Journal of Physiology (1992), **450**, *pp.* 565–579
With 6 figures
Printed in Great Britain

A STUDY OF GLYCOGEN DEPLETION AND THE FIBRE-TYPE COMPOSITION OF CAT SKELETO-FUSIMOTOR UNITS

BY D. BARKER, J. J. A. SCOTT* AND M. J. STACEY

*From the Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE and the *Department of Physiology, University of Leicester, Leicester LE1 9HN*

(*Received 30 May 1991*)

SUMMARY

1. We have used the glycogen-depletion technique, combined with myofibrillar ATPase (mATPase) staining for muscle fibre type, to study the fibre-type composition of four skeleto-fusimotor (β) units in cat peroneus tertius, namely, one β dynamic (βd) unit and three β static (βs) units.

2. Depletion of glycogen was observed in serial cross-sections of thirty-four β-unit extrafusal muscle fibres of various types traced from origin to insertion. No fibre was depleted of glycogen throughout its length; depletion was restricted to a number of zones, usually about five. Oxidative (type I) and oxidative–glycolytic (type IIA) fibres were depleted for a significantly greater proportion of their total length than glycolytic IIB fibres.

3. The fibre-type composition of the βd unit was determined by tracing its fibres from end to end. The muscle unit consisted of one intrafusal bag_1 fibre and ninety-three extrafusal muscle fibres comprising seventy-six type I fibres, eleven IIC fibres, and six fibres that changed from IIC to I during the course of their length (IIC/I fibres). The extrafusal fibre-type composition was thus 81·7% I plus 18·3% IIC and IIC/I.

4. The three βs units ($\beta s1$, $\beta s2$, $\beta s3$) were all fast-contracting and fatigued rapidly. Identification of their extrafusal fibre types, made in 1 mm^2 areas sampled from different parts of each unit, gave mixed compositions as follows: $\beta s1$, IIB+6·7% IIA; $\beta s2$, IIB+5·8% IIA; $\beta s3$, IIB+29·9% IIA. The intrafusal component of each unit included either one or two long chain fibres.

5. In a discussion of the results, the fact that the continuous stimulation of extrafusal muscle fibres does not deplete them of glycogen throughout their length is examined in relation to the work of others who have assumed that it did. With regard to the finding of mixed extrafusal fibre types in the β units, a distinction is drawn between minimal (around 5%) and moderate mixing. It is suggested that minimal mixing may occur in any motor unit as the outcome of endplate degeneration with foreign replacement, but that moderate mixing indicates an on-going process of conversion from one fibre type to another which in the adult may prove to occur only among β units.

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INTRODUCTION

Physiological evidence for the existence of mammalian skeleto-fusimotor axons was first demonstrated by Bessou, Emonet-Dénand & Laporte (1963, 1965) in a deep lumbrical muscle of the cat. They showed that the repetitive stimulation of such axons not only produced extrafusal contraction, but also activated muscle spindles. The axons, later referred to as β axons (Kidd, 1966), were of slow conduction velocity, and the affect of their stimulation upon the spindle was to increase the dynamic sensitivity of its primary ending. The glycogen-depletion method, combined with myofibrillar adenosine triphosphatase (mATPase) staining for muscle-fibre type, showed that the intrafusal contraction produced by these β dynamic (βd) axons was almost exclusively restricted to bag_1 (b_1) fibres, and that the extrafusal contraction was confined to slow oxidative (SO) fibres (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1977, cat tenuissimus and peroneus brevis; Burke & Tsairis, 1977, cat soleus). The existence of fast-conducting β axons had been predicted from observations on silver-stained preparations (Barker, Stacey & Adal, 1970), and was confirmed by glycogen depletion studies on cat peroneus tertius (Harker, Jami, Laporte & Petit, 1977; Jami, Lan-Couton, Malmgren & Petit, 1979). These showed that fast β axons selectively depleted intrafusal long-chain (lc) fibres (Harker *et al.* 1977), were static in action, and activated extrafusal fast oxidative–glycolytic (FOG) fibres (Jami *et al.* 1979).

Jami, Murthy & Petit (1982*a*) shed further light on the extrafusal composition of β motor units in cat peroneus tertius (PT) by determining their fatigue index. They found that out of twenty-four β static (βs) units seventeen were fast contracting, fatigue resistant (FR), six were fast contracting, fatiguable (FF), and one was slow (S); and that out of twelve βd units eleven were S and one was FR. The range of conduction velocities for the βs axons innervating the fast-contracting units was 79–100 m s^{-1}, whereas, with one exception, that for the βd axons was 55–75 m s^{-1}. The βs axon that innervated the S unit had an exceptionally slow conduction velocity of 69 m s^{-1}, whereas the βd axon that innervated the FR unit had an exceptionally fast conduction velocity of 91 m s^{-1}.

Using Brooke & Kaiser's (1970) nomenclature for fibre types, and Burke's (1980) equivalents for muscle-fibre and motor-unit types, the above information about the extrafusal composition of cat hindlimb β motor units may be summarized as follows: (i) βd muscle units are composed of type I fibres, or, rarely, II A fibres; (ii) βs muscle units usually consist of II A fibres, though some consist of II B fibres, or, more rarely, type I fibres.

It was against this background that we used the glycogen-depletion and mATPase fibre-typing techniques to check on the muscle-fibre composition of a few β motor units in cat PT for use as normal controls in an investigation of the restoration of such units after nerve section. We studied four units, one dynamic and three static, and found that each contained a mixture of fibre types, namely, types I and II C in the βd unit, and types II A and II B in the βs units. This was surprising since it had generally been assumed that β motor units, like α motor units, were homogeneous with regard to fibre type, any mixture being entirely intrafusal. However, this assumption was already being called into question as the result of work by Ridge and his colleagues on the development of motor units in the rat's fourth deep lumbrical

(4 DL) muscle (see review by Betz, Ribchester & Ridge, 1990). They have shown that among eight or nine type-specific units in the adult muscle, there are two or three units, smaller than the others, that are composed of two fibre types. At present it is not known whether these are α and β units.

