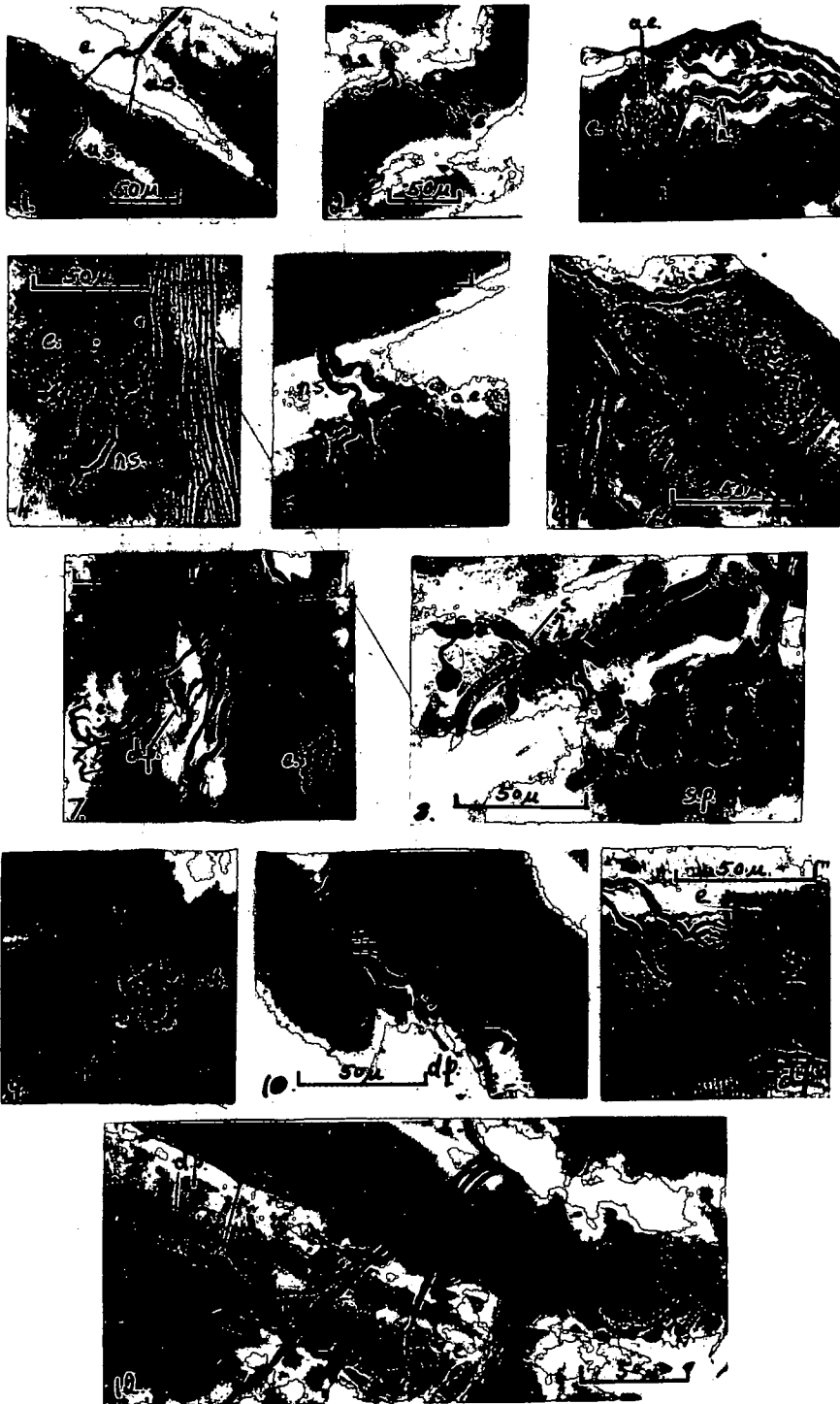Plate 1.

Plate 2.

Photomicrographs taken from teased, whole-mount silver preparations
(modified de Castro's method) of skeletomotor terminals with sprouting in
normal cat, rabbit, and rat muscles.

a. e. accessory ending; d. a. degenerating axon; d. p. degenerating
end-plate; e. end-plate; n. s. nodal sprout; s. sprout; s. p.
sole-plate; u. s. ultraterminal sprout; y. p. young end-plate.

Figure 1. Skeletomotor axon terminal showing nodal and ultraterminal
sprouting in rat intertransversarius (its tracing shown in Text-figure 11 a).

Figure 2, Skeletomotor axon terminal with incipient nodal sprout in
rat intertransversarius (its tracing is shown in Text-figure 11 b).

Figure 3. A nodal sprout forms new terminals (accessory ending) along-
side the old terminal of the parent axon in cat interosseus. Compare with
Figure 5 of this Plate.

Figure 4. A nodal sprout is seen just beginning to form an end-plate
(accessory ending) adjacent to the end-plate of the parent axon. Rabbit
soleus.

