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# MOTOR UNITS OF THE FOURTH DEEP LUMBRICAL MUSCLE OF THE ADULT RAT: ISOMETRIC CONTRACTIONS AND FIBRE TYPE COMPOSITIONS

BY H.-J. GATES, R. M. A. P. RIDGE AND A. ROWLERSON*

*From the Department of Physiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD*

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SUMMARY

1. Isometric twitch and tetanic tensions were recorded from whole muscles and single motor units in fourth deep lumbrical muscles isolated from young adult (60 days) rats. Muscles were superfused with oxygenated Ringer solution at 25 °C except where stated otherwise.

2. It was confirmed that the muscle is supplied most commonly by eleven motor axons, nine via the lateral plantar nerve (LPN), and two via the sural nerve (SN). Motor units whose axons were isolated from either LPN or SN were studied. There was no difference in mean motor unit size.

3. In their unfused tetani most units showed 'sag' and some 'no sag', with no segregation between LPN and SN. 'No sag' units were always small (unit tetanic tension $< 8\%$ whole-muscle tetanic tension), tended to be relatively slowly contracting and relaxing during an isometric twitch, and tended to have relatively low twitch: tetanus ratios. Units showing sag ranged from large to small.

4. In some motor units muscle fibres were depleted of their glycogen by repetitive stimulation at 30 °C in glucose-free Ringer solution, and the muscle and its unstimulated control frozen and sectioned. Neighbouring sections were stained for glycogen and for binding of two myosin-specific antibodies, one specific for slow myosin and the other for type IIA myosin. Myosin ATPase and succinic dehydrogenase histochemistry were also carried out in some muscles.

5. Serial reconstructions showed that all or virtually all extrafusal fibres in the muscle were present in a midbelly section, and that the myosin type of individual fibres did not change significantly along their length. Spindle profiles were seen frequently and in two muscles eight and twelve spindles were identified.

6. Of twenty-six motor units examined twenty contained almost exclusively muscle fibres of the recently described type IIX. All these units showed sag in their isometric tetani.

7. Six units each contained 50% or more of slow myosin-containing fibres (IIC and a few type I). The remaining fibres in these units were IIA. All these units were

* Present address: Department of Physiology, UMDS, St Thomas's Hospital, Lambeth Palace Road, London SE1 7EH.

therefore of mixed fibre composition, and are discussed as IIC/IIA units. In whole muscles slow-myosin-containing fibres were generally distributed evenly (non-randomly) throughout the muscle cross-section.

8. Whole muscles contained on average 970 fibres (S.D. ±70) of which 82 (±9) were slow-myosin-containing. A few muscles from older rats (3–24 months) contained very few such fibres.

9. We conclude that of an average eleven motor units in this muscle two or three are of mixed fibre composition (IIC/IIA), the two constituent kinds of fibre differing in myosin type and cross-sectional area. They probably also differ in developmental origin, and this is discussed.

## INTRODUCTION

Present knowledge of the development of motor units in the rat is largely based on studies of three hindlimb muscles: soleus (e.g. Brown, Jansen & Van Essen, 1976; Thompson, Sutton & Riley, 1984), extensor digitorum longus (EDL; Balice-Gordon & Thompson, 1988) and fourth deep lumbrical muscle (4DL; Betz, Caldwell & Ribchester, 1979; Jones & Ridge, 1987; Jones, Ridge & Rowlerson, 1987*a*, *b*). The motor unit composition of soleus and EDL in normal adults is comparatively well known (Close, 1967; Kugelberg, 1973; Chamberlain & Lewis, 1989), but similar knowledge in 4DL is lacking. For this reason we have examined some of the mechanical properties of single motor units in this muscle, and have identified the histochemical types of extrafusal muscle fibres in the muscle and in single motor units identified by glycogen depletion. Our results are described in this paper. One finding is of an unusual, minority type of motor unit that contains muscle fibres of two histochemical types (IIC and IIA). The possible developmental origin of such units and their constituent muscle fibres is discussed. Preliminary accounts of some of this work have been given previously (Gates, 1988, 1989; Betz, Ribchester & Ridge, 1990).

## METHODS

Four deep lumbrical muscles and their nerve supply were dissected out of the rat (Wistar, 60 days old) which had been killed by stunning and cervical dislocation. The nerve supply included the lateral plantar and sural nerves dissected up to the point near the top of the thigh where the common peroneal nerve branches to give the sural and tibial (and hence lateral plantar) nerves. The composition of the Ringer solution was the following (mM): $Na^+$, 167; $K^+$, 5; $Mg^{2+}$, 1; $Ca^{2+}$, 2; $Cl^-$, 147; $HCO_3^-$, 24; $H_2PO_4^-$, 1; D-glucose, 11. The solution was equilibrated by bubbling with 95% $O_2$, 5% $CO_2$ (pH 7·2). The preparation was superfused at a controlled temperature of 25 °C except during glycogen depletion when the temperature was raised to 30 °C and glucose-free Ringer was used.

Stimulating and recording conditions were the same as those described by Jones & Ridge (1987) except that the semiconductor strain gauge was custom-built (Kulite Ltd) and had a resonant frequency of about 1 kHz and compliance of about 2 μm/mN.

### *Single units*

Motor unit twitches were obtained either from units isolated in a nerve (see below) or from single units obtained by graded stimulation of either sural nerve (SN) or lateral plantar nerve (LPN). Contraction time (time from beginning to peak tension), half-relaxation time (time from peak to half-maximal tension) and peak tension were measured. Tetani from single units (100 or 200 Hz) were obtained only from units isolated in a nerve since graded stimulation is not reliably

controllable under conditions of tetanic stimulation. Single units occurred naturally in SN in some muscles. In others SN or LPN was progressively sectioned until one motor axon only remained in continuity with the muscle. For this to yield complete units the assumption is that axons do not branch central to the point of isolation. Jones & Ridge (1987) found no evidence of axon branching below the peroneal nerve branching point in the thigh of 4-day-old rats. In a few cases the nerves were split to yield a number of filaments that were then stimulated separately via fine suction electrodes. This yielded up to seven separated motor units in the same muscle (e.g. Fig. 3). The criterion of unitary activity was that the response of the muscle to a series of threshold, constant voltage stimuli was all-or-none. All tension recordings were made at optimal length of the muscle for maximal whole-muscle twitch tension. Units were classified as 'sag' or 'no sag' on the basis of the shape of the unfused isometric tetanus (duration 700 ms). If the tension at the end of the tetanus was less than the maximal tension developed during the tetanus that unit was classified as a 'sag' unit. Several frequencies between 15 and 80 Hz were used at 25 °C. Usually 40 Hz gave good separation, but sag at any frequency leading to an unfused tetanus was a sufficient criterion. Units giving no sag at 25 °C were tested at 30 °C (40 Hz tetani). In a few cases this gave rise to sag and the unit was classified as a 'sag' unit. Isometric tetanic tensions for fused tetani were measured for motor units and whole muscles.

Glycogen depletion was achieved by repetitively stimulating the motor unit (or whole-muscle nerve for controls) at 40 Hz for 330 ms every second in glucose-free Ringer solution at 30 °C for 20 min, or until tension developed was reduced to a quarter of the initial tension if this occurred earlier. Stimulation was then stopped for a rest period of 5 min. This regime was continued until the unit developed no tension on stimulation. Each experimental muscle was accompanied throughout the experiment by the contralateral, unstimulated muscle as a control. These showed no apparently glycogen-depleted fibre profiles. A few whole muscles were subjected to this depletion regime by stimulating the muscle nerve. In these cases virtually all muscle fibres were depleted (the few – typically less than ten – that were not depleted were usually surface fibres and were not exclusively either fast or slow fibres).

The rate at which the unit fatigued was measured from a continuous pen recording of tension taken while the unit was undergoing glycogen depletion. Fatigue was expressed as an index, which was the tension produced at 2 min as a ratio of that produced at 0 min. This is the standard fatigue index employed by others that was introduced by Burke, Levine, Tsairis & Zajac (1973) for cat muscle motor units. We also used a second index using 5 min which provided rather better separation of units. In some cases the unit showed some potentiation at the start of the depletion regime. The fatigue index for these units was calculated from the maximum tension and that produced 2 or 5 min later (Kernell, Eerbeek & Verhey, 1983).

*Histology*

Muscles were frozen in isopentane cooled in liquid nitrogen, under moderate stretch, between two pieces of a control limb muscle of known fibre type composition (plantaris or extensor digitorum longus), and the composite block sectioned in a cryostat. Several sections from the midbelly region were taken and close sections were treated in one of three ways: stained with periodic acid Schiff's reagent (PAS) to demonstrate the presence or absence of muscle fibre glycogen; treated with a polyclonal antibody raised against slow type I myosin, or treated with another polyclonal antibody raised against fast IIA myosin. Immunocytochemical staining was achieved by the indirect immunoperoxidase method (see Jones *et al.* 1987*a* for further details). The anti-I myosin antibody was raised against myosin isolated from cat soleus muscle. It is specific for slow myosin heavy chains. Its extraction and the testing of its specificity are described in Rowlerson, Pope, Murray, Whalen & Weeds (1981) and it is the same as that used in our previous study of 4-day-old 4DL muscles (Jones *et al.* 1987*a*, *b*). The anti-IIA myosin antibody was raised against myosin heavy chains from guinea-pig masseter muscle which contains almost exclusively type IIA fibres. Its preparation and testing is given in Carpene, Rowlerson, Veggetti & Mascarello (1982), who found no cross-reactivity with type I fibres, and only a weak cross-reactivity with IIB fibres. Some sections were stained for myosin ATPase, using acid or alkali pre-incubations (Brooke & Kaiser, 1970). Others were stained for succinic dehydrogenase (Nachlas, Tsou, De Sousa, Cheng & Seligman, 1957).