The histological analysis afforded an opportunity to determine the size of a β motor unit. Edström & Kugelberg (1968), who introduced the glycogen-depletion technique, used it to determine the size of α motor units by counting the depleted fibres in the muscle cross-section that had the greatest number. They assumed that the glycogen in the activated extrafusal muscle fibres was depleted from their whole length, and others who have since used the technique for the same purpose (e.g. Burke & Tsairis, 1973; Bodine-Fowler, Garfinkel, Roy & Edgerton, 1990) have made the same assumption. We have traced such fibres from end to end and find that glycogen is not depleted from the whole fibre length, but from a varying number of zones along its length. The proportion of fibre length depleted differs according to fibre type, being greater in oxidative fibres than in glycolytic ones. The muscle cross-section with the greatest number of depleted fibres does not represent the total unit population, but that part of the unit in which there is the greatest number of fibres with coincident depletion zones. In the *βd* unit that we studied the section that contained the greatest number of fibres also contained a further 25% of fibres belonging to the unit that were not depleted, and even the combined total of depleted and non-depleted fibres fell short of the unit's total population.

A preliminary account of some of the results has been published (Barker, Scott & Stacey, 1991).

METHODS

The experiments were performed on four adult cats (mean weight 2·6 kg) anaesthetized with sodium pentobarbitone (Sagatal, 45 mg kg^{-1}, I.V.), which was supplemented as required to maintain an areflexic state with stable blood pressure and heart rate. The core temperature of the animal was maintained at 37 °C by a heating blanket thermostatically controlled via a rectal probe.

The left hindlimb was denervated except for the nerve to PT, which was freed proximally from its point of muscle entry and hooked over a recording electrode. A lumbar laminectomy exposed the L7 and S1 dorsal and ventral roots, which were cut centrally. The skin over the spinal cord and lower hindlimbs was drawn up to form pools that were filled with mineral oil maintained at 37 °C by radiant heat. The distal tendon of PT was freed and attached directly to a tension transducer (Kulite) that was fixed to the shaft of an electromagnetic puller (Ling Dynamic Systems). The puller was operated under feedback control via an in-series length transducer.

The L7 and S1 dorsal roots were then subdivided to isolate filaments that contained single Ia spindle afferents from PT as identified by their characteristic responses (Scott, 1990). Ten such filaments were hooked onto a 10-pole electrode. The ventral roots were subsequently subdivided to isolate single axons.

Each axon was stimulated at frequencies of 40–100 Hz while recording from each afferent. At this stage the period of stimulation was kept as brief as possible to avoid depleting a motor unit of glycogen. The presence of a localized extrafusal contraction showed that the axon belonged either to an α or a β motoneurone; an acceleration in the discharge of one or more Ia afferents provided an initial indication that the axon being stimulated was β type.

Further tests were then made to enable positive identification. These included step changes in stimulation frequency, and evaluation of the affect on the firing rate of the afferent(s) during a ramp-and-hold stretch (Jami *et al.* 1982*a*). The amount of stimulation was again reduced to a minimum and no attempt was made to test for the persistence of the intrafusal effect (Jami *et al.* 1982*a*), or to measure the fatigue index of the motor unit. Similarly, no measure of the maximum tetanic tension of the rapidly fatiguing β units was obtained since this had already started to decline by the time identification of the unit as β type had been confirmed. The measure of

maximum tetanic tension generated by the slow βd unit was carried out during the depletion regime on the assumption that there would have been no significant decline.

The conduction latency of the axon was measured by recording the latency of the orthodromic action potential from the muscle nerve in response to stimulation of the ventral-root filament. The filament was also stimulated supramaximally while monitoring activity in the muscle nerve to ensure that the β axon was completely isolated and that no γ axons were being stimulated.

Having positively identified the axon as belonging to a single β motoneurone, its affect on the spindle afferent(s) it activated was characterized as being static or dynamic. The motor unit was then depleted of glycogen by stimulating for 0·5 s every second, the muscle being given a triangular stretch of 2 mm at 5 mm s^{-1} every 9 s (see Fig. 4). The frequency of stimulation for the βd unit was initially set at 50 Hz for 15 min and then attenuated between 100 and 50 Hz for each subsequent 15 min period, but with 2–5 min of stimulation at 150 Hz every 30 min. After 2·5 h stimulation the frequency was maintained at 120 Hz for the final 30 min to ensure depletion of the intrafusal fibres. For the βs units the stimulation was initially set at 40 Hz, but as soon as fatigue began to develop the frequency was reduced to 20 hz (or 10 Hz later in the stimulus programme) and then attenuated depending on the rate of fatigue and recovery. The last 20 min of stimulation was at 100 Hz.

During this time a diagrammatic sketch of the muscle was made to facilitate orientation during its subsequent sectioning. This showed the points of entry of nerves and blood vessels into PT, together with the position of the unit's focus of contraction, and indicated the distances between these points and the proximal and distal ends of the muscle. At the end of the stimulation regime the muscle was rapidly excised, placed in an aluminium foil boat containing an embedding compound (Cryo-M-Bed), and plunged into a bath of isopentane previously cooled to -160 °C with liquid nitrogen.

The muscle was then serially cross-sectioned in a cryostat at -20 °C. In the first two experiments sections were cut at 25 μm for periodic acid-Schiff (PAS) staining and 15 μm for mATPase activity, but in the others all sections were cut at 15 μm. The sections on every eighth and ninth slide were processed for fibre typing (mATPase profiles), the rest for observing the presence or absence of glycogen (PAS staining). Myofibrillar ATPase profiles were obtained after acid (pH 4·2 and 4·5) and alkaline (pH 10·1) pre-incubation following method A in Snow, Billeter, Mascarello, Carpenè, Rowlerson & Jenny (1982). In the PAS staining only Sigma Schiff's reagent and Sigma periodic acid gave consistently reliable results.

