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A STUDY OF THE RECOVERY OF CAT MUSCLE SPINDLES
AFTER NERVE-CRUSH INJURY.

A thesis presented in candidature for the degree of
Doctor of Philosophy

by

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Durham, August 1982.

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Abstract.

After nerve-crush injury to a muscle nerve the afferent and efferent axons regenerate and reinnervate their peripheral targets. This process has been examined in the muscle spindles of the peroneus brevis muscle of the cat hindlimb. Following reinnervation there was a rapid restoration of the ending structure which clearly demonstrated the presence of a powerful guidance system enabling a high degree of specificity in the reinnervation process.

Although the sensory endings never achieved the full complexity and extent of innervation that characterises the endings of normal spindles, the process of reinnervation was highly successful and the specificity of reinnervation is explained in terms of physical guidance by the basal laminae of the endoneurial tubes and the muscle fibres.

During the early stages of recovery the spindles responded abnormally to ramp-and-hold stretch, often only firing during the rising phase of the ramp. As recovery progressed, so the responses became more normal and this pattern is explained in terms of a gradual reduction in the threshold of the pacemaker site.

Variation of the denervation period showed that on the whole the muscle spindle is resistant to denervation atrophy for at least seven weeks. After all the denervation periods the regenerated endings were fully functional and there was only a slight trend towards slower recovery with increasing denervation period. The morphological appearance of the endings showed a reduction in the regional specificity of reinnervation which is attributed to shrinkage of the intrafusal fibres allowing axons to grow between the basal lamina and the muscle fibre.

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Key to abbreviations

α	alpha skeletomotor axon
b ₁	nuclear - bag ₁ intrafusal muscle fibre
b ₂	nuclear - bag ₂ intrafusal muscle fibre
b ₁ sp	bag ₁ spirals
b t.s.	bag terminal system
c	nuclear - chain intrafusal muscle fibre
c sp	chain spirals
CV	conduction velocity
D	denervation
dev. t.s.	developing terminal system
eq. reg.	equatorial region
γ	gamma fusimotor axon
HFR	held firing rate
Ia	primary afferent
Ia (x2)	hyperinnervated primary afferent
II	secondary afferent
i.t.	irregular termination
l.a.s.	large axonic swelling
mot. ax.	motor axon
NEC	nerve entry crush
NS	normal site crush
P	primary region
PC	post crush
PFR	peak firing rate
PL	peroneus longus
PT	peroneus tertius
per. spa.	periaxial space
RC	repeat crush
RT	reinnervation time
S ₁	S ₁ secondary region
t.e.	terminal expansion
TC	tibial site crush
1st br.n.	first branching node

INTRODUCTION

1.1 Muscle Spindle Structure.

The mammalian muscle spindle is a complex mechanoreceptor located in striated muscle, usually lying between and in parallel with the fasciculi of the muscle. The muscle spindle consists of a small bundle of intrafusal muscle fibres which are enclosed for much of their length in a fibrous cellular capsule. At its extremities this capsule is closely applied to the intrafusal fibres, but swells in the equatorial region to encompass a large periaxial space.

The muscle spindle has a rich motor and sensory innervation, each fibre being innervated by a number of different afferent and efferent axons.

Numerous excellent reviews of the structure and physiology of the muscle spindle have been published in the last decade (Matthews, 1972; 1980; 1981; Barker, 1974; Hunt, 1974; 1978; Laporte, 1978; Kennedy, Poppele & Quick, 1980); therefore I will only deal in depth with those features of the receptor that are of relevance to this study.

1.2 The Intrafusal fibres.

Banks, Harker & Stacey (1977**b**) performed a detailed histochemical and ultrastructural study of the intrafusal fibres which resulted in the full categorisation of the intrafusal fibre types which were designated bag₁, bag₂ and chain (Ovalle & Smith, 1972).

The chain fibres are the smallest of the intrafusal fibres (Boyd, 1962; Barker, Banks, Harker, Milburn & Stacey, 1976**a**) and there is usually a number of these fibres present in the cat spindle, the average being four in tenuissimus spindles (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1976**b**) and six in peroneus tertius (Harker, Jami, Laporte & Petit, 1977).



Structurally and histochemically the chains are the most homogeneous of the intrafusal fibres possessing an M line throughout and staining fairly consistently for glycogen (PAS), ATP'ase and P'ase (Banks et al,1977b; Barker et al,1976a).

The bag₁ is usually larger than the chains (Barker et al, 1976a) and there is usually only one per spindle, although examples of two and very rarely three, have been reported (Barker et al, 1976a; Banks, Barker & Stacey, 1979; 1982).

Both the ultrastructure and histochemistry of the bag₁ change on moving from the equatorial region to the poles. Barker et al (1976a) divided the spindle into three regions: the A region, which extends from the equator to the end of the periaxial space; the B region, which is that part of the poles enclosed by the capsule; and the C region, which is extracapsular.

At the equator the myofibrils are only present round the periphery of the bag₁, the core being taken up by the 'bag' of nuclei (Barker et al,1976a; Banks et al,1977b; Kucera, Dorovini-Zis & Engel,1978) from which the fibre derives its name (Barker,1948). Within the capsule the M line is absent or appears as a faint double M line (Barker et al,1976a), a normal M line only being present in the C region. P'ase, acid pre-incubated ATP'ase and PAS histochemical stains all increase in intensity on moving from the equator to the poles (Banks et al,1977b; Kucera et al,1978). The polar regions of the bag₁ fibre are also characterised by a lack of elastic fibres; these are associated with the bag₂ fibre (Gladden, 1976).

The bag₂ fibre is the largest of the intrafusal fibres (Banks et al,1977b) and is usually single although rare examples of spindles with two bag₂ fibres have been reported (Banks et al,

1979). Ultrastructurally it has a double M line appearance equatorially, the change to a single M line usually occurring at the A/B transition (Barker et al, 1976a; Banks et al, 1977b).

As with the other fibres the equatorial region is the least histochemically reactive part of the fibre (Ovalle & Smith, 1972), although the increase in stain intensity on moving towards the poles is less marked than in the bag₁ (Banks et al, 1977b).

The extreme polar regions of the bag₂ are marked by the prominent elastic fibres, which are absent from the bag₁, and which may reflect a difference in the connective tissue linkages of the two fibres with the extrafusal fibres (Gladden, 1976).

1.3 The Afferent Innervation.

The sensory innervation of the cat muscle spindle usually consists of a single primary ending either alone or with one or more secondary endings which terminate on the intrafusal fibres in the equatorial and juxta-equatorial regions, respectively, Banks et al (1982) examined the afferent innervation of spindles from a number of different muscles of the cat hindlimb, and they reported that in peroneus brevis (PB), of the spindle capsules containing all three types of intrafusal fibre, 8.5% of the spindles had a primary ending only; 44.7% had one primary and one secondary and 25% had a primary and two secondaries. Only 4% of spindles showed a double primary ending. In the case of the b₂c capsules, which are present as the second capsule of a tandem spindle (Banks et al, 1979; 1982), 93% of those examined were innervated by a primary afferent alone.

14. The Primary Ending.

The primary afferent is a large axon having a diameter of 12-22 μ m in the nerve trunk (Adal & Barker, 1962) thereby bringing it within the group I range (Lloyd, 1943). On approaching the spindle the Ia afferent divides to produce two or three first-order branches (Banks, Barker & Stacey, 1977a; 1979; 1982) which further divide such that all the intrafusal fibres are innervated (Boyd, 1962; Cooper & Daniel, 1963).

The annulospiral appearance described by Ruffini (1898) is characteristic of the primary innervation. Banks et al (1982) have analysed the innervation of the different intrafusal fibres and found that the different muscle fibres can be identified by the appearance of the primary innervation. The chain fibres are identifiable by the small spirals associated with their relatively small circumference (Boyd, 1962). On the bag₁ fibre the terminals tend to be close together with a region of irregular arrays at either end. On the bag₂ fibres the terminals are wider apart with less irregularity (Banks et al, 1982).

Banks et al (1977a; 1979; 1982) also examined the distribution of the branches of the Ia axon to the different intrafusal fibres. In the case of PB, 58% of the primaries showed a fully segregated distribution (i.e. the bag₁ fibre was innervated by a first-order branch separate from that supplying the bag₂ and chains). In the case of mixed distributions (42%), the bag₁ shared a first-order branch with the chains, or the bag₂, or both.

At the ultrastructural level the terminals on the bags and chains appear to be similar, with the endings lying in shallow grooves formed by the lips of the sarcolemma partly overlying the

terminals (Landon, 1966; Adal, 1969). The basement membrane of the intrafusal fibres also covers the terminals of the ending (Merrillees, 1960).

In certain areas the chain fibres are closely apposed, and in the equatorial region sensory cross-terminals can occur such that a given sensory terminal can innervate two 'paired' chain fibres (Adal, 1969; Scalzi & Price, 1971; 1972; Banks et al, 1982). This provides anatomical support for the physiological observations of the chain fibres acting as a unit (Boyd, 1976b).

1.5 The Secondary Ending.

The secondary endings are supplied by group II afferents having smaller diameters than the group I axons, lying within the range 4-12 μ m in the nerve trunk (Hunt, 1954). Closer to the spindle there is a marked degree of overlap between the Ia and II axon diameters (Adal & Barker, 1962; Boyd & Davey, 1968). This overlap is even more pronounced if one compares the b_2c primary axonal diameters with those of the secondaries (Banks et al, 1979).

The secondary afferents innervate the regions of the intrafusal fibres adjacent to the primary ending (Boyd, 1962). Boyd (1962) divided the regions adjacent to the primary into a series of zones each 400 μ m long, and classified the secondaries as S_1 , S_2 , S_3 , etc. according to their position.

The distribution of the secondaries is different to that of the primaries in that, although 68% of secondaries show a b_1b_2c distribution (Banks et al, 1982), the chains have a much more extensive innervation than do the bags (Boyd, 1962; Cooper & Daniel, 1963), and the bag_1 fibres usually have about half as much innervation as do the bag_2 fibres (Banks et al, 1979; 1982). The analysis of

physiological data appears to indicate that the bag₂ innervation that is present makes little or no contribution to the overall response pattern of the secondary (Appelberg, Bessou & Laporte, 1966; Boyd, 1981a), and a segregated distribution of the first-order branches is rare (Banks et al, 1982).

As with the primary, the secondary ending is often characterised by the annulospiral appearance (Barker, 1948), although some also end in sprays (Ruffini, 1898; Barker & Ip, 1960).

1.6 The Efferent Innervation.

The intrafusal muscle fibres receive a motor innervation from two classes of efferent axon, the gamma (γ) and beta (β) efferents. The γ efferents are small diameter myelinated axons conducting in the range 15-55m/sec (Kuffler, Hunt & Quilliam, 1951). These efferents were first studied by Leksell (1945) who demonstrated that they probably had no action on the extrafusal fibres. Their exclusively fusimotor action was subsequently confirmed by Kuffler et al (1951).

The β efferents are larger myelinated axons conducting in the range 39-92m/sec, although the majority are between 45-75m/sec (Emonet-Dénand, Jami & Laporte, 1975; Emonet-Dénand & Laporte, 1975). These efferents, unlike the γ axons, branch to innervate both the intrafusal and extrafusal muscle fibres (Bessou, Emonet-Dénand & Laporte, 1965).

1.7 The Gamma Efferent Innervation.

The γ efferents are divided physiologically into two main groups, static and dynamic (Matthews, 1962), on the basis of their effects on the primary afferent response. These static and dynamic γ axons terminate in two very different types of ending termed trail and p₂ plate, respectively (Barker, Stacey & Adal, 1970;

Barker, Emonet-Denand, Laporte, Proske & Stacey, 1973; Barker et al, 1976a, b).

The distribution of these static and dynamic efferents on the different intrafusals has been the cause of much controversy during the last decade (Boyd, 1981a, b). The results of a number of studies from different laboratories with the use of a variety of techniques have indicated that the γ dynamic (γ_d) axons innervate the bag₁ fibres (terminating in p₂ plates) almost exclusively, and the γ static (γ_s) efferents innervate the chains, the bag₂ or both intrafusals fibre types (terminating in the trail endings) (Brown & Butler, 1973; 1975; Barker et al, 1976b (glycogen-depletion technique); Barker, Bessou, Jankowska, Pages & Stacey, 1978 (injection of activated fibres with Procion Yellow dye); Bessou & Pages, 1975; Boyd, Gladden, McWilliam & Ward, 1977a (direct observation of contractions elicited by stimulation of single axons)). The only remaining controversy concerned the presence of γ_s terminations on the bag₁ fibre. This arose as a result of conflicting evidence from the techniques employed. The glycogen-depletion technique indicated that in tenuissimus up to 50% of spindles (Brown & Butler, 1973; Barker et al, 1976b) and 38% of spindles in PB had γ_s innervation on the bag₁ fibre (Emonet-Denand, Jami, Laporte & Tankov, 1980). However, Boyd et al (1977a) observed no activation of the bag₁ fibre by γ_s axons and maintained that the γ_s axons only innervated the bag₂ and chain fibres.

As a result of detailed analysis of teased, silver preparations (Barker & Stacey, 1981) it has been agreed that γ_s terminations are uncommon on the bag₁ fibre. The usual situation therefore is that the γ_d axons innervate the bag₁ fibre and the γ_s axons innervate the bag₂ and chain fibres.

1.3 The Beta Innervation.

The β efferent innervation has also been divided physiologically into static and dynamic groupings (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1977; Banks, Barker, Bessou, Pagès & Stacey, 1978; Harker, Jami, Laporte & Petit, 1977).

The use of degeneration experiments has indicated that the β collaterals terminate in the p_1 plates (Barker et al, 1970; Barker, Emonet-Dénand, Laporte & Stacey, 1980), the β_d efferents innervating the bag₁ fibres (Barker et al, 1977) and the β_s efferents innervating the chains (predominantly the long chain fibres (Harker et al, 1977; Jami, Lan-Couton, Malmgren & Petit, 1978; 1979)).

1.9 Motor-endplate Structure.

Barker (1966) described two types of intrafusal plate ending which were termed p_1 and p_2 (Barker, 1967). The p_1 plate is derived from the collateral branch of the β skeleto-fusimotor efferent (see above). The β collaterals usually terminate as a single plate or group of plates closely adjacent on the same intrafusal fibre (Barker et al, 1970). The plates thus produced quite closely resemble the extrafusal motor endplates, being compressed and having a nuclear sole-plate which also shows a Doyère eminence (Barker et al, 1970). The mean plate length is about $33\mu\text{m}$ (Barker, Emonet-Dénand, Laporte & Stacey, 1979).

The p_2 plate (innervated by the δ_d axons) is an elongated structure, some $73\mu\text{m}$ in length on average, produced by a ramification of the preterminal axon (Barker et al, 1970). The endplate is characterised by a number of knob-like terminals lying in shallow depressions on the sole-plate and there is no Doyère eminence (Adal & Barker, 1967; Barker et al, 1970).

The trail ending is innervated by the δ_s axon which branches at the terminal and preterminal nodes to produce a number

of small ramifications (Barker, 1968). A single γ efferent may branch proximally to the spindle to innervate more than one spindle (Barker, 1968; Barker et al, 1976a; Emonet-Dénand, Laporte, Matthews & Petit, 1977) and it may branch intra-capsularly to innervate both poles of a spindle (Barker, 1968; Barker et al, 1970).

2.1 The Afferent Response.

In 1933, B.H.C. Matthews successfully split a muscle nerve to make the first recordings from a single muscle-spindle afferent. He described four types of ending, two of which (A1 and A2) he believed to originate from the muscle spindle. He attributed the low-threshold response, which paused during muscle contraction, to the flower-spray ending and the higher threshold, less dynamic, response to the annulospiral ending. Subsequent studies have shown that both the A1 and A2 responses are probably those of the annulospiral ending.

Hunt (1954) correlated the afferent responses with the conduction velocities of the afferents. He separated the Ia and II afferents arbitrarily at 72m/sec conduction velocity, at the point corresponding with the trough in the bimodal distribution of conduction velocities (this division is comparable with an axon diameter of 12 μ m by Hursh's conversion factor (Hursh, 1939)) and described both endings as being slowly adapting, the primaries having a lower threshold. Later, Matthews (1963) proposed that afferents conducting at above 80 or below 60m/sec could be classified as Ia or II, respectively, but that more definitive criteria were needed to distinguish between the more intermediate afferents. Cooper (1961) examined the responses of the afferents to constant velocity stretch and demonstrated the classical difference in dynamic sensitivity between the two types of ending, which was quantified for the ramp-and-hold stretch in terms of the 'dynamic response' (Jansen & Matthews, 1962); subsequently the term 'dynamic index' was introduced (Crowe & Matthews, 1964a) which was defined as the difference between the firing rates at the peak of the stretch and at half a second into the hold phase.

The use of the instantaneous frequency display (Jansen & Matthews, 1962) and the instantaneous frequencygram (Bessou, Laporte & Pagès, 1968a) enabled the production of records in which the difference between the two types of afferent are more immediately obvious.

2.2 The Primary Response.

The typical response of the primary afferent to the ramp-and-hold stretch in de-efferented preparations often consists of an 'initial burst' at the start of the ramp followed by a rapid increase in firing up to the peak of the ramp. At the start of the hold phase there is a rapid drop in firing rate followed by a more gradual decline until a new steady level is reached. At the release of the stretch the afferent ceases firing completely as the spindle is unloaded, and then the tonic firing level is gradually restored (see Matthews, 1972; Hunt, 1974).

The initial burst is usually only present in de-efferented preparations (Jansen & Matthews, 1962) and can be abolished by rapid repetitive stretching (Hunt & Ottoson, 1976). Hunt & Ottoson (1976) attributed it to a static frictional force, probably produced by the presence of cross-bridges in the intrafusal fibres which greatly increase the short-range stiffness of the fibres, thereby initially transferring a large proportion of the mechanical displacement to the sensory region (see also Brown, Goodwin & Matthews, 1969; Matthews, 1972).

The increase in firing frequency of the Ia is dependent on the rate and amplitude of the stretch applied. However, the response is proportional to the amplitude of the stretch for movements of only a fraction of a millimetre before a transition in

sensitivity occurs and the response becomes non-linear (Matthews & Stein, 1969; Hunt & Ottoson, 1975; Hasan & Houk, 1975b). The linear portion of the response is the portion associated with the period of initial stiffness of the intrafusal fibres (Hasan & Houk, 1975b).

Working with isolated spindles, Poppele, Kenney & Quick (1979) have shown that the stiffness of the sensory region does not change with changing spindle length, but that the stiffness of the polar regions increases non-linearly. This is probably associated with the paucity of myofibrils in the equatorial region (Barker et al, 1976a; Poppele et al, 1979). Further, Poppele et al (1979) demonstrated that the primary sensitivity (within certain physiological limits) was proportional to the strain ($\Delta L/L$) of the sensory region, therefore any non-linearities in the response must be due to the non-linearities associated with the movements of the polar (myofibrillar) regions of the intrafusal fibres. Recently, Poppele & Quick (1981) have made accurate measurements of the changes in stiffness of the bag₁ myofibrillar regions and the sensory region during slow ramps. The results of these measurements indicate the occurrence of a stretch-induced contraction of the bag₁ which is initiated after the stretch has reached a certain threshold. The effect of such a contraction would be to introduce a delayed amplification of the length changes, thereby possibly accounting for the marked dynamic sensitivity of the primary ending.

Emonet-Denand, Laporte & Tristant (1980) examined the responses of Ia and II endings to slow ramp stretches with small amplitude triangular stretches superimposed. During the ramp, the peak-to-peak response of the primaries to the triangular stretches increased in amplitude at a progressively increasing rate. These

results can be interpreted in the light of the studies by Poppele et al (1979;1981) in that during the slow stretches the polar regions become progressively stiffer and therefore an increasing proportion of the small stretches is transmitted to the sensory region.

Numerous attempts have been made to construct an accurate model for the primary response to the velocity phase of the ramp. Matthews (1964) experimented with some simple models involving springs and dash-pots to recreate the elastic and viscous elements of the intrafusal fibres. Andersson (Lennerstrand,1968a) developed a series of mathematical expressions for the 'slow' and 'quick' elements of the Ia response. Hasan & Houk (1975a,b) derived further expressions for the non-linearity of the dynamic response and more recently Houk, Rymer & Crago (1981a,b) found that the dynamic response was approximately proportional to the stretch velocity raised to the 0.3 power. An accurate approximation could be achieved by combining the $V^{0.3}$ term with a linear length-dependent term.

At the peak of the stretch the primary ending shows a rapid drop in firing rate (Lippold, Nicholls & Redfearn, 1960) before gradually decreasing to a steady level during the hold phase. Smith (1966) observed a slow creep in the 'slow' bag fibre (the bag₁) following the peak of the ramp. This has been attributed to some 'give' in the polar regions of the bag₁ fibre, possibly associated with the lack of elastic fibres (Boyd, 1976b; Gladden, 1976). The observation of a stretch-induced contraction of the bag₁ (Poppele & Quick, 1981) would allow the creep to be explained in terms of a relaxation of the contraction occurring once the stimulus of increasing stretch was removed. The time course of the

creep is comparable with the slow drop in firing level (Boyd, 1976b; Boyd & Ward, 1975; Boyd, Gladden & Ward, 1977b; 1981). In fact Boyd et al (1981), on the basis of measurements of the extension of the sensory region of the bag₁, proposed that the firing rate of the primary at any point during the ramp stretch closely reflects the degree of extension of the spirals on the bag₁ fibre.

The fast drop in firing rate which occurs at the peak of the ramp is too rapid to be explained in terms of mechanical 'give' (Husmark & Ottoson, 1971a, b; Boyd, Gladden, McWilliam & Ward, 1977a). Further, if the velocity of the stretch is increased the fast drop takes the firing rate below the final static level, this drop then being termed the post-dynamic undershoot (Hunt & Ottoson, 1975). This undershoot is attributed to ionic mechanisms (Husmark & Ottoson, 1971a, b) possibly as a result of a voltage-dependent increase in potassium conductance (Hunt, Wilkinson & Fukami, 1978).

At the release of the ramp there is a drop in firing rate as the receptor is unloaded; depending on the rate of release and the extent of the original stretch this drop may be characterised by a post-release undershoot (Hunt & Ottoson, 1975) again attributed to a transient hyperpolarisation as a result of K⁺ conductance changes (Hunt et al, 1978), although measurements of spindle tension do also indicate a small undershoot on release (Hunt & Ottoson, 1975).

2.3 The Secondary Response.

The secondary ending is characteristically less sensitive to the velocity component of the ramp and overall has a higher threshold to stretching (Cooper, 1961; Bessou & Laporte, 1961; Jansen & Matthews, 1962; Matthews, 1963; Lennerstrand, 1968a). The secondary usually shows no initial burst (Matthews, 1963) and responds more smoothly to the rising phase of the ramp. At the peak of the ramp

any pause in firing is small and during the hold phase there is much less adaptation than is shown by the primary (Matthews, 1972; Hunt, 1974). The linear portion of the response to stretch is greater for the secondary than for the primary (Matthews & Stein, 1969).

Many of these differences in response characteristics may be a result of the different position of the secondary ending on the intrafusal fibres as compared with the primary. The secondary ending lies juxta-equatorially and is mainly distributed over the chain fibres (Boyd, 1962; Cooper & Daniel, 1963; Banks et al, 1982).

The chain fibres are more homogeneous along their length than the bag fibres (Banks et al, 1977b) and the juxta-equatorial positioning of the secondary innervation means that the bag innervation is on a region of the fibres having a higher proportion of myofibrils than the equatorial region and therefore is closer in nature to the polar regions. As a result one would expect the secondary to display fewer non-linearities in its pattern of response, especially in the case of S_2 or S_3 secondaries which lie distal to the equator (Boyd, 1962) and tend to show less distribution onto the bag fibres (Banks et al, 1982). There is some indication that this is the case (Poppelle, 1981; Hasan & Houk, 1975a).

The secondary-ending response is usually characterised by an absence of the post-dynamic undershoot and only a relatively small post-release undershoot (Hunt & Ottoson, 1975).

2.4 Fusimotor Stimulation.

Stimulation of fusimotor and skeleto-fusimotor efferent axons causes the intrafusal fibres to contract, thereby transferring a range of mechanical stimuli to the sensory region, depending on which intrafusal fibres are contracting and whether the contraction is restricted to one pole or if both are active.

Stimulation of Gamma Efferents.

Kuffler, Hunt & Quilliam (1951) demonstrated that stimulation of a single γ efferent increased the discharge of spindle afferents in the absence of extrafusal muscle contraction. Matthews (1962) divided the γ efferents into two groups, the γ static and γ dynamic efferents on the basis of their effects on the primary afferent response. Subsequent work has shown that this classification may be too broad since it covers a whole range of intermediate effects (Emonet-Denand et al, 1977); however, it is still a very useful initial division of the gamma populations associated with the recent agreement on a selective pattern of innervation of the intrafusal fibres.

2.5 Gamma Dynamic Stimulation.

The γ_d efferents terminate in the p_2 motor endplates which are almost exclusively restricted to the bag_1 fibre. Bessou & Pages (1972) measured the intracellular potentials evoked by γ_d stimulation and observed that there was no propagated action potential in response to the stimulation. Previously Bessou, Laporte & Pages (1968b) had reported that there was no, or only a very small, increase in the Ia afferent discharge in response to single γ_d shocks and that the response to repetitive stimuli had a slow, smooth rising phase and also a prolonged relaxation phase on cessation of stimulation.

These findings were substantiated by the direct observation studies of Bessou & Pages (1975) and Boyd and co-workers (Boyd, 1976a,b; Boyd et al, 1977a) which showed that the bag_1 fibre contracts slowly in response to repetitive γ_d stimulation and this is reflected in its modulation of the Ia discharge.

When a muscle is stretched during γ_d stimulation the

dynamic response is greatly increased (Matthews,1962; Crowe & Matthews,1964a; Boyd,1981a) due to the contraction stiffening the poles and also potentiating the stretch-induced contraction, thereby transferring a greater proportion of the mechanical displacement to the sensory region (Boyd,1981a).

Following the ramp the rapid fall in firing still persists (Crowe & Matthews,1964a; Lennerstrand & Thoden,1968a), but the slow adaptation is much more marked. This is associated with the greatly pronounced mechanical creep observed during γ_d stimulation (Boyd, 1976b; Boyd et al,1977a; Poppele & Quick,1981).

Throughout the whole cycle, γ_d stimulation increases the absolute firing rate, even when the muscle is relaxed, as well as greatly increasing the dynamic index and dynamic sensitivity when outside the linear range (Hulliger,Matthews & Noth,1977; Crowe & Matthews,1964a; Lennerstrand & Thoden,1968a; Boyd,1981a).

Gamma dynamic stimulation has little effect on the responses of the secondary endings (Appelberg et al,1966; Boyd,1981a). This is in part probably associated with the limited innervation of the bag₁ fibre by the secondary terminals and the relative scarcity of secondaries showing a segregated distribution of their first-order branches (Banks et al,1979;1982).

Where bag₁ contraction does affect the II response, there is not necessarily an increase in the dynamic sensitivity, and any such change is usually masked by an increase in the length sensitivity (Durkovic & Preston,1974; Boyd,1981a).

2.6 Gamma Static Stimulation.

The γ_s efferents terminating in trail endings innervate the bag₂ or the chain fibres, or both (section 1.7).

As with the bag₁ fibre, the bag₂ shows distinct foci of

smooth contraction (Bessou & Pages, 1975; Boyd, 1976a,b; Boyd et al, 1977a) and does not usually respond to single stimuli (Boyd, 1976a,b) although Bessou & Pages (1975) observed very small twitches in response to single shocks. The contraction of the bag₂ fibre is much stronger and more rapid than that of the bag₁ and there is usually no creep associated with the end of the velocity component of the stretch (Boyd, 1976b; Boyd et al, 1977a).

Activation of the bag₂ fibre greatly increases the background discharge (although there is also more marked adaptation of the response), and during the ramp the dynamic index is reduced even though the absolute firing level is elevated. The length sensitivity, when corrected for the adaptation, is also reduced or unchanged (Boyd, 1981a).

As with the bag₁ fibre, contraction of the bag₂ fibre alone has little effect on the response of the secondaries, the overall firing being elevated with little change in the dynamic or length sensitivities (Jami, Lan-Couton & Petit, 1980; Boyd, 1981a). Again, this is probably associated with the predominance of II terminations on the chains rather than on the bag fibres (Boyd, 1962; Banks et al, 1979; 1982).

The nuclear-chain fibres are often activated as a group (Smith, 1966; Bessou & Pages, 1975; Boyd, 1976b; Boyd et al, 1977a) and display rapid, twitch-like contractions. The fusion frequency of the contractions is relatively high, so that up to 60Hz stimulation the contractions will induce 1:1 driving of the primary ending (Brown, Crowe & Matthews, 1965; Boyd, 1976b; Boyd et al, 1977a). This driving completely masks any alterations in receptor sensitivity, and even at higher stimulation frequencies the primary discharge is highly irregular, making measurements of the dependence

of the response on the dynamic and length components of the ramp difficult (Boyd, 1981a).

The secondary ending is rarely driven by activation of the chain fibres and, as with activation of the bag₂ fibre, the dynamic sensitivity is reduced while the length sensitivity is often increased (Jami & Petit, 1978; 1981; Jami et al, 1980; Boyd, 1981a). Jami et al (1980), using the glycogen-depletion technique, demonstrated that only those γ_s axons that innervated several, if not all, of the chain fibres in one or both poles had a marked effect on the II response. Those that activated the bag₂ alone, or the bag₂ and one or two chain fibres had little effect on the II response to ramp stretch.

Emonet-Dénand et al (1977) sub-divided the γ efferents into six classes ranging from 'pure' dynamic action (category I) to 'pure' static (category VI) with the intermediate groups apparently showing some admixture of static and dynamic characteristics which they interpreted in terms of non-selective innervation.

Emonet-Dénand et al (1977) proposed that categories III-V involved some degree of dynamic modification of the static response. However, following the recent histological analyses of Barker & Stacey (1981) and Banks (1981), which revealed only a small proportion of spindles having trail innervation on the bag₁ (17% in PB and 8% in tenuissimus (Barker & Stacey, 1981)), it seems unlikely that 38% of the responses could be due to non-selective innervation as was proposed by Emonet-Dénand et al (1977) on the basis of the physiology and the glycogen-depletion studies (Brown & Butler, 1973; 1975; Barker et al, 1976b). Emonet-Dénand et al (1977) also reported that 23% of the predominantly dynamic category also displayed some static involvement (their category II). Laporte (1979) examined the

group II dynamic axons and proposed that their effect was not due to γ_d innervation of the bag₂ or chain fibres, but rather to the proximity of the contraction site to the equatorial region and the relative strength of the contraction.

A more probable explanation for the variations in γ_s effects is likely to be found in a predominantly selective innervation with the group III effects representing the small proportion of γ_s axons that innervate the bag₁ fibre (Banks, Barker & Stacey, 1981). Categories IV, V and VI could be attributed to γ_s axons that innervate various combinations of either one or both poles of the bag₂ or the chain fibres or both (Barker & Stacey, 1981).

2.7 Beta Skeleto-fusimotor Stimulation.

The co-activation of extrafusal muscle fibres along with the intrafusal fibres by β efferents for a long time masked the augmentation of the afferent response due to the intrafusal-fibre contraction. Bessou, Emonet-Dénand & Laporte (1963; 1965) provided the first clear evidence of the presence of skeleto-fusimotor axons. Working on the first deep lumbrical muscles they demonstrated that the stimulation of certain α axons at frequencies well above the fusion frequency of the extrafusal muscle fibres progressively increased the dynamic response of the spindle primary ending. This increased response was also observed when the extrafusal contraction was blocked by gallamine triethiodide.

Subsequent studies have shown that the β innervation is very common in some muscles, some 70% of PB spindles being innervated by β axons (Emonet-Dénand & Laporte, 1975) and that there are both static and dynamic β efferents (Emonet-Dénand, Jami & Laporte, 1975; Harker et al, 1977; Jami et al, 1978; 1979; Jami, Murthy & Petit, 1982).

Beta dynamic axons innervate the bag₁ fibre almost exclusively (Barker et al, 1977) and, as such, produce effects on the Ia response equivalent to those of the γ_d efferents. Beta static efferents similarly produce effects equivalent to the γ_s axons (Jami, Murthy & Petit, 1980; 1982). Use of the glycogen-depletion technique indicates that these γ_s axons mainly innervate the chains, especially the long chain fibres (Barker et al, 1977; Jami et al, 1978; Banks, 1981).

2.3 Generation of the Afferent Response.

The mechanism of sensory transduction in the muscle spindle is very much open to debate. The only hypotheses at present are purely speculative owing to the lack of hard evidence from experiments. These hypotheses are based on a system of changes in ionic permeability of the terminal membranes coupled to some form of stretch deformation, which could be simple stretching, or flattening, or shearing.

The ionic processes resulting from this deformation are also open to question. Hunt et al (1978), working on decapsulated mammalian spindles, showed that Na^+ was the principal carrier of current associated with the receptor potential. In the absence of Na^+ , Ca^{++} could partially compensate, but the application of Ca^{++} blockers in the presence of normal Na^+ concentrations had no effect on the intra-cellular potentials recorded from the afferent axon.

The role of Ca^{++} conductivity in the response is at present also open to debate. Hunt et al (1978) showed that Na^+ was the principal current-carrying ion and could maintain the full response in the absence of Ca^{++} mobility. Ito and co-workers (Ito & Komatsu, 1979; 1980; Ito, Komatsu & Kaneko, 1980; Ito, Komatsu & Katsuta, 1981) propose a key role for Ca^{++} in the frog spindle in

that application of Ca^{++} blockers suppressed all the responses to stretch. The ionic model they propose involves both Na^+ and Ca^{++} spikes, the pCa^{++} changes producing the receptor potential which drives the pacemakers (Ito et al, 1980).

The post-dynamic and post-release undershoots are associated with hyperpolarisation of the terminals by increased gK^+ (Hunt et al, 1978) apparently by a voltage-dependent coupling, such that the greater the velocity and amplitude of stretch, the greater are the undershoots. Ito, Komatsu, Kaneko & Katsuta (1981) also propose a role for the gK^+ changes which are dependent on the Ca^{++} concentrations and which determine the degree of variability in the discharge of the frog spindle.

The mechanism of combination of receptor potentials to produce the final afferent response is another area for debate. The Ia axon of the primary ending branches at least once and usually several times before the individual branches lose their myelin (Banks et al, 1977a, 1979; 1982). Quick, Kennedy & Poppele (1980) used a ferric ferrocyanide cytochemical stain to show that all the heminodes and some of the preterminal nodes are potential sites of spike generation.

The results of fusimotor stimulation experiments have provided substantial indirect evidence for the presence of a number of pacemaker sites (Crowe & Matthews, 1964a, b; Hulliger, Matthews & Noth, 1977), especially given the apparent occlusion of one pacemaker by increased activity in another (Hulliger & Noth, 1979).

In the frog spindle, analysis of the firing patterns and the arrangement of the myelinated branches has also led to the hypothesis that spikes are produced in several of the branches,

the spike trains then interacting at higher-order nodes (Brokensha & Westbury, 1973; 1975; Ito, Kanamori & Kuroda, 1974). The observation of abortive spikes in some branches further substantiates these theories (Ito, 1969; Ito, 1976; Ito & Ito, 1976) in that it provides evidence that not all the spikes that are generated are actually propagated into the parent axon.

A branch point central to the pacemakers has a key role in the production of the final afferent response. When an action potential arrives at the branch point there is effectively a step increase in diameter due to the larger diameter of the parent axon, plus the diameter of the other branch (or branches). Depending on the relative diameters and the recent history of activity in the axons, the spike may be propagated orthodromically in the main axon and antidromically in the other branch. Another possibility is that it may fail completely as a result of the low safety-factor associated with the increase in membrane area, or it may propagate down the branch and not in the main axon, or vice versa (Eagles & Purple, 1974; Goldstein, 1978; Goldstein & Rall, 1974).

Assuming the action potential is propagated into both parts then the antidromic spike may either invade the pacemaker, thereby resetting it; or collide with an orthodromic spike in the branch. Due to the very short distances involved, such a collision is likely to result in the resetting of both encoders (Eagles & Purple, 1974). The third possibility is that collision will occur without resetting either encoder. In all cases the faster firing pacemaker is likely to occlude the activity of the slower one. The results of Hulliger & Noth (1979) demonstrate the occlusion of the dynamic pacemaker by the static and thus provide support for some combination of these alternatives.

If the spike in one branch fails to propagate past the branch point, the resultant sub-threshold current would depolarise the membrane and hence, for a specific period, enhance the likelihood of propagation of an impulse ascending in the other branch.

If one assumes a system of multiple pacemakers with interaction of spike trains at at least one node, then it becomes apparent that the branching pattern of the afferent is important in determining the characteristics of the response shown by that particular afferent. In a fully segregated Ia ending (i.e. at the first-order branch point, one branch innervates the bag₁ exclusively and the other one innervates the bag₂ and chains) at the first-order branch site there would be interactions between the 'static' train of spikes and the 'dynamic' train. Given such a situation it is possible to envisage occlusion of the dynamic response by the static and vice versa. Hulliger & Noth (1979), however, did not observe any occlusion of the static by the dynamic response and therefore they proposed a model in which there is a group of pacemakers in parallel in each of the first (or second) order branches that feed to a common pacemaker at the main branch point. This final pacemaker may, at certain times of activity, achieve dominance over the lower-order pacemakers and thus modify the simple pattern of spike train interaction. If one assumes a non-segregated branching pattern the picture becomes far more difficult to analyse, the response pattern being dependent on the degree of mixing of static and dynamic inputs prior to the second or first-order pacemakers and on the position of the initial encoder sites which integrate the receptor potentials.

3.1 Response to Nerve Injury.

All peripheral axons have the potential for regeneration following injury, provided that the cell body is undamaged (Kiernan, 1978; 1979), and the processes of degeneration and regeneration have been studied extensively (Young, 1942; Guth, 1956; Mira, 1976; Grafstein & McQuarrie, 1978; Sunderland, 1978; Selzer, 1930; and others).

The brief description of the processes of degeneration and regeneration that follows is based mainly on the material contained in the above reviews.

3.2 Nerve Degeneration.

The first changes following injury to a peripheral nerve axon occur after about 24 hours. Distal to the site of the injury there is a focal swelling of the axon accompanied by fragmentation of the endoplasmic reticulum which mark the first stages of Wallerian degeneration. Over the next two or three days the mitochondria swell and the axon takes on a varicose appearance with discontinuities appearing between the focal swellings.

Although the degenerative changes of the myelin sheath apparently lag behind those of the axon, within two minutes of the injury the myelin retracts from the nodes of Ranvier and there is an increase in the number of Schmidt-Lanterman incisures. Subsequently the myelin lamellae loosen and the myelin fragments, producing characteristic rows of ovoids which are pinched off at the incisures. The sequence of myelin degeneration is well advanced by 36 to 48 hours after the injury.

The Schwann cells proliferate and, with the invading macrophages, phagocytose the axon and myelin debris. The Schwann cells become organised into longitudinal arrays termed Bands of Bungner.

Proximal to the injury site there are retrograde reactions of the axon, although these are not nearly so severe as those distal to the lesion, extending for a few centimetres at the most and more usually being confined to a few millimetres adjacent to the lesion. There is some reduction in diameter and retrograde disruption of the myelin sheath with concomittant Schwann cell proliferation.

The cell body usually shows marked changes in response to injury of its axon, many of which are attributed to changes in preparation for the process of regeneration. The cell body swells, the nucleus moves towards the periphery and the Nissl substance breaks up and becomes dispersed, giving rise to the chromatolytic appearance. There is also an increase in RNA synthesis with a subsequent increase in protein synthesis and also lipid production.

Alterations also occur in the relationship of presynaptic terminals with the cell body, many of these withdrawing contact, and in some cases the functional integrity of other neurons trans-synaptic to the injured cell may be impaired.

3.3 Nerve Regeneration.

The onset and velocity of regeneration is dependent on the severity of the lesion, but there is usually some evidence of sprouting of the proximal tip after 24 hours. Because of the retrograde axonal degeneration, the initial period of growth occurs proximal to the lesion in a segment of relatively undamaged endoneurial tube. However, once the axon tip arrives at the injury site its progress is impeded by the presence of scar tissue in a quantity dependent on the type of injury.

Sunderland (1978) divided the regenerative process into four periods:-

- 1/ The initial delay- the time to the onset of axon growth and for the axon tip to reach the injury site.
- 2/ The scar delay- the time for the axon to traverse the injury site.
- 3/ The growth period- the time for the axon to reach its peripheral target.
- 4/ The period of functional recovery- the time for the axon to re-establish functional connexions.

Outgrowth occurs by the production of a growth cone at the axon tip with fine extensions called filopodia extending from the tip that apparently progress by amoeboid movements. Sprouting also occurs at the nodes of Ranvier preterminal to the axon stump.

Outgrowth is greatly facilitated if the axon grows down a pre-existing endoneurial tube and thus, in crush injuries where most of the tubes remain intact, recovery is swifter than after nerve section. Axons may also grow down between the tubes if their own tubes have been sectioned (Cabaud, Rodkey & McCarrell, 1980).

The Schwann cells proliferate in response to the injury and become organised into longitudinal bands (Bands of Büngner) which provide a degree of orientation of the axon towards the opening of the endoneurial tube (if severed).

Remyelination occurs some seven days after the commencement of outgrowth following nerve crush, but takes longer after section, and the first nodes appear after some fourteen days.

3.4 Rates of Regeneration After Nerve Crush.

A number of different methods has been used to measure the rate of regeneration and the results vary according to the method employed. Growth rates are also affected by the age, condition and species of the animal, and also to a lesser extent on the nerve

injured and the distance of the injury from the periphery.

One of the first, and still widely used, techniques for ascertaining the rate of regeneration is based on the histological identification of the axon tips below the injury site and measuring their rate of advance (Cajal, 1928; Gutmann, Guttmann, Modawar & Young, 1942; Barker & Boddy, 1980).

In Man the rate of advance can be measured by marking the advance of the Tinel sign (Sunderland & Bradley, 1952). In other mammals the same effect can be achieved by exposing the nerve in a lightly anaesthetised animal and noting the most distal point at which pinching the nerve elicits a withdrawal reflex (Gutmann et al, 1942).

Where lesions have been made in cutaneous nerve it is possible to measure regeneration by the reduction in the area of analgesia. This method will, however, give different results to the others since reinnervation of a target organ is a complex process and so the rate is likely to be slower than for direct growth (Gutmann et al, 1942). The situation may also be complicated by the sprouting of intact axons which can invade the denervated area and thereby alter the reinnervation pattern (Livingston, 1947). Recently, though, there has been some evidence to show that sprouting of cutaneous axons does not in fact occur (Devor, Schonfeld, Seltzer & Wall, 1979; Horch, 1981). Gutmann et al (1942) similarly measured the rate of functional completion by monitoring the return of the toe-spread reflex in the rabbit after nerve crush.

Recently, a number of studies has employed the use of radioactively labelled amino acids which are transported axonally and incorporated in the axon tips, such as tritiated proline (Forman & Berenberg, 1978), or tritiated leucine (Bisby, 1978; 1979).

These methods have a great advantage in that as well as providing information about the advance of the fastest growing axons, they also mark the position of the main body of the regenerating axons (Forman & Berenberg, 1973).

After nerve crush of the rat sciatic nerve Forman & Berenberg (1973) measured the positions of the leading wave and the main body of axons and found that they were advancing at 4.4 and 3.0mm/day, respectively. Using the pinch test an overall rate of 3.99mm/day was recorded (Forman, Wood & De Silva, 1979).

In the case of the cat after nerve crush, Konorski & Lubinska (1946) measured growth rates of 4.0 and 4.8mm/day for the peroneal and radial nerves, respectively, using the pinch technique. Barker & Boddy (1980) recorded a rate of advance of 3.2mm/day after an initial delay of 5.8days for the cat common peroneal nerve.

3.5 Factors Affecting Regeneration.

At the site of the injury the extent of the trauma is a key factor in determining the time of onset and the quality of regeneration. If the endoneurial tube remains intact (as is theoretically the case with a crush injury (Sunderland, 1978)) then the disruptive effects of scarring and the problems for the growing axon of negotiating the course from its original endoneurial tube to an open tube distal to the lesion are minimised. Also the effects of shrinkage of the distal tube, which occurs if it remains empty for any period (Sunderland & Bradley, 1950) are reduced, thereby improving the conditions for maturation of the axon.

Even after nerve crush there is evidence of substantial branching of the damaged axons (Shaw, 1955), but it appears that in the majority of cases the parent axon will only support one

branch, the others degenerating over a period of 150 days following the injury (Gutmann & Sanders, 1943; Devor & Govrin-Lippmann, 1979a; Horch & Lisney, 1981a). After nerve section a number of these branches may persist (Esslen, 1960; Fullerton & Gilliatt, 1965; Horch & Lisney, 1981a). Since in practice a perfect crush is not possible, and some of the endoneurial tubes are always severed (Gutmann & Young, 1944), the possibility must be entertained of the persistence of a small number of multiple sprouts in the distal segment of a crushed nerve.

The restoration of functional connexions with the peripheral target is of great importance for the maturation of the regenerating axon (Sanders & Young, 1946), although the restoration of the appropriate connexions is not essential (Aitken, 1949; Aitken, Sharman & Young, 1947).

3.6 Properties of Regenerated Axons.

Following a crush injury, the fibre diameter decreases proximally to the lesion (Gutmann & Sanders, 1943). This is due to a reduction in axon diameter accompanied by a slight increase in myelin sheath thickness (Sanders, 1948). After 300 days the myelin sheaths are still larger than normal and the axon diameters have recovered to a great extent (Sanders, 1948).

In the distal stump of rabbits Gutmann & Sanders (1943) observed that the fibres recovered their normal diameters after a year, and Sanders (1948) reported that the myelin sheaths were thicker than normal. In contrast, Schröder (1972) found that in rats and dogs, even after a year, the distal axons were only 95% and 82% of their normal diameters, respectively, and the myelin sheaths were 79% and 57%, respectively, of normal. Cragg & Thomas (1964) also reported a slight reduction in fibre diameter a year after nerve crush.

In normal nerve fibres the inter-node distances tend to be largest in the thickest axons and to decrease towards the periphery (Quick, Kennedy & Donaldson, 1979). This could be due to the Schwann cell being stretched as growth of the animal proceeds (Lubinska, 1959). Following regeneration, all the inter-nodal distances are of uniform length (Vizoso & Young, 1943) and this length is independent of axon diameter (Jacobs & Cavanagh, 1969).

A reduction in conduction velocity (CV) is characteristic of regenerated axons. Sanders & Whitteridge (1946) observed that in the rabbit CV's did not return to normal till 400-500 days post-crush in the distal segment, but were increased by up to 10% of normal proximally. In contrast, Cragg & Thomas (1964) reported that after 16 months the CV's in the distal segment of rabbit peroneal nerves were only 75% of normal and in the proximal segment they were 80% of normal after 150 days, but had recovered to normal after 200 days. Erlanger & Schoepfler (1946), working on the phrenic nerve of cats, found that after a year the CV's had recovered to only 62% of normal. Horch & Lisney (1981a) also working on cats reported that after nerve crush, near-normal CV's were restored after 6 months; however, even after 18 months following nerve section the CV's were only 50% of normal.

Some of these changes in CV may be associated with the reductions in fibre diameter. However, as can be seen, they can persist after the diameters have apparently recovered to their normal size, and the ratio of axon diameter to myelin thickness is also nearly normal (Sanders, 1948). The reduction in inter-node distance is not considered to be a major factor in causing these reductions in CV (Cragg & Thomas, 1961; 1964; Jacobs & Cavanagh, 1969; Schröder, 1972). Schröder (1972) proposed that

these reductions are attributable to a reduction in myelin thickness which he observed, but which was not reported in previous studies.

As there tends to be a proportional restoration of fibre diameter (Sanders & Young, 1944) so also the CV's recover proportionally such that the fastest conducting axons, given equal conditions for regeneration, give rise to the fastest conducting sprouts (Devor & Govrin-Lippmann, 1979b).

During the early stages when the myelin sheath and regenerated axon are relatively thin, the safety-factor for spike transmission is very low. A spike propagating orthodromically in an afferent axon will pass through a number of regions which, because of their geometry, may act as filters.

The fine regenerated axon will act as a low-pass filter since the refractory period varies inversely with fibre size (Paintal, 1967) (always assuming that the ability to conduct spikes has been restored). Thulin (1962) reported a three-to-five fold increase in the absolute refractory period in regenerated axons which persisted for up to 44 weeks after the lesion. The site of the lesion will also act as a site of low safety-factor for transmission. This is because there is a pronounced increase in axon diameter on going from the regenerated portion to the parent axon (Waxman, 1980). This increase is further pronounced if more than one axon sprout persists. Any such step increase in the area of membrane to be depolarised reduces the probability of normal spike transmission (Goldstein, 1978; Goldstein & Rall, 1974; Spiro, Yarom & Parnas, 1976; Parnas, Hochstein & Parnas, 1976; Smith, 1980a, b).

3.7 Specificity of Reinnervation of Target Structures.

The problem of the degree of specificity of innervation and reinnervation of target tissues and organs has been under examination for a long time with evidence favouring both selective and non-selective patterns of reinnervation.

In 1917, Elsberg attempted to demonstrate that when the original and a foreign nerve were made available for the reinnervation of a muscle, the original nerve always predominated. Weiss & Hoag (1946), in experiments in which the two nerves were given more equal opportunities to reinnervate the target tissue, found that on average there was no preference for the original compared with the foreign nerve. In the individual cases, though, one or other nerve always strongly predominated, which indicated that which ever nerve arrived first at the distal stump blocked the growth of the other one.

Bernstein & Guth (1961) provided further evidence for non-selectivity of reinnervation after nerve section or crush by a comparison of the ratio of functional innervation of the soleus and plantaris muscles by the L4 and L5 spinal roots before and after injury. In normal rats, the contraction produced by stimulation of L4 relative to L5 is significantly greater in plantaris than in soleus. Following reinnervation, no such difference was observable for either type of injury.

Normal extrafusal muscle fibres are arranged in a checker-board pattern of different histochemical types depending on their motor innervation, and they can be classified according to their ATP'ase, P'ase and succinate dehydrogenase activities (Stein & Padykula, 1962; De Reuck, Van Der Eecken & Roels, 1973). Following reinnervation after nerve section the arrangement is changed such

that fibres having the same histochemical properties tend to be grouped together (Karpoti & Engel, 1968; De Reuck et al, 1973). Further evidence against selective reinnervation is provided by the fact that the variation in size of the reinnervated motor units is much larger than in control muscles (Kugelberg, Ekstrom & Abbruzzesse, 1970; Bagust & Lewis, 1974).

Despite these findings there is strong evidence favouring a degree of specificity, and that is in the reinnervation of the original motor endplates by the returning axons. This has been demonstrated in the frog (Miledi, 1960; Dennis & Miledi, 1974b) and in rabbits (Bennett, McLachlan & Taylor, 1973).

In the case of specialised sense organs there is also evidence for a more selective process of reinnervation, especially after nerve crush. Studies on the reinnervation of cutaneous sense organs indicate that after crush or cut regenerating afferents retain their original functional specificities either by reinnervating, or reforming the same type of receptor as they originally innervated (Burgess & Horch, 1973; Burgess, English, Horch & Stensaas, 1974; Horch, 1979; Horch & Burgess, 1980; Dykes & Terzis, 1979; Terzis & Dykes, 1980).

There is also apparently minimal change in the central synaptic organisation to indicate a change in sensory modality (Horch, 1976), although the retraction and reformation of central synaptic connexions must allow a certain degree of plasticity (Horch & Burgess, 1980; Horch & Lisney, 1981b). After nerve section there is evidence of much greater reorganisation of central projections than after crush (Brushart & Mesulam, 1980; Brushart, Henry & Mesulam, 1981).

Mapping of the afferent receptive fields and cutaneous receptor distributions before and after injury shows that following nerve crush the pattern is restored quite accurately; however, after nerve section the pattern is markedly changed (Burgess et al., 1974; Horch, 1979; Horch & Burgess, 1980; Dykes & Terzis, 1979; Terzis & Dykes, 1980). These results provide evidence that the axons regenerating after nerve crush are mainly confined to their original endoneurial tubes which act as a guidance system (Sunderland, 1973). After nerve section, the chances of an axon entering its original tube are very slim and this is reflected in the reduction of selectivity of reinnervation which can lead to faulty localisation and mis-representation of stimuli (Horch & Burgess, 1980; Hallin, Wiesenfeld & Lindblom, 1981).

4.1 Morphological and Histological Effects of Denervation of the Muscle Spindle.

The effects of short periods of denervation on the gross morphology of the muscle spindle are relatively slight. Even after three months of denervation, De Reuck et al (1973) reported no significant change in the mean diameter of the intrafusal fibres, although Kubota et al (Kubota, Sato, Masegi, Kaboyashi & Shishido, 1978) recorded a reduction in intrafusal diameter down to half normal size and Schröder, Kemme & Scholz (1979) observed a reduction in the mean transverse area. Arendt & Asmussen (1976a, b) also reported an increase in the length of the spindle along with some atrophy of the chain fibres. After more prolonged periods of denervation marked atrophy and degenerative changes become apparent (Tower, 1932; Swash & Fox, 1974; Schröder et al, 1979).

One of the most striking effects of denervation is the increase in the number of the intrafusal muscle fibres in the spindle (Schröder, 1974a; Schröder et al, 1979; Arendt & Asmussen, 1976b; Kucera, 1977a). These apparently arise either by a maturation of satellite cells (Schröder, 1974a) or by splitting of an intrafusal fibre (Schröder, 1974a; Kucera, 1977a). This occurs very rarely with the chain fibres (Arendt & Asmussen, 1976b), but is quite common with the bag₁ (Kucera, 1977a). The new fibre usually runs from the juxta-equatorial region to the poles and only rarely enters the sensory regions (Kucera, 1977a). Schröder (1974a) found such an increase in the intrafusal fibre population in 20% of reinnervated rat spindles.

At the ultrastructural level the effects of denervation on the intrafusal fibres are slight over the first three months,

with a decrease in the number of mitochondria and an increase in the number of intrafusal nuclei. There is also an increase in the number of Schwann cells and in the quantity of connective tissue within the capsule (Schröder, 1974a; Schröder et al, 1979). Within five to seven days of denervation the surface of the intrafusal fibres becomes irregular with numerous undulations (Schröder et al, 1979; Kubota et al, 1978).

The histochemical effects of short-term denervation are more marked with reductions in ATP'ase, P'ase and succinate dehydrogenase activities occurring within two weeks of denervation (De Reuck et al, 1973). Histochemically the intrafusal fibres respond to denervation in the same way as the extrafusal fibres (De Reuck et al, 1973) as compared with their relative resistance to structural change (De Reuck et al, 1973; Arendt & Asmussen, 1976a, b; Schröder et al, 1979).

4.2 Reinnervation.

The process of afferent and efferent reinnervation arrests the atrophy and degeneration of the muscle spindle, although most of the morphological and ultrastructural changes to the intrafusal fibres persist (Schröder, 1974a; Schröder et al, 1979; Arendt & Asmussen, 1976a, b; Kucera, 1977a). Since after crush injury the denervation period is likely to be relatively short (in the experiments to be described the longest denervation period was 50 days), then any structural changes are minimised by the early return of the nerve supply.

After reinnervation the majority of the intrafusal fibres show a return to their normal histochemical profiles, although occasional abnormalities occur. These tend to be localised in an

individual fibre with the rest of the fibre appearing normal. As such these may be due to the local effects of reinnervation by an inappropriate motor axon (Kucera, 1977b).

4.3 Restoration of the Motor and Sensory Endings.

In 1900, Huber reported observations on the degeneration and regeneration of rabbit motor and sensory endings after nerve crush and reported that both could be restored completely. Subsequently, Tello (1907) carried out a similar study of spindle reinnervation after nerve section in the rabbit and he described in great detail the courses of the regenerating motor and sensory axons leading to their relocation on the intrafusal fibres. As well as observing apparent abnormalities in the endings produced, he also observed a number of axons which entered the spindle capsule and left again without making an ending. These axons proceeded to form a motor endplate on one of the extrafusal fibres.

Ip & Vrbová (1973) found that the motor reinnervation proceeded more rapidly than the sensory reinnervation after nerve crush in kittens such that by the time the efferent innervation was almost fully restored, the afferent innervation was still very poor. Ip, Vrbová & Westbury (1977) further demonstrated that successful restoration of the sensory endings is strongly dependent on the prior restoration of the efferent supply. This was shown by sectioning the ventral roots at the same time as the peripheral lesion was produced. Under these conditions the sensory endings failed to achieve a near-normal configuration.

Eventual, though slow, restoration of the sensory endings in the rat was reported by Zhenevskaya & Umnova (1965) after crush with the axons ramifying to produce progressively richer

innervation which results in an apparently normal or near-normal sensory innervation.

In contrast to these observations, Barker & Boddy (1980) found that even 36 weeks after nerve crush all the regenerated primary endings were defective to some extent, one of the most common abnormalities being for one or other of the bag fibres to receive very little, and sometimes no, innervation. Similarly, all the secondary endings examined were also abnormal.

Barker & Boddy (1980) also examined the motor endings and observed that the p_1 plates are the first to achieve a recognisable appearance followed by the p_2 and trail endings. All the motor endings appeared to be restored on the same fibre types as in the normal spindle, the only marked abnormality being a degree of hyperinnervation (this may also occur with the sensory endings).

Barker & Boddy confirmed Tello's (1977) observation of motor axons which course through the spindle and leave to produce extrafusal endplates (Barker & Boddy, 1980). In some instances they noted that these visiting axons made aberrant connexions with the intrafusal fibres en route and they termed these axons 'α invaders'.

Despite the abnormalities that have been reported, the over-riding feature of the reinnervation of the muscle spindle after nerve-crush injury is the high degree of specificity, with the majority of Ia axons only terminating in the equatorial regions, II axons making their endings juxta-equatorially and the motor axons reinnervating their original fibre types (Barker & Boddy, 1980).

At the ultrastructural level certain abnormalities occur in the organisation of the sensory terminals. Schröder (1974b) described these as consisting of a) an alteration in the nature of the contact between the terminals and the intrafusal fibres with some endings only making contact in small areas, the other regions being separated by intervening basement membrane. b) abnormalities in the shape and structure of the terminal, possibly due to the surface irregularities of the intrafusal fibres which occur as a result of denervation (Schröder et al, 1979; Kubota et al, 1973). c) abnormal associations between the terminals and the Schwann cells, with the Schwann cell processes being closely applied to the nerve terminals.

The motor endings examined by Schröder (1974b) appeared to be normal although Schwann cell processes associated with the endings were more numerous than in the controls. These observations were, however, made 24 months after the lesion; there are no descriptions of the endings at earlier times.

4.4 Responses of Reinnervated Spindles.

Thulin (1960) excised a 10mm portion of the tibial nerve in cats and allowed regeneration to proceed for up to 30 weeks. The subsequent experiments indicated that the spindle afferents achieved functional connexions at about the same time as the skeletal-motor efferents, but the small gamma efferents were markedly delayed. Thirty months after nerve section, Bessou, Laporte & Pages (1966) located functional primary endings which could be activated by γ stimulation.

Homma (1969) also reported that the Ia and α axons achieved functional connexions at the same time after section and suture

of the tibial nerve in cats. After freezing the sciatic nerve in cats with dry ice, Takano (1976) found that the afferent responses from the triceps surae muscles were rare before four months had elapsed and he could find no evidence of restoration of the gamma innervation.

In contrast to these results Ip et al (1977) reported that sensory reinnervation could not proceed successfully without prior reinnervation of the intrafusal fibres by the γ axons. Seventy-three days after section of the medial popliteal nerve combined with a ventral root section in cats, they found that 66% of the spindle afferents that they isolated from the soleus muscle displayed abnormal responses, these being characterised by a rapid adaptation or total failure of firing during the hold phase of a ramp-and-hold stretch. Similar increased adaptation rates were observed by Fukami (1972) during the early stages of reinnervation of snake spindles after nerve crush or section, and also in the responses of afferents in new-born kittens (Skoglund, 1960).

Brown & Butler (1976) carried out a more detailed study of the physiology of reinnervated cat spindles. During the early stages of recovery after nerve crush (up to six weeks), although the majority of the functional afferents responded normally, some abnormal afferents were recorded, these becoming less prevalent as recovery progressed. Brown & Butler (1976) attributed such abnormalities to incomplete reinnervation which was rectified if the animal was allowed longer to recover.

As was stated by Ip et al (1977), the most common abnormality involved a lack of the normal response during the hold phase (Brown & Butler, 1976; Fukami, 1972; Hyde & Scott, 1981). These

abnormal afferents can, however, be activated by δ fusimotor stimulation and then show apparently normal patterns of response (Hyde & Scott, 1981).

Brown & Butler (1976) isolated a number of fusimotor and skeleto-fusimotor axons and found that where an efferent innervated more than one spindle its static or dynamic nature was consistent for each spindle. As with the histological findings (Ip & Vrbova, 1973; Ip et al, 1977; Barker & Boddy, 1980), these results indicate a very high degree of selectivity in the restoration of the afferent and efferent endings, this being especially true after nerve crush.

Brown & Butler (1976) also observed an increase in the number of β axons, especially after nerve section, which could be attributed to abnormal branching of axons at the site of the lesion. These extra axons were predominantly static in nature, which is the probable case if these β axons represent the α invaders described by Barker & Boddy (1980) as a motor axon making random, aberrant endings on the intrafusal fibres would be more likely to land on a fibre having a static action.

4.5 The Present Study.

The studies to be described were designed to examine in greater detail the functional and morphological characteristics of the reinnervated muscle spindles and to attempt to evaluate the effects of different periods of denervation on the quality of recovery after nerve-crush injury.

The system under examination was the cat peroneus brevis muscle with its muscle nerve. This system was selected for a number of reasons:-

1/ A great deal is already known about the normal structure and function of cat peroneus brevis muscle spindles and the peroneal system has been used in some of the previous studies on reinnervation (Brown & Butler, 1976; Barker & Boddy, 1980) although most of the work was on peroneus longus rather than brevis. For this study brevis was chosen in preference to longus because there is a greater length of muscle nerve available for the studies involving crushing at different sites.

2/ Injury to the peroneus brevis muscle nerve or even to the common peroneal nerve has little adverse effect on the mobility of the animal.

3/ The common peroneal nerve and brevis muscle nerve are easily accessible over much of their lengths, thereby minimising the amount of dissection involved in the initial operations and so reducing the risk of damage to other muscles, disruption of the blood supply or excessive scarring.

The crush injury is defined as a second degree injury (Sunderland, 1978) in which the axon is severed but the endoneurial tubes and structures of the nerve trunk including the blood supply are preserved. This type of injury should therefore allow the greatest possible specificity in the restoration of the connections with the peripheral targets (see sections 3.7 & 4.2). Because of this the crush injury forms a useful starting point for studies to examine the effectiveness of different nerve repair techniques; the muscle spindle being an ideal subject for such studies because it has both motor and sensory innervation.

Because recovery is relatively swift after nerve crush compared with more drastic injuries, a further series of experiments

was performed to examine the effects of different periods of denervation on the recovery. The original crush site was mid-way along the common peroneal nerve (selected because it was the most easily accessible of all the sites) so some variation in the denervation period was achieved by crushing closer or further from the muscle. The increased denervation period achieved by the latter procedure was limited by the maximum length of the common peroneal nerve which, half-way up the thigh, joins with the tibial nerve to form the sciatic nerve. Crushes of the sciatic nerve were not employed because the sciatic is much larger in cross section than the common peroneal and so total severance of all the axons might not always have been achieved. Also, injury to the sciatic nerve would have affected a much greater proportion of the limb musculature. Instead, therefore, the common peroneal nerve was crushed and, after a given period, re-crushed. Although this procedure also introduces other factors such as the increased probability of some axons being cut rather than crushed, damage to the supporting structures of the nerve and an alteration in the cell body response to the lesion, these were considered to be less critical than the effects of crushing the sciatic nerve.

The physiological and histological study of reinnervated spindles was concentrated on examination of the sensory endings, mainly the primary endings. The motor innervation was only looked at in a superficial fashion to ascertain its presence and the general restoration of motor functions.

METHODS

5.1 The Initial Operations.

The experiments were performed on adult cats having an average weight of 2.2kg. The animals were anaesthetised with an intra-peritoneal injection of sodium pentobarbitone (Sagatal: May & Baker Ltd; 45mg/kg) which was supplemented with halothane (Fluothane: B.P.) administered orally as required to maintain the animal in an areflexic state. All the initial operations were performed under aseptic conditions.

5.2 Common Peroneal Nerve Crush.

The common peroneal nerve of the left hindlimb was exposed and crushed at the knee, approximately 4mm above the point where it passes through the lateral head of gastrocnemius muscle. This site was selected because it entailed the minimum of dissection, thereby reducing the risk of scar tissue adversely affecting recovery, and also because it could always be located accurately.

The nerve was crushed between the tips of a pair of specially ground fine forceps, the same pair being used for all the operations. The technique was the same as that employed by Barker & Boddy (1980), the nerve being crushed for one minute from each side. The effectiveness of this technique has been demonstrated histologically (A.Boddy, personal communication) and physiologically in that after the shortest recovery periods (20 days) peroneus brevis muscle could not be made to twitch by stimulation of the muscle nerve at 20 times the threshold of α skeleto-motor axons in control animals and no functional afferents could be located in the dorsal root filaments.

After the operation the skin was stitched with surgical silk, the area of the incision was dusted with anti-biotic powder

and then sealed with plastic skin (Nobecutane: Astra Chemicals Ltd.).

5.3 Nerve-entry Crush.

In order to examine the effect that the period of denervation has on the quality of reinnervation, the crush operation was performed at different sites along the nerve. For the nerve-entry crush operations (the NEC experiments) a small incision was made mid-way along the calf of the hindlimb, and soleus, peroneus longus (PL) and tibialis anterior muscles were separated to expose the superficial peroneal nerve. The muscular branch to peroneus brevis (PB) and peroneus tertius (PT) was dissected free from the surrounding tissues and crushed approximately 2mm distal to its point of separation from the superficial peroneal nerve. The same crush technique was employed as for crushing the common peroneal nerve.

5.4 Delayed Return Experiments.

An increased period of denervation was achieved in two different ways. The first approach was to crush the common peroneal nerve at the knee (as in 5.2) and then repeat the procedure at weekly or fortnightly intervals, crushing at the same point each time, thereby retarding the progress of regeneration (the RC experiments).

The alternative method was to crush the common peroneal nerve more proximally (the TC experiments). A longitudinal incision was made mid-way down the thigh and the biceps femoris muscle was retracted to expose the nerve which was crushed just distal to its point of separation from the tibial nerve. Lesions at more proximal sites were not employed since these would have involved crushing the sciatic nerve (see section 4.5).

5.5 Measurements of Reinnervation Time.

Measurements of the regeneration rates of the axons have been obtained by histological means (Barker & Boddy, 1980) and used to calculate the time taken for the axons to grow from the crush site to the muscle. Using these measurements it is possible to compare the results obtained from crushing the nerve at different sites and from repeating the crush operations.

Barker & Boddy (1980) reported that after a reorganisation time of 5.8 days the fastest growing axons advanced at a rate of 3.2mm/day. The average distance from the normal crush site (at the knee) to PB was 51mm, so the fastest growing axons would have taken approximately 22 days to reach the muscle.

In the case of the NEC experiments (nerve-entry crush), the crush site was 38mm closer to PB than was the normal site; therefore, given the same regeneration rates, the fastest growing axons would only take 10 days to reach the muscle. The more proximal site was 75mm from the muscle and so the fastest growing axons would take 29 days to reach the muscle.

From these measurements one can calculate the 'Reinnervation Time' (RT) which was defined by Barker & Boddy (1980) as being 'the time that the fastest-growing axons are calculated to have entered and been reinnervating the muscle'. All times will be given in terms of days post-crush (days PC) and days reinnervation time (days RT).

5.6 Acute Experiments.

The animals were allowed to recover for periods of 20 to 140 days after the injury before an acute experiment was performed to assess the extent of reinnervation.

5.7 The Dissection.

The animals were anaesthetised with an intra-peritoneal injection of Sagatal and the relevant skin surfaces were shaved. The trachea was cannulated to allow forced ventilation should it prove necessary.

The left carotid artery was cannulated and connected to a pressure transducer to provide continuous monitoring of the blood pressure and heart rate. The arterial cannula was filled with a solution of heparin (10 μ g/ml) in 0.9% saline to prevent clotting. The radial vein of the right forearm was also cannulated to permit injection of supplementary doses of Sagatal (6mg/ml) as required to maintain the depth of anaesthesia throughout the experiment.

The operated hindlimb was extensively denervated to leave only the muscle nerve to PB intact. This was achieved via incisions at three levels:-

1/ In the groin the femoral nerve and branches of the obturator nerve were sectioned.