Camera lucida drawings were made of midbelly sections of the muscles. The midbelly was identified as the part of the muscle length which contained the largest number of fibres in

transverse sections, together with nerve bundles in the perimysium and no proximal or distal tendon in the section periphery. Those fibres completely without the pink PAS stain for glycogen were marked on the drawing as depleted. Each fibre in the muscle was then assigned to a type depending on whether it bound either or both antibody (type I, anti-I positive; type IIC, anti-I and anti-IIA positive; type IIA, anti-IIA positive; and type IIX (see Discussion) negative for both antibodies) in serial sections. The muscle fibre profiles in the entire cross-section were then digitized and fibre type, whether depleted or not, and cross-sectional areas were stored in a data array.

*Mean values*

All mean values throughout this paper are given as ± one standard deviation.

## RESULTS

### *The number of motor units in 4DL muscle*

In a sample of normal muscles we counted 11·4 ± 1·2 (mean ± S.D.; $n = 44$) motor units by counting the number of repeatable and voltage-dependent increments in twitch tension as the voltage of the stimulus applied separately to the lateral plantar (LPN) and sural nerves (SN) was varied between subthreshold and supramaximal. This value is similar to that obtained in adult rat muscle by Betz *et al.* (1979; 11·3 ± 1·9) and Jones & Ridge (1987; 11·2 ± 0·9), and in neonatal (3–5 day) rat muscles by Jones & Ridge (1987; 10·9 ± 1·3), using the same method.

The number of motor units in the muscle was also deduced from estimates of mean motor unit size obtained by tension measurement and by histology. Motor unit size (as a percentage whole-muscle tetanic tension) was measured from each unit isolated by nerve section from LPN or SN, or occurring naturally as a single unit in SN. Mean motor unit sizes were similar in these three cases. They were respectively: 6·7 ± 4·8 %, $n = 48$; 6·3 ± 3·3 %, $n = 74$ and 6·2 ± 3·4 %, $n = 25$. These values are equivalent to mean motor unit numbers per muscle of 14·9, 15·9 and 16·1. In units depleted of their glycogen, unit size was calculated as percentage of fibre numbers or cross-sectional areas of the whole muscle. In these cases for isolated LPN or naturally single SN units mean values ranged from 6·3 to 7·5 %, equivalent to 15·9 to 13·0 motor units per muscle respectively.

There is thus the possibility that an assessment of the number of motor units in a muscle by counting of tension increments gives an underestimate, perhaps because small motor units are missed. The question of the mean number of motor units per muscle is considered further in the Discussion section. For the time being we conclude that in the rats used in these experiments its value was between eleven and sixteen.

### *Distribution of motor units between the lateral plantar and sural nerves*

In the normal muscle sample in which 11·4 motor units per muscle on average was found by tension increments, LPN supplied 9·3 ± 1·7 ($n = 44$) and SN supplied 2·4 ± 1·7 ($n = 46$). However, there was some bias towards sural units in this sample, since some muscles with no SN units were rejected. A second sample was without sampling bias, but in these animals the contralateral muscle had been partially denervated at birth, by section of LPN, for a different series of experiments. Motor unit numbers in this sample were: total number, 11·2 ± 1·1 ($n = 35$); via LPN, 9·1 ± 1·5; and via SN, 2·1 ± 1·7. These values are very close to those found by Betz *et al.* (1979). As no differences in motor unit number or motor unit size were found

between muscles from normal animals and contralateral controls to partially denervated muscles they have been pooled. A few of the motor units studied were from contralateral control muscles.

*Whole-muscle twitches and tetani*

The mean isometric twitch tension was $33{\cdot}8 \pm 7{\cdot}9$ mN ($n = 85$) and time-to-peak contraction was $22{\cdot}7 \pm 1{\cdot}3$ ms. Mean fused (maximal) tetanic tension was

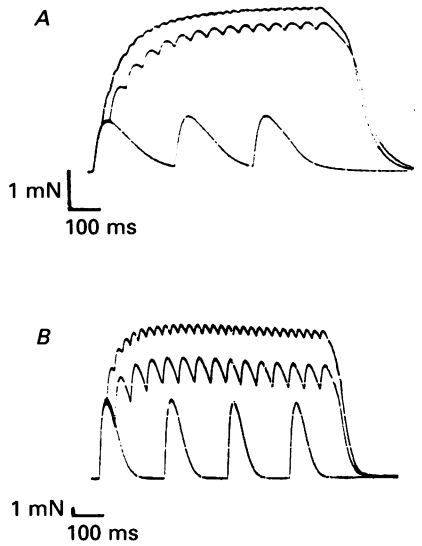


Fig. 1. Unfused tetani of 'no sag' unit (*A*) and 'sag' unit (*B*). Tetani at 5, 20 and 40 Hz for 700 ms.

$119{\cdot}8 \pm 26{\cdot}5$ mN. Mean twitch: tetanus ratio was $0{\cdot}28 \pm 0{\cdot}05$. This is equivalent to a mean specific tension of 22·6 N/cm$^2$, based on the mean fibre numbers in whole muscles and fibre cross-sectional areas given below.

*Motor unit tetani: 'sag' and 'no sag' units*

The shape of the unfused tetanus has been found to be a good criterion for separating motor units with respect to fibre type composition. Burke *et al.* (1973), who introduced this criterion, found that in the cat gastrocnemius muscle units showing no sag consisted of slow fibres, on the basis of their myosin ATPase histochemistry, whereas units showing sag did not. Examples of records of unfused tetani from a 'no sag' and a 'sag' unit are shown in Fig. 1. The difference between 'no sag' and 'sag' units in 4DL was quite clear, though the degree of sag was generally less pronounced than in the cat gastrocnemius units recorded by Burke *et al.* (1973).

*Motor unit twitches*

In a few muscles mechanical responses from a high proportion of the motor units in the muscle were recorded separately by stimulating filaments dissected from LPN and SN. Records of unit twitches from such a muscle are shown in Fig. 2. This muscle

had twelve motor units (by counting tension increments). There were seven in LPN (one increment was not repeatable and was therefore apparently due to causes other than voltage-dependent unit recruitment) and five in SN (an unusually large number in SN, though the maximum number of SN motor units seen in one muscle was seven).

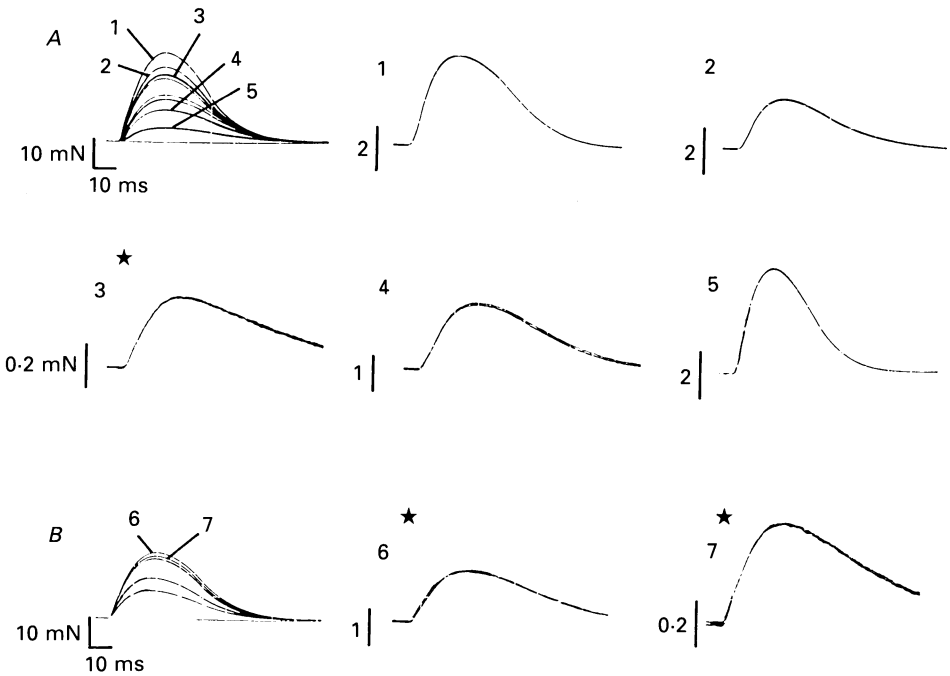


Fig. 2. Twitches recorded from a single muscle. *A*, in response to graded stimulation of the lateral plantar nerve. Seven voltage-dependent and repeatable increments were counted (an eighth increment was not repeatable). Of these seven motor units five were separately isolated by nerve splitting. Individual unit twitches are shown in records 1–5, and increments corresponding to each unit are labelled. *B*, records obtained by stimulating the sural nerve. Here five units were counted, of which two (Nos. 6 and 7) were isolated. Time scale bar 10 ms for all records. Starred units showed no sag of the unfused tetani (all others showed sag).

Five units were isolated in LPN and two in SN. There is a wide range of unit twitch tensions, and three of the small units were found to be 'no sag' units (one in LPN and two in SN).

From this one muscle one can conclude the following: (1) there is no exclusive selection of units by size or sag property in SN; (2) there can be more than one 'no sag' unit in a muscle; and (3) though the 'no sag' units are small there are also small units (as well as large units) that show 'sag'.

