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TYPES OF INTRA- AND EXTRAFUSAL MUSCLE FIBRE INNERVATED BY DYNAMIC SKELETO-FUSIMOTOR AXONS IN CAT PERONEUS BREVIS AND TENUISSIMUS MUSCLES, AS DETERMINED BY THE GLYCOGEN-DEPLETION METHOD

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SUMMARY

1. The types of intra- and extrafusal muscle fibre innervated by dynamic skeleto-fusimotor (β) axons were determined by using a modification of the glycogen-depletion method of Edström & Kugelberg (1968) combined with histochemical tests for various enzyme reactions. A single β axon was prepared in each of the experiments, which were carried out on six peroneus brevis and two tenuissimus muscles.

2. The intrafusal distribution of dynamic β axons is almost exclusively restricted to bag_1 fibres. The bag_1 fibre was depleted in each of twenty-four β-innervated spindle poles; the only fibres of a different type depleted intrafusally were a bag_2 fibre in one pole and a long chain in another.

3. Depletion in the bag_1 fibres was usually restricted to one zone in one pole, generally in a mid-polar location.

4. The extrafusal muscle fibres depleted by dynamic β axons belong to the slow oxidative type as defined by Ariano, Armstrong & Edgerton (1973). The number of such fibres in each motor unit could not be accurately determined, but is almost certainly small.

5. The slow oxidative muscle fibres innervated by dynamic β axons were not depleted over their entire length. Since there is no reason to assume that they are not twitch fibres, it would seem that the localized depletions result from the conditions required to obtain glycogen depletion, i.e. long periods of motor stimulation applied during the occlusion of the muscle's blood supply. Under similar experimental conditions depletion of

glycogen was also restricted to portions of fibres in fast oxidative–glycolytic motor units, but extended over most of the length of the fibres in fast glycolytic units.

INTRODUCTION

The aim of the present study was to use the glycogen-depletion method of Edström & Kugelberg (1968) to determine the types of intra- and extrafusal muscle fibre innervated by dynamic skeleto-fusimotor (β) axons. The activation of such axons elicits the contraction of both intra- and extrafusal muscle fibres and increases the dynamic sensitivity of primary endings. Their significant contribution to the motor innervation of cat muscle spindles has recently been reviewed by Laporte & Emonet-Dénand (1976).

Mammalian muscle spindles are composed of nuclear-chain muscle fibres and two types of nuclear-bag muscle fibre, namely bag_1 and bag_2 fibres, as has been shown by recent histochemical and ultrastructural studies (Ovalle & Smith, 1972; Banks, Barker, Harker & Stacey, 1975; Banks, Harker & Stacey, 1977). Both types of bag fibre are present in every spindle, usually one of each type. In cat spindles, the bag_1 fibre, as compared with the bag_2, is shorter and thinner; its glycogen content is less; and the alkaline ATPase reaction is weaker producing a lighter stain. In a recent study of the sites of glycogen depletion produced by stimulating dynamic γ axons, we found that they were almost exclusively located in bag_1 fibres (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1976*b*). The present study has shown that this also applies to the fusimotor distribution of dynamic β axons. In a preliminary account of the work (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1975) we referred to the bag_1 fibres as 'intermediate bag fibres', a term since abandoned (see Banks *et al.* 1977).

The three types of mammalian extrafusal muscle fibre are referred to in this paper as 'fast glycolytic', 'slow oxidative', and 'fast oxidative–glycolytic' following the classification of Ariano *et al.* (1973). This avoids the confusion caused by the often contradictory use of letters and Roman numerals by different authors for the same types of fibre (see review by Close, 1972). The extrafusal muscle fibres innervated by dynamic β axons were found to be of the slow oxidative type.

METHODS

The experiments were carried out on eight adult cats anaesthetized with I.P. pentobarbital (Nembutal, Abbott Laboratories; 40 mg/kg). The essentials of the experimental methods were similar to those fully described by Emonet-Dénand, Jami & Laporte (1975) and Barker *et al.* (1976*b*).

In each experiment a single dynamic β axon supplying the tenuissimus or peroneus brevis muscle was prepared and repetitively stimulated during several periods of occlusion of the blood supply to the muscles so as to produce glycogen depletion in the muscle fibres of its motor unit. The muscle portions containing the activated spindles were then quick-frozen, cut serially, processed for glycogen staining and examined for glycogen depletion.

Identification and stimulation of dynamic β axons. After extensive denervation of the hind limb, in which only the nerve to tenuissimus (in two experiments) or to peroneus brevis (in six experiments) was preserved, single Ia afferents innervating spindles in the experimental muscle were prepared by splitting dorsal root filaments. Afferents from peroneus brevis were identified as Ia axons only if their discharge was also accelerated by the stimulation of γ axons. Single motor axons were isolated by splitting ventral roots until orthodromic stimulation of a filament elicited an all-or-none action potential in the muscle nerve together with an all-or-none action potential of the motor unit. Great care was paid to the detection of γ axon potentials in order to ensure than no γ axon was present in the filament containing the β axon selected for repetitive stimulation.