Since the glycogen in muscle fibres occurs as granules located mainly around the terminal cisternae of the sarcoplasmic reticulum, the presence of a PAS-positive reticulum was regarded by us, as by Burke & Tsairis (1973) and Burke, Levine, Sakman & Tsairis (1974), as indicating the presence of glycogen. A few fibres of each type were traced in normal muscle to become familiar with their normal glycogen profiles and range of variation. Glycogen was considered to be depleted from a muscle fibre when its sarcoplasmic reticulum was invisible, or partly or wholly visible but blanched. Sections in which the reticulum was only faintly stained, or was part-stained and part-blanched, were regarded as not depleted. Depletion was monitored at approximately 100 μm intervals in thirty-four β unit extrafusal muscle fibres of various types (ten type I, five IIa, twelve IIB, four IIC, three IIC/I) traced for their whole length through serial cross-sections. Cross-sectional areas of different fibre types were obtained by measuring their shortest and longest axes at intervals of 0·5 to 1·0 mm over fibre lengths of 5 to 8 mm.

The number of fibres in the βd unit was determined by tracing their course from origin to insertion examining sections at intervals of 0·9 mm. The position and type of each fibre were recorded at these intervals in photographs of adjacent PAS- and mATPase-stained sections. In the case of the βs units fibre type was determined with the aid of photographs of similarly adjacent sections, the proportions of IIA and IIB fibres being ascertained in two or three 1 mm^2 areas sampled in different parts of the unit.

RESULTS

Depletion of glycogen in different types of extrafusal muscles fibres

The adult cat PT is 3–4 cm long and its extrafusal muscle fibres are mostly about 1 cm long (range 0·6–1·7 cm). The results of monitoring glycogen depletion during end-to-end tracing of different fibre types are summarized in Table 1 and Fig. 1. All types of fibre had several zones of depletion, usually about five. Oxidative (type I)

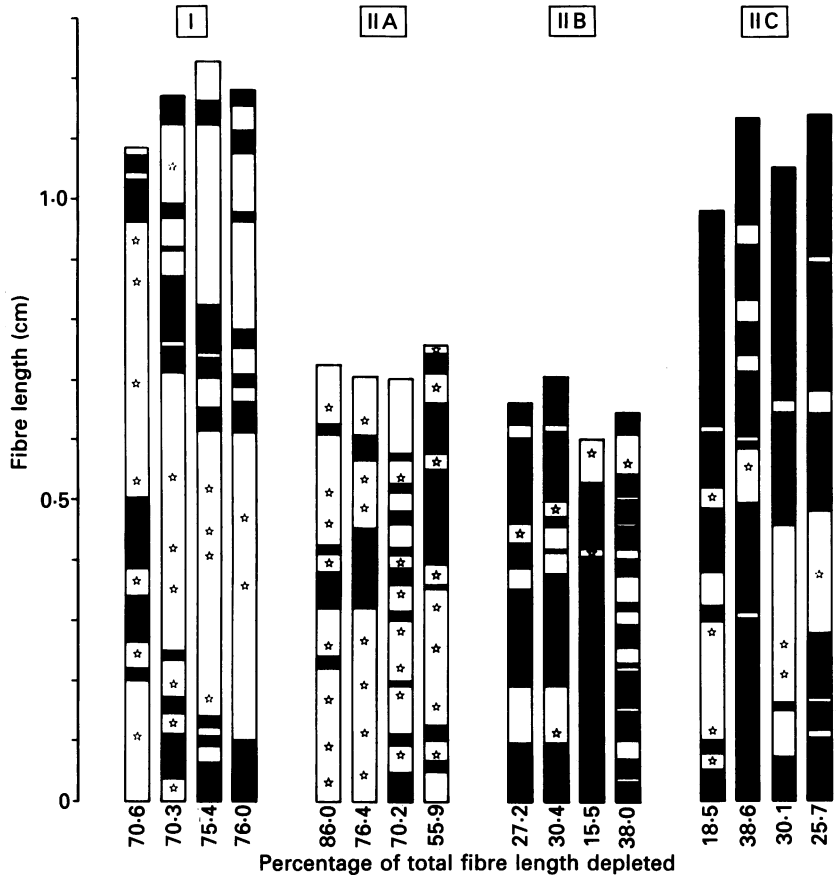

Fig. 1. Schematic representation of the depletion of glycogen from extrafusal muscle fibres belonging to two peroneus tertius β units. The type I and IIC fibres belonged to a dynamic β unit, the IIA and IIB fibres to a static β unit (β*s*1). Depleted zones are shown white, non-depleted black. White indicates that the sarcoplasmic reticulum was partly or wholely visible, but blanched; stars indicate the midpoints of regions up to 0·5 mm long where the sarcoplasmic reticulum was invisible. Compare with Table 1.

TABLE 1. Depletion of glycogen in different types of extrafusal muscle fibre belonging to β motor units in cat PT

Fibre type	Number of fibres	Percentage of total length depleted		Depletion zones				
				Length (mm)			Number	
		Range	Mean ± S.E.M.	Range	Mean minimum	Mean maximum	Range	Median
I	10	56·2–77·7	70·0 ± 2·04	0·10–5·08	0·14	4·05	4–14	6·5
IIA	5	52·8–86·0	68·3 ± 6·23	0·08–3·29	0·37	2·21	3–9	5·0
IIB	12	7·5–55·5	29·3 ± 4·50	0·08–2·10	0·13	0·75	2–11	5·5
IIC	4	18·5–38·6	28·2 ± 4·20	0·17–2·93	0·17	1·94	3–6	5·0
IIC/I	3	27·5–45·7	37·8 ± 5·39	0·10–2·78	0·17	1·98	4–9	5·0

and oxidative–glycolytic (type II A) fibres were depleted for a significantly greater proportion of their total length than glycolytic II B and other fibre types ($P < 0.01$, C test: Scheer, 1986), the difference being about two-thirds as against one-third. This difference was expressed by their depletion zones being of greater length, e.g. a mean

