Journal of Physiology (1992), **445**, *pp.* 457–472
With 6 figures
Printed in Great Britain

THE IMPORTANCE OF COMPETITION BETWEEN MOTONEURONES IN DEVELOPING RAT MUSCLE; EFFECTS OF PARTIAL DENERVATION AT BIRTH

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

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

(*Received 11 March 1991*)

SUMMARY

1. The number of motor units in developing fourth deep lumbrical muscles was reduced by unilateral partial denervation of the muscle at birth, by cutting the lateral plantar nerve. A minority of the motor axons arrive via the sural nerve, and were thus not cut. Those muscles that contained one motor unit (one-unit muscles) after partial denervation developed in the absence of competition between motoneurones. Muscles with two motor units had little competition. A few four-unit muscles were studied for comparison.

2. Isometric twitch and tetanic tensions of single motor units were recorded *in vitro* at 60 days of age in response to stimulation of the sural nerve. On average, units in partially denervated muscles generated more tension than normal units. The isometric tension characteristics of the units in the one-unit and two-unit muscles were different from the normal units (e.g. slower contracting and more fatiguable). The units of four-unit muscles had properties similar to those of normal muscles.

3. Fibres of an individual unit were identified by glycogen depletion and S (slow) fibres were identified in cross-section that bound a polyclonal antibody to slow type I myosin. Those fibres that did not bind the antibody were designated F fibres. The units of one-unit muscles had the same total number of fibres and fibre type composition (both S and F fibres in the same unit) as estimated from previous work to exist at birth. The units of two-unit muscles contained the same total number of fibres, but apparently fewer S fibres, though this may have been as a result of incomplete glycogen depletion of some fibres.

4. It is concluded that competition between motoneurone terminals is necessary for the withdrawal of mismatched connections on muscle fibres present at birth; or, alternatively, that such withdrawal cannot take place if it would result in denervation of the muscle fibre.

INTRODUCTION

The rat fourth deep lumbrical muscle (4DL) consists mainly of fast muscle fibres (F fibres) with a minority, around 9%, which contain slow myosin (S fibres). Motor units in neonatal 4DL muscle are composed of muscle fibres of both types (Jones,

Ridge & Rowlerson, 1987*a*), whereas the majority of motor units in the adult muscles are virtually pure in fibre type containing only the fast fibres (Gates, Ridge & Rowlerson, 1991). As there is no change in the number of S fibres in the muscle during development it has been postulated that the pure, fast motor units are produced by elimination of terminals from mismatched fibres rather than by fibre type conversion (Jones, Ridge & Rowlerson, 1987*b*). Slow fibres (mainly of type IIC) in the adult occur in small motor units that also contain fast fibres of a type (type IIA) distinct from the majority fast type (type IIX) in the muscle. These units have been called IIC/IIA units (Gates *et al.* 1991). Synapse elimination from 4DL has been shown to involve competition between nerve terminals on individual muscle fibres (Betz, Caldwell & Ribchester, 1980). It might be expected therefore that motor units in muscles that develop with no competition would keep their neonatal mixed fibre composition.

In the present study competition was reduced or removed by partially denervating the muscles at birth. This was done by cutting one of the nerves to the muscle, the lateral plantar nerve. The muscles were then supplied only by the sural nerve which carries few, and sometimes only one, motor axons to the muscle (Betz *et al.* 1980). Tension development by single motor units was recorded *in vitro*, and the muscle fibres of the unit identified histologically by glycogen depletion (Edstrom & Kugelberg, 1968). Their histochemical type was determined using polyclonal myosin-specific antibodies (Jones *et al.* 1987*a*). The main finding was that, in the absence of competition, motor units in adult rats maintained their neonatal muscle fibre composition. We conclude, therefore, that competition is required for the withdrawal of mismatched motoneuronal connections from muscle fibres during development.

Preliminary accounts of some of this work have been given (Gates, 1988, 1989, 1990). Some results have also been included in a recent review (Betz, Ribchester & Ridge, 1990).

METHODS

Partial denervation was performed in rats less than 12 h after birth, and the acute experiment at 60 days of age.

Partial denervation

Newborn male Wistar rats were anaesthetized by inhalation of Penthrane (methoxyflurane, BP, Abbott Laboratories). The fourth deep lumbrical muscle of one hindlimb was partially denervated by cutting the lateral plantar nerve immediately above the ankle (see Betz *et al.* 1980 for methods). The operations were performed under sterile conditions. A short length (1·5 mm) of the nerve was removed in order to ensure that there was no regeneration. Therefore the muscles were supplied by zero to five motoneurones whose axons arrived via the sural nerve. The contralateral leg was left unoperated as a control. The animals were returned to the litter and reared as normal.

Tension recording

The acute experiments were performed at 60 days postnatal age (see Gates *et al.* 1991 for details). Muscles and their nerve supply were dissected out and mounted in a chamber. Isometric tension recordings were made at 25 °C from both the contralateral control and the partially denervated muscle. A custom-built strain gauge (Kulite) was used that had an unloaded resonant frequency of 1 kHz and a compliance of about 2 μm/mN. The number of motor units in both muscles was assessed by counting the number of repeatable increments in tension occurring as the stimulus strength was increased progressively. If the partially denervated muscle contained one motor unit only (here called a one-unit muscle) the maximal isometric twitch and tetanic tensions were

recorded. The fibres of the unit were then depleted of their glycogen by repetitive stimulation using a 40 Hz stimulus for 330 milliseconds per second. Rest periods were given when the tension had fallen to half of the original tension. This regime was then repeated until no tension developed on stimulation. This allowed us, in sections cut from the muscle, to separate innervated fibres from denervated fibres that were present, though greatly atrophied. Where more than one motor unit remained after partial denervation a single unit was isolated by progressive section of the sural nerve until an all-or-none twitch response was obtained on stimulating the nerve. After isometric twitches and tetani had been recorded such isolated units were also subjected to the glycogen depletion regime. During the glycogen depletion regime a fatigue index was obtained for every unit studied. This was the ratio of the tension produced at 2 min to that produced at the start of the depletion regime.