A motor axon was provisionally identified as β if: (i) its stimulation at 100/sec elicited activation of extrafusal muscle fibres together with an increase in the discharge frequency of one or more of the prepared Ia afferents; and (ii) it exerted a dynamic action on the response of the same afferents to rapid ramp stretches of 2–5 mm (see Text-fig. 1), or, as in two peroneus brevis experiments, to small (0·5 mm) sinusoidal stretch at a frequency of 1–2 Hz. Additional evidence in favour of identifying a motor axon as β was provided by the observation that the stimulation of such axons at 10–20/sec often produced a pause in the discharge of the activated spindle, thus revealing an unloading effect produced by the contraction of the innervated extrafusal muscle fibres (see Bessou, Emonet-Dénand & Laporte, 1965). The best proof of β identity, of course, lay in the histological examination of the experimental muscle and the finding of glycogen depletion in both intra- and extrafusal muscle fibres. Standard procedures for β identification, based on blocking neuromuscular transmission in extrafusal muscle fibres (Bessou *et al.* 1965; Emonet-Dénand & Laporte, 1975), were not used since it was feared that incomplete reversal of the block might reduce the efficiency of the subsequent stimulation used to produce extrafusal glycogen depletion. All testing procedures were carried out as quickly as possible in order to avoid the possibility of depleting muscle fibres other than those innervated by the presumed β axon, and as a further precaution the animal was left to rest for an hour before starting the stimulation procedures used to produce depletion.

The regime of stimulation and blood occlusion used to produce glycogen depletion was the same as that reported in our γ-depletion study (Barker *et al.* 1976*b*), except that during its application the muscle action potential was monitored as well as the discharge from the activated primary endings. A rapid decrease in amplitude of the muscle action potentials was interpreted as an indication of unwanted neuromuscular block rather than muscle-fibre fatigue. In that event, the stimulation frequency was reduced and the total number of stimulation periods increased up to eighteen.

The nerve action potentials were continuously and carefully monitored throughout the stimulation periods so as to be certain that no stray γ axon was being excited. This may occur owing to the recovery of a γ axon damaged during the root-splitting process. Since small action potentials of slow γ axons may be overlooked when recorded together with large amplitude muscle action potentials, a further control on the absence of γ stimulation was provided, after removal of the muscle for histology, by recording the action potential of the stimulated β axon from the cut nerve stump.

If present, the monophasic action potential of even the slowest γ axon would become apparent and the muscle discarded.

During the course of the investigation the unexpected observation was made that the glycogen depletion produced in extrafusal muscle fibres by the stimulation of β axons does not extend over their whole length. Since the muscle fibres are presumably twitch fibres, the factors responsible for this localized depletion are not clear. We therefore decided to study the depletion produced in other types of extrafusal muscle fibre, isolating single α axons and applying the same regime of stimulation and blood occlusion as was used with the β axons. Ten experiments involving the three types of motor unit were carried out on peroneus brevis muscles.

Histological procedures. The two tenuissimus muscles were processed exactly as described by Barker *et al.* (1976*b*). After excision and quenching in isopentane cooled to $-160°$ C, the pieces of muscle containing the activated spindles were fixed in absolute ethanol during freeze-substitution at $-40°$ C for 2 or 3 days and then embedded in plasticized paraffin wax (Paramat). The paraffin blocks were despatched from Paris to Durham where serial transverse 10 μm sections were cut, stained for glycogen using the periodic acid–Schiff (PAS) method and examined for depletion.

The six peroneus brevis β experiments, including the histological analysis, were carried out in Paris during three visits made by D.W.H. The muscles were sectioned frozen and pairs of sequential slides were processed for glycogen (PAS) and either alkaline ATPase (Guth & Samaha, 1970), P'ase (Eränkö & Palkama, 1961), or succinate dehydrogenase (Nachlas, Tsou, de Souza, Cheng & Seligman, 1957). Comparison of sections stained for glycogen with adjacent sections stained for the enzyme reactions enabled the histochemical profiles of the depleted fibres of the skeleto-fusimotor unit to be identified. The same histological procedures were applied in the ten peroneus brevis α experiments.

Serial reconstructions were made of each experimental spindle in order to map the zones of glycogen depletion in the intrafusal muscle fibres. The three types of fibre were identified on the basis of length, diameter, equatorial nucleation, glycogen content and (for fresh-frozen material) histochemical profile, according to the criteria established by Banks *et al.* (1977).

The reasons why no precise correlation could be made in tenuissimus between a depleted spindle and a physiologically studied spindle are discussed by Barker *et al.* (1976*b*). In peroneus brevis the distribution of the spindles is such as to make it impossible to locate a particular spindle, other than very approximately; depleted spindles and β-activated spindles were therefore only collectively correlated.