### *Motor unit sizes and contraction times*

Contraction times (twitch times-to-peak) of single units ranged from 18 to 36 ms. Relationships between unit size and contraction times and half-relaxation times are shown for 'sag' and 'no sag' units in Fig. 3 *A* and *B*. Among 'sag' units small units

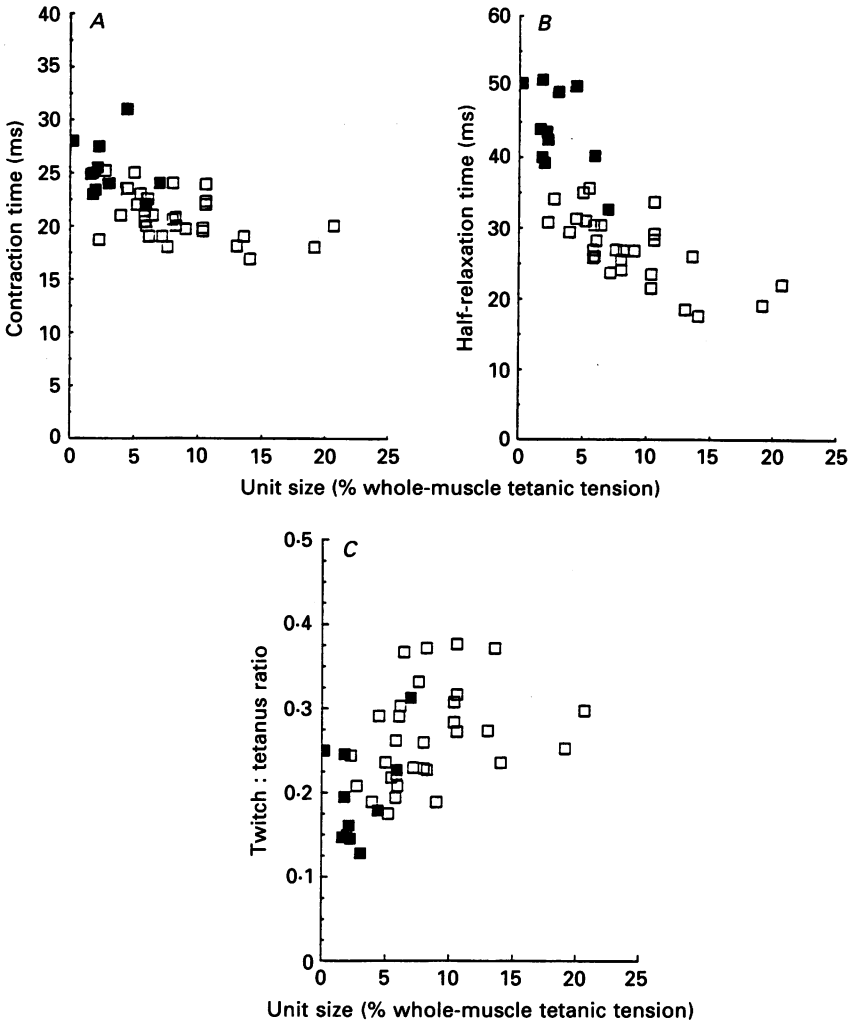


Fig 3. Unit size (% whole-muscle tetanic tension) plotted against contraction time (*A*), half-relaxation time (*B*) and twitch:tetanus ratio (*C*). ■, 'no sag' units. □, 'sag' units.

TABLE 1. A comparison of mechanical properties of 'sag' and 'no sag' motor units in 4DL

| | Unit size (% whole-muscle tetanus) | Unit twitch contraction time (ms) | Unit twitch half-relaxation time (ms) | Twitch: tetanus ratio | Fatigue index | |
|---|---|---|---|---|---|---|
| | | | | | 2 min | 5 min |
| 'Sag' | 7·6±3·6 (37) | 21·4±2·3 (37) | 28·4±5·5 (35) | 0·27±0·07 (37) | 0·85±0·10 (30) | 0·64±0·25 (30) |
| 'No sag' | 3·0±1·8 (15) | 25·9±3·7 (15) | 45·3±7·4 (15) | 0·20±0·05 (15)s | 0·92±0·06 (14) | 0·83±0·17 (14) |
| | $P < 0·001$ | $P < 0·001$ | $P < 0·001$ | $P < 0·001$ | $0·01 < P < 0·05$ | $0·01 < P < 0·05$ |

Mean values±S.D. Number in parentheses. $P$ from Student's $t$ test.

tended to have longer contraction times and, more clearly, longer half-relaxation times than larger units. 'No sag' units tended to be small and slowly contracting and relaxing. Although there was some overlap with the population of 'sag' motor units in all these properties, mean values for 'sag' and 'no sag' units were significantly

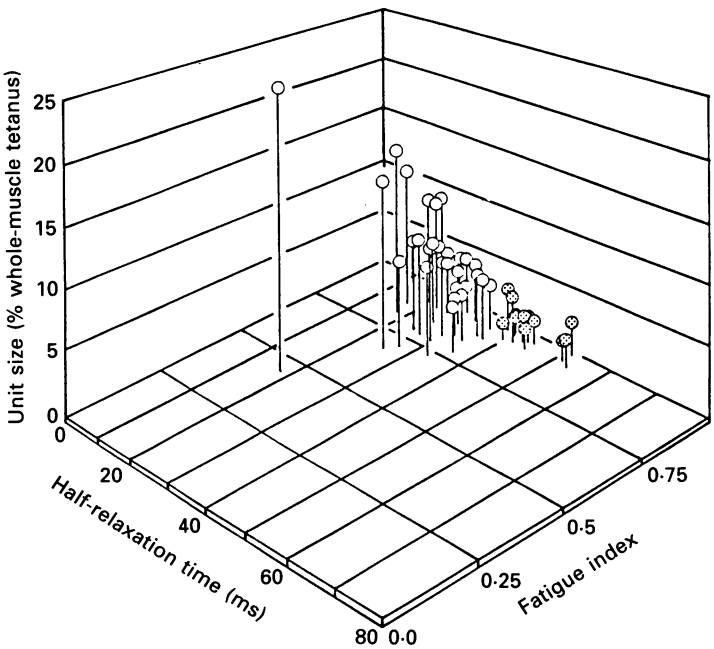


Fig. 4. The interrelationship between unit size (% whole-muscle tetanic tension), twitch half-relaxation time and fatigue index. 'No sag' units are stippled. Comparison with Fig. 5 of Burke *et al.* (1973) shows that there are no FF units in rat 4DL.

different in all three. Mean values are given in Table 1. It is apparent from Fig. 3 that 'no sag' units have long contraction times in association with their small size, but they separate from 'sag' units more clearly on the basis of half-relaxation times.

### *Motor unit twitch: tetanus ratios*

Twitch:tetanus ratios varied directly with unit size, as shown in Fig. 3*C*. 'No sag' units had a significantly smaller twitch:tetanus ratio than 'sag' units (see Table 1), and this is largely in association with their small size.

### *Motor unit fatigue*

In most cases tension development was recorded continuously during glycogen depletion of the motor unit by repetitive stimulation. The rate of fatigue was expressed as a fatigue index (the proportion of maximal tension maintained after 2 and 5 min of stimulation – see Methods). Although there was a tendency for 'no sag' units to be less fatiguable, mean values of fatigue indices were not significantly different. Values are given in Table 1.

*Physiological profiles of motor units*

To facilitate comparisons with the data of Burke *et al.* (1973) from motor units in cat gastrocnemius (their Fig. 5) a three-dimensional plot is given in Fig. 4. Here we plot half-relaxation time (since this separated 'sag' and 'no sag' (stippled) units better than contraction time), percentage of whole-muscle tetanic tension, and

TABLE 2. Muscle fibre types and specific antimyosin antibody binding

| Fibre type | Anti-I | Anti-IIA |
|---|---|---|
| I | + | − |
| IIA | − | + |
| IIC | + | + |
| IIX | − | − |

fatigue index (at 2 min). The main difference between the rat lumbrical units and the cat gastrocnemius units is that the rapidly fatiguing and fast contracting units (FF of Burke *et al.* 1973) of the cat muscle are not present in the rat lumbrical muscle. This point is discussed further below.

*Types of muscle fibres in whole muscles: histochemistry*

In some control muscles a systematic examination of the histochemical properties was performed for myosin ATPase activity after a wide range of treatments at different pH (9·4–10·8 and 5–4·1), succinic dehydrogenase and also α-glycerophosphate dehydrogenase activities. 4DL was included in composite blocks with extensor digitorum longus or plantaris (which acted as controls for fibre typing), frozen and sectioned in a cryostat. In other muscles neighbouring midbelly sections were simply pre-incubated at about pH 4·2 and 10·7 (actual values were empirically determined for each batch of sections), and then stained for myosin ATPase. In all cases adjacent sections were also tested for specific antibody binding. Two antibodies were used, one raised against slow myosin and the other against type IIA myosin (see Methods). For reasons discussed below the antibody binding was used for classification of muscle fibre types in preference to myosin ATPase staining. Four types of extrafusal muscle fibres were recognized (derived from histochemistry by Brooke & Kaiser, 1970), as shown in Table 2. The majority of fibres in a cross-section of 4DL did not bind either antibody. These fibres were probably IIX (Schiaffino, Gorza, Sartore, Saggin, Ausoni, Vianello, Gundersen & Lomo, 1989; Gorza, 1990) rather than the more generally known IIB, because they had high levels of succinic dehydrogenase (see below), and on myosin ATPase staining showed very high alkali stability and moderate acid stability. The high level of succinic dehydrogenase correlates with the fact that there were no FF type motor units in 4DL (see Fig. 5). A second type of fibre was recognized that bound *both* antibodies, and was alkali-stable in myosin ATPase staining. This was therefore type IIC. In some muscles there were a few type I fibres that were positive for antislow but negative for the anti-IIA antibody, and in these fibres myosin ATPase was alkali-labile. However, type I

fibres were either a small minority only, or absent altogether. A third type of fibre bound the IIA antibody only. In this paper we will refer to these fibres as IIA, but histochemically they were not typical. These fibres showed a wide range of staining intensity for the IIA antibody, and although a few showed the classical acid-labile myosin ATPase staining, most showed acid-stable myosin ATPase staining like that of IIX fibres (alkali and partially acid stable). Possibly, therefore, these fibres synthesize both IIA and IIX type myosins in variable ratio. Because of their variable IIA staining intensities it was not possible to make accurate counts of their numbers in whole-muscle sections.