Muscle fibre type identification

The control and experimental muscles were frozen together and cut in cross-section at a thickness of 10 μm. Glycogen-containing fibres were identified using a periodic acid–Schiff stain and in neighbouring sections the fibre type was determined by the binding of a polyclonal myosin-specific antibody raised against slow type I myosin (Rowlerson, Pope, Murray, Whalen & Weeds, 1981) and in some cases against fast IIA myosin (Carpene, Rowlerson, Veggetti & Mascarello, 1982). In normal adult 4DL muscle most fibres that bind the type I myosin antibody also bind the IIA antibody and are therefore described as IIC fibres (Gates *et al.* 1991). There are also some fibres that bind the IIA antibody alone (type IIA fibres) and others that bind neither of these antibodies (type IIX fibres; Gates *et al.* 1991). In the partially denervated muscles it was found that IIA antibody binding was very variable and difficult to interpret. Because of this, binding of the antibody has not been used in classifying the muscle fibres which are described here as slow-myosin-containing (S) and slow-myosin-free (F) muscle fibres. This is the classification of 4DL muscle fibre type that was used in a study of the muscle at 4 days postnatal age (Jones *et al.* 1987*a*).

Camera lucida drawings were made of a mid-belly section of the partially denervated muscle. The glycogen-depleted fibres of a motor unit were identified, along with the fibre type of the individual fibres in adjacent sections. This drawing was then digitized to give the fibre counts and cross-sectional area measurements. Some partially denervated muscles and totally denervated muscles were also frozen prior to glycogen depletion as controls. All of the fibres in these muscles were found to contain glycogen.

Normalized motor unit sizes

In order to allow comparison with normal motor units, the sizes of the motor units in the partially denervated muscles were expressed as unit tension as percentage maximal tetanic tension of the contralateral control muscle. Motor units were classified as 'sag' or 'no sag' units on the basis of the shape of the unfused tetanus (Burke, Levine, Tsairis & Zajac, 1973). The sample of normal units with which comparisons were made is the sample described by Gates *et al.* (1991). Normal unit size is expressed as unit tension as percentage of whole-muscle maximal tetanic tension of the muscle containing the unit. Most of these units were from muscles in unoperated rats, but a few were obtained from contralateral muscles of rats where the ipsilateral muscle had been partially denervated. These were included as, in all characteristics measured, they fell within the range found in muscles of unoperated rats and the mean values were not significantly different. Characteristics compared were: unit size (tension) as percentage whole contralateral muscle tetanic tension, time to peak twitch tension (TTP), half-relaxation time (HRT), the ratio of TTP to HRT, the ratio of twitch to tetanic tension and fatigue index at 2 min. Motor unit sizes were also estimated from histology of the glycogen-depleted unit. For normal units they are expressed as area as percentage total muscle fibres cross-sectional area, and for units in partially denervated muscles as area as percentage total muscle fibre cross-sectional area of the contralateral muscle.

In all cases the level of statistical significance was obtained by Student's t test. Mean values are given $\pm$ one standard deviation.

Calculated levels of polyneuronal innervation

The aim of this study was to reduce competition between motoneurones during postnatal development, by partial denervation of the muscle at birth. There is no competition where only one motor unit remains, but as this state applies in only about a quarter of partially denervated

muscles, it was of interest to estimate the level of competition in the two-unit muscles. As competition will occur at polyneuronally innervated endplate sites the probability that a fibre is innervated by both of the motoneurones remaining in these muscles after the operation was calculated.

The probability that a fibre is innervated by a particular motoneurone, P_i, is given by $P_i = a/T$ where a is the average number of muscle fibres in a motor unit at birth (120; Betz, Caldwell & Ribchester, 1979) and T is the total number of muscle fibres in the muscle at birth (404; Jones *et al.* 1987*b*). Therefore $P_i = 120/404 = 0{\cdot}3$.

In a muscle with two motoneurones remaining after the operation the probability that a muscle fibre will be innervated by both of the motoneurones, P_2, will be $P_2 = P_i^2 = 0{\cdot}09$.

Therefore in a partially denervated muscle with two motor units remaining 91% of the muscle fibres develop without competition at their endplates. This probability would be altered by muscle fibre production after birth. In fact it is known that in normal 4DL muscle almost half the muscle fibres present in adult muscles are generated postnatally (Betz *et al.* 1979), and partial denervation at birth has shown that the number of such muscle fibres is dependent on the number of remaining motoneurones (Betz *et al.* 1980). This effect, though, becomes obvious only when there are four motoneurones or more present (see Fig. 6 of Betz *et al.* 1980), and so it is safe to assume that insignificant numbers of muscle fibres are generated postnatally in two-unit muscles.

RESULTS

Physiology

Whole muscles: number of units

The mean number of motoneurones carried in the sural nerve to 4DL muscles in control animals was $2{\cdot}27 \pm 1{\cdot}67$ ($n = 52$). The mean values for operated animals were not different (at the 10% level) from those for control unoperated animals (partially denervated muscles $1{\cdot}87 \pm 1{\cdot}67$, $n = 53$; contralateral muscles $1{\cdot}80 \pm 1{\cdot}35$, $n = 56$).

Motor unit sizes, estimated from tension developed

The frequency of stimulation that produced maximal tetanic tension in whole muscles and units varied between different partially denervated muscles, and was often lower than that in normal muscles: 80 Hz as compared to up to 200 Hz. On average, the units were larger in the partially denervated muscles (expressed as percentage contralateral muscle tetanic tension) than in the normal muscles, as found previously by Betz *et al.* (1980). This was the case for units in one-unit and two-unit muscles. Values were $28{\cdot}3 \pm 14{\cdot}1$ ($n = 14$) and $27{\cdot}4 \pm 13{\cdot}2$ ($n = 18$) respectively (normal units $6{\cdot}6 \pm 4{\cdot}2$; $n = 123$).

Motor unit twitches and tetani

There are two types of motor unit in normal 4DL muscles, separable by the shape of their unfused tetani: fast units in which the tension shows sag and slow units which do not show sag (Gates *et al.* 1991). The motor units in partially denervated muscles also could be divided on the basis of 'sag' and 'no sag'.