Figure 5. A nodal sprout forms new terminals (accessory ending)
within the same sole-plate borne by the same parent axon. Rabbit per-
oneal I (see also Text-figure 11 f).

Figure 6. On the right is shown an extrafusal double motor ending and
on the left an axon terminal bearing an accessory ending alongside it.
Cat interosseus.

Figure 7. Degenerating extrafusal motor end-plate (apparently a double

Plate 2 (cont.).

motor ending) and axon among a group of well-stained, 'normal' end-plates and axons in cat tenuissimus (its tracing is given in Text-figure 8 a).

Figure 8. Sprouting has obviously re-innervated an abandoned, empty old plate from a sprout which has then grown beyond it and terminated as a ball of axoplasm at the tip. Rat gastrocnemius.

Figure 9. An extrafusal ending evidently shows degenerating end-plate with swollen terminals (on the right) lying next to a new plate with slender and solid terminals (on the left) formed by a nodal sprout from the parent terminal axon. This is a particular interesting instance interpreted as showing degeneration and replacement going on simultaneously in the same end-plate. Cat tenuissimus (see also its tracing in Text-figure 8 b).

Figure 10. Degenerating extrafusal motor end-plate with swollen axons and terminals. Rabbit peroneal I.

Figure 11. A retracted, degenerating end-plate (below) lies in the neighbourhood of a well-stained, apparently 'normal' end-plate (above). Cat tenuissimus (see its tracing in Text-figure 8 d).

Figure 12. An extrafusal motor end-plate and terminal axon branch in the early stages of degeneration (on the left) lies in the neighbourhood of two apparently 'normal' end-plates and terminal axons. Rat gastrocnemius.

Plate 2.

Plate 3.

Photomicrographs taken from teased, whole-mount silver preparations (modified de Castro's method) of fusimotor terminals in normal (n) and de-afferentated (d/a) spindles of cat and rabbit.

Figure 1,   The terminal branches of an active replacing fusimotor axon innervating the polar region of a d/a cat spindle from C167 flexor digitorum longus, mesial head.   It is given as an evidence of showing that replacement process is going on at the time of its fixation (see text on p. 35 and its tracing in Text-figure 10 f).

Figure 2,   A fusimotor end-plate innervating the polar end of a d/a cat C165 peroneal II spindle has an ultraterminal sprout apparently growing away from the spindle and making connexion with a nearby capillary (see also text on p. 34 and its tracing in Text-figure 9 j).

Figure 3,   A presumably 'dying back' end plate supplied by two fusimotor fibre branches of different diameters terminating on the polar region of a normal rabbit tenuissimus spindle.   Note the similarity between its retracted terminals with those found in the extrafusal degenerating end-plates (compare, for instance, Figures 9 & 11, Plate 2).

b. a. ball of axoplasm; b. v. blood vessel; c. l. capsule lamellae; d. p. degenerating end-plate; e. end-plate; e. t. escaping terminal; o.p. old end-plate; u.s. ultraterminal sprout.

1.

b.a.   U.S.   o.p.   u.s.

e.t.   e.l.   50μ

2.   b.   u.s.

50μ

3.   60μ
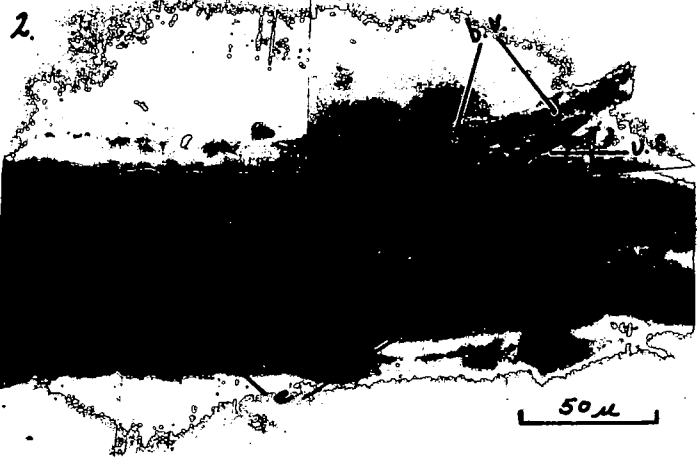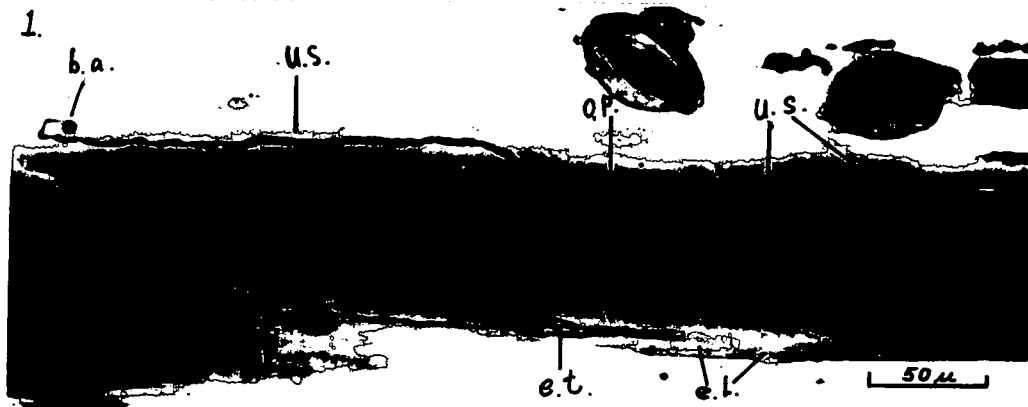
Plate 3.