2/ On the lateral side of the hip the gluteal and cordo-femoralis muscles were reflected and denervated and piriformis was removed allowing access to the sciatic nerve and its branches innervating the deeper muscles of the hip region. The tail was denervated by blunt dissection and the caudal-femoral cutaneous nerve was sectioned. The muscular branch to the hamstrings and the branch to tenuissimus were also sectioned.

3/ An incision was made in the lateral surface of the lower hindlimb from the dorsal venous arch up to the knee. The tibial and sural nerves were sectioned close to the point of

separation from the common peroneal nerve. The branches to extensor digitorum longus, PF, PL and tibialis anterior were sectioned as also were the deep peroneal nerve and the superficial peroneal nerve distal to the branch to PB.

The effectiveness of the denervation was tested by stimulating the sciatic nerve and watching for contractions occurring in muscles other than PB.

The tendon of PB was cleared to its insertion in the foot and the maximum physiological length of the muscle in situ was ascertained by flexion of the foot. This length was marked by a thread which was tied to the tendon in line with one fixed to the lateral surface of the malleolus. The tendon was then cut and clipped directly to the shaft of an electromagnetic puller (Ling Dynamic systems, V201) positioned so as to pull the muscle along its normal path and so that a ramp stretch of 1.8mm amplitude brought the muscle up to its maximum length. The average physiological range of muscle movement was 4.1mm from being slack to full extension, so at the chosen resting length (1.8mm short of the maximum length) the muscle was maintained under a reasonable degree of tension.

In the case of two of the 26 day animals, at the time of the crush operation, an incision was made over the ankle and the maximum length and range of movement were measured and marked with nylon thread as described above. This was done to attempt to confirm that no changes in muscle length or range of muscle movement occurred as a result of denervation. Unfortunately, in both cases the insertion of the stitches induced the formation of excessive amounts of scar tissue which completely obscured the

stitches and also greatly restricted the movements of the tendons around the malleolus, so no valid results were obtained.

A lumbar laminectomy was performed from S1 to L⁴ and the L7 and S1 dorsal roots were freed from the nerve cord, along with the ventral roots, and cut as far centrally as possible.

The skin flaps over the cord and leg muscles were raised to form pools which were filled with warm mineral oil and maintained at 37°C with radiant heat. The core temperature of the animal was maintained at 37-38°C by a heating blanket (Ealing) controlled by a rectal probe.

5.8 Recording procedures.

The L7 and S1 dorsal roots were sub-divided on a black glass plate to produce filaments containing functionally single afferents whose responses were recorded via extra-cellular platinum electrodes. Conduction latency measurements were made by stimulating the muscle nerve and recording the orthodromic afferent impulse in the dorsal root. The length of the nerve was measured in situ post mortem.

Afferents were identified as originating from muscle spindles on the basis of their response to ramp-and-hold stretch and by their characteristic pause and rebound to muscle twitch. In the majority of cases it was also possible to distinguish between primary and secondary afferents by their responses to the ramp and their relative conduction velocities (this was not the case for the shorter recovery periods).

The ramps employed were of 1.3mm amplitude at velocities of 2.5, 5 and 10mm/sec with a hold of 1 or 3 seconds.

5.9 Gamma Stimulation.

In a number of experiments the L7 ventral root was similarly divided to produce functionally single γ efferents. These were stimulated at 70 or 100Hz while recording the responses of the spindle afferents. When an effect was observed the γ axon was classified as static or dynamic on the basis of its effects on the peak and hold phase firing rates of the afferent to ramp-and-hold stretch (Crowe & Matthews, 1964a; Boyd et al, 1977a).

5.10 Numbers of Animals Used.

Twenty-six animals were used in the main series of experiments (the NS series) plus five unoperated controls. A further fourteen were used in the fusimotor stimulation experiments plus three unoperated controls. The numbers of experiments are shown below:-

Time (d PC)	21	26	33	40	47	61	75	96	140	controls
Passive	2	2	2	1	3	2	4	8	2	5
γ Expts	-	-	-	2	5	1	2	4	-	3

A total of 14 animals were used in the NEC experiments:-

Time (d PC)	15	19	26	33	40
Passive	2	3	2	3	2
γ Expts	-	2	-	-	-

A further ten animals were used for the delayed return experiments with two animals at each time period. A small number of animals were involved in experiments which were not successful and these have not been included in the figures given above.

5.11 Histological Staining procedure.

At the termination of the experiments the PB muscle was removed from the leg and processed for silver staining using a modification by A. Boddy & F. Diwan (personal communication) of the

Barker & Ip (1963) silver method. The protocol is as follows:-

- 1/ Fix for a minimum of 5 days in a freshly prepared solution of:-

10gm Chloral hydrate.
450ml 95% Alcohol.
500ml Distilled Water
10ml HNO_3 (conc.).

- 2/ Wash for 24hrs with a flow rate of 1 litre/hr of:-

30l Distilled Water
6gm $\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$.
Made up to pH 9 with sat. NaOH.
Keep cool with running tap water.

- 3/ Place for 30hrs in Ammoniacal alcohol:-

1 litre of 95% Alcohol.
0.9ml 33% NH_3

- 4/ Coat with agar as follows:-

Dissolve 10mg agar powder in 1 litre of distilled water. Bring to boil. As it cools dip the muscles into the solution repeatedly. Leave to gel.

- 5/ Incubate in the dark, in a shaking water bath at 37°C for 7 days in freshly prepared 1.5% AgNO_3 .

- 6/ Remove the agar coat.

- 7/ Reduce for 24hrs in:-

20gm Quinol.
250ml 93% Formic acid.
750ml Distilled Water.

- 8/ Rinse in distilled water.
- 9/ Clear in glycerol for a minimum of 2 days.
- 10/ Soak spindles individually and mount in glycerol.

RESULTS

The Histology of Reinnervated Muscle Spindles.

6.1 General Observations.

The silver stain has proved highly capricious throughout this and other studies despite numerous attempts to improve it. The variations on the basic technique employed for most of this study usually impregnated the efferent innervation successfully, but the staining of the afferents was very variable. As a result, the amount of raw data obtained for the different recovery periods was variable and interpretation of the appearance of the endings had to be tempered by an assessment of the quality of the staining. In spite of these conditions a great deal of valuable data did emerge from the histological analyses and this will be used to complement the results of the physiology (given in subsequent chapters) in an attempt to derive an overall picture of the process of reinnervation.

During the early stages of recovery there was a rapid improvement in the histological appearance of the spindles, but after about 40 days post-crush there was little overall change in the extent or quality of the reinnervation. All the endings, both afferent and efferent, developed into their characteristic configurations; however, at no time was there any difficulty in recognising spindles that had been reinnervated. In every case the sensory endings failed to achieve the degree of complexity and organisation that is typical of normal afferent endings.

The main features that stand out from these results are the high degrees of specificity of reinnervation of the endings. In all cases the primary endings were confined to the nuclear bag and myotube regions of the intrafusal fibres (the P region) as

is the case for normal primary endings. Similarly, the secondaries were located on the adjacent regions of the intrafusal fibres in the S_1 , S_2 and, rarely, S_3 regions (see section 1.5) and the motor axons terminated in the polar regions. Where an axon was misplaced and made contact with the intrafusal fibres in the wrong region it was apparently incapable of forming a recognisable ending in that region.

6.2 The Primary Ending.

At 20 days PC (-2 days RT) none of the spindles examined had a primary ending, indeed, at this stage only one or two spindles had any innervation at all and this was in the form of very fine motor axons in the polar regions that had not as yet produced any recognisable endings. Numerous fine axons were visible in the intra-muscular nerve trunk, though these, of course, could not be identified.

The first reinnervation by primary axons was observed at 26 days PC (4 days RT). All the axons were still relatively fine and of the four spindles that were reasonably stained only two had primary endings and both of these were very incomplete (Figs. 1, 2, Plate 1). In Fig. 1 the only part of the ending that has been formed is a spiral around one of the chain fibres. Fig. 3 (Plate 1) shows part of the equatorial region of a spindle at 26 days PC which lacked a Ia axon. The group of axons that is visible coursed through from one pole to the other and these are presumably motor in origin. One axon, also from one of the poles, can be seen entering the equatorial region and then turning back towards the pole from which it came.

By 33 days PC (11 days RT) the majority of the spindles

Table 1.

The proportions (%) of spindles lacking a primary ending after each of the recovery periods in the NS study.

Time (days PC)	Time (days RT)	% of spindles with no Ia	Total examined
26	4	50.0	4
33	11	22.2	13
40	18	33.3	30
47	25	11.1	27
61	39	0.0	25
75	53	0.0	60
96	74	3.6	28
140	118	5.2	19

Table 2.

The numbers of fully restored primary endings after each of the recovery periods compared with those that failed to re-innervate one or more intrafusal fibre types. Only those capsules that contained chain fibres and one bag₁ and one bag₂ are included.

Time (days PC)	Time (days RT)	b ₁ b ₂ c endings	b ₁ or b ₂ c endings
33	11	11	0
40	18	23	0
47	25	21	1
61	39	19	0
75	53	54	3
96	74	15	2
140	118	2	2
Totals		145	3

were innervated by primary afferents (Table 1) and many of these were relatively well restored (Figs. 4, 5, Plate 2) with some terminals on all the intrafusal fibre types (Table 2).

From 33 days PC onwards almost all spindles examined had a primary ending (Table 1). Of 205 spindles examined 33-140 days post-crush (11-118 days RT), only 10 (4.9%) lacked a primary ending. Most of these uninnervated spindles were found during the early recovery periods up to and including 47 days PC (25 days RT). Over this initial period some 13% of spindles lacked a primary ending, whereas from 61 days PC (39 days RT) onwards this figure dropped to 1.5%. This drop in the number of uninnervated spindles after 47 days PC (25 days RT) corresponds with the timing of the second wave of reinnervation described by Barker & Boddy (1980), which they attributed to the late return of those afferents whose endoneurial tubes were severed by the crush operation. It is quite likely that the small proportion of spindles still lacking primary innervation after the return of the second wave would remain permanently uninnervated.

During the early stages of recovery many of the endings were characterised by the incompleteness of the annulospiral formations. In the endings shown in Figs. 5 and 6 (Plate 2) a number of the terminals on the bag fibres are only partly formed giving rise to an irregular appearance. These irregular portions of the endings should be distinguished from the peripheral regions of the bag endings which are often irregular in appearance in normal primary endings (Figs. 41, 42, Plate 14; Figs. 43, 44, Plate 15). Similar irregular areas, often characterised by small terminal swellings were common features of the regenerated endings (Figs. 8, 9, Plate 3). As recovery progressed the endings

were rapidly completed so that by 40 days PC (18 days RT) the formations were complete (Figs. 15, 16, Plate 5) whereas the peripheral irregularities were still present throughout recovery (Fig. 16, Plate 5; Fig. 32, Plate 11).

After each of the recovery periods there was a very wide range of primary-ending configurations from those which were fairly close to normal spindles (Plates 14, 15) in appearance, such as those in Fig. 4 (Plate 2, 33 days PC); Figs. 15, 16 (Plate 5, 40 days PC); Fig. 23 (Plate 8, 47 days PC); Figs. 32, 33 (Plate 11, 75 days PC) and Fig. 36 (Plate 12, 96 days PC), to those which show poor restoration and sometimes marked abnormalities (Figs. 7, 8, 11, Plate 2; Figs. 17, 18, Plate 6; Fig. 21, Plate 7; Figs. 24, 25, Plate 3; Figs. 27, 29, Plate 9; Fig. 37, Plate 12 and Fig. 38, Plate 13), with a whole range of intermediate conditions. The plates are arranged chronologically to provide a picture of the range of ending configurations at each time period.

In some cases the range of quality of reinnervation could be associated with the location of the spindles within the muscle. There would inevitably have been a difference in the length of time spindles at the proximal end of the muscle had been reinnervated compared with those at the distal end purely as a result of the time taken for the axons to grow from one end of the muscle to the other. This would only have been of importance during the very early stages of reinnervation when the four days, or so, taken by the axons to reach the distal part of the muscle from the point of nerve entry would noticeably delay the reinnervation of the distal spindles compared with those situated more proximally.

Provided the primary axon made contact with the intrafusal bundle in the equatorial region it almost always reinnervated all

the intrafusal fibre types to some extent (Table 2). Of the 153 endings in b_1b_2c capsules that were analysed, only in 8 cases (5.2%) did the primary afferent fail to reinnervate all three types of fibre. These endings reinnervated one of the bags and the chain fibres, the other bag fibre remaining uninnervated (Fig. 29, Plate 9; Fig. 37, Plate 12).

It was impossible to determine whether all the chain fibres of a given spindle had been reinnervated, so the assessment was made on the basis of observation of terminals on at least one chain fibre. It was often possible to distinguish between the bag_1 and bag_2 fibres. This could be done on the basis of the presence of elastic fibres around the extreme polar regions, dissociation of the fibres at the equatorial region, the type of motor innervation present in the poles and the distribution of secondary endings. The two latter criteria are dependent on the assumption of a specific restoration of the endings, but where all the criteria have been available these have been in agreement and there is evidence to support such an assumption.

The data given in Table 2 show that there was no time-dependent reduction in the proportion of endings failing to reinnervate a bag fibre, such as might indicate that these endings had been analysed while growth was in progress and before the terminals had reached one of the fibres. Indeed, as can be seen from Table 2, most of these incomplete endings occurred in preparations after the longer recovery periods after the return of the second wave of reinnervation. There is no evidence of there having been a sequential process of reinnervation. The Ia axon branches at least once before reaching the intrafusal fibre bundle and each of the individual branches would probably reinnervate the different

fibres simultaneously. Thus, even at the very early stages (Plates 2, 3, 5, 6), where there is a recognisable ending, all the fibre types have been reinnervated to some extent.

Hyperinnervation of the spindle by Ia axons was noted in a number of instances (Fig. 7, Plate 3; Fig. 18, Plate 6; Fig. 30, Plate 10; Fig. 39, Plate 13). This is defined as being the reinnervation of the P region by several Ia axons contained within the same endoneurial tube, which can be traced back, as separate axons, for at least 1000 μ m (1mm) from the centre of the P region. As such, hyperinnervation can be distinguished from double primary innervation where two separate primaries innervate one spindle capsule (Fig. 19, Plate 6). Double primary innervation occurs in some 4.4% of normal spindles (Banks et al, 1982) whereas hyperinnervation is unique to the regenerated condition. The other difference between a hyperinnervated spindle and one with a double primary is that the hyperinnervating axons tend to innervate separate muscle fibres and rarely branch; together, therefore, they produce a single fairly typical primary ending (Fig. 18, Plate 6). The two primary axons of the double primary each form a complete primary ending and, although there is often some overlap, the two endings mainly lie adjacent to each other (Fig. 19, Plate 6).

The data given in Table 3 show that hyperinnervation is relatively uncommon; overall some 12.4% of b_1b_2c capsules were hyperinnervated. Further, there is no indication of a time-dependent trend in the proportions of hyperinnervated spindles.

There might have been inaccuracies in the assessment of the frequency of hyperinnervation because of the possibility of the spindle nerve branch being broken during teasing distal to the first-order branch point. This is most likely to occur where the branch

Table 3.

The frequency of occurrence of hyperinnervation of the primary region after each of the recovery periods. See text for further details.

Time (Days RT)	No. of Ia axons.				Totals	% occurrence
	1	2	3	4		
4	2	0	0	0	2	0.0
11	12	1	0	0	13	7.7
18	22	4	1	0	27	18.5
25	21	0	0	0	21	0.0
39	21	1	1	1	24	12.5
53	39	5	1	0	45	13.3
74	21	2	0	0	23	8.7
118	14	0	4	0	18	22.2
Totals	148	13	7	1	169	12.4

Figure 45. Histograms showing the distributions of the distances (μm) from the first branching node to the centre on the primary innervation for normal and regenerated primary axons. There is no significant difference between the two populations ($P > 0.01$ Student's 't' test).

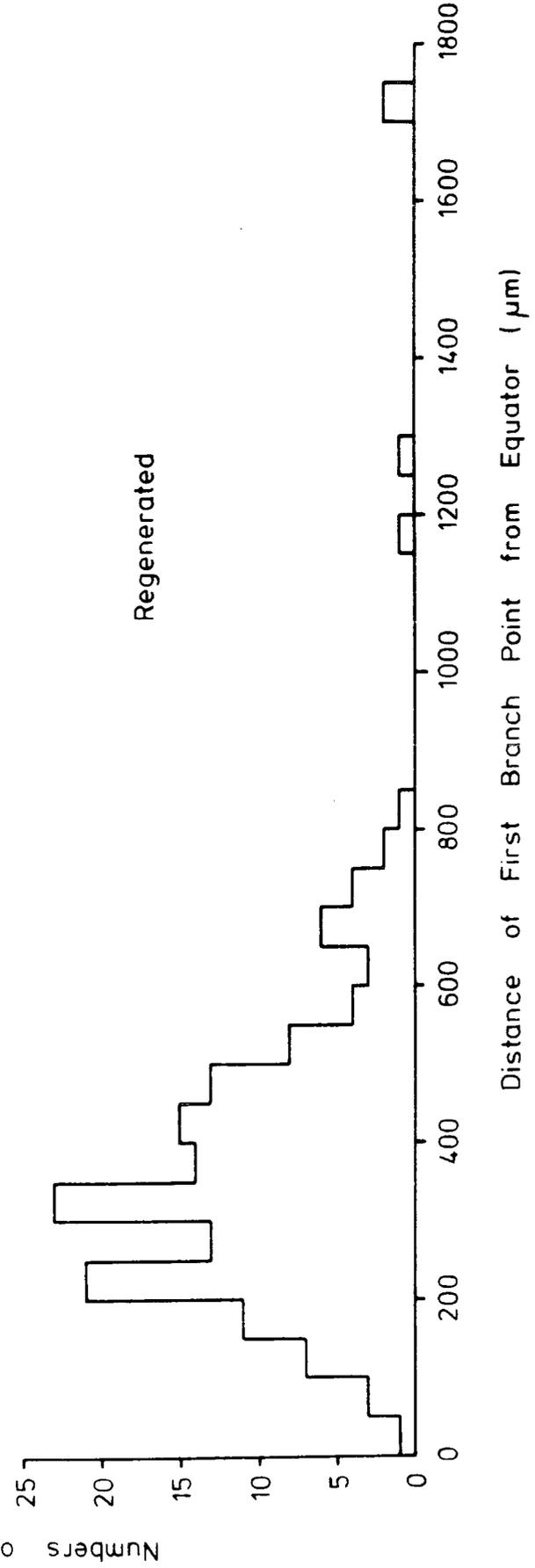
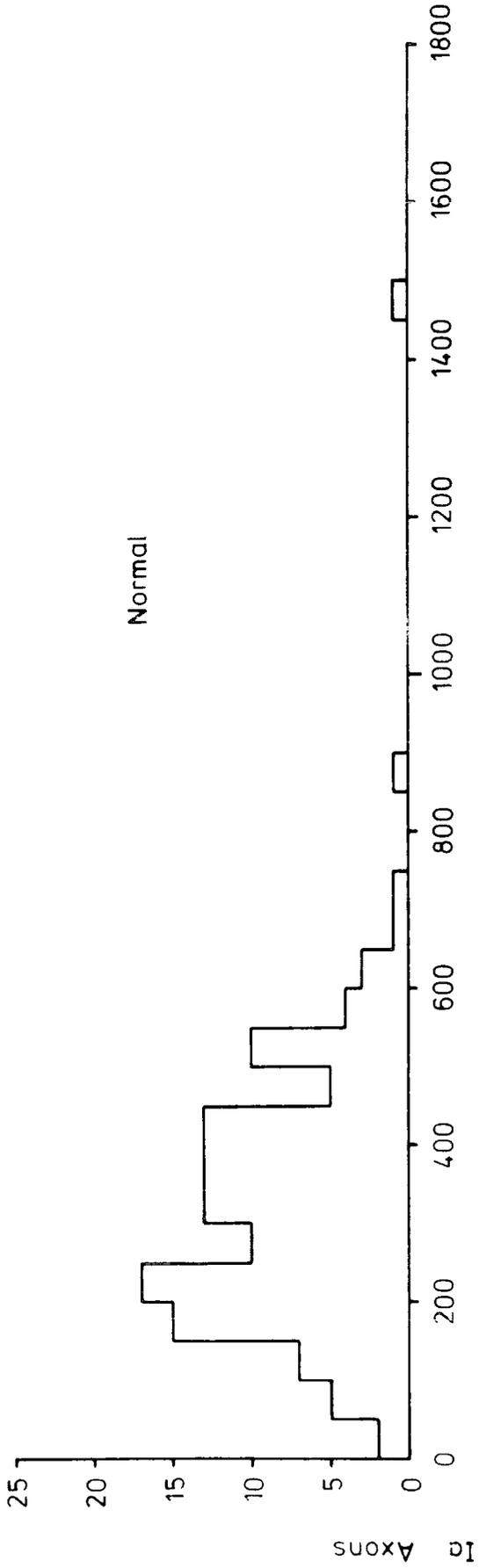


Table 4.

The mean number of bands visible on the nuclear-bag fibres in the P region after each recovery period.

Time (Days PC)	Time (Days PC)	Mean no. of bands	Range	Total no. of bag fibres
33	11	6.9	2-12	3
40	13	7.6	2-20	23
47	25	7.5	1-13	31
61	39	7.9	2-14	18
75	53	6.2	2-12	86
96	74	10.6	5-14	25
140	113	6.0	1-9	5
Controls		14.0	5-27	42

Table 5.

The mean lengths (μm) of the primary endings after each of the recovery periods. The means are given with their standard errors.

Time (days PC)	Time (days RT)	Mean length of ending (μm)	Range (μm)	No. of endings
33	11	248.6 ± 17.3	148-333	12
40	18	258.6 ± 14.9	90-438	24
47	25	288.7 ± 15.6	171-462	23
61	39	269.9 ± 10.1	171-333	18
75	53	276.8 ± 13.3	181-438	26
96	74	256.3 ± 12.3	133-338	20
140	118	297.5 ± 21.2	219-390	6
Controls		314.8 ± 11.6	166-476	33

point was far removed from the spindle. To minimise the risk of over-estimation of the frequency of hyperinnervation, spindles were only regarded as being hyperinnervated if the axons could be traced back for at least 1000 μ m without any sign of the first-order branch point. Conversely, absence of hyperinnervation was only recorded for spindles where the branch point was located; if the axon was broken close to the spindle, distal to the first-order branch point, then no result was recorded.

The distance between the first branching node and the centre of the primary innervation was measured in both operated and normal animals. The distributions of these distances are shown in the histograms of Fig. 45 and there is no significant difference between the two populations ($P > 0.05$, Student's 't' test). Only 11% of the regenerated and 5.7% of the normal axons had first-order branch points more than 600 μ m from the equatorial region and this provides evidence for the figures for the occurrence of hyperinnervation being of the right order.

At no stage did any of the primary endings achieve the complexity or extent of innervation that characterises the appearance of normal spindles (Plates 14, 15). One of the most easily recognisable deficiencies was in the extent of the regular annulospiral terminals on the bag fibres of reinnervated spindles. The numbers of spirals and rings produced by the primary afferent on the bag fibres were counted and the results are given in Table 4 (the number of spirals and rings was determined by counting the number of bands of the axon terminals visible on one (usually the upper) surface of the muscle fibres). For all the recovery periods the number of bands is markedly lower than in normal spindles and, except for the initial period of development of the ending, there is no trend towards an

increase in the complexity of the innervation with increasing time.

The mean length of the primary endings was also ascertained by measuring the length of the intrafusal fibres bearing primary innervation from one extreme end to the other (Table 5). There is a slight reduction in the length of the endings in the reinnervated spindles compared with the controls, although after two of the recovery periods (47 and 140 days PC) the mean ending length is not significantly different from that in normal spindles. Again, there is no clear trend towards a larger ending with increasing recovery period.

Even some of the endings that appear to be extremely well restored such as those shown in Figs. 15 and 16 (Plate 5) and Fig. 23 (Plate 8) are all less extensively innervated than the average normal spindle. The primary in Fig. 15 formed 20 bands on the bag_2 but only 4 on the bag_1 ; similarly, the spindle in Fig. 16 has 16 bands on the bag_2 but only 5 on the bag_1 and the primary shown in Fig. 23 supplied 9 bands to the bag_1 and 6 to the bag_2 . The best reinnervated of all the spindle primary regions to be found in this study is that shown in Fig. 36 (Plate 12) in which the bag_1 has 13 bands and the bag_2 has 12. In contrast, the average normal primary forms 12.8 bands on the bag_1 and 15.3 on the bag_2 (Table 4).

The feature that is apparent from the results given in Tables 4 and 5 and from examining the spindles in chronological order is that the ending is reconstituted very rapidly after the initial contact of the Ia axon with the intrafusal fibres. At 20 days PC there were no spindles with primary innervation; by 26 days PC (4 days RT) the first primary axons had made contact and had started forming endings (Plate 1), while by 33 days PC (11 days RT)

most of the endings were apparently approaching completion (Plates 2, 3) with little sign of any further increase in the extent of the primary innervation (Tables 4, 5).

In a number of instances the equatorial region was invaded by axons which, from their inability to form endings and their route into the equatorial region, were presumably motor in origin. This invasion took two forms. In the first case, branches of fusimotor axons from the polar regions could invade the equatorial region. These either appeared to terminate in large ball-like swellings (Fig. 21, Plate 7) or, more commonly, they traversed the sensory region to the other pole. Such crossings of the equatorial region by preterminal fusimotor axons are also reasonably common in the normal material and these axons sometimes form swellings en route which are termed vesicular axonic swellings (Barker et al, 1970). Occasionally, as in Fig. 3 (Plate 1) the axon would enter the equatorial region and then double back returning towards the same pole.

In the absence of a primary axon the Ia endoneurial tube often contained one or more axons which entered the primary region. Some of these axons formed large ball-like swellings in the primary region (Fig. 20, Plate 7) similar in form to those produced by the fusimotor axons. Others failed to make any terminations and left the spindle either via the poles or by the same nerve trunk in which they had arrived (Figs. 13, 14, Plate 4; Fig. 22, Plate 7).

Occasionally the primary axon itself formed swellings within the primary region. An example of such an ending is shown in Fig. 24 (Plate 8). All the branches have formed some spirals on the intra-fusal fibres and one branch also produced a ball-like swelling with the branch then continuing on from the swelling.

The branches of the primary axons always appeared to be confined within the primary region indicating some degree of regional specificity which prevented them from entering the secondary zones. Where part of the primary did encroach upon the S_1 regions it either petered out after attempting to form some sort of ending (Fig. 25, Plate 8), or the branch entered the S_1 region and then turned back to the primary region to terminate normally (Fig. 38, Plate 13).

6.3 The Secondary Ending.

The secondary afferents, like the primaries, succeeded in reinnervating the spindles to form easily recognisable endings within the normal regions. The first secondaries arrived back at about the same time as the first primaries, although on the whole they tended to regenerate more slowly and the time span for the restoration of the II population was longer than that for the primaries. This is expressed in terms of the mean number of secondaries per b_1b_2c capsule and the proportion of spindles lacking secondary innervation (Table 6). Despite the slight trend of increasing secondary reinnervation, the mean number of secondaries per spindle was consistently lower than that for normal spindles. Much of this reduction is evident in the greater proportion of spindles with only primary innervation and also fewer spindles showing multiple secondary innervation. (Table 6).

Because the secondary ending is normally fairly irregular in appearance (Plates, 14, 15), often with little or none of the regular terminations characteristic of the primary, it was difficult to assess the quality of reinnervation. In the main, however, even the best restored of the secondaries appeared to be less well developed than normal secondaries as can be seen by comparison

Table 6.

The numbers of afferent endings per spindle after each of the recovery periods expressed as a percentage of the total number of b_1b_2c capsules examined.

Type of innervation	Time (days RT)										Controls
	4	11	18	25	39	53	74	118			
0.	25.0	11.1	3.3	7.4	0.0	0.0	3.6	5.3			0.0
CS_1	25.0	11.1	0.0	3.7	0.0	0.0	0.0	0.0			0.0
P	25.0	22.2	40.0	18.5	28.0	26.0	21.1	52.6			8.7
PS_1	25.0	33.3	40.0	44.4	40.0	50.0	46.4	26.3			45.7
PS_1S_2	0.0	11.1	0.0	3.7	4.0	33.3	0.0	5.3			13.0
S_1PS_1	0.0	11.1	13.3	22.3	28.0	16.6	25.0	10.5			13.0
$S_1PS_1S_2$	0.0	0.0	3.3	0.0	0.0	33.3	3.6	0.0			17.4
$S_2S_1PS_1S_2S_3$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			2.2
Total no. in sample	4	18	30	27	25	60	28	19			46

Cont'd overleaf.

Table 6 (cont'd).

The mean number of secondaries per b_1b_2c capsule.

Time (days RT)	4	11	18	25	39	53	74	118	Controls
Mean no. of secondaries.	0.5	0.8	0.76	1.0	1.04	1.0	1.07	0.58	2.0
Proportion (%) of the spindle population lacking a secondary ending.									
Time (days RT)	4	11	18	25	39	53	74	118	Controls
Prop. of spindles with no secondary	50.0	33.3	43.4	25.9	28.0	26.6	25.0	57.9	8.7

Table 7.

The distribution of the terminals of S_1 secondary endings on the different intrafusal fibre types expressed in terms of the frequency of occurrence of each type of secondary ending.

The data for the normal control spindles was taken from Banks et al (1982).

Time (Days PC)	Time (Days RT)	Distribution of secondary ending			Total no. of endings
		$b_1 b_2 c$	b_1 or $b_2 c$	c only	
33	11	44.4	44.4	11.2	9
40	18	0.0	66.7	33.3	3
47	25	13.3	60.0	26.7	15
61	39	22.2	55.6	22.2	9
96	74	75.0	18.7	6.3	16
140	118	60.0	40.0	0.0	5
Controls		81.8	17.8	0.4	253

Table 8.

The frequency of occurrence of secondary invasion of the primary region and the site of termination of the invading axons after each of the recovery periods.

Time (days PC)	Time (days RT)	Course of invading branch Ends in P region	Traverses P region to fade / make ending	S_1 in other pole present / absent	No. of secondaries	% of II's invading P.
26	4	0	1	0	2	50.0
33	11	2	0	1	14	14.3
40	18	0	0	0	24	12.5
47	25	3	0	0	19	15.8
61	39	1	0	0	23	4.4
75	53	4	4	6	56	19.6
96	74	4	1	4	22	45.5
140	113	3	1	1	10	40.0

of those shown in Fig. 28 (Plate 9), Figs. 30 and 31 (Plate 10), Fig. 34 (Plate 11) and Figs. 38 and 41 (Plate 13) with the normal secondaries shown in Plates 14 and 15.

Most of these well-restored secondaries were observed after the longer recovery periods (47 days PC onwards). During the earlier stages the endings were poorly developed and often only terminated on the chains and one bag fibre (Figs. 7, 8, 10, Plate 3; Fig. 12, Plate 4). The distributions of the terminals of S_1 secondaries to the different intrafusal fibre types were examined and compared with those of normal spindles (Table 7). Despite the paucity of data, certain trends are apparent, namely that during the early recovery periods up to 61 days PC (39 days RT) a much greater proportion of the S_1 secondaries terminated on one bag (usually the bag₂) and the chain fibres, or on the chain fibres alone, than is normally the case. From 75 days PC (53 days RT) onwards the pattern changed with many more of the S_1 secondaries supplying all three fibre types. Even so, the proportion of regenerated II afferents forming b_1b_2c endings never achieved the levels seen in normal spindles.

A number of secondaries produced branches that invaded the primary region (Table 8). These branches either terminated in the primary region without forming an ending (sometimes a small ball-like termination was visible which could have represented a growth cone) (Fig. 12, Plate 4; Fig. 28, Plate 9) or, more commonly, they traversed the primary region and terminated in the S_1 region of the opposite pole, sometimes producing a small ending (Fig. 15, Plate 5; Fig. 27; Plate 9; Fig. 31, Plate 10).

In almost every spindle where this secondary invasion was observed the spindle had been reinnervated by a primary axon, so

this phenomenon cannot be attributed to the absence of primary innervation. Further, in a number of cases the S_1 region on the far side of the equator was already occupied by a secondary afferent (Table 8).

As can be seen from Table 8, the numbers of secondaries producing such branches increased with increasing recovery time which indicates that the II afferents in question were still growing for much of even the longest recovery periods. It is noteworthy that these branches are unique to the regenerated condition, never having been reported in normal spindles (see, for example, Banks et al, 1982).

6.4 Efferent Reinnervation.

A detailed analysis of the efferent reinnervation was not attempted in this study. General observations indicated that the motor endings were present after all the recovery periods, even prior to the return of the sensory axons. All three types of motor ending: p_1 , p_2 (Figs. 47 to 52, Plate 16; Figs. 53, 54, Plate 17) and trail (Figs. 62 to 65, Plate 18) were observed, although during the early stages they were undeveloped and difficult to identify. After longer recovery periods their appearance was very close to that of normal intrafusal motor endings (Figs. 51, 52, Plate 16; Figs. 53, 54, Plate 17; Figs. 63, 64, 65, Plate 18). Skeletomotor (\propto) endplates were also observed after all the recovery periods on the extrafusal fibres and these also achieved near-normal appearance as recovery progressed.

Plate 1.

Photographs of the equatorial regions of teased, silver preparations of PB muscle spindles 26 days after the nerve-crush injury (4 days RT). Abbreviations: Ia, primary axon; c sp., primary ending spirals on the chain fibres; dev.t.s., developing terminal system; eq.reg., equatorial region; if.m.f., intrafusal muscle fibres; mot.ax., motor axons; per.sp., periaxial space; 1st br.n., first branching node of the sensory axon.

Figure 1. The primary axon has only just made contact with the intrafusal fibres in the equatorial region and only part of the ending has been formed, consisting of a spiral round one of the chain fibres. The equatorial region can be identified, in the absence of the primary innervation, by the periaxial space and the presence of the 'bags' of nuclei in the nuclear-bag fibres (not in focus in this figure).

Figure 2. As with Fig. 1, the Ia axon has only just made contact with the intrafusal fibres and the terminal systems are in the earliest stages of development.

Figure 3. There is no sensory axon in the equatorial region. A number of presumed motor axons course through part of the region from the lower to the upper poles. One motor axon (mot.ax.) enters the equatorial region from the lower pole and doubles back without attempting to form an ending.

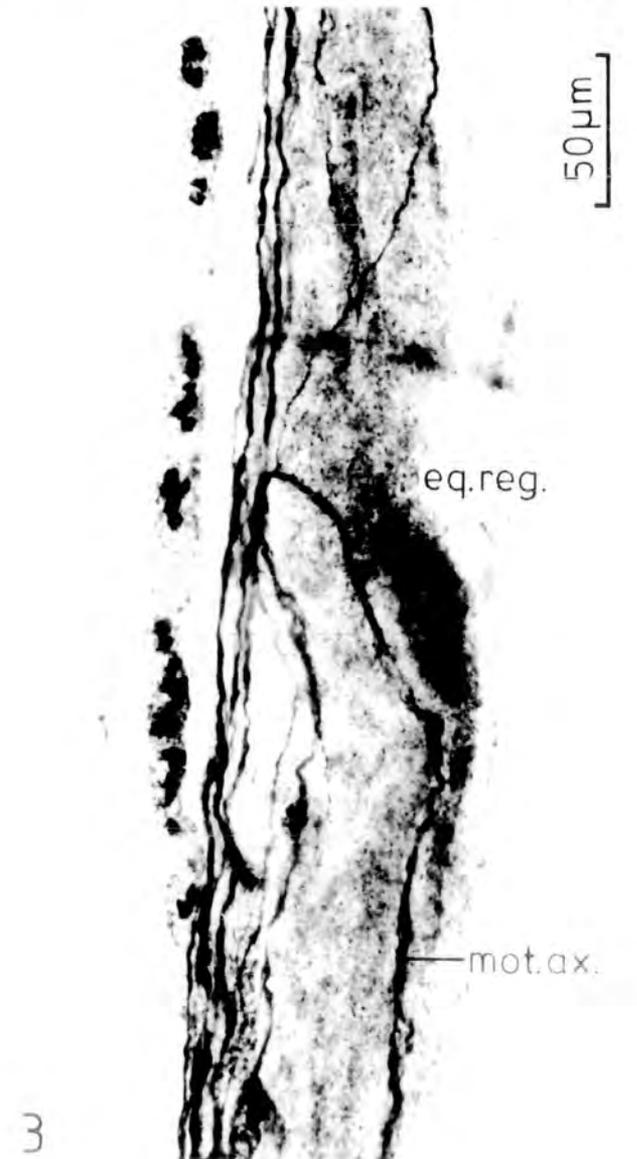
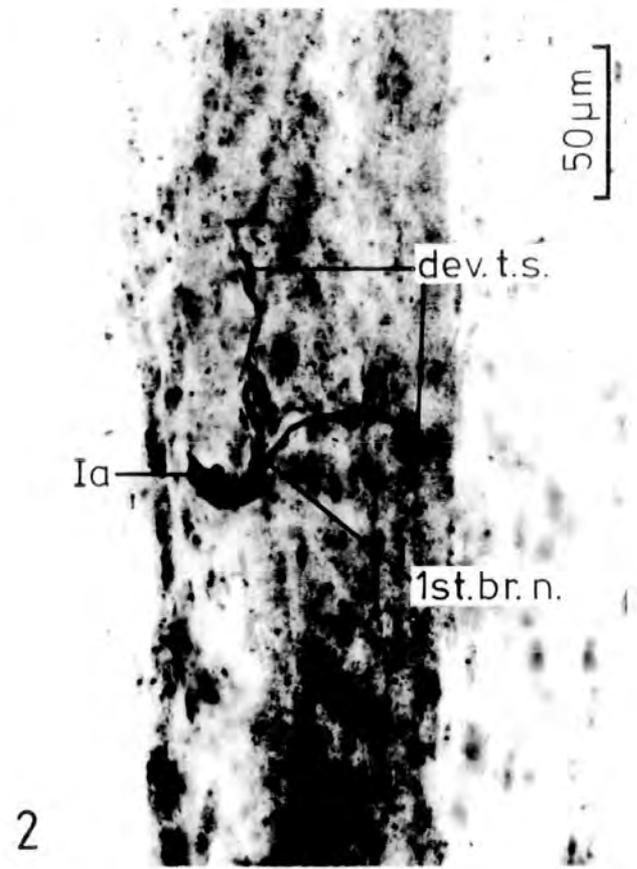
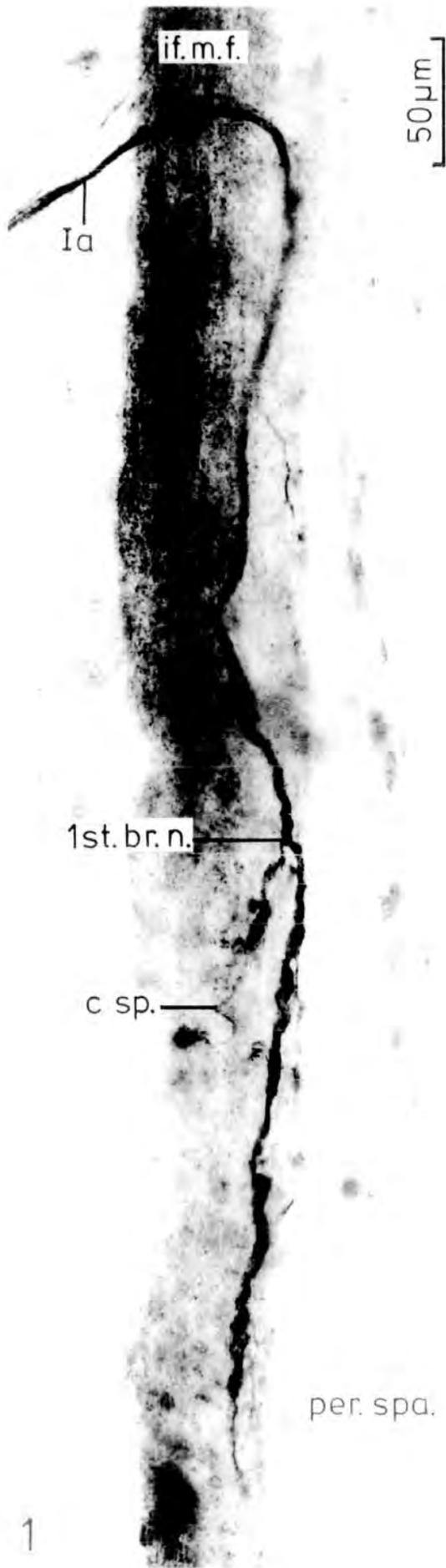


Plate 2.

Photographs of silver preparations at 33 days PC (11 days RT).

Abbreviations: b ts., bag terminal system; i.t., irregular terminations; P, primary ending; t.e., terminal expansions. Other abbreviations as for Plate 1.

Figure 4. A well-restored primary ending. The bottom nuclear-bag fibre is invested with distinct annulospirals and complete rings characteristic of a bag terminal system. Its relative size also indicates that it is a bag fibre, though it was not possible to identify it as a bag₁ or bag₂. The peripheral portions of the ending are irregular as occurs in normal spindles (Plates 14, 15), although in this spindle the region of irregularity is quite extensive.

Figure 5. Both bag fibres are innervated and are supplied by separate first-order branches of the Ia axon. It was not possible to distinguish between the two bag fibres. The innervation of the lower bag is not complete; the arrows mark some of the rings that are not fully developed.

Figure 6. A high-power photograph of the right-hand half of the primary shown in Fig. 5. A number of small swellings, or terminal expansions, are visible on the tips of the terminals. These expansions are also common in normal primary endings, especially in the peripheral irregular regions.

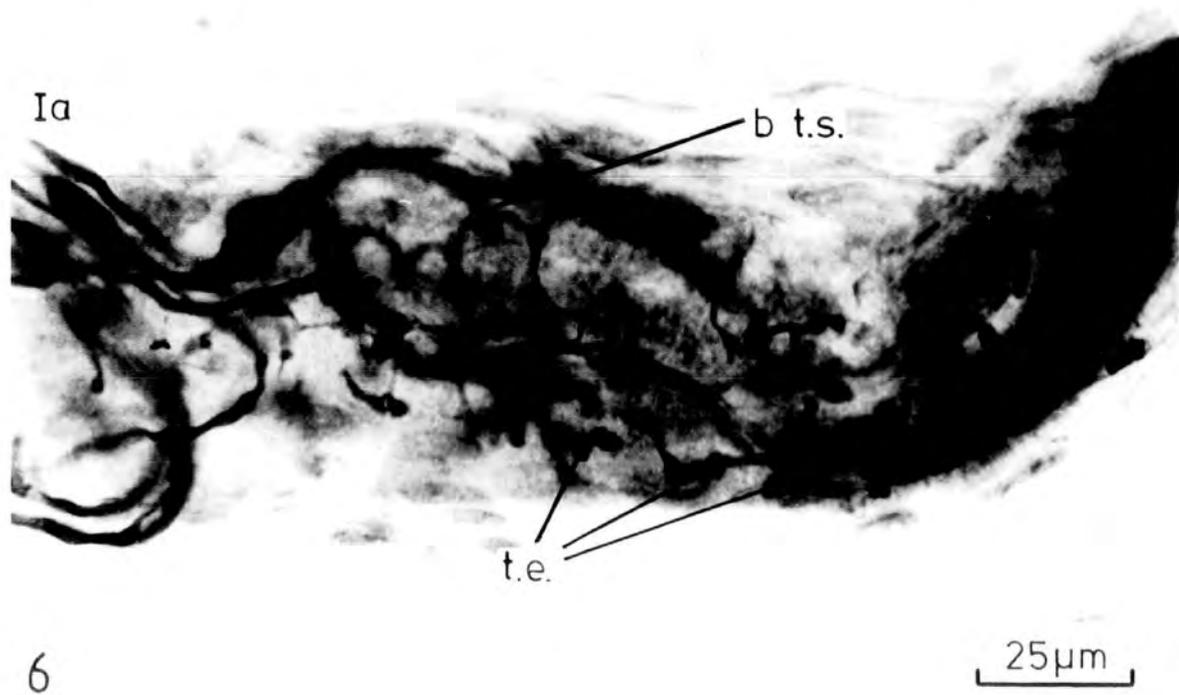
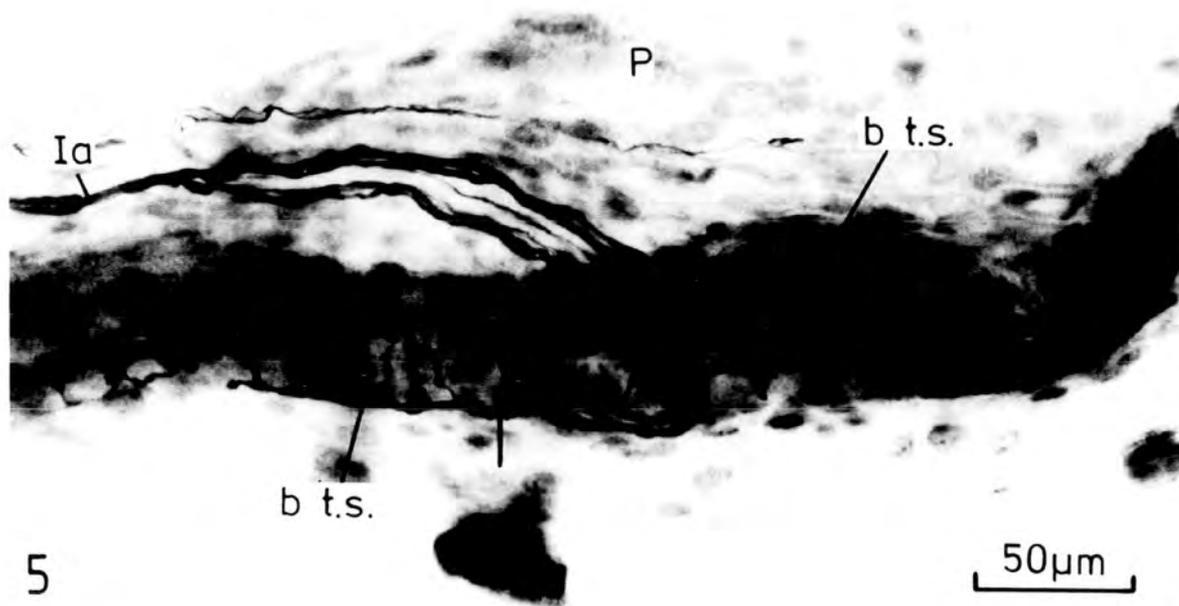
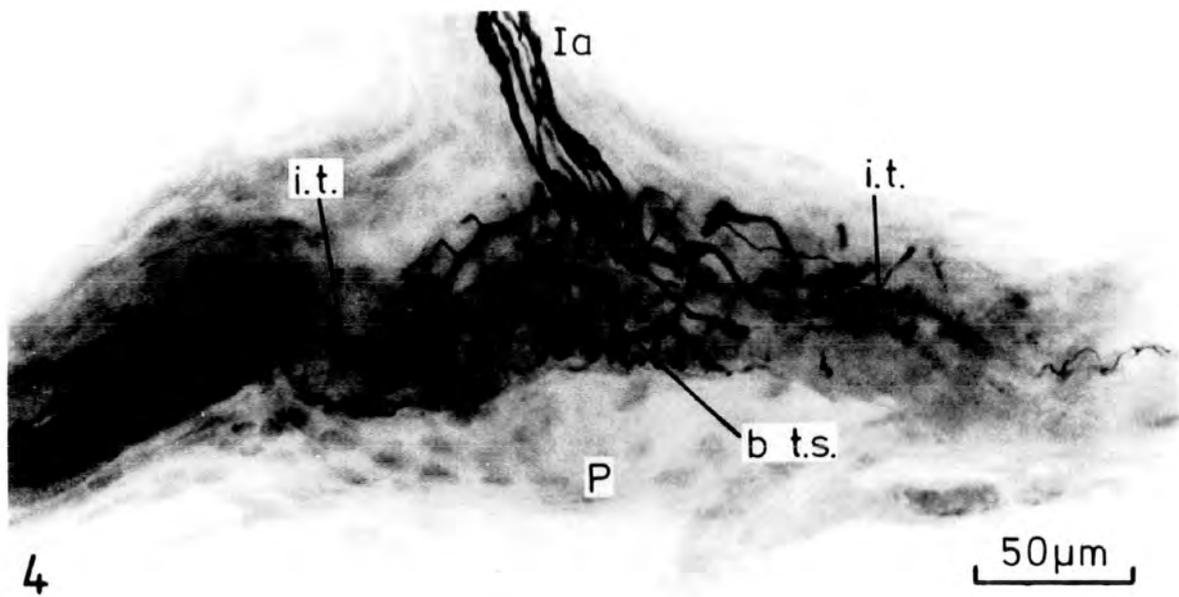


Plate 3.

Photographs of silver-stained primary and secondary endings at 33 days PC (11 days RT). Abbreviations: Ia(x2), a hyperinnervated primary with two axons in the same endoneurial tube; II, secondary axon; b_1 the bag₁ intrafusal fibre; b_2 , the bag₂ fibre; c, a chain fibre; S_1 , the secondary ending adjacent to the primary. Other abbreviations as for Plates 1 and 2.

Figure 7. An instance of hyperinnervation of the primary region; the two axons were traced back 1225 μ m and remained separate, though within the same tube (at this point the axons were broken as a result of the teasing). The primary ending shows a lack of organisation with very little spiralling. The II axon terminates close to the primary ending and produces only a very limited ending mainly confined to the chain fibres.

Figure 8. The bag₁ and bag₂ fibres were identified by their motor innervation in the polar regions (see section 6.2). The bag₁ is sparsely innervated, receiving only two spirals (b_1 sp.). A well-developed system of spiralling is visible on one of the chain fibres in the upper part of the primary ending. The S_1 secondary innervation (partly out of focus here) is very limited in extent.

Figure 9. A high-power view of the lower extremities of the primary ending in Fig. 8 to show the terminal expansions and incomplete spirals characteristic of the ends of primary endings.

Figure 10. A developing secondary ending; the arrow marks the only recognisable terminal yet formed.

Figure 11. A poorly-developed primary ending with very sparse terminations on the intrafusal muscle fibres; despite this, all three fibre types are innervated to some extent.

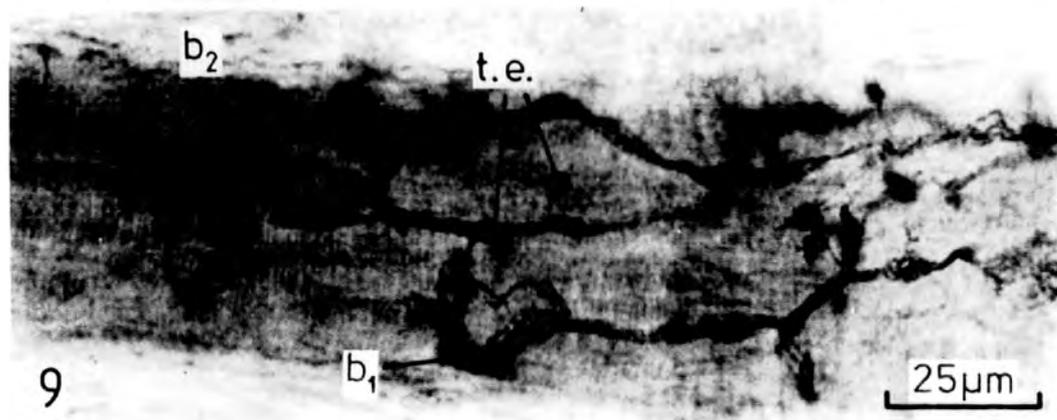
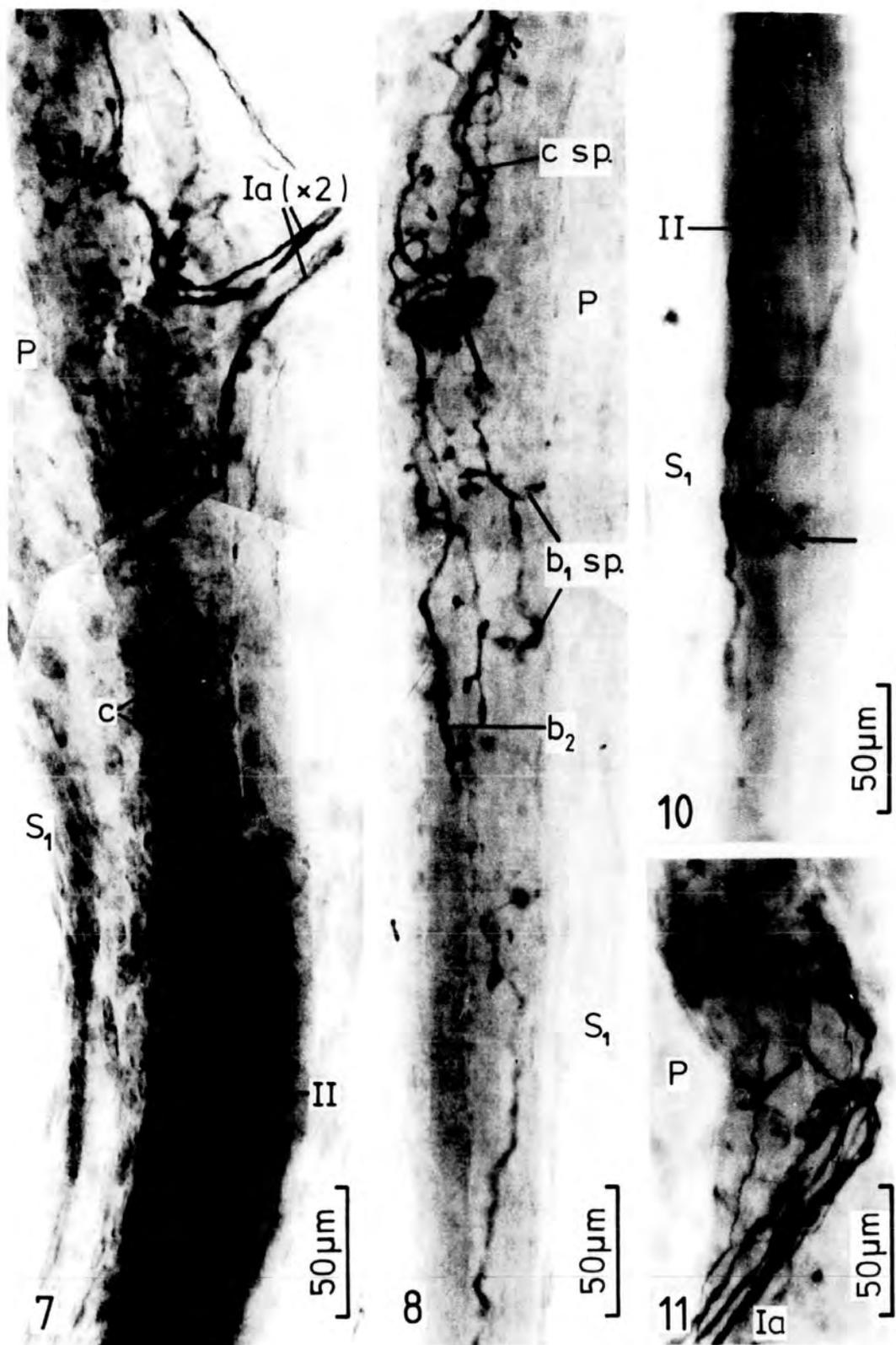


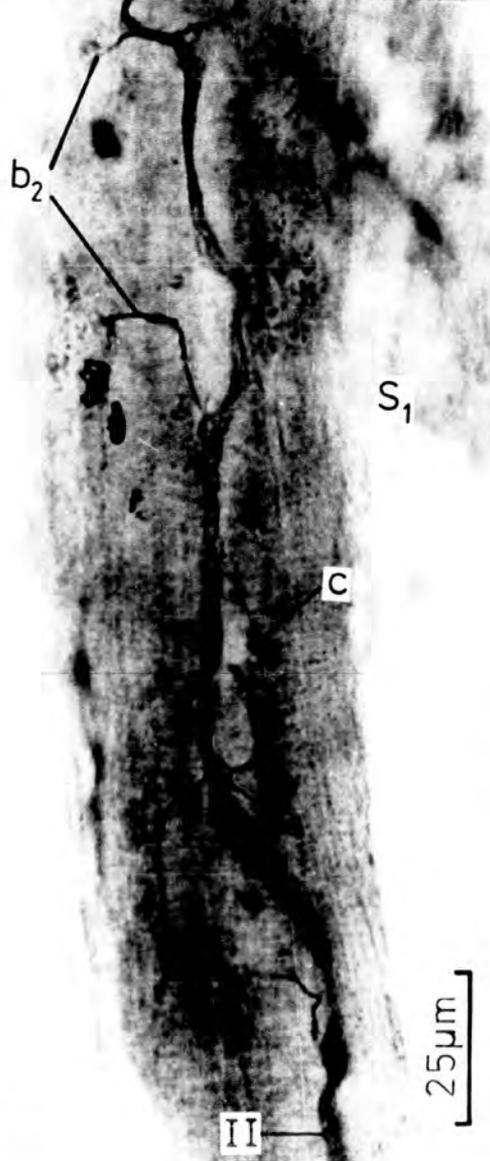
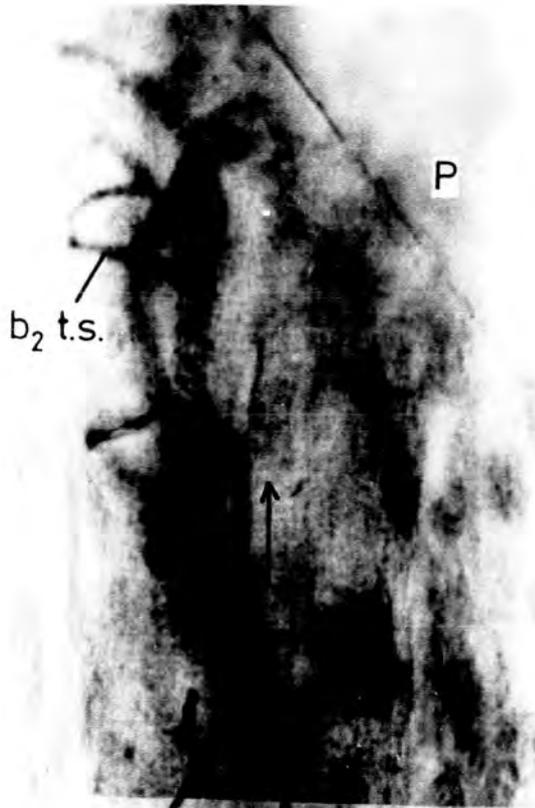
Plate 4.

Silver-stained muscle-spindle equatorial regions at 33 days PC (11 days RT). Abbreviations: Ia tube, the endoneurial tube normally containing the Ia axon. Other abbreviations as for previous plates.

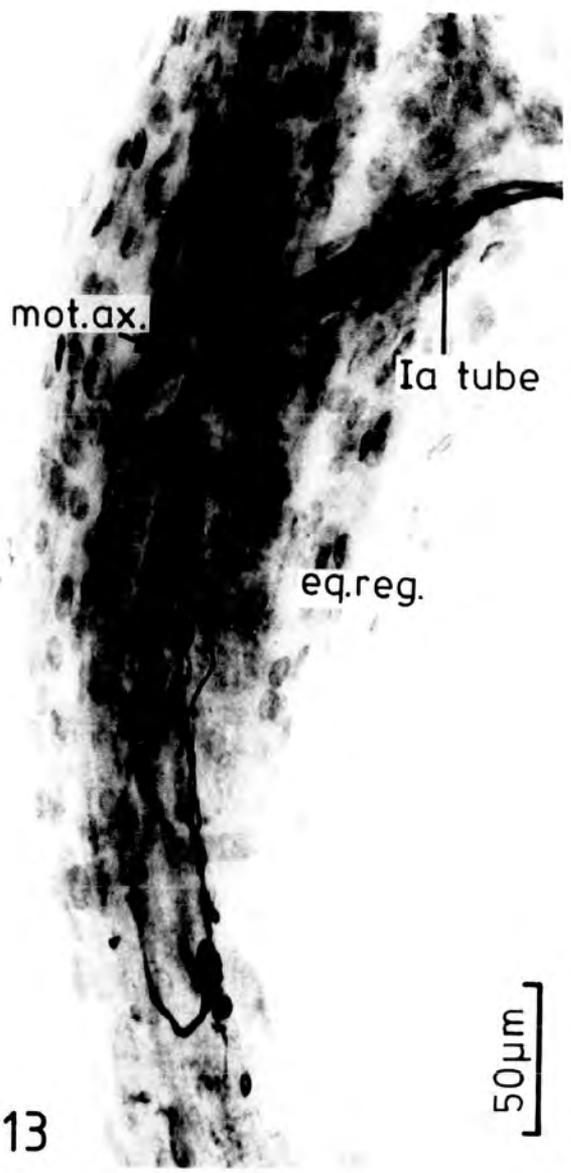
Figure 12. The secondary ending produces terminals in the bag₂ and chain fibres in the S₁ region and then enters the primary region passing the bag₂ primary terminals as shown by the arrow. It subsequently terminated in a ball-like swelling near the centre of the primary ending (not shown here).

Figure 13. There is no Ia axon in evidence in the equatorial region. The Ia tube contains a number of fine axons which enter the equatorial region and disperse, growing towards the poles. On this basis they are presumed to be motor axons.

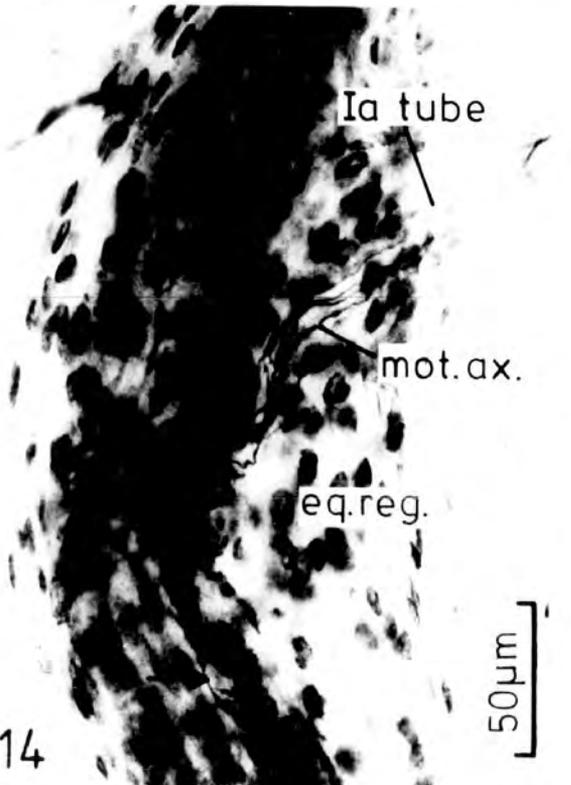
Figure 14. As with Fig. 13, the Ia tube contains a number of fine axons which, like the one marked with the arrow, proceed towards the poles of the spindle and are therefore assumed to be motor in origin.



12



13



14

Plate 5.

Silver-stained primary endings of muscle spindles 40 days after nerve-crush injury (18 days RT). Abbreviations as for previous plates.

Figure 15. A very well-restored primary ending with well-developed terminal systems on all the intrafusal fibre types. The bag₁ innervation is relatively sparse with only four well-formed bands. The four arrows mark the path of an S₁ secondary terminal branch which originated in the lower pole (marked S₁) and courses through the primary region to terminate in the upper S₁ region (not shown here).

Figure 16. Another very well-restored primary ending with well-developed terminal systems on all the fibre types. As with the ending in Fig. 15, the bag₁ innervation is relatively limited compared with that of the bag₂ fibre.

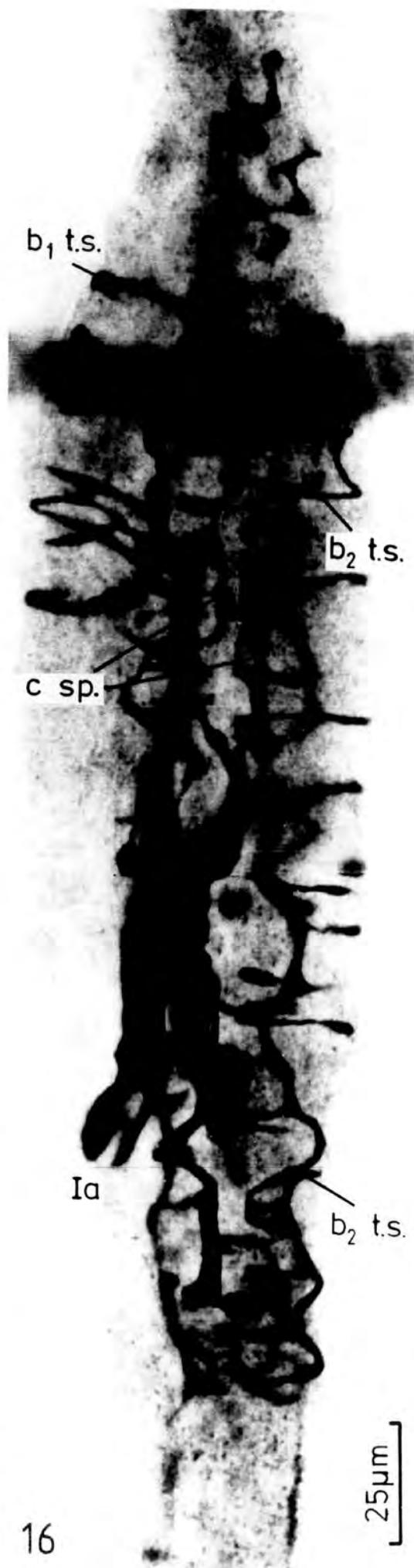


Plate 6.

Photographs of poorly-developed or abnormal primary endings at 40days PC (18 days RT). Abbreviations as for previous plates.

Figure 17. The Ia axon produces a series of irregular terminals on the intrafusal fibres towards the periphery of the equatorial region; the central region is apparently lacking any innervation.

Figure 18. There are two Ia axons in the primary tube which contribute to the primary ending. The axons were still separate 1450 μ m from the ending. The innervation is very limited and poorly organised, although terminals are visible on all fibre types.

Figure 19. In comparison with Fig. 18, this is an example of a double primary ending. The two Ia axons (Ia_1 and Ia_2) are contained in separate endoneurial tubes and form endings which, although they overlap slightly, are clearly distinct as compared with the ending shown in Fig. 18. This spindle was also innervated by two S_1 secondaries (not shown here), one either side of the primary region. Both were poorly developed.

The two primaries were identified as such (as opposed to one of them being an S_1 secondary) on the basis of the form of the endings, both of which consisted of a central region of regular banding on the bag fibres bounded by irregular regions.