All of the one-unit muscles tested showed no sag, whereas only 31% of the units from two-unit muscles showed no sag, while the remainder showed sag. These data are shown in Fig. 1, where the frequency distributions of unit size from tetanic tension measurements are shown for the two types of unit in one-unit and two-unit muscles, and for units in normal muscles. It will be seen in Fig. 1*A* and *B* that in the partially denervated muscles the property of 'sag' or 'no sag' did not correlate with

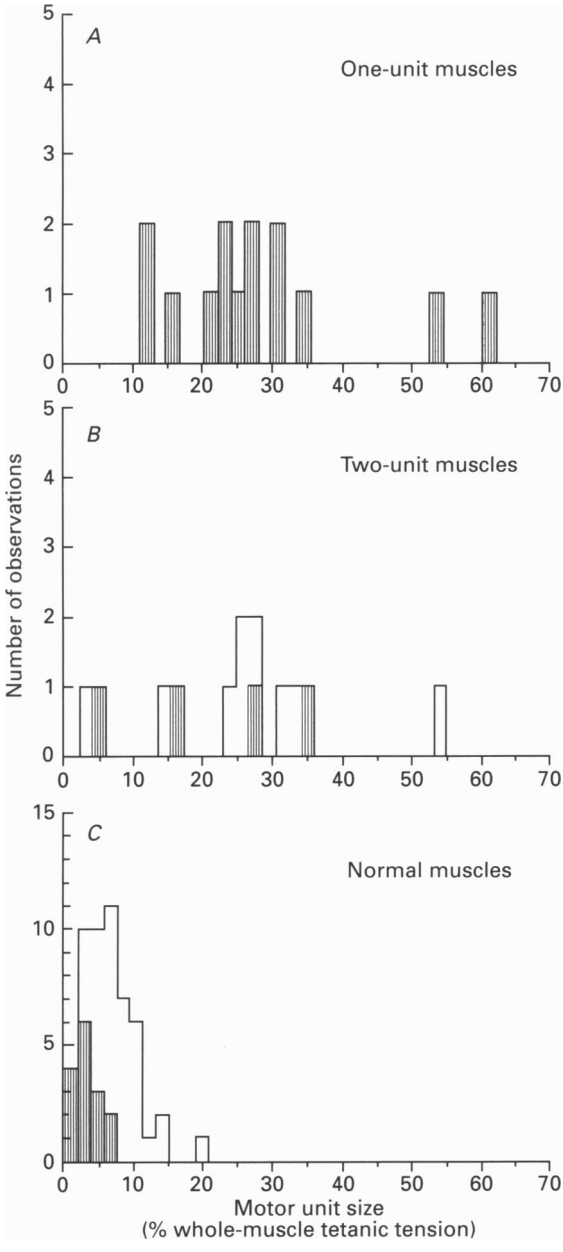

Fig. 1. Distribution of the size of motor units isolated from: *A*, one-unit muscles; *B*, two-unit muscles (expressed as percentage contralateral control whole-muscle tetanic tension); and *C*, normal muscles. Filled columns, units showing no sag (see text) in their unfused tetani.

unit size, in contrast to motor units in normal muscles where all 'no sag' units are small (Fig. 1*C*).

Data for other physiological properties are summarized in Table 1. The one-unit

muscles had longer times to peak twitch tension (TTP) than either the 'sag' or 'no sag' units of normal muscle, but their half-relaxation times (HRT) were not proportionately as long as were those of the normal 'no sag' units (Table 1). When there were more motor units remaining in the muscles the units were found to be faster contracting than units of one-unit muscles, but the ratios of TTP to HRT were

TABLE 1. Motor unit isometric tension data

	Partially denervated muscles: number of units			Normal muscles: type of unit		
	1	2	4	All	'Sag'	'No sag'
TTP (ms)	27·11 ± 4·02* 15	25·03 ± 4·62† 30	21·50 ± 1·50 5	22·89 ± 4·28 140	21·36 ± 2·28 37	25·85 ± 3·74** 15
HRT (ms)	40·44 ± 10·85* 12	31·65 ± 6·34 27	26·20 ± 1·21 5	32·47 ± 9·16 121	28·42 ± 5·46 35	45·29 ± 7·39** 15
TTP HRT	0·71 ± 0·11 12	0·82 ± 0·15* 27	0·82 ± 0·06* 5	0·73 ± 0·13 122	0·77 ± 0·11 35	0·58 ± 0·08** 15
Tw/Tet	0·27 ± 0·04 14	0·29 ± 0·06 21	0·26 ± 0·03 3	0·27 ± 0·07 121	0·27 ± 0·07 37	0·20 ± 0·05** 15
FI2	0·73 ± 0·08* 9	0·63 ± 0·19** 13	0·86 ± 0·11 2	0·86 ± 0·11 49	0·85 ± 0·10 30	0·92 ± 0·06† 14

Mean data for isometric tension recordings and fatigue measurements of units isolated from partially denervated and normal muscles. The data are pooled according to the number of units remaining in the partially denervated muscles. Mean data are given for normal motor units which showed 'sag' and 'no sag' in their unfused tetani as well as mean values for all normal units. Mean values (± S.D.) for each group are given with sample size. Abbreviations are: time to peak twitch tension, TTP; half-relaxation time, HRT; ratio of maximal twitch tension to maximal tetanic tension, Tw/Tet; and fatigue index at 2 min, FI2 (see text). Student's t test to compare properties of units isolated from partially denervated muscles with normal units, and normal units that showed no sag with those that showed sag, gave the following P values: $^{**}P = 0{\cdot}001$, $^{*}0{\cdot}001 < P < 0{\cdot}01$, $\dagger 0{\cdot}01 < P < 0{\cdot}05$.

unaffected (Table 1). For all of the partially denervated muscles the mean values for the twitch to tetanus ratios were not significantly different from those of the normal units. The one-unit muscles and the isolated units from the two-unit muscles were more sensitive to fatigue than both the normal sag' and 'no sag' units (see Table 1). The motor units of the four-unit muscles had properties similar to those of normal motor units

There was no relationship between the size of the motor units as percentage contralateral control muscle tension and any of the measured physiological properties of the unit (TTP, HRT, ratio of TTP to HRT, ratio of twitch to tetanic tension and fatigue index). The property of 'sag' or 'no sag' also did not correlate with any of the other physiological properties measured. Thus it was not possible to distinguish sub-populations of unit types (contrast with normal units; Gates *et al.* 1991).