Plate 4.

Photomicrographs of teased, whole-mount modified de Castro's silver preparations of intrafusal motor end-plates exhibiting sprouting at various stages in both normal (n) and de-afferentated (d/a) spindles from various hindlimb muscles of the cat. Figures 1 to 4 are examples of incipient nodal or ultraterminal sprouts (compare with tracings in Text-figure 9); and 5 to 8 are examples of nodal and ultraterminal sprouts well advanced in development into accessory endings (a.e.; also compare with tracings in Text-figure 10). They are all intrafusal motor end-plates taken from:

<u>Figure1</u> cat C159 interosseus (d/a) showing two nodal sprouts one of which is out of focus (see also its tracing in Text-figure 9 <u>g</u>).

<u>Figure 2</u>, cat C169 superficial lumbrical (n) exhibiting a well-advanced nodal sprout obviously terminating on another intrafusal muscle fibre.

<u>Figure 3</u>, cat C167 peroneal I (d/a) sending off a long ultraterminal sprout towards the end of the pole. This end-plate is definitely terminating on a nuclear-chain intrafusal muscle fibre.

<u>Figure 4</u>, cat C159 soleus (d/a) terminating on a nuclear-chain muscle fibre with an ultraterminal sprout.

<u>Figure 5</u>, cat C169 peroneal III (n) with an accessory ending formed from its nodal sprout (see also its tracing in Text-figure 10 <u>b</u>).

<u>Figure 6</u>, cat 1st deep lumbrical (d/a). A well-developed young end-plate (y.p.) is seen sprung off from the ultraterminal sprout of the end-plate (see also its tracing in Text-figure 10 <u>d</u>).

<u>Figure 7</u>, cat C167 peroneal I (d/a) with an accessory ending formed from its nodal sprout (its tracing given in Text-figure 10 <u>e</u>).

<u>Figure 8</u>, cat C159 peroneal I (d/a) with an accessory ending and an ultraterminal with a ball of axoplasm (b.a.) at the tip.

a. e. accessory ending; b. a. ball of axoplasm; e. end-plate; u. s. ultraterminal sprout; x. u. s. ultraterminal from another terminal passing through the top of the end-plate; y. p. young end-plate.
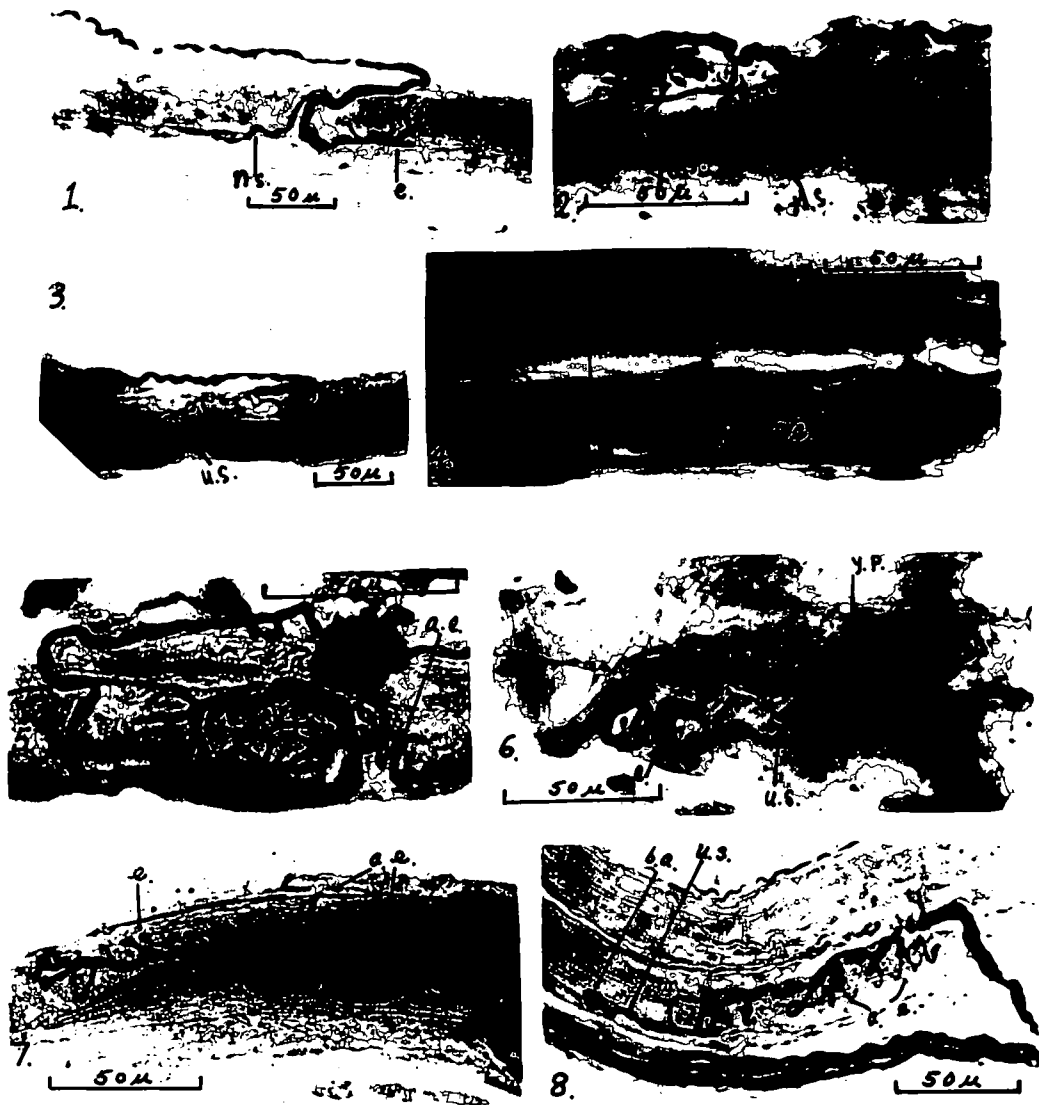
Plate 4.