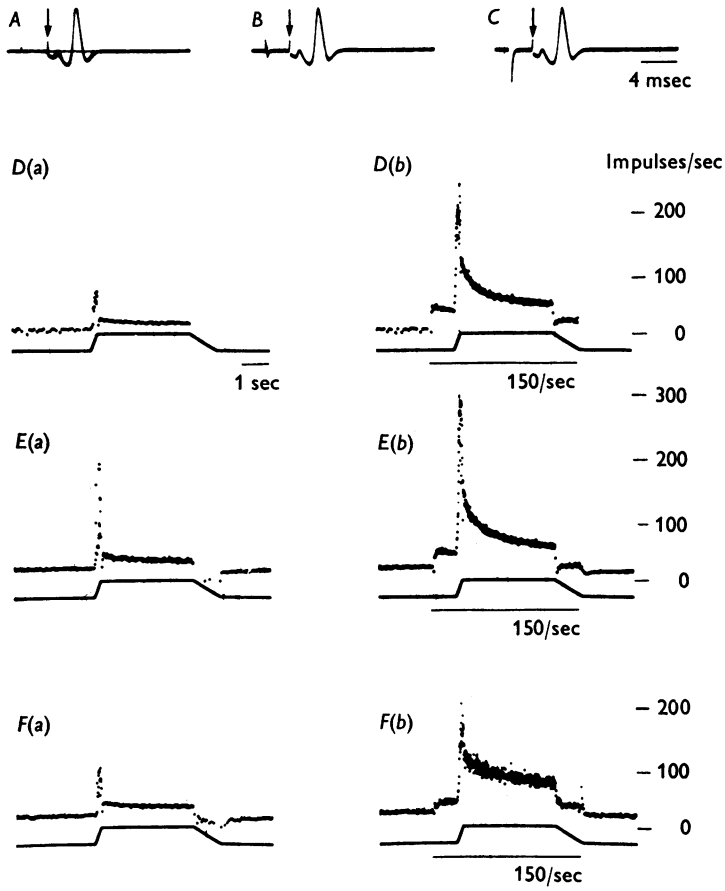
RESULTS

Intrafusal depletion

Of the eight single dynamic β axons studied (conduction velocity range 51–77 m/sec), three were observed to accelerate the discharge of one spindle primary afferent, three accelerated two primaries and two accelerated three. Histological analysis of the eight muscles revealed that two muscles had one spindle showing glycogen depletion, three had two, two had three, and one had four.

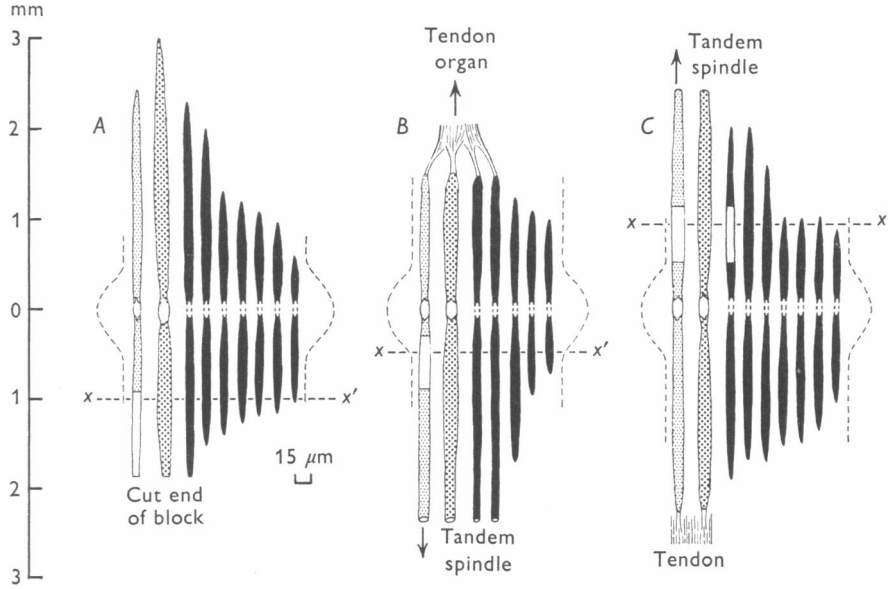
In one particularly successful experiment carried out on a peroneus brevis muscle the isolated β axon accelerated the discharge of three out of seven prepared single Ia afferents, and subsequent examination of the

muscle correspondingly revealed glycogen depletion in three spindles. Text-fig. 1 shows the increase in dynamic sensitivity of each of the three spindles following stimulation of the β axon. In two spindles the dynamic



Text-fig. 1. Dynamic action exerted by a single β axon on three primary endings in peroneus brevis. *A–C*, nerve and muscle action potentials recorded after stimulation of a L7 ventral-root filament. The first recording electrode was placed under a small nerve branch innervating the muscle; the second electrode on the surface of the muscle. Superimposed sweeps. Since a relatively slow sweep speed was used, the action potential of the motor axon appears in each record as a vertical bar; this is indicated by an arrow. *A*, near-threshold stimulation showing the all-or-none behaviour of the potentials. *B*, threshold stimulation; the conduction velocity of the axon was 77 m/sec. *C*, stimulus strength approximately 100 × threshold, showing that the motor filament did not contain any high-threshold slow-conducting γ axon. *D–F*, (*a*) passive responses of the three primary endings to ramp-and-hold stretch of 3 mm. *D–F* (*b*), modifications of the responses due to repetitive stimulation of the β axon at 150/sec. Note the very large increase in dynamic index in records *D* (*b*) and *E* (*b*).