Histology

The histological appearance of the partially denervated muscles was very different from that of the normal muscles (Figs 2 and 3; compare with the normal muscles of

Gates *et al.* 1991). The one-unit muscles contained a small number of very hypertrophied fibres with atrophied muscle fibres principally around the edge of the muscle section (Fig. 2). The degree of hypertrophy and atrophy in the two-unit muscles was extremely variable from muscle to muscle. Some had an appearance very similar to the one-unit muscles with greatly hypertrophied fibres and many atrophied fibres whereas others had few atrophied fibres with the remainder of the fibres not greatly hypertrophied (Fig. 3). Again the atrophied fibres were generally found around the edge rather than in the body of the muscle. The four-unit muscles had an appearance similar to the normal muscles although they contained a reduced number of fibres.

All of the muscles contained fibres which showed positive reactions with slow myosin antibody. These S fibres were distributed fairly evenly throughout the muscle cross-section (Figs 2*B* and 3*B*). The fibre types of the completely denervated fibres could also be identified by slow myosin antibody labelling. The staining with the fast IIA antibody was extremely variable (not shown).

Whole muscle; total numbers of muscle fibres

The total number of muscle fibres (innervated + denervated) in the partially denervated muscles was much reduced as compared with the normal muscles. The one-unit muscles contained fewer fibres (343 ± 115, $n = 8$) than were probably present at the time of the operation (about 400–500; Betz *et al.* 1979; Jones *et al.* 1987*b*; Schmalbruch, 1990). In these one-unit muscles the presumed denervated fibres were greatly atrophied so that by 60 days they were often difficult to identify. It is possible that some had completely degenerated or did not extend through the midbelly where counts were made. The two-unit muscles contained approximately the same number of fibres (426 ± 80, $n = 12$) as was present at the time of the operation, only a few of which appeared atrophied.

Muscle fibre type

The major difference in the total number of muscle fibres (innervated + denervated) in partially denervated muscles as compared with the normal muscles was in the number of F fibres, which was greatly reduced in the one-unit and two-unit muscles. The number of S fibres was also reduced, but by a smaller proportion. Values are given in Table 2. The reduction in the number of S fibres probably reflected the degeneration of some denervated S fibres.

Motor unit muscle fibre number

There was a wide range of motor unit sizes in the partially denervated muscles (Fig. 4), as is the case in neonatal muscle (Jones & Ridge, 1987). The mean number of fibres in the motor units of the one-unit muscles (123 ± 65, $n = 8$) and the two-unit muscles (122 ± 57, $n = 12$) was higher than in the normal motor units (68 ± 24, $n = 27$). The mean number of fibres in the units of the one-unit and two-unit muscles was similar to that estimated to be present at birth (120; Betz *et al.* 1979).