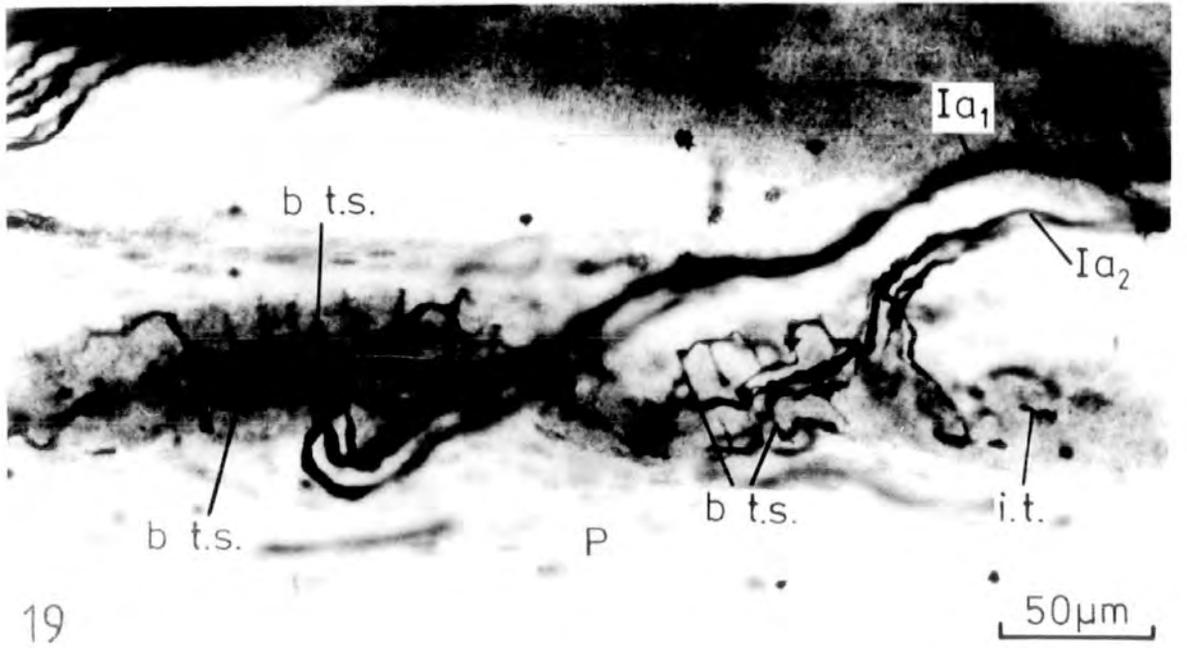
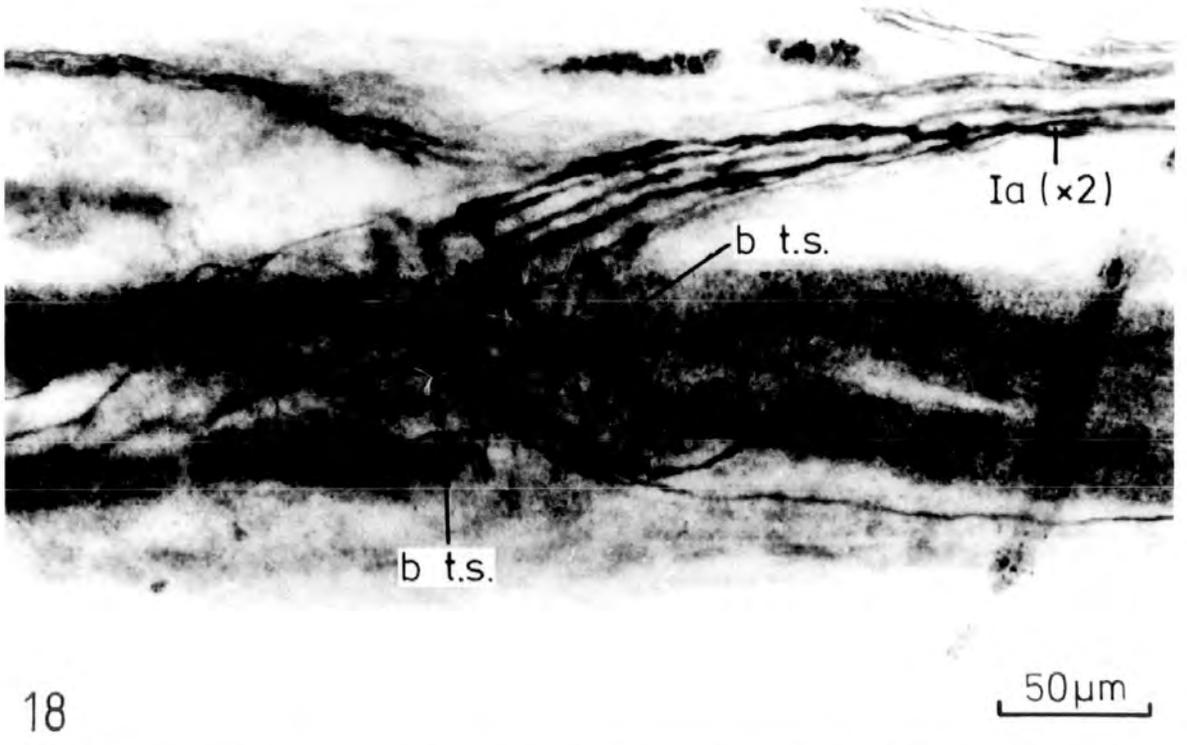
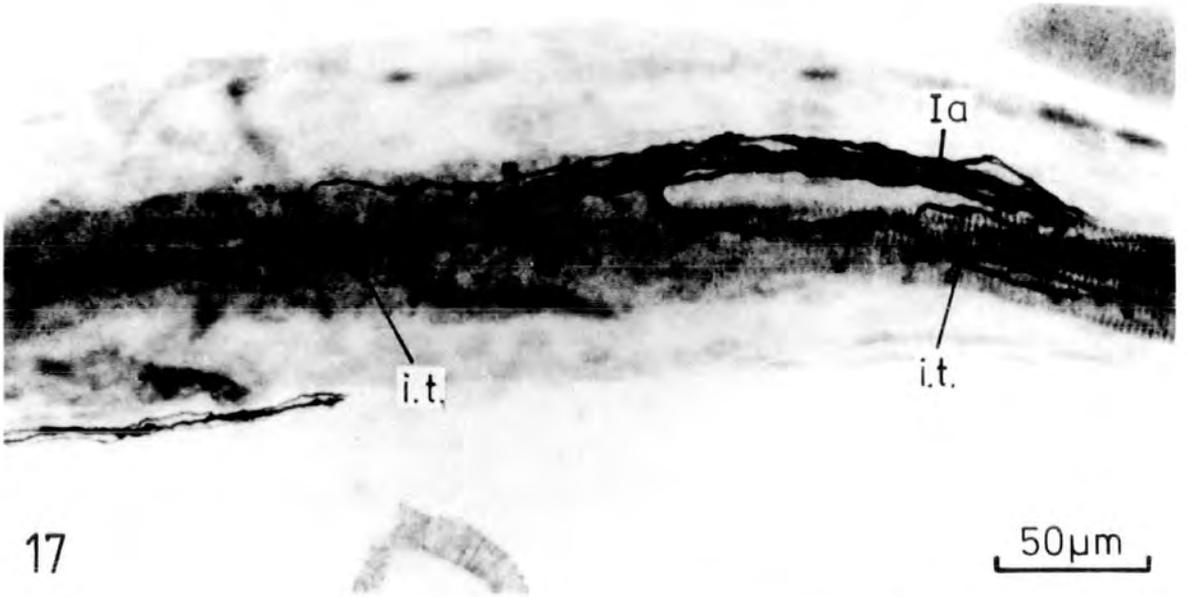


Plate 7.

Photographs of primary regions which have been invaded by foreign axons (presumably motor) from different sources. These spindles were observed in the sample at 40 days PC (18 days RT). Abbreviations: l.a.s., large axonic swelling. Other abbreviations as for previous plates.

Figure 20. There is no Ia present in the equatorial region. The only axon is a presumed motor axon which entered the spindle via the route normally taken by the Ia. This axon failed to branch and formed the large ball-like swelling in the centre of the equatorial region.

Figure 21. The primary axon produces a very sparse, disorganised innervation of the intrafusal fibres. Some motor axons from the poles have invaded the region and are seen to terminate in large axonic swellings.

Figure 22. The Ia tube contained a single axon (presumably motor) which can be seen entering the equatorial region, whereupon it doubles back and leaves the spindle via the same nerve trunk. It gives rise to some fine branches but none of these attempt to form an ending.

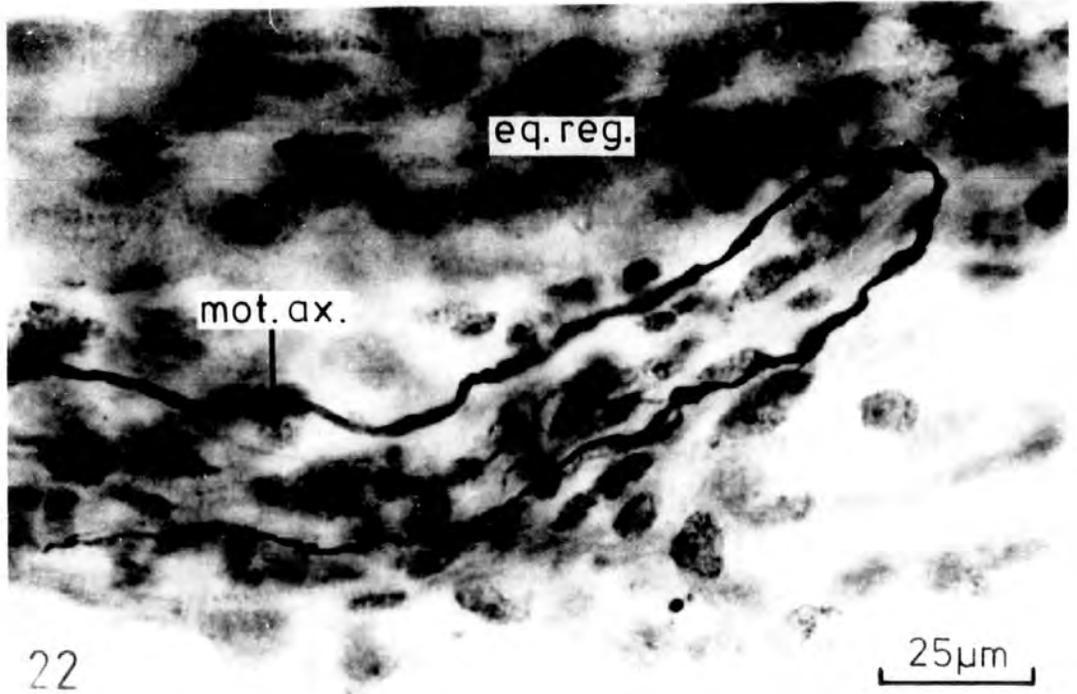
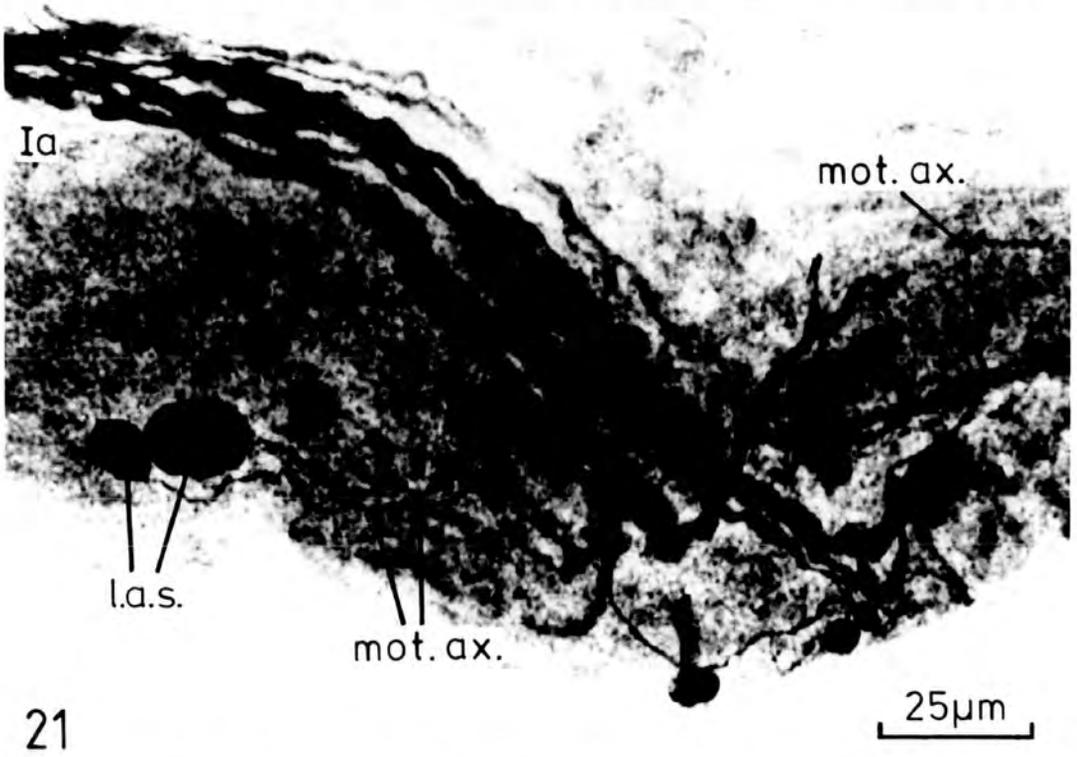
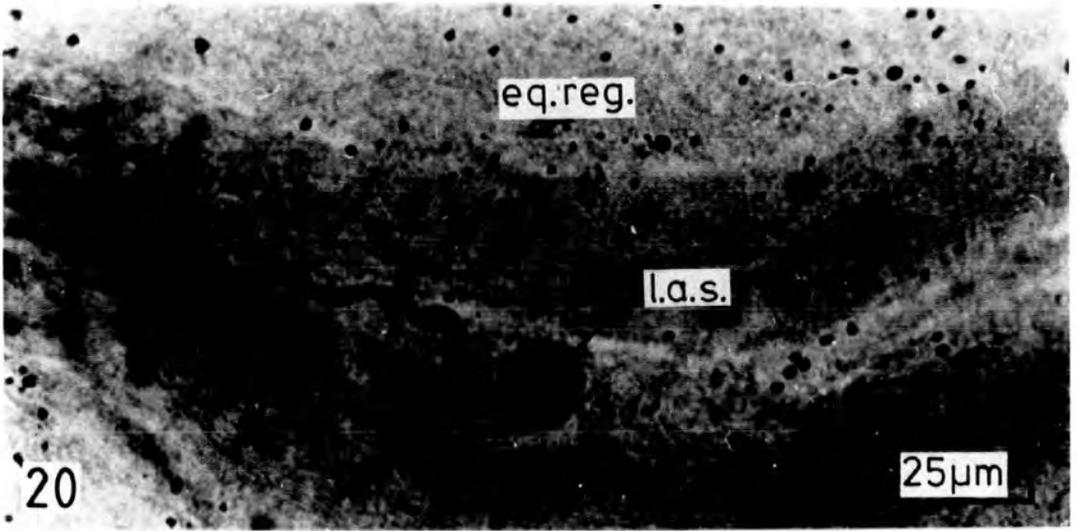


Plate 8.

Photographs of silver-stained primary endings at 47 days PC (25 days RT). Abbreviations as for previous plates.

Figure 23. The primary ending is very well restored with well-developed terminal systems on all the intrafusal fibre types. Both bag fibres show extensive innervation. The bag₂ has a long region of irregular innervation at one end.

Figure 24. The Ia axon produces a very small ending (only 175 μ m in length). There is very little banding, much of the ending consisting of irregular terminations. The spirals that are present on one of the bag fibres terminate in a large ball-like swelling as indicated by the arrow.

Figure 25. There is a well-formed terminal system on the bag₁ fibre which is separated into two portions with a gap in the middle. The bag₂ lies beneath the bag₁ and is not visible in the photograph but it too was well innervated. One branch of the primary (arrowed) encroaches on the edge of the S₁ region where it peters out after attempting to make an ending.

Figure 26. The primary innervation is well restored though of limited extent. The bag₁ fibre is innervated by two first-order branches which are distinct from the one that supplies the bag₂ and chains.

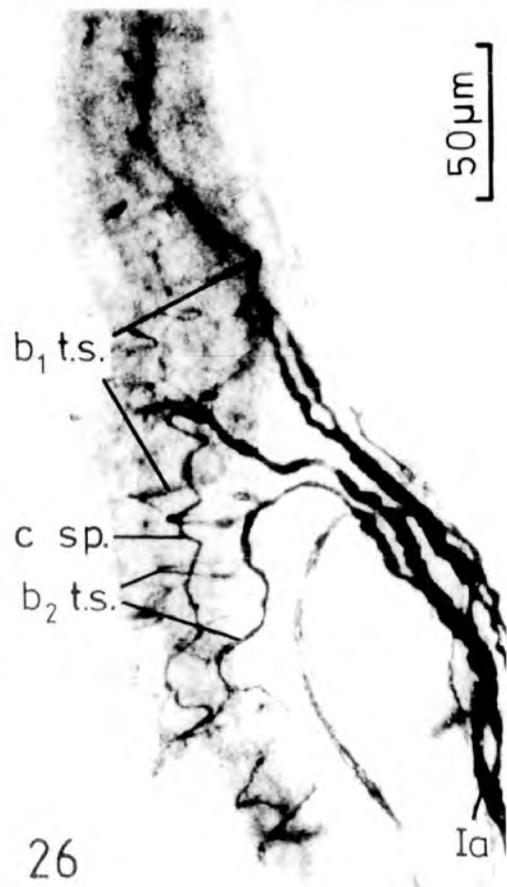
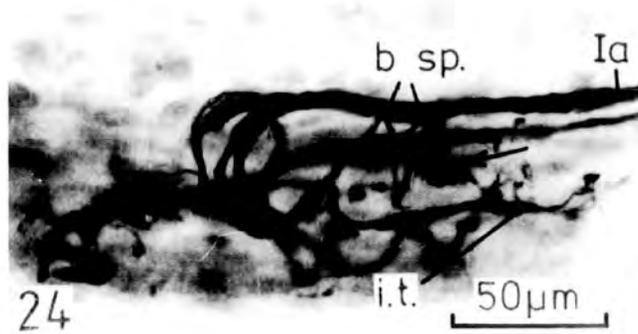
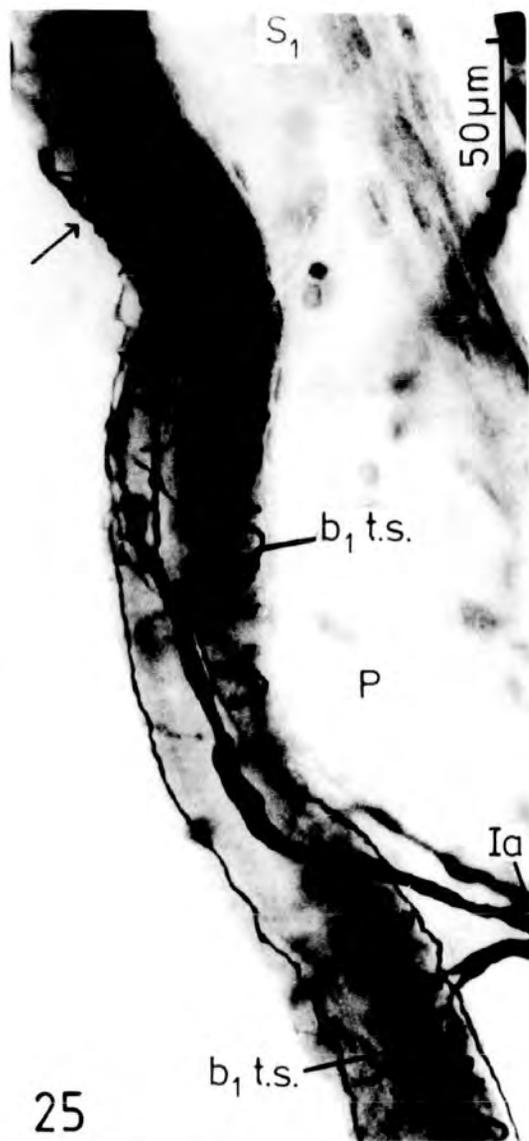
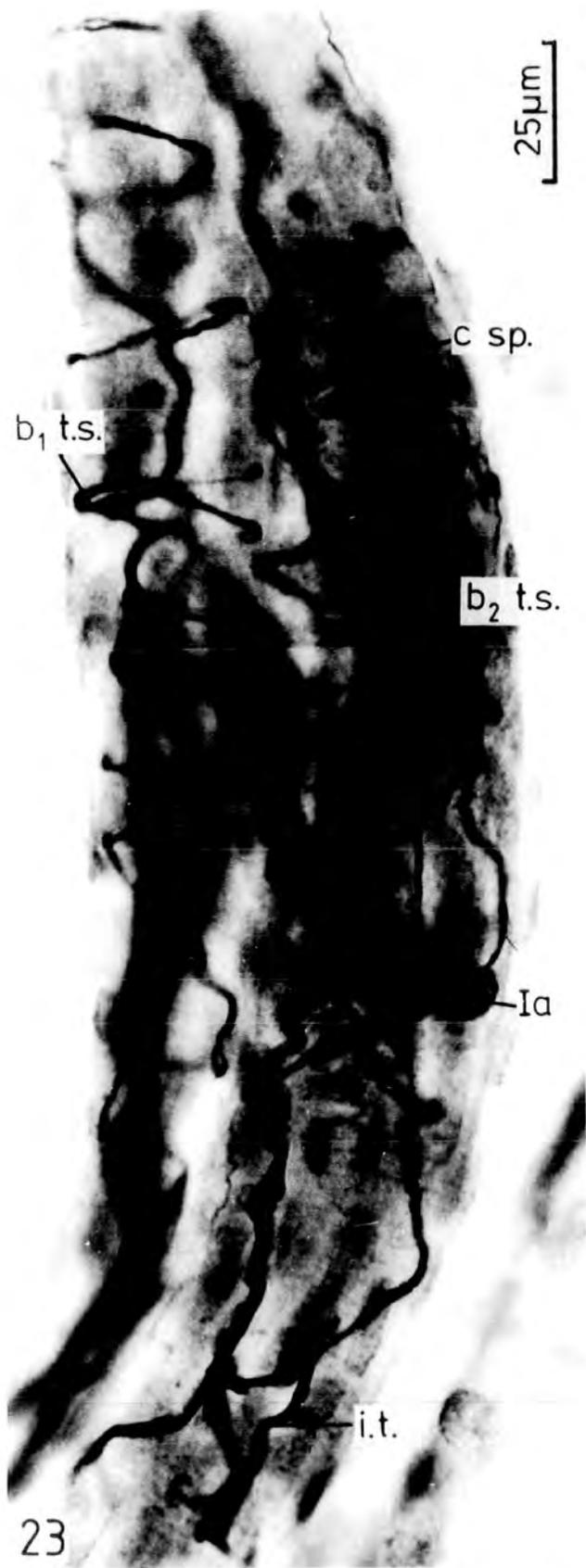


Plate 9.

Photographs of primary and secondary endings at 47 days PC (25 days RT), showing some of the abnormalities of reinnervation observed at this time. Abbreviations as for previous plates.

Figure 27. The primary ending is mainly irregular with few regular spirals. The secondary ending innervates the bag₂ and chain fibres in the upper pole and produces a branch (arrowed) that traverses the primary region to terminate in the lower S₁ region.

Figure 28. There is no primary axon present in this spindle, the secondary axon produces some terminals in the S₁ region and also invades the primary region but does not give rise to any terminals.

Figure 29. The primary innervation consists solely of a number of spirals on one of the chain fibres with a single terminal on a bag fibre. None of the other intrafusal fibres is innervated.

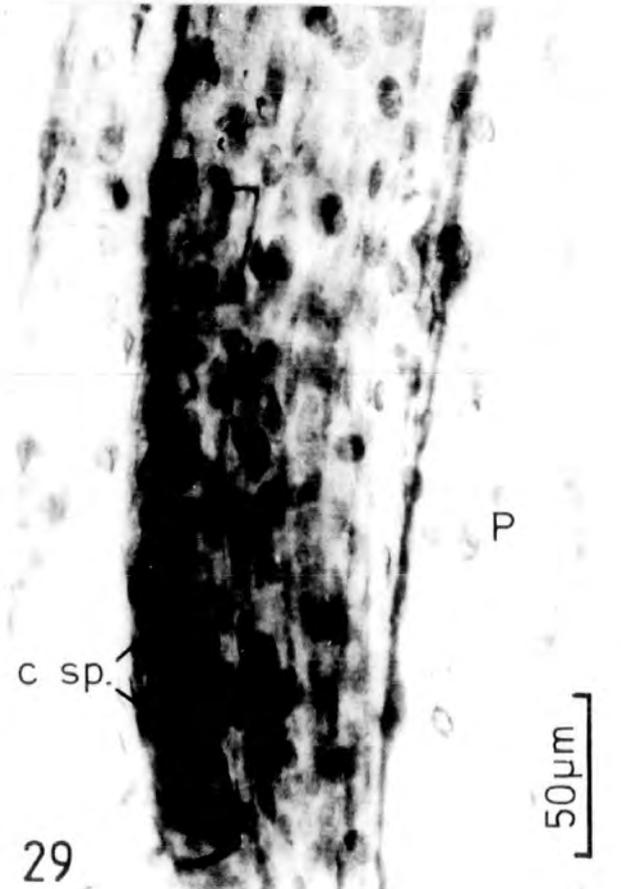
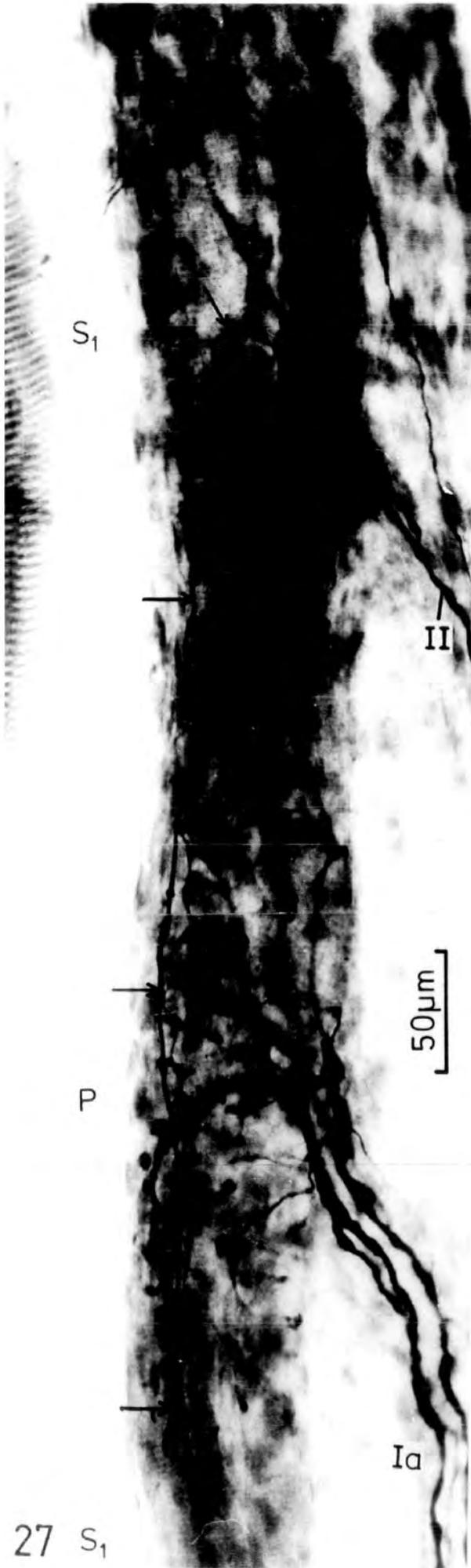
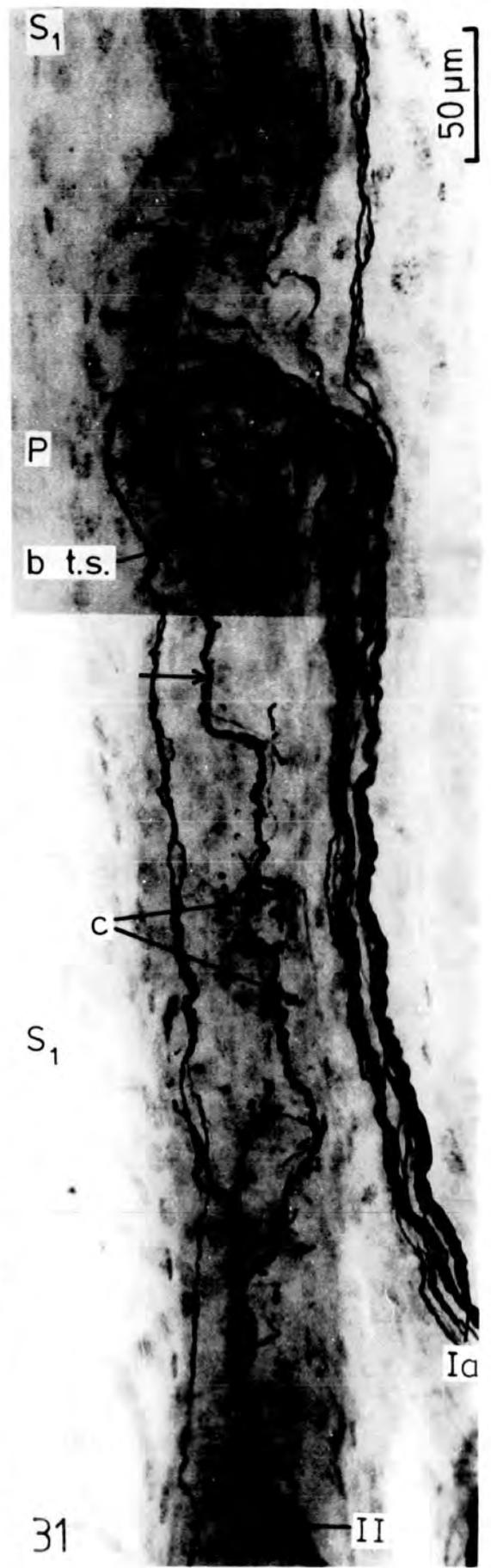
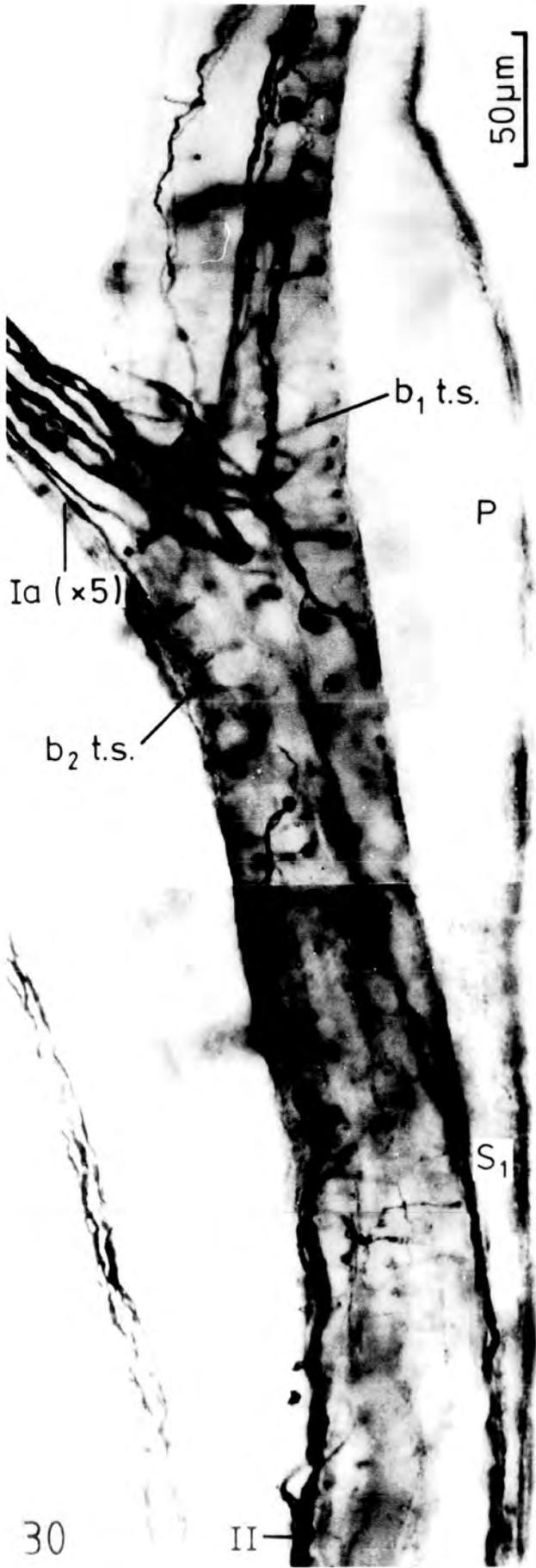


Plate 10.

Photographs of silver-stained primary and secondary endings at 61 days PC (39 days RT) and 75 days PC (53 days RT). Abbreviations as for previous plates.

Figure 30. This spindle, examined 61 days after the crush injury is hyperinnervated by five Ia axons contained within a single Ia endoneurial tube. The axons were traced back 1500 μ m and were still clearly separate at this point. The intrafusal fibres are well reinnervated and each fibre type is supplied by a separate axon which does not give branches to any other fibre type. The secondary ending also innervates all three fibre types and, although it closely approaches the primary ending, does not overlap with it.

Figure 31. This is an example of a reasonably well-reinnervated spindle at 75 days PC (53 days RT). The primary axon has reinnervated all three fibre types, although only to a limited extent. The secondary axon in the lower S₁ region forms endings only on the chain fibres. One branch (arrowed) traverses the primary region and peters out in the upper S₁ region.



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Plate 11.

Photographs of primary and secondary endings at 75 days PC (53 days RT). Abbreviations as for previous plates.

Figure 32. The primary ending is well reinnervated with extensive terminal systems on all three types of intrafusal fibre. Numerous terminal expansions are in evidence on the outer terminals on the lower bag fibre. The S₁ secondary is well restored and is mainly restricted to the chain fibres.

Figure 33. Another example of a reasonably well-restored primary ending, although the right-hand bag fibre is only sparsely innervated.

Figure 34. A photograph of a well-restored secondary ending. All three fibre types are reinnervated, but it was not possible to distinguish between the bag fibres.

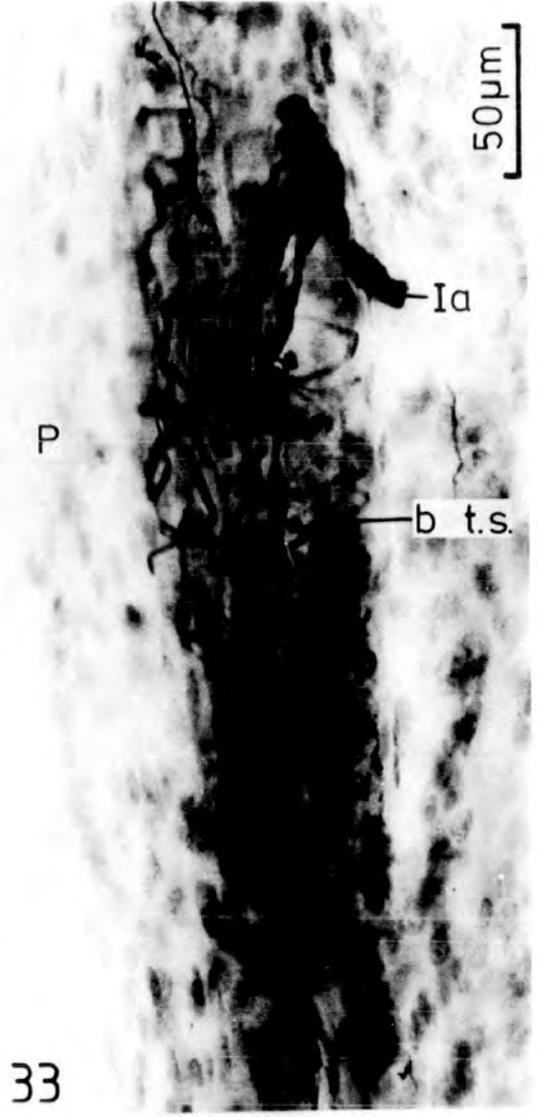
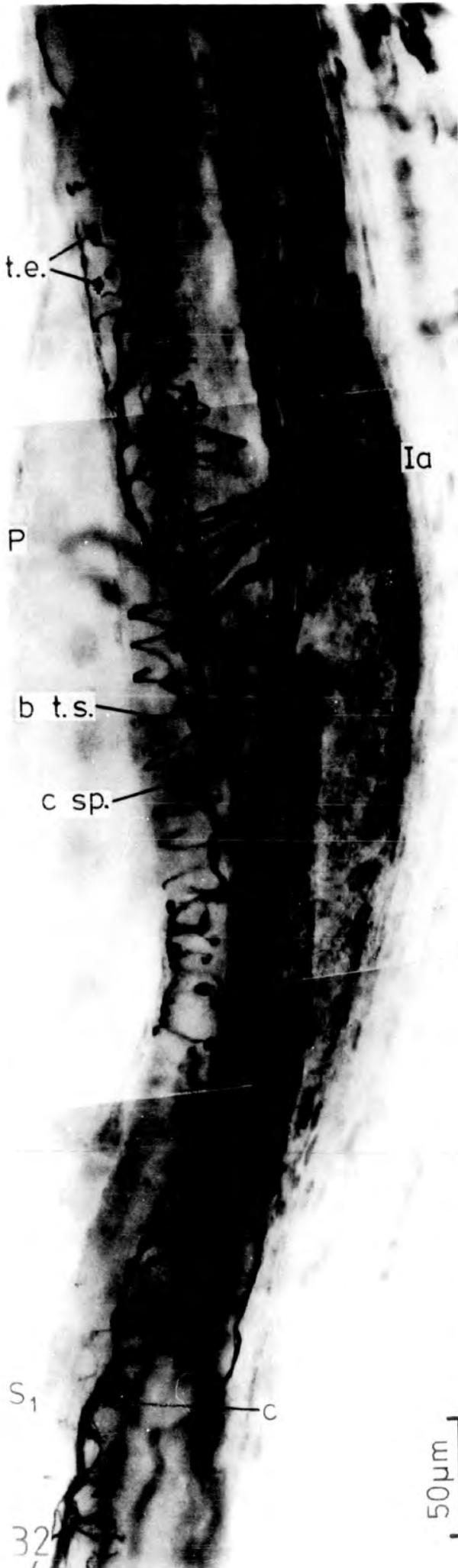


Plate 12.

Photographs of silver-stained primary endings at 96 days PC (74 days RT). Abbreviations as for previous plates.

Figure 35. A reasonably well-restored primary ending with regular terminations on both bag fibres.

Figure 36. A very well-restored, near normal-looking primary with extensive reinnervation of both bag fibres, the bag₁ having 13 bands and the bag₂ 12. The bag₁ fibre primary innervation was segregated from that to the bag₂ and chains.

Figure 37. An example of an abnormal primary which fails to supply any innervation to the bag₁ fibre.

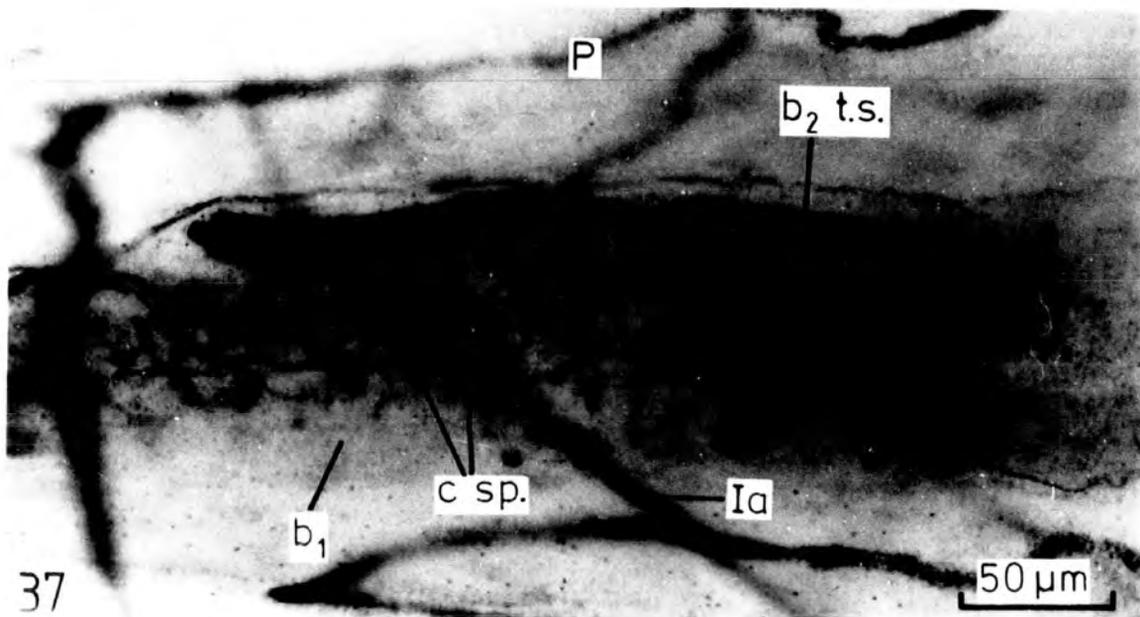
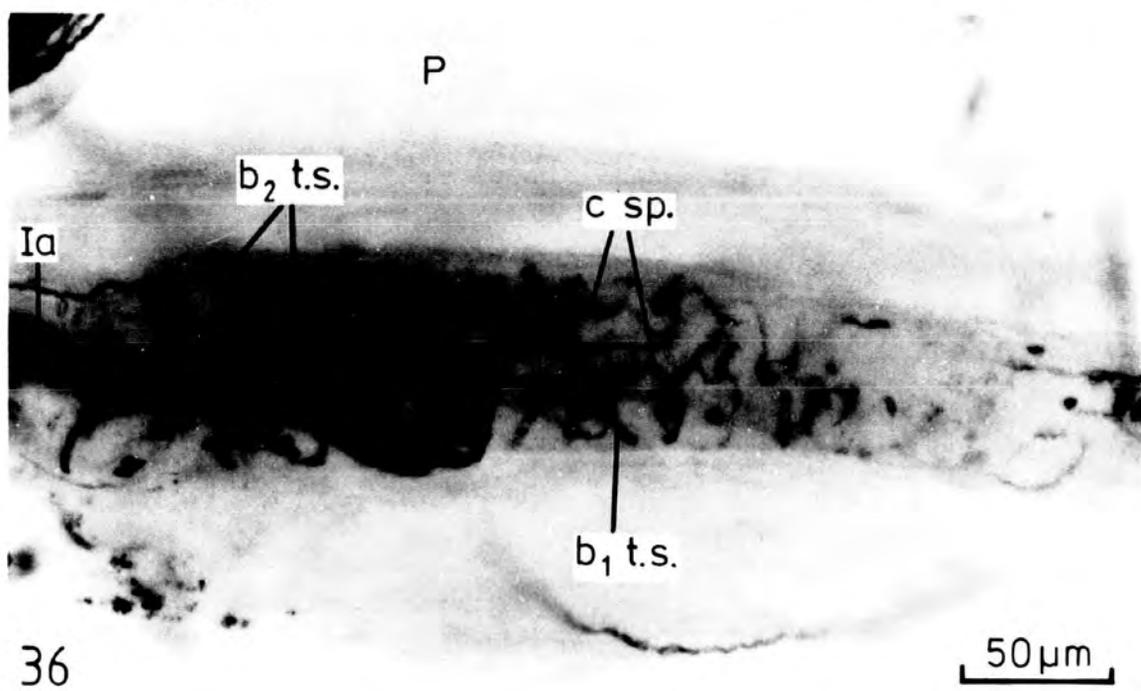
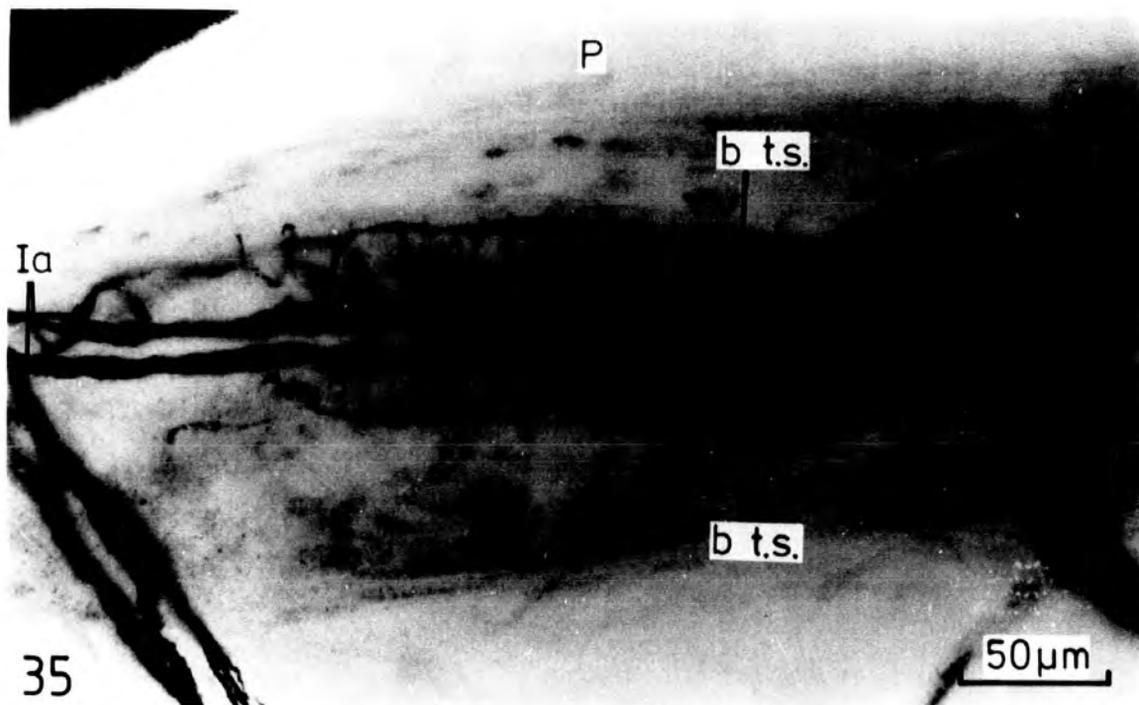


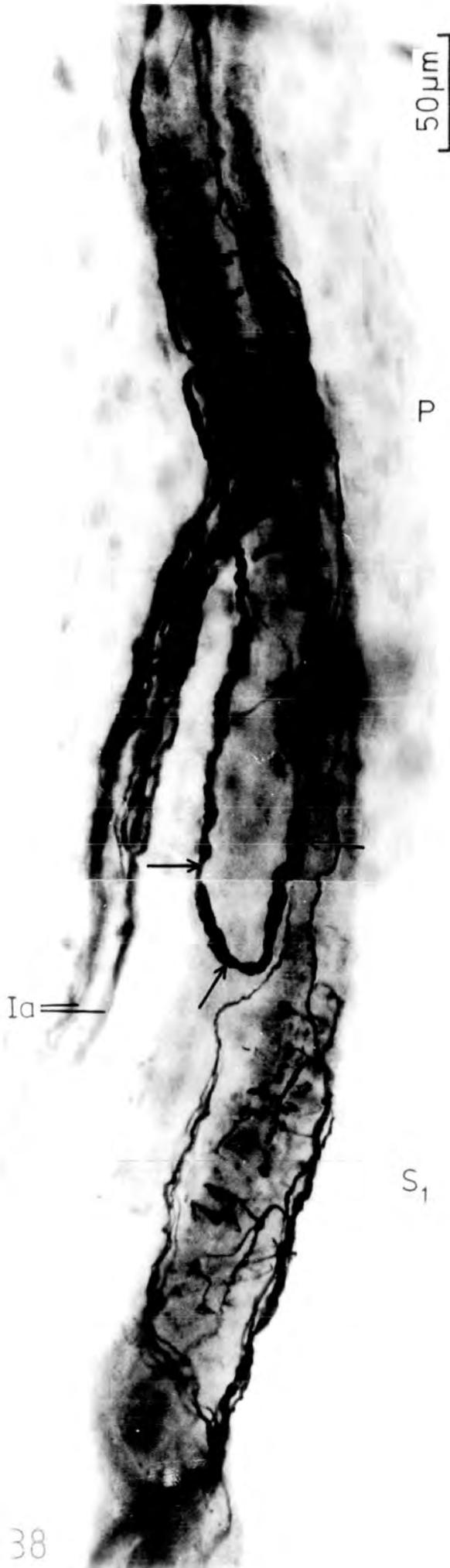
Plate 13.

Photographs of silver-stained primary and secondary endings after 140 days of recovery (118 days RT). Abbreviations as for previous plates.

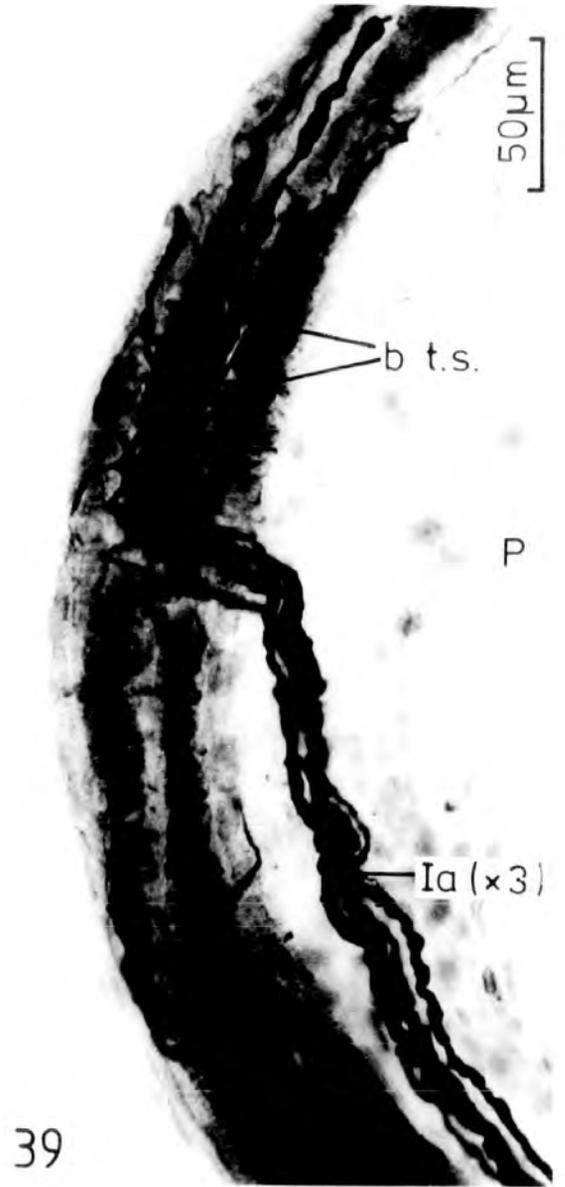
Figure 38. This primary ending is poorly restored, the main feature being the large branch (arrowed) which encroaches upon the limit of the S_1 region and then turns back to end in the P region. The S_1 secondary is relatively well restored.

Figure 39. An example of hyperinnervation by three primary axons each of which contributes to the overall configuration of the primary ending.

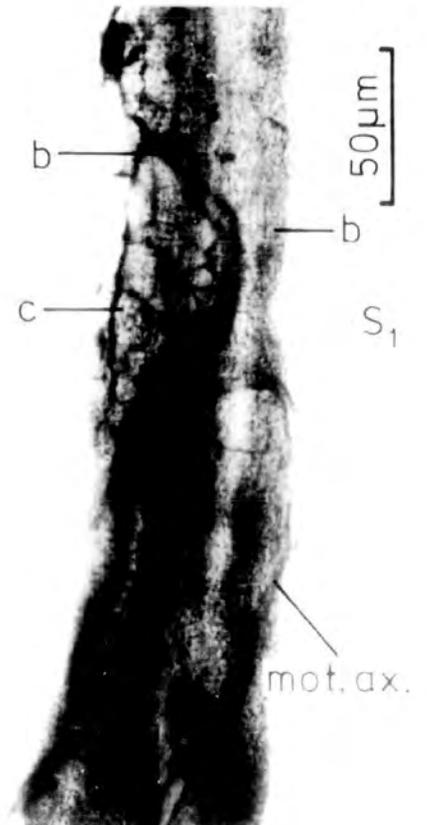
Figure 40. A well-restored secondary ending mainly confined to the chains and one bag fibre; the right-hand bag fibre passes through without being innervated.



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Plate 14.

Silver-stained preparations of normal primary and secondary endings from the PB muscle of an unoperated animal. Abbreviations as for previous plates.

Figure 41. A normal primary ending with a long region of regular annulospiral innervation on each of the intrafusal fibres. The bag₁ has 12 bands and the bag₂ has 15. The section of regular ending is bounded at either end by an irregular region which is more extensive on the bag₁ than on the bag₂ fibre.

Figure 42. A primary and S₁ secondary endings from an unoperated animal. The bag₁ fibre lies apart from the bag₂ and chain fibres and is innervated by a separate first-order branch of the Ia axon. The bag₁ has 13 bands and the bag₂ has 14. The S₁ secondary innervates all the intrafusal fibres but is distributed mainly on the bag₂ and chain fibres.

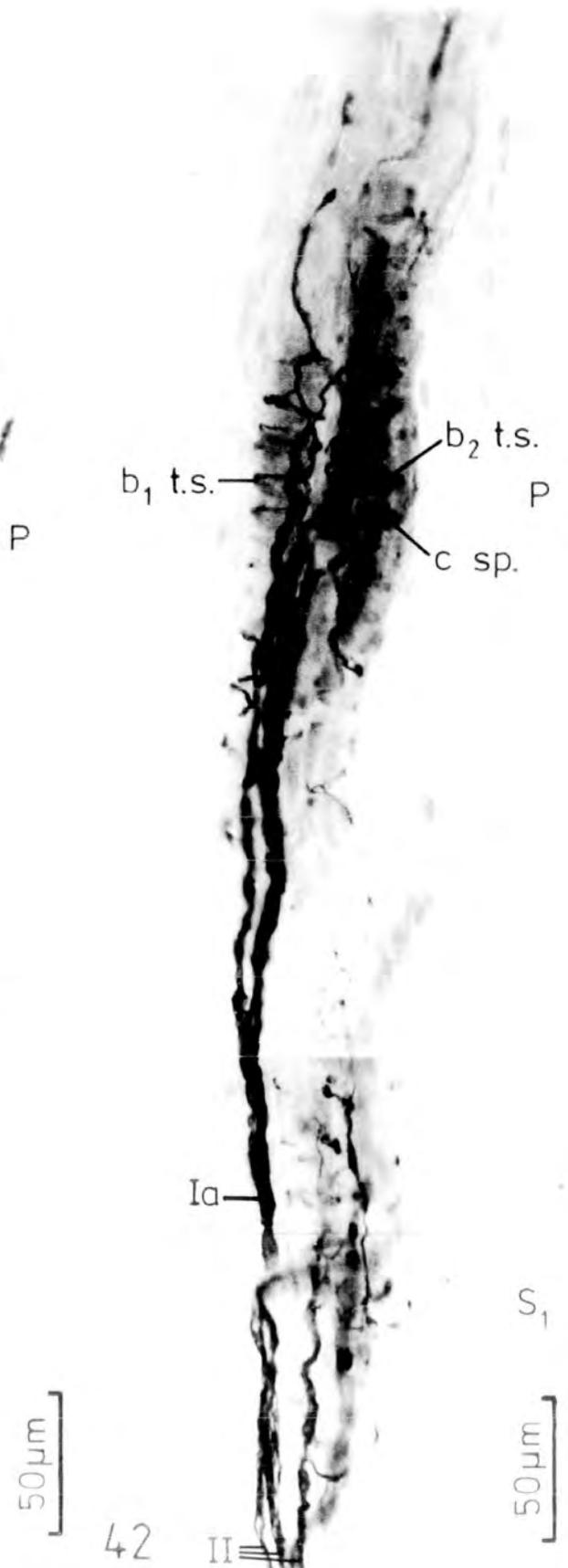
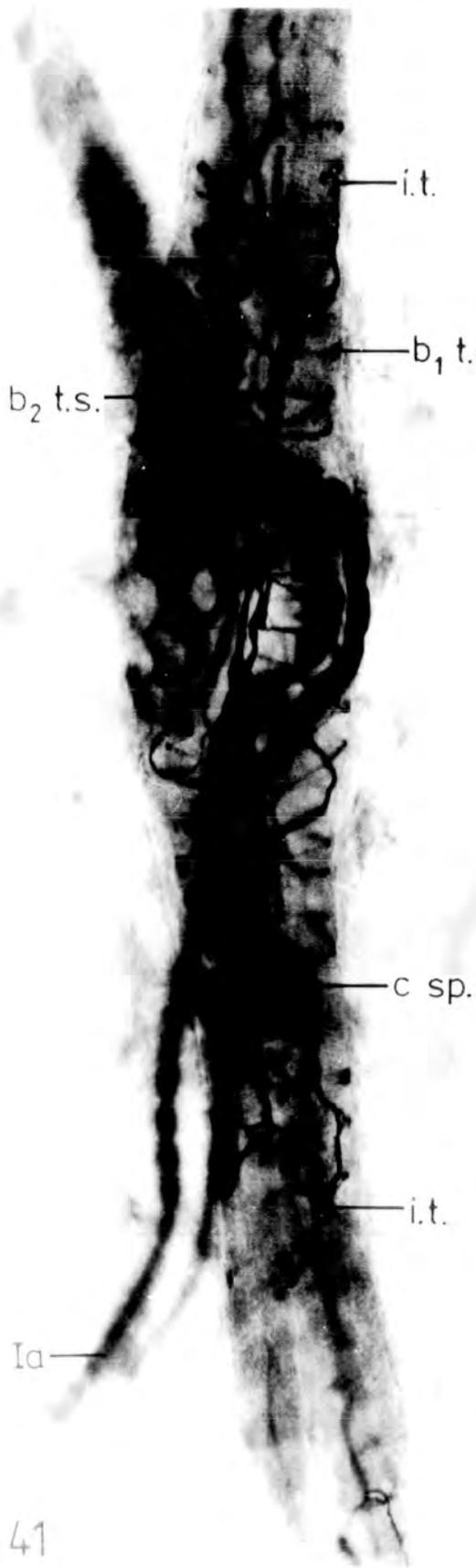


Plate 15.

Photographs of silver-stained primary and secondary endings from normal PB muscle spindles. Abbreviations as for previous plates.

Figure 43. A primary ending with S_1 and S_2 secondary endings. The primary axon supplies 8 bands to each of the bag fibres. The S_1 secondary innervates all the intrafusal fibres, although the bag₁ only receives one small branch. The S_2 secondary is distributed only to the bag₂ and chain fibres.

Figure 44. A very extensively-innervated primary region and an S_1 secondary ending. The bag₁ has 13 bands and the bag₂ has 22. The S_1 secondary innervates all the intrafusal fibres but is mainly confined to the bag₂ and chain fibres.

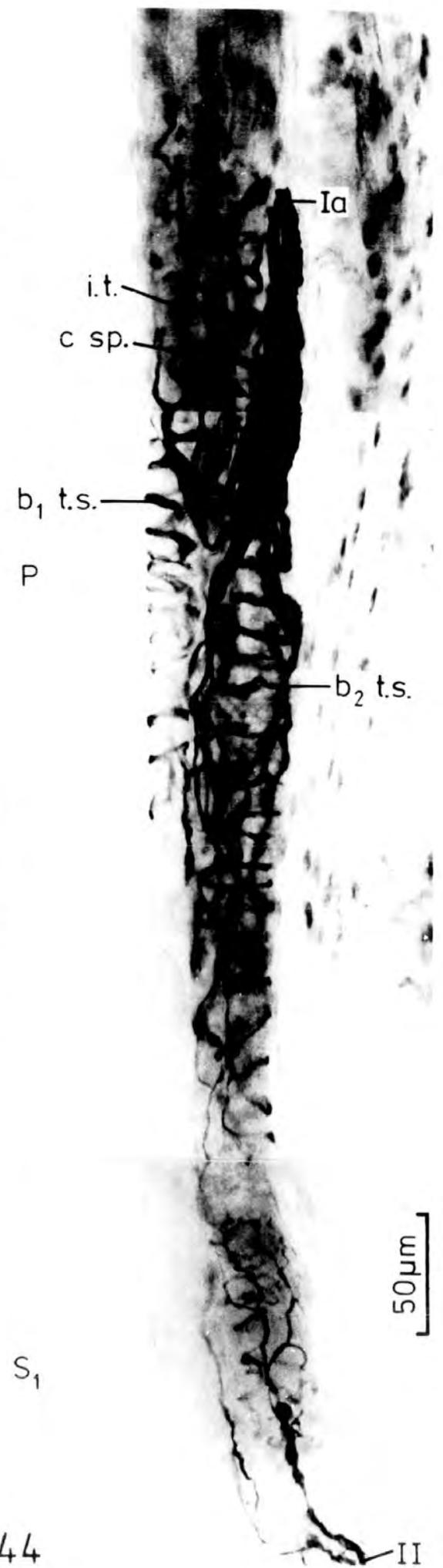


Plate 16.

Photographs of silver-stained intrafusal motor endplate in chronological order of recovery.

Figure 46. A developing motor endplate at 26 days PC (4 days RT); because of the very limited state of development of the reinnervation of the spindle it was not possible to identify either the type of the motor ending or on what muscle it was lying.

Figures 47, 48 and 49. Developing motor endplates at 33 days PC (11 days RT). Identification of the motor endplate type was not possible.

Figure 50. A p_1 plate on a bag_1 fibre at 40 days PC (18 days RT). Note the Doyere eminence.

Figure 51. A p_2 plate on a bag_1 fibre at 75 days PC (53 days RT).

Figure 52. A double p_1 plate on a bag_1 fibre at 75 days PC (53 days RT). Two motor axons (possibly branches of the same axon, unfortunately the axons were broken) each form a p_1 ending.

50



25µm

49



25µm

51



25µm

48



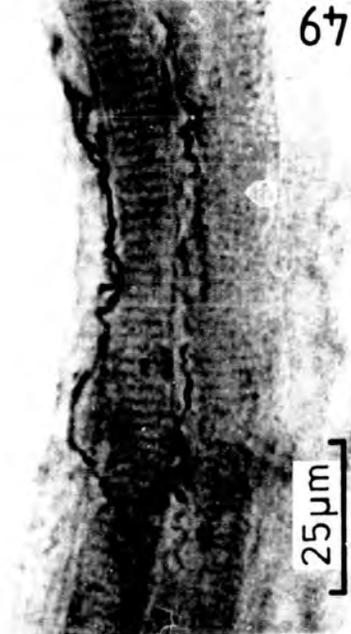
25µm

52



25µm

47



25µm

47, 25µm



Plate 17.

Photographs of silver-stained motor endings after different recovery periods.

Figure 53. A p_1 plate on the bag_1 fibre of a normal spindle.

Figure 54. A p_2 plate on the bag_1 fibre of a normal spindle.

Figure 55. Developing extrafusal (α skeletomotor) innervation after 26 days of recovery (4 days RT).

Figures 56 and 57. Developing α motor endplates on extrafusal muscle fibres at 33 days PC (11 days RT).

Figures 58 and 59. Alpha motor endplates at 40 days PC (18 days RT).

Figure 60. An α extrafusal motor endplate at 47 days PC (25 days RT).

Figure 61. A normal α motor endplate. Note the concentration of nuclei under the terminals.

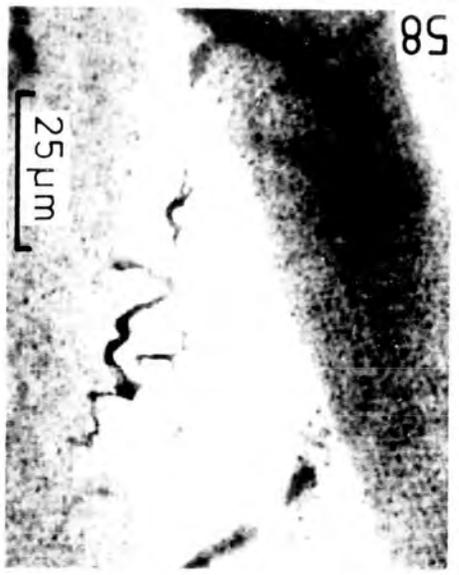
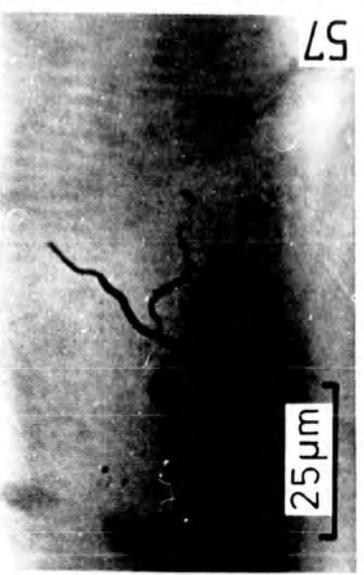
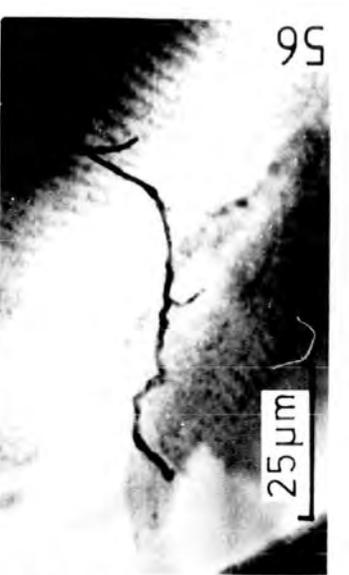
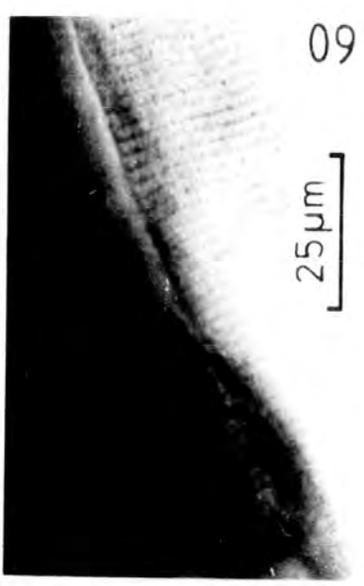


Plate 18.

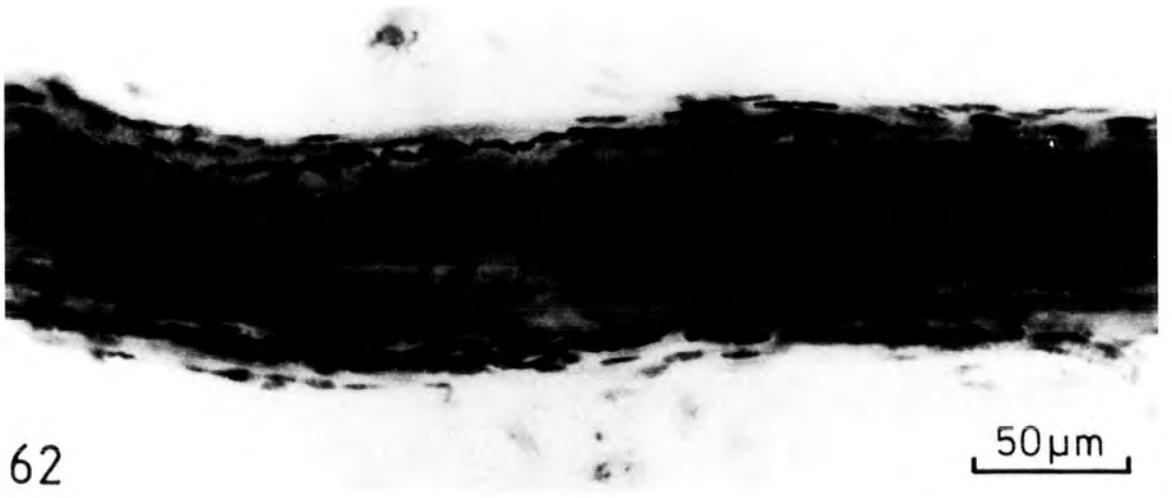
Photographs of silver-stained trail endings in reinnervated and normal muscle spindles.

Figure 62. Trail innervation on the chain fibres and a little on the bag₂ fibre of a spindle at 75 days PC (53 days RT).

Figure 63. Trail innervation at 140 days PC (118 days RT).

Figure 64. A high-power photograph of the trail innervation on a chain fibre at 140 days PC (118 days RT). The concentrations of nuclei under the terminals are clearly visible.

Figure 65. An example of trail innervation in a normal spindle. The innervation is mainly confined to the chains with a small branch to the bag₂ fibre.



The Physiology of Reinnervated Muscle Spindles.

7.1 General Observations.

The first afferent responses were recorded after 26 days recovery (4 days RT), although at this time the majority of the afferents that could be stimulated in the muscle nerve were unresponsive to muscle stretch which indicates that they had yet to achieve functional connexions with the intrafusal fibres. This corresponds with the physiological findings of Brown & Butler (1976) and the histological observations of chapter 6 that the first Ia axons arrived in the spindle capsule at about 4-5 days RT.

Previous to this, at 20 days post-crush (-2 days RT) no direct responses were observed to stimulation of the muscle nerve at up to 20 times the threshold of control α axons, which demonstrates that all the afferents had degenerated after the crush injury.

It should always be borne in mind when examining the results of the physiological experiments given below that they represent a sample of the population of functional units present at that time and, further, it must be assumed that a small proportion of the afferents never regenerate successfully to reinnervate the spindles. After the first recovery periods relatively few afferents were isolated in each preparation which indicates that a high proportion of the afferents had yet to achieve functional connexions and this continued until the return of the second wave of reinnervation (section 6.2, 7.3). After the return of the second wave there would still have been a residual population of uninnervated spindles.

During the early stages, up to 47 days PC (25 days RT), there were far fewer secondaries than primaries isolated in the

dorsal root (of those units that could be identified as Ia or II); after the longer recovery periods the ratios became more even indicating that the secondaries probably regenerate more slowly. In all the samples, however, including the controls, fewer secondaries were isolated than primaries. This is because the sampling technique was slightly biased against secondaries since, having small axons, they are more likely to be damaged during splitting and also the signals are of smaller amplitude and therefore more likely to be missed.

Overall the results show a trend of increasing recovery of normal function with increasing time after the injury.

7.2 Conduction Velocities.

The conduction velocity (CV) distributions of the afferent and efferent populations are indicative of the progress of regeneration at any given period. As the time allowed for recovery was increased, so the CV distributions approached normality (Figs. 66, 67). This gradual increase in CV corresponds with the increasing axonal diameter and myelin thickness of the regenerating axons.

As the stimulating electrodes were placed distal to the crush site (Fig. 68), the CV measurements cover two regions of the nerve: the proximal portion over which the CV is only slightly reduced (see the NEC experiments) and the distal portion between the crush site and the stimulating electrodes over which there is a marked reduction in CV.