response was typical (see Text-fig 1*D*(*b*) and *E*(*b*)), but the response of the third spindle (Text-fig. 1F(*b*)) was different. The discharge was irregular suggesting that a fast-contracting muscle fibre was involved. Subsequent histological examination confirmed this. Zones of glycogen depletion were found in the bag_1 fibres in each of the three spindles, but in one of them



Text fig. 2. Schematic reconstructions of three peroneus brevis spindles showing patterns of glycogen depletion elicited by stimulation of a single dynamic β axon (same experiment as in Text-fig. 1). Intrafusal fibre types have been standardized for diameter and glycogen level as follows: bag_1 fibres, medium diameter, light shading (medium glycogen); bag_2 fibres, large diameter, coarse shading (medium/high glycogen); chain fibres, small diameter, filled (high glycogen). The chain fibres in each pole are arranged in order of decreasing length and do not necessarily correspond. The proximal poles are those at the top of the Text-figure. The bag_1 fibre shows depletion (blank stretch) in each of the three spindles; in spindle *C* a chain fibre is also depleted. $x - - - x'$, level of transverse sections illustrated in Pl. 1.

there was also depletion in a long chain fibre (i.e. a chain fibre whose course extends well outside the capsule; see Barker, Banks, Harker, Milburn & Stacey, 1976*a*). Schematic reconstructions of these spindles (two of them were parts of tandem spindles) are shown in Text-fig. 2 and photographs of sections representative of the depletion in each spindle are illustrated in Pl. 1. Each bag_1 fibre was depleted in one zone and one pole only.

The eight single dynamic β axons produced zones of glycogen depletion in fifteen whole spindles and three half-spindles (i.e. spindles cut in two when the muscle portion was excised so as to leave only one complete pole). The patterns of depletion in these spindles are summarized in Text-fig. 3.

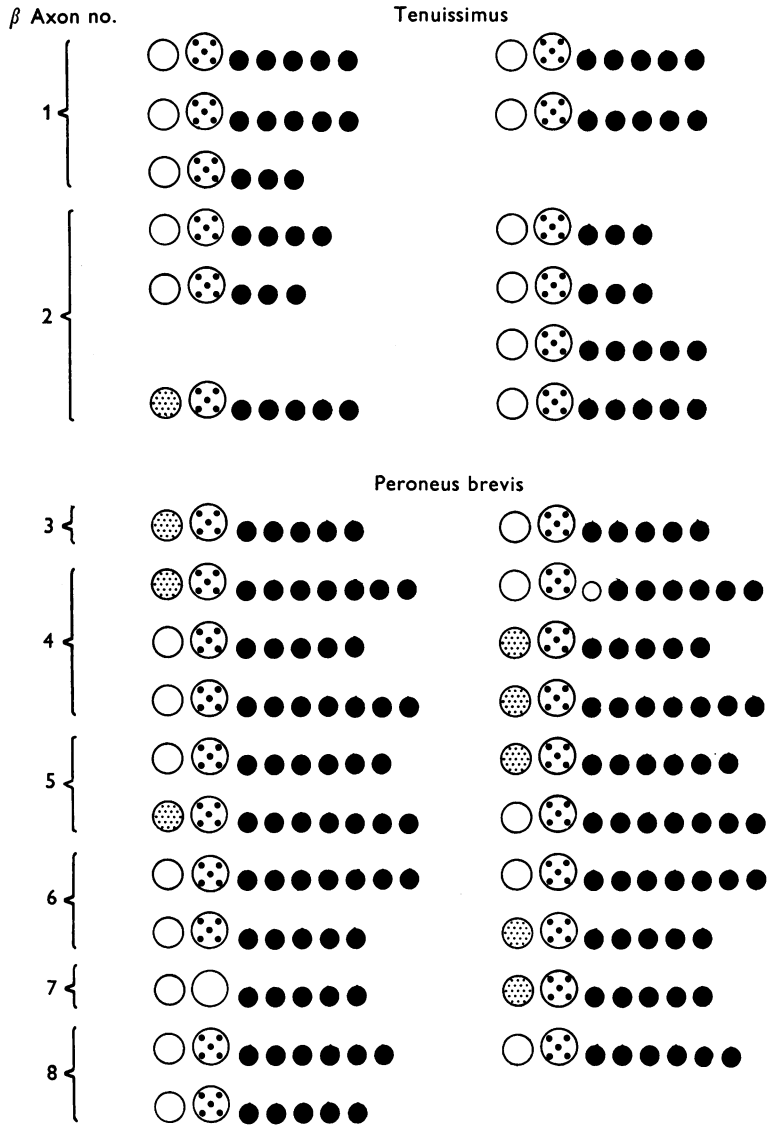
The fifteen whole spindles contained thirty bag fibres (one per spindle of each type) and eighty-two chain fibres (mean chain-fibre complement, 5.46). Depletion was almost exclusively restricted to the bag_1 fibres. In thirteen whole spindles and the three half spindles this was the only fibre type depleted. In the remaining two whole spindles other fibre types were depleted in addition, in one a bag_2 fibre, in the other a long chain fibre. In terms of the twenty-four spindle poles depleted, bag_1 fibres were activated in twenty-four, a bag_2 fibre in one, and a long chain fibre in one.