Photomicrographs showing peculiar arborizations on the tendinous pole of normal (n) and de-afferentated (d/a) cat and rabbit spindles (Figures 1-4) and characteristic ball of axoplasm at tip of growing sprout (Figure 5).   Teased, whole-mount modified de Castro's silver preparations taken from:

Figure 1,   cat C159 peroneal III (d/a), see text on p. 36;

Figure 2,   rabbit soleus (n), see text on p. 36-37;

Figure 3,   cat C159 interosseus (d/a), see text on p. 37;

Figure 4,   cat interosseus (n), see text on p. 37;

Figure 5,   cat C165 peroneal III (d/a).

a. o.   axonic growth;   b. a.   ball of axoplasm;

c. m.   contact on muscle;   c. t.   contact on tendon;

e.   intrafusal motor end-plate;   fu' a   fusimotor plate fibre branch;   fu' b   fusimotor trail fibre branch;

t. a. 1 & 2   tendinous arborizations;

n. s.   nodal sprout;   u. s.   ultraterminal sprout;
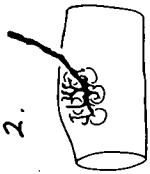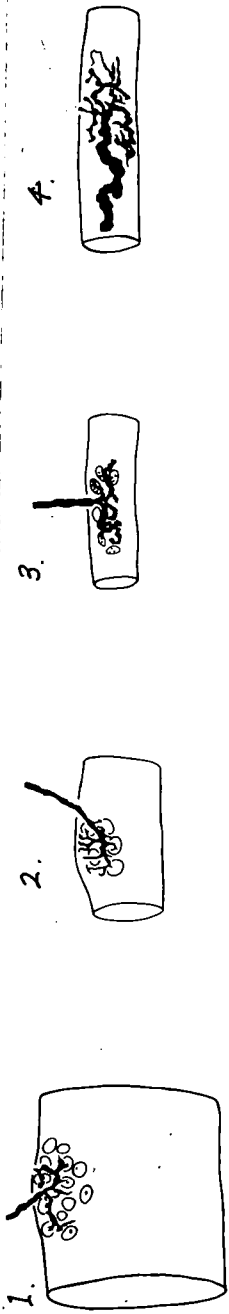
x.   point of branching.
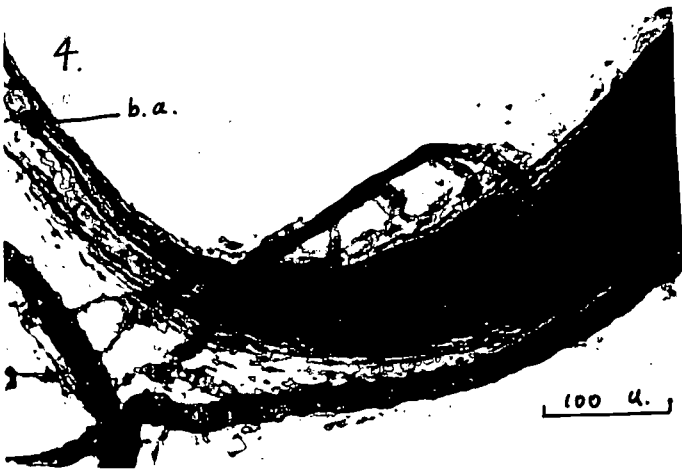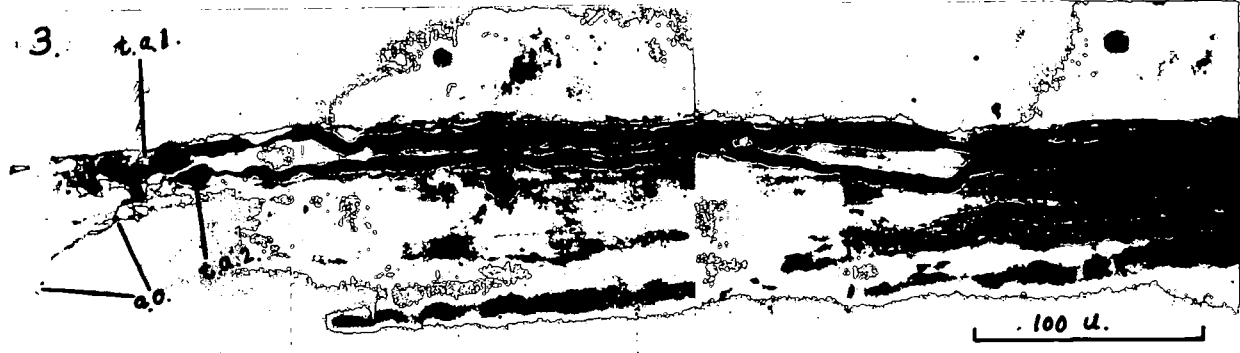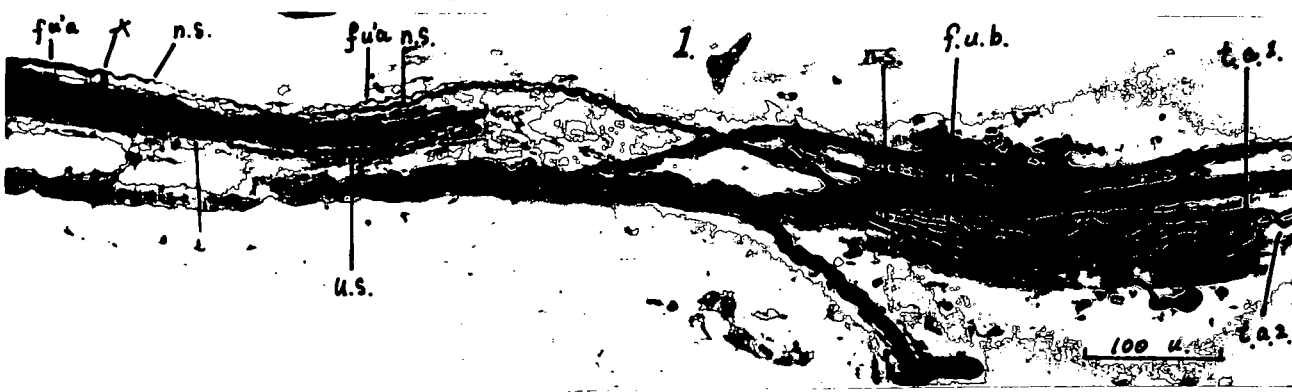
Plate 5.

Plate 6.