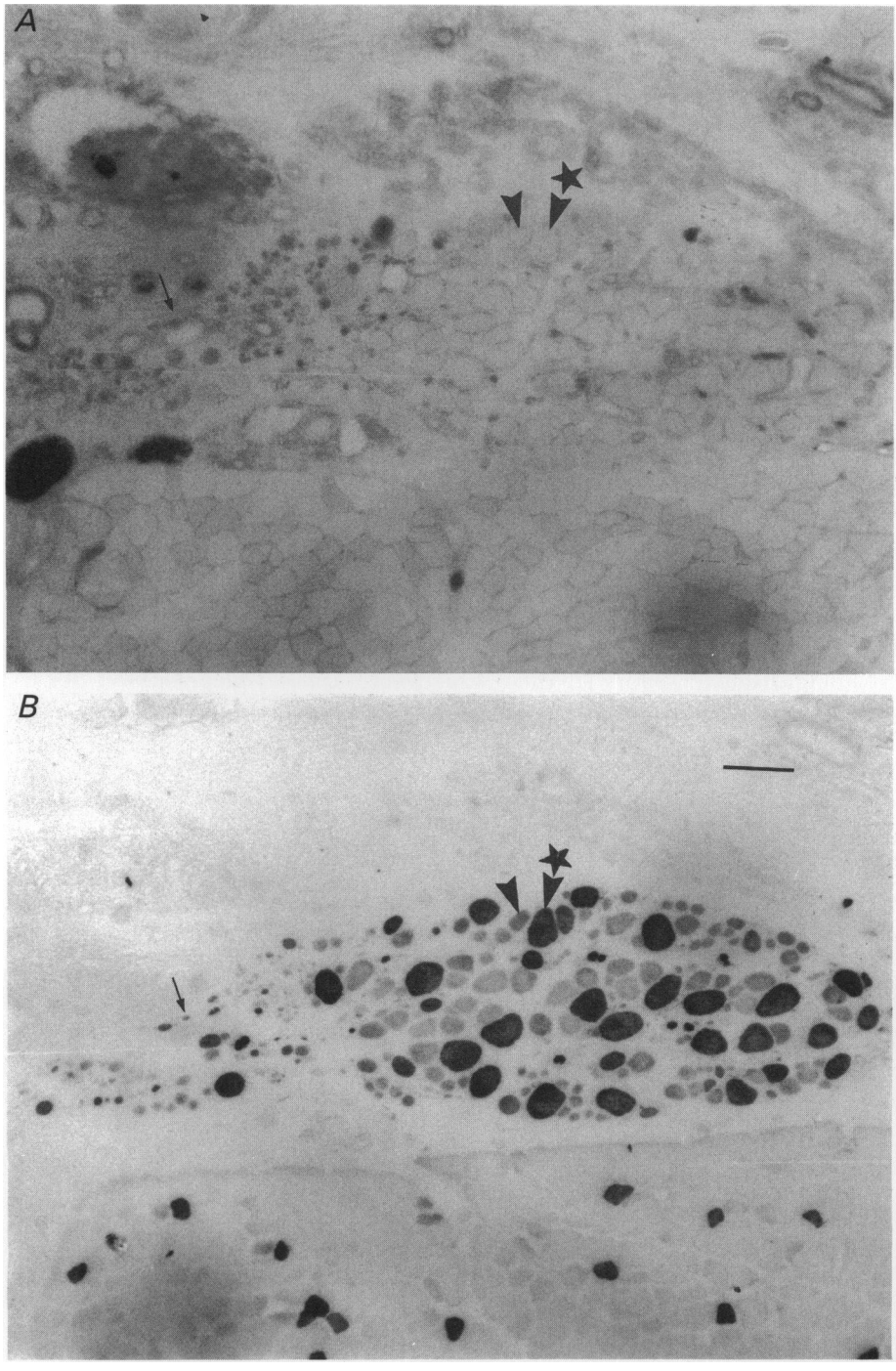

Fig. 2. Adjacent sections from a muscle (mid-belly) containing one motor unit 60 days after partial denervation (by cutting the lateral plantar nerve) at birth. *A*, periodic acid–Schiff stain for glycogen. Fibres in the unit were depleted of their glycogen by repetitive stimulation. Note that only atrophied fibres (example with small arrow) have not been depleted of their glycogen. *B* , slow-myosin-specific antibody. The unit contains

Motor unit muscle fibre type

One-unit muscles. None of the motor units in the one-unit muscles was pure in fibre type (Fig. 4), whereas 80% of normal motor units were found to be pure or virtually so (Gates *et al.* 1991). On average there were 24 ± 11 ($n = 8$) S fibres per unit.

Two-unit muscles. Glycogen-depleted units in the two-unit muscles apparently contained significantly fewer S fibres (11 ± 7, $n = 12$, $P < 0{\cdot}01$) as compared to the one-unit muscles (Fig. 4); two of the units in the sample were mixed only to the extent found in some normal muscles (4%; see units in Gates *et al.* 1991). This was despite there being no difference in the total fibre number in the motor unit (122 ± 57, $n = 12$) to that found in the one-unit muscles. Possibly not all of the S fibres in the unit were depleted of glycogen. If this were so, then the total number of fibres in the unit was also underestimated. Fibres were taken as depleted only if they were unstained by the periodic acid–Schiff reagent; thus those fibres that had been partially depleted were not counted in the unit. There is further indication that the count of S fibres was not complete. The two-unit muscles contained many S fibres that were not depleted with the unit but were obviously innervated; that is they had not atrophied or they had hypertrophied. Counts of such fibres in two-unit muscles, where they could be made, gave a mean count of 44 ± 11 ($n = 10$). If these were divided evenly between the two units in the muscle, then there would have been about twenty-two S fibres per unit. Thus it seems most likely that incomplete glycogen depletion accounted for the apparent reduction of S fibres per unit in the two-unit muscles.

Fibre cross-sectional area and distribution of innervated fibres

One-unit muscles. The depleted fibres in the one-unit muscles were greatly hypertrophied (Fig. 2). The hypertrophy of the S fibres was greater than that of the F fibres, the S fibres having twice the cross-sectional area of the F fibres (S fibres 1542 ± 526 μm^2 and F fibres 714 ± 335 μm^2, $n = 8$). Mean cross-sectional areas of fibres in normal muscles at 60 days were: slow (IIC+I) 393 ± 68 μm^2 and fast (IIX) 582 ± 80 μm^2.

Two-unit and four-unit muscles. The degree of hypertrophy of the innervated fibres in the two-unit muscles varied considerably from muscle to muscle. Generally the hypertrophy of the innervated S fibres was less than in the one-unit muscles (cross-sectional area 1120 ± 656 μm^2, $n = 12$, for S fibres in the two-unit muscles). Possibly this is due to more severe functional overload in one-unit than two-unit muscles. However the cross-sectional areas of F fibres in one-unit and two-unit muscles were similar (806 ± 241 μm^2, $n = 12$, for two-unit muscles). A total of three units were isolated from four-unit muscles and successfully depleted of glycogen. The S fibres in these units were hypertrophied as compared to those in the normal muscles but there was no difference between the mean cross-sectional areas of the S (601 ± 63 μm^2) and the F fibres (604 ± 127 μm^2).

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both slow-myosin-containing, S, fibres (starred arrow-head) and slow-myosin-free, F, fibres (plain arrow-head). Part of an EDL muscle (control for antibody) is at the bottom of the pictures (in which in *A* many fibres are depleted of glycogen. This is probably due to prolonged hypoxia, as this muscle was not maintained in the muscle bath). Bar = 100 μm.