The CV distribution of the afferent population from the unoperated controls is distinctly bimodal with a trough at 50-60m/sec (Fig. 66). This is associated with the separation of the spindle afferent population into primary and secondary afferents (section 2.1). After recovery from the nerve crush, this bimodality

is no longer obvious as a result of an increase in the overlap between the Ia and II afferents. This occurs because of variations in the delay time at the crush site and in the regeneration rate of the individual axons. This increased overlap made it impossible to distinguish between Ia and II afferents on the basis of CV measurements alone. In most cases, though, where firm identification was possible on the basis of the response characteristics, it was found that the II afferents in the regenerated nerves conducted more slowly than did the primaries.

At 26 days PC (4 days RT) the mean afferent CV was $23.4\text{m/sec} \pm 1.68$ (S.E. of mean) compared with $69.5\text{m/sec} \pm 2.21$ for the unoperated controls. At 4 days RT the fastest afferent isolated conducted at only 35.9m/sec compared with 104.5m/sec in the control sample.

With increasing recovery time there was a steady increase in the afferent CV's (Fig. 66). Thus at 33 days PC (11 days RT) the mean CV was $41.2\text{m/sec} \pm 1.24$ and by 40 days PC (18 days RT) it had risen to $47.4\text{m/sec} \pm 1.85$ with a range of $25.7\text{--}66.1\text{m/sec}$. At 47 days PC (25 days RT) there was a slight drop in the mean CV to $44.2\text{m/sec} \pm 1.26$ with an increase in the proportion of afferents conducting at the lower velocities such that the range had expanded to $14.5\text{--}68.2\text{m/sec}$. This increase in the numbers of small-diameter axons is probably associated with the second wave of re-innervation that occurred at 25 days RT (section 6.2).

After 47 days PC (25 days RT) there was a continued increase in the mean CV's although this slowed down after the longer recovery periods. At 61 days PC (39 days RT) the mean CV was $50.1\text{m/sec} \pm 2.01$ and at 75 days PC (53 days RT) it was $55.5\text{m/sec} \pm 2.54$. This increase continued up to 96 days PC (74 days RT)

Figure 66. The distributions of conduction velocity of the control and regenerated spindle afferent populations. The times are given in terms of days post-crush. The CV's were obtained from measurements of the time taken for the action potentials to travel from the stimulating electrodes distal to the lesion to the recording electrodes on the dorsal root filaments. The length of nerve between these points was measured in situ at the end of the experiment.

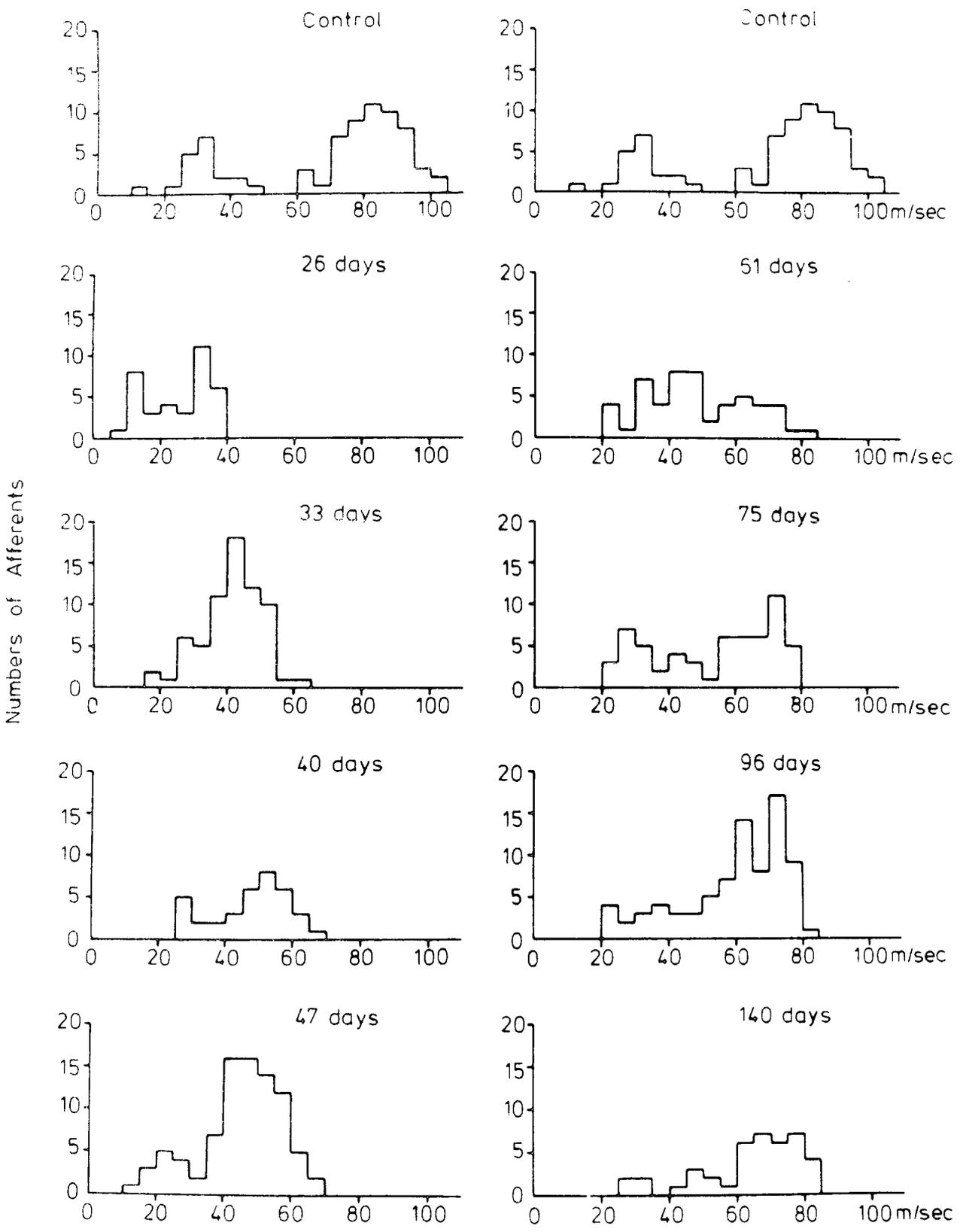


Figure 67. Histograms of the CV's of control and regenerated efferent axons. Measurements are as for Fig. 66. The bimodal distribution of each population marks the separation of the δ and α (plus β) sub-populations.

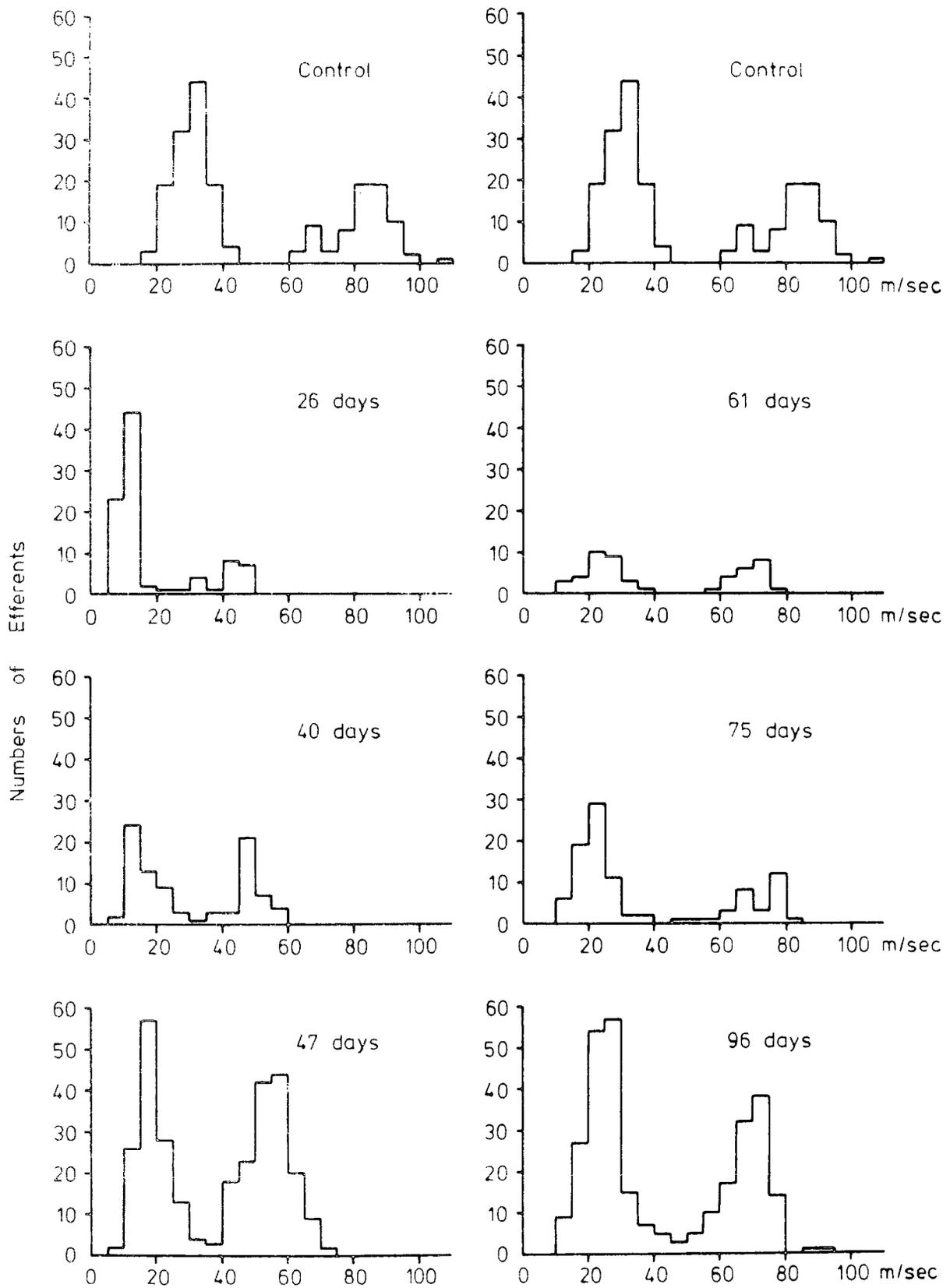
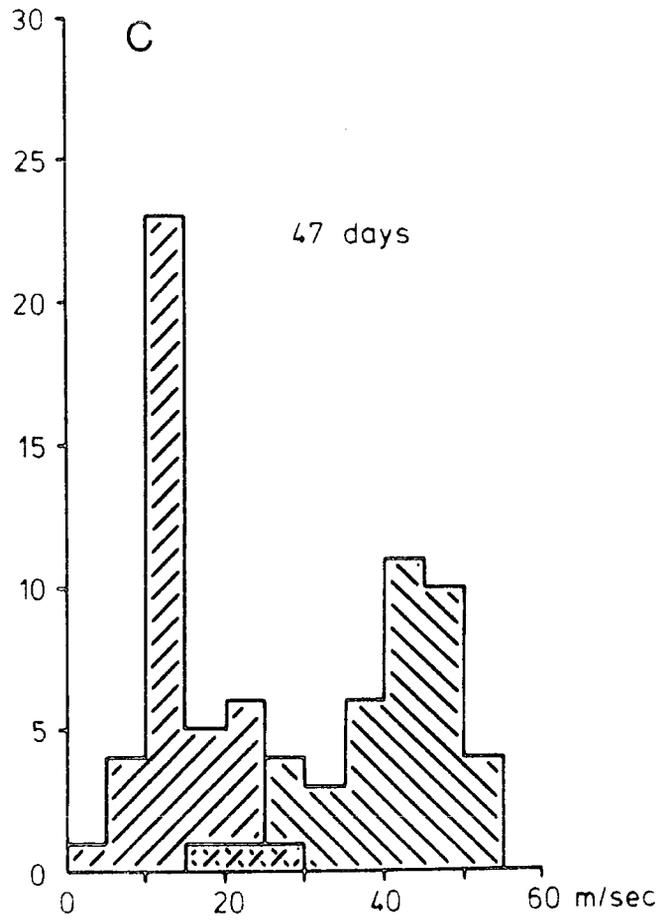
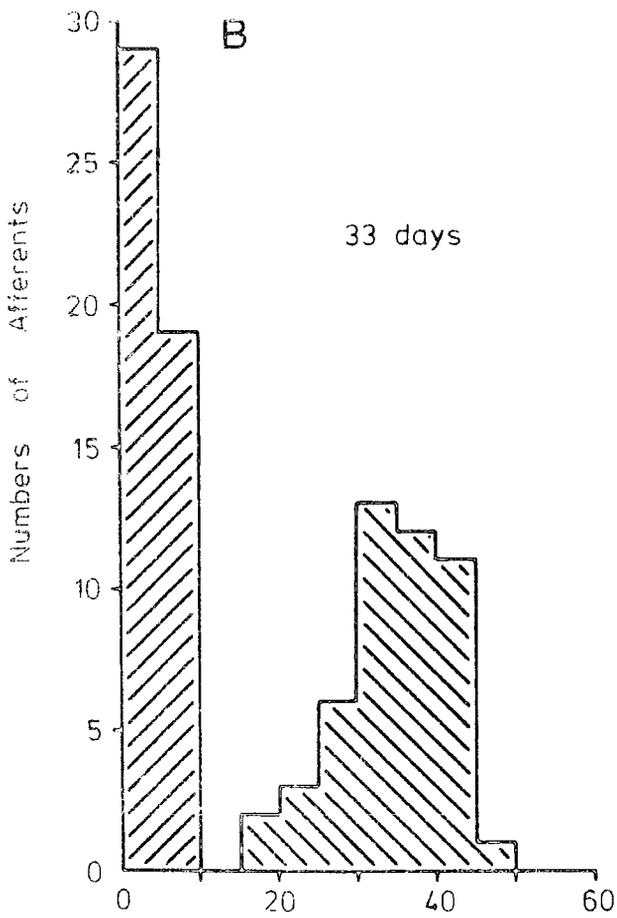
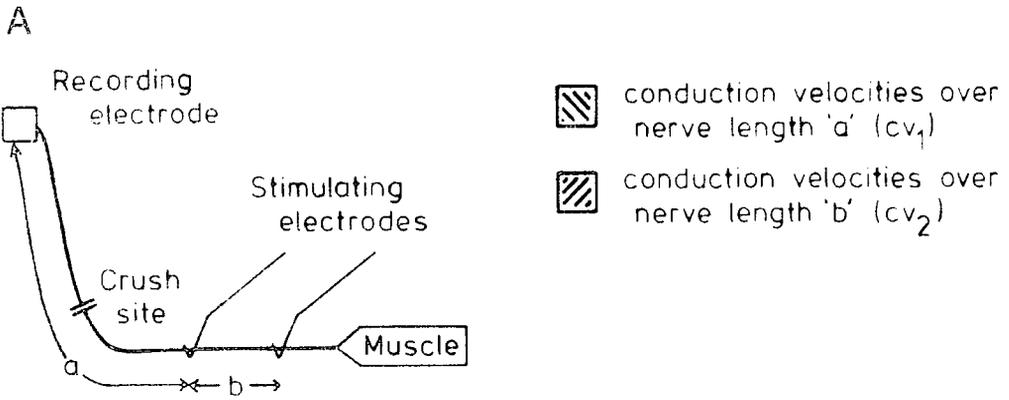


Figure 68. A comparison of the spindle afferent CV's over two sections of the nerve. A, arrangement of the stimulating and recording electrodes in relation to the crush site and the muscle. The length of nerve over which the measurements were made for the histograms in Figs. 66 and 67 is that marked 'a'. B, histograms of the afferent CV's over nerve lengths 'a' (CV_1) and 'b' (CV_2) at 33 days PC. C, the same measurements made at 47 days PC.



when the mean CV was $61.3\text{m/sec} \pm 1.63$, however, by 140 days PC (118 days RT) it had only risen to $63.3\text{m/sec} \pm 2.47$. Neither of these last two results is significantly different from the CV's of the afferent population in the unoperated controls ($P < 0.05$, Student's 't' test) which had a mean of $69.5\text{m/sec} \pm 2.21$.

Although there was no significant difference between the CV's at 96 and 140 days PC and those of the controls, the fastest conducting regenerated afferents still conducted much slower than the fastest control afferents. At 61 days PC the fastest afferent conducted at 80.4m/sec , at 96 days PC it conducted at 80.7m/sec and at 140 days PC the fastest afferent had a CV of 82.5m/sec compared with 104.5m/sec in the controls. Thus it seems unlikely that the regenerated afferents would ever achieve the full range of conduction velocities.

As recovery progressed the greatest changes in CV occurred over the distal portion of the nerve between the crush site and the muscle (i.e. over the regenerated portion of the nerve). Fig. 68 shows the CV measurements over two sections of the nerve (CV_1 and CV_2) at 33 (11 days RT) and 47 days PC (25 days RT) for the afferent populations. At 33 days PC, the mean of CV_2 was $4.5\text{m/sec} \pm 0.24$ which was 13% of CV_1 ($34.2\text{m/sec} \pm 0.96$). By 47 days PC, CV_2 ($13.4\text{m/sec} \pm 0.74$) had risen to 33% of CV_1 ($40.4\text{m/sec} \pm 1.40$).

Fig. 67 shows the CV distributions of the efferent populations and the same trends are observable as for the afferents. The distinct bimodality of the distributions marks the separation of the α (and β) from the γ sub-populations of the muscle nerve.

7.3 Classification of Responses.

A number of abnormally responding afferents was observed, especially during the early recovery periods. These abnormal

afferents were characterised by a total or partial failure to maintain firing during the hold phase of the ramp-and-hold stretches. In order to provide a convenient means of assessing recovery, the afferents have been classified into four classes on the basis of the degree of abnormality shown, and examples of their responses to a 5mm/sec ramp stretch are shown in Figs. 69, 70, 71, 72 and 73. Because of the nature of these abnormalities it was often difficult to distinguish between primary and secondary afferents (especially in the more extreme cases). Since it was found that, where firm identifications could be made, both afferents showed these abnormalities, they will be considered together for the purposes of classification of their responses.

The class 1 afferents (Fig. 69) respond only to the velocity phase of the ramp and never have a background discharge. At the end of the stretch these afferents fall silent and show no response to the hold phase. As such they may appear similar to tendon organs, except that many give an initial burst at the start of the ramp and they all fire during the relaxation phase of a muscle twitch, remaining silent during the contraction itself. These class 1 afferents can also be identified by their response to intra-venous injections of succinylcholine (section 7.5).

Fig. 70 shows the class 2 afferents. These respond to the velocity component of the ramp and also fire for some, but not all, of the hold phase, sometimes displaying an irregular series of spikes which subside over three seconds. Three seconds was chosen as the arbitrary hold-phase firing period to differentiate between the class 2 and class 3 units (see below) since it was found that the majority of units that maintained firing for more than three seconds of the hold phase would still be firing after twenty seconds of maintained stretch.

The class 3 afferents (Fig. 71) display an apparently normal response pattern to the ramp-and-hold stretch, maintaining firing throughout the hold phase. The distinguishing feature is the lack of any background discharge at the resting length of the muscle. A few such units were also observed in the unoperated control animals at the resting length of the muscle but they represent a much smaller proportion of the population than in even the longest term operated animals (Table 9).

Fig. 72 shows the responses of the class 4 units which are sufficiently similar to the control units (Fig. 73) to be described as afferents that have recovered to normal function. As such, they display a resting discharge at the resting length of the muscle and fire throughout the ramp-and-hold stretch. For further comparison, two examples of each class of unit are shown in Fig. 74.

Because it might be expected that some of these responses (especially the class 3) could be dependent on muscle length, a control experiment was performed on an unoperated animal in which the resting length of the muscle was varied in an attempt to recreate some of the abnormal patterns of response. Eight primaries and seven secondaries were tested with ramp-and-hold stretches starting from a range of resting lengths. The initial length was the standard resting length used in all the experiments (1.8mm short of the maximum physiological length) down to a length 4mm short of this, at which point the muscle was totally slack and part of the ramp stretch was taken up with straightening the muscle.

All the primaries showed responses similar to the class 4 units (i.e. they had a background discharge and fired throughout the ramp-and-hold stretch) at the normal resting length and six maintained these full responses irrespective of the muscle length (Fig. 75)

(following each change of resting length two minutes was allowed to elapse before testing to allow the spindle to reset to the new muscle length). Two primary endings did lose their tonic discharge and became similar to the class 3 units but this only occurred when the muscle was held at a length 2.5mm short of the resting length normally used. Since this was 4.3mm short of the maximum physiological length, then the muscle would not have been under any appreciable tension (the average maximum range of movement in situ was 4.1mm).

The tonic discharge of the secondaries was more dependent on the muscle length. Six of the seven secondaries had a background discharge at the normal resting length but three of these became similar to class 3 units when the resting length was reduced by more than 1mm (Fig. 76). Whatever the resting length the endings always fired throughout the hold phase so the class 1 and 2 responses could not be recreated by varying the resting length in normal muscles. In the case of some units, it is apparent that the class 3/4 separation could be dependent on the length of the muscle. Even with these, however, the resting length had to be reduced by more than 1mm to create this effect (i.e. by some 25% of the maximum range of movement of the muscle). It therefore seems most improbable that any variations in the range of movement occurring as a result of the denervation, or any small errors in the technique employed to measure the muscle length, could account for the abnormal responses that were observed.

As was also found by Brown & Butler (1976), the proportion of abnormal afferents was greatest during the early recovery periods. In this study, by 96 days PC (74 days RT), 79.7% of afferents responded normally to the ramp stretch, being classified as class 3 or 4 (60.9% and 18.8%, respectively) (Table 9). By 140 days PC

Figure 69. Four examples of the instantaneous frequency records generated by class 1 afferents in response to a ramp-and-hold stretch of 1.8mm amplitude at 5mm/sec with a hold of 1sec (all the stretches are similar for subsequent figures unless stated otherwise). The CV's of the afferents were: A, 44.5m/sec. B, 60.3m/sec. C, 55.6m/sec. D, 45.6m/sec.

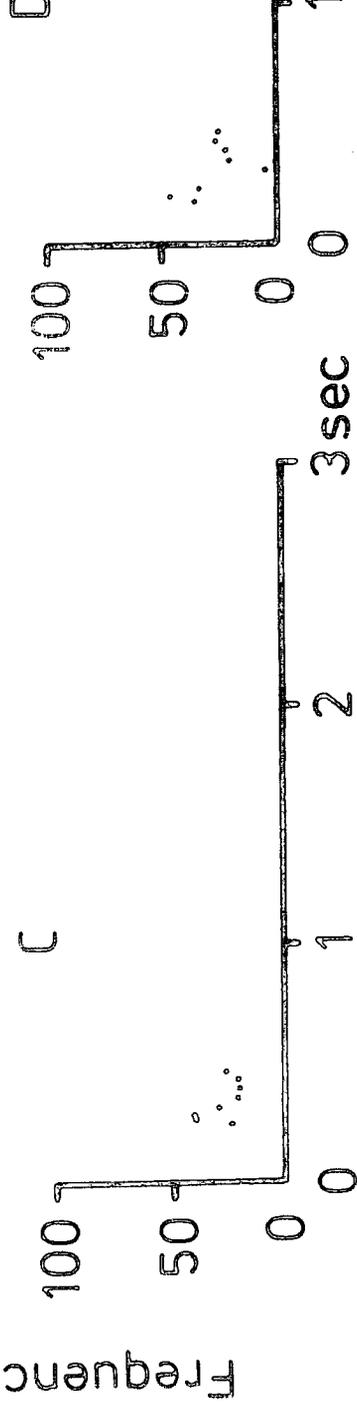
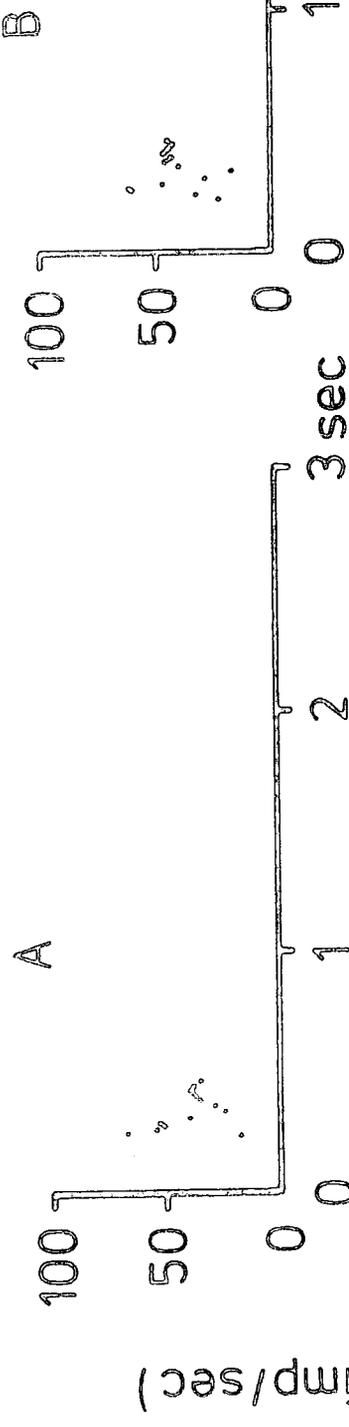
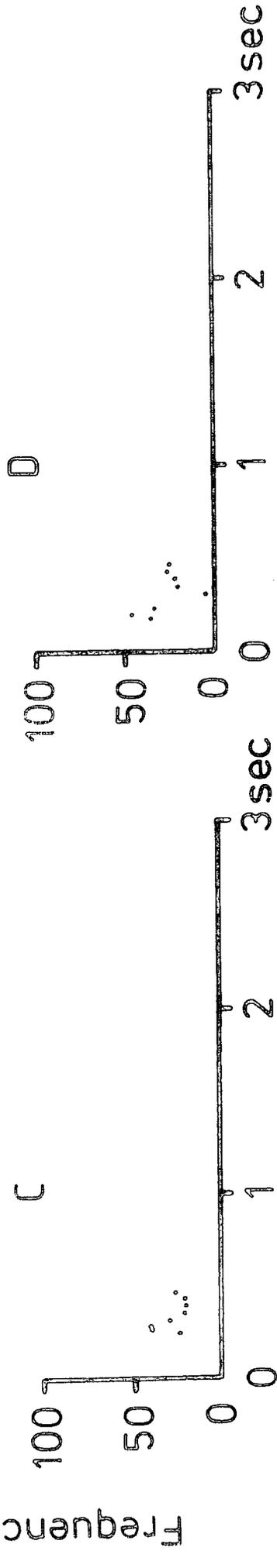
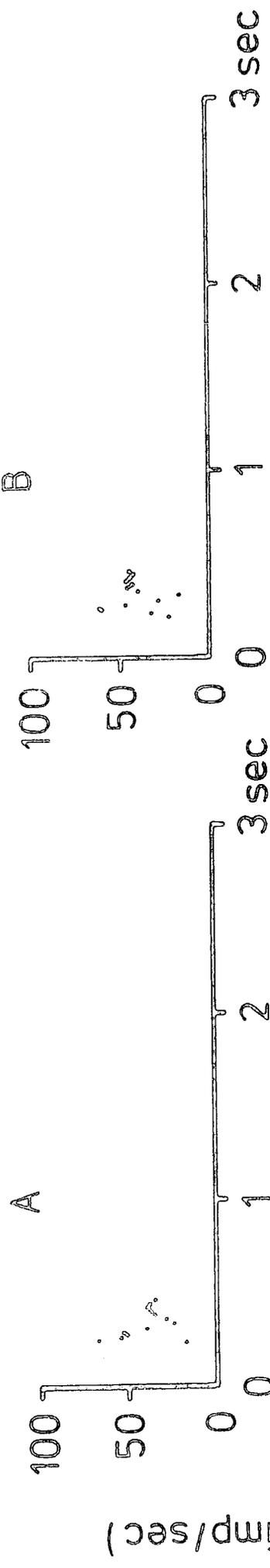


Figure 70. Records of four class 2 afferents. Ramps as for Fig.

69. Afferent CV's:- A, 55m/sec. B, 46.8m/sec. C, 44.5m/sec.

D, 52.3m/sec.

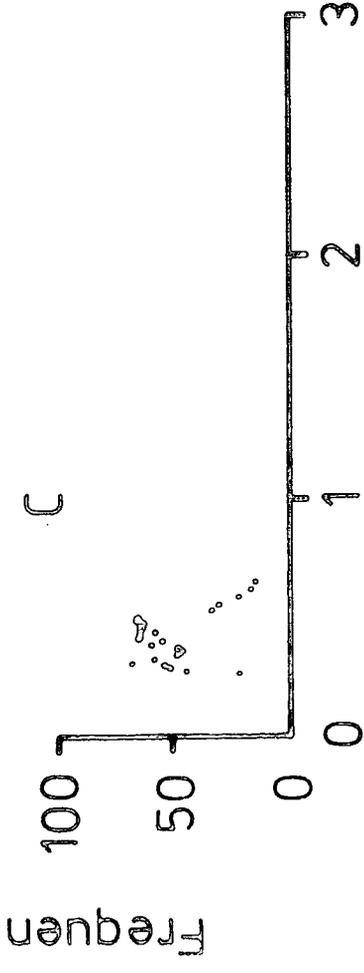
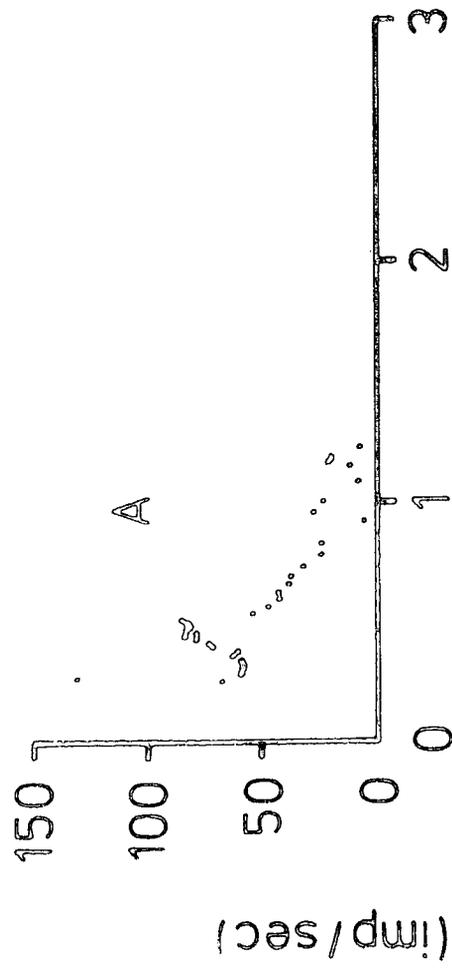
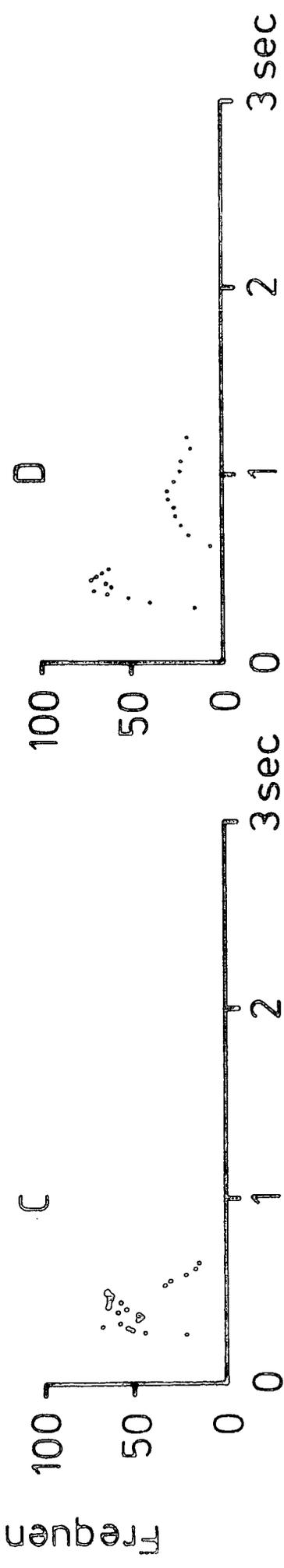
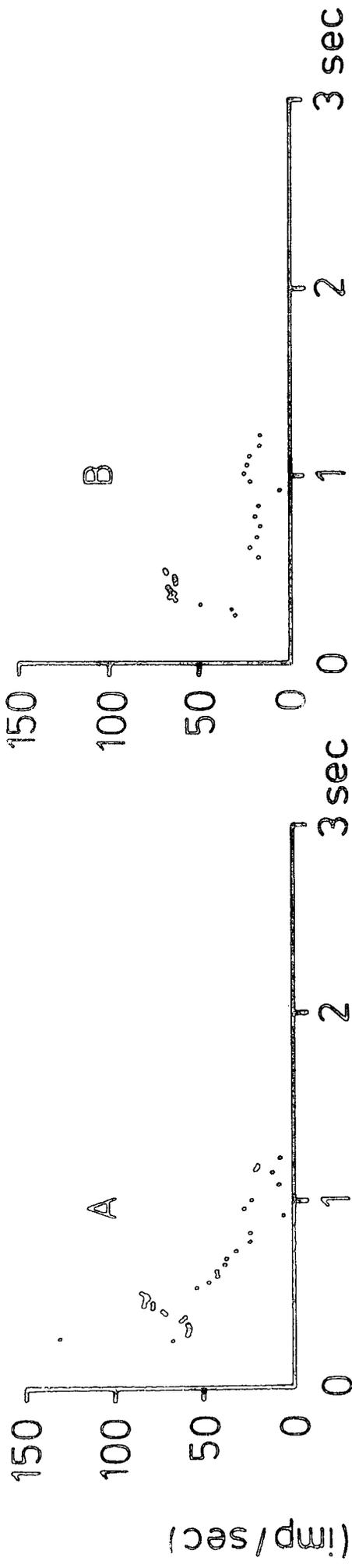


Figure 71. Records of four class 3 afferents. A and B are primaries, C and D are secondaries. Afferent CV's:- A, 51.9m/sec. B, 44.5m/sec. C, 23.4m/sec. D, 25.5m/sec.

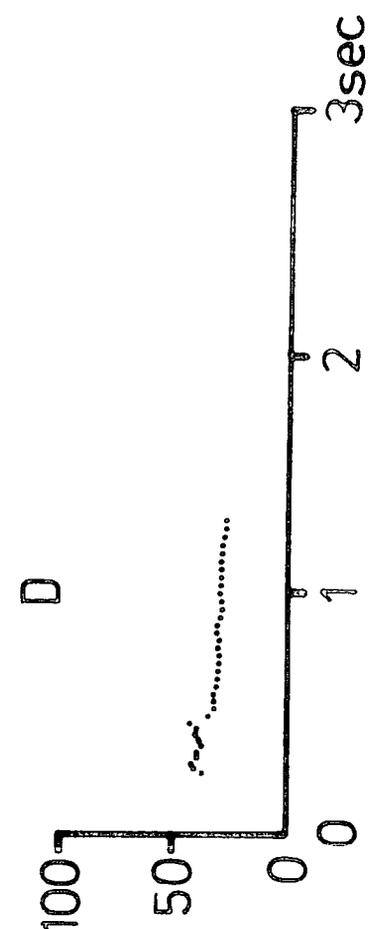
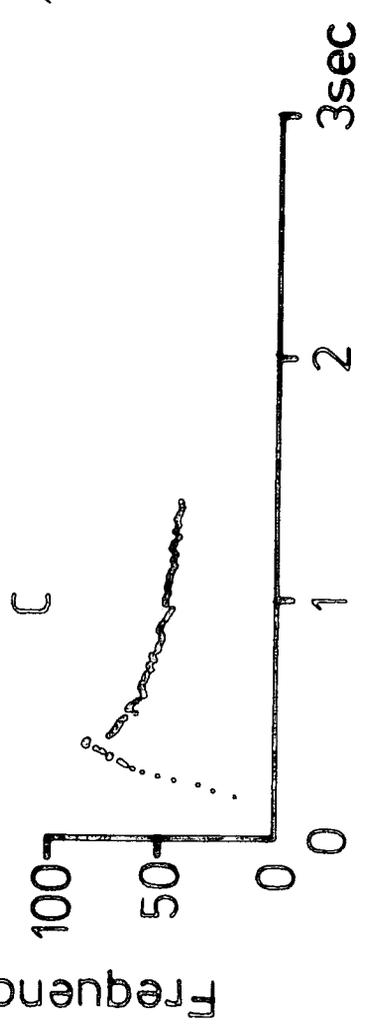
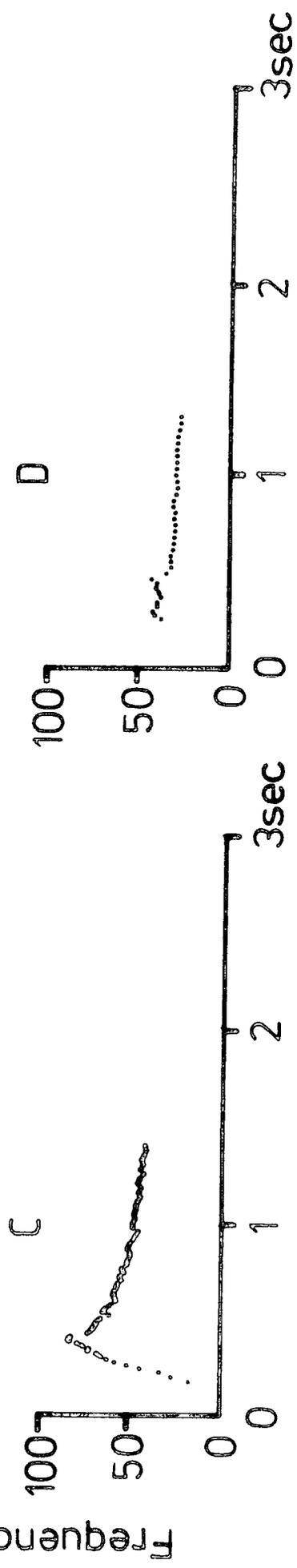
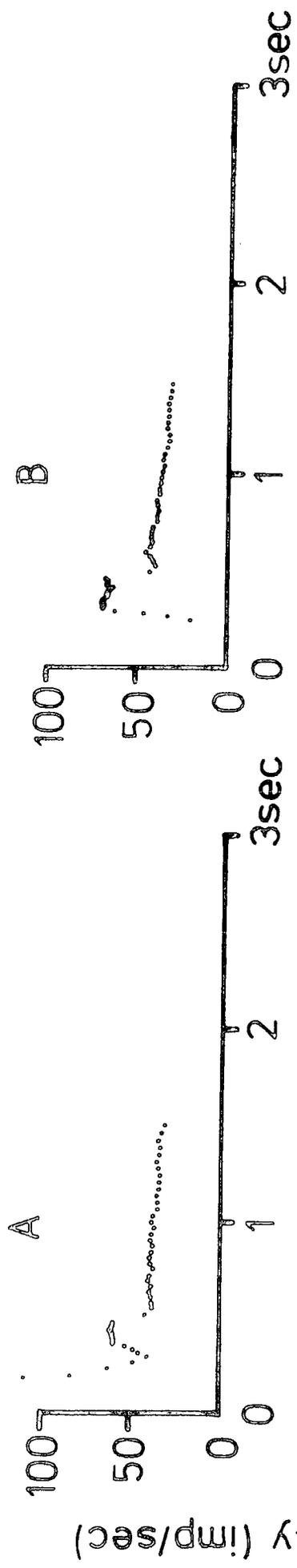


Figure 72. Records of four class 4 afferents. A and B are secondaries, C and D are primaries. Afferent CV's:- A, 25.5m/sec. B, 17.8m/sec. C, 34.6m/sec. D, 51.0m/sec.

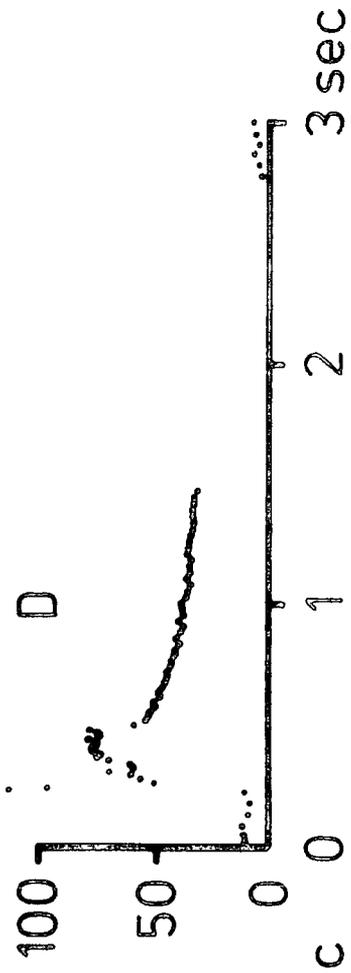
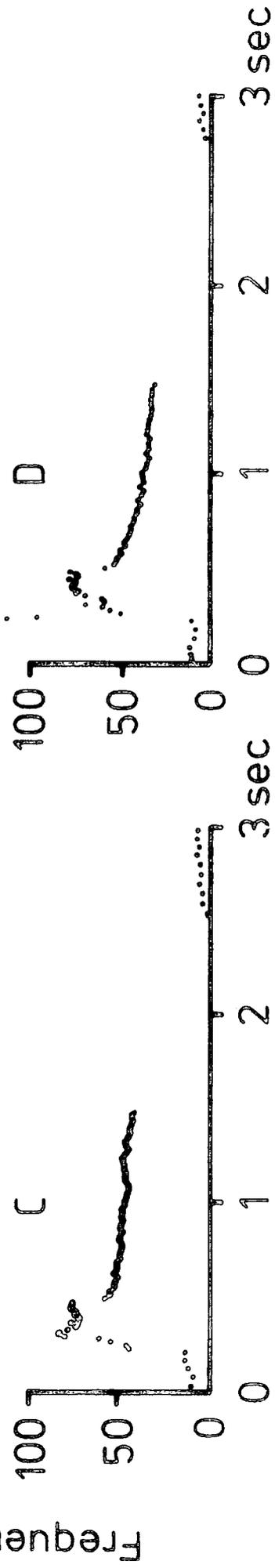
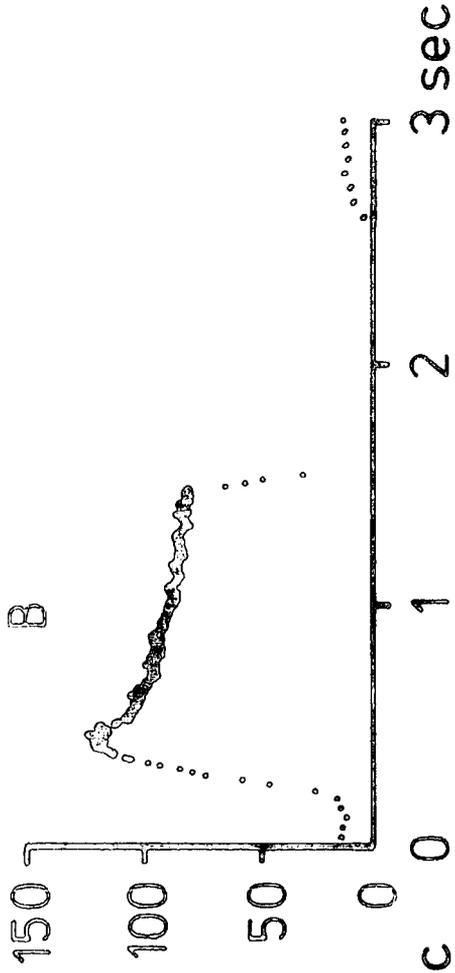
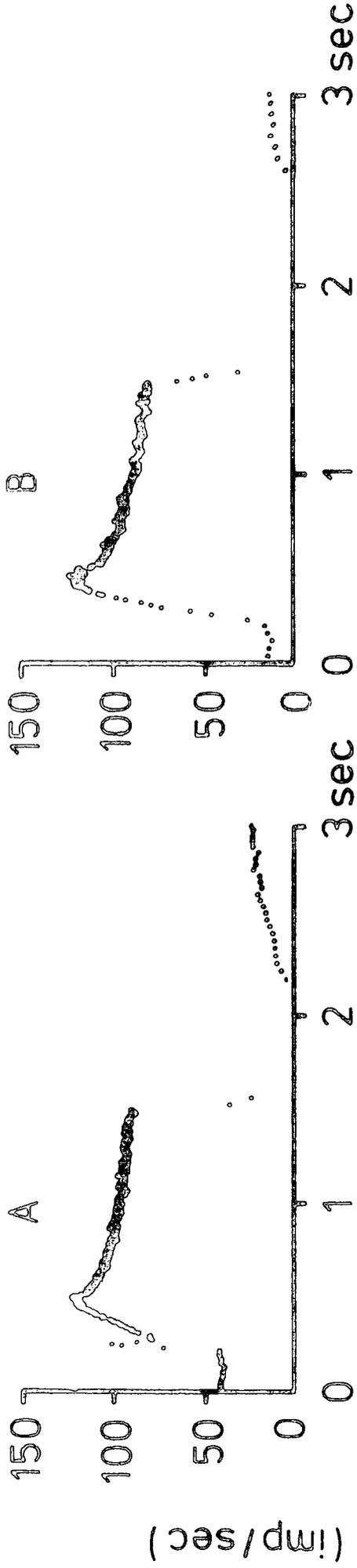


Figure 73. Records of the responses of four control afferents from unoperated animals to ramp-and-hold stretch. A and C are primaries, B and D are secondaries. Afferent CV's:- A, 73m/sec. B, 47.5m/sec. C, 76m/sec. D, 25m/sec.

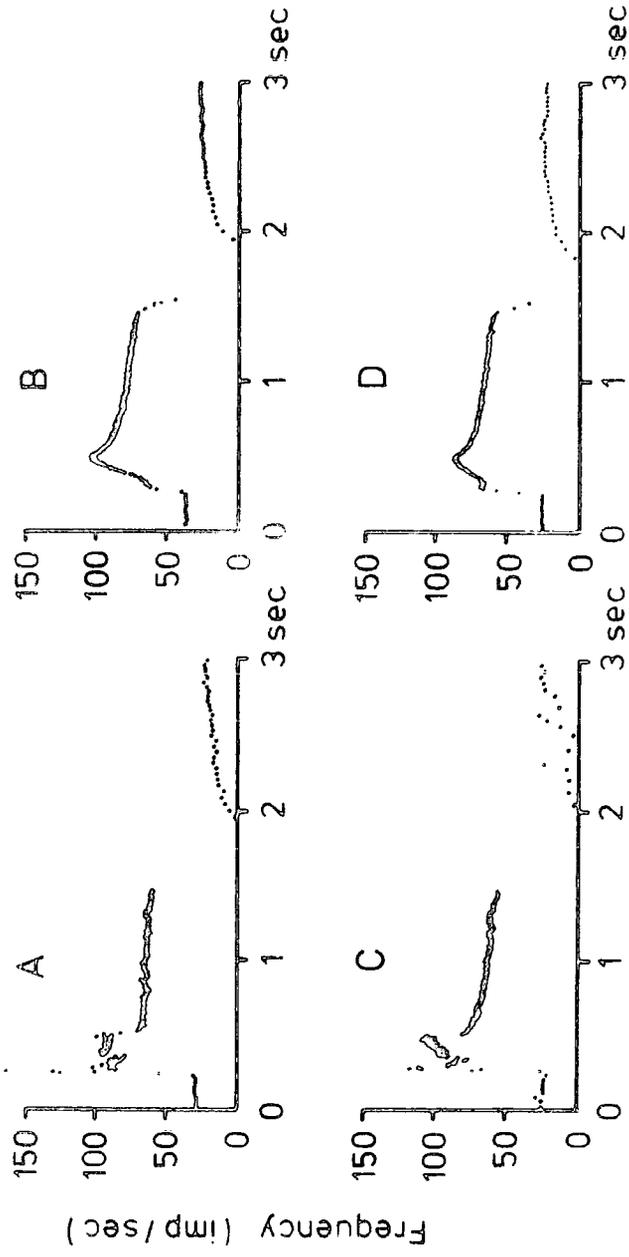


Table 9.

The proportions (as a percentage of the total population) of the four classes of regenerated spindle afferent after different periods of regeneration. The recovery times are given in terms of days PC and days RT. In this and subsequent tables the data for primary and secondary units have been combined.

Time (days PC)	Time (days RT)	Afferent classes				Total no. in sample
		1	2	3	4	
26	4	7.0	62.0	27.5	3.5	29
33	11	13.0	50.0	16.5	20.5	54
40	18	2.8	48.5	32.5	16.2	37
47	25	18.5	29.2	42.5	9.8	113
61	39	10.6	15.0	59.5	14.9	49
75	53	13.6	4.5	63.6	18.3	44
97	84	1.5	18.8	60.9	18.8	69
140	118	0.0	2.6	18.4	79.0	38
Controls		0.0	0.0	10.2	89.8	89

Figure 74. Two examples of each class of afferent response to passive stretch. A, class 1 afferents. B, class 2 afferents. C, class 3 afferents. D, class 4 afferents.

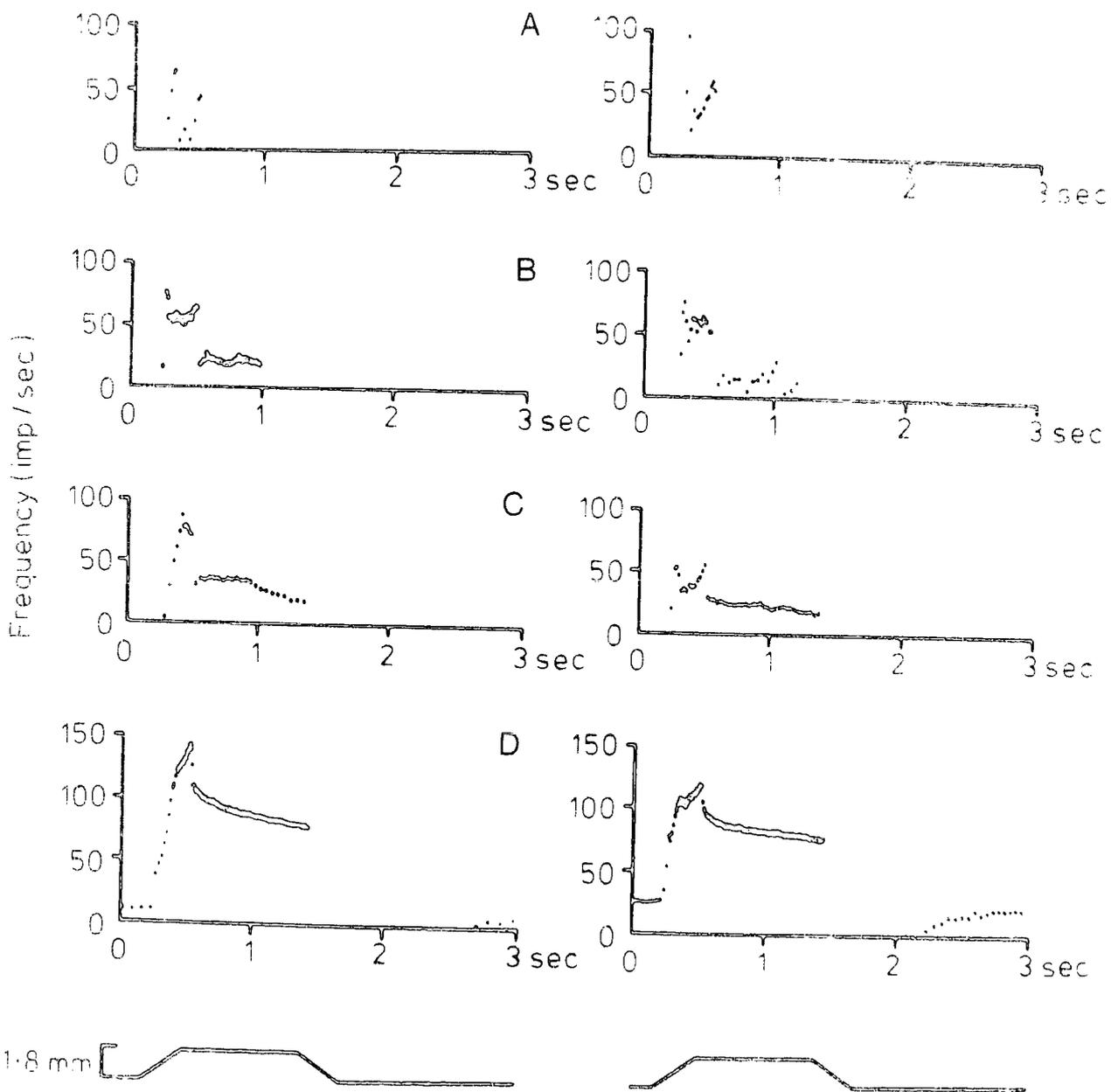


Figure 75. Effects of varying the resting length on the responses of a class 4 control primary afferent to ramp-and-hold stretch. The ramp was 1.8mm in amplitude with a velocity of 5mm/sec and a hold of 1sec. The initial resting length (r.l.) was taken to be the length of the muscle 1.8mm short of the maximum physiological length; for subsequent stretches the resting length was reduced by 0.5 or 1mm as indicated on the figure. A period of 2 minutes was allowed to elapse to allow the spindle to reset in between each change of resting length.

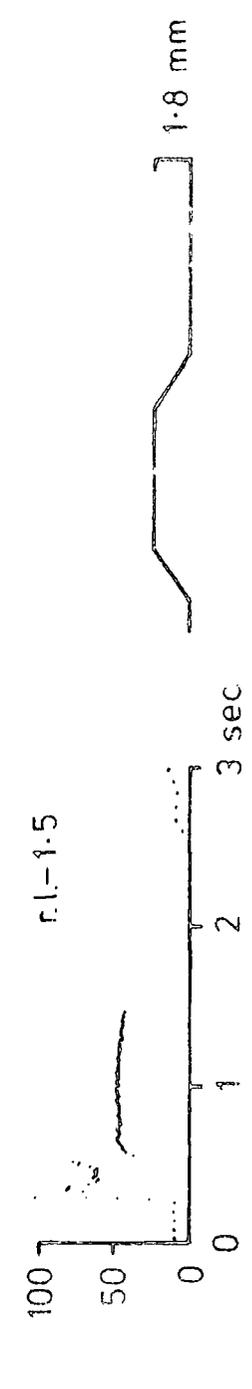
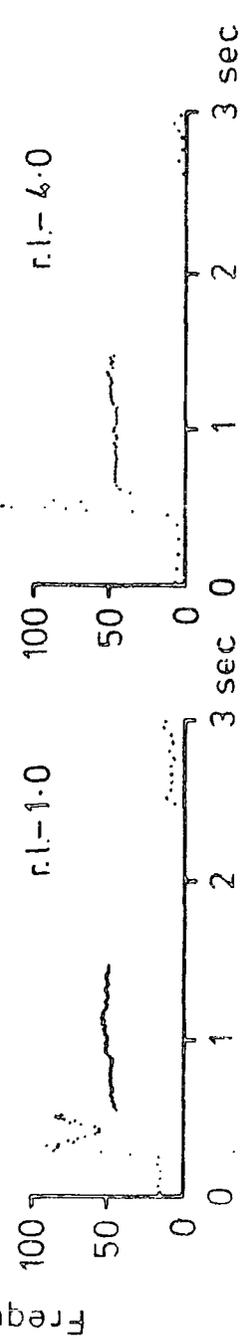
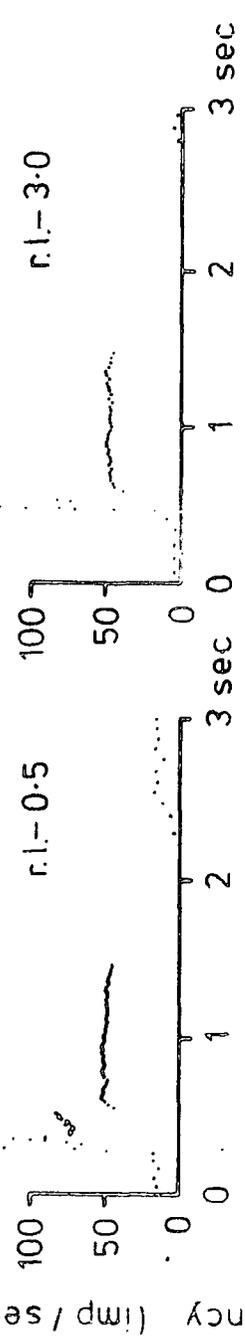
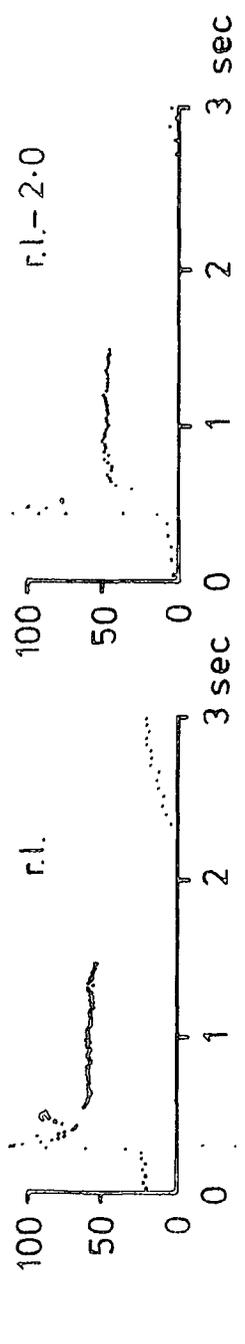
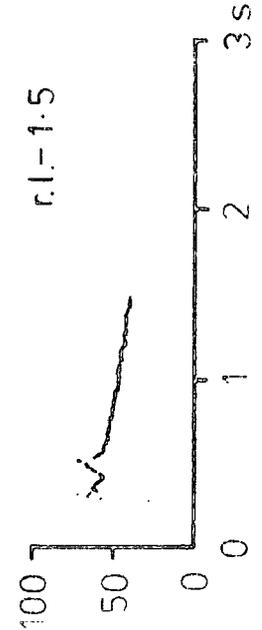
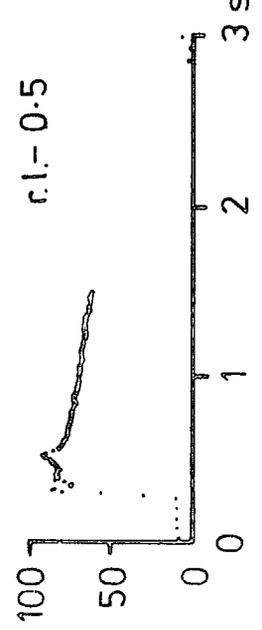
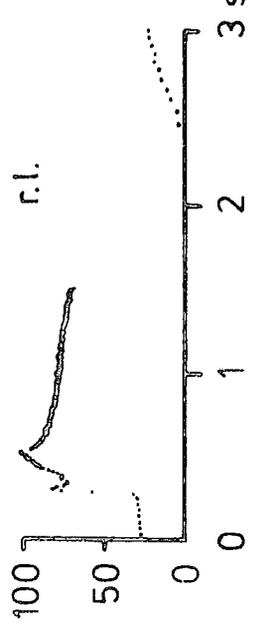
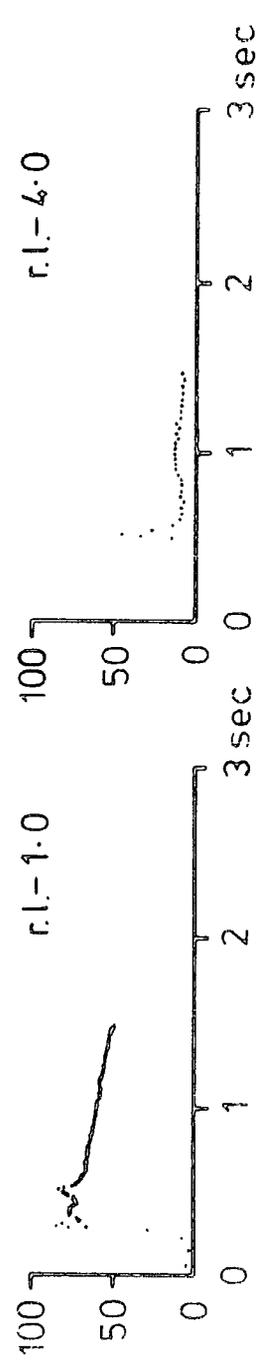
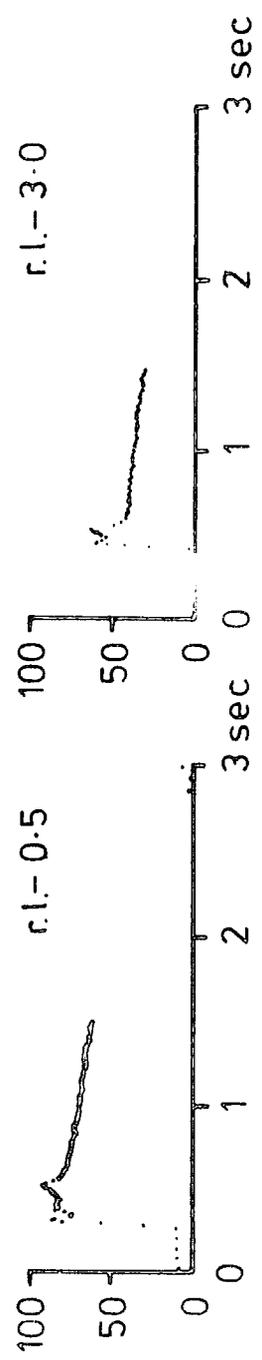
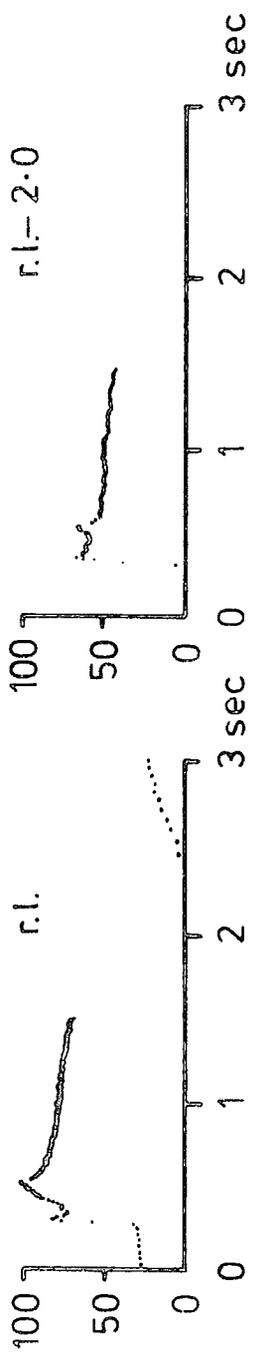


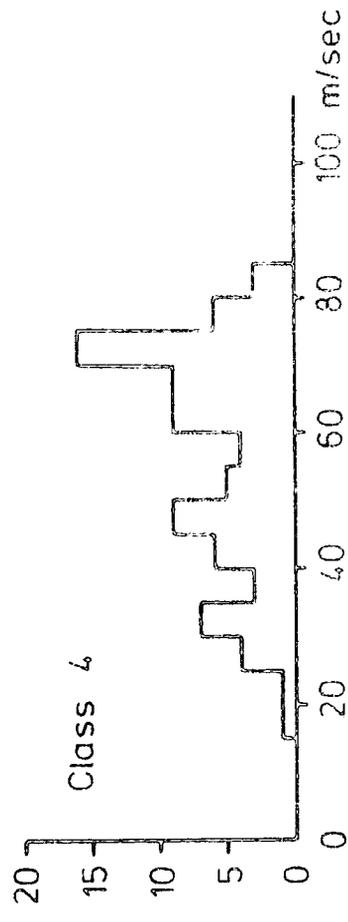
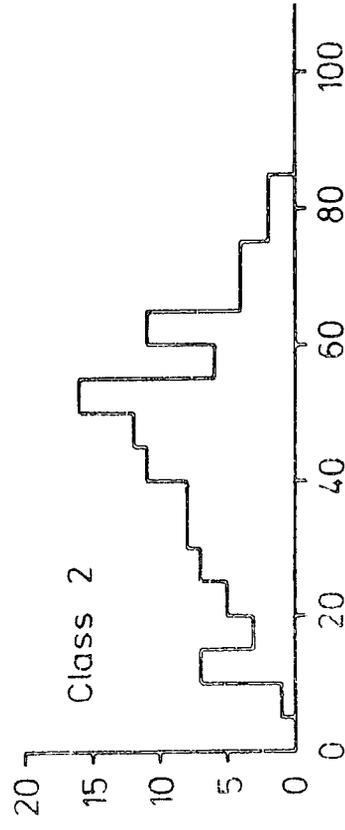
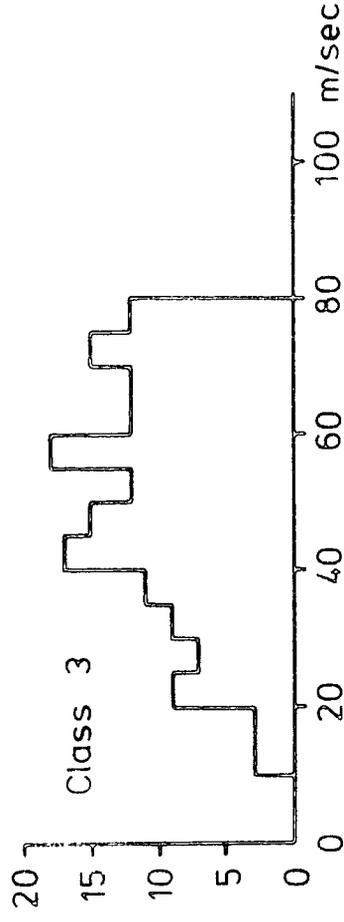
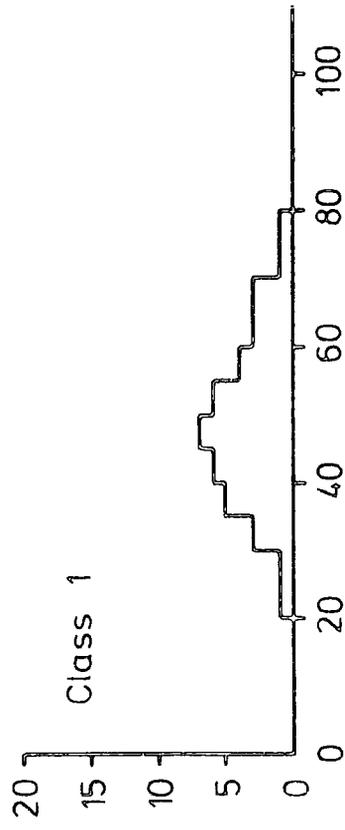
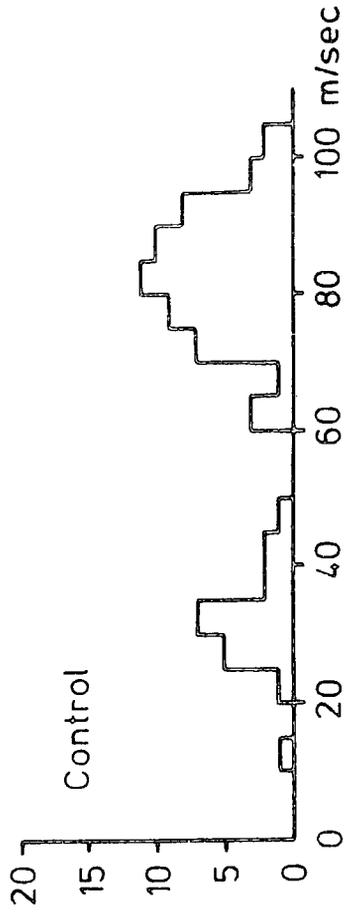
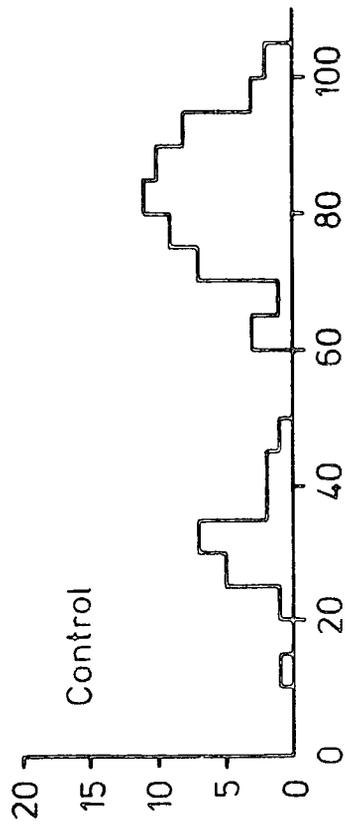
Figure 76. The effect of varying the resting length on the responses of a class 4 type control secondary to passive ramp-and-hold stretch. Procedure as for Fig. 75.



Frequency (imp/sec)

Figure 77. Histograms showing the CV distributions of the four classes of afferent over all the recovery periods compared with the control populations from unoperated animals.

Numbers of Afferents



(118 days RT) the proportion of class 4 afferents had risen to 79% compared with 39.8% in the controls.

At 47 days PC (25 days RT) there was a marked increase in the proportion of class 1 afferents compared with 40 days PC (18 days RT) (Table 9). This higher level persisted for the 61 (39 days RT) and 75 (53 days RT) day recovery periods before dropping to zero. This could be due to the delayed return of that proportion of afferents whose endoneurial tubes were cut during the crush operation and which constitute a second wave of reinnervation of the muscle spindles.