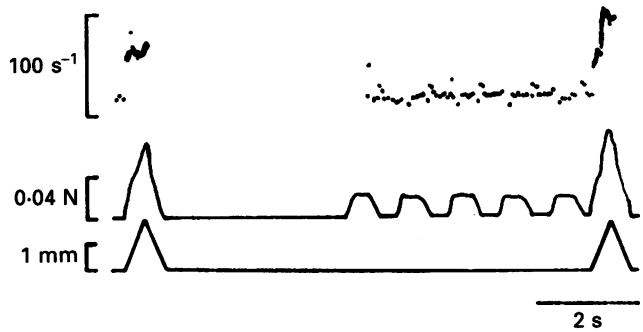

Fig. 2. Instantaneous frequency record of a Ia afferent during glycogen depletion of the dynamic β unit. The β axon was stimulated at 100 Hz for 0·5 s s^{-1} while the muscle was being given a triangular stretch of 2 mm every 9 s. For the purposes of this record the stimulator was switched off for the first 4 s of the sweep to reveal the passive responses. Upper trace, instantaneous frequency; middle trace, muscle tension; lower trace, muscle length.

maximum length of 4·05 mm in type I fibres as compared with 0·75 mm in II B fibres. Although the zones of depletion were short in II B fibres, there were long stretches of reduced glycogen, as indicated by faint PAS staining, on either side of a zone, a feature not shown by type I or II A fibres.

One depletion zone in a fibre was often longer than the others (see Fig. 1). It may have included the site of the motor endplate; on the other hand, variations in the pattern of depletion among different fibre types, as well as among different fibres of the same type, suggest that there is unlikely to be any consistent relationship between length of depletion zone and site of endplate.

Analysis of βd motor unit

Stimulation of the βd axon produced a focus of contraction located 10 mm from the proximal end of the muscle on its antero-lateral surface 2 mm proximal to the entry of the muscle nerve. The contraction was extremely resistant to fatigue; there was no sag in the tetanic tension (maximum 0·02 N) indicating that it was generated by a slow motor unit (Burke, Levine, Tsairis & Zajac, 1973). The conduction velocity of the axon was 72 m s^{-1}. Its stimulation affected a single Ia afferent, evoking a substantial increase in the dynamic response during muscle stretch (Fig. 2). The responses at the resting length were relatively slight, and the afferent displayed a marked re-extension discharge at the onset of motor unit relaxation.

The muscle unit consisted of one b_1 fibre and ninety-three extrafusal muscle fibres comprising seventy-six type I fibres, eleven II C fibres, and six fibres that changed from II C to I during the course of their length (II C/I fibres). Fibres belonging to the unit (unit fibres) were distributed throughout the proximal two-thirds of the muscle,

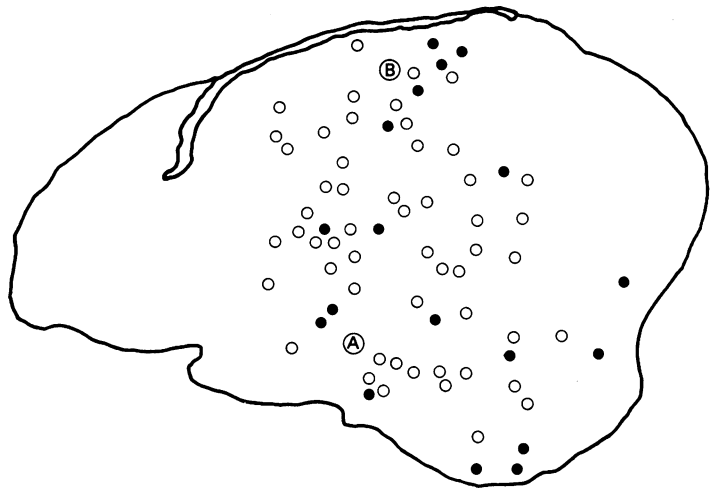

Fig. 3. Glycogen depletion of extrafusal muscle fibres belonging to a dynamic β unit in cat peroneus tertius. Outline drawing of a PAS-stained cross-section (no. 434) through the unit at the level of maximum depletion shows fifty-four depleted fibres (open circles) and eighteen fibres that belong to the unit but are not depleted in this section (filled circles). A and B represent muscle spindles; a depleted bag_1 fibre was present in B.

TABLE 2.Number of extrafusal muscle fibres present at different levels of a glycogen-depleted dynamic β unit in cat PT

Section		β unit muscle fibres	
Number	Interval (mm)	Depleted	Not depleted
10	—	1	1
54	1·06	7	5
90	0·88	16	9
142	1·26	21	7
214	1·76	42	18
286	1·68	46	24
360	1·71	53	20
434	1·77	54	18
504	1·59	53	15
578	1·71	33	22
648	1·69	24	14
720	1·72	18	5
794	1·79	10	2
870	1·86	4	1
938	1·66	2	1
990	1·26	2	0

Total motor unit population: 93.

whose length was 4 cm. The numbers of unit fibres present at different levels of the muscle are shown in Table 2. The focus of the unit's contraction corresponded to a region situated between sections 360 and 434. These sections had the highest numbers of unit fibres (seventy-three and seventy-two) and were located in the middle of an area lying 5·2 to 13·6 mm from the proximal end of the muscle where

all levels sectioned contained over fifty unit fibres and had high numbers of fibres with coincident depletion zones. In these sections (Nos. 214 to 578 in Table 2) the proportion of non-depleted unit fibres present averaged 30%, and the difference between the numbers of depleted fibres and the total unit population averaged 50%.