Depletion was usually restricted to one zone in one pole; three bag_1-fibre poles had two zones of depletion and one had three. Six of the fifteen whole spindles were depleted in both poles. This occurred more frequently among tenuissimus spindles (four of five spindles depleted in both poles) than peroneal ones (two of ten spindles depleted in both poles). The spindles in peroneus brevis also differed in being shorter (by about 2 mm) and more often linked in tandem.

The mean length of the depleted zones in bag_1 fibres was 485 μm in tenuissimus spindles (16 zones; range 150–1400 μm) and 646 μm in peroneal spindles (13 zones; range 300–1260 μm). In the single bag_2 fibre activated the depleted zone measured 330 μm, and in the long chain 528 μm. In terms of the three regions between equator and pole extremity distinguished by Barker *et al.* (1976*a*) the centres of the depleted zones were located as follows: three (9·7 %) in region A (that part of the equatorial region extending from the equator to the equatorial end of the periaxial space); seventeen (54·8 %) in region B (that part of the pole lying between region A and the end of the capsule); and eleven (35·5 %) in region C (extracapsular part of the pole). The centres of the depleted zones were thus mostly (64·5 %) intracapsular. In the tenuissimus spindles the mean distance of the depleted zone centres from the equator was 1653 μm; this compares with a mean distance from the equator of 1732 μm for the centres of zones depleted by dynamic γ axons in tenuissimus spindles in our γ-depletion study (Barker *et al.* 1976*b*).

Extrafusal depletion

The extrafusal muscle fibres depleted by the stimulation of single β axons were studied in five peroneus brevis experiments. The fibres do not closely surround the spindle but are found scattered among several muscle bundles, as in the case of ordinary motor units. Part of a transverse section of the muscle from the same experiment illustrated in Text-figs. 1 and 2 is seen in Pl. 1 A. It shows one of the three depleted spindles, together with some of the extrafusal fibres depleted by the same β axon. Histochemical tests, which gave light staining with alkaline ATPase and dark with succinate dehydrogenase, showed that the fibres were of the slow oxidative type (see



Text-fig. 3. Summary of the glycogen depletion produced in cat tenuissimus and peroneus brevis spindles by stimulating single dynamic β axons during periods of blood occlusion. Dynamic action identified with ramp stretches (axons 1–6) or sinusoidal stretch (axons 7–8). Both poles of the affected spindles are shown, except when one was lost. Fibres that contained one or more zones depleted of glycogen in a particular pole are shown as open circles; bag_1 fibre, ◎; bag_2 fibre, ⊛; chain fibre, ●.

Pl. 2*b*), and this was also true of the extrafusal fibres in the four other β motor units.

The number of depleted extrafusal fibres in the β motor units was highly variable from one experiment to another (range 5–40), in poor correlation with the amplitude of the recorded muscle action potential. Since slow muscle fibres are very resistant to glycogen depletion (Burke, Levine, Tsairis & Zajac, 1973), probably only a fraction of the fibres belonging to the stimulated β motor unit were depleted in each experiment. There were some fibres that appeared paler than normal, suggesting that glycogen had been partially depleted in them. However, following our previous practice (Barker *et al.* 1976*b*) we ignored these and counted only those fibres in which complete blanching was observed.

An unexpected finding was that the affected extrafusal fibres were not depleted over their entire length. The zones of glycogen depletion measured from 0·5 to 2 mm, whereas the length of extrafusal muscle fibres in peroneus brevis ranges from 4 to 8 mm. Fibre length was measured after teasing acid-macerated muscles (Adrian, 1925) into separate bundles. Further teasing of these bundles, which all contain the three types of fibre, showed that fibres of different diameter had the same length, extending from one aponeurotic or tendinous insertion to the other.

Restriction of the depletion of glycogen to part of the total length of the fibre was not specific to β units. Three motor units innervated by axons conducting at 67, 70 and 82 m/sec were also composed of fibres of the slow oxidative type in which depletion was similarly restricted to a length of only 0·5–1·5 mm. In three other motor units (axonal conduction velocities 77, 86 and 86 m/sec) the fibres were of the fast oxidative–glycolytic type and depletion was again restricted, the lengths blanched being 1·5–2·5 mm. Finally, in four motor units (axonal conduction velocities 97, 100 and 104 m/sec) the fibres were of the fast glycolytic type and depletion was extensive, the zones measuring 3–5 mm long and occupying most of the fibre. Counts of the number of fibres with totally blanched zones comprising these ten motor units ranged from twenty to eighty for the slow oxidative type, from twenty to 250 for the fast glycolytic type, and averaged fifty for the fast oxidative–glycolytic type. These figures do not represent total populations, since they exclude fibres that appeared to have zones of incomplete depletion.