Fig. 77 shows the CV distributions of the different afferent classes for all the regeneration periods. There is no significant difference between the CV distributions of any of the classes which indicates that the axons of the more abnormal afferents were at an equivalent stage of regeneration as those of the more normal units.

7.4 Fusimotor Stimulation.

Functional γ fusimotor axons could be found at every stage of recovery and all the classes of afferent, even the most abnormal could be activated by γ_s and γ_d stimulation.

Figs. 78, 79, 80 and 86 show examples of each class of afferent responding to passive stretch and to γ_s stimulation at 100Hz applied during the ramp stretch. In unoperated animals γ_s stimulation increases the background and hold-phase firing of both primary and secondary afferents with a reduction in the dynamic index (Fig. 81; section 2.6). The same holds for the class 3 and 4 regenerated afferents (Figs. 80, 86). Gamma static activation of a class 1 afferent restores the static sensitivity (Fig. 78) so that it fires throughout the hold phase and often displays a background discharge at

the resting length of the muscle. It also causes the class 2 afferents to maintain firing during the hold phase (Fig. 79).

Stimulation of γ_s efferents had the same effects on the dynamic indices of both the control and regenerated primary afferents irrespective of the degree of abnormality shown. The unoperated controls had a mean dynamic index (DI) of $49.3 \text{ imp/sec} \pm 4.5$ (S.E. of mean) for a 5 mm/sec stretch which was reduced to $29.3 \text{ imp/sec} \pm 4.29$ when γ_s efferents were stimulated at 100 Hz during the ramp-and-hold stretch. The class 2 regenerated afferents had a mean DI of $43.7 \text{ imp/sec} \pm 3.95$ for the passive stretch, which was reduced to $32.05 \text{ imp/sec} \pm 4.81$ during γ_s stimulation. Similarly the class 3 afferents had mean DI's of $41.75 \text{ imp/sec} \pm 4.49$ for the passive stretch and $28.5 \text{ imp/sec} \pm 3.21$ during γ_s stimulation. Similar comparisons could not be made for the class 1 afferents as they did not fire during the hold phase of the passive ramp stretch and therefore it was not possible to measure the DI's.

Stimulation of γ_d axons causes the bag_1 fibre to contract (section 2.5). This contraction evokes a large increase in the dynamic response of the primary ending and smaller increases in the resting and hold-phase firing (Fig. 85). For all the classes of regenerated afferent γ_d stimulation greatly elevated the dynamic response to the ramp stretch (Figs. 82, 83, 84, 86).

During γ_d stimulation the class 1 and 2 primary afferents were caused to fire during the hold phase as well as during the stretch itself. Some also displayed a resting discharge (Figs. 82, 83). The hold phase firing rate decayed with a time course of $0.5\text{--}2 \text{ sec}$ which is comparable to the time course of $0.5\text{--}2.5 \text{ sec}$ reported by Boyd et al (Boyd, 1976; 1981a; Boyd, Gladden & Ward, 1977; 1981) for the mechanical creep of the bag_1 fibre during γ_d stimulation.

In control spindles γ_d stimulation greatly increases the dynamic response as quantified by the dynamic index. The mean DI of the control primaries to passive stretch was $49.3\text{imp/sec} \pm 4.5$, which was increased to $109.6\text{imp/sec} \pm 15.39$ during γ_d stimulation at 100Hz. Similar increases were noted for the regenerated afferents. The class 2 units had a mean DI of $43.7\text{imp/sec} \pm 3.95$ to passive stretch which was elevated to $91.3\text{imp/sec} \pm 8.06$ by γ_d stimulation and the mean passive DI of the class 3 afferents which was $41.7\text{imp/sec} \pm 4.49$ was raised to $96.8\text{imp/sec} \pm 12.38$.

The effects of both γ_s and γ_d stimulation therefore were very consistent in both normal and reinnervated spindles, the alterations in DI being very similar irrespective of the type of afferent being examined. For further comparison of these effects Fig. 86 shows the responses of a class 1 primary and a class 4 primary to passive ramp stretch and to stimulation of γ_s and γ_d efferents during the ramp stretch.

During the early recovery periods (up to 61 days PC) some gamma axons were isolated whose activation of a sensory ending failed rapidly during stimulation. This failure resulted in a bursting discharge owing to the irregular contractions of the intrafusal fibres (Fig. 87). This could be caused by conduction failure in the regenerated axon close to its termination where it is very fine and therefore has a low safety-factor for conduction, or to failure of neuromuscular transmission. After resting the axon for two minutes, impulse transmission was sustained for longer periods than if the gap between stimulation sequences was only ten seconds. Also, the onset of transmission failure could be accelerated by increasing the stimulus frequency (special care was taken with such axons to ensure that they were fully isolated from any α axons which might have produced a somewhat similar effect).

Figure 78. The responses of two class 1 afferents to γ_s stimulation during a ramp-and-hold stretch. A, responses to passive stretch, the same ramp stretch was used as for the previous figures. B, responses to γ_s stimulation at 100Hz. Stimulation was commenced 0.4sec before the start of the ramp and was maintained for the full duration of the record. Afferent CV's (from left to right):= 68.9m/sec and 55.8m/sec. the corresponding γ_s CV's were 22.9m/sec and 17.9m/sec, respectively.

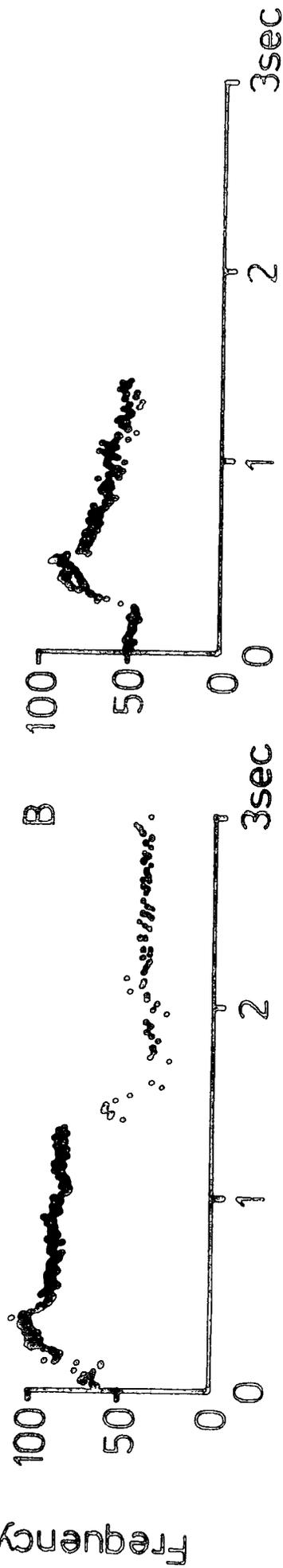
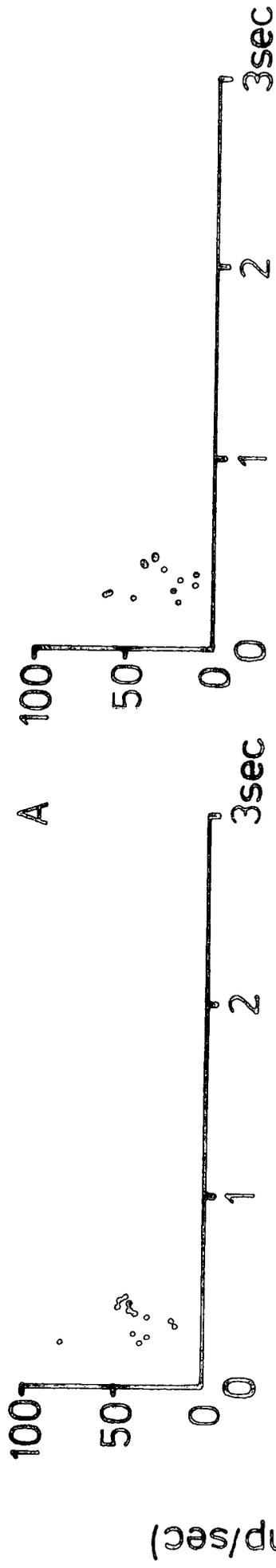
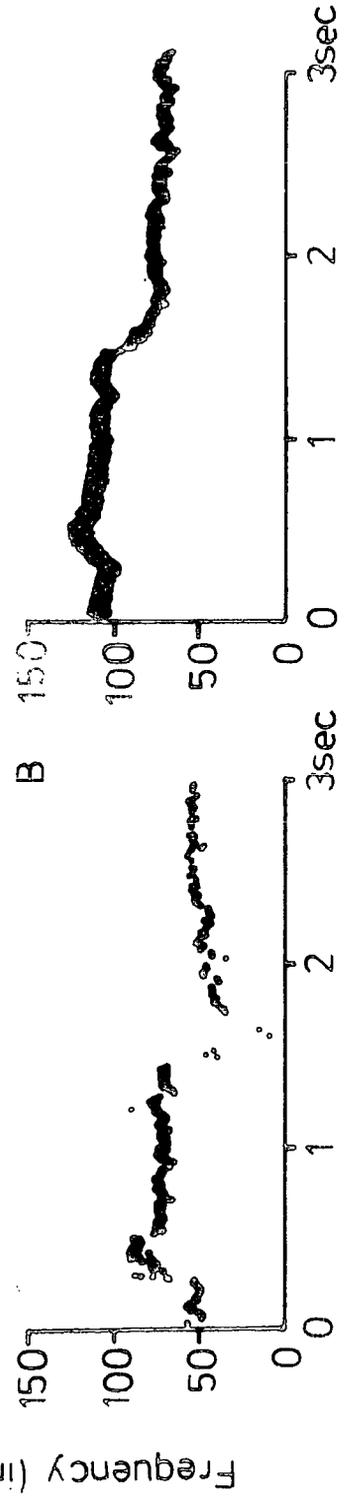
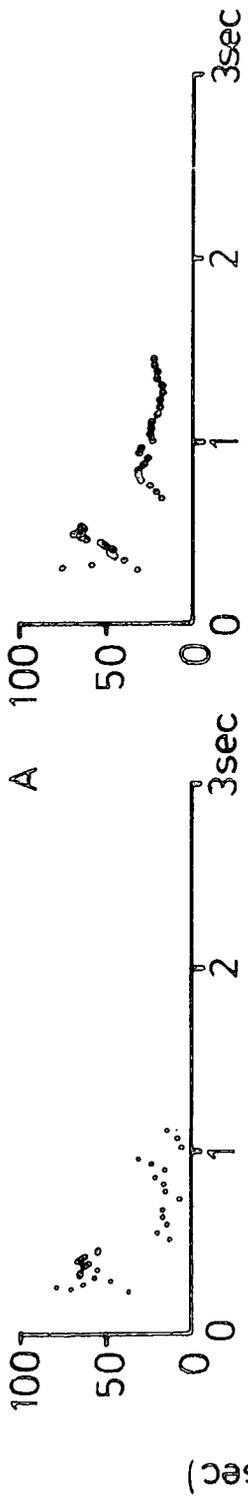


Figure 79. Responses of two class 2 primary afferents to γ_s stimulation. Recording procedure as for Fig. 78.

Afferent CV's:- 59.1m/sec and 54.0m/sec.

γ_s CV's:- 11.9m/sec and 24.3m/sec.



1.8mm [

Figure 80. Responses of two class 3 units, a secondary and a primary to γ_s stimulation. Procedures as for Fig. 78.

Afferent CV's := 38.2m/sec and 77.0m/sec.

γ_s CV's := 15.9m/sec and 20.1m/sec.

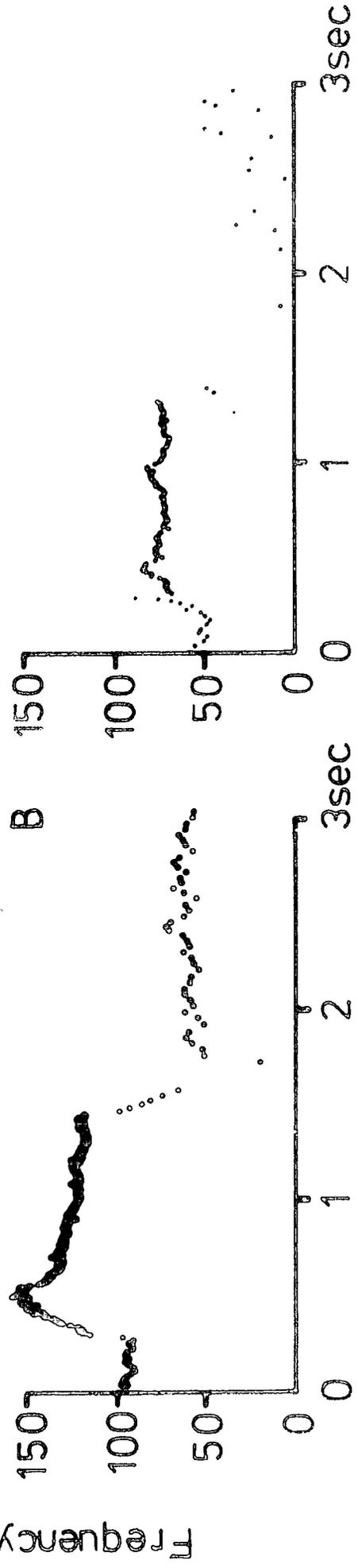
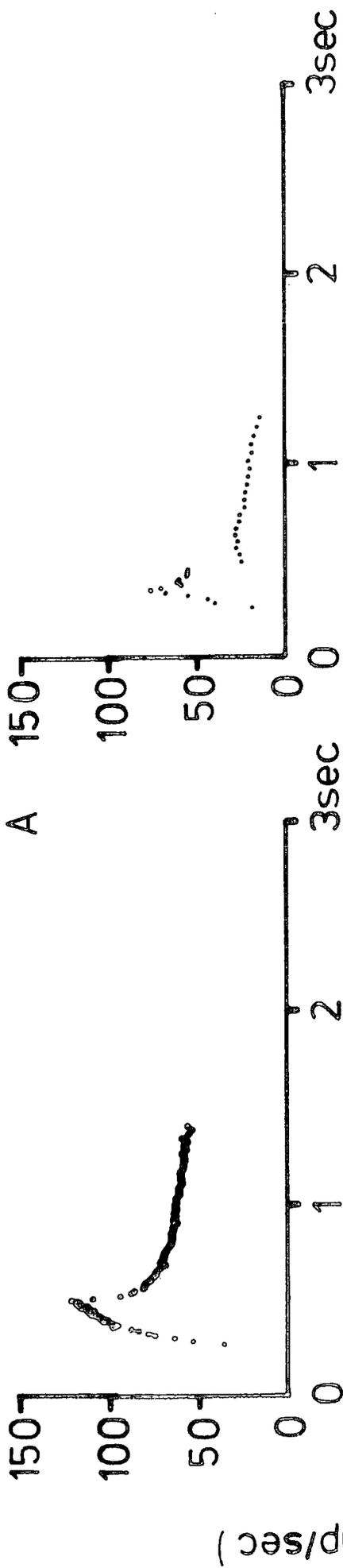


Figure 81. Responses of a primary and a secondary control afferents to γ_s stimulation at 100Hz, as for previous figures.

Afferent CV's:- 100m/sec and 35.7m/sec.

γ_s CV's:- 24.0m/sec and 35m/sec.

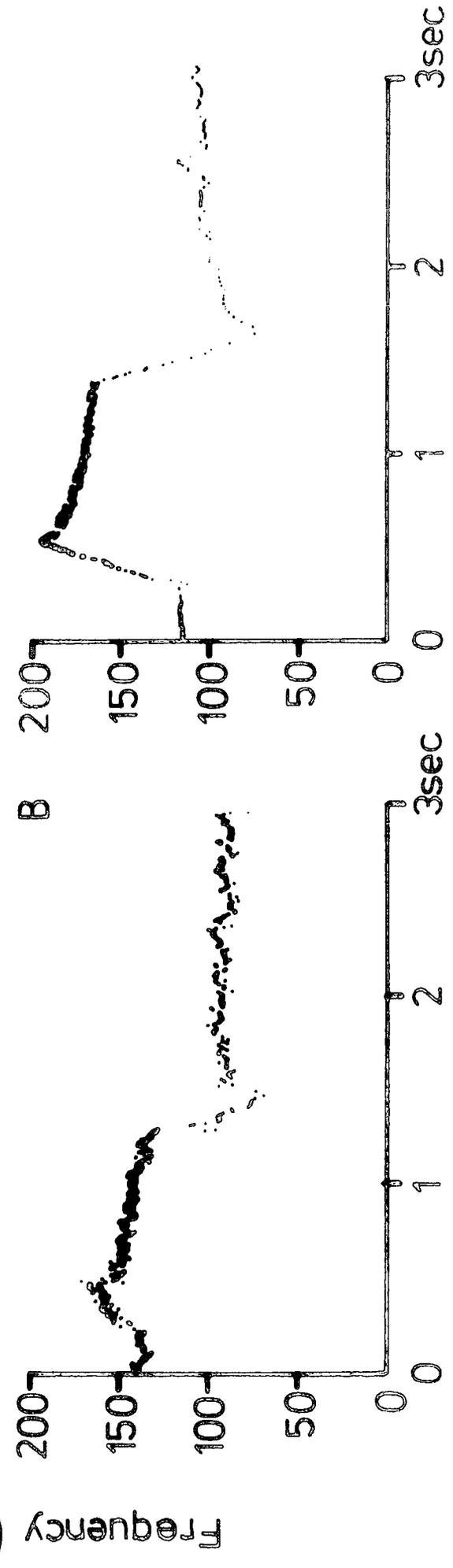
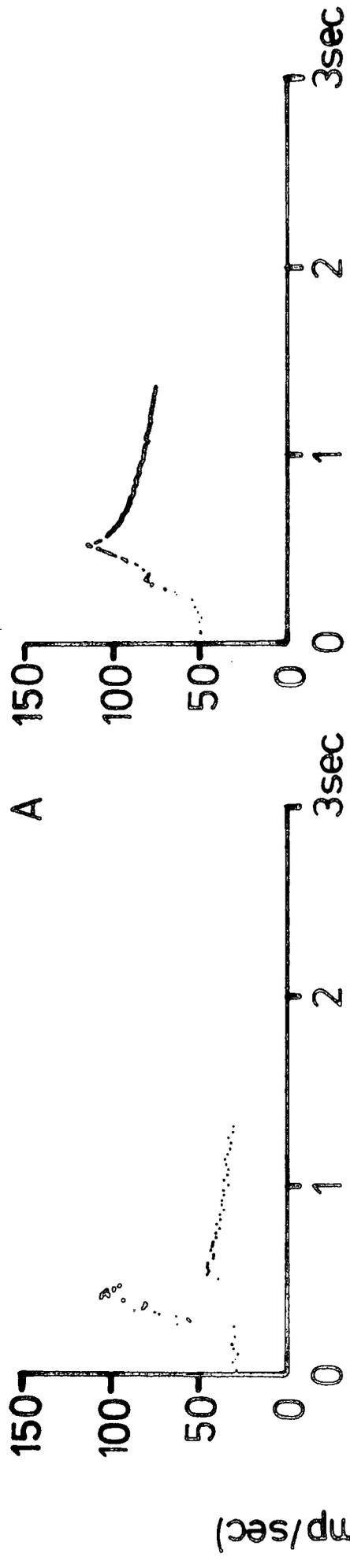


Figure 82. The effect of γ_d stimulation at 100Hz on the reponses to ramp-and-hold stretch of two class 1 primary afferents. A, passive ramp stretch. B, ramp stretch with γ_d stimulation at 100 Hz. Stimulation procedure as for Fig. 78.

Afferent CV's:- 71.5m/sec and 42.3m/sec.

γ_d CV's:- 27.5m/sec and 20.9m/sec.

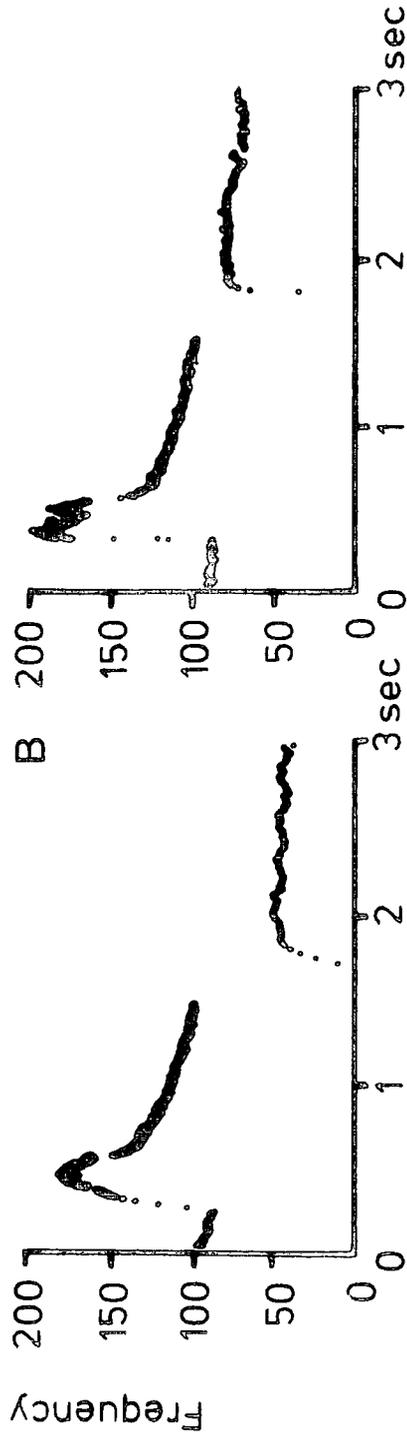
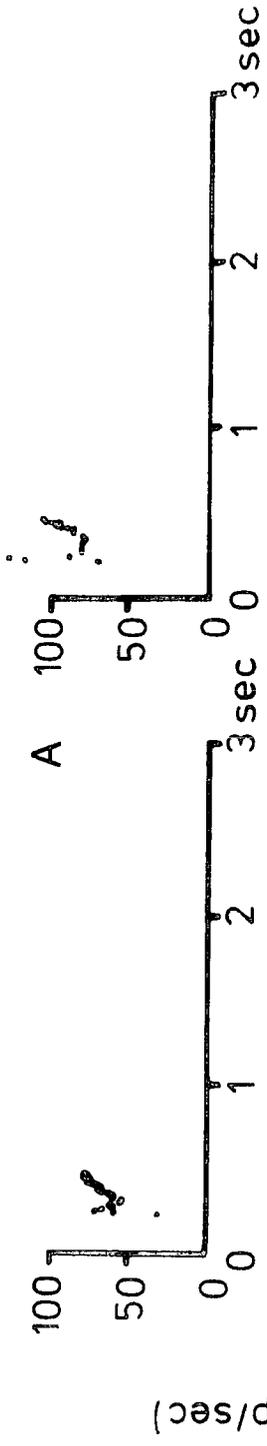


Figure 83. Responses of two class 2 primary afferents to γ_d stimulation at 100Hz.

Afferent CV's:- 62.1m/sec and 45.4m/sec.

γ_d CV's:- 21.3m/sec and 14.2m/sec.

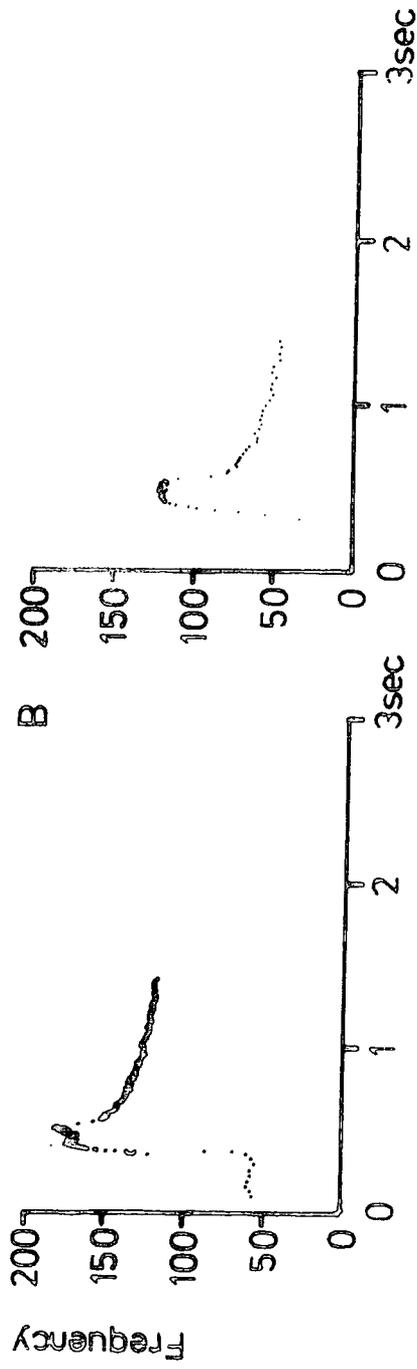
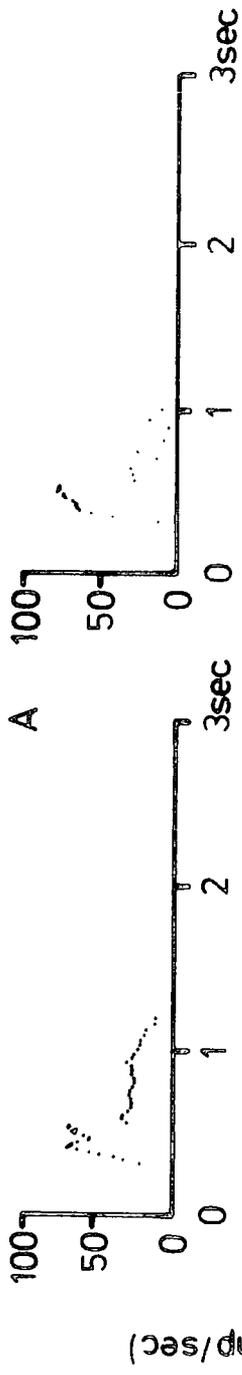


Figure 84. Responses of two class 3 primary afferents to γ_d stimulation.

Afferent CV's:- 62.0m/sec and 66.1m/sec.

γ_d CV's:- 25.9m/sec and 19.7m/sec.

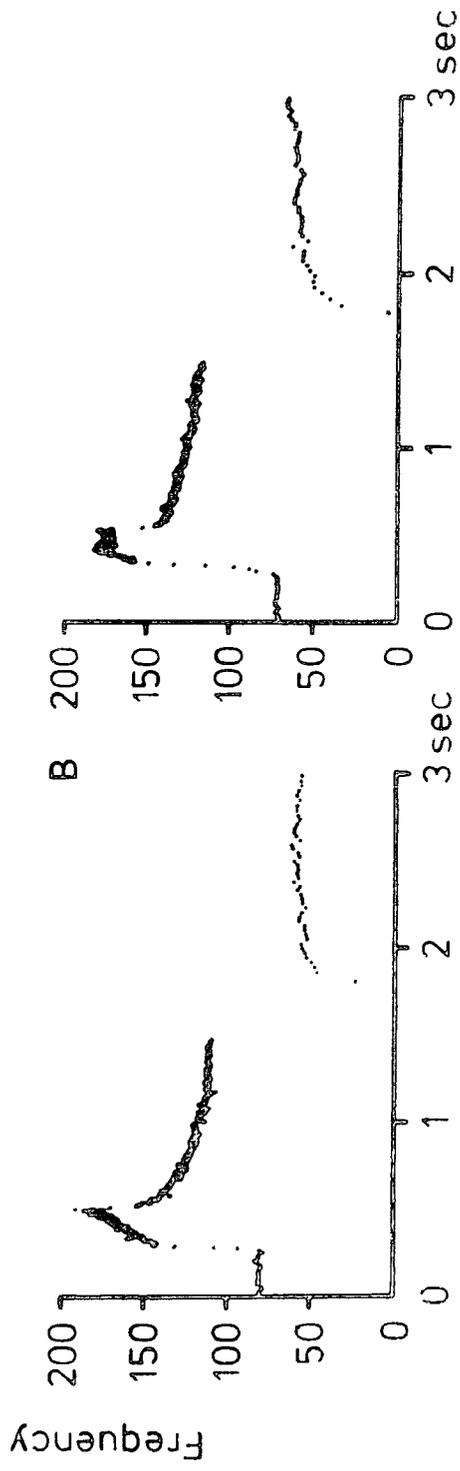
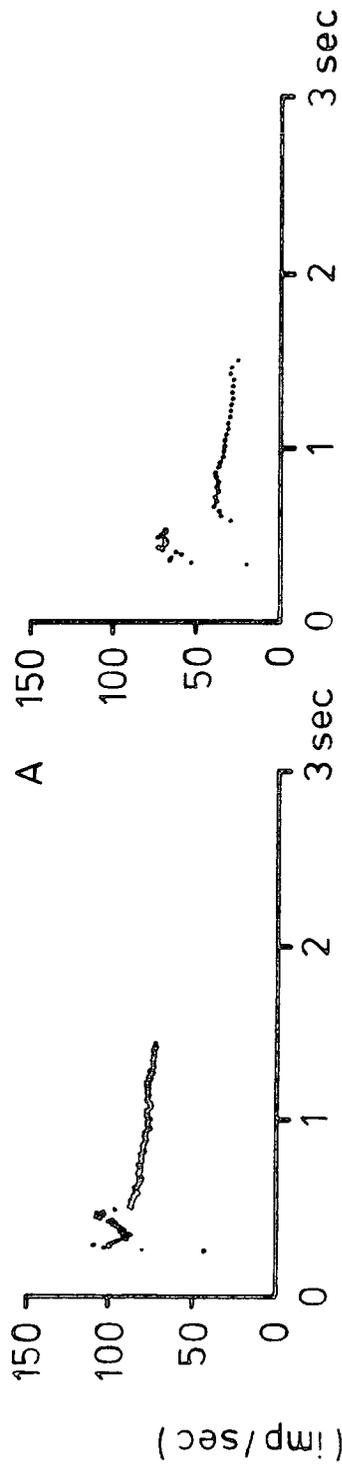


Figure 85. Responses of two control primary afferents from unoperated animals to γ_d stimulation at 100Hz.

Afferent CV's:- 95.2m/sec and 86.1m/sec.

γ_d CV's:- 35.7m/sec and 33.5m/sec.

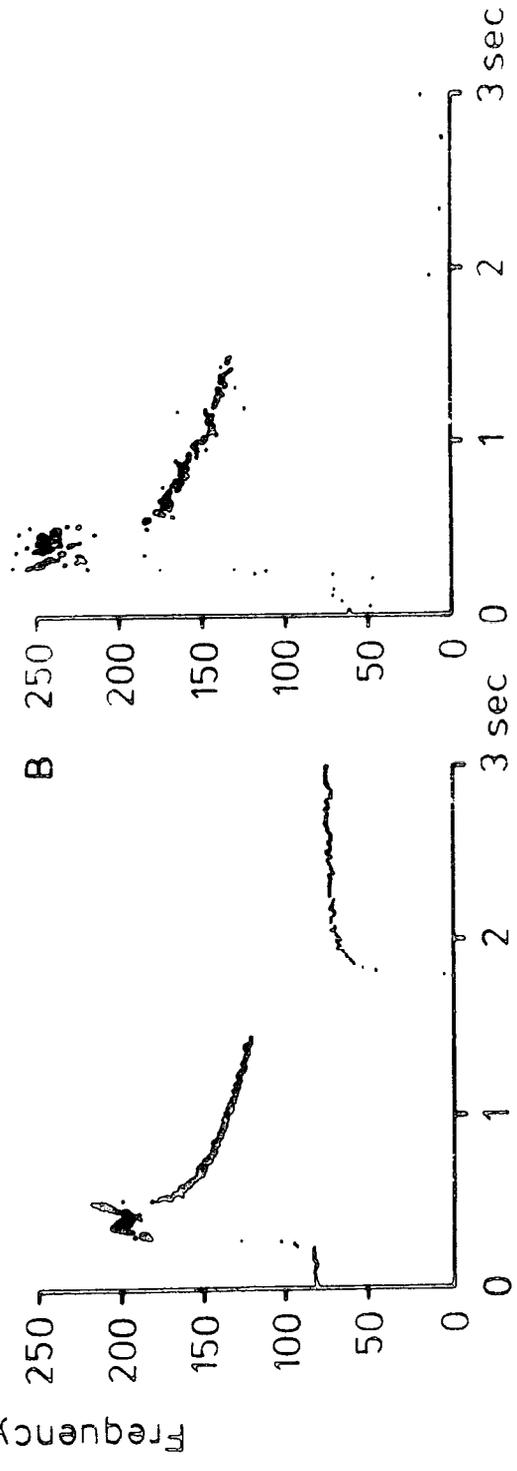
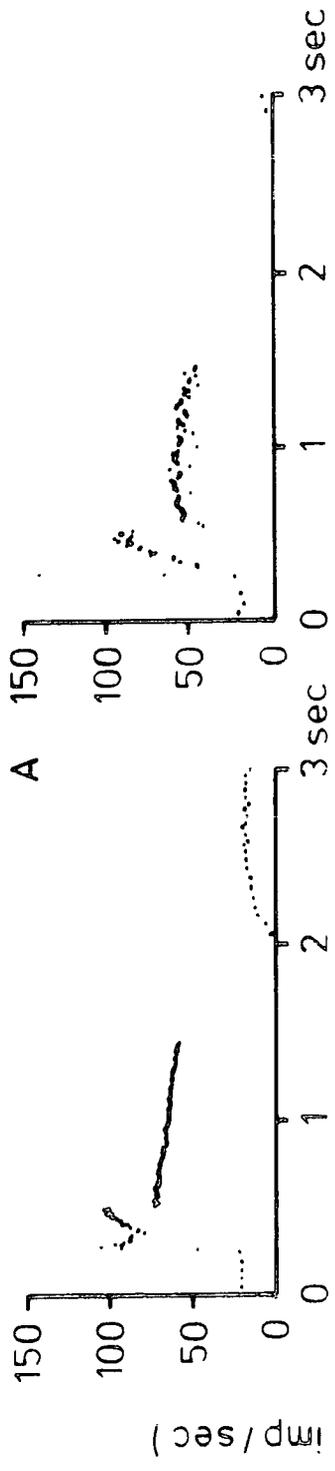


Figure 86. A comparison of the responses of a class 1 and a class 4 regenerated primary afferents to fusimotor stimulation.

A, passive ramp stretch. B, γ_s stimulation at 100Hz. C, γ_d stimulation at 100Hz. Stimulation procedures as for Fig. 78.

Afferent CV's:- 57.1m/sec and 82.2m/sec.

γ_s CV's:- 22.2m/sec and 31.2m/sec.

γ_d CV's:- 26.3m/sec and 33.5m/sec.

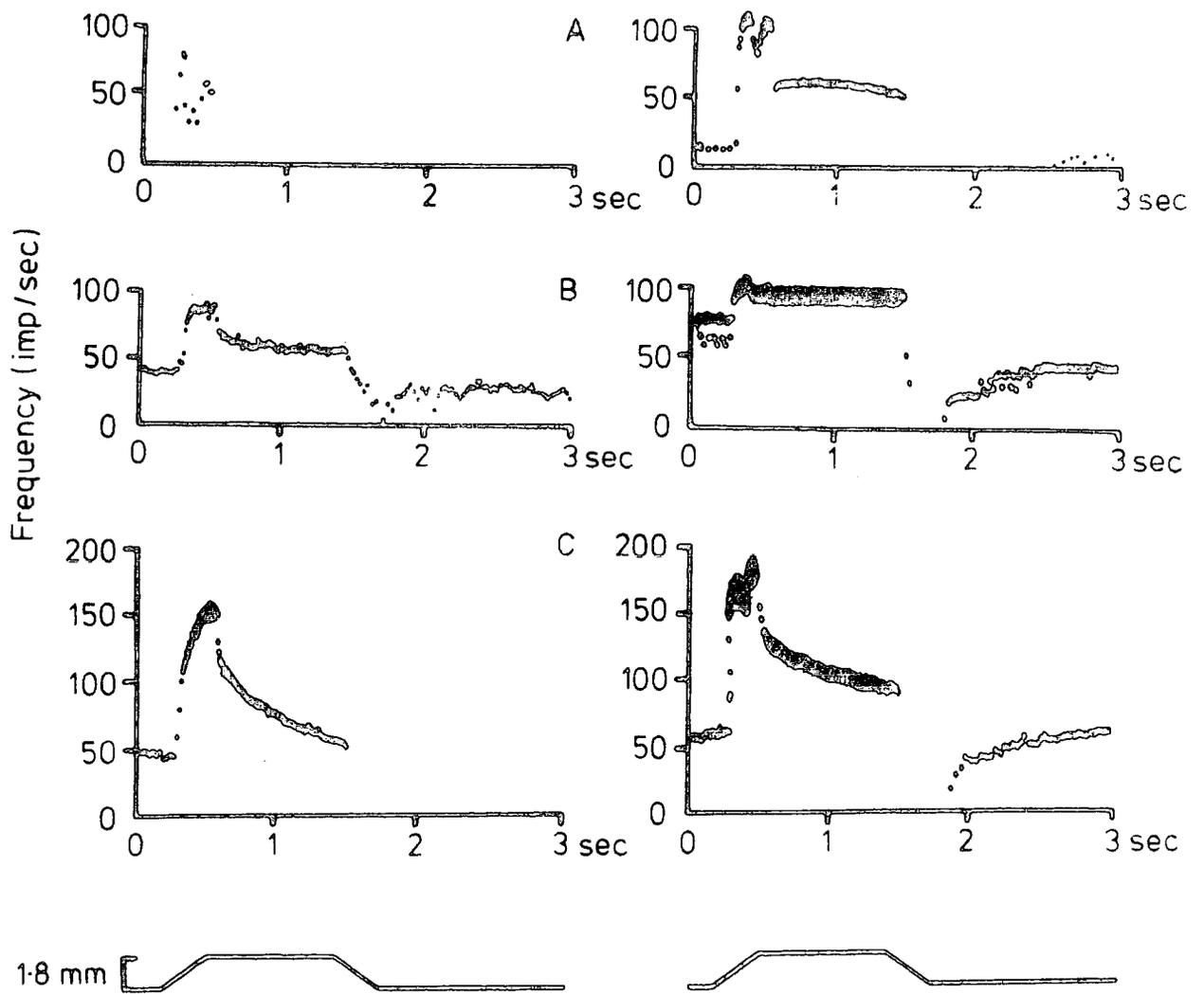


Figure 87. Failure of a γ_s axon to maintain secure contraction of the intrafusal fibres. A, response to passive stretch of a class 3 primary afferent. B, ramp stretch with γ_s stimulation at 100Hz after resting the γ axon for 2 minutes. C, recorded after B with only a 10sec break between stimulation sequences. Afferent CV:- 59.1m/sec.

γ_s CV:- 18.9m/sec.

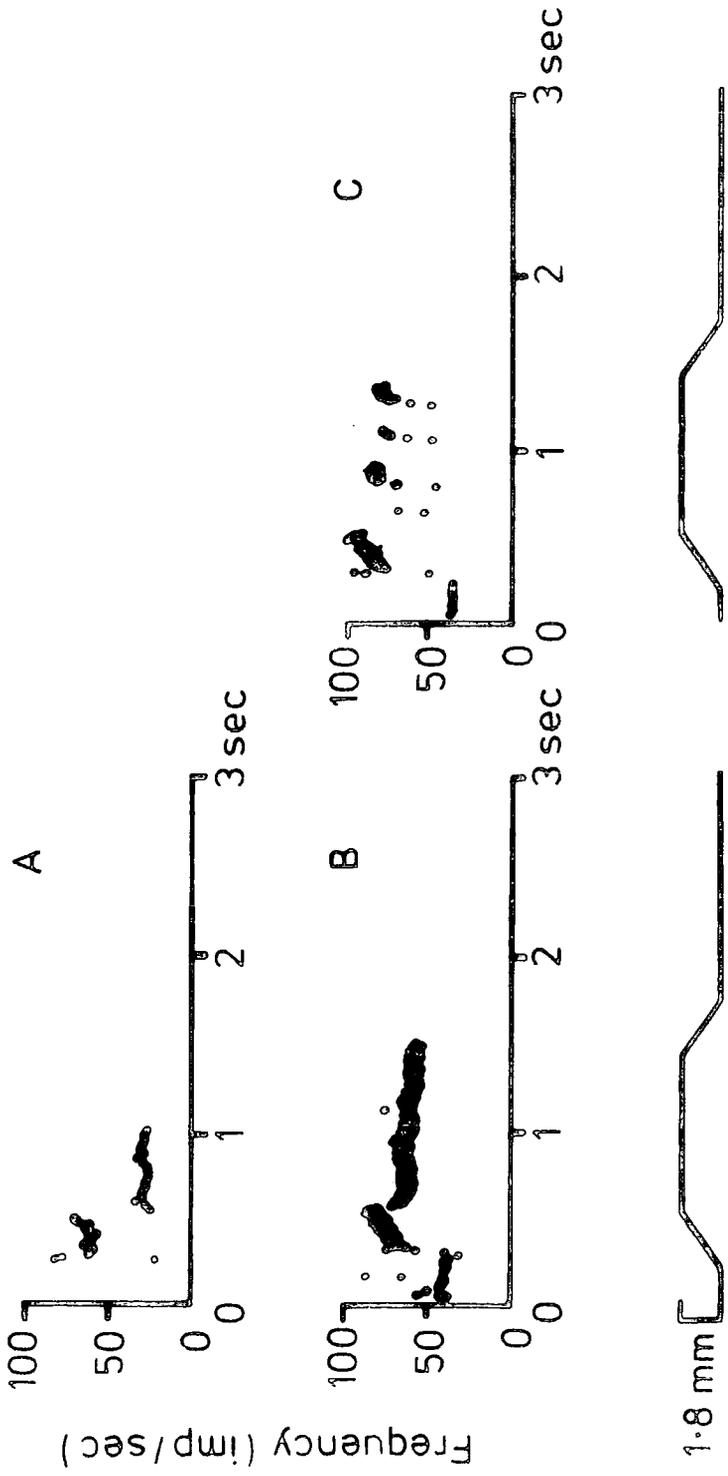


Figure 88. Confirmation of the static nature of a regenerated γ_s axon by its effect on two class 3 primaries. A, responses of the two primaries to passive stretch. B, responses of the two primaries to stretch during stimulation of the same γ_s axon at 100Hz.

Afferent CV's:- 68.9m/sec and 56.7m/sec.

γ_s CV:- 22.9m/sec.

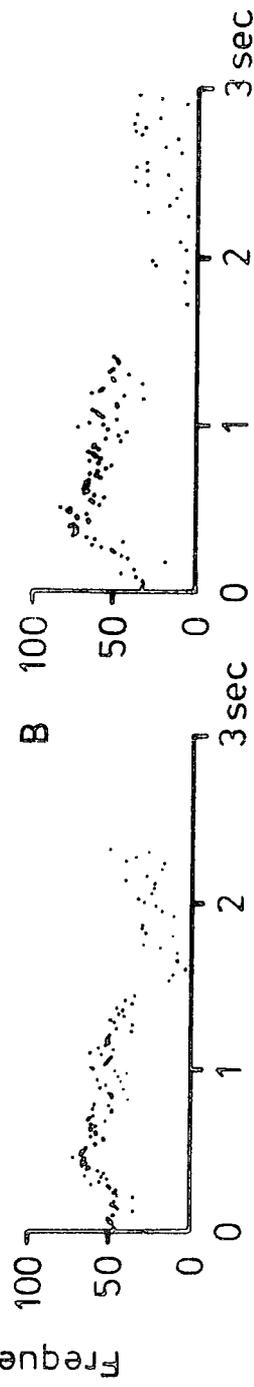
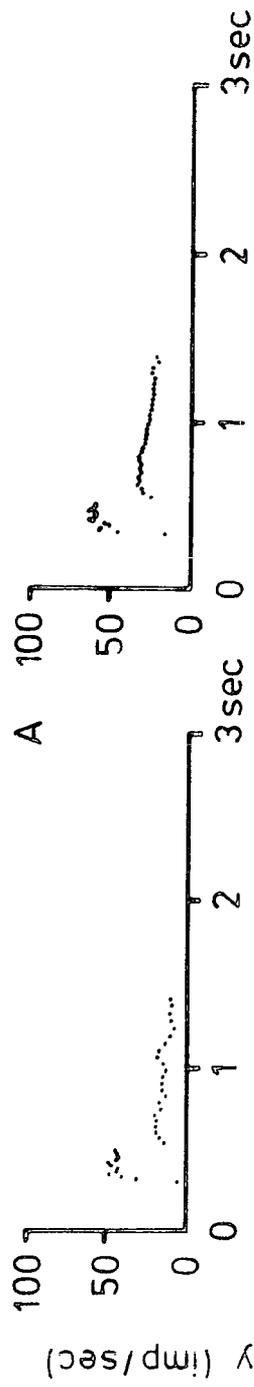


Table 10.

General data on the γ fusimotor stimulation experiments. The upper half shows the numbers of each class of afferent that were activated by either γ_s or γ_d axons or both. The lower half shows the total numbers of each type of γ axon tested and the numbers of axons that had their actions confirmed on more than one spindle.

Unit class	Total no. of units.	No. of γ_s axons	No. of γ_d axons	No. driven by both.
1	12	12	6	4
2	25	30	13	10
3	48	64	16	7
4	5	9	3	1
Controls	15	24	11	7
Regenerated axons		No. of γ axons tested.	No. of γ axons driving 2 units	No. of γ axons driving 3 units.
Gamma static		91	13	6
Gamma dynamic		26	3	4
Control axons				
Gamma static		21	2	0
Gamma dynamic		7	1	1

A number of both γ_s and γ_d axons were isolated which had an effect on more than one spindle (Table 10). Nineteen γ_s and seven γ_d regenerated efferents had their actions confirmed on more than one afferent ending. No examples were found of a gamma axon having mixed effects (i.e. acting like a dynamic axon in one spindle and a static in another). Fig. 88 shows an example of two class 3 primaries isolated at 47 days PC (25 days RT) which were both activated by stimulation of the same γ_s efferent.

7.5 Effects of Succinylcholine.

Succinylcholine (Sch) is a depolarising drug which produces a sustained contraction of the intrafusal fibres (Smith, 1966; Rack & Westbury, 1966; Dutia, 1980), thereby increasing the primary and secondary discharge rates. The effect on the primary is especially marked, being similar to the effects of strong dynamic fusimotor stimulation (Rack & Westbury, 1966; Dutia, 1980).

The effects of an intra-venous injection of Sch (BDH; 0.125mg/kg) on the response to ramp stretch of a class 1 afferent are shown in Fig. 89. ^{Presumably} The contraction of the bag₁ fibre (which has a lower threshold to Sch than the other fibres (Dutia, 1980)) elicited a marked increase in the peak firing rate and a concomitant elevation of excitation during the hold phase. As the effects of the drug subsided, so the primary returned to being a class 1 unit. If the responses shown in Fig. 89 are compared with those in Fig. 82, the similarity between the effects of γ_d stimulation and Sch can be seen.

7.6 Firing Rates.

Comparison of the responses of the regenerated with the control afferents to passive stretch (Figs. 69, 70, 71, 72, 73, 74) indicates that the regenerated afferents (especially the class 1

units) display a reduced response to the ramp-and-hold stretch.

The distributions of the peak firing rate (PFR's) (i.e. the firing rate at the end of the velocity phase of the ramp) of the four classes of afferent to passive stretch at 10mm/sec are shown in Fig. 90 and compared with the control data. The PFR's of the class 1 and 2 afferents are significantly lower than those of the controls ($P < 0.01$, Student's 't' test). There is no significant difference between the PFR's of the class 4 and the control afferents.

The mean PFR of the class 1 afferents is $64.3 \text{ imp/sec} \pm 5.9$ (S.E. of mean) compared with $137.6 \text{ imp/sec} \pm 4.48$ for the controls (Table 11). The mean firing rate of the controls 0.5sec into the hold phase is $69.4 \text{ imp/sec} \pm 2.28$, a reduction of 68.2 imp/sec from the PFR (this reduction is the value of the dynamic index). A comparable subtractive reduction in the firing rates of the class 1 afferents could account for the lack of a static response. The data given in Table 11 also shows that the dynamic indices are roughly consistent for all the classes with primaries and secondaries considered together and also for the primaries taken separately (this could only be done for the class 3 and 4 units and the controls because of the nature of the abnormalities of the class 1 and 2 afferents which made it difficult to distinguish between primaries and secondaries).

The data for the PFR's is also expressed in terms of the time after the injury in Fig. 91 and Table 12. After the first three recovery periods there was a wide dispersion of the data (Fig. 91) with a small proportion of units firing at very high rates to the passive stretch (see section 9.5). In the unoperated animals 6.5% of the afferents reached a peak firing rate in excess of 200 imp/sec . At 26 days PC (4 days RT) 34.5% of the afferents fired at more than

200imp/sec. By 33 days PC (11 days RT) this proportion had dropped to 3.7%. The effect of these highly responsive afferents on the mean PFR's can be seen in Table 12. At 26 days PC the mean PFR was $174.5 \text{ imp/sec} \pm 16.8$ (note the large standard error), which is, in fact, significantly higher than that of the normal afferents ($137.6 \text{ imp/sec} \pm 4.48$; $P < 0.05$, Student's 't' test).

There was a progressive decline in the mean PFR's up to 47 days PC (25 days RT), which is associated almost entirely with a reduction in the number of highly responsive units as can be seen from Fig. 91, with little change occurring in the responses of the main bulk of units. From 47 to 75 days PC the range of responses was remarkably constant, as were the mean PFR's (Table 12) and then there was a gradual increase in firing rate towards the control levels. Excluding the data for 26 days PC (4 days RT) all the mean PFR's are significantly lower than those of normal units ($P < 0.002$, Student's 't' test), except at 140 days when there is no significant difference.

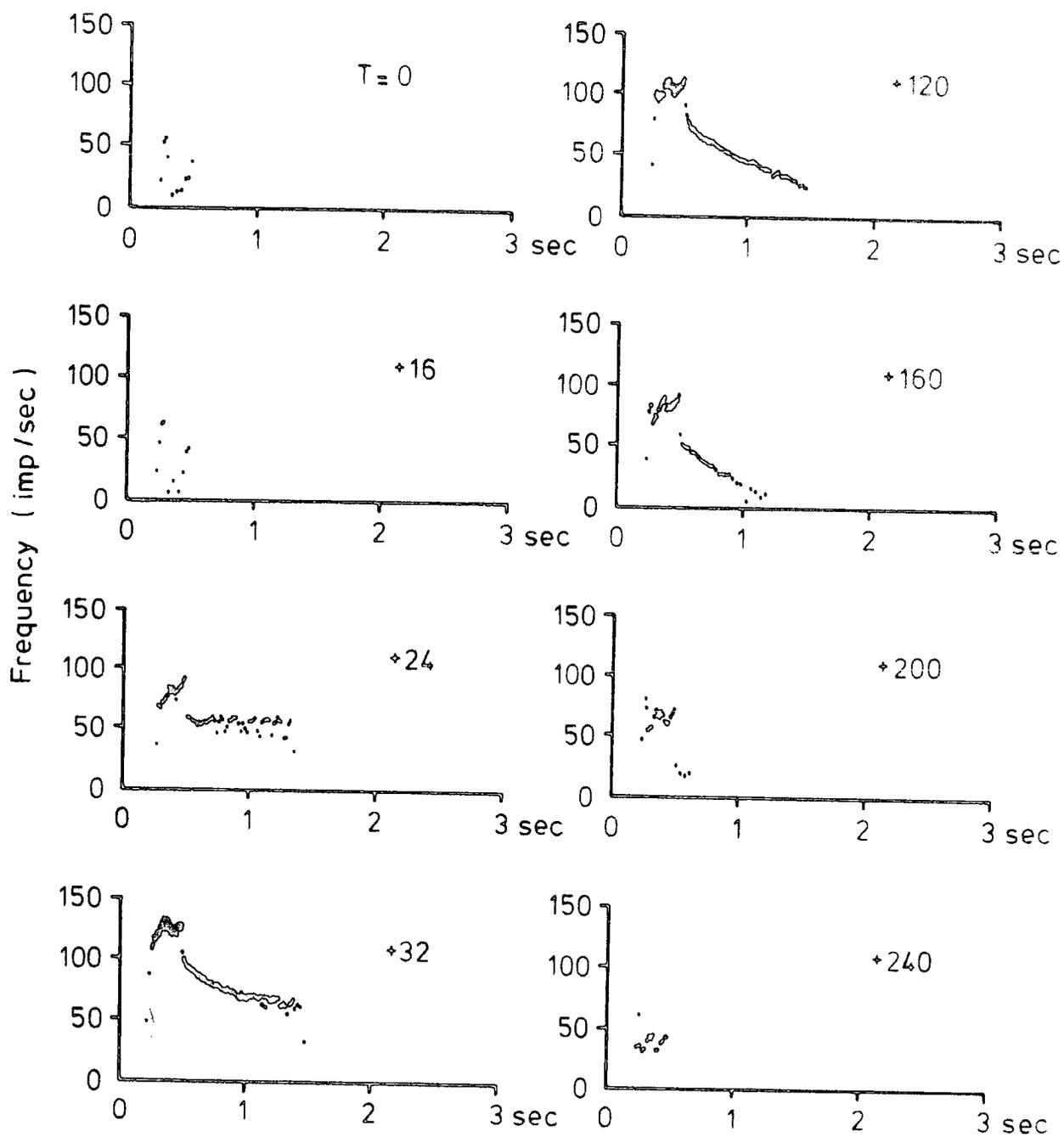
7.7 Following Capacity.

The ability of the regenerated afferent axons to carry high-frequency trains of action potentials was examined in two animals at 33 days PC (11 days RT). This period was chosen because there was a high proportion of class 1 units in the afferent population and the axons were still relatively fine without the stimulus threshold being excessively high over the distal regions of the regenerated segment. The muscle nerve was stimulated for 2sec at each of a range of frequencies close to its point of entry into PB. A total of ten afferents was tested; four of these were class 1 units, four class 2 units and two were class 3.

All the afferents followed the stimulation for frequencies

of up to 150Hz and six of them followed at frequencies of up to 300Hz (including two of the class 1 afferents). The inability to follow at 200Hz could be partially rectified by raising the stimulus intensity to five times threshold (these axons being relatively fine the threshold for single shocks was well above that for normal axons anyway and the stimulus was set at three times threshold for the first stages of the following test) such that all the afferents then successfully transmitted the spike trains at frequencies up to 200 Hz without any sign of conduction block or of any spikes dropping out.

Figure 89. The effects of an intra-venous injection of Sch (0.125mg/kg) on the responses of a class 1 primary afferent to ramp-and-hold stretch. The time, in seconds, after the injection is given for each record.



1.8 mm

Figure 90. Histograms of the peak firing rates (PFR's) of the four classes of regenerated afferent compared with the control afferents. The firing rates were measured at the end of the velocity phase of a 10mm/sec ramp stretch of 1.8mm amplitude.

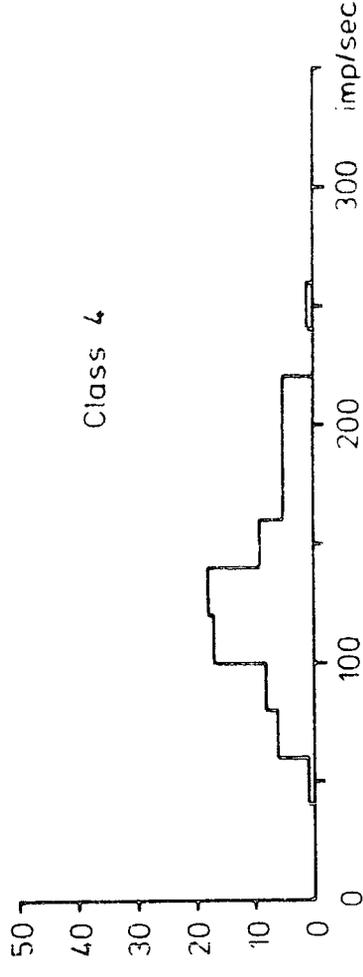
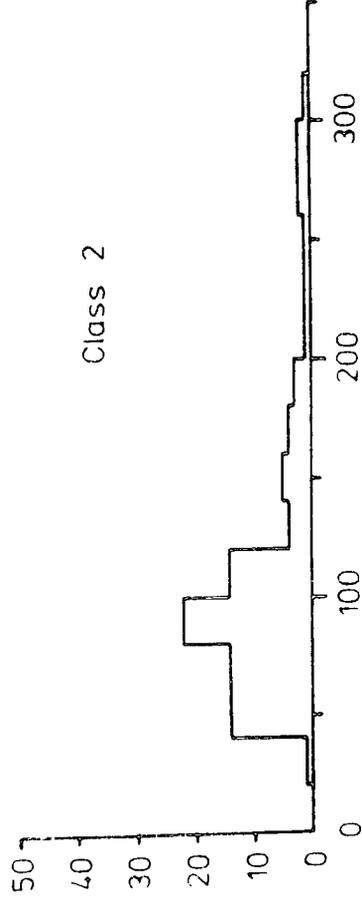
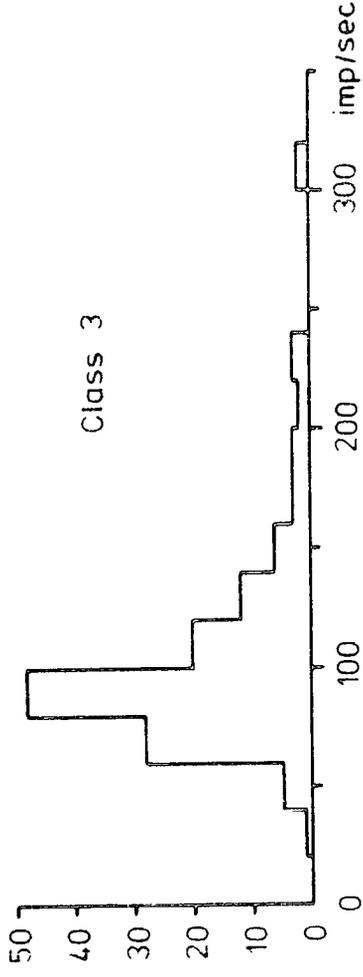
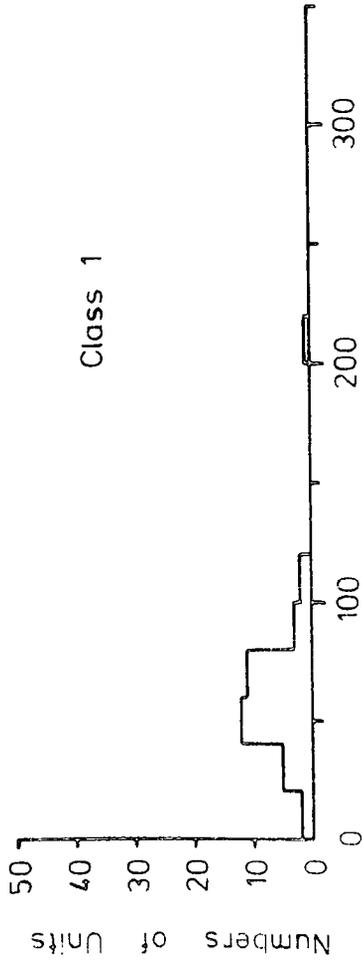
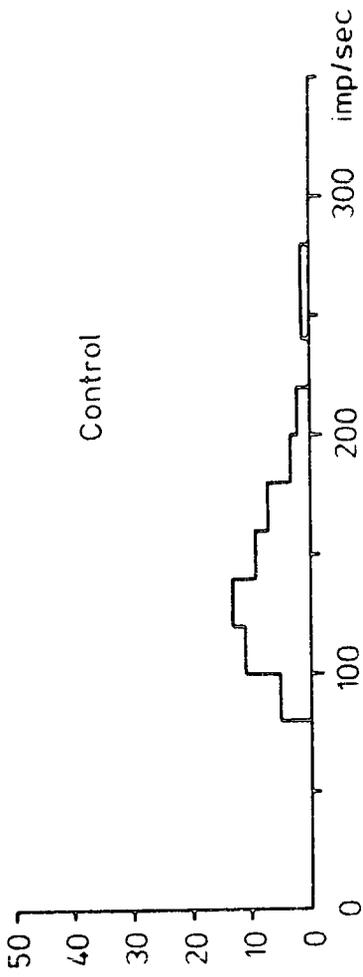
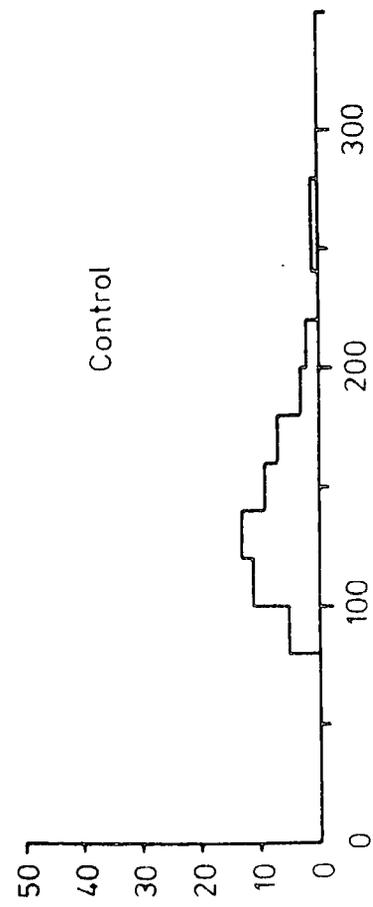


Table 11.

The mean peak and held firing rates for passive stretch of the four classes of afferent with their standard errors. Except where stated, data for primaries and secondaries have been combined. The figures for the primaries alone are only for those primaries that were definitely identified on the basis of CV and response pattern.

Class	Total no.	PFR (imp/sec)	HFR (imp/sec)	Dynamic index
1	47	64.3 ± 5.90	0.0	≤ 64.3
2	72	100.3 ± 6.06	33.4 ± 1.89 [@]	66.8
3	105	107.1 ± 4.4	49.4 ± 1.78	57.7
3 (Ia's)	84	99.5 ± 4.06	42.2 ± 1.84	57.3
4	75	129.5 ± 4.45	63.8 ± 2.61	65.7
4 (Ia's)	40	134.1 ± 6.22	65.9 ± 3.90	68.2
Controls	87	137.6 ± 4.48	69.4 ± 2.28	68.2
C (Ia's)	59	141.1 ± 4.76	64.1 ± 2.45	77.0

@ Not all the class 2 units fired for 0.5sec of the hold phase and these units have been excluded from the data given here.

Figure 91. Histograms of the PFR's of the afferent populations at given times after the crush operation compared with the controls. The times are given in terms of days PC.

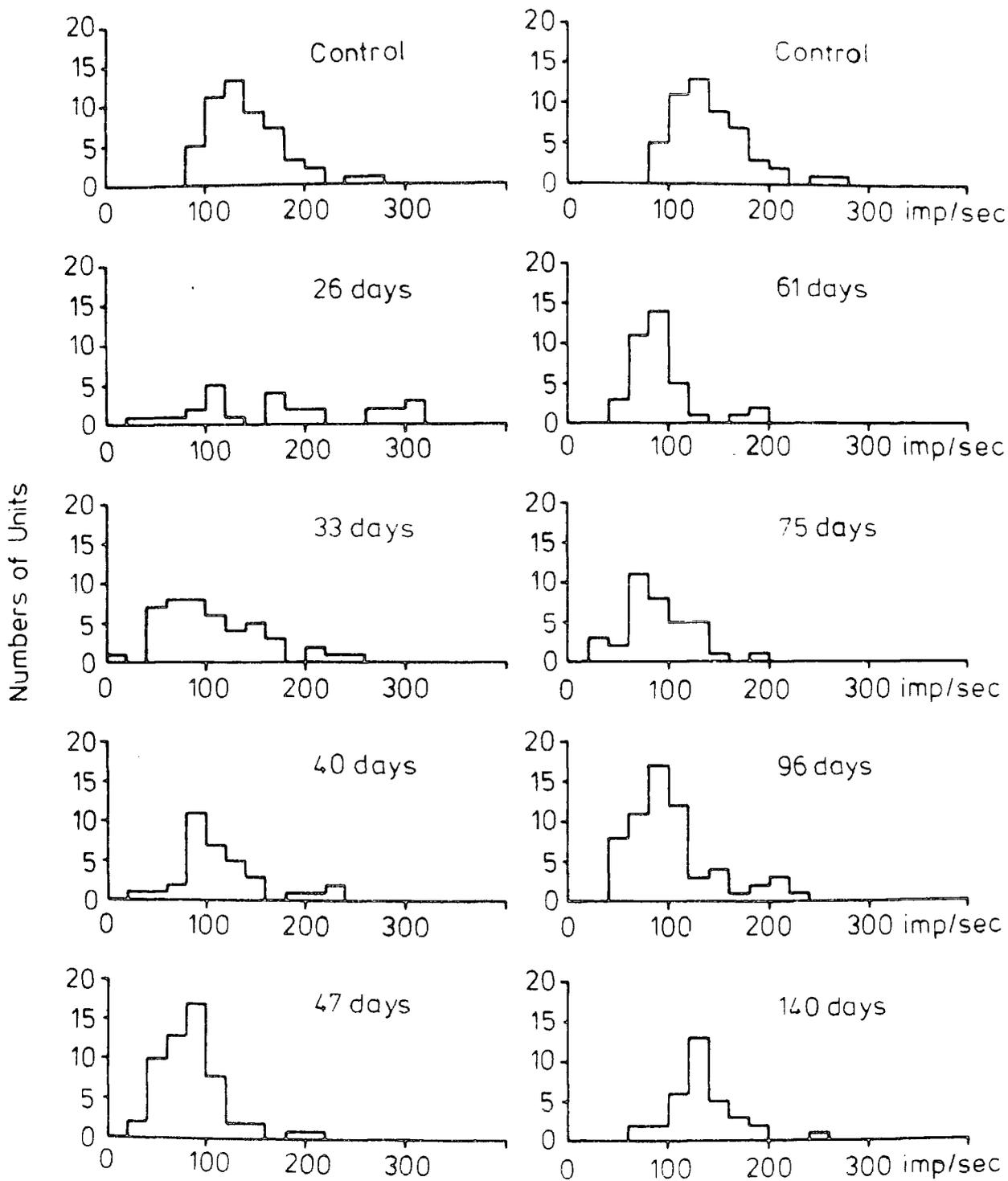


Table 12.

The mean peak firing rates (PFR's) with their standard errors for the afferent populations after different periods of recovery.

Time (days PC)	Time (days RT)	No. of units	PFR (imp/sec)
26	4	26	174.5 \pm 16.80
33	11	46	107.5 \pm 7.74
40	18	34	115.1 \pm 7.68
47	25	85	88.7 \pm 3.70
61	39	38	88.8 \pm 5.31
75	53	36	88.9 \pm 5.21
96	74	63	102.2 \pm 5.49
140	118	34	135.0 \pm 5.59
Controls		87	137.6 \pm 4.48

The Histology of Muscle Spindles Reinnervated After Short-term Denervation.

8.1 General Observations.

Following crush injury close to the muscle (NEC), the regeneration and subsequent restoration of both sensory and motor endings was rapid and successful. By 9 days RT most of the endings appeared nearly complete, although the restoration of the secondaries lagged slightly behind that of the primaries. As with the NS series (chapter 6), no spindles were found which had been reinnervated by sensory axons without motor endings also being present, although the reverse condition was observed in a small proportion of each sample.

8.2 The Primary Ending.

The first primary endings were in evidence by 15 days PC (5 days RT) although at this time most of the spindles lacked sensory innervation. By 19 days PC (9 days RT) almost all of the spindles had been reinnervated by a primary afferent (Table 13), many of them showing a high quality of restoration. Throughout the subsequent recovery periods a small proportion of spindles lacked Ia reinnervation (Table 13). There was no indication of a second wave of reinnervation. This might have been expected at about 26 days RT (section 6.2), although its apparent absence could be a result of the small size of the samples.

By 9 days RT nearly all of the primary axons that had reinnervated the spindles had produced well-restored endings, some examples of which are shown in Figs. 92, 93 (Plate 19) and Figs. 96, 97 (Plate 20). In each of the endings shown in these figures all the fibres have been reinnervated and all the bag fibres show regular banding. This pattern of successful restoration was observed in

the great majority of spindles observed at this time (Tables 14, 15, 16). A few Ia's were observed in spindles located at the distal end of the muscle which had not yet developed endings as the axons apparently had yet to make contact with the intrafusal muscle fibres although they had, in some cases, divided into first-order branches.

The majority of endings examined after longer periods of recovery (of which examples are shown in Plates 22-24) show a similar high quality of restoration although at each time period there were some abnormal endings (for example Figs. 108, 109, Plate 24). In every spindle where a primary ending had been formed, all three types of intrafusal fibre were reinnervated to some extent (Table 14) and most of the bag fibres showed at least 3 bands, the average being 7-8 bands compared with 14 in normal PB spindles (Table 15).