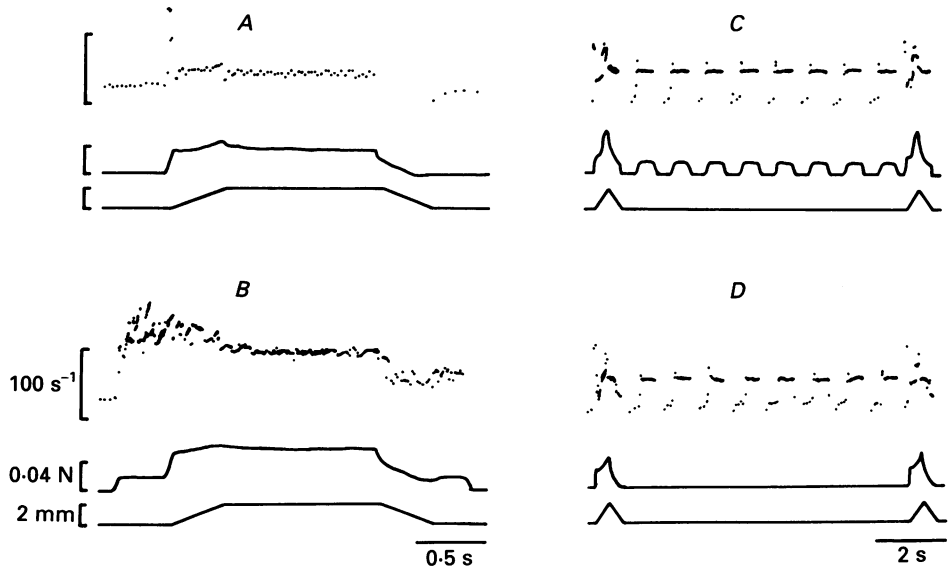

Fig. 4. Instantaneous frequency records of a Ia afferent during activation of the static β unit $\beta s2$. *A*, response to ramp-and-hold stretch at 5 mm s^{-1}. *B*, ramp stretch with stimulation of the axon at 100 Hz; the afferent discharged approximately 1:1 during the held phase. *C* and *D*, two sequences of 60 Hz stimulation; (i) at the start of the glycogen-depletion regime (*C*), and (ii) following fatigue of the extrafusal component of the unit after 2·5 h of stimulation (*D*). The Ia afferent responded 1:1 in both cases. Upper trace, instantaneous frequency; middle trace, muscle tension; lower trace, muscle length.

Note that the total of fifty-four depleted fibres in the section with the highest number of such fibres (no. 434 in Table 2) would be regarded by many (see Discussion) as representing the total unit population, but fell short of this by thirty-nine fibres (see Fig. 3). The unit fibres in this section comprised fifty-eight type I, eight IIC, all six IIC/I fibres, and the b_1 fibre in the activated spindle. Among the depleted extrafusal fibres, the sarcoplasmic reticulum was invisible in twenty-one fibres, blanched in thirty-one, and partly visible and blanched in two. The eighteen non-depleted unit fibres included seven in which the sarcoplasmic reticulum was partly or wholly PAS positive, though only faintly stained. We found profiles of this kind to be characteristic of fibres sectioned near the borders of a depletion zone, and since such sections contained glycogen, albeit at a reduced level, we regarded fibres in these locations as not depleted. Burke *et al.* (1974) regarded the depletion of such fibres as 'questionable', but nevertheless, accepted them as depleted.

The IIC and IIC/I fibres were smaller than the type I fibres, had a finer sarcoplasmic reticulum, and stained more intensely with PAS. The mean cross-sectional area of type I fibres was $2143 \pm 86\ \mu m^2$ S.E.M. ($n = 11$) compared with