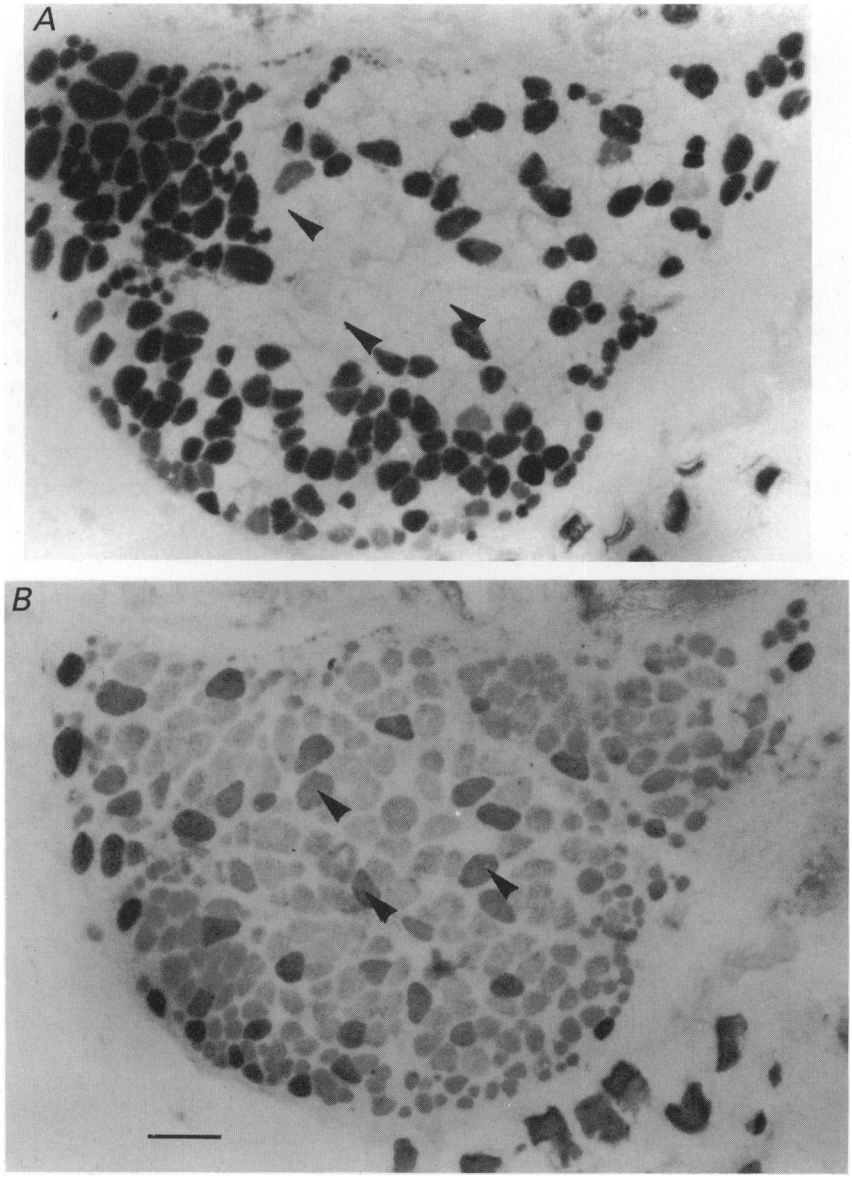

Fig. 3. Adjacent sections from a muscle containing two motor units at 60 days after partial denervation at birth. The muscle fibres in one unit have been depleted of glycogen by repetitive stimulation. *A*, periodic acid–Schiff stain. *B*, slow-myosin-specific antibody. The depleted unit contains both S (examples indicated with arrow-heads) and F muscle fibres. Bar = 100 μm.

There was marked grouping of the fibres belonging to individual units in the two-unit muscles (e.g. Fig. 3*A*). This probably reflects the situation in the neonatal muscles where grouping is often seen (Jones *et al.* 1987*a*). This grouping is not seen in normal adult 4DL motor units (Gates *et al.* 1991; H.-J. Gates & W. J. Betz, submitted).

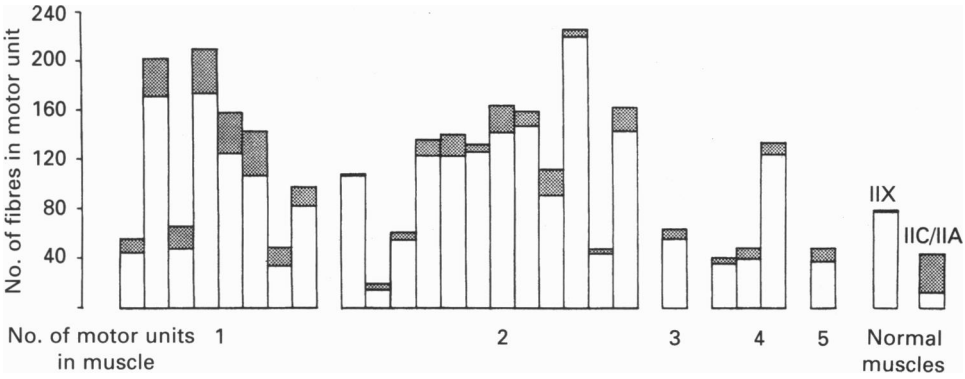

Fig. 4. The numbers of fibres of each type in individual motor units identified by glycogen depletion and reaction with the slow-myosin-specific antibody; S fibres stippled, F fibres open columns. The numbers of motor units remaining in the muscles after partial denervation are given below the histograms. The two bars to the right represent the mean fibre type composition of the two types of normal units: fast (IIX) units and slow (IIC/IIA) units. The mean values with standard deviations for these units are: IIX units ($n = 20$); anti-slow negative $74{\cdot}5 \pm 21{\cdot}6$: anti-slow positive $0{\cdot}7 \pm 1{\cdot}0$: IIC/IIA units ($n = 6$); anti-slow negative, $12{\cdot}2 \pm 6{\cdot}8$; anti-slow positive $30{\cdot}3 \pm 12{\cdot}9$.

TABLE 2. The number of F (slow-myosin-free) and S (slow-myosin-containing) muscle fibres (innervated + denervated) in partially denervated and normal muscles

Number of motoneurones remaining	Number of muscle fibres ± S.D. (n)	
	F	S
1	299 ± 105* (8)	44 ± 12** (8)
2	373 ± 76* (12)	53 ± 10** (12)
Normal	887 ± 67 (27)	82 ± 9 (27)

Student's t test to compare the number of fibres of each type in partially denervated muscles with that in normal muscles gave the following P values: $^{**}P = 0{\cdot}001$, $^{*}P = 0{\cdot}01$.

Relationship between motor unit tension development and histology

The unit size calculated from tetanic tension was similar to that calculated from fibre cross-sectional area. This is shown in Fig. 5. The calculated specific tensions developed by the fibres within the units were not different from that developed by most normal muscle fibres. Values (N/cm^2) were: in one-unit muscles $25{\cdot}7 \pm 5{\cdot}8$, $n = 8$; two-unit muscle $25{\cdot}9 \pm 6{\cdot}5$, $n = 12$; normal muscle fibres from IIX units $24{\cdot}0 \pm 5{\cdot}3$, $n = 20$.

The physiological properties of the motor units in the partially denervated muscles were not related to the proportions of S and F muscle fibres that the unit contained. For each unit, the percentage of all the fibres that were of S type, and the percentage of total fibre cross-sectional area contributed by S fibres, were plotted against unit TTP, HRT, the ratio of TTP to HRT, the ratio of twitch to tetanic tension, and the fatigue index measured at 2 min. In no case was a relationship statistically significant at the 5% level.