The formation of the endings was very rapid and after 19 days PC (9 days RT) there was little apparent improvement in the quality of reinnervation over the subsequent periods (Tables 15, 16) so that the endings never achieved the same length or complexity of innervation displayed by normal endings. Thus at 19 days PC (9 days RT) the average length of the primary endings was $265.7\mu\text{m} \pm 13.3$ and at 40 days PC (30 days RT) it was unchanged at $257.6\mu\text{m} \pm 24.3$ (Table 16) compared with a mean length of $314.8\mu\text{m} \pm 11.6$ for the normal primary endings. Similarly, the average number of bands on the bag fibres was 8.8 at 19 days PC (9 days RT) and 8.5 at 40 days PC (30 days RT) compared with 14.01 bands supplied by the normal primary axons (Table 15). Even the best restored of the primary endings showed fewer bands than the average for the normal endings. The primary shown in Fig. 93 (Plate 19) has 8 bands on the bag₁ and 16 on the bag₂; of the primaries shown in Plate 21, the one in Fig. 99 has 10 bands on one bag fibre but only 3 on

Table 13.

The numbers of spindles lacking primary innervation after the nerve-entry crush (NEC series).

Time (days PC)	19	26	33	40
Time (days RT)	9	16	23	30
No. of spindles examined.	38	21	8	15
No. of spindles with no Ia.	3	2	0	1
% of spindles with no Ia	7.8	9.5	0.0	6.6

Table 14.

The distribution of terminals of the Ia afferent axon to the different fibre types (NEC series).

Time (days PC)	19	26	33	40
Time (days RT)	9	16	23	30
No. of b_1b_2c endings	32	14	8	9
No. of bc endings	0	0	0	0
Total no. of endings.	32	14	8	9

Table 15.

The mean number of bands visible on the bag fibres after different periods of recovery from nerve-entry crush (NEC series).

Time (days PC)	Time (days RT)	Mean no. of bands	Range	Total no. of bag fibres
19	9	8.8	2-17	35
26	16	6.7	2-14	22
33	23	7.2	3-12	12
40	30	8.5	3-13	10
Controls		14.01	5-27	87

Table 16.

The mean length of the primary endings restored after the nerve-entry crush injury (NEC).

Time (days PC)	Time (days RT)	Mean length of ending (μm)	Range (μm)	No. of endings
19	9	265.7 ± 13.3	176-405	22
26	16	252.0 ± 18.6	100-376	15
33	23	246.8 ± 23.5	124-333	7
40	30	257.6 ± 24.3	153-343	10
Controls		314.8 ± 11.6	166-476	33

the other; and in Fig. 100 the primary supplies 11 bands to the bag₁ and 12 to the bag₂.

Hyperinnervation by more than one Ia axon was only observed in 3 spindles (4.7%) out of a total sample of 63 primary endings. Two of these were in the sample at 19 days PC and one at 26 days PC (16 days RT) but, as with the NS series (section 6.2), there is probably no relationship between the occurrence of hyperinnervation and the length of the recovery period.

A few abnormal patterns of reinnervation of the P region were observed. One such, after 40 days recovery (30 days RT) is shown in Fig. 109 (Plate 24). A number of fine axons are enclosed in the Ia tube and enter the primary region together. Some of the axons leave the region in favour of the poles and are presumably motor in origin. A few others attempt to form endings in the region and some faint spirals are visible round the intrafusal fibres, all of which receive some form of termination although the overall restoration is very poor.

Motor invasion of the primary region was also observed in other spindles both in the presence (Fig. 99, Plate 21) and absence (Fig. 103, Plate 22) of the primary ending. In each case the axons either terminate in swellings (Fig. 99, Plate 21) or leave the region without forming an ending (Fig. 103, Plate 22). There was never any invasion of the S₁ or more polar regions by branches of the primary ending.

8.3 The Secondary Ending.

The first secondary endings were present at 15 days PC (5 days RT) having arrived back at about the same time as the first primaries. By 19 days PC (9 days RT) most of the secondaries had returned and successfully reinnervated the intrafusal fibres

(Fig. 98, Plate 20). The number of secondaries per spindle never reached the levels found in normal PB spindles (Table 17). This was due to a persistently greater proportion of spindles having no secondary innervation and a reduction in the number of spindles innervated by more than one secondary.

At 19 days PC (9 days RT) a relatively high proportion of the S_1 secondaries had reinnervated only the chains (16%) compared with 0.4% in normal spindles (Table 18). There was also a greater proportion of S_1 secondaries innervating the chains and one bag fibre compared with the controls (28% compared with 17.8%). This was also noted in the NS study and possibly reflects a relatively slow rate of ending restoration by the secondaries. After the subsequent recovery periods the proportions of S_1 secondaries innervating all three fibre types rose to near-normal levels (Table 18).

Most of the secondaries were well restored (Fig. 98, Plate 20; Fig. 99, Plate 21; Fig. 104, Plate 23) although a few apparently abnormal endings occurred in each sample (Fig. 105, Plate 23). Invasion of the P region by branches of the S_1 secondaries occurred in 11% (out of a total sample of 36 endings) of spindles with the majority of these occurring at 23 and 30 days RT.

8.4 Motor Reinnervation.

All the spindles examined from 15 days PC (5 days RT) onwards were reinnervated by efferent axons and in every case the motor endings, both intrafusal and extrafusal, were rapidly restored to near normality (Figs. 94, 95, Plate 19).

Table 17.

The numbers of afferent endings per spindle after different periods of recovery following a nerve-entry crush injury. The proportions of each type are expressed in terms of percentages of the total population of b_1b_2c capsules in the muscle at each time.

Type of innervation	9	16	23	30	Controls
0	7.9	9.5	0.0	6.6	0.0
OS ₁	0.0	0.0	0.0	0.0	0.0
P	18.5	28.6	12.5	26.6	8.7
PS ₁	36.8	47.6	62.5	66.6	45.7
PS ₁ S ₂	10.5	9.5	0.0	0.0	13.0
S ₁ PS ₁	15.8	0.0	25.0	0.0	13.0
S ₁ PS ₁ S ₂	5.3	0.0	0.0	0.0	17.4
S ₁ PS ₁ S ₂ S ₃	2.6	0.0	0.0	0.0	0.0
S ₂ S ₁ PS ₁ S ₂ S ₃	0.0	0.0	0.0	0.0	2.2
S ₂ S ₁ PPS ₁ S ₂	0.0	4.8	0.0	0.0	0.0
PP	2.6	0.0	0.0	0.0	0.0
Total no. of capsules in sample.	38	21	8	15	46

Cont'd overleaf.

Table 17 (cont'd).

The mean number of secondaries per $b_1 b_2 c$ capsule.

Time (days RT)	9	16	23	40	Controls.
Mean no. of secondaries.	1.15	0.86	1.12	0.66	2.0
Proportion (%) of the spindle population lacking a secondary ending.					
Time (days RT)	9	16	23	40	Controls
Prop. of spindles with no secondary	29.0	38.1	12.5	33.2	8.7

Table 18.

The distribution of terminals of S₁ secondaries to the different fibre types after regeneration following a nerve-entry crush injury (NEC series). The frequency of occurrence of each type of secondary ending is expressed as a percentage of the total number of S₁ secondaries at that time. The control data was taken from Babks et al (1982).

Time (days PC)	Time (days RT)	b ₁ b ₂ c endings	b ₁ or b ₂ c endings	c only endings	Total no. of secondaries
19	9	56.0	28.0	16.0	25
26	16	71.4	28.6	0.0	7
33	23	100.0	0.0	0.0	5
40	30	66.7	33.3	0.0	3
Controls		81.8	17.8	0.4	253

Plate 19.

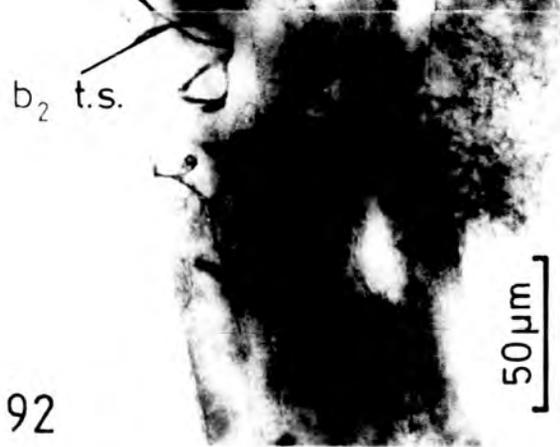
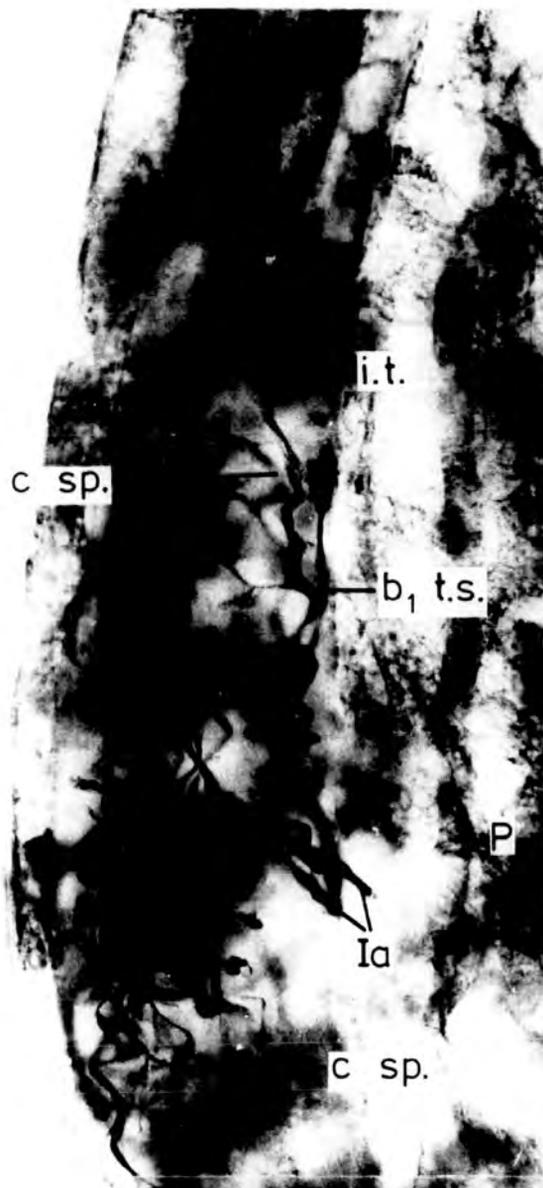
Photographs of silver-stained muscle-spindle preparations at 19 days PC (9 days RT) after a nerve-entry crush (NEC series). Abbreviations as for previous plates.

Figure 92. A well-restored primary ending at 9 days RT. All the intrafusal fibres are reinnervated; the bag₁ has 5 bands on it and the bag₂ has 6. The overall configuration of the primary is fully restored with a central region of regular annulospiral ending on the bags bounded by irregular sections and extensive regular spiralling on the chains.

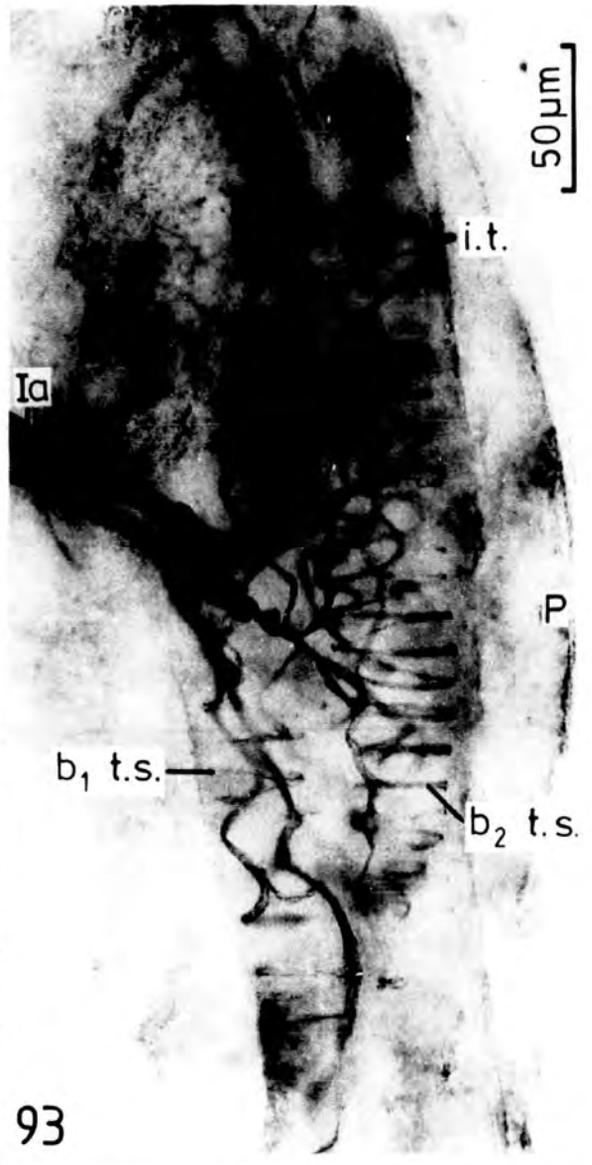
Figure 93. The primary afferent has produced extensive innervation on all the intrafusal fibres with 8 bands on the bag₁ and 15 on the bag₂. As with Fig. 92, the overall configuration of the ending is clearly restored.

Figure 94. A reinnervated p₂ plate motor ending on a bag₁ fibre. All the motor endings were well restored by this time.

Figure 95. A double p₁ plate. One axon entered the pole from the intra-muscular nerve trunk and branched to form these two plates on the bag₁ fibre. Note the concentrations of nuclei beneath the axon terminals.



92



93



95



94

Plate 20.

Photographs of silver-stained primary and secondary endings 19 days (9 days RT) after a nerve-entry crush injury (NEC series). Abbreviations as for previous plates.

Figure 96. A well-restored primary ending with extensive reinnervation of all the intrafusal fibres. A number of expansions occur on the tips of the terminals. The S_1 secondary is less well restored and probably only innervates the chains. One branch of the secondary (arrowed) approaches the P region but does not overlap with the primary.

Figure 97. A small but well-reinnervated primary with terminals on all fibres. The left-hand bag fibre (b ts.) has 11 bands and the right-hand one (out of focus here) has 9.

Figure 98. A well-restored b_1b_2c secondary ending showing some spiral terminations.

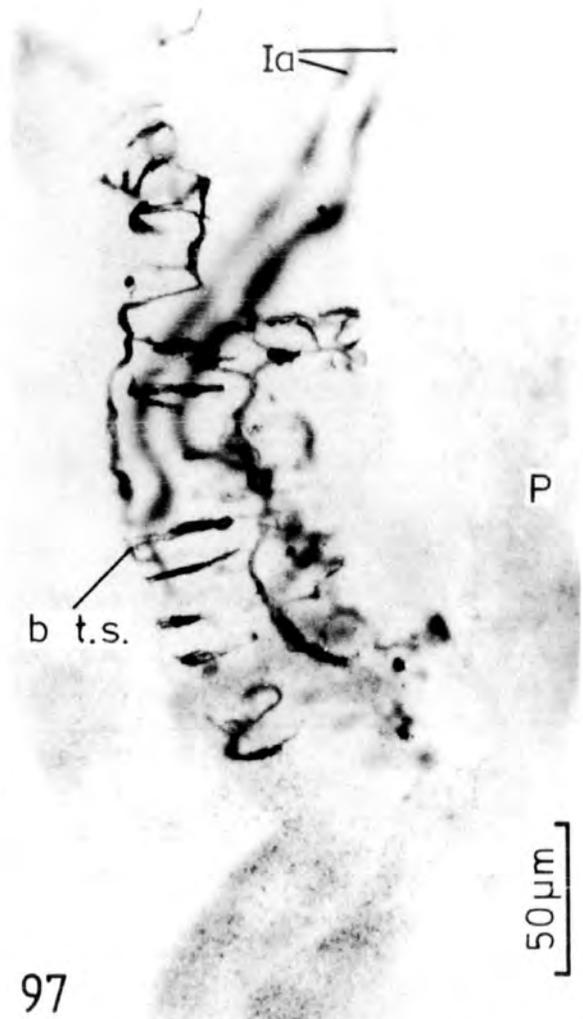
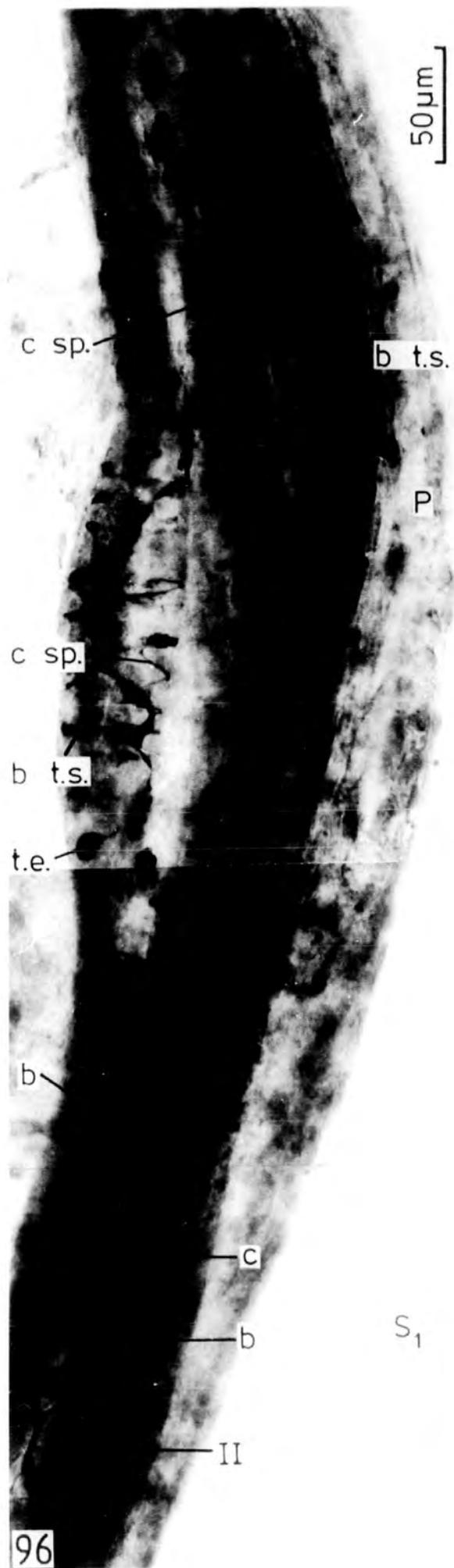


Plate 21.

Silver-stained primary and secondary endings at 26 days PC (16 days RT) after nerve-entry crush (NEC series). Abbreviations as for previous plates.

Figure 99. A well-developed primary ending with extensive spiralling on the chain fibres. One bag fibre has 10 bands but the other has only three. The S_1 secondary in the lower pole is well restored with terminals on all the fibres. In the upper pole some small motor axons enter the P region and form ball-like swellings (arrowed).

Figure 100. A high-power photograph of a very well-restored primary ending. Extensive, regular innervation is evident on all the intrafusal fibres.

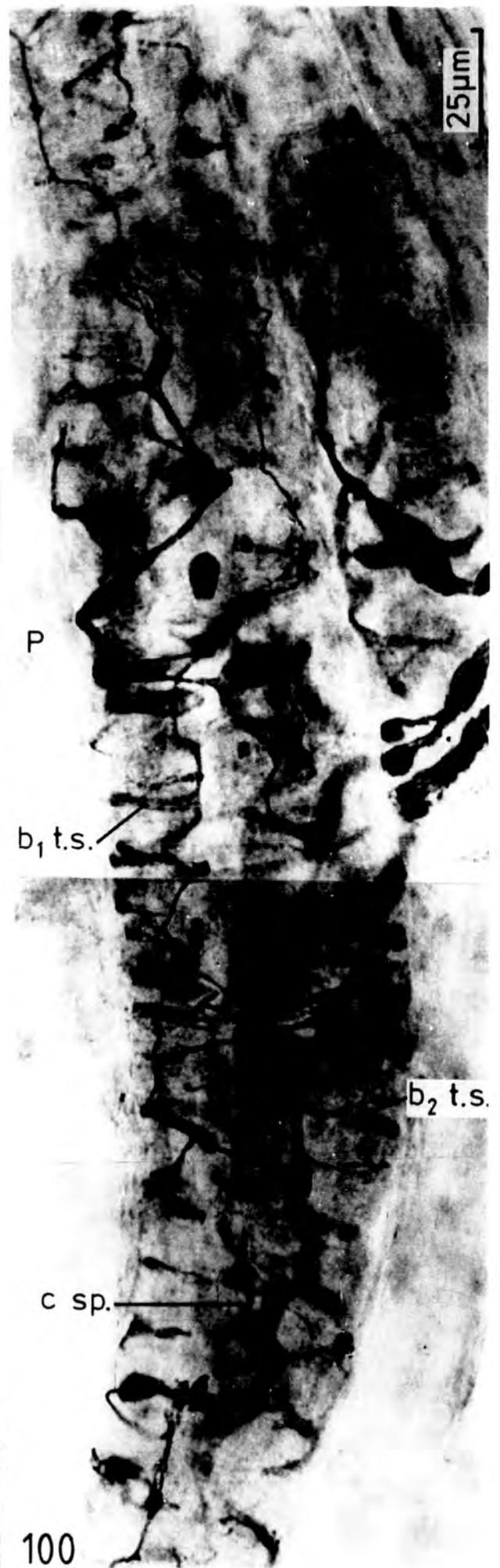
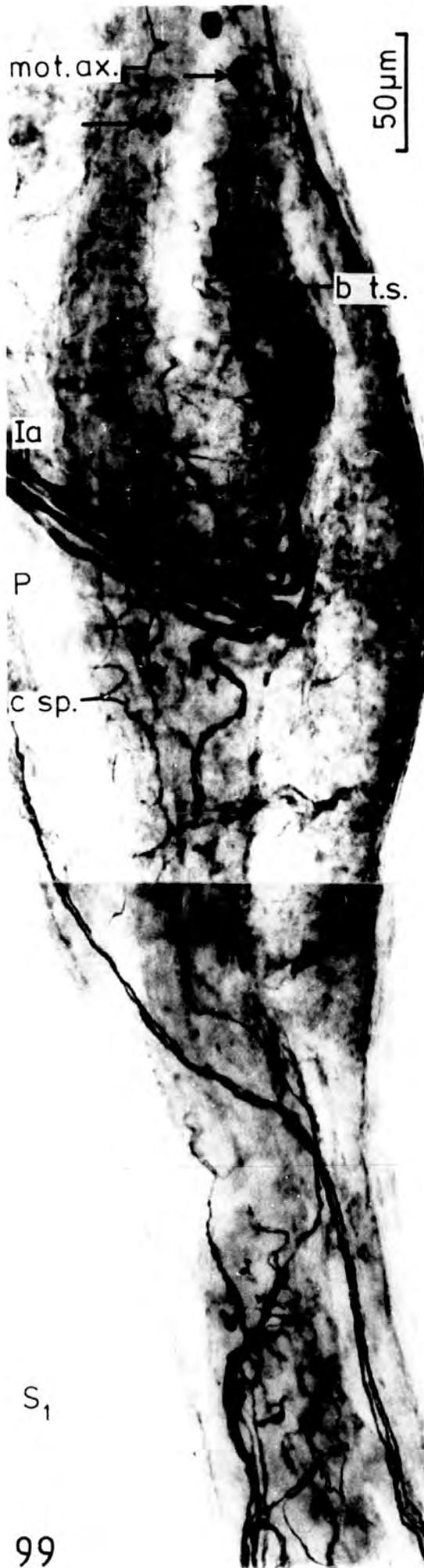


Plate 22.

Photographs of silver-stained primary and secondary endings at 26 days PC (16 days RT) in the NEC series. Abbreviations as for previous plates.

Figure 101. A well-restored primary ending with regular innervation on all the fibre types. The S_1 secondary is well developed but only innervates the bag_2 and chain fibres. The bag_1 is free of secondary terminations, the axons associated with it being fusimotor in origin.

Figure 102. All fibre types are reinnervated by the Ia axon although the chain innervation is limited. The S_1 secondary supplies terminals to all the fibres and approaches, but does not overlap the edge of the primary ending.

Figure 103. There is no primary present, the only axon in the equatorial region came from the lower pole and is presumably motor in origin. In the S_1 region it gives off a branch that terminates in a small expansion (arrowed), the rest of the axon carries on into the P region giving off a few branches which also do not form any recognisable endings. Towards the centre of the P region the axon doubles back and returns to leave the spindle via the lower pole; its course is indicated by the two arrows.

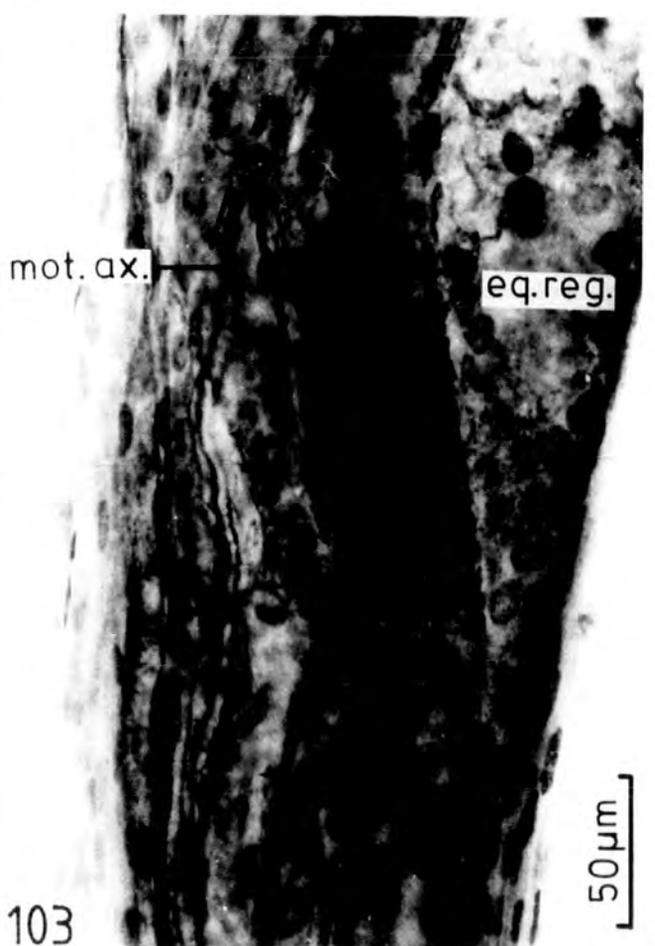
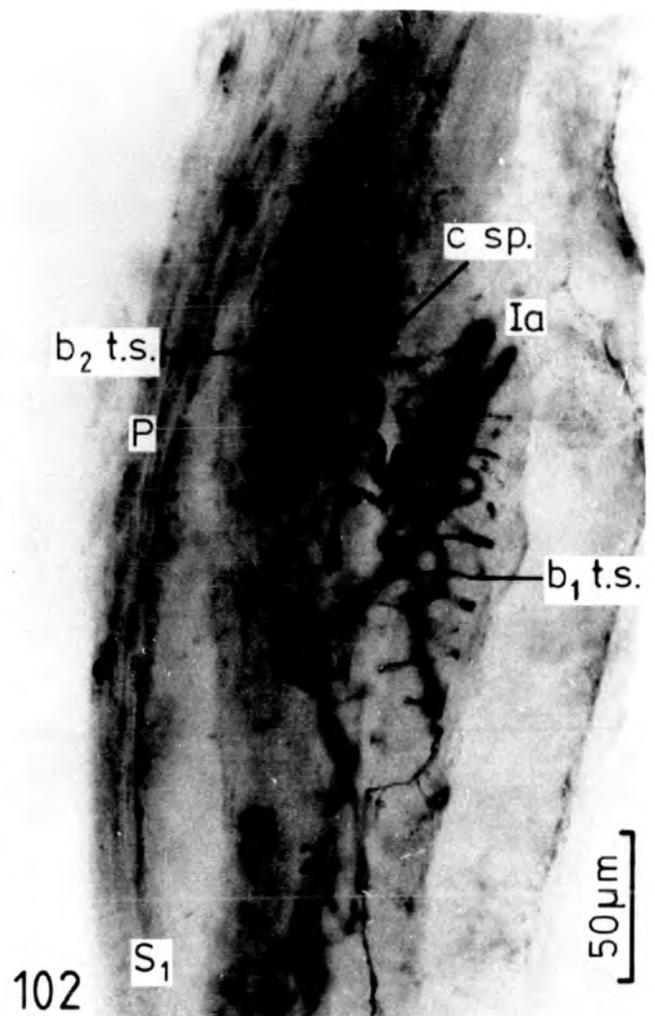
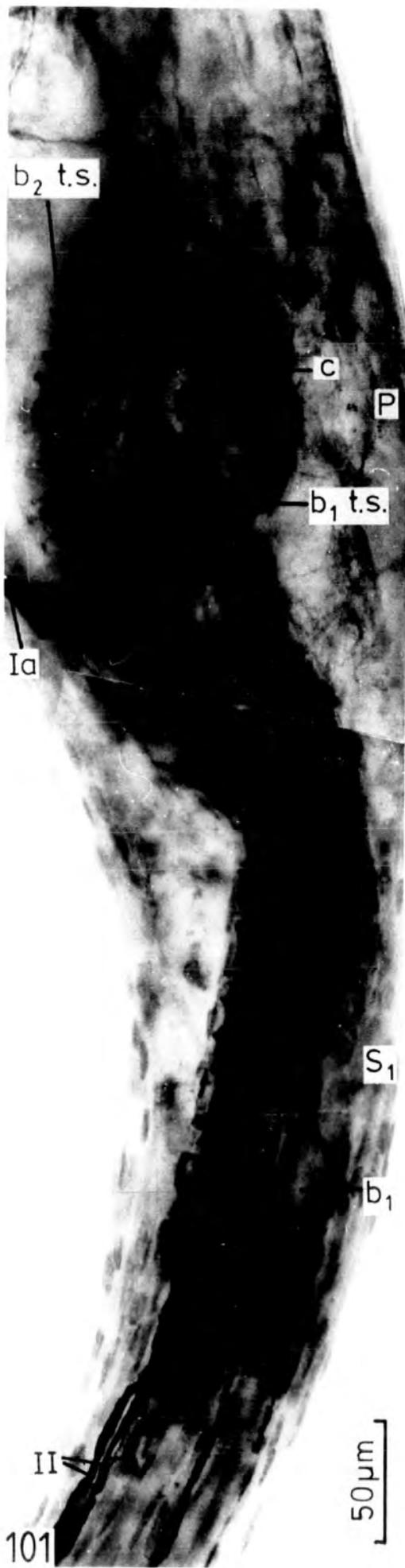


Plate 23.

Photographs of silver-stained primary and secondary endings at 26 (16 days RT) and 33 (23 days RT) days PC in the NEC series.

Abbreviations as for previous plates.

Figure 104. Examples of S_1 and S_2 secondary endings at 26 days PC.

The S_1 secondary only supplies terminals to the bag₂ and chains.

The S_2 has only reinnervated the chains. In both cases some spirals are visible.

Figure 105. A poorly-restored S_1 secondary at 33 days PC (23 days

RT). There are few recognisable terminations, with most of the branches ending in the large axonic swellings.

Figure 106. A well-restored primary ending with regular innerv-

ation on all the fibres. A branch (arrowed) of the left S_1 secondary enters the P region and crosses it (out of focus here) to terminate in the opposite S_1 pole.

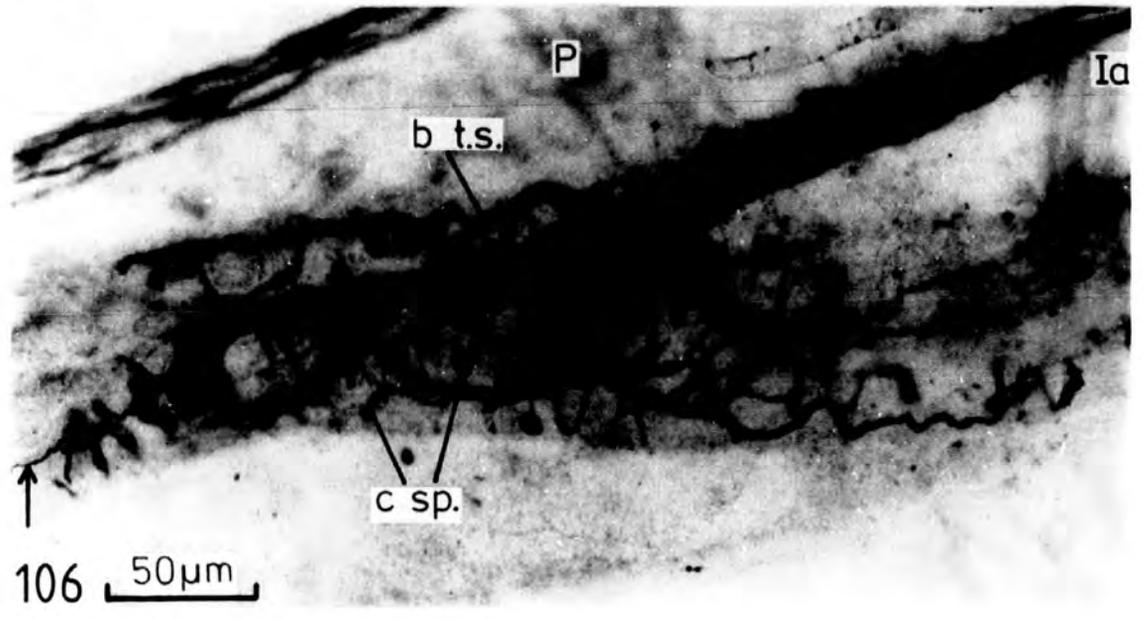
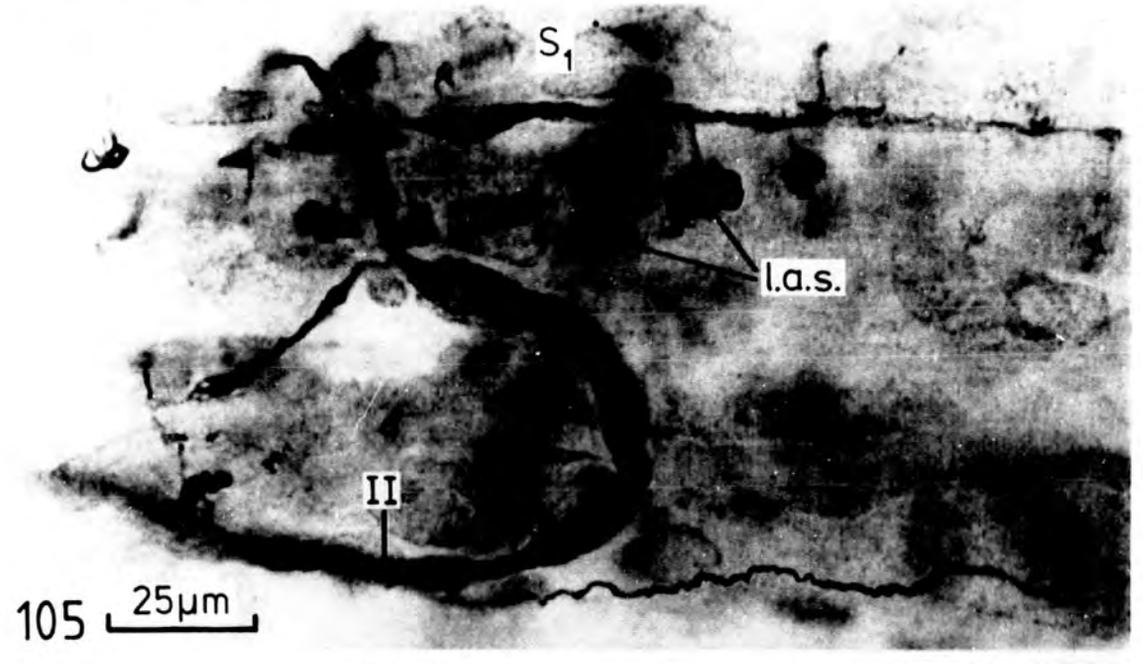
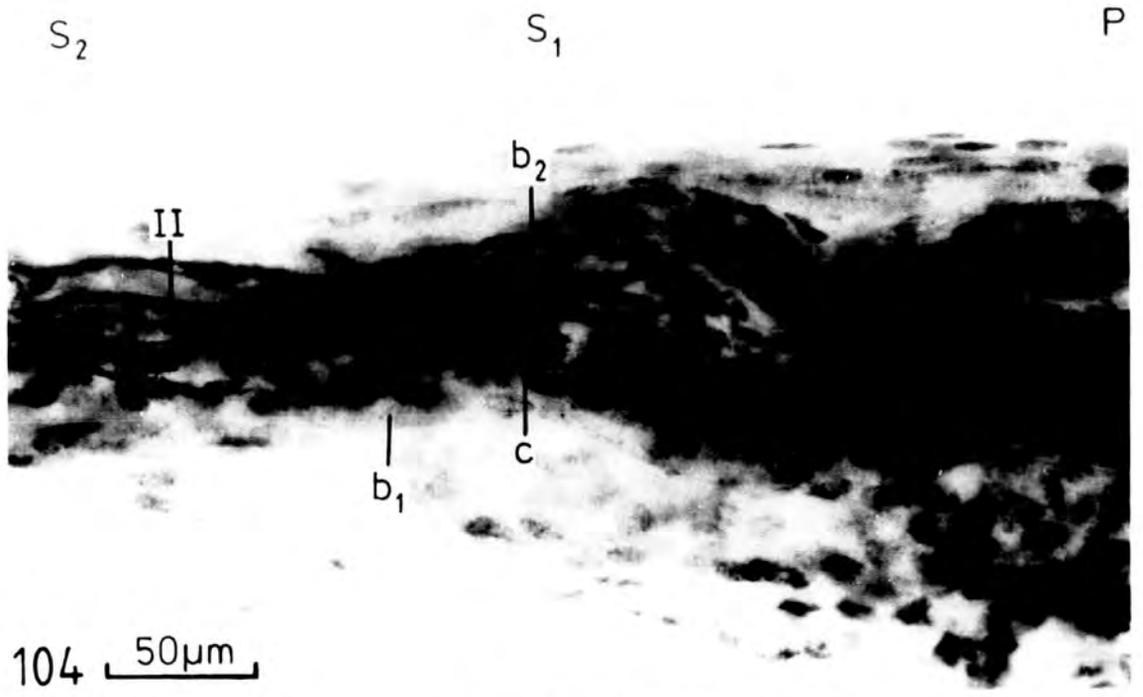


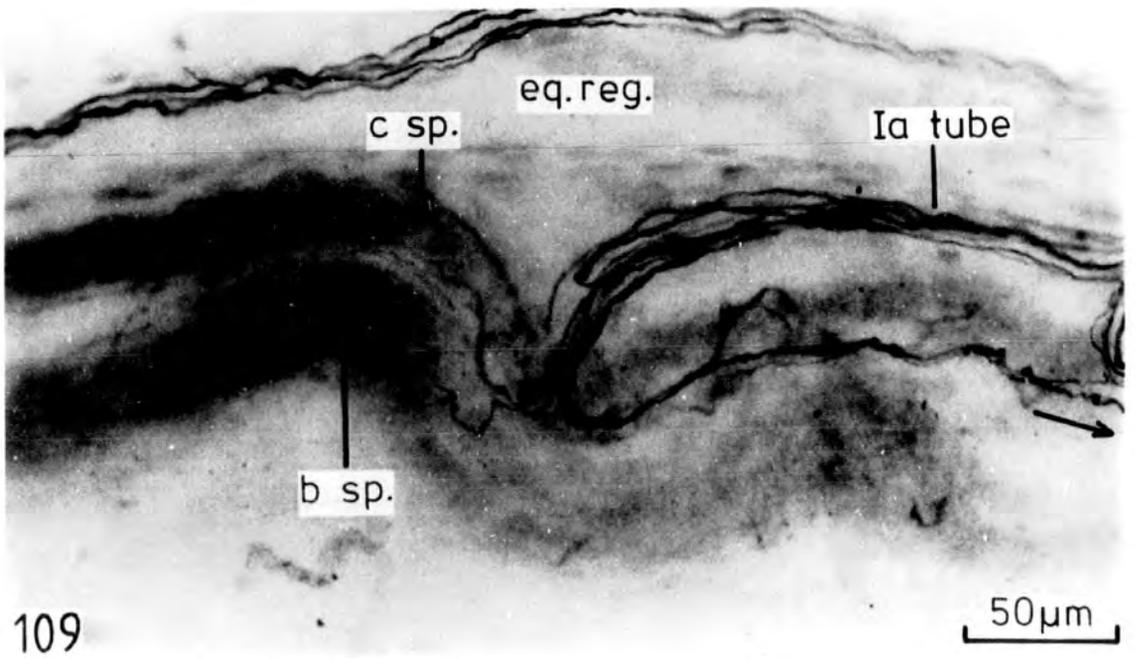
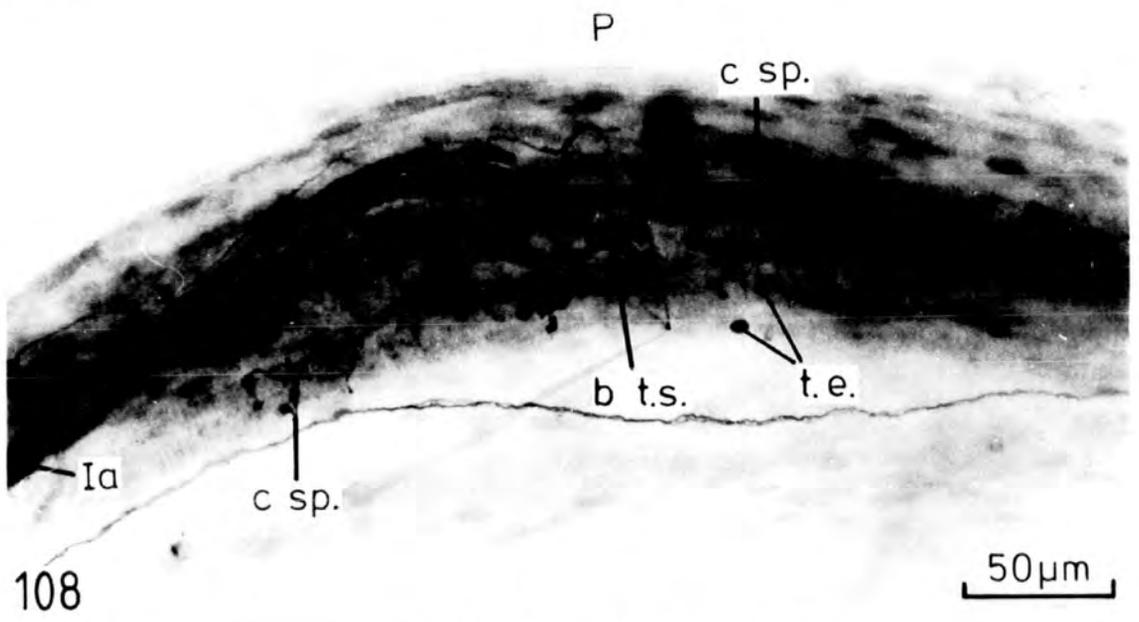
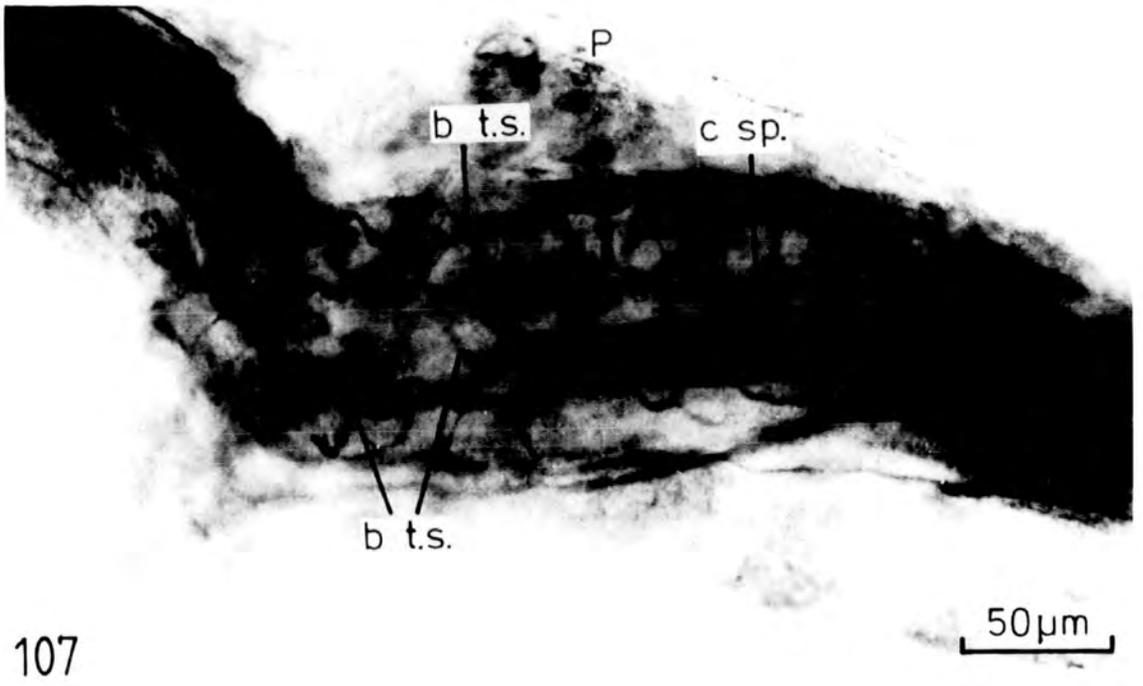
Plate 24.

Silver-stained primary endings after 40 days recovery (30 days RT) following a nerve-entry crush injury (NEC series). Abbreviations as for previous plates.

Figure 107. An example of a spindle with three nuclear-bag fibres. All the fibres are well reinnervated; it was not possible to distinguish between the bag fibres.

Figure 108. A fairly irregular primary ending with numerous expansions on the ends of the terminals.

Figure 109. An abnormally reinnervated spindle. The Ia tube contains a number of small axons most of which leave the equatorial region for the poles. Some of the branches, however, form a few spirals around the intrafusal fibres. Unfortunately these have not stained very well but it is possible to see some terminations on each fibre type.



The Histology of Muscle Spindles Reinnervated After long-term Denervation.

8.5 General Observations.

This experimental series includes two types of crush operation; (i) a proximal crush at the point of separation of the tibial nerve from the common peroneal (the TC series) and (ii) the repeat crush experiments (the RC series) in which the crush operation was performed at the knee (as in the NS series) and then repeated once or twice after intervals of 7 or 14 days. Unfortunately, although a complete set of physiological results was obtained (see chapter 9), the staining of the material from this series was generally very poor and so the sample sizes are low.

The overall impression derived from the histological analysis is that as the period of denervation increased there was a comparable increase in the degree of disorganisation shown by the endings. Even after 50 days denervation (50 days D), however, many of the endings were restored successfully and even the most disorganised endings were still recognisable.

8.6 The Primary Ending.

The capricious nature of the stain in this series prevented assessment of the proportion of spindles lacking sensory innervation, so only those spindles that had visible innervation were analysed. A total of 7 spindles were analysable from the TC study; these had been denervated for 29 days and were examined at 32 days RT (the spindles in the NS study were denervated for 22 days). A further 7 spindles were analysable from the repeat crush study; these had also been denervated for 29 days and were examined at 39 days RT. Thirty-eight spindles were also examined from the double-repeat crush series and these had been denervated for 50 days and were examined at 39 days RT.

After 29 days D at 32 and 39 days RT all the primary afferents had reinnervated all the intrafusal fibre types to some extent, albeit sparsely in some cases (Fig. 111, Plate 25). After 50 days D, 2 out of 35 primaries (5.7%) failed to reinnervate one of the bag fibres.

After 29 days D all the primary endings showed some degree of disorganisation, often with the terminals dispersed irregularly on some, if not all, of the intrafusal fibres (Figs. 110-112, Plate 25; Fig. 113, Plate 26). The degree of disorganisation was greatly increased after 50 days D with many of the endings showing little, or no, regular annulospiral innervation (Figs. 116-122, Plates 27-29). On 56 bag fibres which showed some banding, the mean number of bands was 5.5 (with a range of 1-12); a further 8 bag fibres, although bearing some terminals showed no annulospiral innervation at all (Fig. 123).

Of 24 primary endings examined at 50 days D, 8 produced branches that invaded the neighbouring S_1 regions. Some of these attempted some form of ending and then turned back into the P region (Fig. 117, Plate 27; Fig. 120, Plate 29), whereas others formed endings in the S_1 region (Fig. 118, Plate 28) or showed signs of traversing the S_1 region completely (Fig. 123). These abnormalities were unique to the 50 days D material, the only fairly similar observation being that of a Ia axon at 118 days RT (22 days D) in the NS series which produced a branch that entered the S_1 region and then turned back without attempting to form an ending (Fig. 38, Plate 13).

Despite the abnormalities displayed as a result of the increased denervation period, the physical dimensions of the primary endings still remained fairly consistent (Table 19). The apparent increase in average length of the endings after 50 days D

Table 19.

The mean lengths of the primary endings (with their standard errors) after different periods of denervation and reinnervation.

Time (days D)	Exptl. series	Time (days RT)	Mean length of endings (μm)	Range (μm)	Total no. of endings.
10	NEC	30	257.6 ± 24.3	153-343	10
22	NS	39	269.9 ± 10.1	171-333	18
29	TC	32	309.0 ± 30.5	176-447	7
29	RC	39	227.4 ± 12.1	200-281	7
50	RC	39	326.8 ± 14.5	190-500	32
Controls			314.8 ± 11.6	166-476	33

is probably due to the presence in the sample of those endings that invaded the S_1 regions. The measurements were made on the basis of the presence of terminals, so for an ending such as that shown in Fig. 120 (Plate 29), where the invading branches did not form an ending within the S_1 region, the measurement only covers that length of the intrafusal fibres bearing terminals.

Hyperinnervation of the primary region was noted although, as with the NS and NEC series, it was fairly uncommon. It was absent from the 14 spindles denervated for 21 days but, occurred in 5 out of 21 (23.8%) spindles after 50 days D.

8.7 The Secondary Ending.

The secondary endings reinnervated the spindles in about the same numbers as in the NS and NEC studies despite the increased periods of denervation. After 29 days D there were 0.7 and 1.0 secondaries per spindle (at 32 and 39 days RT, respectively) and after 50 days D there were 0.97 secondary endings per spindle.

The quality of restoration of the endings was very poor. After 50 days D, of 15 S_1 secondaries only 2 (13.3%) innervated all three fibre types; 6 (40%) innervated the chains and one bag fibre and 7 (46.7%) innervated the chains only. Even after only 29 days D the secondary restoration was very poor in every case with only a few irregular terminals being formed (Figs. 114, 115, Plate 26; Fig. 117, Plate 27). Overlap with the primary innervation was common after 29 days D (Fig. 117, Plate 27) and 50 days D (Fig. 119, Plate 28; Fig. 123), where of 24 S_1 secondaries examined, 9 (37.5%) produced branches that invaded the P region.

8.8 Motor Reinnervation.

The restoration of the motor endings appeared little affected by the increased periods of denervation. As with the previous

studies, all the spindles that had been reinnervated by sensory axons were also reinnervated by motor axons which gave rise to well-restored and fully recognisable p_1 , p_2 and trail endings. In some spindles, though, there was evidence of disorganisation of the motor innervation in that numerous axons could be traced coursing through the poles and doubling back on themselves.

8.9 Comparison of the Effects of Different Periods of Denervation.

The experimental series that have been described covered a range of denervation periods from 10 to 50 days. The spindles that had been denervated for the shortest time generally showed the best restoration of the sensory innervation although there was not much difference between the NEC (10 days D) and the NS (22 days D) series in terms of the speed or quality of reinnervation.

In both series the first primary axons reached the spindles by 4-5 days RT and by 9-11 days RT most of the spindles examined had primary endings, though there were fewer uninnervated spindles in the NEC (Table 13) than in the NS samples (Table 1) until after the return of the second wave of reinnervation at about 25 days RT in the NS study.

In every spindle examined in the NEC study, provided the primary axon had made contact in the P region, there was successful reinnervation of all the fibre types (Table 14). The same was true in the NS series over the same recovery periods (up to 30 days RT) with one exception (Table 2). After this time some primary endings were observed that failed to reinnervate one of the bag fibres (the NEC study was not continued over these longer time periods). The quality of restoration in the two series also appears to be equivalent in terms of the number of terminal bands on the bag fibres (Tables 4, 15) and the overall length of the primary ending

(Tables 5, 16). Hyperinnervation of the P region, though, was more common in the NS series where 12% of spindles showed hyperinnervation than in the NEC series where only 4.7% of spindles were hyperinnervated. There was a further increase in hyperinnervation after 50 days denervation when 23.8% of the spindles were hyperinnervated.

The restoration of the primary endings after longer periods of denervation showed increasing degrees of disorganisation and a reduction in the marked regional specificities shown in the NEC and NS series. Although there was no increase in the proportion of primaries failing to reinnervate all the intrafusal fibres in either the TC (proximal-site crush) or RC (repeated crush) studies compared with the NS series, there was less regular innervation and fewer bands were visible around the bag fibres after 50 days D than after 22 days D. The main difference between the spindles reinnervated after 50 days D and those reinnervated after shorter denervation periods was the breakdown, in some cases, of the regional restrictions on the disposition of primary axon terminals on the intrafusal fibres. Thus some primary endings were found in which branches of the Ia afferent entered the S_1 region and formed some sort of ending well within the S_1 region.

The pattern of restoration of the secondary endings showed similar trends to that of the primary, with little difference between those on spindles that had been denervated for 10 or 22 days. There was, however, a small difference in the rate of restoration in that although in both cases the secondaries arrived back at the same time (in both cases the first II's returned to the spindle at about 5 days RT) the secondaries in the NEC series were more rapid in their reinnervation of all the fibre types than were the NS secondaries (Tables 7, 18). After longer periods of denervation there

was an increase in the proportion of S_1 secondaries that did not reinnervate all the intrafusal fibre types and also in the proportion of S_1 secondaries that invaded the primary region.

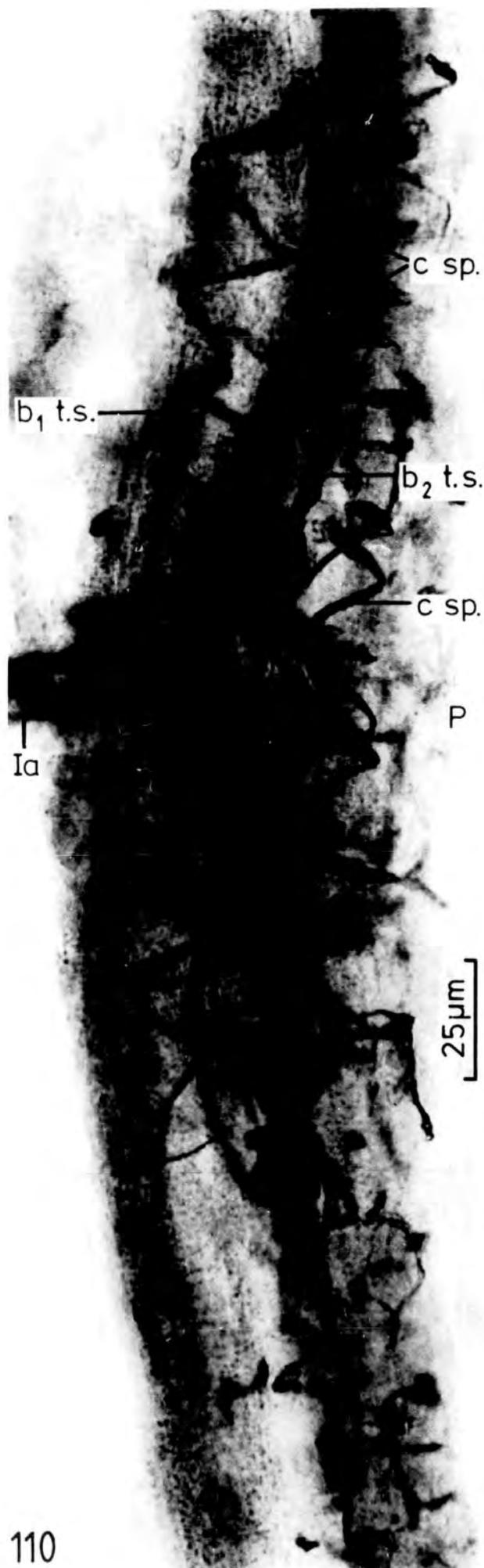
Plate 25.

Photographs of silver-stained primary endings after recovery from a proximal nerve-crush injury (at the point of separation of the common peroneal nerve from the tibial: the TC series). Time scales:- 61 days PC, 25 days RT, 29 days D. Abbreviations as for previous plates.

Figure 110. A reasonably well-restored primary ending with the chains extensively reinnervated. The bag innervation is mainly irregular with the bag₁ receiving only five distinct bands and the bag₂ only two.

Figure 111. A poorly restored, irregular primary ending with minimal visible banding on any fibre, although all fibre types have some innervation.

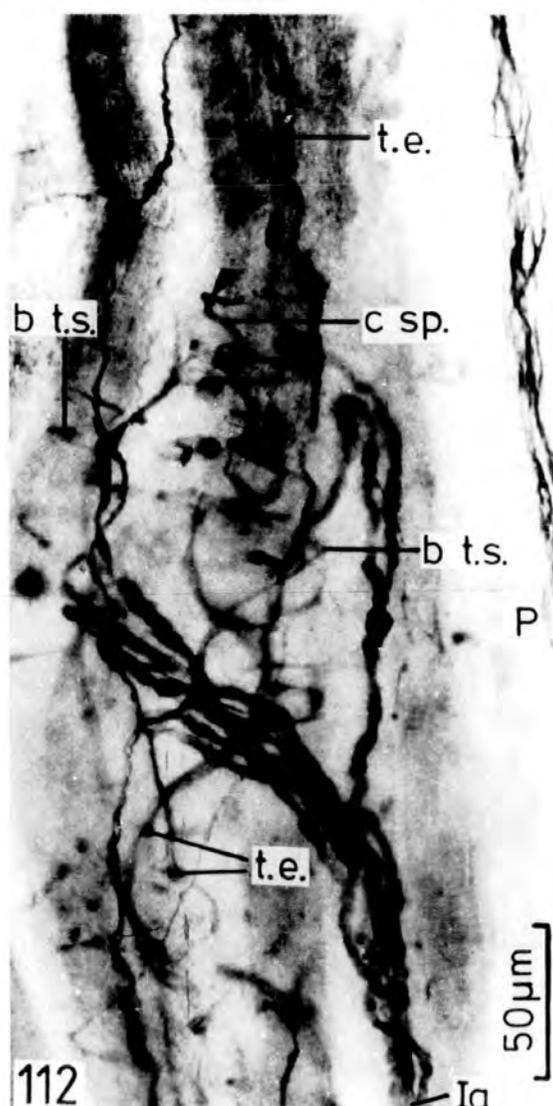
Figure 112. This spindle shows fairly extensive, but poorly organised, primary innervation. There is some regular innervation of the bag fibres but little on the chains. There are several expansions on the axon terminals.



110



111



112

Plate 26.

Photographs of silver-stained primary and secondary endings after proximal nerve injury (TC series). Time scales:- 61 days PC, 25 days RT, 29 days D. Abbreviations as for previous plates.

Figure 113. A very extended primary ending which is largely irregular with little banding on any fibre type.

Figure 114. An S_1 secondary which gives very limited innervation to the intrafusal fibres. The bag_1 shows no reinnervation.

Figure 115. Another S_1 secondary which also only innervates the bag_2 and chain fibres and these with only a few terminals.

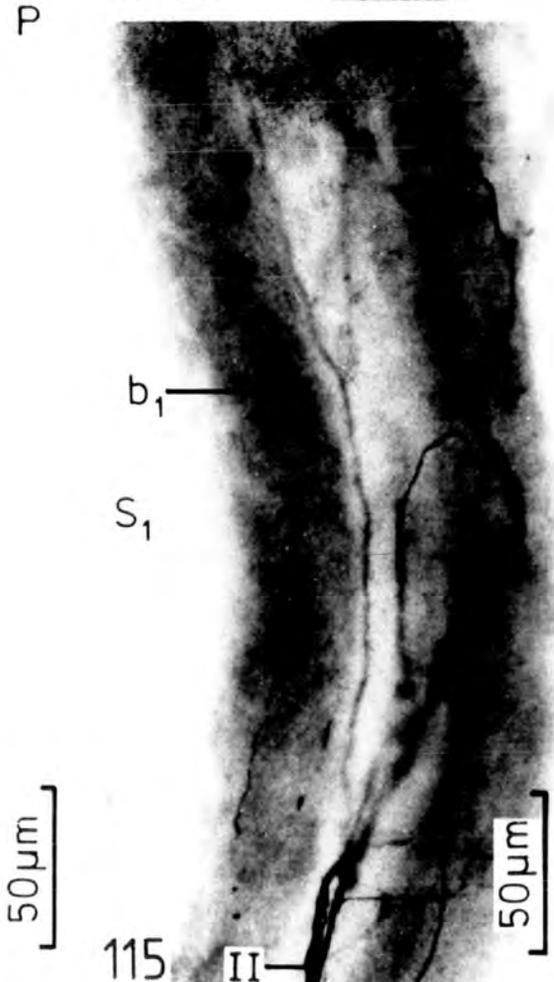
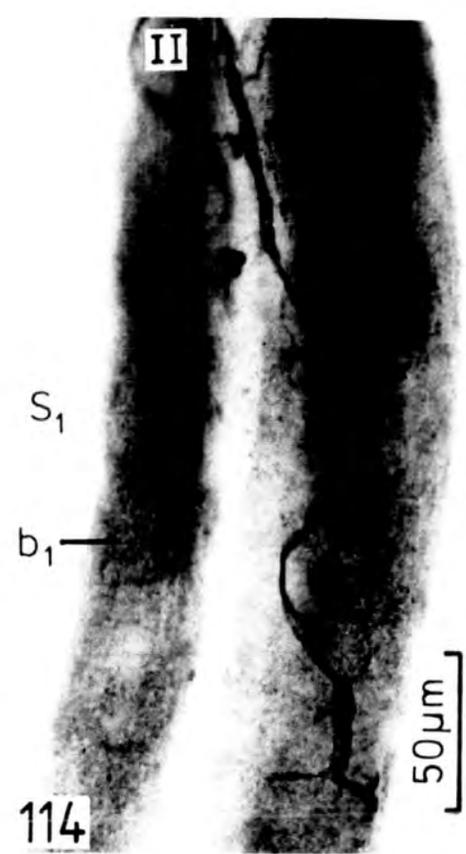
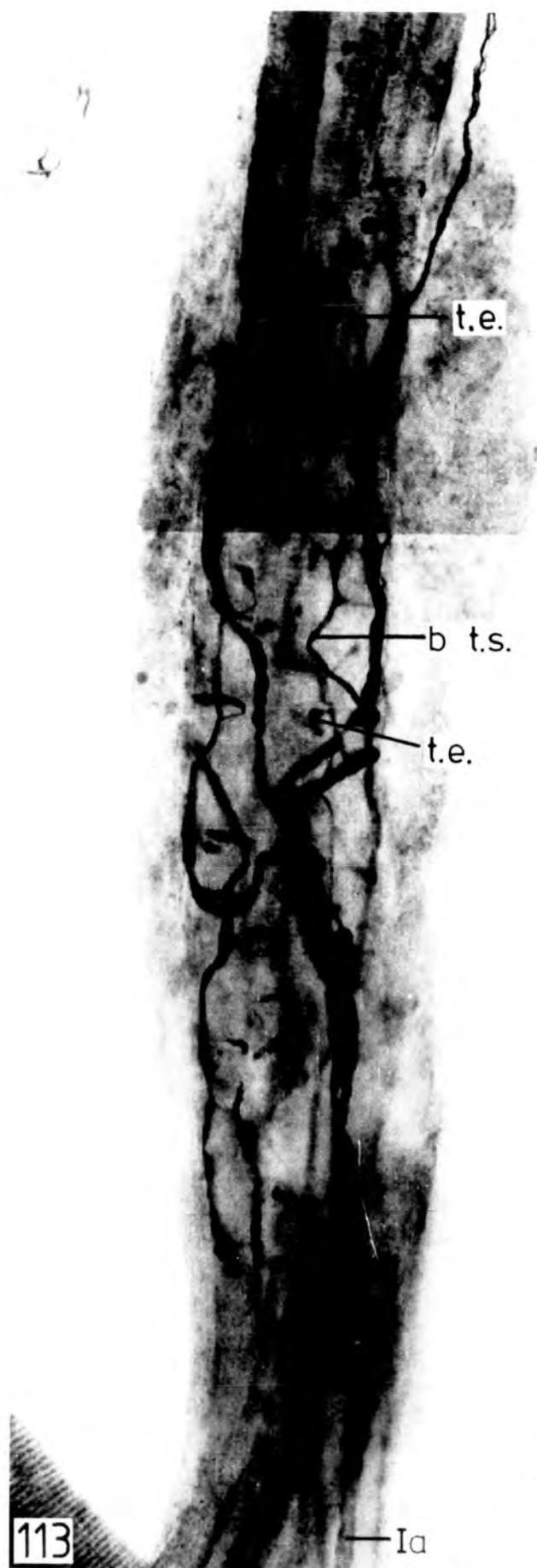
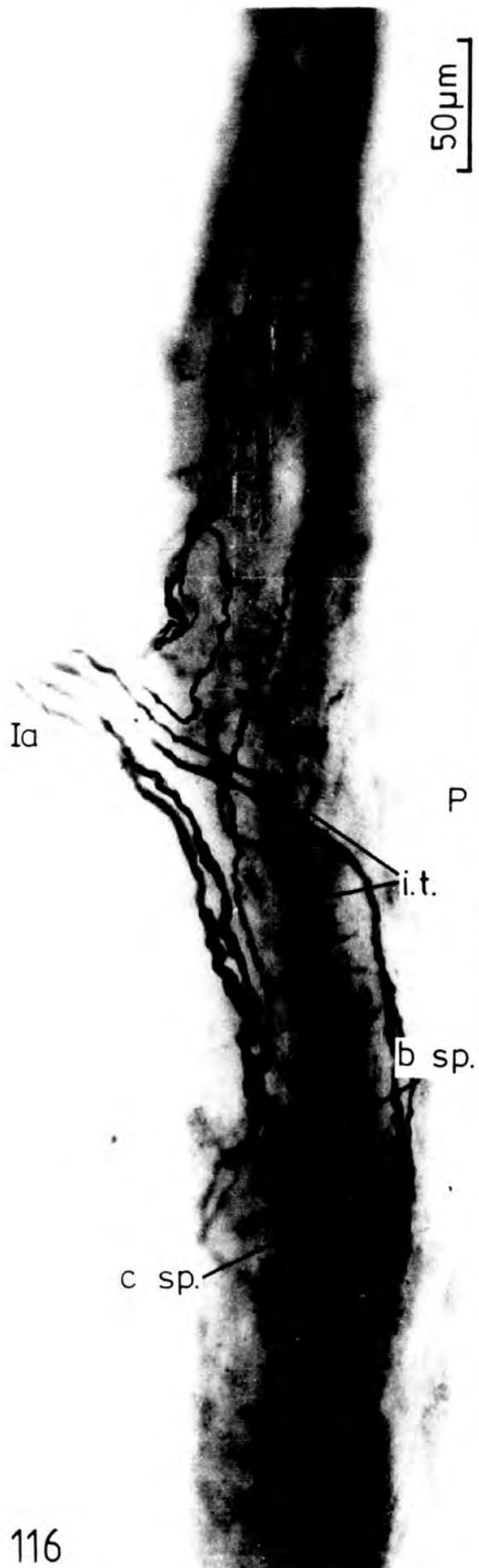


Plate 27.