Tracings of photomicrographs of motor end-plates from teased, whole-mount, silver preparations (modified de Castro method) from various normal (n) as well as de-afferentated (d/a) muscles of cat and rabbit. They are all intrafusal motor end-plates except in Figure 1 or 2, an extrafusal motor end-plate from rabbit and cat respectively is given for comparison. Motor end-plates in Figures 3-8 are terminating on nuclear-bag muscle fibres whereas those in Figures 9-11 and 13 are terminating on nuclear-chain muscle fibres and those in Figure 12 supplied by one fusimotor fibre branch are terminating on both types of intrafusal muscle fibres. Photomicrographs of Figures 3, 7, and 11 are reproduced in Text-figure 12 a & b, and Plate 9; while those of Figures 5 and 6 are reproduced in Text-figure 3 a & b respectively.

They are taken from the following muscles;

Figure 1, rabbit Rb7 soleus (n);   Figure 2, cat C154 interosseus (n); Figures 3, 4, 7, 8, and 10, cat C159 interosseus (d/a);   Figure 5, rabbit Rb7 vastus intermedius (n);   Figure 6, cat C167 peroneal I (d/a);   Figures 9 and 11, cat C159 peroneal II (d/a);   Figures 12 and 13, cat C159 soleus (d/a).

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.
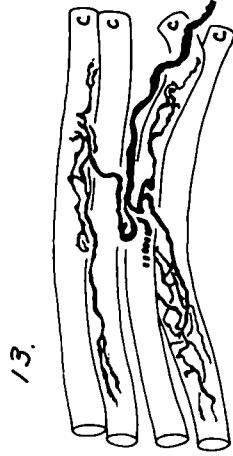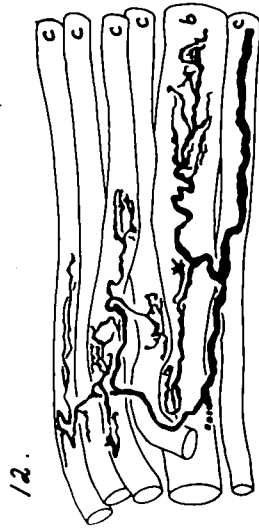
11.