DISCUSSION

The present study has shown that dynamic skeleto-fusimotor (β) axons innervate extrafusal muscle fibres of the slow oxidative type and that their collateral branches distributed to muscle spindles innervate bag_1 fibres almost exclusively. The pattern of intrafusal depletion produced by

dynamic β axons is strikingly similar to that produced by dynamic γ axons. In our γ-depletion study (Barker *et al.* 1976*b*) we found that the depletion produced by four dynamic γ axons was likewise almost exclusively restricted to bag_1 fibres. This was the only fibre type depleted in thirteen spindles and one half-spindle. In the remaining three spindles activated, other fibre types were depleted in addition, in one a bag_2 fibre, in two a long chain fibre. An almost selective distribution of dynamic γ axons to bag fibres has also been reported by Brown & Butler (1973) in their glycogen depletion study of tenuissimus spindles. In a sample of seven spindles, all had depleted bag fibres; only one spindle had a chain fibre depleted in addition. In cinematographic analysis (Bessou & Pagès, 1973, 1975; Boyd, Gladden, McWilliam & Ward, 1975; Boyd & Ward, 1975) only bag-fibre contraction has been observed on stimulating a single dynamic γ axon and the 'dynamic bag fibre' distinguished by Boyd *et al.* (1975) is most probably the same as the histochemically defined bag_1 fibre in our studies. The common innervation of the same spindle by both β and γ dynamic axons has been demonstrated in various muscles (Bessou *et al.* 1965; Emonet-Dénand & Laporte, 1975) and the fact that they share the same intrafusal distribution is further evidence that dynamic effects are produced through the activation of a specific type of intrafusal muscle fibre, namely the bag_1 fibre (see discussion in Barker *et al.* 1976*b*).

Direct evidence in support of the assumption that the fusimotor collaterals of β axons terminate in p_1 plates (Barker, 1967) was provided by an instance of this being observed in a tenuissimus muscle in which all other motor axons except one β axon had degenerated (Barker, Emonet-Dénand, Laporte, Proske & Stacey, 1971). Apart from this, the evidence is circumstantial and depends on the fact that after section of a muscle nerve, extrafusal end-plates and p_1 plates degenerate simultaneously and faster than other fusimotor endings (Barker, Stacey & Adal, 1970). Since nerve endings apparently degenerate at rates that differ according to the diameter of their stem axon (those supplied by the largest axons being the first to disappear), this observation suggests that the axons supplying extrafusal end-plates and those supplying p_1 plates have comparable diameters and that they are larger than γ axons. Such large axons contributing to spindle innervation might either belong to a purely fusimotor component in the α range of conduction velocity, or constitute a distinct skeleto-fusimotor group. The former alternative (see, for example, Barrios, Haase & Heinrich, 1967) has been ruled out by the results of an extensive study showing that stimulation of single motor axons in the 50–100 m/sec range always activates extrafusal muscle fibres (Ellaway, Emonet-Dénand, Joffroy & Laporte, 1972).

The location of the depleted zones in spindles activated by the

stimulation of dynamic β axons is consistent with their representing sites of p_1 innervation. Studies of teased, silver preparations by Barker *et al.* (1970) have shown that the axons supplying p_1 plates are predominantly distributed to bag fibres and that most of them enter a spindle pole to terminate in a single plate, either in the midpolar region or towards the end of the pole. They observed two instances in which the same axon supplied p_1 plates to both bag and chain fibres. These observations are matched in the present study by the intra- and extracapsular location of the depleted zones in bag fibres (specifically bag_1); the depletion in a spindle usually being restricted to a single zone in one pole; and the occasional occurrence in a spindle of both bag_1 and chain depletion. A similar close correlation also occurs between the location of the depleted zones in spindles activated by dynamic γ axons, and the distribution of p_2 plates as observed in silver preparations (Barker *et al.* 1976*b*). In fact, it may be said of the two types of dynamic axon, β and γ, that the only differences between them at receptor level lie in the nature of their terminals and the axons supplying them.

In a sample of seventeen peroneus brevis spindles Barker *et al.* (1970) found that eleven (65 %) received a p_1 innervation. In a physiological investigation of peroneus brevis Emonet-Dénand & Laporte (1975) found that a similar proportion of spindles was activated by β axons, namely 72 % (twenty-three in a sample of thirty-two). For comparison, the proportion of spindles that received a p_1 innervation in the other peroneal muscles studied by Barker *et al.* (1970) was 26 % in peroneus longus and 83 % in peroneus digiti quinti. (In their paper Emonet-Dénand & Laporte (1975) erroneously compared the proportion of β-innervated spindles with the proportion of spindles *poles* (34·5 %) reported by Barker *et al.* (1970) as receiving p_1 innervation in a sample of eighty-four peroneus brevis poles. The proportion is, of course, lower in terms of poles than spindles since usually only one pole of a spindle receives p_1 innervation.)