*Succinic dehydrogenase*

Staining sections for the oxidative mitochondrial enzyme succinic dehydrogenase revealed that all fibre types in 4DL showed a comparatively high level of staining, and that those fibres not binding either antibody were distributed throughout the entire range of staining for succinic dehydrogenase. An example is shown in Fig. 9*D*. No fibres were therefore typical IIB fibres in this regard.

## *Serial reconstruction*

*Spindles and intrafusal fibres*

Spindles were often identified in muscle cross-sections either by the presence of a capsule surrounding a small group of intrafusal fibres, or by small fibres occurring in a group that we assumed to be intrafusal fibres. Such fibres were not included in counts of extrafusal fibres. Typically there were from one to four spindles in each midbelly muscle section.

In two muscles from one rat serial reconstructions of muscle spindles were made throughout the entire muscle length. In one muscle twelve spindles were recognized, and in the other eight. The positions of their mid-equatorial regions and the extent of the bag fibres along the muscle length in one muscle are shown diagrammatically in Fig. 5.

W. J. Betz & G. S. Bewick (personal communication) have found that the fluorescent dye RH795 (Molecular Probes) stains spindle capsules and primary endings. They have counted up to eleven spindles in whole mount, living 4DL from 8-day-old animals.

In the remainder of this study all muscle fibre counts were made from transverse sections cut in the midbelly region of the muscle. The criteria for deciding that a section was from the midbelly were that its cross-sectional area appeared to be no smaller than that of other sections, and that no recognizable tendon was situated in the muscle periphery. The serially reconstructed muscles showed that such sections could be found occupying about one millimetre of the middle region of the muscle length (see Fig. 5). Beyond this region some tendinous material appeared in the muscle periphery corresponding to the beginning of the myotendinous insertion of the proximal or distal tendon. Within the tendon-free length the total number of muscle fibres per section was greatest and remained virtually constant, and so it is likely that we were seeing all the muscle fibres in the muscle in sections from this region. Muscle fibre numbers fell off either side of the tendon-free region. The numbers

of slow-myosin-containing (IIC and I) and of IIA fibres remained approximately constant throughout this region also. When followed along their length, individual fibres did not change from the fast category (IIX, IIA) to the slow category (I, IIC) but there were occasional instances where a fibre would apparently shift from one type to the other within the fast or slow categories.

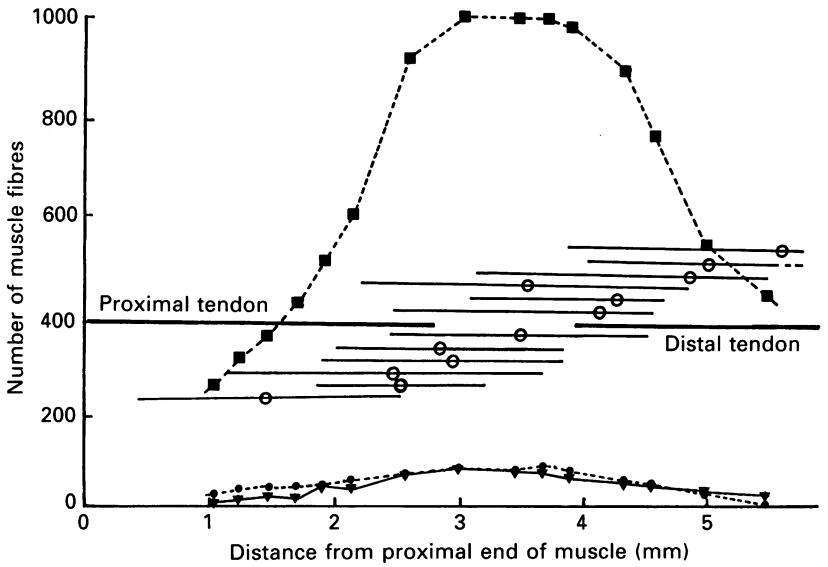


Fig 5. Results of serial reconstruction of one adult (60 days) rat 4DL muscle. Total numbers of muscle fibres (■), those staining for the antislow myosin antibody (●) or anti-IIA antibody (▼) are plotted along the length of the muscle. Each spindle capsule at the mid-equatorial position (○) and the extent of the longest intrafusal fibre (line) along the length of the muscle are indicated, as is the presence of tendon in the section (thick line; proximal or distal).

*Numbers and cross-sectional areas of muscle fibres in whole muscles*

In twenty-seven muscles all the extrafusal fibres were classified into types using specific antibody binding, counted and their cross-sectional areas measured. Mean counts per muscle (±S.D.) were: IIX, 818±81; IIC+I, 82±9; IIA, 69±35, (the large variation in numbers of IIA fibres probably reflects the difficulty in classification due to the graded intensity of staining). The mean total fibre count of 970±72 is close to that reported by Betz *et al.* 1979 (938±79), and the number of slow-myosin-containing fibres (IIC+I) is close to those reported by Jones *et al.* (1987*b*) for rats less than 10-day-old (85·3±7·9) and more than 10 days (82·1±6·7). We also compared the mean cross-sectional areas of the different fibre types. Mean values for each muscle were then averaged (since total fibre counts for each muscle are broadly similar this gave values close to a true mean for each fibre type). Resulting values ($\mu m^2$, mean±S.D.) were: IIX, 582±80; IIC, 393±68; IIA, 320±90. However, the reliability of these values is limited by the fact that the muscles were frozen at

lengths that were not controlled in relation to optimal length. Some of the intermuscle variation will therefore be artifactual. To control for this we normalized the mean cross-sectional area of the IIX fibres in each muscle to 1·0, and expressed the mean cross-sectional area of IIC and IIA fibres in each muscle as a ratio to 1. The

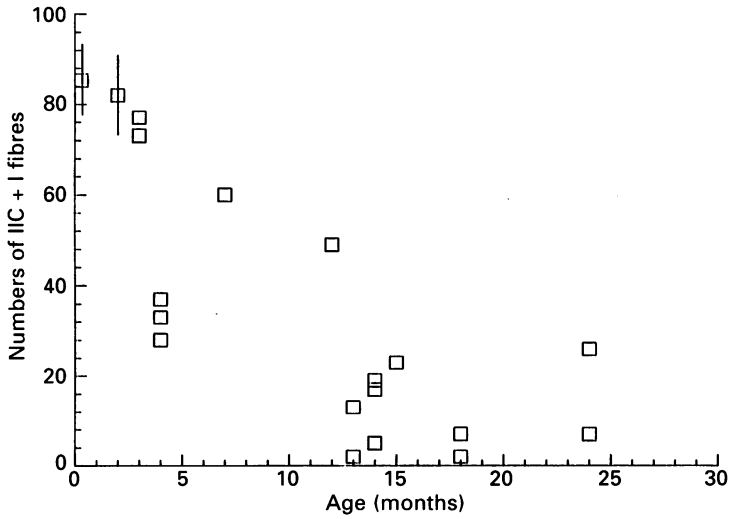


Fig. 6. The number of IIC+I fibres in 4DL muscles from rats of different ages.

means ± S.D. ($n$) of these ratios for all the muscles are: IIC/IIX = 0·674 ± 0·078 (27); IIA/IIX = 0·566 ± 0·057 (26). When compared this way the cross-sectional area of each fibre type is significantly different from that of the other two fibre types (IIX > IIC > IIA, $P = 0·001$ for all three comparisons; two-tailed $t$ test).

It can therefore be concluded that IIA fibres are on average the smallest fibres. This point is discussed further below.

*Muscles from older rats*

Since IIC fibres in rat soleus have been considered to be transitional fibres (Kugelberg, 1976) it was of interest to know if their number in 4DL, which remains constant from near birth to 60 days, remains constant in older rats. We therefore examined 4DL in a number of older rats and counted total fibre numbers and numbers of IIC+I fibres. Muscles from rats ranging in age from 3 months to 2 years were studied, and the data for IIC+I fibres are shown in Fig. 6. At 3 months the number of muscle fibres positive for the antislow myosin antibody was not significantly different from that in neonates (Jones *et al.* 1987*b*). Thereafter this number declined over the first 12 months or so. Beyond 12 months there appeared to be random variation rather than a further progressive decline. There was no significant change in the total number of fibres in the muscle (total fibre counts could not be made in the sections from the 2-year-old rat but they did not appear atrophic).

In most cases adjacent sections were treated with the anti-IIA antibody. Full counts were not made, but in all cases the majority of fibres positive for antislow were

also positive for anti-IIA (that is, they were IIC fibres). In all cases there was a variable number of other fibres that were positive for anti-IIA, but negative for anti-I.

### *Muscle fibre composition of single motor units*

#### *Unit tensions and cross-sectional areas*

In twenty-seven muscles a single motor unit in each was stimulated and its isometric mechanical properties recorded. It was then subjected to the glycogen-

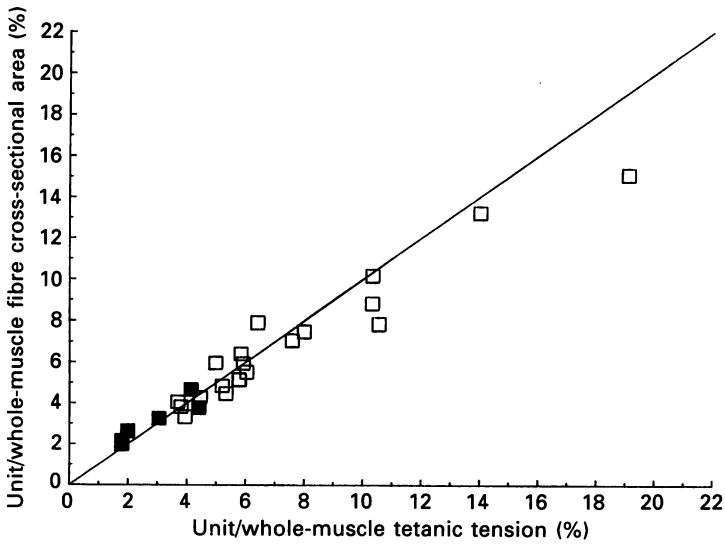


Fig. 7. Comparison of unit sizes calculated from histology (sum of cross-sectional areas of muscle fibres in the unit as percentage sum of cross-sectional areas of all fibres in the muscle) and tension development (unit tetanic tension as percentage of whole-muscle tension). The line of equality is shown. ■, IIC/IIA units.

depletion regime, frozen, sectioned and stained. The muscle fibres in each motor unit were then classified with respect to type and their cross-sectional areas measured. It was found that maximum tetanic tension developed by the unit was related to its cross-sectional area, and that points for all the units were close to the line of equality (with the possible exception of the larger units), as shown in Fig. 7. We conclude that glycogen depletion of these units was generally successful, there being neither large scale variations in the effectiveness of glycogen depletion nor significant apparent depletion of fibres outside the motor unit.