12.

13.

100μ

Plate 6.

Plate 7.

Tracings of photomicrographs of trail ramifications on intrafusal muscle fibres from teased, whole-mount silver preparations (modified de Castro method) from various normal (n) as well as de-afferentated (d/a) cat and rabbit spindles. In most cases, particularly in normal spindles, it is very difficult to decide whether the ramifications are terminating on nuclear-bag or nuclear-chain muscle fibres. But wherever possible, the type of muscle fibres they innervate is indicated in the brackets. Photomicrographs of Figure 4 (plus terminations from another trail ending), and Figures 10, 11, and 12 are reproduced in Figures 1 and 2, Plate 8; and Text-figure 17 a & b respectively.

They are taken from spindles of the following muscles:

Figure 1, rabbit Rb11 peroneal IV (d/a); Figure 2, cat C165 peroneal II (n); Figures 3 & 5, cat C169 1st deep lumbrical (n); Figures 4 & 9, cat C167 peroneal I (d/a; on nuclear-chain fibres); Figure 6, cat C165 tenuissimus (n); Figures 7 & 8, cat C169 peroneal III (n); Figure 10, cat C167 flexor digitorum longus, mesial head (d/a; on nuclear-chain fibres); Figure 11, cat C159 peroneal I (d/a; on nuclear-bag fibres); Figures 12 & 13, cat C159 interosseus (d/a).

3.

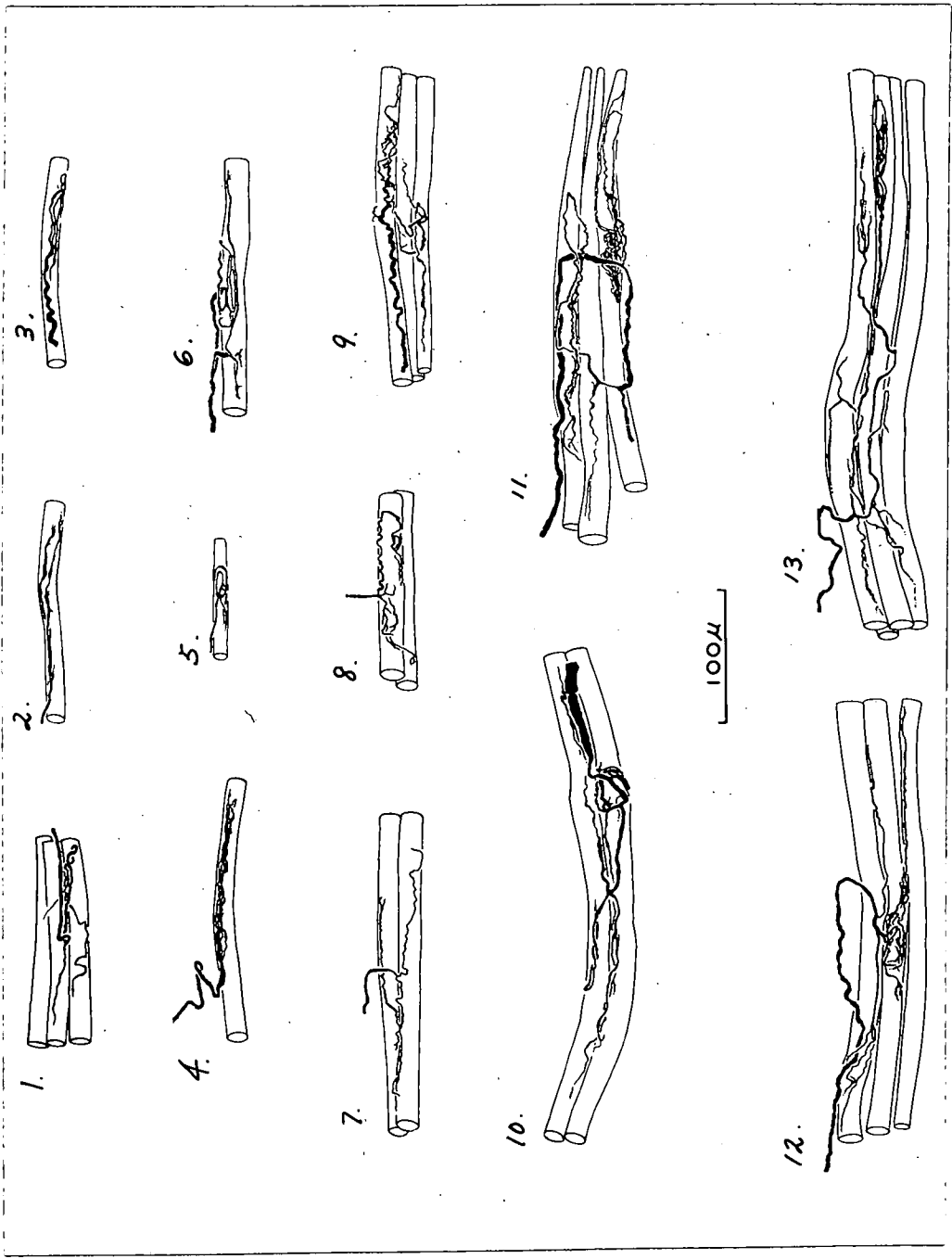6.