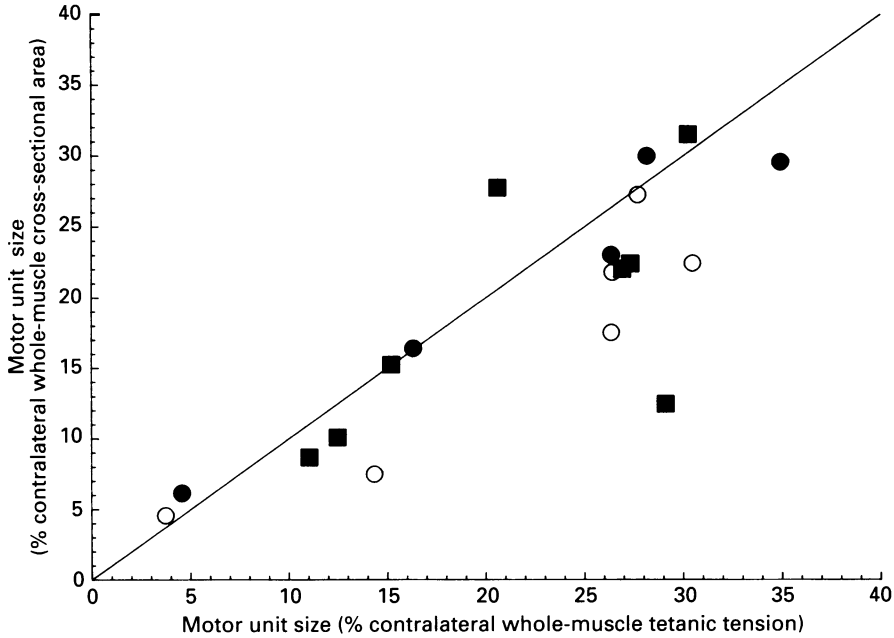

Fig. 5. Motor unit size expressed as percentage total fibre cross-sectional area in contralateral control muscles plotted against the unit size expressed as percentage of the contralateral control whole-muscle tetanic tension. The line of equality is shown. One-unit muscles: □; two-unit muscles: ○. Filled symbols, units showing no sag in unfused tetanus.

DISCUSSION

Normal motor unit development

In a normal developing muscle, neonatal polyneuronal innervation is reduced to the adult pattern of one synaptic input per muscle fibre by synapse elimination. Approximately in parallel with this, the mixed motor units of the neonate (rat soleus: Thompson, Sutton & Riley, 1984; rat 4DL: Jones *et al.* 1987*a*) change to motor units each consisting of one type of muscle fibre only (though a minority of motor units in adult 4DL do not conform to this; Gates *et al.* 1991). In rat 4DL the reduction of motor unit size resulting from synapse elimination appears to be entirely dependent upon competitive interaction of some sort between synaptic inputs (Betz *et al.* 1980). There is evidence in 4DL that the transformation of mixed motor units to pure motor units is brought about by selective withdrawal of mismatching contacts between motoneurones and muscle fibres, as part of the overall process of synapse elimination, rather than conversion of muscle fibres under the influence of maturing motoneurones (Jones *et al.* 1987*a*, *b*). Is the process of mismatch withdrawal, which could only constitute a minority part of synapse elimination, dependent on competition ? The experiments described in this paper were undertaken to answer this question.

Fibre composition of units in muscles partially denervated at birth

Partial denervation of 4DL at birth by section of the lateral plantar nerve leaves some motor axons intact, as these reach the muscle via the sural nerve. In about a quarter of cases one motor axon only survives, and in these cases there can be no competition at the neonatal neuromuscular junctions. Where two axons survive it can be calculated that the probability of competition at a particular neuromuscular junction is small (see Methods). Under these conditions the hypothesis that mismatch withdrawal is dependent on competition leads to certain predictions. Initially we will consider the case of the one-unit muscles.

Firstly, the remaining motoneurone might induce a change of myosin type in mismatched fibres so that the resulting motor unit in the muscle of the adult was homogeneous with regard to fibre type, though of expanded size in comparison to normal adult motor units. In our experiments this did not happen, which was quite surprising in view of the well-known plasticity of adult muscle fibre types under a changing influence of the motor supply (e.g. Buller, Eccles & Eccles, 1960). Recently it has been suggested that control of the motoneurone over the properties of the muscle fibres it innervates is not always complete (e.g. Foehring, Sypert & Munson, 1987; Ausoni, Gorza, Schiaffino, Gundersen & Lomo, 1990). Under conditions of partial denervation fibre type conversion may not occur, as the pattern of impulses in the motoneurones is not normal (Huizar, Kuno, Kudo & Miyata, 1977).

Alternatively, in the absence of fibre type conversion, the neonatal motor unit would remain essentially unaltered in the one-unit muscle of the adult both regarding the number of muscle fibres within it and its mixed fibre type composition. It is known already from the experiments of Betz *et al.* (1980) that in one-unit muscles, motor unit size is unaltered from that estimated at birth. Is the ratio of S to F fibres in the unit of one-unit adult muscle similar to that for units in newborn muscle? In fact, the composition of units *at birth* is not known for 4DL, and would be very difficult to ascertain directly because of electrical coupling between muscle fibres. However, data at 4 days postnatal are available (Jones *et al.* 1987*a*), at which time some units are known to consist of S and F fibres in much the same ratio as holds for the total fibre population of the muscle, indicating that at this stage motoneuronal contacts can be non-selective with regard to fibre type. The number of S and F fibres in 4 day units (Jones *et al.* 1987*a*) are plotted in Fig. 6*A*. A ratio of whole muscle S to F fibres was calculated from the average number of S and F fibres in whole 4 day muscles: $87/617 = 0{\cdot}14$ ($n = 12$; from Jones *et al.* 1987*b*). This ratio is represented by the dashed line drawn in Fig. 6*A*, *B* and *C*. If many motor units are also non-selective at birth their numbers of S and F fibres should approximate to a different ratio, because there are fewer fibres overall at birth than at 4 days, but the same number of S fibres (Jones *et al.* 1987*b*). The ratio at birth is $85/319 = 0{\cdot}27$ (one muscle; Jones *et al.* 1987*b*). This ratio is represented as the continuous line in Fig. 6*A*, *B* and *C*. It is assumed that most of the units are not selectively innervated at birth so that the ratio within them is the same as that in the whole muscle. Clearly the units of one-unit muscles (Fig. 6*B*) fall close to this line, which lends support to the idea that these units have remained unchanged from birth.