*Motor unit types.* We found two distinct types of motor unit. Twenty units were virtually homogeneous with regard to fibre type, none or very few of the fibres binding either specific antibody. These were therefore IIX units. An example is shown in Fig. 8 (*A*, PAS; *B*, anti-I; *C*, anti-IIA). All of these units showed sag of the unfused tetanus and data from them are included in Figs 3 and 4.

Six units contained both IIC (including a few type I) and IIA fibres. IIC fibres were the more common fibre type except in one unit which contained the same number of

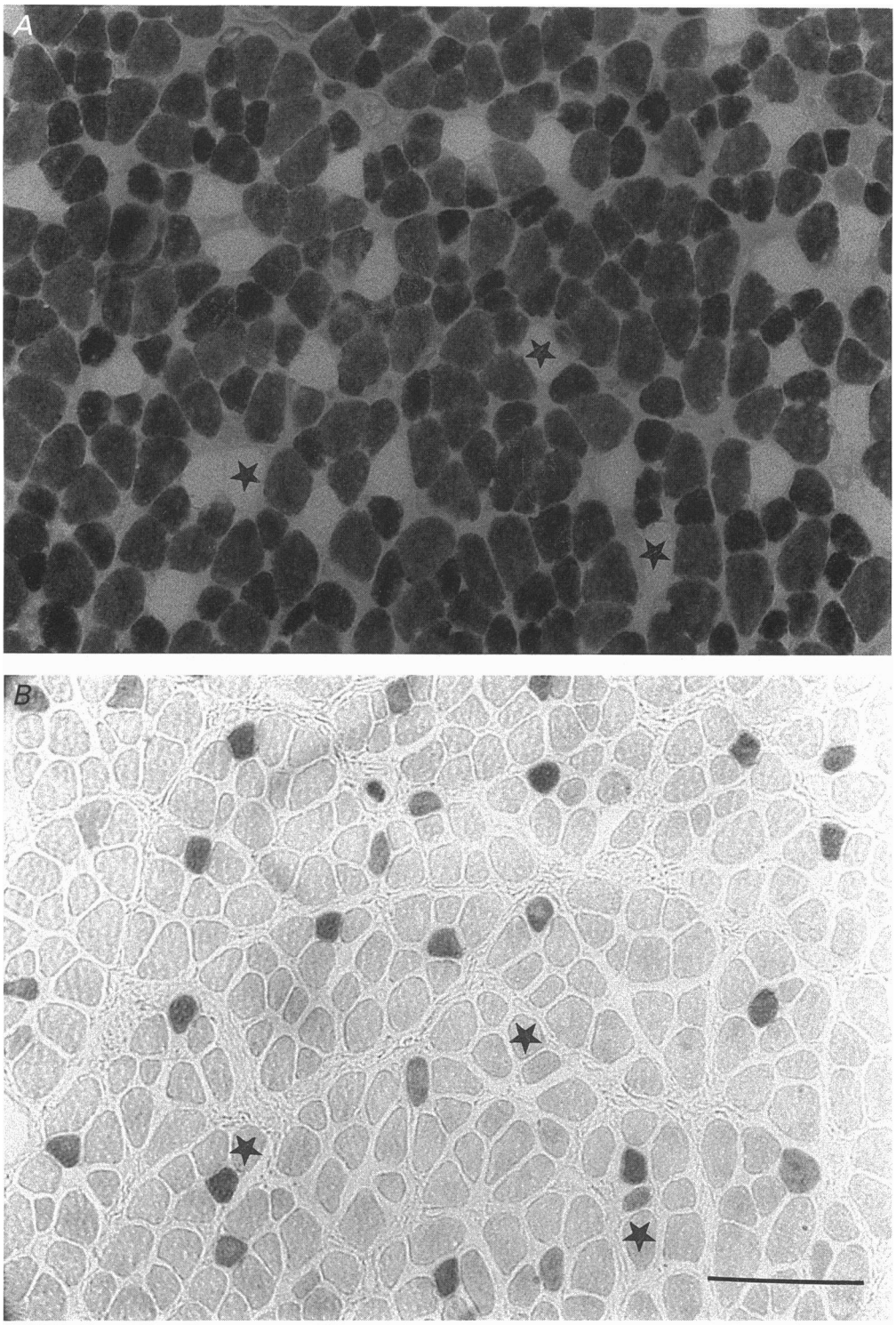


Fig. 8(*A*, *B*). For legend see facing page.

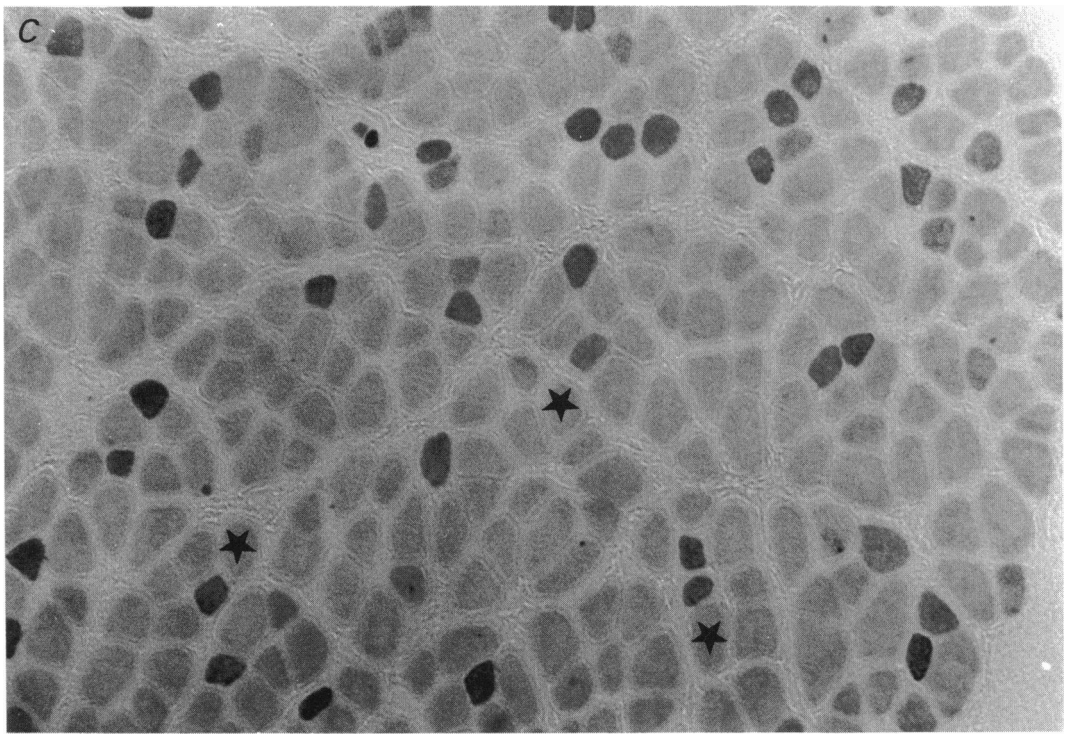


Fig. 8. Corresponding fields from sections of a muscle in which a IIX motor unit was depleted of glycogen. *A*, PAS for glycogen. *B*, anti-I antibody. *C*, anti-IIA antibody. All the depleted fibres in the field (three are starred) are negative for both antibodies. Bar = 100 $\mu$m.

IIC and IIA fibres. An example of a IIC/IIA unit is shown in Fig. 9 (*A*, *B* and *C* being stained as in Fig. 8; *D*, SDH). None of the IIC/IIA units showed sag in the unfused tetanus, and data from them are included in Figs 3 and 4. The numbers of fibres of each type in all the units studied is shown in Fig. 10.

A further 'unit' (No. 27 in Fig. 10) appears anomalous. Possibly two units were depleted inadvertently together, one being IIX and the other IIC/IIA. However, this would mean that the IIC/IIA was biased in favour of IIA, unlike the other six IIC/IIA units. Alternatively this may be a genuine unit. If so it would appear to be of a rare type probably not always present in the muscle; or it could have been a IIA unit, since IIA staining intensity is very variable, and it is difficult to separate all IIA from IIX fibres.