9.

11.

2.

5.

8.

13.

100μ

1.

4.

7.

10.

12.

Plate 7.

Plate 8.

Photomicrographs of trail ramifications on intrafusal
muscle fibres found in de-afferentated (d/a) spindles of cat.
Teased, whole-mount silver preparations; modified de Castro's
technique.   Note that it is interesting to find that some
trail ramifications as in Figures 4 & 5 appear plate-like and
only by following the other terminations of their branches
that they can be identified as trail ramifications.

They are teased out from the following muscles:

Figure 1,   Cat C167 peroneal I (d/a, terminating on nuclear-
chain intrafusal muscle fibre) showing two trail ramifications,
part of its tracing is reproduced in Figure 10, Plate 7.

Figure 2,   Cat C167 flexor digitorum longus, mesial head (d/a,
terminating on nuclear-chain muscle fibres), it tracing is
reproduced in Figure 11, Plate 7.

Figures 3, 4, & 5,   Cat C159 interosseus (d/a, terminating on
both nuclear-bag and nuclear-chain muscle fibres).

Figure 4,   Being a part of Figure 5 to show the plate-like
trail ramification more clearly at a higher magnification.
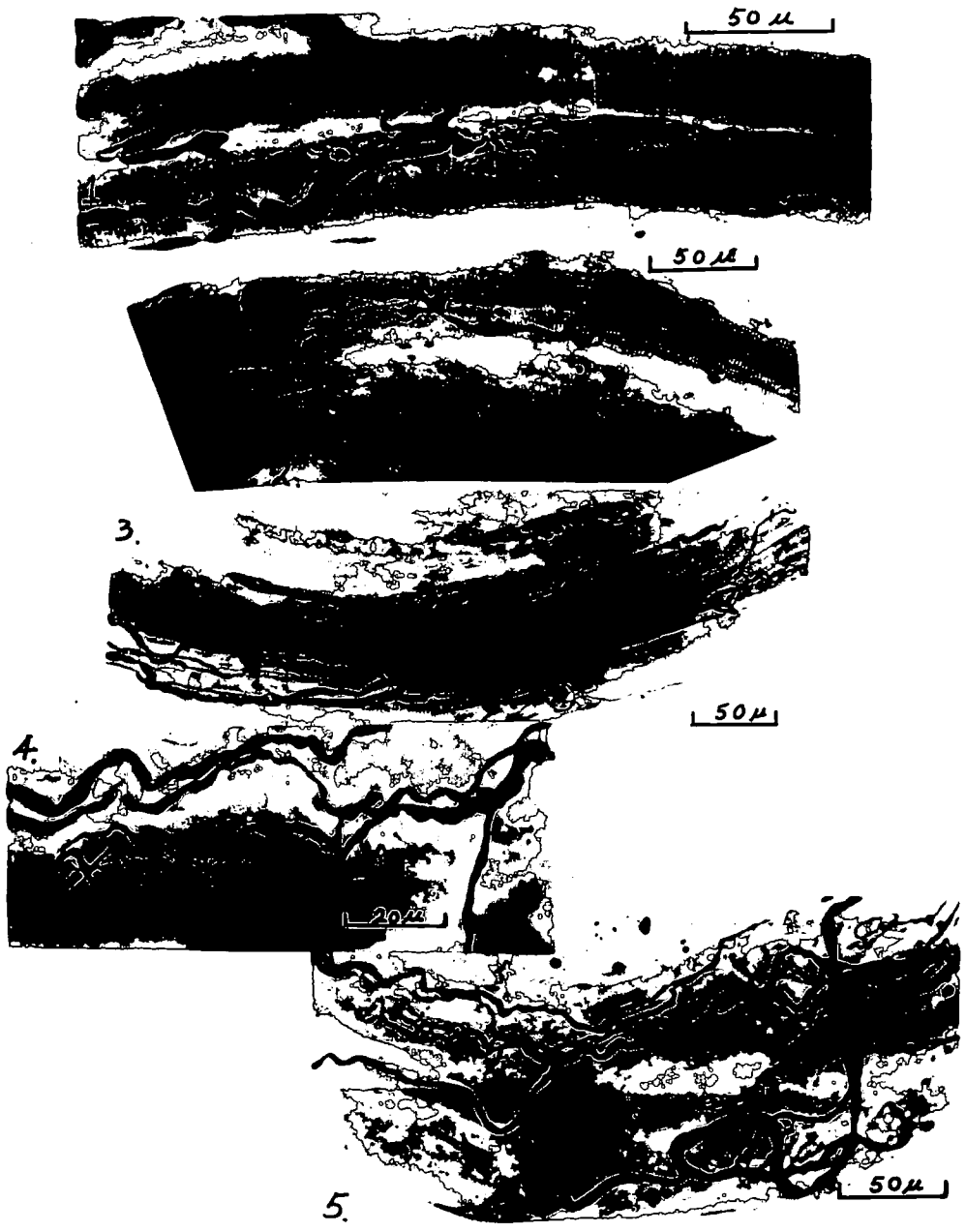
50 µ