In our experiments with β axons only one chain fibre among a total of eighty-two showed glycogen depletion, whereas Barker *et al.* (1970) found that 25 % of the p_1 innervation was distributed to chain fibres. However, it is probable that most of this innervation belongs to the fast-conducting (above 90 m/sec) β axons recently found by Harker, Jami & Laporte (1976) to be innervating 27 % of the spindles in peroneus digiti quinti (referred to by them as peroneus tertius). The glycogen depletion produced by the stimulation of these axons was almost entirely restricted to one or two of the longest chain fibres in any spindle pole. The function of these β axons, and the type of extrafusal muscle fibre they innervate, remain to be determined.

Accurate estimation of the size of motor units innervated by dynamic β

axons is not possible from the results of glycogen depletion experiments because of the occurrence of fibres with incomplete depletion but they are almost certainly small. The relatively slow conduction velocity of dynamic β axons indicates that they innervate small motor units. Motor units in peroneus brevis innervated by α axons in the same range of slow conduction velocity develop mean tetanic tensions of between 1 and 5 g (Jami & Petit, 1975). It has been demonstrated histologically by Adal & Barker (1965) that the distribution of some β axons is predominantly fusimotor, whereas that of others is predominantly skeletomotor. This factor may have contributed to the high variability of the counts made of the extrafusal muscle fibres in our depleted β motor units.

It is difficult to understand why the depletion of glycogen should have been restricted to short lengths in extrafusal muscle fibres of the slow oxidative and fast oxidative–glycolytic types, since there is no reason to suppose that these are not twitch muscle fibres. It will be appreciated that the concurrence of blood occlusion with unphysiological rates of stimulation during periods of one or several minutes creates highly abnormal conditions for muscle contraction. We can only state that the different types of muscle fibre are unequally sensitive to these experimental conditions and that the muscle fibres most dependent on oxidative metabolism are those in which propagation of action potential, or contraction, or both, are probably most vulnerable. It would be interesting to know whether the localized sites of depletion are centred around motor end-plates.

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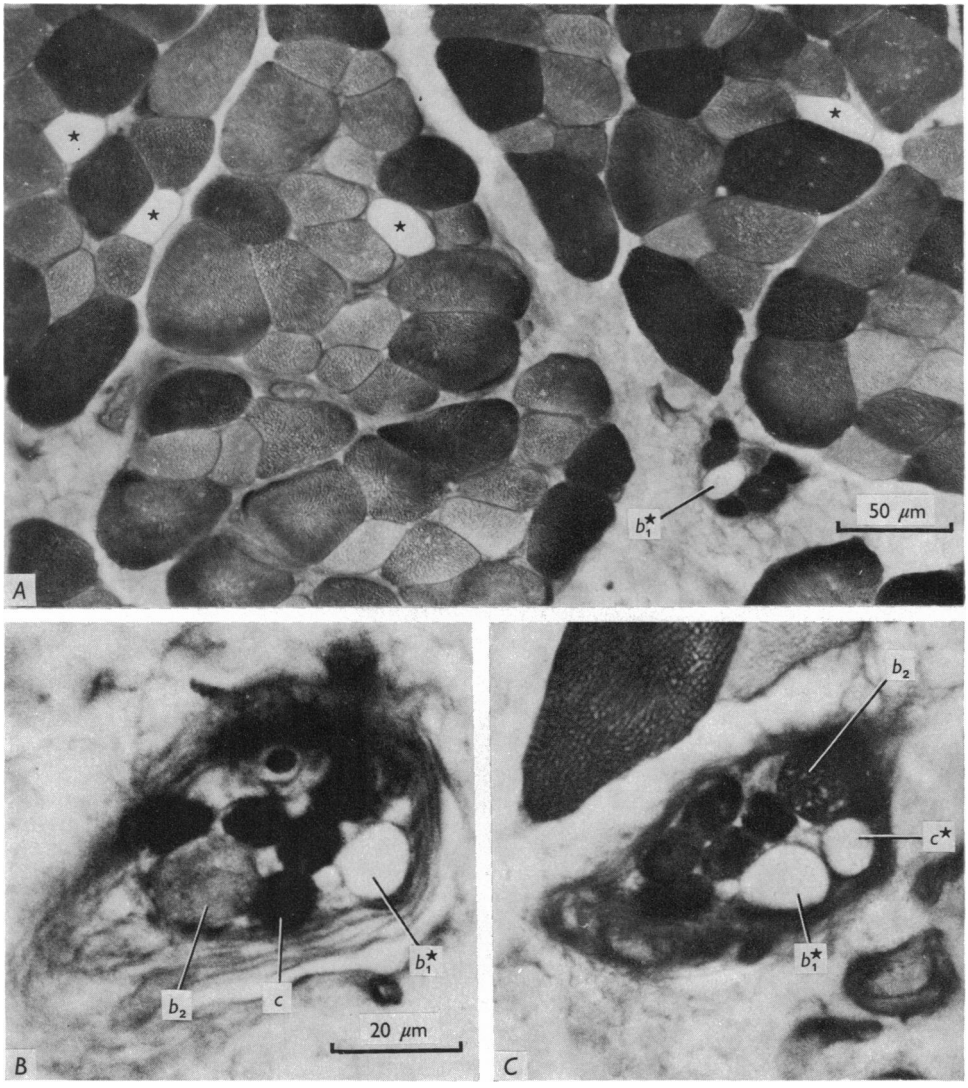
EXPLANATION OF PLATES