*Muscle fibre cross-sectional areas in IIC/IIA units*

On average IIA fibres were the smallest in cross-sectional area of extrafusal fibres in whole-muscle sections. This was found to be the case in four of the six IIC/IIA motor units studied. In the two remaining units in which this was not clear, one had a small number of muscle fibres (No. 26 in Fig. 11) and the other had very few IIA fibres (No. 23). The difference was clearest in the largest unit (No. 25), and the

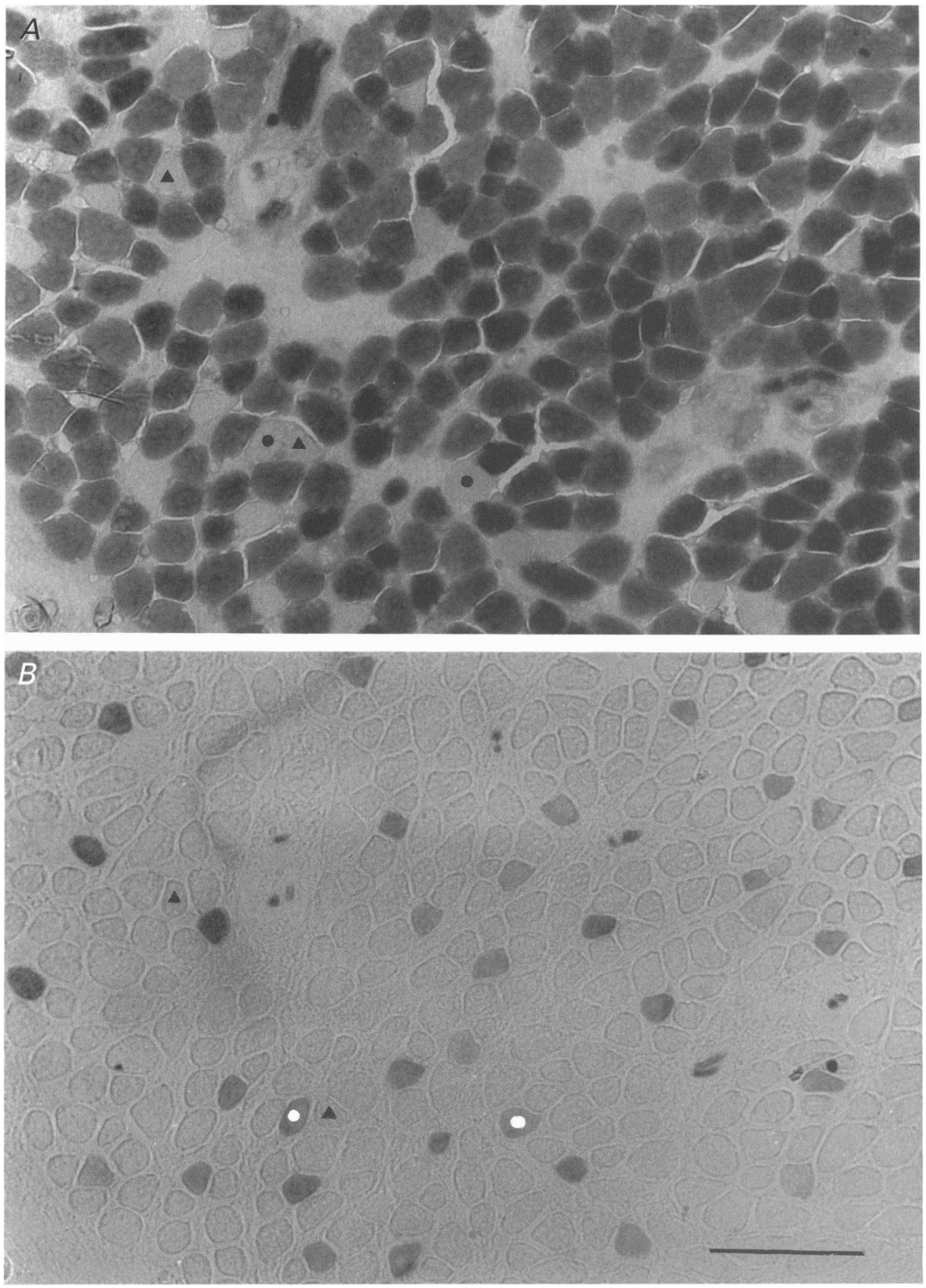


Fig. 9(*A*, *B*). For legend see facing page.

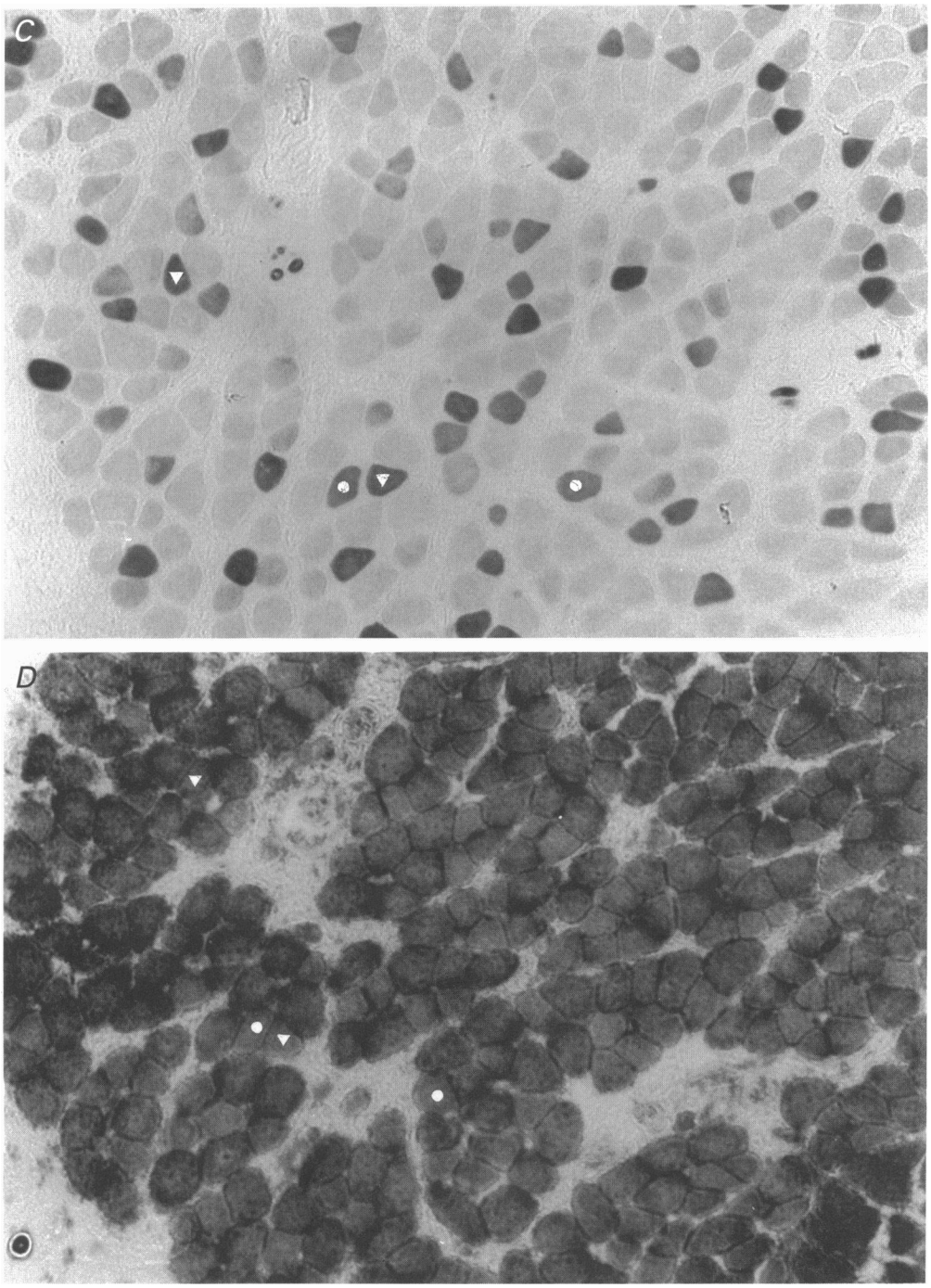


Fig. 9. As in Fig. 8 but for a IIC/IIA motor unit. *A*, PAS. *B*, anti-I. *C*, anti-IIA. *D*, succinic dehydrogenase. Two IIC fibres marked (○ or ●) and two IIA fibres marked (▲ or △). Note in *D* that all fibres stain intensely for succinic dehydrogenase. Bar = 100 $\mu$m.

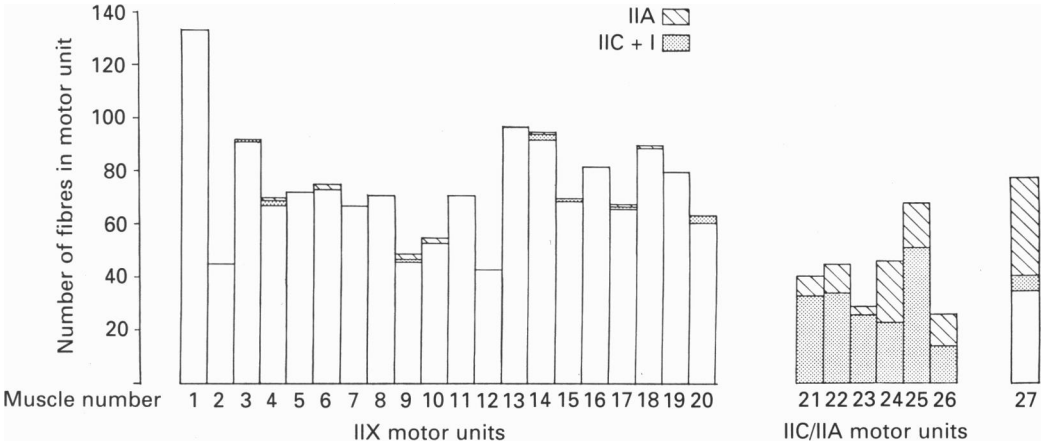


Fig. 10. The numbers of fibres of each type in the motor units analysed immunohistochemically. Note that there were no pure IIC or IIA motor units.