50 µ

3.

50 µ

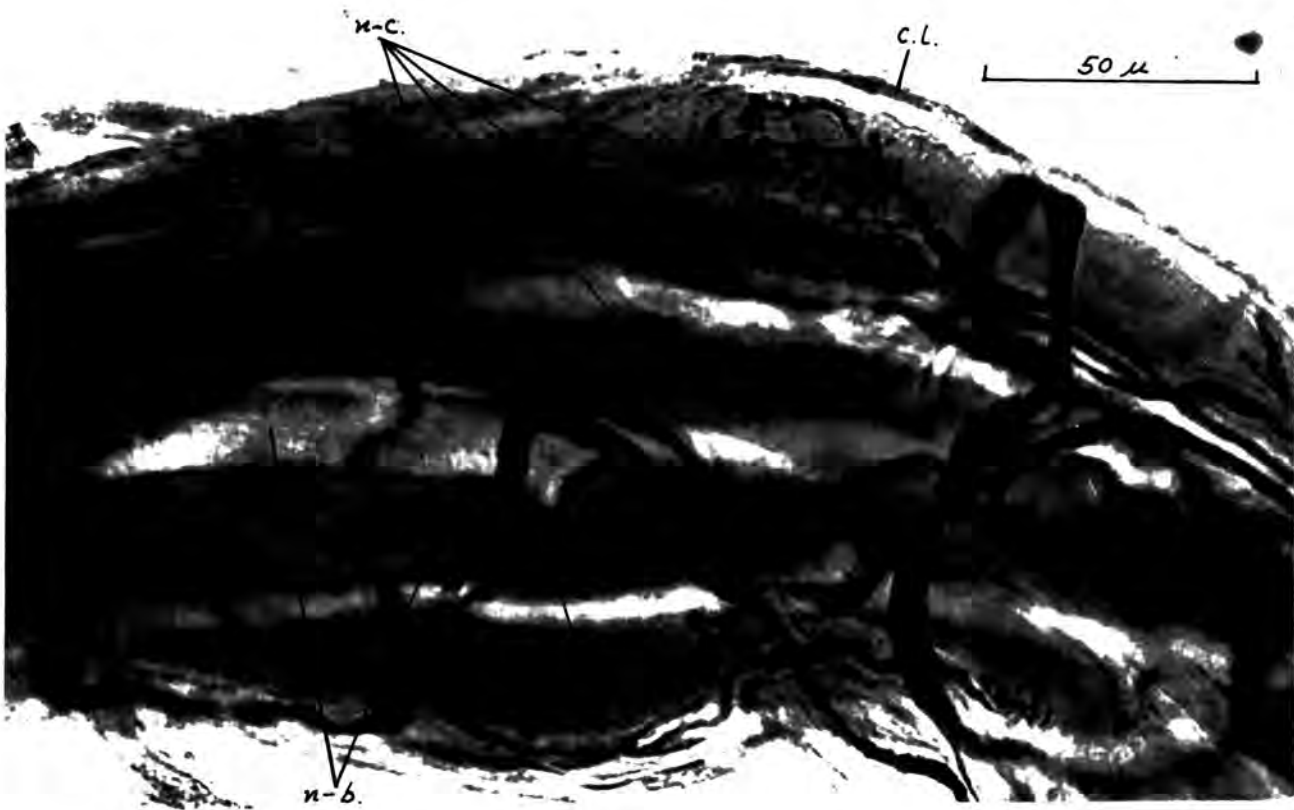4.

20 µ

5.

50 µ

Plate 8.

Plate 9.

Photomicrograph of a part of a de-afferentated (d/a) cat
spindle pole showing a typical motor end-plate terminating
definitely on a nuclear-chain intrafusal muscle fibre. A
gentle press was applied to this spindle to spread out the
muscle fibres in order to make their identification easier.
In fact, this pole comes from the same spindle as that in
Text-figure 5 so that the two nuclear-bag fibres could be
identified and traced through with certainty.   Modified
de Castro's silver preparation teased from cat C159 peroneal
II (d/a).   c. l. capsule lamellae; n-b. nuclear-bag;
and n-c. nuclear-chain intrafusal muscle fibres.