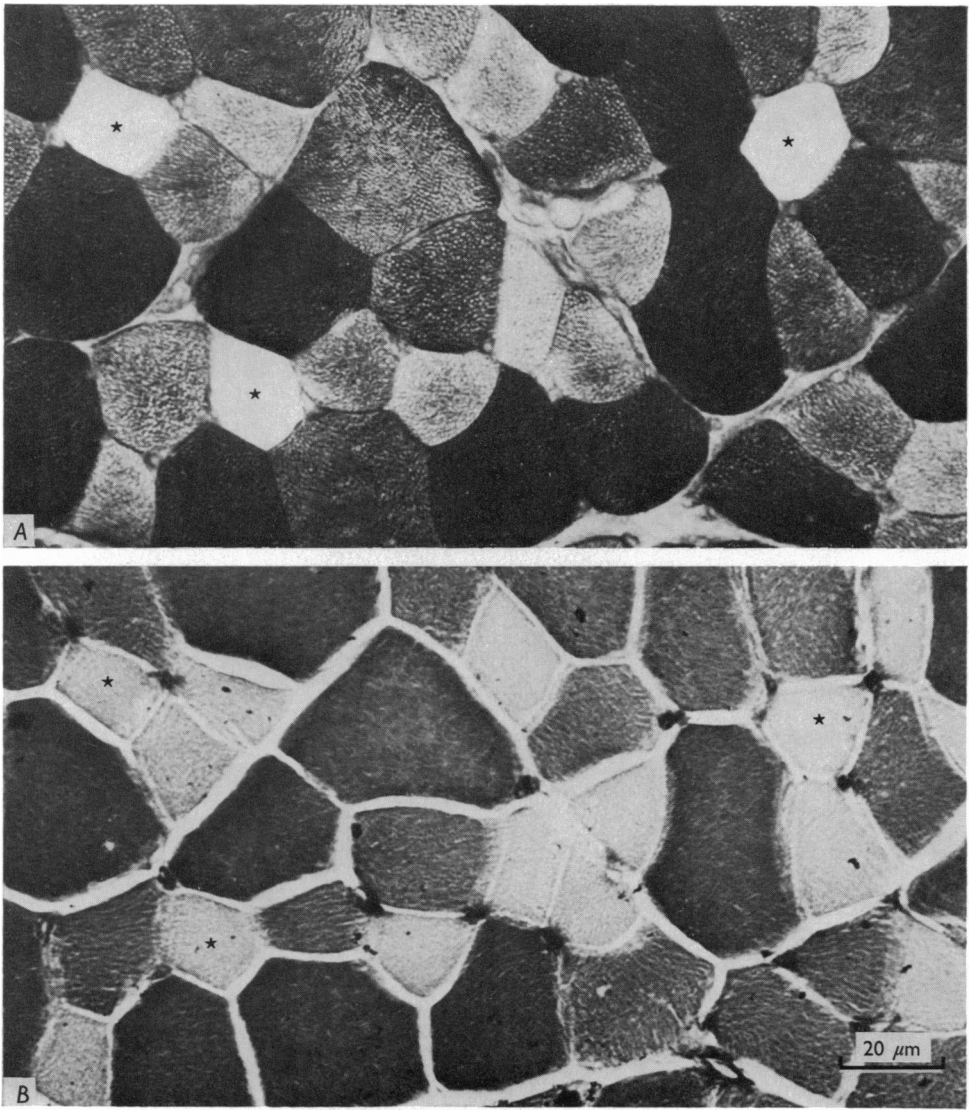
PLATE 1

Photographs of 10 μm frozen transverse sections of cat peroneus brevis muscle stained for glycogen (PAS method). The muscle is from the same experiment as that illustrated in Text-figs. 1 and 2. The spindles shown in Figs. *A*, *B*, *C* correspond with spindles *A*, *B*, *C*, respectively, in Text-fig. 2. b_1, bag_1 fibre; b_2, bag_2 fibre; *c*, chain fibre. Depleted muscle fibres indicated by asterisks. *A*, glycogen depletion in four extrafusal muscle fibres and one bag_1 fibre (spindle in lower right quadrant). *B*, spindle with a depleted bag_1 fibre. *C*, spindle showing depletion in the bag_1 fibre and one chain fibre.

PLATE 2

Same experiment as Pl. 1. Successive 10 μm transverse sections demonstrating the actomyosin ATPase profile of three extrafusal muscle fibres belonging to the motor unit innervated by the dynamic β axon. *A*, the section illustrated, stained for glycogen (PAS method), shows three depleted extrafusal muscle fibres. The following section, illustrated in *B*, has been stained for alkaline ATPase (Guth & Samaha, 1970) and shows that the actomyosin ATPase content of the depleted fibres is low.


A
$b_1^\star$
50 μm
B
b_2
c
$b_1^\star$
20 μm
C
b_2
$c^\star$
$b_1^\star$


A
B
20 μm