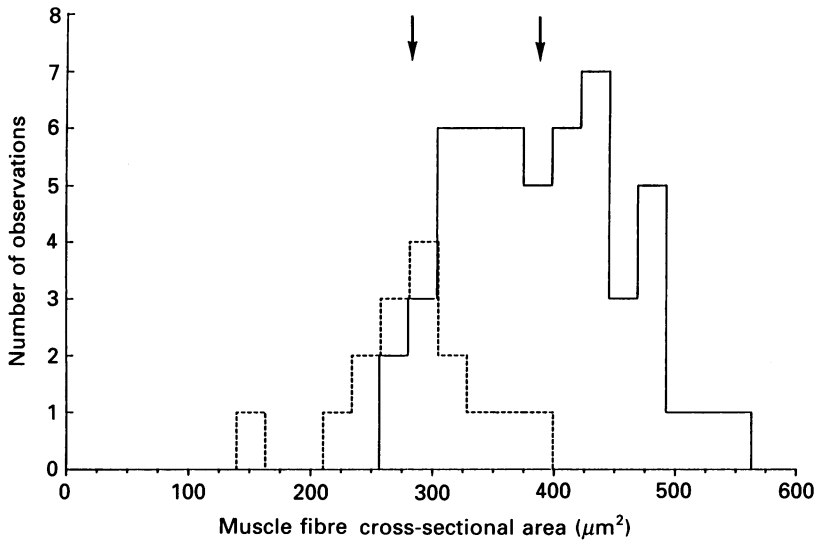


Fig. 11. Frequency distribution of cross-sectional areas of IIC and IIA (dashed line) fibres in a IIC/IIA unit (No. 25 of Fig. 9). Arrows indicate areas ($\mu m^2$, mean $\pm$ S.D. ($n$)): IIC = $309 \pm 69(52)$; IIA = $284 \pm 57(16)$.

frequency distribution is shown in Fig. 11. It is interesting and surprising that muscle fibres under the influence of the same motoneurone can be differentiated both on the basis of myosin composition and fibre cross-sectional area.

## DISCUSSION

### *Types of muscle fibres in 4DL*

Using two specific antimyosin antibodies (anti-I and anti-IIA) we have found three main extrafusal fibre types in 4DL. One type binds both antibodies, and, on this basis and its myosin ATPase staining properties (alkali and partially acid

stable), conforms to type IIC of Brooke & Kaiser (1970). This type, together with occasional type I fibres (positive for anti-I only), occurs in quite constant numbers from muscle to muscle (about 80) and corresponds to the eighty or so slow-myosin-containing fibres described by Jones *et al.* (1987*a*, *b*) in neonatal 4DL.

A second type of fibre binds anti-IIA only, and because of this is referred to as type IIA in this paper, though these fibres are not the same as the well-known IIA fibres of, for example, rat gastrocnemius (Brooke & Kaiser, 1970) and soleus (the minority fibre type in this muscle; Kugelberg, 1976), since in most of them the myosin ATPase staining pattern (alkali-stable, weakly acid-stable) is characteristic of IIX and IIB fibres (Brooke & Kaiser, 1970; Gorza, 1990). It is possible that these fibres contain both IIA and IIX type myosins which have been shown to co-exist in fibres of some other muscles (Gorza, 1990). Another feature of the IIA fibres in the present study is the very wide range of staining intensities found for anti-IIA, ranging continuously from intense to just above background. This limits the accuracy of counts of this type of fibre in whole-muscle sections, since classification is uncertain at the low intensity end of the range. Our fibre counts (about 70) gave values similar to those of IIC+I. Interestingly these type IIA fibres have the smallest cross-sectional areas of the three extrafusal types.

The third, and far the most common, type of fibre binds neither of the specific antimyosin antibodies used in this study. On this basis, and their myosin ATPase staining, we refer to these fibres as IIX fibres rather than IIB. The distinction between IIB and IIX fibre types was made recently by Schiaffino and his colleagues. In the rat, IIB and IIX fibres are indistinguishable using acid pre-incubation with ATPase activity, but differ in their alkali–ATPase stability, succinic dehydrogenase intensity and reactivity with some myosin heavy chain specific antibodies (Schiaffino, Saggin, Viel, Ausoni, Sartore & Gorza, 1986; Gorza, 1990). Although we do not have the myosin heavy chain antibodies used by Schiaffino *et al.* and Gorza, the overall histochemical profile (and lack of reactions with anti-I and anti-IIA) shown by the majority of fibres in 4DL is clearly that of the IIX type. In cat, IIB fibres are easily fatigued and have low levels of succinic dehydrogenase staining (Burke *et al.* 1973). In rat, before the recognition that the 'IIB' category also included IIX, IIB fibres and motor units were found to range from fatiguable to fatigue-resistant, and to exhibit a wide range of succinic dehydrogenase staining intensities (e.g. Kugelberg & Lindegren, 1979). In 4DL we have found the IIX motor units to be comparatively fatigue resistant, consistent with their relatively high succinic dehydrogenase activity.

*Types of motor units*

Surprisingly the twenty-six motor units studied by glycogen depletion were found to be classified into two types and not three. This is because all the units (6) containing IIC fibres also contained IIA fibres, no pure IIC or IIA units being encountered. This motor unit type we refer to as IIC/IIA. None of these six units contained any fibres that were obviously IIX, though the wide range of the IIA antibody staining intensity makes it impossible to be certain of this. (A 27th unit was anomalous and is discussed briefly in Results).

IIC fibres have been demonstrated in young ($< 5$ weeks) rat soleus (most slow

fibres here being type I) by Brooke & Kaiser (1970) and by Kugelberg (1976) who considered them to be in transition from type IIA to type I (there is a large-scale conversion of IIA to type I fibres in rat soleus between birth and 34 weeks). Kugelberg also found a number of intermediate motor units between types IIA and I, some of which contained muscle fibres in the same motor unit with differing histochemical profiles, and some of these were of the type IIC/IIA. Unfortunately no numerical data are presented in the study about the frequency of occurrence of such units with age (5- and 34-week-old rats were studied), or about the ratio of IIC and IIA fibres in such units. It is possible that the IIC/IIA units in 4DL are also transitional, but if so they represent transition from slow to fast and not fast to slow as in soleus. This condition derives from the following: first that the IIC fibres almost certainly are the slow-myosin-containing fibres of the neonate and thus most probably derived from primary myotubes; (Jones *et al.* 1987*b*; Harris, Fitzsimons & McEwan, 1989), and second that 4DL muscles from old rats show a reduction in the number of IIC fibres, never an increase. We found in older rats that between 3 and 4 months there is a marked reduction in the number of IIC+I fibres. From 4 months onwards there is no clear, progressive further reduction, and there are still such fibres present at 2 years of age (Fig. 6). It is noticeable over this age range that there is considerable variation between animals, and even between the two muscles of the same animal. This observation would be consistent with conversion of one or two (but not all) of the two or three IIC/IIA units present originally.

Half the IIX motor units contained a *small* number of IIC and/or IIA fibres (see Fig. 10). It is most unlikely that all of these were errors of identification, and why they should exist is unknown. Possibly small numbers of fibres are relinquished naturally by motoneurones and subsequently incorporated into other motor units. If one of these were of inappropriate type to the adopting unit it would not be converted to the new type for at least 50 days, judging from results for units sprouting after partial denervation (Burke, Ridge, Rowlerson & Weedon, 1990). The finding that in older rats the number of IIC+I fibres is often reduced could be accounted for by the same mechanism, but after sufficient time for fibre conversion and accumulation of significant numbers by this probably slow process. Alternatively, loss of a few motoneurones followed by sprouting of the remaining terminals to reinnervate denervated fibres could lead to changes in the numbers of IIC-I fibres by neuronally mediated fibre conversion. Since only a reduction, and never an increase, in IIC+I fibres was observed, this would imply either that such conversions are only one way (IIC+I → IIX) or that only IIC/IIA motoneurones are lost with advancing age.

Mechanical recording showed that the shape of the unfused tetanus was diagnostic of motor unit type. All IIX units showed sag, and all IIC/IIA units showed no sag.

*The number of motor units in the muscle*

Estimates of the proportion of motor units that are IIC/IIA range from 0·18 to 0·29. Of the total sample of units studied mechanically 13/45 were 'no sag', = 0·29; of the sural units naturally occurring as single units, the proportion was 5/28 = 0·18; of all units analysed histologically, the proportion was 6/27 = 0·22. Thus if there are on average eleven motor units per muscle (from tension increments – see Results), we would expect two or three of them to be IIC/IIA. However, if there are as many as

sixteen units on average in the muscle (derived from the mean size of isolated units – see Results) then there could be as many as five IIC/IIA units. For the six IIC/IIA units in the histochemically analysed sample the mean number of IIC fibres (±S.D.) per unit was 30·3±12·9, which would give an average of three IIC/IIA units per muscle, since on average there are about eighty slow fibres in the muscle.

*Developmental origins of the different types of muscle fibre*

Previously (Jones *et al.* 1987*b*) it has been argued that the slow-myosin-containing fibres of the neonatal 4DL are the developing primary myotubes of the muscle primordium. It now seems likely that these fibres represent about 4/5ths of the original primary myotubes, since Ross, Duxson & Harris (1987) have shown that normally about a hundred primary myotubes appear in the embryonic muscle. Recently Condon, Silberstein, Blau & Thompson (1990) have described how about twenty primary myotubes in 4DL cease to express slow myosin between embryonic days 18 and 21. These fibres are situated near the edge of muscle sections.

A small margin of error exists in these numbers due to the first-forming nuclear bag fibres of spindles, which are primary myotubes developmentally modified by the presence of the primary afferent ending (Milburn, 1984). At 4 days these intrafusal fibres appear to be of similar cross-sectional area to extrafusal slow-myosin-containing fibres (Jones *et al.* 1987*b*) and would have been included in counts of such fibres at this age unless the spindle capsule was visible in the section and allowed their identification. Since in the adult, spindle capsules are not even confined to the midbelly of the muscle, it is likely that at 4 days several of these intrafusal fibres would not have been identified as such. In adult muscle, on the other hand, it is unlikely that significant numbers of nuclear bag fibres were counted as extrafusal since they are so much smaller. The fact that the mean number of slow-myosin-containing fibres at 4 days and in adults (IIC+I) is similar implies that this error is small.