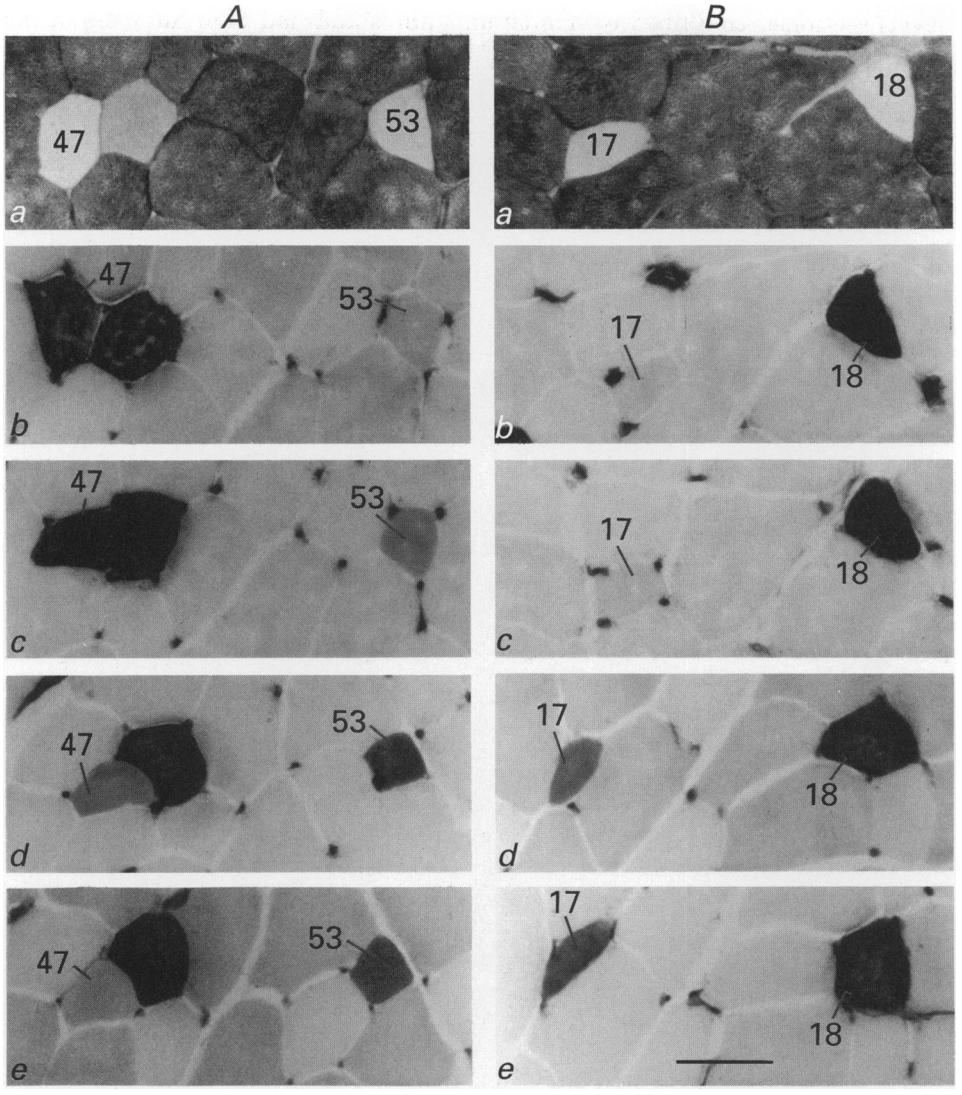

Fig. 5. Photographs illustrating type I and type IIC/I fibres belonging to the dynamic β unit. The IIC/I fibres are shown switching fibre type over a length of a few millimetres. *Aa*, PAS-stained 25 μm cross-section shows fibres 47 and 53 depleted of glycogen. *b–e*, myofibrillar–ATPase activity after acid pre-incubation (pH 4·15–4·20) in 15 μm serial cross-sections sampled over a length of 5·5 mm shows that the type I profile of fibre 47 in *b* and *c* changes to a type IIC profile in *d* and *e*. Similarly the type IIC profile in fibre 53 seen in *a* and *b* changes to type I in *d* and *e*. Approximate distances between sections: *a* to *b*, 0·5 mm; *b* to *c* and *c* to *d*, 2 mm; *d* to *e*, 1 mm. *Ba*, PAS-stained 25 μm cross-section shows depletion of glycogen in fibres 17 and 18. *b–e*, myofibrillar–ATPase activity after acid pre-incubation (pH 4·15–4·20) in 15 μm serial cross-sections sampled over a length of 4·05 mm shows that fibre 18 is type I, whereas the profile of fibre 17 changes from type IIC to type I. Approximate distances between sections: *a* to *b*, 50 μm; *b* to *c*, 1 mm; *c* to *d*, 2 mm; *d* to *e*, 1 mm. Scale bar in *Be* indicates 50 μm and applies throughout.

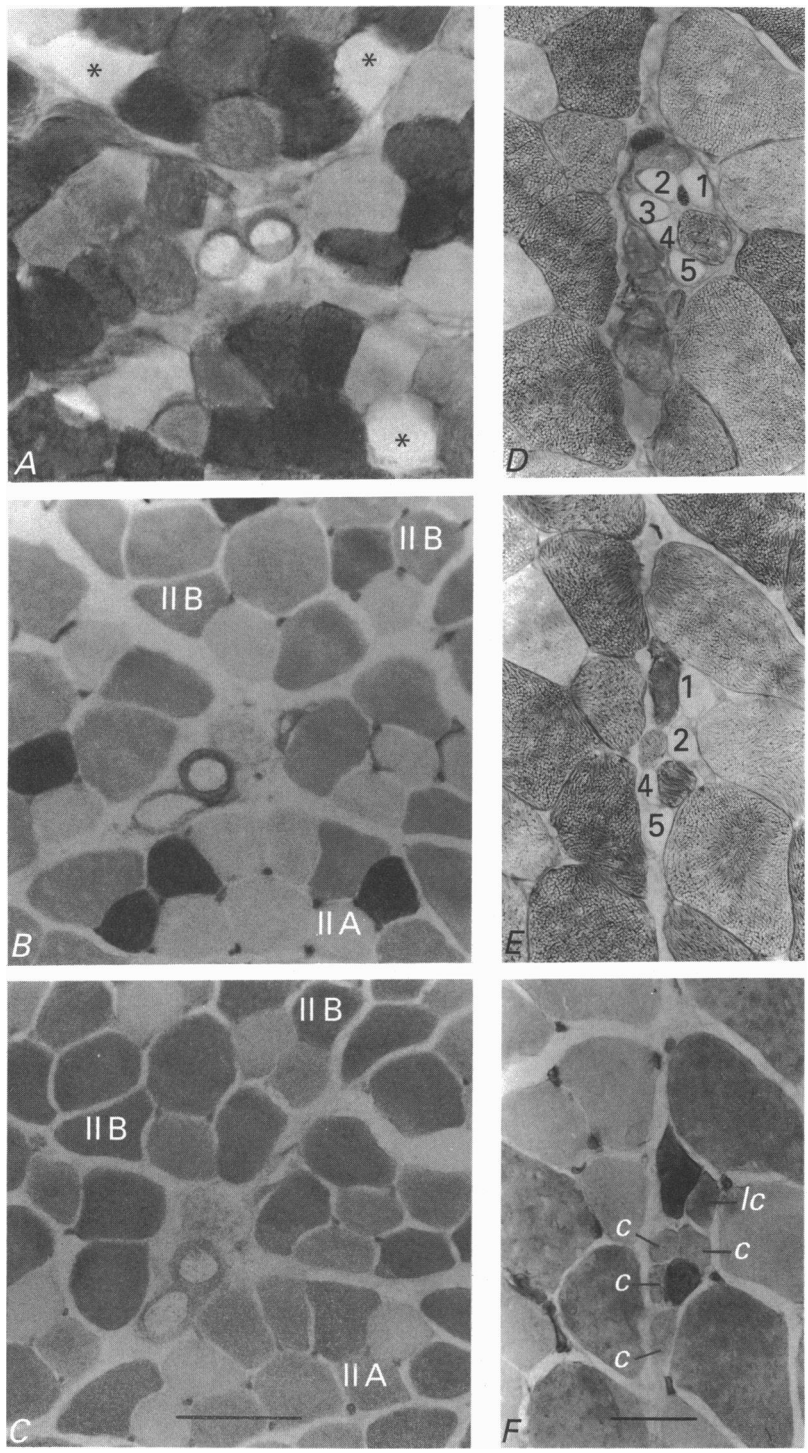

Fig. 6. *A–C*, photographs illustrate fibre types belonging to the static unit $\beta s1$ whose fibre-type composition was IIB+6·7% IIA. *A*, PAS-stained 25 μm cross-section shows depletion of glycogen in three muscle fibres (asterisked) whose types are revealed in *B* and *C*.