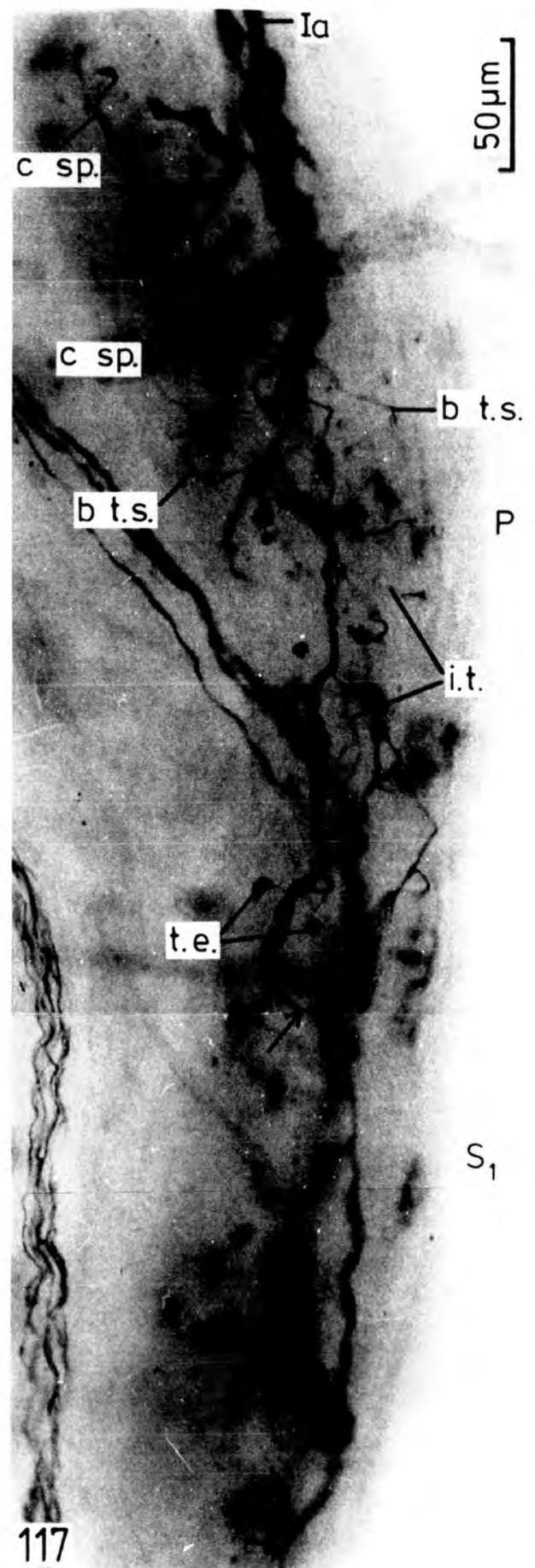
Silver-stained primary and secondary endings at 39 days RT after repeated crushing of the nerve (RC series) to give a denervation period of 50 days. Abbreviations as for previous plates.

Figure 116. An abnormal primary ending which, although extensive, produces little regular innervation on any of the intrafusal fibres. The regular bands that are visible are restricted to the periphery of the ending, the central region being irregular.

Figure 117. A poorly-organised primary ending with little regular banding. One branch (arrowed) enters the S_1 region and overlaps with the S_1 secondary innervation (which was poorly restored) before turning back into the P region. En route it gives off small terminals which end in ball-like expansions.



116



117

Plate 28.

Silver-stained primary endings at 39 days RT after 50 days denervation (RC series). Abbreviations as for previous plates.

Figure 118. A relatively well-restored primary ending with 7 bands on each of the bag fibres. One branch (arrowed) leaves the P region and invades the lower S_1 region where it forms some irregular terminals on the chain fibres.

Figure 119. The primary ending is mainly irregular and disorganised but does supply terminals to all the intrafusal fibres. The S_1 secondary is also poorly organised and there is a long region where it overlaps with the lower end of the primary ending.

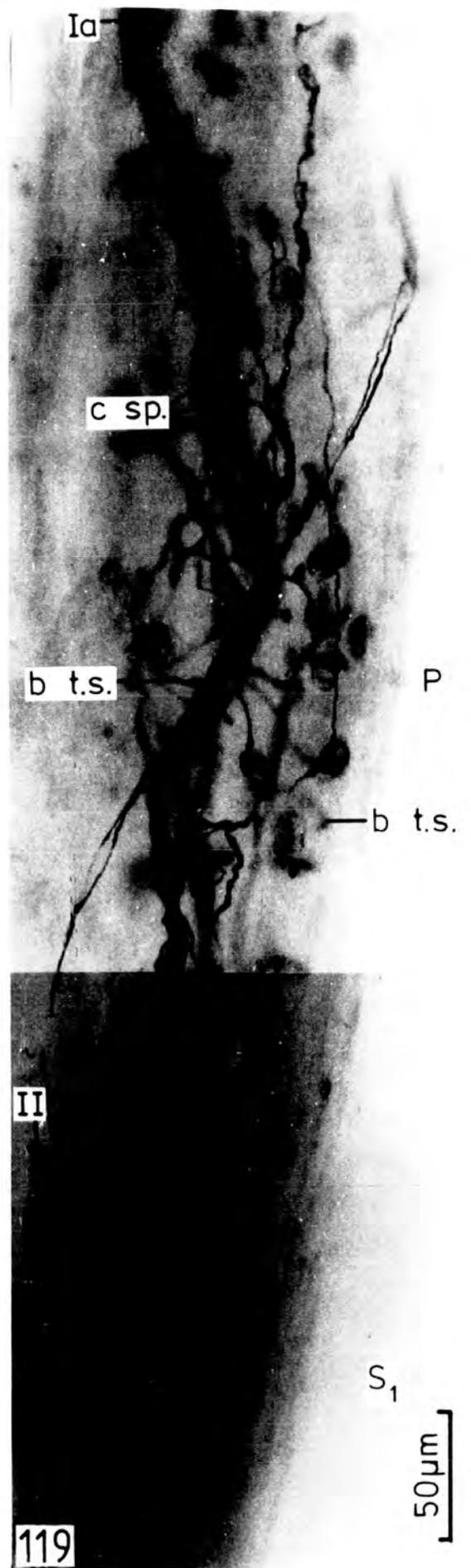
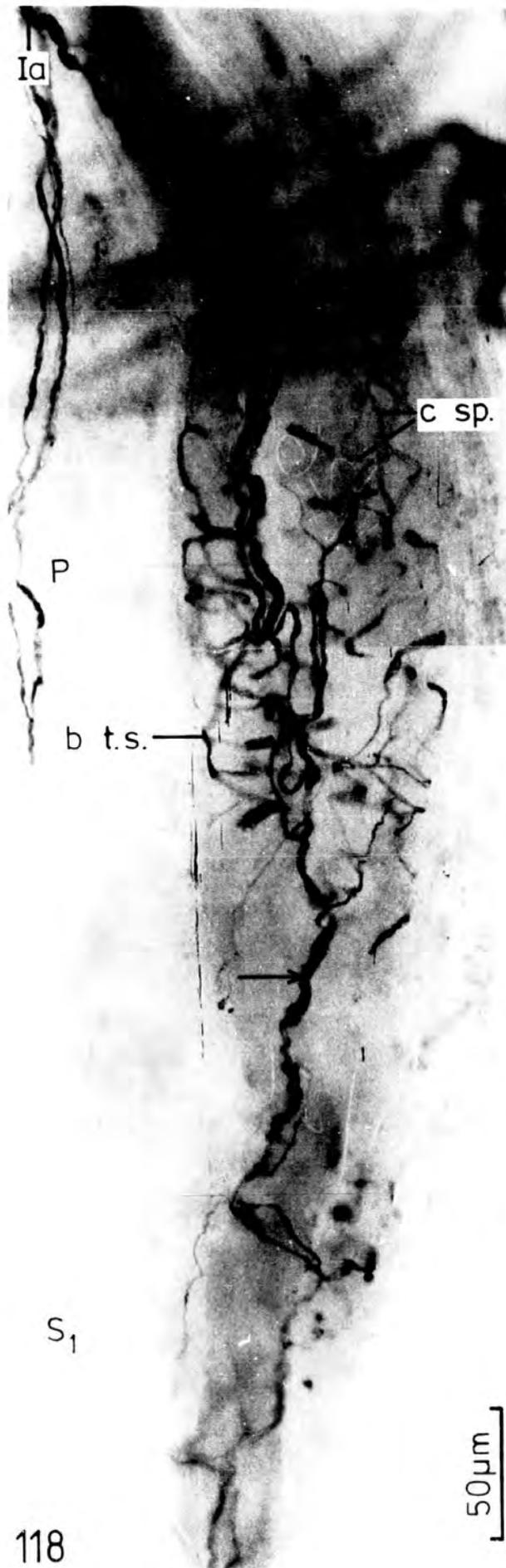


Plate 29.

Photographs of silver-stained primary endings at 39 days RT after 50 days denervation (RC series). Abbreviations as for previous plates.

Figure 120. All the fibres are reinnervated by the primary but only the chain fibres show regular banding, the rest of the ending being irregular. One branch leaves the P region and then doubles back to produce terminals on the chain fibres.

Figure 121. A well-restored primary with regular spirals on both bag fibres (9 on the bag₁ and 10 on the bag₂) bounded by more irregular regions.

Figure 122. The primary ending shows good restoration, though the ending is relatively small.

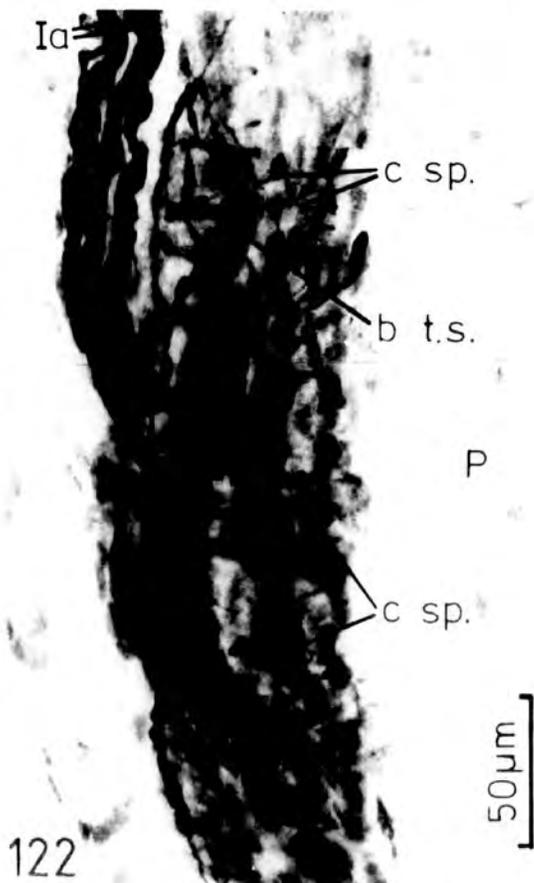
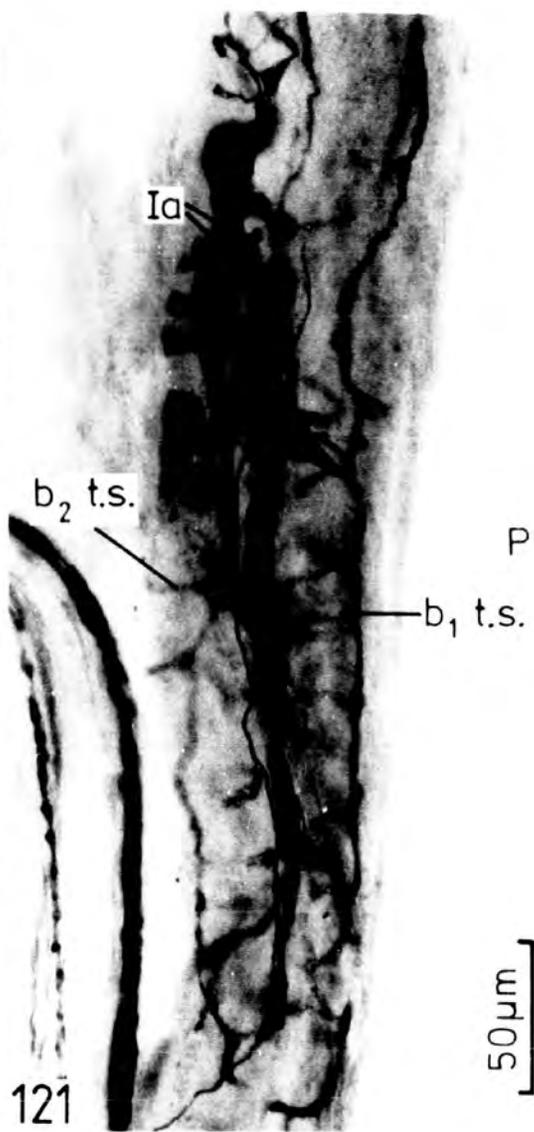
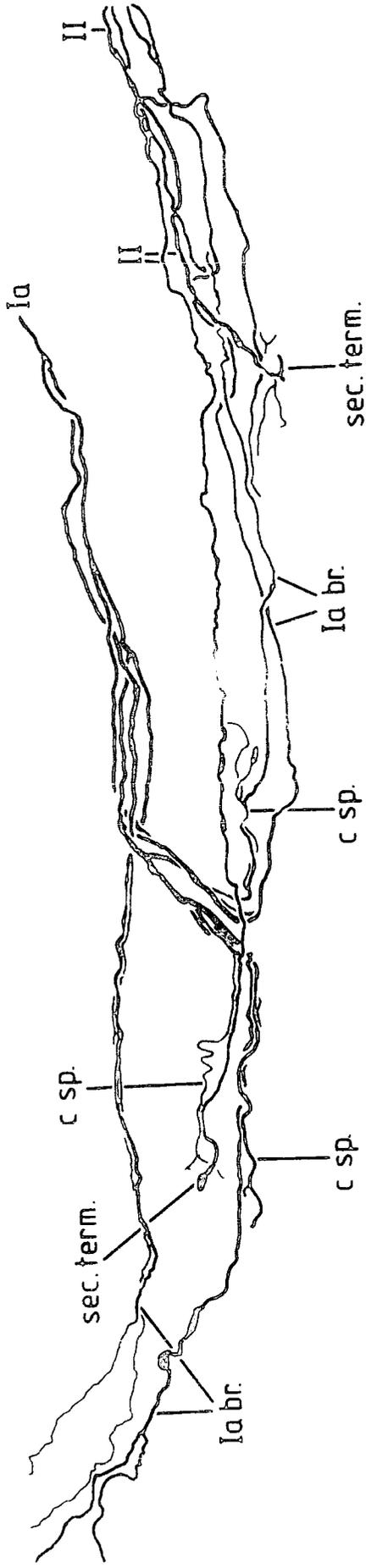


Figure 123. A camera lucida drawing of a silver preparation of a primary and secondary endings at 39 days RT after 50 days denervation (RC series). There appears to have been almost a complete breakdown of the regional specificity with branches of the primary invading both S_1 regions and branches of the S_1 secondary invading the P region. The only spiral endings produced by the primary lie on the chain fibres, all the other branches run in parallel with the intrafusal fibres with little obvious attempt at forming endings.



S₁

P

S₁

100 μm

9.1 Muscle-spindle Physiology After Reinnervation Following Different Periods of Denervation.

A number of procedures was employed to examine the effect that the period of denervation has on the quality of reinnervation (sections 5.2-5.4). Using these procedures the effects of denervation and subsequent reinnervation periods were examined physiologically over the following time periods:-

Expt'l series	Denervation period (Days D)	Reinnervation period (days RT).
NEC	10	5-30
NS	22	4-118
TC	29	25-32
RC	29-50	39

9.2 The NEC series Afferent Conduction Velocities.

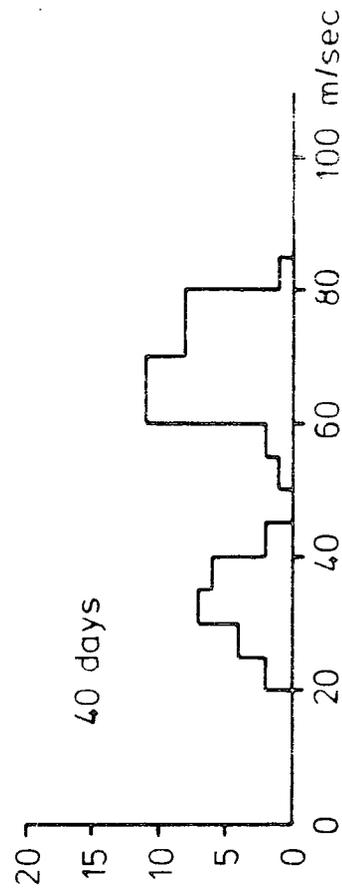
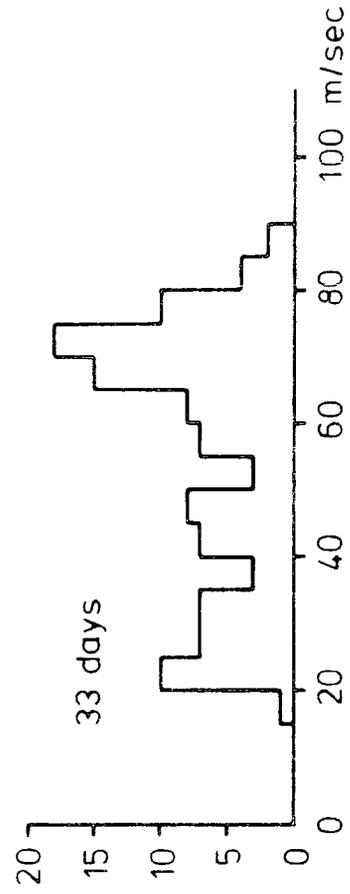
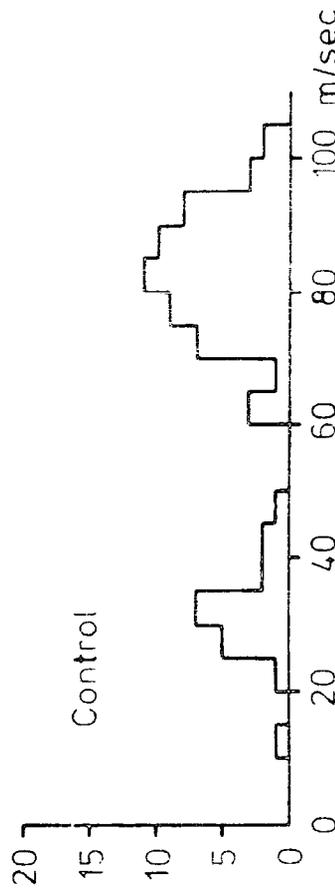
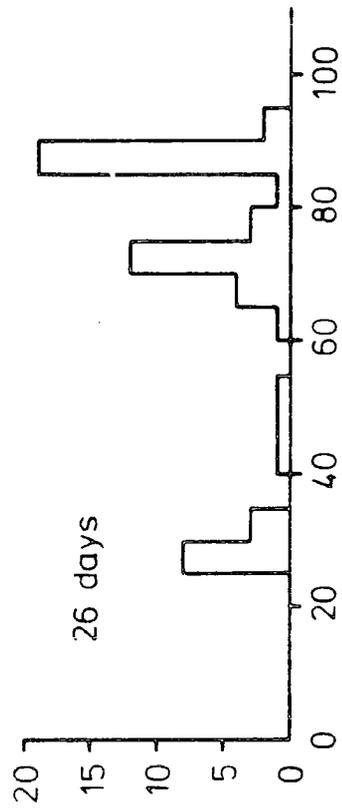
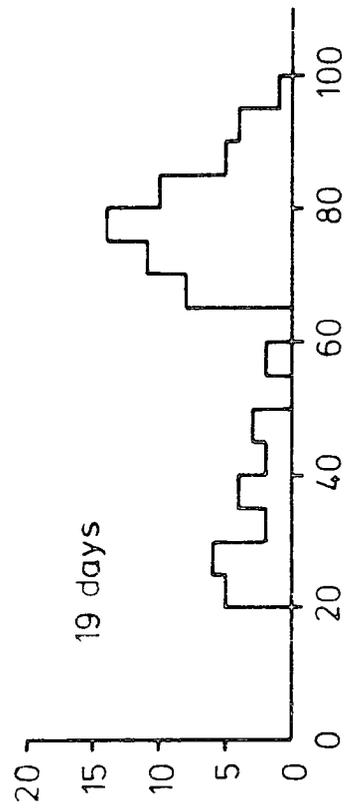
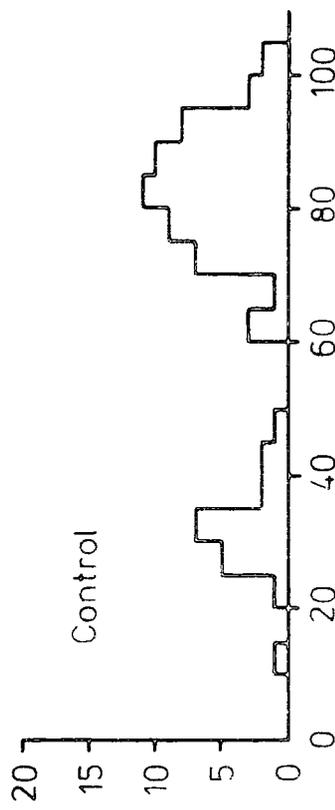
The first responsive units were found at 5 days RT but they were sufficiently rare to make it impractical to try to record a significant number for analysis.

The CV's of the functional afferents from 19-40 days PC (9-30 days RT) are shown in Fig. 124. Because the lesion was made very close to the muscle the stimulating electrodes on the muscle-nerve were placed central to the injury site. These CV histograms, therefore, cannot be compared with those from the other series of experiments because the measurements were only made over the proximal segment of the nerve.

The histograms in Fig. 124 show that over the proximal segment there was initially only a small reduction in the afferent CV's. At 19 days PC (9 days RT) and 26 days PC (16 days RT) the mean CV's were $64.7\text{m/sec} \pm 2.48$ and $69.7\text{m/sec} \pm 3.36$, respectively,

Figure 124. The conduction velocities of regenerated primary and secondary muscle-spindle afferents after increasing periods of recovery following a nerve=entry nerve=crush injury (NEC series). The times are given in terms of days PC and the spindles were denervated for about ten days. Because the injury site was close to the muscle the stimulating electrodes had to be placed on the muscle=nerve central to the lesion and so the CV measurements are for the proximal segment of the nerve only.

Numbers of Afferents



compared with a mean of $69.5\text{m/sec} \pm 2.21$ for the afferents from the unoperated control animals. Over the subsequent recovery periods there was a reduction in the afferent CV's (Fig. 124). At 33 (23 days RT) and 40 days PC (30 days RT) the mean CV's were $55.6\text{m/sec} \pm 1.92$ and $56.2\text{m/sec} \pm 2.26$, respectively, these being significantly different from the controls ($P < 0.001$, Student's 't' test). This trend of falling CV's is the reverse of that occurring in the distal segment of the nerve (Fig. 66).

9.3 Response Classification.

The proportions of the four classes of afferent response in the afferent populations after the different recovery periods are given in Table 20. It is apparent that recovery of class 4 characteristics was a relatively rapid process so that by 30 days RT there were no class 1 or class 2 units in the sample and 58.7% of the afferents were class 4 in nature. Even at 9 days RT, 41.1% of the afferents were class 4.

At 25 days RT there was a large increase in the proportion of class 1 and 2 afferents in the population. This is similar to the increase in class 1 and 2 units observed at 25 days RT in the NS series and reflects the return of the second wave of reinnervation. The second wave was not evident from the histological analysis of the NEC material (section 8.2) but this absence was probably due to the relatively small size of the sample examined at this period.

Comparison of the proportions of abnormally responding afferents in the NS and NEC series after corresponding reinnervation times shows that the NEC afferents recovered normal function much more rapidly (Tables 9, 20). Although the first functional

Table 20.

The proportion of the four classes of regenerated spindle afferent in the populations of the afferents from the nerve-entry crush (NOC experiments) after different periods of recovery. The proportions are expressed as a percentage of the total population.

Days PC	Days RT	Classes of afferent				No. of units
		1	2	3	4	
19	9	3.8	19.2	35.9	41.1	78
26	16	0.0	8.5	57.4	34.1	47
33	23	18.4	12.8	41.3	27.5	109
40	30	0.0	0.0	41.3	58.7	63
Controls		0.0	0.0	10.2	89.8	89

Table 21.

The mean peak and held firing rates (imp/sec) of the NEC series afferents with their standard errors for a 10mm/sec ramp stretch.

Class	Total no.	PFR	HFR	Dynamic index
1	23	65.1 \pm 11.9	0.0	>65.1
2	32	119.9 \pm 12.4	25.4 \pm 3.94	93.7
3	126	114.3 \pm 6.38	43.9 \pm 1.92	70.4
4	115	129.5 \pm 5.93	59.2 \pm 2.17	70.3
Controls	87	137.6 \pm 4.48	69.4 \pm 2.28	68.2

afferents were observed at about 4 days RT in both cases, by 30 days RT in the NEC series 58.7% of afferents were class 4 in nature compared with 14.9% at 39 days RT after crushing at the knee (the NS series). Whereas the class 1 afferents were absent from the NEC populations by 30 days RT, they were still present in the NS study at 84 days RT.

9.4 Fusimotor Stimulation.

In two experiments at 9 days RT in the NEC series ¹⁵ functional γ axons were isolated and tested against the afferent units. As with the NS series, both γ_s and γ_d axons were isolated and appeared to function normally, as far as normal function can be inferred from their effects on the afferent responses. All classes of afferent were activated by γ axons and no γ axons were isolated that had different effects on afferents from different spindles.

9.5 Firing Rates.

The mean peak (PFR) and held (HFR) firing rates of the four classes of afferent in the NEC series are given in Table 21 and can be compared with those from the NS series (Table 11). There is a marked similarity between the average firing rates of the corresponding afferent classes from the two populations and at no point is there any significant difference between the corresponding classes.

The NEC afferents also show the same consistency in the dynamic indices of the different classes of afferent. There is, however, some variation with the class 2 units which have a DI of 93.7imp/sec compared with 70.4 and 70.3imp/sec for the class 3 and 4 units, respectively. This variation is attributable to the presence of a greater proportion of highly dynamic units than were

present in the NS populations at the equivalent periods. The results given in Table 21 also give greater weighting to these units since the last recordings were made at 30 days RT rather than allowing recovery to continue for up to 118 days RT as in the NS series.

The data is expressed in the form of a histogram (Fig. 125) which can be compared with Fig. 90 for the NS series (note the different scales). In both series of experiments there were some extremely dynamic units, some of which reached PFR's of about 400imp/sec (Fig. 125). Two class 4 units that fired at more than 400imp/sec had dynamic indices of 290 and 255imp/sec compared with the average index for class 4 afferents of 70.3imp/sec (Table 21).

The majority of these highly dynamic units were found during the early stages of recovery and disappeared quite rapidly from the afferent populations (Figs. 91, 126). The presence of such units in the population greatly elevates the mean PFR's (Table 22), so that at 9 days RT in the NEC series the mean PFR was 177.0 ± 10.04 compared with 137.6 ± 4.48 for the controls. Despite the large standard errors associated with such a wide dispersal of data points, there is a significant difference between these two populations ($P < 0.002$, Student's 't' test).

In the control animals only 6.5% of the afferents reached a PFR in excess of 200imp/sec for a 10mm/sec ramp stretch. In the NEC experiments, at 9 days RT, 35% of afferents exceeded this figure and at 4 days RT in the NS series 34.5% fired at more than 200imp/sec, though by 11 days RT this had dropped to 8.7%. At 16 days RT only 10.6% of the NEC afferents still exceeded 200imp/sec at the peak of the ramp.

Gamma static axons were isolated which activated several of these highly dynamic afferents and the responses obtained

were characteristic of δ_s activation; no δ_d axons were found that would affect the responses of these units.

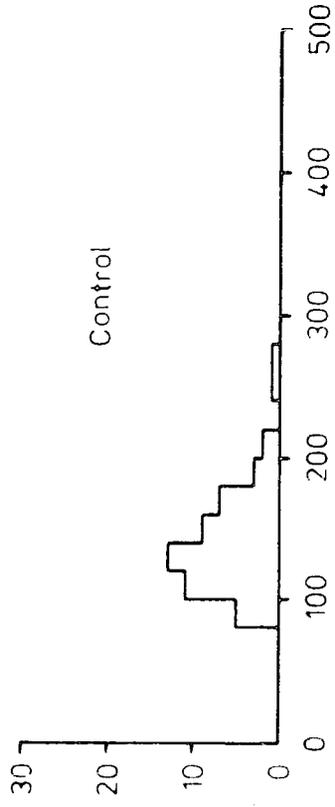
The response records to ramps of three velocities (2.5, 5, and 10mm/sec) for two of these highly dynamic afferents are shown in Fig. 127. Both these units display a clear break in firing at the peak of the two faster ramps. Although this break was not universal for this type of unit, it was very common, especially for those that reached very high firing rates.

The break for unit B (Fig. 127) occurs immediately after the peak of the ramp, at the same time as the normal post-dynamic undershoot of a primary ending (Fig. 73). Unit A shows a different response in that the break occurs before the peak of the ramp is reached. The PFR of Unit A for the slow ramps was 255imp/sec and this is the peak reached in the two faster ramps as well, with firing apparently being cut off at this point in both cases. This is very different from the greatly increased response of Unit B to increased ramp velocity.

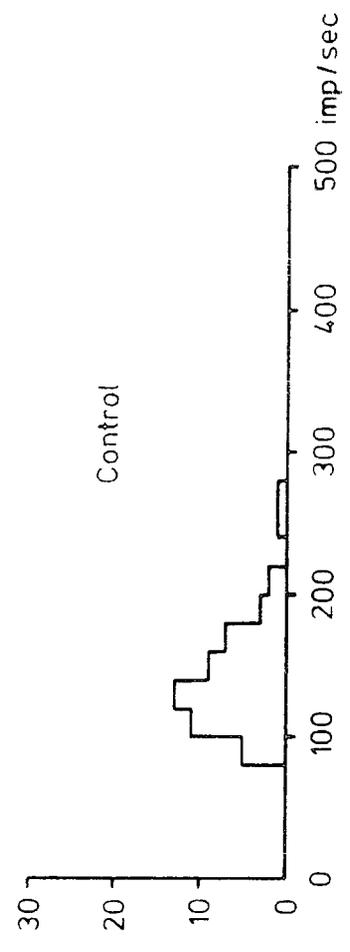
As recovery progressed there was a reduction in the excitability of the highly dynamic units leading to a corresponding reduction in the mean firing rates (Table 22). This decline followed the same time course as in the NS series (Table 12) so that at about 25 days RT (the time of arrival of the second wave) the mean PFR's of both populations reached their lowest point, being $83.7\text{imp/sec} \pm 3.67$ and $88.7\text{imp/sec} \pm 3.7$ for the NEC and NS series, respectively. The PFR's of the NS series remained at this level till after 53 days RT; the firing rates of the NEC afferents, however, showed an immediate recovery back towards normal levels.

Thus the spindles of the NEC series that were denervated for only 10 days appeared to recover to normal function, both in

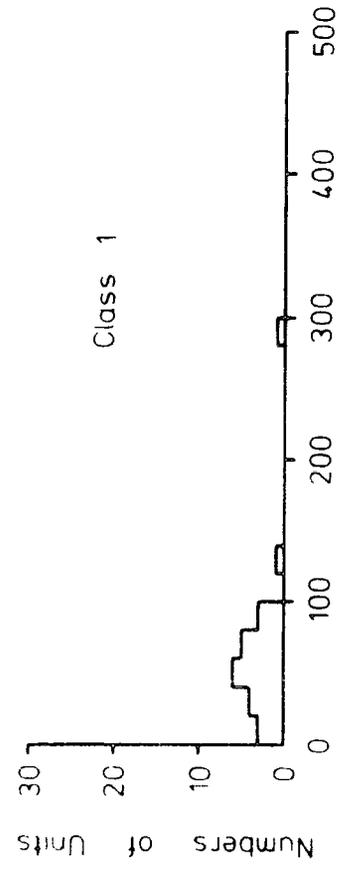
Figure 125. The peak firing rates (PFR's) of the four classes of afferent present in the afferent populations over the complete range of recovery periods after nerve-entry crush injury (NEC series). The ramp velocity was 10mm/sec. Data for primary and secondary afferents has been combined.



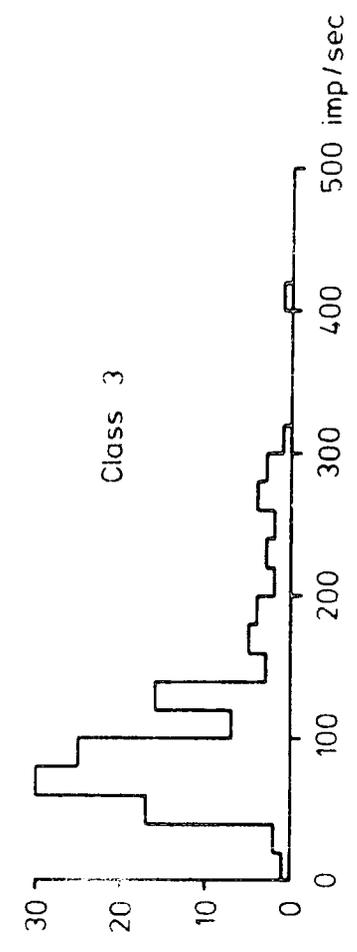
Control



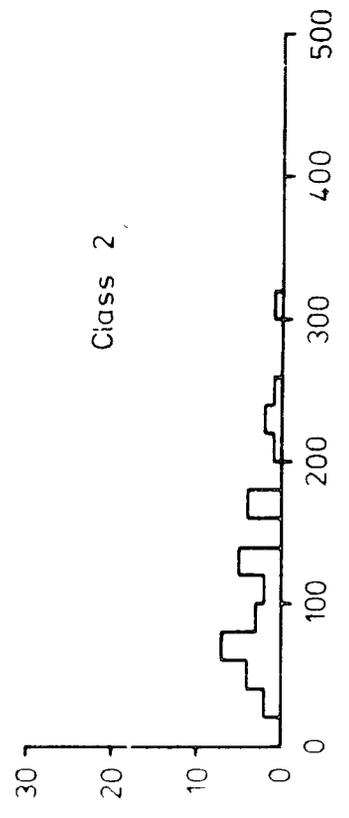
Control



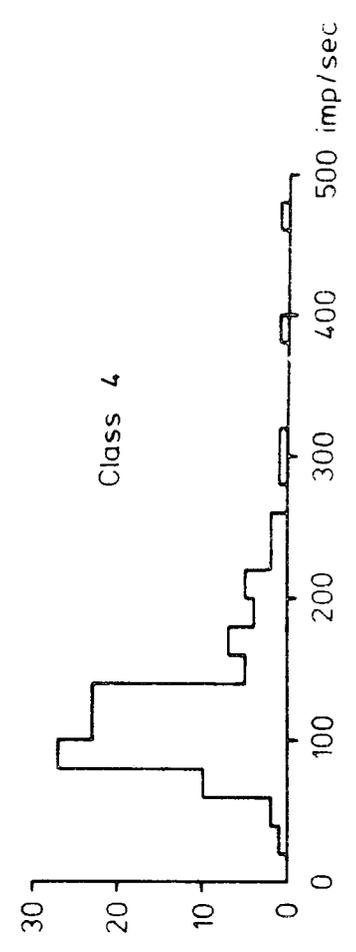
Class 1



Class 3



Class 2



Class 4

Table 22.

The mean peak firing rates (imp/sec) of the NEC regenerated afferents after different periods of recovery. The data for primary and secondary afferents has been combined.

Days PC	Days RT	PFR	No. of units
19	9	177.0 \pm 10.1	77
26	16	111.1 \pm 8.35	47
33	23	83.7 \pm 3.67	107
40	30	106.8 \pm 4.79	63
Controls		137.6 \pm 4.48	87

Figure 126. The peak firing rates (PFR's) of regenerated primary and secondary afferents after different recovery in the NEC series of experiments. The recovery times are given in days PC. Ramp velocity was 10mm/sec and data for primary and secondary afferents have been combined.

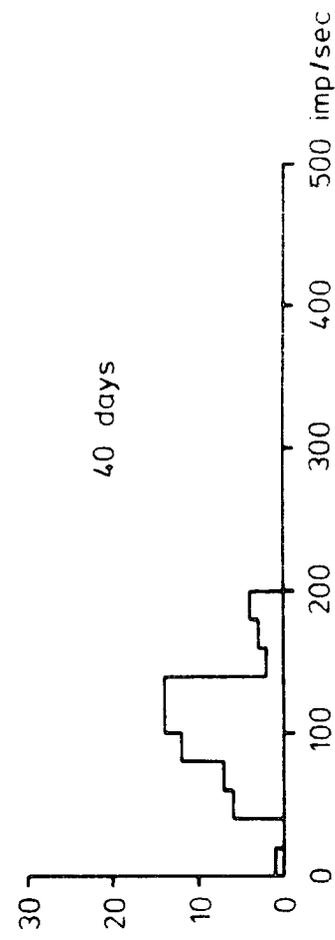
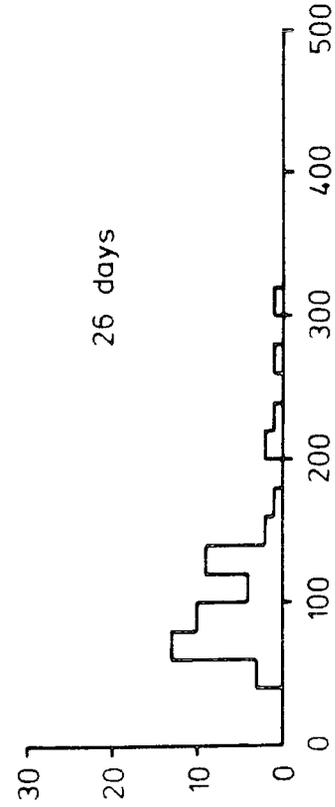
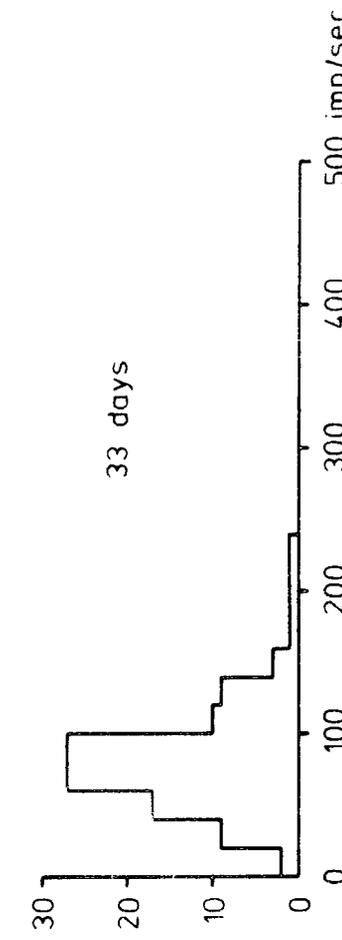
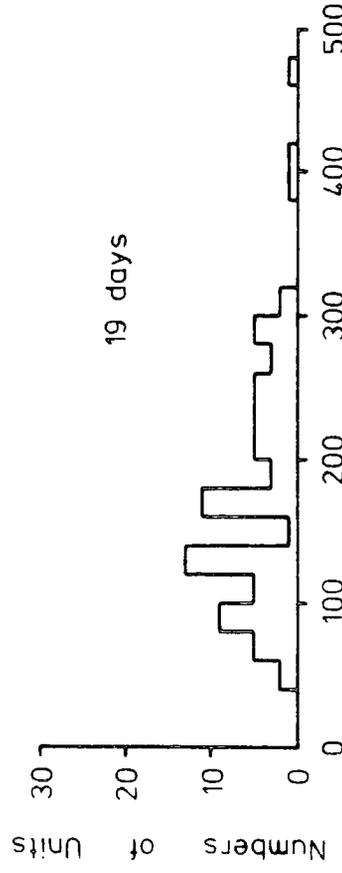
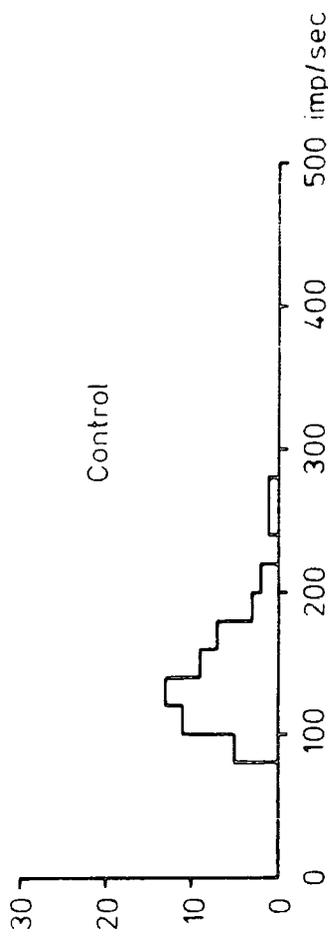
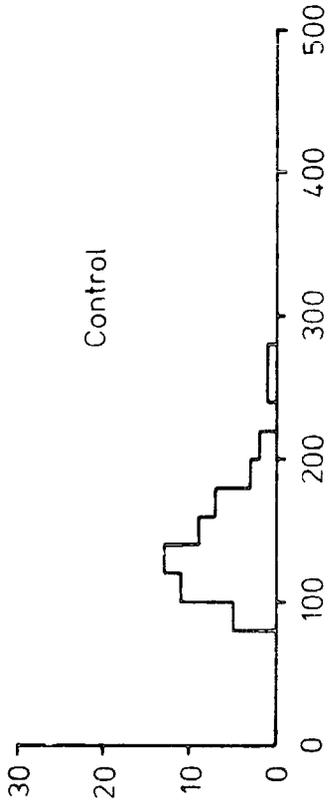
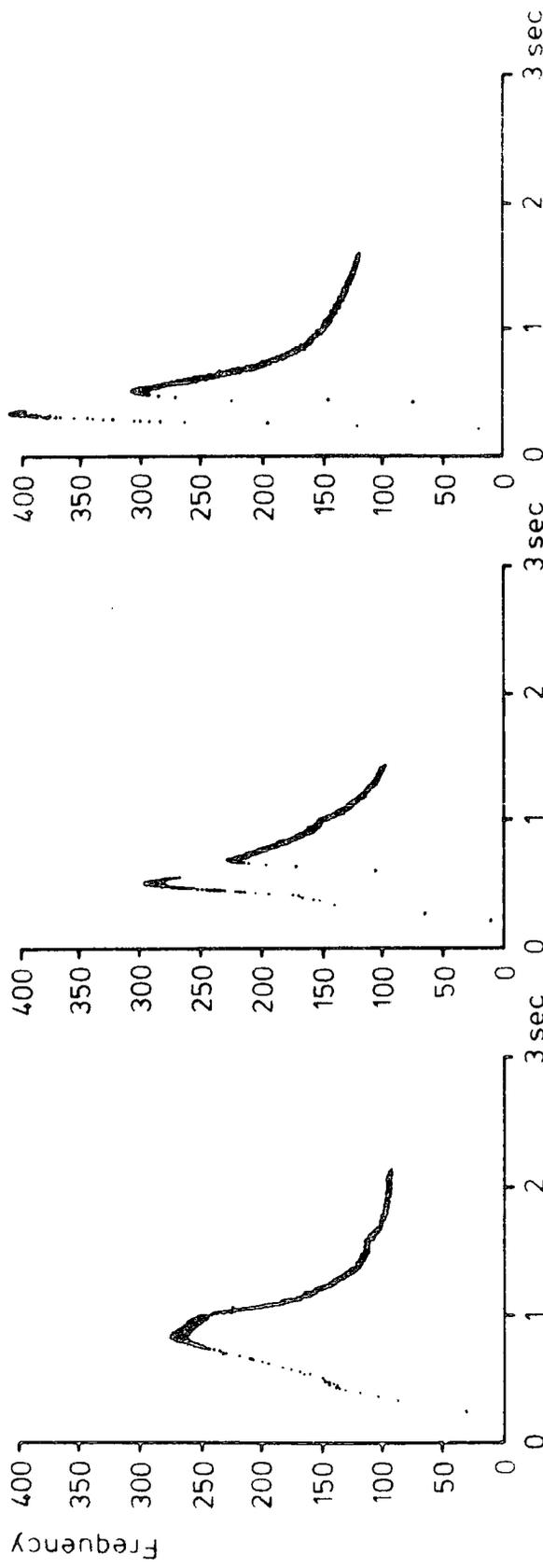
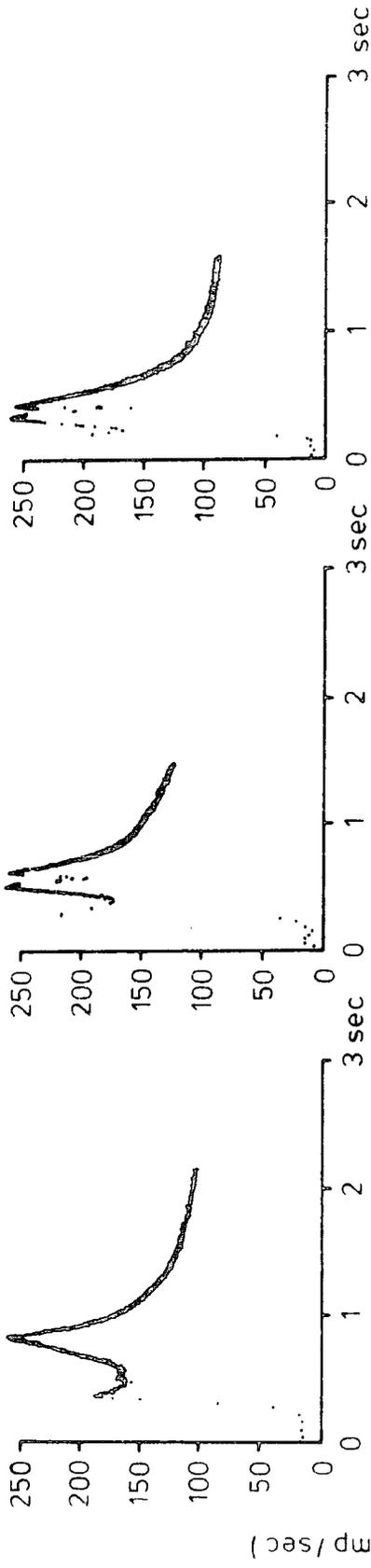


Figure 127. The instantaneous frequency records of two highly dynamic muscle-spindle afferents from the NEC series. The ramps were applied at three velocities: - 2.5, 5 and 10mm/sec; the stretch was 1.8mm in amplitude with a hold of 1sec before the release.



terms of classification of their responses and in their peak firing rates, substantially faster than those of the NS series that were denervated for 22 days.

9.6 Conduction Velocities of Delayed-return Afferents.

The afferent CV distributions following recovery from the longer periods of denervation are shown in Figs. 128 (RC series) and 129 (TC series). All the mean CV's are significantly different from the normal controls ($P < 0.001$, Student's 't' test). There is, however, no significant difference between the CV distributions of the NS series and those of even the longest denervation periods after comparable periods of reinnervation, although there is a very slight progressive decrease in the CV's of the repeat-crush afferents with increasing denervation time (Fig. 128). At 39 days RT after 22 days denervation (the NS series) the mean afferent CV was $50.6\text{m/sec} \pm 2.0$ (S.E. of mean); after 29 days D (at 39 days RT) the mean CV was $48.5\text{m/sec} \pm 1.8$; the mean CV for 36 days D was $46.7\text{m/sec} \pm 2.1$ and for 50 days D it was $44.8\text{m/sec} \pm 2.52$. Although these differences are not significant, the trend appears to be consistent.

In the case of the TC series (Fig. 129) there is a significant difference ($P < 0.001$, Student's 't' test) between the afferent CV's after 22 (NS) and 29 (TC) days D with a reinnervation time of 25 days in each case. This is attributable to the increased length of the distal segment of the nerve in the TC series because the crush was carried out at a more proximal site (in the NS series some 13% of the total nerve length used for CV measurements was distal to the crush site, whereas for the TC series it was 25%). At 32 days RT (29 days D) the TC afferents showed the expected increase in mean CV compared with 25 days RT

(36.7m/sec \pm 1.52 and 33.8m/sec \pm 1.31, respectively. This can be compared with 44.1m/sec \pm 1.27 for 25 days RT (22 days D) for the NS series).

9.7 Response Classification.

The proportions of the four classes of regenerated afferent unit in the afferent populations after the different periods of denervation are given in Table 23. For the purposes of comparison the results are quoted both in terms of the denervation periods (days D) and of the recovery periods (days RT).

After the 23-25 days RT periods there is no marked difference between the spindles that have been denervated for 10, 22 or 29 days, although if anything, the afferents in the NS series (22 days D) appear to be the slowest to recover, with fewer class 4 units than both the TC and NEC series and more class 1 units than the TC population (29 days D).

After the 30-32 days RT periods the more rapid recovery shown by the NEC afferents becomes apparent compared with the TC afferents which have progressed more slowly.

At 39 days RT the most striking feature is the apparent absence of any adverse effects as a result of increasing the denervation periods from 22 to 50 days. In the RC series there is a slight trend towards slower recovery with increasing denervation time which is apparent in the increase in numbers of class 1 units; however, the NS afferents after only 22 days would appear to be the slowest to recover.

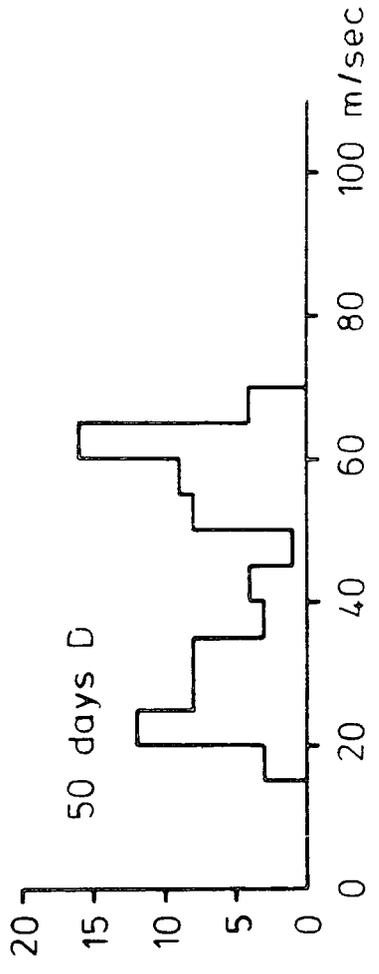
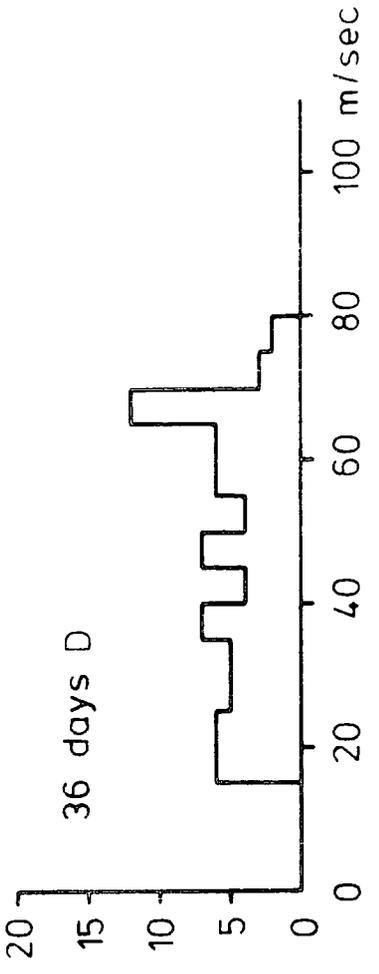
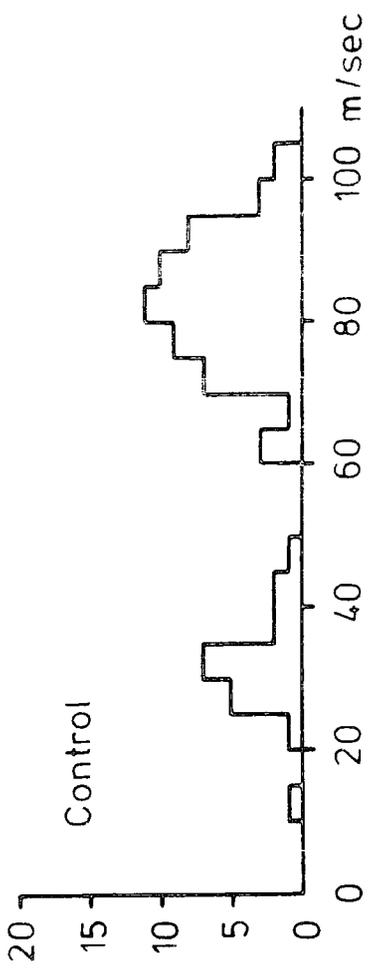
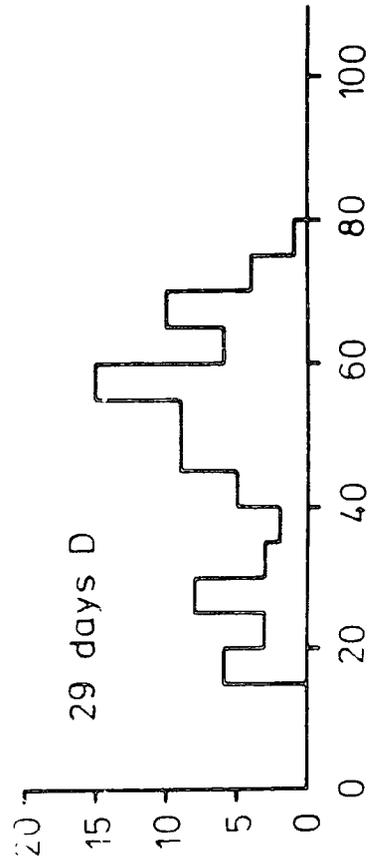
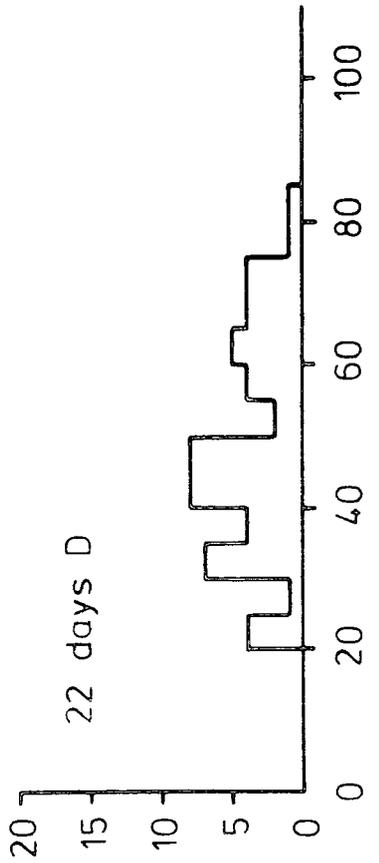
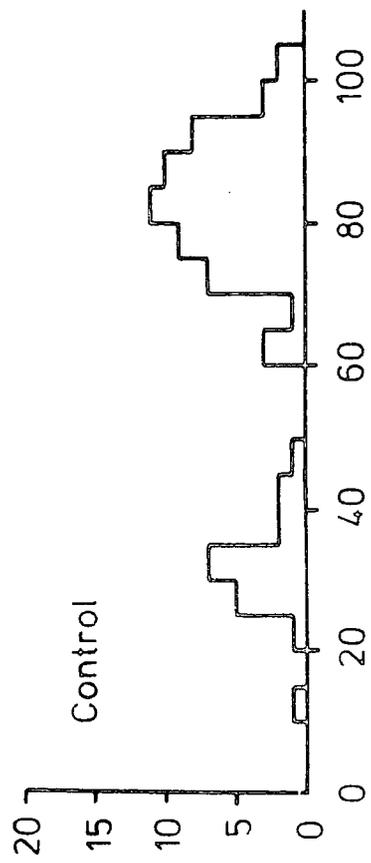
9.8 Firing Rates.

The mean peak firing rates (PFR's) are given in Table 24 in terms of denervation and reinnervation periods and the data is presented in histogram form in Figs. 130, 131. The striking feature

of these results is their close similarity despite the variations in denervation time. The only results that show any difference are those of the NEC series which show an increase in the mean PFR's at 30 days RT. This marks their more rapid recovery of normal function compared with the afferents of the NS and longer denervation series.

Any adverse effects due to the increased periods of denervation after 22 days are nearly negligible as far as the amplitude of the response to a given mechanical stimulus is concerned. There is a very small trend towards lower PFR's in the RC series (Table 24) but the difference is not significant, as is also evident from the histograms in Fig. 131.

Figure 128. The conduction velocities of regenerated spindle afferents after different periods of denervation (days D), achieved by repetition of the crush injury (the RC series), compared with the data from the NS series (22 days D) and from the unoperated control afferents. All the recordings of the regenerated afferents for this figure were made at 39 days RT.



Numbers of Afferents

Figure 129. The conduction velocities of regenerated spindle afferents after recovery from different periods of denervation, achieved by making the lesion more proximally (the TC series), compared with the data from the NS series and the unoperated controls. Note that because the lesion of the two 29 days D populations was made more proximally this increased the ratio of distal segment : proximal segment over the length of nerve used for these measurements.

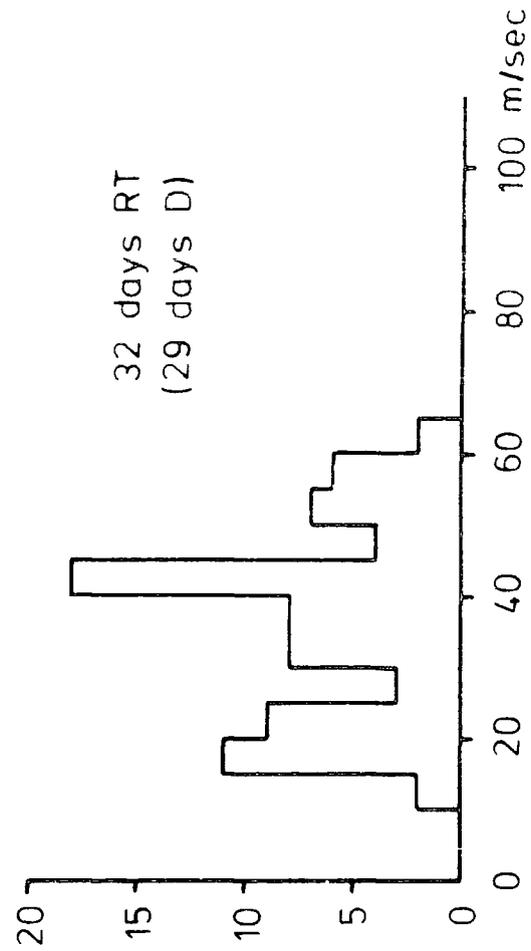
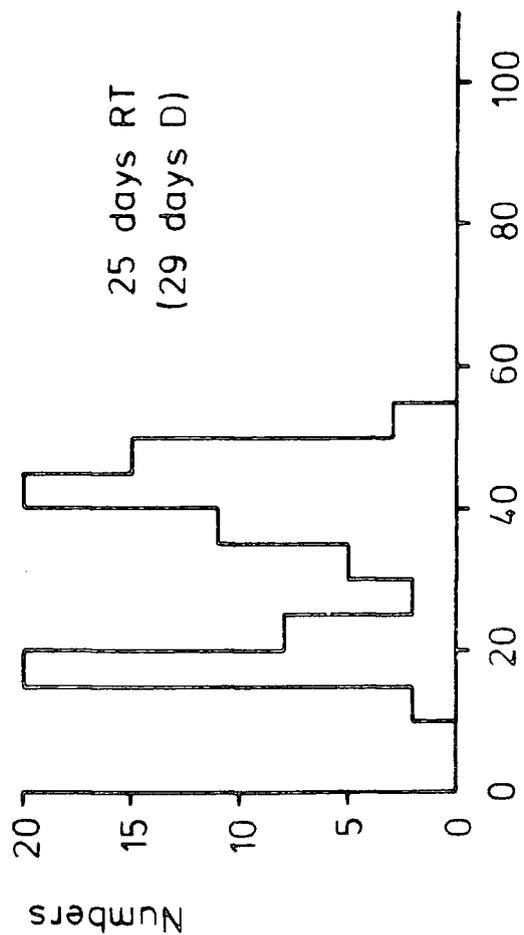
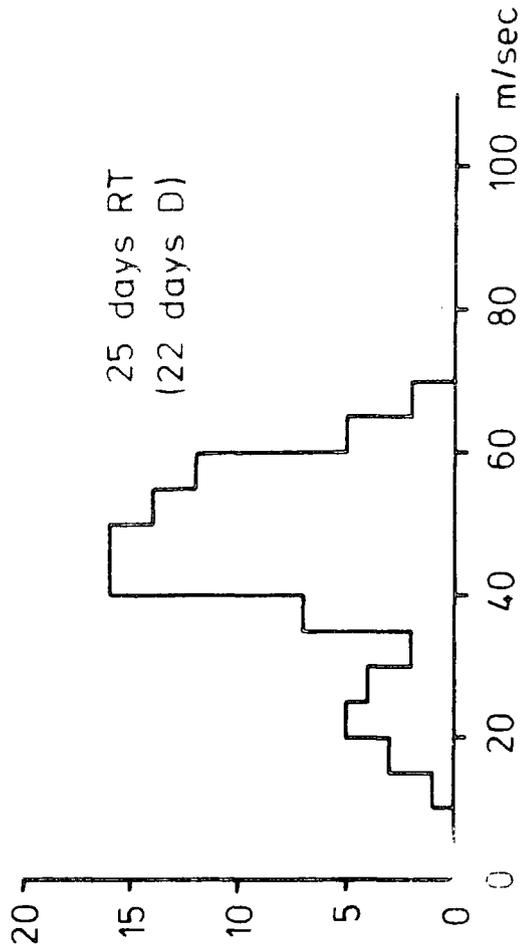
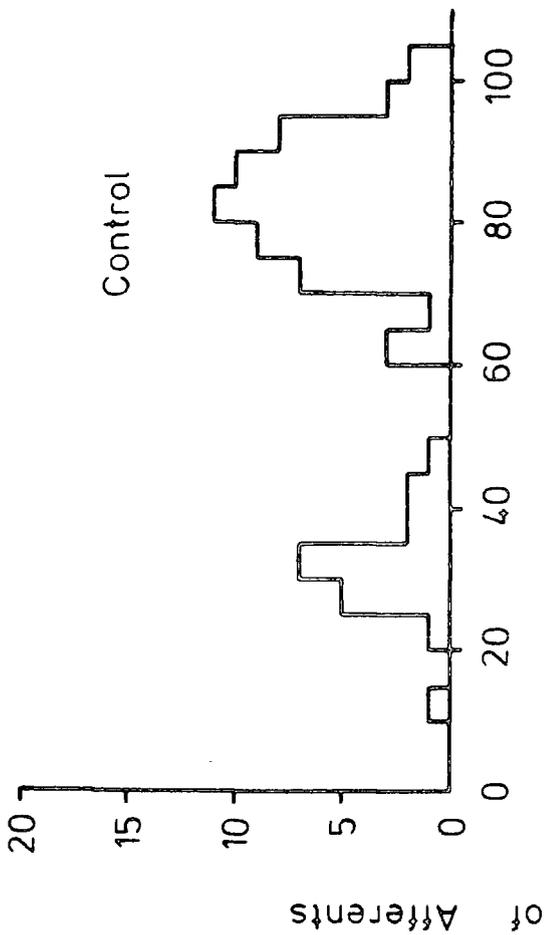


Table 23.

The proportions (expressed as a percentage of the total population) of the four classes of regenerated afferent in the populations after different periods of denervation.

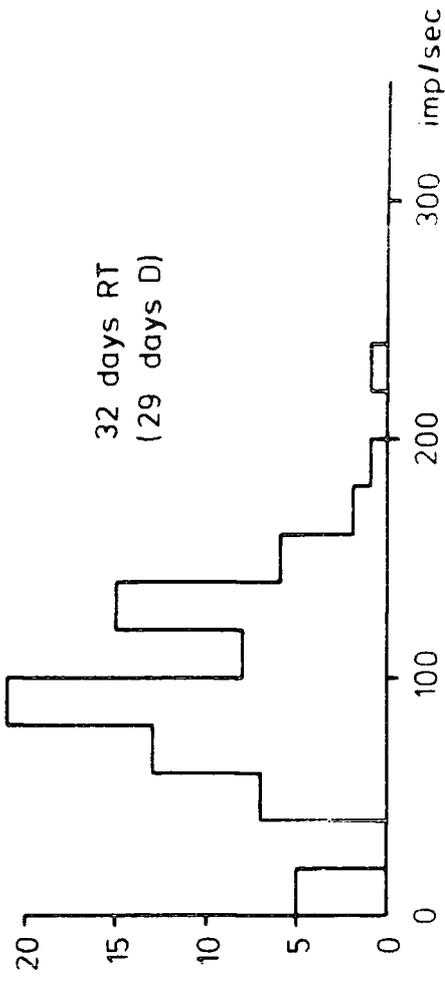
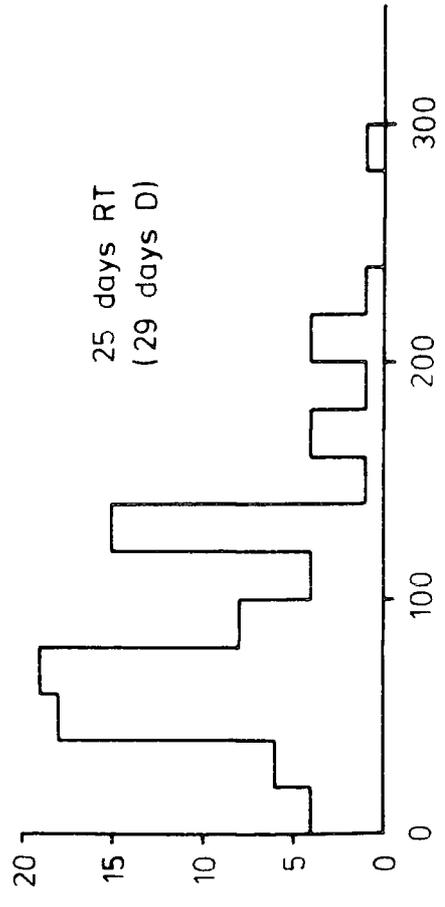
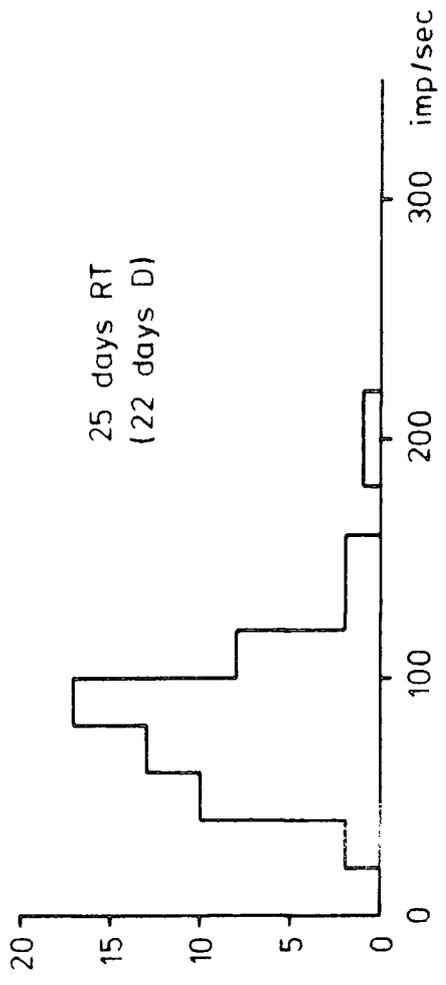
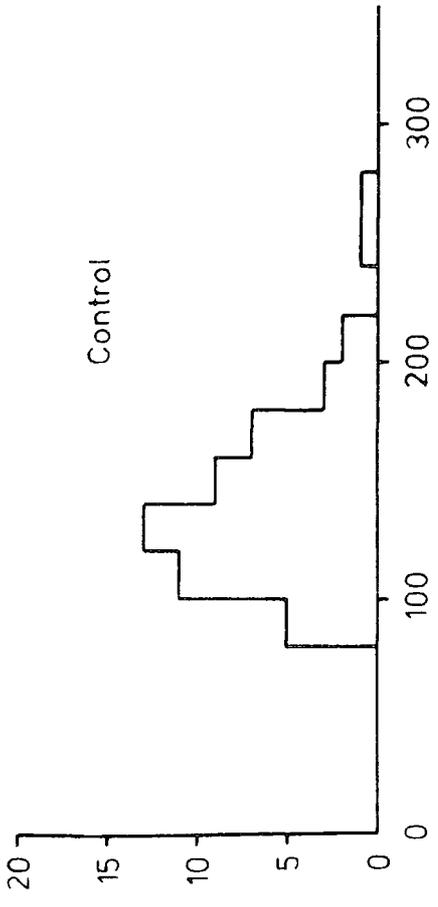
Days D	Expt'l series	Days RT	Classes of Afferent				Total no. of units.
			1	2	3	4	
10	NEC	23	18.4	12.8	41.3	27.5	109
22	NS	25	18.5	29.2	42.5	9.8	113
29	TC	25	10.5	30.5	41.9	17.4	86
10	NEC	30	0.0	0.0	41.3	58.7	63
29	TC	32	5.1	21.5	58.2	15.2	79
22	NS	39	10.6	15.0	59.5	14.9	47
29	RC	39	1.3	10.1	78.5	10.1	79
36	RC	39	2.7	9.6	75.4	12.3	73
50	RC	39	7.9	13.2	53.9	25.0	76

Table 24.

The mean peak firing rates (imp/sec) of the regenerated afferents after different periods of denervation and reinnervation. The means are given with their standard errors.