Since the number of IIC+I fibres in 60 day muscles is very similar to the number of slow-myosin-containing fibres in neonates, and since they are distributed comparatively evenly throughout the muscle section (in contrast to the near-random distribution of fibres in a motor unit; H.-J. Gates & W. J. Betz, unpublished observation), as are the neonatal slow-myosin-containing fibres, it is reasonable to assume that the adult IIC+I fibres are indeed the same fibres, and so were primary myotubes in early development. IIX fibres, about half of which are generated after birth (Betz *et al.* 1979; Jones *et al.* 1987*b*), must be very largely derived from secondary myotubes. IIA fibres could be derived from two lineages: a small number from primary myotubes (the few primaries that do not become I/IIC) and the rest from the secondary population (Hoh, Hughes, Hugh & Pozgaj, 1989).

Rowlerson (1988) found that type I fibres began to be converted to IIC (IIA myosin made its first appearance) at about 1 month, but that the first IIA fibres appeared by day 16. In the adult, IIA fibres are the smallest type in cross-sectional area (see Fig. 11). Interestingly we have found small, fast (slow-myosin-free) fibres in two units biased towards slow fibres, in young rats below the age at which the IIA population can be identified as such. One of these units was in a 10 day muscle (authors' unpublished observation) and the other in a 4 day muscle (unit 12 of Jones *et al.* 1987*a*). Thus the subpopulation of small, fast fibres that will become type IIA

fibres may be differentiated and already incorporated into future IIC/IIA motor units early in life. Why or how IIA fibres become associated within the same units as the slow-myosin-containing fibres derived from primary myotubes is unknown. It is also surprising that these fibres are not converted to IIC or I fibres under neuronal influence, or alternatively that IIC/I fibres are not converted early to IIA (but see Ausoni, Gorza, Schiaffino, Gundersen & Lomo, 1990).

The reason for the formation and maintenance of the IIC/IIA units is unknown. The 4DL muscle is very well endowed with muscle spindles (we found twelve in one muscle). Skeletofusimotor supply ($\beta$-innervation) has been demonstrated anatomically in this muscle (Porayko & Smith, 1968), and slowly contracting motor units have been shown physiologically to be $\beta$-units in another small rat muscle (caudal muscle of the midtail region: Andrew & Part, 1974). It would be interesting to know if the IIC/IIA motor units of 4DL are $\beta$-units, and whether this is related in some way to their mixed extrafusal fibre composition.

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## REFERENCES

ANDREW, B. L. & PART, N. J. (1974). The division of control of muscle spindles between fusimotor and mixed skeletofusimotor fibres in a rat caudal muscle. *Quarterly Journal of Physiology* **59**, 331–349.

AUSONI, S., GORZA, L., SCHIAFFINO, S., GUNDERSEN, K. & LOMO, T. (1990). Expression of myosin heavy chain isoforms in stimulated fast and slow muscles. *Journal of Neuroscience* **10**, 153–160.

BALICE-GORDON, R. J. & THOMPSON, W. J. (1988). Synaptic rearrangements and alterations in motor unit properties in neonatal rat extensor digitorum longus muscle. *Journal of Physiology* **398**, 191–210.

BETZ, W. J., CALDWELL, J. H. & RIBCHESTER, R. R. (1979). The size of motor units during postnatal development of rat lumbrical muscle. *Journal of Physiology* **297**, 463–478.

BETZ, W. J., RIBCHESTER, R. R. & RIDGE, R. M. A. P. (1990). Competitive mechanisms underlying synapse elimination in the lumbrical muscle of the rat. *Journal of Neurobiology* **21**, 1–17.

BROOKE, M. H. & KAISER, K. K. (1970). Muscle fiber types – how many and what kind? *Archives of Neurology* (Chicago) **23**, 369–379.

BROWN, M. C., JANSEN, J. K. S. & VAN ESSEN, D. (1976). Polyneuronal innervation of skeletal muscle in new-born rats and its elimination during maturation. *Journal of Physiology* **261**, 387–422.

BURKE, P., RIDGE, R. M. A. P., ROWLERSON, A. & WEEDON, M. C. (1990). Fibre compositions of motor units in muscles partially denervated in the adult rat. *Journal of Physiology* **423**, 83*P*.

BURKE, R. E., LEVINE, D. N., TSAIRIS, P. & ZAJAC, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *Journal of Physiology* **234**, 723–748.

CARPENE, E., ROWLERSON, A., VEGGETTI, A. & MASCARELLO, F. (1982). Preparation of type-specific antimyosin antibodies and determination of their specificity by biochemical and immunohistochemical methods. *Italian Journal of Biochemistry* **31**, 330–341.

CHAMBERLAIN, S. & LEWIS, D. M. (1989). Contractile characteristics and innervation ratio of rat soleus motor units. *Journal of Physiology* **412**, 1–21.

CLOSE, R. (1967). Properties of motor units in fast and slow skeletal muscle of the rat. *Journal of Physiology* **193**, 45–55.

CONDON, K., SILBERSTEIN, L., BLAU, H. M. & THOMPSON, W. J. (1990). Development of muscle fiber types in prenatal rat hindlimb. *Developmental Biology* **138**, 256–274.

GATES, H.-J. (1988). Motor units in a lumbrical muscle of adult rat. *Pflügers Archiv* **411**, suppl. 1, R195.

GATES, H.-J. (1989). Competition is necessary for normal sorting of neuromuscular connections in a rat lumbrical muscle. *European Journal of Neuroscience* suppl. 1, 209.

GORZA, L. (1990). Identification of a novel type 2 fiber population in mammalian skeletal muscle by combined use of histochemical myosin ATPase and anti-myosin monoclonal antibodies. *Journal of Histochemistry and Cytochemistry* **38**, 257–265.

HARRIS, A. J., FITZSIMONS, R. B. & MCEWAN, J. C. (1989). Neural control of the sequence of expression of myosin heavy chain isoforms in foetal mammalian muscles. *Development* **107**, 751–769.

HOH, J. F. Y., HUGHES, S., HUGH, G. & POZGAJ, I. (1989). Three hierarchies in skeletal muscle fibre classification: allotype, isotype and phenotype. In *Cellular and Molecular Biology of Muscle Development*, ed. KEDES, L. E. & STOCKDALE, F. E., pp. 15–26. Alan R. Liss, New York.

JONES, S. P. & RIDGE, R. M. A. P. (1987). Motor units in a skeletal muscle of neonatal rat: mechanical properties and weak neuromuscular transmission. *Journal of Physiology* **386**, 355–375.

JONES, S. P., RIDGE, R. M. A. P. & ROWLERSON, A. (1987*a*). The non-selective innervation of muscle fibres and mixed composition of motor units in a muscle of neonatal rat. *Journal of Physiology* **386**, 377–394.

JONES, S. P., RIDGE, R. M. A. P. & ROWLERSON, A. (1987*b*). Rat muscle during post-natal development: evidence in favour of no interconversion between fast- and slow-twitch fibres. *Journal of Physiology* **386**, 395–406.

KERNELL, D., EERBEEK, O. & VERHEY, B. A. (1983). Motor unit categorisation on basis of contractile properties: An experimental analysis of the composition of the cat's m. peroneus longus. *Experimental Brain Research* **50**, 211–219.

KUGELBERG, E. (1973). Histochemical composition, contraction speed and fatiguability of rat soleus motor units. *Journal of Neurological Science* **20**, 177–198.

KUGELBERG, E. (1976). Adaptive transformation of rat soleus motor units during growth. Histochemistry and contraction speed. *Journal of Neurological Science* **27**, 269–289.

KUGELBERG, E. & LINDEGREN, B. (1979). Transmission and contraction fatigue of rat motor units in relation to succinate dehydrogenase activity of motor unit fibres. *Journal of Physiology* **288**, 285–300.

MILBURN, A. (1984). Stages in the development of cat muscle spindles. *Journal of Embryology and Experimental Morphology* **82**, 177–216.

NACHLAS, M. M., TSOU, K. U., DE SOUSA, E., CHENG, C. S. & SELIGMAN, A. M. (1957). Cytochemical demonstration of succinate dehydrogenase by the use of a new *p*-nitrophenyl substituted ditetrazole. *Journal of Histochemistry and Cytochemistry* **5**, 420–463.

PORAYKO, O. & SMITH, R. S. (1968). Morphology of muscle spindles in the rat. *Experientia* **24**, 588–589.

ROSS, J. J., DUXSON, M. J. & HARRIS, A. J. (1987). Formation of primary and secondary myotubules in rat lumbrical muscles. *Development* **100**, 383–394.

ROWLERSON, A. (1988). Fibre types in the rat fourth deep lumbrical muscle during post-natal development. *Journal of Physiology* **406**, 68*P*.

ROWLERSON, A., POPE, B., MURRAY, J., WHALEN, R. G. & WEEDS, A. G. (1981). A novel myosin present in cat jaw-closing muscles. *Journal of Muscle Research and Cell Motility* **2**, 415–438.

SCHIAFFINO, S., GORZA, L., SARTORE, S., SAGGIN, L., AUSONI, S., VIANELLO, M., GUNDERSEN, K. & LOMO, T. (1989). Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. *Journal of Muscle Research and Cell Motility* **10**, 197–205.

SCHIAFFINO, S., SAGGIN, L., VIEL, A., AUSONI, S., SARTORE, S. & CORZA, L. (1986). Muscle fiber types identified by monoclonal antibodies to myosin heavy chains. In *Biochemical Aspects of Physical Exercise*, ed. BENZI, G., PACKER, L. & SILIPRANDI, N. pp. 27–34. Elsevier, Amsterdam.

THOMPSON, W. J., SUTTON, L. A. & RILEY, D. A. (1984). Fibre type composition of single motor units during synapse elimination in neonatal rat soleus muscle. *Nature* **309**, 709–711.