1268 ± 40 μm^2 ($n = 11$) for IIC fibres and 1350 ± 92 μm^2 ($n = 6$) for IIC/I fibres. In the IIC/I fibres the switch-over from IIC to I in mATPase profile occurred at a place in the fibre such that the greater part of its length was usually IIC. Examples of IIC/I switch-overs are illustrated in Fig. 5.

Analysis of three βs units

We shall for convenience refer to these units as *βs*1, *βs*2 and *βs*3. Their axons all had fast conduction velocities (*βs*1, 90 m s^{-1}; *βs*2 and *βs*3, 95 m s^{-1}), and the tetanic tension records of their muscle units all initially displayed the sag typical of fast-contracting motor units (Burke *et al.* 1973). All three units fatigued relatively rapidly and were therefore stimulated at low frequencies (20–40 Hz) to prevent extrafusal conduction block, with brief periods at higher frequencies (60–100 Hz) to ensure depletion of the intrafusal component.

Each axon evoked 1:1 driving of the discharge of one Ia afferent, and the *βs*1 and *βs*3 axons also elicited small increases in the discharge rates of a second afferent that were not due to extrafusal contraction. The actions of the *βs*2 axon are illustrated in Fig. 4. This shows, in *B*, that stimulating at 100 Hz during a ramp-and-hold stretch produced a pronounced static response with driving during the hold phase; *C* and *D* compare responses to repeated triangular stretches recorded soon after the isolation of the axon and the beginning of the depletion regime (*C*), with responses recorded after $2\frac{1}{2}$ h stimulation near the end of the depletion regime (*D*). Stimulation at 60 Hz produced 1:1 driving of the afferent discharge in both *C* and *D*, but in *D* the extrafusal contraction has failed, confirming that the driving was generated by intrafusal contraction.

Extrafusal muscle-fibre types were identified in 1 mm^2 areas sampled from different parts of each unit as follows: *βs*1, eighty-three IIB, six IIA (6·7%); *βs*2, eighty-one IIB, five IIA (5·8%); *βs*3, 101 IIB, forty-three IIA (29·9%). Two spindles were activated in both *βs*1 and *βs*3, but only one spindle in *βs*2. The intrafusal depletions were: *βs*1, $b_1 + lc$ in one spindle, one chain fibre (*c*) in another; *βs*2, $b_1 + lc$; *βs*3, $lc + 4c$ in one spindle, *lc* in another.

Fibres belonging to all three units were distributed throughout most of the muscle. In each unit the activated spindle(s) lay within the area of maximum depletion. In *βs*1 the focus of contraction coincided with this area, but in the other units it lay several millimetres apart.

Examples of extrafusal and intrafusal depletions are illustrated in Figs 5 and 6.

B, myofibrillar–ATPase activity after acid pre-incubation (pH 4·35) in an adjacent 15 μm section reveals that the fibres depleted are two type IIB and one type IIA. *C*, another adjacent section shows myofibrillar–ATPase activity after alkaline pre-incubation (pH 9·8) that confirms the fibre typing in *B*. Scale bar in *C* indicates 100 μm; *A* and *B* at same scale.

D, *E*, photographs of 15 μm thick PAS-stained serial cross-sections through one of two spindles activated in the static β unit *βs*3 showing depletion of glycogen in one long chain fibre (1) and four chain fibres (2–5). The sections were cut approximately 450 μm apart through the spindle's proximal pole.

F, section adjacent to *E* showing myofibrillar–ATPase activity after acid pre-incubation (pH 4·35). Intrafusal muscle-fibre identification made on the basis of histochemical profile (PAS, mATPase) as well as length and position of fibres. *lc*, long chain fibre; *c*, chain fibre. Scale bar indicates 50 μm; *D* and *E* at same scale.

DISCUSSION

The glycogen-depletion technique enables the physiological characteristics of a muscle fibre to be correlated with its histochemical profile, and this has proved most useful in advancing knowledge of motor units and muscle spindles. The technique has also been used, most recently by Bodine-Fowler *et al.* (1990), to estimate the innervation ratios and territories of motor units, and to study the spatial distribution of their muscle fibres. In all these studies it has been assumed that an extrafusal muscle fibre 'marked' by depletion was thus marked throughout its length. Although various stimulation regimes have been devised to make certain of this, nobody, apart from ourselves, has traced extrafusal muscle fibres from end to end to find out whether this was so. We have shown that the depletion of glycogen in such fibres is, in fact, not end-to-end but regional, as has already been demonstrated in intrafusal muscle fibres (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1976; Barker *et al.* 1977; Harker *et al.* 1977; Jami *et al.* 1979). Any cross-section of a muscle unit depleted of glycogen is therefore likely to contain some fibres belonging to the unit which are not depleted in that particular section (see Fig. 3). In our *βd* unit the sections with the highest numbers of depleted fibres also included, on average, a further 30% of unit fibres that were not depleted. If, as seems likely, the depletion of glycogen achieved by others has been similarly regional, it follows that their innervation ratios will have been underestimates (see e.g. those given by Edström & Kugelberg, 1968; Doyle & Mayer, 1969; Burke *et al.* 1974; Kugelberg, 1976; Bodine-Fowler *et al.* 1990). Moreover, if the innervation ratio has been obtained simply by counting the depleted fibres in the muscle cross-section with the greatest number, as has usually been the case, the further assumption has to be made that such a section will include virtually all the fibres belonging to the unit. The internal architecture of most muscles makes this very unlikely. In our *βd* unit the relevant section (no. 434 in Table 2) did not include 23% of the total unit population.