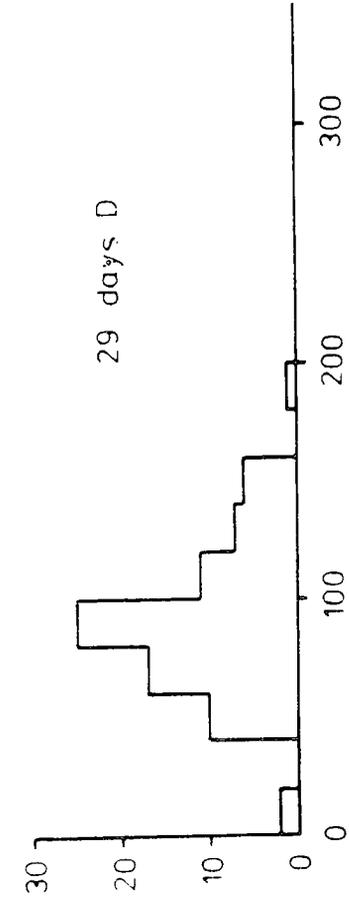
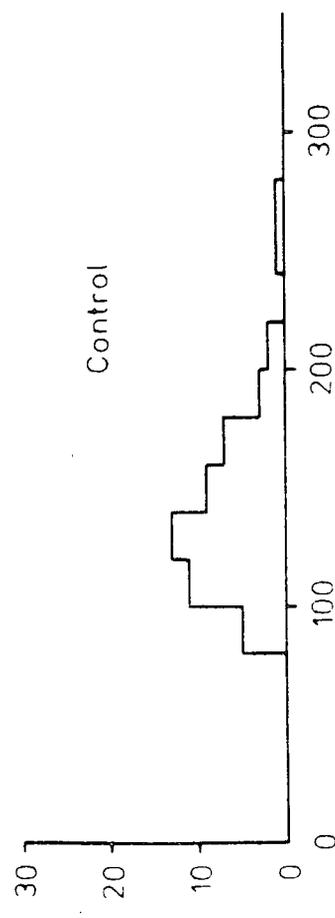
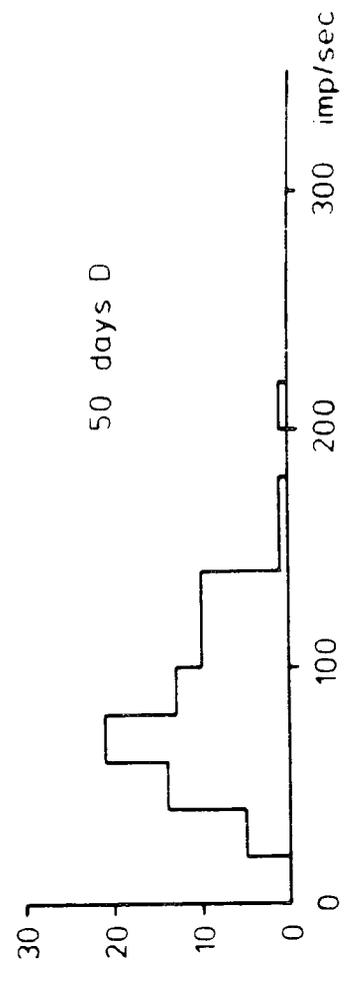
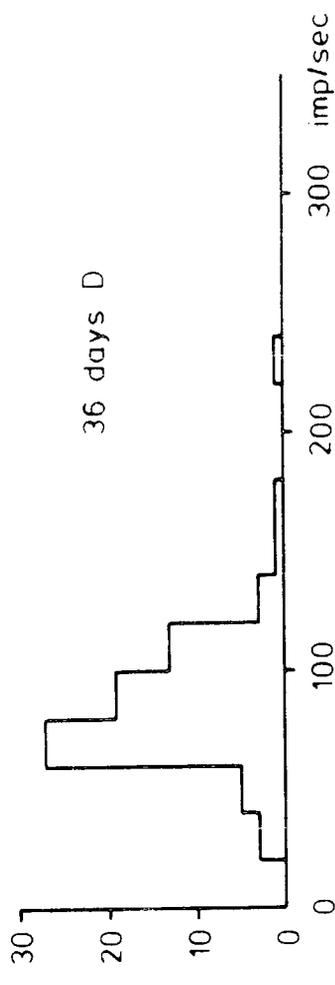
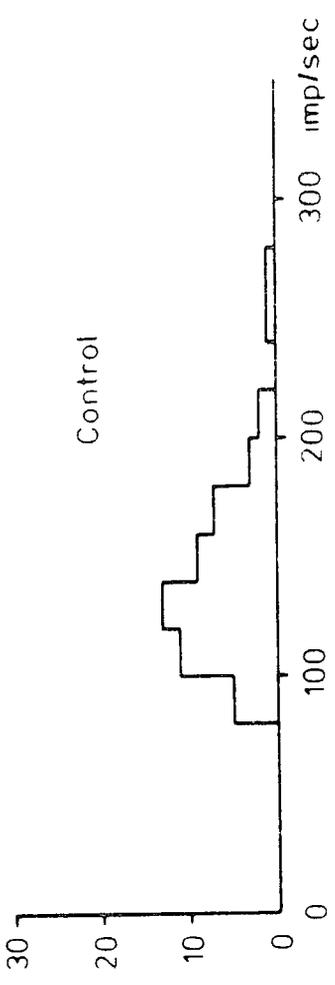
Days D	Expt'l series	Days RT	PFR	Total no. of units
10	NEC	23	83.7 \pm 3.67	107
22	NS	25	88.7 \pm 3.70	85
29	TC	25	91.2 \pm 6.00	86
10	NEC	30	106.8 \pm 4.79	63
29	TC	32	96.7 \pm 4.38	79
22	NS	32	88.8 \pm 5.31	38
29	RC	39	89.7 \pm 3.40	79
36	RC	39	86.6 \pm 3.53	73
50	RC	39	83.8 \pm 3.86	76

Figure 130. The mean peak firing rates (PFR's) of the primary and secondary afferents after different periods of denervation and reinnervation in the NS (22 days D) and TC series. Ramp velocity was 10mm/sec.



Numbers of Units

Figure 131. The mean peak firing rates (PFR's) of the primary and secondary afferents at 39 days RT after different periods of denervation (days D) in the NS and RC series of experiments. Ramp velocity was 10mm/sec.



Numbers of Units

DISCUSSION

Restoration of the Structure of the Sensory Ending.

10.1 General Observations.

The histological and physiological results described in chapters 6 and 7 from the NS series reveal a pattern of successful reinnervation by both the sensory and motor axons. There appears to have been a high degree of specificity in the reinnervation process both in the guidance of the regenerating axons back to the correct sites and in the formation of the endings, which often appeared quite similar to those found in normal spindles. The results of this accurate reinnervation are reflected in the restoration of the near-normal function of the endings.

10.2 Primary-ending Structure.

The earliest examples of primary endings were observed at 4 days RT which is in close agreement with the findings of Barker & Boddy (1980). As they and Ip & Vrbová (1973) observed, this was after the return of the efferent axons which is surprising in that there is evidence that large-diameter axons regenerate faster than smaller ones (Thulin, 1960; Homma, 1969; Wall & Devor, 1978).

There is some evidence that sensory axons are more adversely affected by nerve injury and atrophy more rapidly than motor axons (Gutmann & Holubar, 1949; Hoffer, Stein & Gordon, 1979; Hoffer, Gordon & Stein, 1980). Huber (1900) reported that motor axons both degenerated and regenerated faster than sensory axons. In contrast, a number of authors have reported that there is no difference in the rates of regeneration (Gutmann et al, 1942; Homma, 1969; Bisby, 1979), although Bisby (1979) did suggest that there might be differences in the modes of regeneration of the two types of axon. Even if there are slight differences in regeneration rate it seems unlikely that these could account for the earlier return of the relatively small γ fusimotor axons.

The apparent dependence of successful Ia restoration on the prior reinnervation of the spindle by the γ axons (Ip & Vrbová, 1973; Ip et al., 1977) is also surprising in that in development the Ia is the first axon to make contact with the developing myotubes and plays a key role in the differentiation of the intrafusal muscle fibres (Zelena, 1964; Zelena & Soukup, 1973; Milburn, 1973). Some evidence has been presented suggesting that after certain types of injury the primary axons do return before the γ axons (Thulin, 1960 (resection of part of the nerve); Takano, 1976 (freezing of the nerve with dry ice); Homma, 1969 (nerve section and suture)). All these latter injuries, however, are far more traumatic than simple crush and therefore inevitably result in greater disruption of the regeneration process.

Most of the Ia axons reach the spindle between 0 and 18 days RT, with the arrival of the second wave at about 25 days RT (Tables 1, 9). Barker & Boddy (1980) attributed the second wave of reinnervation to the delayed return of that proportion of afferents whose endoneurial tubes were severed during the crush operation and which therefore had to traverse a region of scar tissue to reach the distal opening of the tube. Severance of some of the tubes would also account for the failure of some axons to return, since these might fail to locate the distal part of their original endoneurial tube and thus would not be directed back to the muscle.

Once the axons make contact with the intrafusal fibres the formation of the ending proceeds very rapidly. There is no evidence of a sequential formation of the endings on the different fibre types; the results given in Table 2 indicate that all the fibres are reinnervated at about the same time. This is not surprising as the Ia axon branches at least once before making contact with the

intrafusal fibres, so the individual branches would presumably land on different fibres and commence forming an ending at about the same time.

The ending is formed within a few days of the axons making contact and after this period there is little further growth (Tables 4, 5) despite the fact that the endings generally have fewer bands and some are shorter than normal primary endings. During the period of restoration it appears that the terminals grow longitudinally along the fibres and produce the helical spiral formations, the rings are produced from some of these longitudinal branches towards the periphery of the ending and are formed after the branch is complete. During the early stages there was a greater proportion of incomplete rings than were observed later on (Figs. 5, 6, Plate 2).

The endings examined had nearly always achieved a final configuration similar to that of normal spindles, characterised by central regions of regular banding on the bag fibres bounded by irregular terminations. The chains showed mainly regular spiralling (Figs. 41-44, Plates 14, 15; Figs. 15, 16, Plate 5). This suggests that there is a powerful guidance system controlling the growth of the axon terminals to produce this pattern of reinnervation. That the system is not perfect is evident from the poorly restored endings that were observed in every population of spindles and also from the reduction in the number of bands visible in even the best restored of the endings (Table 15). The average length of the endings was also reduced (Table 16), although this reduction was not always significant and probably reflects the presence in each sample of some poorly restored endings rather than a consistent reduction in the length of all the endings.

Almost without exception the primary endings examined displayed some degree of abnormality that identified them as regenerated endings, and this agrees with the observation of Barker & Boddy (1980) and also with the observations of ultrastructural abnormalities (Schröder, 1974b). These findings are, to a certain extent, in conflict with those of Ip et al (1977) who stated that 'most of the sensory endings of spindles that had only their peripheral nerves crushed had a normal appearance'. It should be borne in mind, however, that in their study they were comparing these endings with the much more abnormal endings produced as a result of chronic de-efferentation combined with the nerve crush and therefore they were working with a different code of reference.

In a small proportion of spindles the primary afferent failed to reinnervate all the intrafusal fibre types. The majority of such incompletely reinnervated spindles were noted after the longer periods of recovery from 75 days PC (53 days RT) onwards (Table 2). In every case the uninnervated fibre was a bag fibre so the primary innervated either the bag₁, or the bag₂ and the chains. No spindles were found which completely lacked chain-fibre innervation; however, it was not possible to ascertain whether all the chain fibres had been reinnervated in every spindle. Also it was not possible to identify the bag₁ and bag₂ fibres sufficiently frequently to determine whether either fibre was more likely to be missed by the primary axon. Even if both bag fibres were reinnervated it was quite common for one bag fibre to receive relatively few bands (Figs. 15, 16, Plate 5) as was also observed by Barker & Boddy (1980).

Most of the incomplete primary endings were observed in spindles after the longer recovery periods. This suggests that



these might be spindles whose primary axons were delayed in regeneration such that when the afferent did return, not all the intrafusal fibres were receptive to the developing terminals. This might be an intermediate stage in the development of a total inability of the Ia to reinnervate the intrafusal fibres as can occur after nerve section (A. Boddy, personal communication). This proposal, however, is not borne out by the findings of the delayed-return experiments (Chapter 8) since even after 50 days denervation almost all of the regenerating primaries reinnervated all the intrafusal fibres. The other possibility is that these primaries are not axons that originally innervated b_1b_2c capsules but could, for example, be primary axons from b_2c capsules that, as a result of endoneurial tube severance, entered the wrong endoneurial tubes and were guided to the wrong primary regions.

Where hyperinnervation occurred the restoration of the ending was as good as that effected by primaries whose first-order branch point was close to the spindle (Fig. 18, Plate 6; Fig. 30, Plate 10; Fig. 39, Plate 13). In every case the axons were traced back as far as possible and hyperinnervation was not presumed if they could not be traced for at least 1000 μ m, thereby minimising the risk of the axons having been broken just distal to the branch point. The individual axons contained within the endoneurial tubes often did not branch and were therefore restricted to reinnervating one fibre type in the spindle. This suggests that these axons do in fact represent the branches of a single parent axon which has branched at a point far removed from the spindle. The alternative hypothesis is that these axons are indeed the regenerated sprouts of two separate parent axons which, because of endoneurial tube severance at the crush site, have by chance both entered the same

distal tube. This is unlikely for two reasons: firstly, it is improbable that sprouts belonging to two separate Ia axons would happen to enter the same endoneurial tube and, secondly, if they were sprouts of separate Ia axons then each might be more likely to attempt to form a complete ending, as occurs with the double primary endings (Fig. 19, Plate 6) rather than being restricted to one fibre type, as was often the case.

If it is assumed that these axons represent the branches of a single Ia axon that has branches at a very distant point it seems likely that this point would be removed from any trophic influences of the spindle that might induce branching. The most probable site would therefore be the crush site itself. If this is so then it might be that, despite the evidence to the contrary from other sources (Gutmann & Sanders, 1943; Devor & Govrin-Lippmann, 1979b; Horch & Lisney, 1981a), in a small proportion of cases the parent axons are capable of supporting more than one axon sprout at the crush site. Alternatively, the occurrences of hyperinnervation could be the result of severance of the axon and its endoneurial tube leading to more than one sprout being maintained (and assuming that the sprouts are successful in locating the same distal tube) as has been noted after the transection of cutaneous nerves (Horch, 1976; Horch & Lisney, 1981a; Burgess & Horch, 1973). In either case the occurrence of hyperinnervation would be independent of the recovery time and would probably be dependent on the inevitable variations in the force with which the crush was effected. Therefore the occurrence of hyperinnervation is probably representative of the degree of disruption produced at the crush site. Barker & Boddy (1980), as well as observing primary hyperinnervation, also reported its occurrence in some secondary and motor endings.

10.3 Secondary-ending Structure.

The restoration of the secondary endings after nerve-crush injury appears on the whole to be successful, as was also found by Barker & Boddy (1980). As with the primary, however, all the endings displayed some degree of abnormality, the main features being a reduction in the complexity and extent of the reinnervated endings compared with the normal secondary endings.

The secondary axons generally regenerated more slowly than the primaries and the endings took longer to achieve full restoration (Tables 6, 7). This is in line with the results of other authors which show that the rate of regeneration is, at least in part, dependent on the size of the parent axon (Thulin, 1960; Takano, 1976) and also that the onset of sprouting is dependent on axon diameter (Devor & Govrin-Lippmann, 1979a).

The results also indicate that fewer secondary axons regenerate successfully compared with the primaries (Tables 1, 6) since there was an increase in the number of spindles without secondary innervation, whereas almost all of the spindles were reinnervated by primary axons (after the return of the second wave). There was also a reduction in the number of spindles showing multiple secondary innervation. This, again could be related to the size of the axons such that the larger sensory axons might be more likely to regenerate successfully.

The distributions of the S_1 secondaries to the different intrafusal fibres, as given in Table 7, show that proportionally fewer of the S_1 secondaries reinnervate all three fibre types compared with normal secondaries. Until 75 days PC (53 days RT) the proportion of b_1b_2c secondaries was much lower than in normal spindles, the distribution becoming nearer to the normal situation

over the subsequent recovery periods. Although confident identification of the bag_1 and bag_2 fibres was not always possible, it was apparent that the majority of the secondaries that were distributed to only one bag fibre and the chains did, in fact, innervate the bag_2 rather than the bag_1 . The finding that the bag_1 fibre was most likely to be uninervated and that there were no S_1 secondaries that failed to reinnervate the chains is a reflection of the normal distribution of S_1 secondary terminals to the different intrafusal fibres. Thus, in normal spindles the chains are the most extensively innervated and the bag_1 usually receives the least innervation or, in 17.8% of spindles, is not innervated at all (Banks et al, 1982).

The relatively slow, sequential process of reinnervation by the secondaries is different from the more rapid, nearly simultaneous process displayed by the primaries. These differences could be related to the different branching patterns of the two afferents. The primary axon divides at the first branching node to produce two or three first-order branches (Banks et al, 1977; 1979; 1982) one of which often innervates only the bag_1 fibre while the others innervate the bag_2 and chain fibres. Each of these myelinated branches usually further sub-divides before giving rise at the heminodes to the unmyelinated pre-terminal axons which innervate the muscle fibres. In contrast, the pre-terminal axons that produce the secondary endings may be derived from both the heminodes and the penultimate nodes and a segregation of the first-order branches (i.e. one branch supplying the bag_1 alone, the other the bag_2 and chains) is comparatively rare, only occurring in 22.7% of S_1 secondaries as opposed to 58% (Banks et al, 1982) of peroneus brevis primary endings. These differences in the derivation of the

pre-terminal axons in the two types of ending could explain the more sequential process of reinnervation displayed by the secondary afferents (especially if combined with a slower rate of regeneration) given the chronological differences in the formation of the pre-terminal axons.

10.4. Specificity of Reinnervation.

The results of both the histology and physiology indicate a very high degree of specificity of reinnervation after nerve-crush injury, as was also observed by Brown & Butler (1976) and Barker & Boddy (1980). This specificity is evident for both the sensory and motor axons in that each type of ending was reformed in its original region. Thus the primary axons entered the spindle capsule and made direct contact with the intrafusal fibres in the P region, usually without any sign of 'searching' for the correct site (Figs. 4, 5, Plate 2). In the rare event of terminal branches approaching the S_1 region, these either doubled back into the P region (Fig. 38, Plate 13) or failed to produce an ending (Fig. 25, Plate 8). Similarly, the branches of secondary or motor axons that encroached upon the P region either coursed through the region (Fig. 3 Plate 1; Fig. 15, Plate 5) or formed large ball-like swellings (Figs. 20, 21 Plate 7). Further evidence for specificity comes from the finding that γ axons which reinnervated more than one spindle retained their functional characteristics in all the spindles they innervated (Table 10; see also Table 1 of Brown & Butler, 1976).

McMahan et al (Letinsky, Fishbeck & McMahan, 1976; Sanes, Marshall & McMahan, 1978; McMahan, Edgington & Kuffler, 1980a; McMahan, Sargent, Rubin & Burden, 1980b) and Miledi (1960) have shown that, in the frog, motor axons regenerating after nerve section

reinnervate their original sites on the extrafusal muscle fibres (this has also been demonstrated in the rabbit (Bennett et al,1973)). They have further demonstrated that this is due to the regional specificities of the basal lamina of the muscle fibres. This lamina is resistant to degeneration and can persist for at least five weeks in the absence of both the nerve and the muscle fibre (Sanes, Marshall & McMahan,1978).

In normal muscle spindles the basal lamina (which lines the endoneurial tube) of the motor axons is fused with that of the muscle fibre, the lamina of the muscle fibre being continuous in the synaptic cleft between the axoplasm and the underlying muscle cells (Merillees,1960; Barker et al,1970; Banker & Girvin,1971). The relationship between the sensory terminals and the intrafusal fibres is different in that the terminals are applied directly to the muscle surface, lying in shallow grooves (Adal,1969)with the basal lamina running over the terminals and being continuous with that of the endoneurial tube of the axon (Merrillees,1960; Banker & Girvin,1971). Given the persistence of both the basal lamina of the muscle fibre after denervation (Sanes et al,1978; McMahan et al,1980a) and that of the nerve, despite degeneration of the axon (Thomas,1964; Haftek & Thomas,1968), it seems probable that, after a crush injury, most of the axons will not only be guided back to the spindles they originally innervated, but also back to their original sites on the intrafusal fibres. The restoration of the somatotopic organisation of cutaneous afferents after nerve crush lends support to this hypothesis (Burgess et al,1974; Horch,1979; Horch & Burgess,1980; Dykes & Terzis,1979).

If one assumes that each type of axon has a unique

relationship with the underlying intrafusal muscle fibre, which is probable given the different natures of the different motor and sensory axons and the regional variations of the intrafusal fibres, then it is likely that the intrafusal basal laminae are specific for the different types of axon. This could account for the observation that when the regenerating efferent axons branch and invade other regions of the spindle they are apparently unable to form endings within those regions.

The distribution of the sensory axons is also likely to be limited physically by the basal lamina of the intrafusal fibres because the terminals lie beneath the lamina. Thus, in regions where there have not previously been sensory endings the basal lamina would be closely applied to the surface of the muscle fibres and so it would be unlikely that the terminals of a sensory axon could establish an ending.

Despite some minor abnormalities, in each case the restoration of the primary ending was very accurate. Even if there were fewer terminal bands than normal (Table 4) the overall length of the ending was usually only marginally reduced (Table 5) and the characteristic form of the ending was often fully restored. This accuracy would thus be the result of the persistence of the basal lamina which would maintain the shape of the original ending so the regenerating axon terminals would simply follow the original paths. The absence of portions of the ending (Fig. 25, Plate 8) or of some of the banding could be due to the failure of the returning axon to branch at all the branch points of the basal lamina tracks.

Schröder (1974b) reported that after nerve crush in the rat some of the sensory terminals were separated from the intrafusal

fibres by intervening processes of basal lamina. Sanes et al (1978) observed that when a muscle fibre shrinks as a result of denervation atrophy the basal lamina folds and new lamina is produced which cuts off the folds. This could also occur to seal off some of the sections that previously contained a sensory ending. It is also possible that, in the absence of a sensory axon, the lamina of the muscle fibre would grow across the surface of the fibre and close off the route of access of the sensory axon. Therefore if the return of the sensory axon were sufficiently delayed it might become impossible for it to reinnervate the muscle fibres. The absence of muscle spindles in regenerated muscles of the rat has been attributed to the persistence of the old, or formation of new, basal laminae around the myotubes preventing the sensory axons from establishing the contact required to promote myotube differentiation (Zelena & Sobotkova, 1971).

The main question raised by this scheme for highly specific reinnervation is why, after nerve crush, there is not a similar restoration of original connexions between the regenerating α axons and the extrafusal muscle fibres (Karpati & Engel, 1968; Kugelberg et al, 1970; De Reuck et al, 1973; Bagust & Lewis, 1974). One possible explanation is that the α axons do regenerate down their original tubes and return to an original muscle fibre (presumably the most proximal one). At this point, the trophic effects of the neighbouring denervated muscle fibres could induce collateral sprouting by the axons so that they would reinnervate the block of fibres around the muscle fibre with which they first made contact. There is some evidence for this type of reinnervation in that following reinnervation, muscle fibres of the same histochemical type are

clumped together rather than distributed in mosaic fashion throughout the muscle (Dubowitz, 1967; Kugelberg et al, 1970; De Reuck et al, 1973). Cross-union experiments have further demonstrated that the reinnervating α axons can change the nature of the fibres they reinnervate (Buller, Eccles & Eccles, 1960; Dubowitz, 1967), which indicates that the extrafusal fibres do not maintain their integrity to the same extent as the intrafusal fibres (De Reuck et al, 1973).

10.5. Invasion of the Primary Region.

Invasion of the P region took three forms:- (i) a foreign axon could enter the region via the Ia endoneurial tube in the absence of the primary (Figs. 13, 14, Plate 4; Figs. 20, 22, Plate 7); (ii) a motor axon from the poles, travelling in parallel with the intrafusal fibres could enter the P region (Fig. 3, Plate 1; Fig. 21, Plate 7); (iii) a branch of an S_1 secondary could invade the P region (Fig. 12, Plate 4; Fig. 15, Plate 5; Fig. 27, Plate 9; Fig. 31, Plate 10).

The foreign axons that entered the P region via the Ia tube either formed large ball-like swellings (Fig. 20, Plate 7) or left the region for the poles (Figs. 13, 14, Plate 4; Fig. 22, Plate 7) and were presumably motor in origin. Some of these axons, especially the large diameter axons (Fig. 20, Plate 7) could represent the α invaders described by Barker & Boddy (1980). These are α axons which, as a result of the endoneurial tubes being severed, enter the wrong tube and grow down into the spindle where they may attempt to form an ending if they arrive in the polar regions. If they travel down a Ia tube then they will presumably be guided beneath the basal lamina of the muscle fibres. Once there they would be incapable of forming an ending because motor endings are formed

basal lamina of the

applied to the muscle fibre. The progress of the axon may well then be impeded, if not totally prevented. It is possible that the axon shown in Fig. 20 is an example of this condition, the large axonic swelling in this case representing the damming up of the axoplasm (see Weiss, 1944; his Fig. 1). The fine axons that have been observed leaving the P region might be small enough to grow beneath the lamina where it has become slightly separated from the underlying muscle fibre as a result of atrophy.

Motor axons coursing through the P region from one pole to the other are also common features of the normal spindle (Barker et al, 1970) and are usually the branches of fusimotor axons. It is also quite common for these axons to produce axonic swellings as they pass through these regions (see Figs. 23-25, Plate 61 of Barker et al, 1970), or they may terminate in such swellings in the sensory regions. Barker et al (1970) suggested that these swellings represent attempts to form synaptic contacts which are inhibited by the different regional specificity of the sensory region compared with the poles.

Invasion of the primary region by branches of the secondary axons is unique to the regenerated condition and becomes more prevalent with increasing recovery time (section 6.3) indicating a continuing growth process. The apparent end of such axon branches is to form an ending in the S_1 region of the opposite pole, irrespective of whether there was an S_1 secondary already present or not (Table 8). It is presumed that these branches must be able to grow beneath the basal lamina to reach the opposite pole, so again this depends on there having been some atrophic reduction in intrafusal fibre diameter to allow their passage.

This abnormality shows that the physical guidance system

of the basal lamina of the endoneurial tubes and muscle fibres is not the only operative system for directing the axons. There is substantial evidence for trophic factors produced by denervated extrafusal muscle which guide the regenerating axons and, in partly denervated muscle, provide a stimulus for the formation of collateral sprouts (Diamond, Cooper, Turner & MacIntyre, 1976; Brown, Holland & Hopkins, 1981). It is probable that similar factors are released by the intrafusal fibres lacking motor or sensory innervation. Thus in a spindle that was originally innervated by two S_1 secondaries, if one should regenerate before the other it would be possible that the first one back would respond to the trophic factors emitted by the still-denervated S_1 region of the opposite pole. These factors would induce the formation of a sprout which would grow towards that S_1 region and possibly form an ending there despite (in some cases) the subsequent return of the S_1 secondary that originally innervated that region.

There is also evidence for regional chemical specificities in that, although both the motor axons and the secondary axons were able to grow through the P region, neither were able to form recognisable endings there. This shows that the P region is highly specific for Ia axons since secondary sensory axons, also growing beneath the basal lamina were unable to form endings in the primary region.

The Restoration of Muscle-spindle Function.

11.1 General Observations.

All the physiological results presented for the NS series (Chapter 7) indicate a process of continuous improvement in the functioning of the sensory units, from the early stages of abnormal responses, to the restoration of apparently normal response patterns and the attainment of near-normal axonal conduction velocities for both the afferents and efferents.

The gamma efferents apparently functioned normally throughout (except for some early on which were unable to maintain the contraction of the intrafusal muscle fibres), although such normality of function can only be inferred from their effects on the afferent responses. These results also provide evidence to confirm the histological observations (section 6.4) that the γ axons certainly reform their connexions with the intrafusal fibres at least as early as the first sensory axons. At no point was there any evidence of a lack of specificity in the restoration of the gamma endings on the different types of intrafusal fibre.

11.2 Afferent Conduction Velocities.

The progressive increase in both the afferent and efferent CV's during recovery agrees with the results of many other authors (Sanders & Whitteridge, 1946; Cragg & Thomas, 1961; 1964; Horch & Lisney, 1981a; and others) all of whom observed that distal to the crush site the axons never achieved a full return to normal conduction velocities. The results described in section 7.2 are for measurements of CV over a length of nerve comprising portions of both the distal and the proximal segments. By 140 days PC (118 days RT) the mean CV's had recovered to near-normality (Fig. 66).

Overall there was evidence of a proportional restoration of the CV's with the primary afferent axons conducting faster than the secondary group and the α efferent axons conducting faster than the δ group. This is also in agreement with the findings of other authors (Devor & Govrin-Lipmann, 1979b) and with the histological analyses that show a proportional restoration of the fibre diameters (Sanders & Young, 1944).

11.3 Possible Sites of Generation of the Abnormal Responses.

A range of responses was described for the regenerated afferents (section 7.3) from those units that only responded to the velocity phase of the ramp through an intermediate series to those units that responded within the normal range.

Afferents showing only the phasic portion of the response to ramp stretch (the class 1 units) have been recorded from reinnervated cat spindles by a number of workers (Brown & Butler, 1976; Ip et al, 1977; Hyde & Scott, 1981) and also from reinnervated snake spindles (Fukami, 1972). Such responses were also recorded during development (Skoglund, 1960). Phasic responses and elevated stimulus thresholds were also reported from cutaneous receptors during the early stages of reinnervation (Burgess & Horch, 1973; Dykes & Terzis, 1979; Terzis & Dykes, 1980).

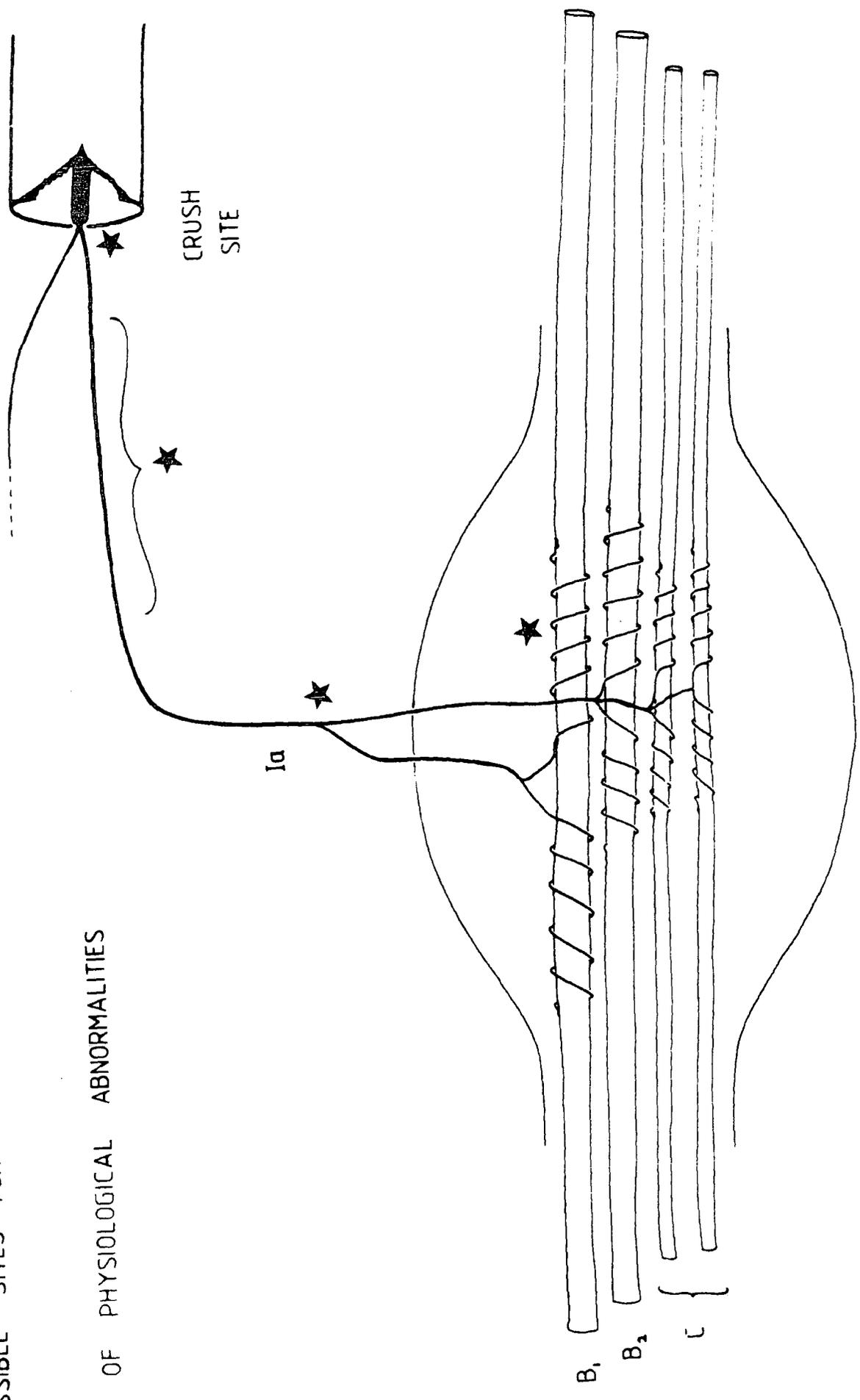
There has been a number of suggestions as to the cause of these abnormal responses. Fig. 132 illustrates the proposed sites at which such abnormalities might be generated. The possible causes are as follows:-

A/ Failure of secure impulse transmission at the crush site or along the fine regenerated sensory axon and its pre-terminal branches.

Figure 132. A schematic diagram of a muscle spindle and its primary axon. The asterisks mark the possible sites of generation of abnormalities in the response patterns. These are: 1/ The crush site. 2/ The regenerated axon. 3/ The pacemaker site. 4/ The axon terminals.

POSSIBLE SITES FOR THE GENERATION

OF PHYSIOLOGICAL ABNORMALITIES



B/ Incomplete reinnervation of the intrafusal muscle fibres.

C/ Failure of the sensory terminals to form secure attachments to the underlying muscle fibres leading to slippage.

D/ A reduction in, or rapid adaptation of the receptor potential possibly as a result of changes in the ionic conductance properties of the terminal membranes.

E/ An increase in the threshold of the pacemaker site.

A/ Failure of Impulse Transmission.

Both the regenerated axon and the crush site are regions having a low safety-factor for spike transmission and therefore they could act as low-pass filters (section 3.6) thereby modifying the afferent response.

The capacity of the regenerated axons (including those of class 1 units) to follow stimulation at frequencies of up to 200Hz without conduction block after only a relatively short recovery period (33 days PC) indicates that the axons themselves are unlikely to be the cause of the abnormalities since the majority of class 1 units never exceeded 100imp/sec in response to passive ramp stretch. Further, the ability of the axons to transmit high frequency spike trains during fusimotor stimulation is conclusive evidence that impulse conduction is not a limiting factor. The only occasion where conduction failure might be influencing the response pattern was during the transmission of the very high frequency spike trains generated by the highly dynamic units (section 9.5), especially when they appear to reach a cut-off peak frequency as illustrated by the unit in Fig. 127.

B/ Incomplete Reinnervation.

The possibility that incomplete reinnervation might be the

underlying cause of the phasic-only responses was postulated by Brown & Butler (1976), and was given further support by Barker & Boddy (1980) who observed that the most common abnormality in the restoration of the primary ending was for one or other of the bag fibres to receive very few terminals and sometimes none at all.

In this study only 5.2% of primaries failed to reinnervate all three types of muscle fibre (Table 2) although it was more common for one bag fibre to receive only one or two terminals (Table 4) while the other bag and the chains were well innervated. However, the incompletely restored endings were only present after the longer recovery periods and showed no signs of improvement whereas the abnormally responding units were isolated early on and became increasingly rare. Moreover, stimulation of γ_s and γ_d efferents restored the hold phase and background discharges of even the most abnormally responding afferents and gave rise to response patterns similar to those of normal primaries under the same conditions (Fig. 86). The restoration of the static response of a class 1 ending by γ_s stimulation indicates that the bag₂ or the chains, or both, must have been reinnervated by the regenerating primary afferent even though this was not evident from the responses to passive stretch alone.

Brown & Butler (1976) and Ip et al (1977) also suggested that the lack of a functional efferent innervation might be a factor in reducing the ability of the afferent axons to reform fully functional connexions with the intrafusal fibres. The analysis of the silver-stained spindles from the experimental animals shows that in every spindle where a primary was present motor reinnervation had also occurred. These motor axons were fully functional even in those spindles whose afferents generated the most abnormal responses.

C/ Terminal Slippage.

Mechanical slippage of loosely attached sensory terminals could result in a greatly increased rate of adaptation of the response in a manner similar to the effect of the mechanical creep of the bag₁ fibre (Boyd et al, 1977b; 1981). Schröder's (1974b) observations of abnormalities in the contact relationship between the afferent terminals and the muscle fibres, where portions of the basal lamina of the muscle can be interposed between the terminals and the muscle (section 4.2) lend some support to this theory.

Fukami (1972) tested for terminal slippage in reinnervated snake spindles by examining the responses to mechanical and electrical stimuli. He observed that spindles which showed a phasic-only response to the ramp stretch gave a similar response to externally applied electric current indicating that the abnormality was not mechanical in origin. This also militates against the possibility of some alteration in the mechanical properties of the intrafusal fibres being responsible.

If terminal slippage occurred, it would also be expected to manifest itself in the response to fusimotor stimulation and depolarisation by Sch, which was not the case.

D/ Reduction or Adaptation of the Receptor Potential.

Comparison of the responses of class 1 and class 4 regenerated afferents (Fig. 74) shows that the peak firing rates of the class 1 afferents at the end of the velocity phase of the stretch are markedly less than those generated by the class 4 afferents for an equivalent stimulus. This reduction in firing rates is further illustrated in Fig. 90 and Table 11. The data given in Table 11 also show that there is an increase in the hold-phase firing (where present) as the units recover from being

class 1 to class 4 in nature.

These figures indicate that the abnormal responses are associated with a decrease in the amplitude of the response to a given mechanical stimulus. This lowered response could be attributed to a change in the ionic conductance of the terminal membranes thereby leading to a reduced receptor potential. Such a change might occur as a result of a relative scarcity of functional Na^+ channels (or possibly Ca^{++} channels) during the early stages of recovery leading to a reduction in the current flow. Such a reduction in the receptor potential was proposed by Fukami & Ridge (1971) as one possible explanation for the increased adaptation observed in the responses of denervated snake spindles.

As both the peak and hold-phase firing rates increase as recovery progresses, the difference between them (the dynamic index) remains roughly constant (Table 11). If the abnormal responses occurred as a result of a lowering of the receptor potential due to the inability of the terminal membranes to sustain the normal receptor potential and to carry the normal levels of current flow, then the effect would be one of a proportional reduction in the response. If this were so, the dynamic index of a class 2 unit would be proportionally less than that of a class 4 unit, which was not the case (Table 11).

Another possibility is the presence of a rapid time-dependent change in the conductance characteristics of the terminal membranes. Fukami (1972) demonstrated that there was a rapid adaptation of the response to stretch of reinnervated snake spindles, which he attributed to the electrical properties of the terminal membranes. He further proposed that this effect was related to that observed by Fukami & Ridge (1971) during the

degeneration of function of snake spindles as a result of denervation.

Such an adaptation of the receptor potential is unlikely to be the cause of the reduced peak firing rates because the shapes of the instantaneous frequency records of the class 1, 2, and 3 units for the velocity phase of the stretch are similar to those of the class 4 and normal control units (Fig. 74).

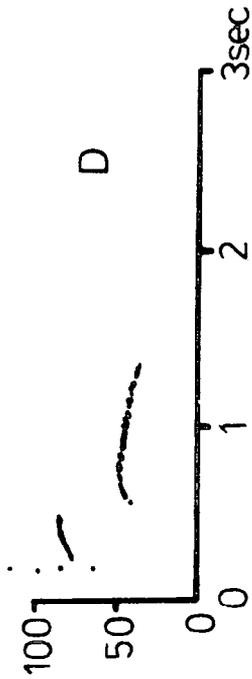
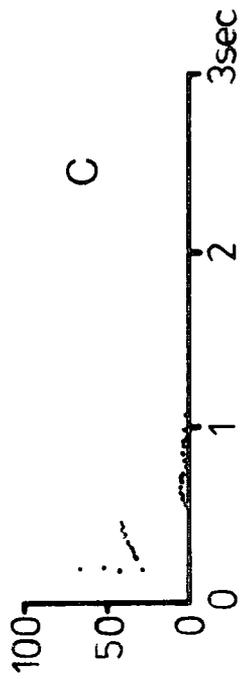
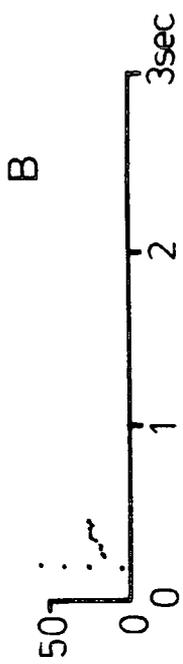
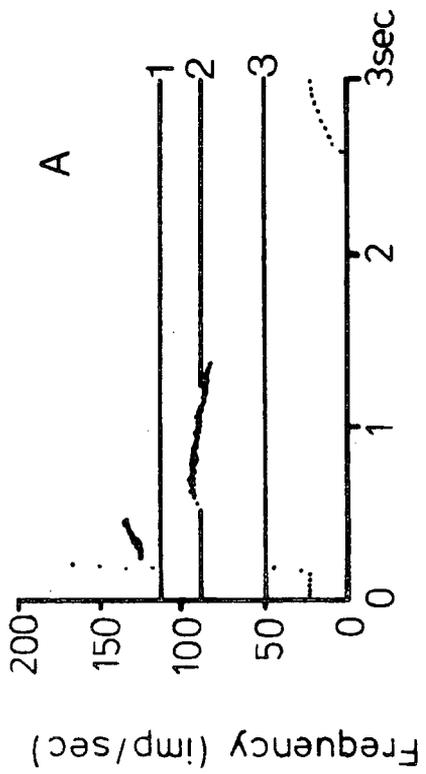
Adaptation of the response could account for some of the responses shown by class 2 units (Fig. 70) where the firing ceases during the hold phase of the stretch. Even here, though, it seems unlikely to be a major factor because normal primaries show an adaptation of the response during the first few seconds of the hold phase associated with the mechanical creep of the bag₁ fibre (Boyd et al, 1977b; 1981) and any marked increase in the adaptation would lead to an increase in the dynamic index. Further, during γ_s stimulation the responses of class 1 and 2 afferents (Figs. 78, 79) show little if any of the adaptation that would be expected if it was the result of adaptation of the receptor potential.

E/ Increase in Pacemaker Threshold.

The abnormal responses appear to be associated with a reduction in the amplitude of the response to all phases of the ramp-and-hold stretch, probably with little, if any, contribution from an increased rate of adaptation (of either electrical or mechanical origins).

Fig. 133 illustrates the effects of an increased pacemaker threshold on the response of a normal primary ending. Fig. 133A shows the instantaneous frequency record of a normal primary afferent. Superimposed on this picture are three lines to represent the effects of an increased pacemaker threshold. If the threshold

Figure 133. A scheme to explain the abnormal primary-ending responses in terms of an increased pacemaker threshold. A, the instantaneous frequency record of a typical primary ending in response to a ramp-and-hold stretch. Superimposed on this are three base-lines to show the effect of raising the pacemaker threshold on the response pattern evoked by the stretch. B, a response pattern similar to that of a class 1 unit created by raising the pacemaker threshold to base-line 1. C, a class 2 unit response, being that part of the primary response in A lying above base-line 2. D, a class 3 unit response created by raising the threshold to base-line 3.



1.8 mm [

is set at line 1, then the response that would be observed consists of that part of the record lying above line 1. This is illustrated in Fig. 133B. The response generated is similar to the class 1 records illustrated in Fig. 69, consisting of the peaks of the afferent response that occur at the start of the ramp (the initial burst) and during the latter part (if not all) of the velocity phase of the ramp.

The record shown in Fig. 133C is of a class 2 unit, with the response failing part-way through the hold phase. Such a response is generated by setting the threshold base-line along line 2 (Fig. 133A). An increased rate of adaptation might also need to be invoked to create some of the class 2 responses (Fig. 70), although in the main these could probably be accounted for by the normal hold-phase adaptation of the afferent response.

Base-line 3 (Fig. 133A) similarly sets the level for the class 3 response in which the response to the ramp is normal (though of slightly reduced amplitude) but the background discharge is absent (Fig. 71). Obviously in reality, these shifts in the pacemaker threshold would be part of a continuous variation in threshold rather than being set at specific levels as described here.

The increased pacemaker threshold, by producing a subtractive reduction in the response amplitude, can generate the different patterns of response and can also account for the constant dynamic index and the normal appearance of the responses to fusimotor stimulation. Whether this model could also account for the phasic-only responses recorded from kitten spindles (Skoglund, 1960) is a matter for further experiment.

Because of the nature of this threshold effect, it seems likely that most of the class 1 units are primary afferents. This

is probable because primary afferents usually show a large drop in firing rate from the peak of the ramp to the start of the hold phase compared with the more gradual decline shown by secondaries. Thus, the secondaries would be more likely to display class 2 or 3 responses even during the early periods of recovery. This could, in part, explain the lack of difference between the CV's of the class 1 afferents and those of the other regenerated classes (Fig. 77) since the class 1 group would be strongly weighted towards the Ia range of CV's for the specific regeneration period.

11.4 Failure of Gamma Transmission.

The records in Fig. 87 (section 7.4) illustrate the failure of a γ_s axon to secure maintained contraction of the intrafusal muscle fibres when stimulated repetitively for more than a few seconds.

There are two probable sites of failure (given that the stimulus employed in the ventral root was of sufficient amplitude to ensure secure following at 100Hz); either conduction failure in the regenerated portion of the axon, or failure of neuromuscular transmission.

During the early stages of regeneration there is a three to five-fold increase in the absolute refractory period of the axon (Thulin, 1962) combined with a reduction in the safety-factor of transmission, especially past branch points (Waxman, 1980). These factors mean that the capacity of the axon to carry high-frequency spike trains is greatly reduced. This could result in intermittent failure, or even total blockage (section 3.6).

At the relatively low frequencies employed, however, it is more likely that failure of neuromuscular transmission would be the cause of failing contraction. There is evidence that newly

regenerated synaptic terminals, although functional, may evoke smaller ~~miniature~~ endplate potentials than normal (Dennis & Miledi, 1974b; Bennett et al, 1973) which could be attributed to the small size of the newly regenerated terminals since there is a correlation between terminal size and quantal content (Kuno, Tukanis & Weakly, 1971). This could lead to a failure of secure transmission, especially during relatively long periods of stimulation.

11.5 Highly Dynamic Afferents.

Afferents with a peak firing rate of 200imp/sec or more were present during the earliest recovery periods in both the NS and NEC series (sections 7.6 and 9.5). In both cases they constituted some 35% of the afferent population compared with 6.5% in the controls (section 9.5). As recovery progressed the proportion of such afferents declined rapidly in both the NS and NEC afferent populations.

Almost all of the regenerated afferents firing at more than 200imp/sec, and all those that exceeded 250imp/sec, were well within the primary range of conduction velocities and their response patterns (Fig. 127) closely resembled those of primary afferents during γ_d stimulation (Fig. 85) or depolarisation by Sch (Fig. 89). The response pattern thus consisted of a very fast rising phase with a rapid fall at the peak of the ramp followed by a very marked adaptation during the hold phase.

After chronic de-efferentation, the response to stretch of spindle afferents is greatly enhanced (Hník, 1970; Hník & Lessler, 1973; Arutyunyan, 1981). Hník (Hník, 1970; Hník & Lessler, 1973) found that the dynamic responsiveness was greatly increased and that the secondary response was more affected than that of the

primaries. Arutyunyan (1981) also reported a marked increase in the dynamic response of primary afferents followed by an enhanced adaptation but he found little augmentation of the secondary response.

Hník & Lessler (1973) proposed that this effect might be due to changes in the visco-elastic properties of the spindle as a result of de-efferentation, or to an alteration in the membrane properties of the sensory ending. Arutyunyan (1981) also proposed some changes in the visco-elastic properties that would increase the transmission of stretch to the sensory region because of an increase in the stiffness of the poles.

Hník (Hník, 1970; Hník & Lessler, 1973) found that the enhanced response was fully developed within six days of de-efferentation and then remained unchanged for at least two months. This argues against an atrophic change in the stiffness of the poles since it has been shown that the spindle is resistant to atrophy for some weeks after total denervation, with a subsequent period of progressive atrophy of the intrafusal fibres (De Reuck et al, 1973; Tower, 1932).

Most of the changes as a result of denervation, therefore, are relatively slow to become apparent and subsequently follow a progressive course, whereas the onset of the augmented response is rapid and, once developed, shows little subsequent change. Further, it seems unlikely that an increase in the stiffness of the poles, possibly as the combined result of muscle-fibre atrophy and an increase in the amount of connective tissue, as proposed by Arutyunyan (1981), could account for both the increased sensitivity to stretch and the increased adaptation during the hold phase.

An alternative explanation might be that the stretch-induced contraction of the bag₁ fibre (Poppele & Quick, 1981) is enhanced by the absence of a functional fusimotor innervation which normally acts partially to suppress the stretch activation. During the very early stages of recovery, it is possible that the sensory ending might become fully functional before the γ_d endings, or before the presence of the γ_d endings had fully suppressed the enhanced stretch activation. Dennis & Miledi (1974a) showed that during regeneration some muscle fibres can be reinnervated by motor fibres that are initially non-functional and they termed this the 'non-transmitting stage' of regeneration. As the γ_d endings mature they would rapidly suppress the enhanced stretch activation. In contrast, any effects due to atrophy, or the increased deposition of connective tissue, might be expected to be longer lasting after the restoration of the spindle innervation. Also, in the first place such changes would probably affect most, if not all, of the spindles, rather than the 35% noted at the earliest stages of restored function.

The Histology of Muscle Spindles Reinnervated After Short Periods of Denervation.

12.1 Restoration of Primary Endings in the NEC and NS Series.

The NEC and NS series of experiments both covered the recovery periods from 4 to 30 days RT after periods of denervation of 10 and 22 days, respectively. The restoration of the primary endings proceeded at the same rate in both cases; with the first afferents having returned at 4-5 days RT and most of the spindles possessing primary endings by 9-11 days RT. There were, however, fewer uninnervated spindles in the NEC series than in the NS series until the return of the second wave of reinnervation at about 25 days RT in the NS series (Tables 1, 13). This second wave was not apparent from the histological analysis of the NEC material, though this could have been due to the relatively small samples examined since there was some evidence of a second wave from the physiological results (section 9.3).

Barker & Boddy (1980) proposed that the second wave of reinnervation marks the return of those primary axons whose endoneurial tubes were severed during the crush operation. It also seems likely that the frequency of occurrence of hyperinnervation could reflect the degree of disruption at the crush site in terms of axonal and endoneurial tube transection (section 10.2).

It would be expected from Laplace's law (Laplace's law states that the tension in the wall of a cylinder is proportional to the difference between the internal and external pressures multiplied by the radius) that the disruption caused by an equivalent crush operation at the knee would be greater than that caused by crushing the nerve close to its point of entry into the muscle because of the differences in the cross-sectional areas of the nerve at these

two points. Thus it is probable that a greater proportion of endoneurial tubes would be severed by the NS crush operation than by the NEC operation and the axons that would be most damaged in each case would be the large-diameter group I and A axons (Strain & Olson, 1975).

As a result it would be expected that the second wave of reinnervation would be more pronounced in the NS series and that this does not necessarily reflect any effects due to the increased denervation period. Similarly, there were higher levels of hyperinnervation observed in the NS material (12.4% of P regions were hyperinnervated) compared with the NEC spindles, of which only 4.7% showed primary hyperinnervation.

There was little difference in the time scales of recovery of the primary endings (in terms of days RT) between the two series of experiments, or in the quality of restoration of the endings. The primary endings showed the same numbers of terminal bands (Tables 4, 15) and the endings were of equivalent lengths (Tables 5, 16). The absence of any clear differences probably reflects the resistance of the spindle to atrophy during short-term denervation (De Reuck et al, 1973). De Reuck et al (1973) found that there was no significant reduction in the diameters of the intrafusal fibres of rat spindles even after three months of denervation, although they did observe reductions in the enzyme activities beginning as soon as 14 days after the lesion. In contrast, Kubota et al (1978) reported that within 7 days of denervation the muscle fibres from spindles in the snout muscles of the mole were reduced to two-thirds of their normal diameter and after 40 days they were reduced to half normal size. Schröder et al (1979), however, reported only a small decrease in the diameter of the intrafusal fibres of rat

spindles after three months denervation after taking into account the effects of the splitting of some of the muscle fibres on the spectrum of fibre diameters. Overall, therefore, it seems likely that gross structural atrophy is relatively slight over the first three weeks of denervation, although there is a progressive loss of histochemical activity.

12.2 Restoration of the Secondary Endings.

The S_1 secondaries in the NEC material showed a more rapid recovery than did those of the NS series. There was no clear difference in the time of return of the secondary afferents (Tables 6, 17), but the NEC secondaries reinnervated all the fibre types more rapidly than did the NS secondaries (Tables 7, 18). Over the first 30 days RT, 65.8% of the NEC secondaries (out of a total of 41 endings) reinnervated all three fibre types, whereas over the first 39 days RT in the NS series only 26.2% of secondaries (of a total of 42) reinnervated all three fibre types, and 52.4% were distributed to the chains and one bag fibre. Only after the longer recovery periods did the majority of NS S_1 secondaries reinnervate all three fibre types.

Since the secondary axons appeared to regenerate at the same rate irrespective of whether the crush was made at the knee or close to the muscle, it is difficult to determine why the process of reinnervation should be slower after a longer period of denervation. In both series there is evidence for a sequential process of reinnervation with the chains being reinnervated first followed by the bag fibres in turn (usually the bag_2 was reinnervated before the bag_1). These results suggest that the process of branching at the heminodes or penultimate nodes is slowed by the increased period of denervation. This might be due either to some effect

that the period of separation from the muscle has on the axon or, conversely, that the bag fibres (especially the bag₁) become less receptive as a result of some changes in specificity as might be indicated by the alterations in enzyme activity that begin around 14 days D (De Rueck et al, 1973).

12.3 The Effects of Longer Periods of Denervation on Reinnervation.

After the longer periods of denervation there was evidence of the beginnings of a breakdown in the marked regional specificities that were in evidence in the NEC and NS series. This was most obvious in the spindles that had been denervated for 50 days.

If it is assumed that the configurations of the basal lamina over the intrafusal fibres act as a physical guidance system for the returning sensory axons, which form their terminals underneath the lamina (section 10.4), then the progressive atrophic reduction in the intrafusal fibre diameter (however slight initially) would, with time, reduce the effectiveness of such a guidance system. Thus, as the fibres atrophied and shrank they would become separated from the lamina, thereby allowing small (but progressively larger) axon branches to grow between the muscle and the lamina. This would be further exacerbated by the folding of the basal lamina, as atrophy occurred and the subsequent cutting off of those folds by the growth of new lamina (Sanes et al, 1978).

Therefore purely in terms of the reduction in the effectiveness of the physical guidance system, a breakdown in the regional limitations imposed on the endings would be expected. This physical guidance system is unlikely to be the only mechanism controlling the distribution of the axon terminals on the intrafusal fibres (section 10.5). There is also likely to be a system of regional chemical specificities that controls the formation of endings.

These chemical specificities may be postulated in the NS study where there were limitations on the abilities of axons to form endings in foreign regions (section 10.5). Similar limitations are also evident from the RC series material in those spindles where there has apparently been a breakdown in the track guidance and branches of the Ia enter the S_1 region. Some of these branches double back into the P region (Fig. 117, Plate 27; Fig. 120, Plate 29; see also Fig. 38, Plate 13) and it seems likely that this may be due to the chemical influences of the S_1 region inhibiting their growth.

As the period of denervation lengthens there is also likely to be a breakdown in the regional specificities, which would further reduce the accuracy of reinnervation. This is beginning to become apparent at 50 days D when the branches of the Ia axons in some spindles were able to form endings in the S_1 regions (Fig. 118, Plate 28; Fig. 123). De Reuck et al (1973) reported that after two weeks of denervation the ATP'ase, P'ase and succinate dehydrogenase activities begin to decrease and as a result there would be a loss of at least some of the regional variations characteristic of the normal spindle. After four weeks denervation De Reuck et al (1973) found that the histochemical activities were so decreased that it was not possible to identify the fibres on the basis of their histochemistry alone.

After the repeat crush operations (RC) used to achieve the longest period of denervation (50 days D) there was an increase in the occurrence of hyperinnervation (section 8.6) to 23.8% of the population. This, again, is probably associated with the transection of the endoneurial tubes which is likely to be more frequent after repeated crushing of the nerve.

The Effect of the Denervation Period on the Recovery of Function.

13.1 General Observations.

The range of denervation periods studied was from 10 to 50 days. The results described in Chapter 9 show that an increase in the denervation period has most marked effects for the short periods from 10 to 22 days D. This is apparent not so much from comparisons of the relative rates of reformation of functional connexions, which are similar in both series (and are also similar histologically), but from comparisons of the rate of recovery of the normal response pattern once connexions have been re-established.

The longer periods of denervation show little in the way of marked effects although there was a slight trend towards slower recovery with increasing denervation time. The longest denervation period was 50 days and most of the histological and biometrical studies, ^{by others.} indicate that, although there is some atrophy of the intra-fusal fibres over this period and a reduction in enzyme activities, the atrophy is not very pronounced until the spindle has been denervated for more than 84 days (De Reuck et al, 1973; Schröder et al, 1979).

13.2 Afferent Conduction Velocities.

Not all the data on conduction velocities can be compared directly because of the differences in the siting of the stimulating electrodes with respect to the crush site.

The results of the NEC series show that initially there was only a slight reduction in the afferent CV's proximal to the injury site (Fig. 124). As the period for recovery increased, however, there was a progressive reduction in the CV's over the proximal segment which is the reverse of the trend seen in the distal segment (Fig. 60). Cragg & Thomas (1961) reported similar reductions

in CV in the proximal segment commencing some 25-30 days after nerve crush. They found that by 50 days the CV's had dropped to 30% of normal and remained so for another 100 days before gradually returning to normal. They correlated these findings with alterations in the axon diameters and myelin sheath thicknesses that occurred over extensive regions of the proximal segment. They found a clear correlation between the CV's and the diameters of the axons and also a relationship with the thickness of the myelin sheath (section 3.6).

The greatest changes in CV occurred in the distal segment (Fig. 68) where the axons were actively growing. The results illustrated in Fig. 128 show that delaying regeneration by repeating the crush injury had little effect on the restoration of the afferent CV's. This indicates that axonal regeneration as a whole was little hindered by the repeated lesions or by the increased period when the axons were deprived of the peripheral connexions which are important in regulating their maturation (Sanders & Young, 1946; Aitken, 1949; Aitken et al., 1947).

13.3 Recovery of Response Pattern and Firing Rates.

The afferents recorded in the NEC experiments showed the most rapid recovery after the injury but, as with the CV results, there is apparently little to distinguish between the results of the NS series after 22 days denervation and those even after 50 days D (the RC series).

The conclusions that can be drawn from these findings are that during the first three weeks of denervation certain changes take place in the spindle, or the regenerating axon, which, although not affecting the rate of reformation of the connexions, reduce the rate at which the endings mature. De Reuck et al (1973) reported

that the histochemical effects of denervation did not become apparent till after two weeks of denervation when the enzyme activities of the intrafusal fibres started to decrease. In the NEC experiments the denervation period was only 10 days, so there would have been little, if any reduction in the enzyme activities by the time the motor and sensory axons returned and arrested the progress of atrophy. In the NS series, however, these changes would have been well under way by the time the axons returned and, even if such changes had no effect on the restoration of the connexions, they might well have affected the rate of maturation of the endings.

After the first three to four weeks there was apparently some degree of stabilisation such that the denervation period up to 50 days had little effect on the restoration of spindle function. It must be assumed that if the denervation period were to be extended still further, into the period when marked atrophic changes occur (Tower, 1932; De Reuck et al, 1973; Swash & Fox, 1974; Schröder et al, 1979), then the formation of functional connexions and their rate of maturation would be impaired (Gutmann & Young, 1944).

If an axon is injured once, and then injured again after one or two weeks have elapsed, then the rate of axonal regeneration is increased as a result of accelerated sprout formation and outgrowth (McQuarrie, 1979; McQuarrie & Grafstein, 1973; McQuarrie, Grafstein & Gershom, 1977). These effects are attributed to an enhancement of the cell body response to the second lesion as a result of the effects of the initial conditioning lesion (McQuarrie et al, 1977).

Verma (1979) reported that, in the frog, restoration of the extrafusal neuromuscular connexions was accelerated as a result of repeating the lesion, despite the increased period of denervation. This indicates that the initial increase in the rate of growth is maintained for most, if not all, of the regeneration period (Verma, 1979). Verma (1979) further noted that the reformation of the endplates themselves was also accelerated and he suggested that the initial denervation induced the development of some latent capacity for regeneration developed by both the nerve and the muscle.

If an increased growth rate occurred and was maintained in the repeat crush experiments then these spindles would have had longer periods of reinnervation time and would have been denervated for shorter periods than was predicted by assuming a fairly constant rate of regeneration. This increased period of reinnervation might have offset any slight reductions in the rate of ending maturation that might have occurred as a result of the increased denervation period.

The increased denervation period achieved by making the lesion more proximally (the TC series) also had little effect on the rate of recovery, there being little difference between the quality of function of the afferent populations in the TC and NS series. This lends support to the hypothesis that after the first three weeks there is some degree of stabilisation such that increasing the denervation period has little effect on the recovery of function over the periods examined.

It should also be borne in mind, however, that these measurements were made at a time when the units in the NS series were showing little progress towards normal function. The results shown in Tables 9 and 12 indicate that over the period of 25-53

days RT the recovery of the afferent population as a whole was very slow with no changes occurring in the mean peak firing rates. This is probably partly due to the return and subsequent gradual saturation of the afferents returning in the second wave of reinnervation. The effect of this plateau could be to mask some of the effects of the increased denervation periods because it is not possible to determine whether the delayed-return afferent population was actually at the same point on the plateau as the RC afferents. Thus, at 74 days RT, when the NS population showed a marked improvement, the afferents that were in the RC populations, especially those in the 50 days D sample, might have lagged behind and not shown any improvement till after a longer period of recovery. There is a need for further experiments to clarify this point.

14.1 Comparison of the Histological and Physiological Results.

In the NS series there was a close correlation between the histological and physiological results. Both sets of results indicated that the first afferents were restored by 4 days RT and that the restoration of the secondaries lagged slightly behind that of the primaries. There was also clear agreement on the time of return of the second wave of reinnervation (Tables 1, 3).

The configurations of the endings were completed very rapidly with little apparent change occurring after 33 to 40 days RT (section 6.2). The time period for restoration of normal function was more extended with a relatively high proportion of afferents still showing class 1 or 2 responses after 84 days RT (Table 9). These differences probably reflect the different processes involved. The restoration of the structure of the ending is a matter of axon growth directed by the presumed physical and trophic guidance systems. The restoration of ending function is based on a process of maturation of the terminal and pacemaker membranes that is probably dependent on the prior restoration of contact between the axon terminals and the intrafusal fibres. Therefore the restoration of normal function would inevitably lag behind the physical restoration of the ending, especially as the rate of maturation of the pacemaker sites appears to be relatively slow (Table 9).

An important disparity between the histological and physiological results of the NS series is that whereas the physiological results showed an eventual return to normal function, there was never a full recovery in terms of the appearance of the endings which always showed a marked reduction in the number of terminal bands formed around the intrafusal fibres. This raises the question as to the extent to which morphological abnormality results in physiological dysfunction.

It could be argued that the ramp-and-hold stretch, with or without δ fusimotor stimulation at set frequencies, is an insufficiently rigorous test of spindle function and is too crude to pick up the more subtle physiological abnormalities that may be present. A further problem is that, because of the nature of the system under examination in these experiments, a direct correlation between morphology and function in an individual spindle was not possible. Despite these drawbacks it can be concluded that in the normal spindle there is probably a degree of redundancy in the elaborate configurations of spirals, rings and irregular terminals formed by the afferent axons on the intrafusal fibres, certainly as far as can be determined by the restoration of apparently normal function as seen in these experiments.

The histological and physiological results of the NEC experiments also show a correlation of time scales (except over the return of the second wave of reinnervation; see section 12.1). In both sets of results the first endings were located at 5 days RT with a much greater proportion having returned by 9 days RT. The results also show a comparably rapid restoration of morphology and function, although, as with the NS series, the restoration of function lagged slightly behind the restoration of the ending structure. Again, after the longer recovery periods, normal function was apparently restored without the full restoration of normal morphology.

The greatest disparity between the histological and physiological results occurred in the RC and, to a lesser extent, the TC experimental series. Whereas the histological data showed a progressive deterioration in the complexity and organisation shown by the regenerated endings as the denervation period was increased

(section 8.5), the physiological results showed only a very slight trend towards lower firing rates and reduced CV's (section 9.7, 9.8). Some of this absence of functional deterioration might be attributed to the slightly increased reinnervation period due to the increased rate of regeneration after the repeated lesions (section 13.3). The problem still remains, however, of determining to what extent normal (or apparently normal) function is dependent on the restoration of morphological normality and to what extent the structure of the ending is redundant. In this context it would have been of especial interest to have been able to examine the response patterns of individual spindle endings (such as those from the spindle in Fig. 123) and subsequently to analyse the distributions of the endings on the intrafusal fibres.

This question is one that can only be begun to be answered by experiments in which single spindles are tested with a range of mechanical stimuli (ramp stretches, sine-wave stretching, vibration, etc) in the presence and absence of both γ and β stimulation, followed up by thorough histological or ultrastructural analysis of the complete spindle. What the results that have been described do show is that there may be some degree of redundancy in the elaborate configurations that characterise primary and secondary endings. Under the experimental regimes that were employed, regenerated endings with only half the normal number of terminal bands were apparently capable of functioning normally.

14.2 Final Conclusions.

All the results that have been described indicate that both morphologically and physiologically the prognosis for spindle re-innervation is very good after nerve-crush injury even if the spindles remain denervated for some weeks. There is a powerful guidance system which is probably a physical track provided by the basal lamina of both the axons and the muscle fibres, supplemented by the trophic attractions and regional specificities of the denervated intrafusal muscle fibres. Together these systems enable a highly successful restoration of the spindle innervation and, consequently, of spindle function.

Mistakes do occur in this system; the ones that were most common in these studies came about either as a result of severance of the endoneurial tubes, or because atrophy of the muscle fibres caused the basal lamina to become separated from the surface of the fibres and also reduced the chemical specificities of the different regions.

Severance of the endoneurial tubes was a direct result of the mechanical injury and, in some cases, the correct axon was unable to locate the opening to the distal segment or else an axon of the wrong type entered the tube. In either eventuality the correct innervation was not restored and this obviously would have had detrimental effects on the functioning of the spindle.

Separation of the muscle fibre from its basal lamina as a result of muscle atrophy would reduce the effectiveness of the track guidance thereby allowing the axon branches to invade other regions. This would, therefore, be detrimental to the organised distribution of the terminals. With increasing periods of denervation these effects became more pronounced and there was also

evidence of a reduction in the regional specificities which further reduced the quality of reinnervation.

The results of these experiments have important implications for studies on recovery after nerve section and on the effectiveness of different nerve-repair techniques. If the guidance system operates as has been proposed, then a primary axon is only likely to reinnervate the primary region of a spindle if it is successful in relocating the distal stump of a Ia endoneurial tube. This success at the injury site is especially critical for sensory axons since they form their endings beneath the basal lamina of the muscle fibres and access to this site, other than by growing down the correct tube, would be severely restricted. The problem for motor axons is not so great because they form their endings on the surface of the lamina of the muscle fibres. In their case, therefore, local trophic attractions would probably enable the eventual reinnervation of an endplate by a motor axon even if it originally arrived in the wrong region of the spindle.

These differences in the requirement for successful location of the distal stump probably account for the findings of many surgeons that although there is often reasonable restoration of gross motor control after nerve repair, the sensory systems are always less successfully restored (Hudson & Domisse, 1977; Tallis, Staniforth & Fisher, 1978; Haase, Bjerre & Simesen, 1980; Hallin et al, 1981). The ultimate (though probably never fully attainable) objectives of nerve repair must therefore be near-perfect alignment of the proximal and distal stumps, achieved with the minimum of disruption (to avoid excessive scar tissue), the repair being effected as soon after the occurrence of the injury as possible.

The results obtained from these series of experiments form a useful starting point for analysing the reinnervation of the muscle spindle after other forms of nerve injury. Because after the crush injury almost all the axons regenerate to reinnervate their old sites, these results can be used to identify what effects are due to the period of denervation itself as opposed to those due to incorrect reinnervation. Further work is also necessary to examine the effects of even longer periods of denervation on the success of the subsequent reinnervation, as the periods of denervation that were examined were only long enough to show the start of the breakdown of the specificities of reinnervation.

The results that have been described do indicate that successful restoration of function is not dependent on the perfect restoration of the morphology of the endings. This conclusion provides some hope for the restoration of spindle function after more serious nerve injuries even if the period of denervation has been such that the ending is very poorly restored in terms of its physical appearance.

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