A similar picture would be predicted for two-unit muscles, since little competition

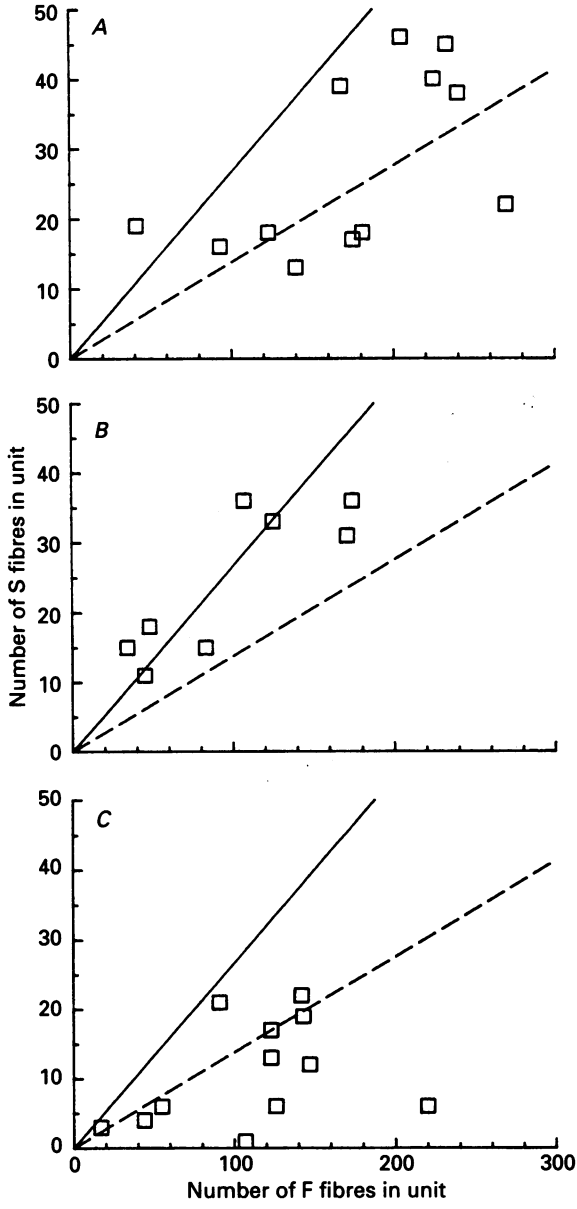

Fig. 6. The number of S fibres in depleted motor units plotted against the number of F fibres in the unit. Units in *A*, normal 4 day muscles (data from Jones *et al.* 1987*a*); *B*, one-unit muscles; and *C*, two-unit muscles. The dashed line in *A*, *B* and *C* represents the ratio of S to F fibres (0·14) found in normal 4 day whole muscles ($n = 12$; Jones *et al.* 1987 *a*). The slope of the continuous line in *A*, *B* and *C* derives from the ratio of S to F fibres in normal newborn whole muscle (0·27, one muscle; Jones *et al.* 1987*b*). Note S to F fibre ratios for adult one-unit muscles (*B*) fall close to this line. If muscle fibres in the units at birth had been innervated non-selectively and no change had occurred subsequently this relationship would be expected.

between the two motoneurones is predicted (see Methods) and from previous work (see Betz *et al.* 1980) few, if any, muscle fibres are generated postnatally in such muscles. However, as shown in Fig. 6*C*, a different picture emerged in two-unit muscles. Here a wide variation in the ratio of S to F fibres is apparent, and the data points all fall below the predicted ratio at birth (and closer to that at 4 days). Part of this effect could be explained by incomplete glycogen depletion of the S fibre population leading to an underestimate of the number of S fibres in the unit, rather than mismatch withdrawal from S fibres. We present evidence that this may have occurred to some extent. It is possible that fibre production has occurred in some units which would also contribute to a reduction of S to F ratio, since F fibres only are produced postnatally (Jones *et al.* 1987*b*). However, the unit size in the two-unit muscles remains unaltered at around 120 fibres. Again it is possible the number of fibres has been underestimated due to incomplete depletion of glycogen. Despite these problems it is still certain that these units contain mismatched fibres.

In summary, therefore, the main conclusion from the experiments described in this paper is that in rat 4DL withdrawal by motoneurones of synaptic contacts on mismatched fibres, which normally occurs during developmental synapse elimination, is dependent upon competition. As a consequence, where competition is removed at birth (in one-unit muscles produced by partial denervation), the resulting motor unit in the muscle of the adult contains the same muscle fibres that it contained at birth, no synapse elimination or mismatch withdrawal having taken place. Alternatively, it is possible that synapse elimination cannot occur if it would leave the muscle fibre denervated. Few if any muscle fibres become denervated in normal developmental synapse elimination. If neonatal muscle fibres exert a force that resists their denervation by contact withdrawal, then such a mechanism could, by itself, prevent postnatal change in one-unit muscles. Our experiments do not allow us to decide between these two alternatives.

We thank the SERC and the Wellcome Trust for supporting the research (H.-J. Gates was an SERC Research Student), NIH for some of the equipment; Dr A. Rowlerson for the donation of the antibodies and for performing the immunohistochemistry; Dr W. J. Betz for writing the computer programs used; Drs W. J. Betz, G. S. Bewick, D. M. Lewis and A. Rowlerson for their comments on an earlier draft of the manuscript; Mrs G. Goodacre and Mrs P. East for typing; and Mrs A. Emerton and Mr P. Robbins for photography.

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