The careful studies of glycogen-depleted motor units by Burke and his colleagues (Burke & Tsairis, 1973; Burke *et al.* 1974) appear to be the only ones to have allowed for this, and their study of slow α motor units in cat soleus (Burke *et al.* 1974) offers some close comparisons with our own. The soleus unit illustrated in their Fig. 3 had seventy-eight depleted fibres comprising fifty-nine counted in the section of maximum depletion, and nineteen located elsewhere. According to our results a further 30% of fibres should be added to allow for unit fibres that were not depleted in the particular sections studied, but this is offset to some extent by their having recognized some fibres as depleted that we would not (see Results). A reduction of the corrective factor by about 10% would allow for this and result in their innervation ratio for this unit being raised from 1:78 to 1:94.

It is not clear why glycogen should only be depleted from a few regions of a muscle fibre rather than from its whole length. It may simply reflect a pattern of glycogen distribution which is subject to regional variation. Biochemical analyses of the glycogen content of muscle fibres have shown that it varies widely both along the length of individual muscle fibres (Hintz, Chi, Fell, Ivy, Kaiser, Lowry & Lowry, 1982) and among fibres of the same type (Essen & Henriksson, 1974), and this accords with our own experience of tracing PAS-stained normal fibres.

In an earlier glycogen-depletion study (Barker *et al.* 1977) we reported that the depletion of extrafusal muscle fibres was restricted to a single zone. In that study the main histological objective was to track down the sites of depletion in spindles that had been activated in *βd* units. Depletion was noted in type I fibres belonging to the unit and located near the activated spindles(s), and a few were traced to determine the length of a depletion zone. Several α muscle units of different types were also studied to ascertain the lengths of single depletion zones. A fibre that showed depletion in a section was traced in either direction until it was judged not to be depleted. No extrafusal fibre was traced beyond the limits of a single depletion zone. In IIB fibres we reported depletion zones up to 5 mm long, but in the light of the present study it is clear that these included the long stretches of reduced glycogen that lie on each side of the depletion zones.

The maximum tetanic tension of 0·02 N developed by our *βd* motor unit is at the lower end of the range of 0·25–5·0 g given by Jami *et al.* (1982*a*) for the maximum tetanic tensions of twelve β-innervated S units in PT. A similar range of 0·3–4·5 g is given by Jami, Murthy, Petit & Zytnicki (1982*b*) for the maximum tetanic tensions of eleven PT motor units classified as S on the basis of various physiological tests but without distinguishing the innervating axon as α or β. This suggests that a muscle unit composed of type I fibres (S unit) is of similar size whether innervated by an α or a β motoneurone. The same conclusion becomes evident on comparing the tetanic tension ranges for FF and FR β units in PT (Jami *et al.* 1982*a*) with those of units belonging to the same physiological types in α/β samples (Jami *et al.* 1982*b*). For example, the highest maximal tetanic tension given for β-innervated FF units was 62·5 g, that for FF units in the α/β sample 62·0 g.

Lastly, there is the finding of extrafusal fibre types being mixed in the β units we studied. The degree of mixing was either minimal, as in the β static units *βs*1 (IIB + 6·7 % IIA) and *βs*2 (IIB + 5·8 % IIA), or moderate, as in the β static unit *βs*3 (IIB + 29·9 % IIA and the *βd* unit (I + 18·3 % IIC and IIC/I). Minimal mixing was in fact noticed by Edström & Kugelberg (1968) who reported that three of seven IIB units in the rat anterior tibial muscle had a few IIA fibres, and that three of the six IIA units had a few IIB fibres. Similar minimal mixing of small numbers of IIC, or both IIC and IIA, fibres was found by Gates, Ridge & Rowlerson (1991) in ten of twenty IIX motor units belonging to rat 4DL muscles. They suggest that this may be due to small numbers of fibres being 'relinquished naturally by motoneurones and subsequently incorporated into other motor units.' Such a turnover could be part of the continuous cycle of endplate degeneration and replacement proposed by Barker & Ip (1965, 1966) and now widely accepted to be a feature of normal muscle (see review by Cotman, Nieto-Sampedro & Harris, 1981).

However, this process is unlikely to account for the moderate mixing that we have encountered. It may be that this is brought about by the units being in transition from one type to another. This appears to be happening to the smaller motor units described by Gates *et al.* (1991) in rat 4DL muscle. These consisted of a mixture of IIC and IIA fibres, the proportion of IIC fibres varying between 50 and 89 % in 3-month-old rats, but being greatly reduced by 4 months. They suggest that these units may be in the process of converting into IIA units. In rats aged between 4 and 24 months the conversion appears to be complete in some, but not all, of the IIC/IIA

units originally present. It may be that our I/IIC βd unit was in a similar state of conversion into a fully type I unit, there still being some IIC fibres present as well as a few fibres in course of transition from IIC to I. The existence of similar βd units in rat deep masseter is suggested by the fact that in a muscle otherwise consisting entirely of IIA fibres, there are a few type I and IIC fibres that surround a cluster of about forty spindles (Rowlerson, Mascarello, Barker & Saed, 1988), some of which have β-innervated plates on b_1 fibres (Banks, Barker, Saed & Stacey, 1988). Future work may show that β units develop differently from α units because of their afferent connexions. It is known that Ia afferents suppress the growth of muscle fibres in spindle development (Barker & Milburn, 1984). Perhaps they are also capable of influencing the fibre-type composition of β muscle units by changing the rules of synaptic competition during their development.

We wish to thank David Hutchinson for photographic assistance, and the Medical Research Council (Grant G8814879N) for financial support.

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