Journal of Physiology (1989), **412**, *pp.* 1–21
With 6 text-figures
Printed in Great Britain

CONTRACTILE CHARACTERISTICS AND INNERVATION RATIO OF RAT SOLEUS MOTOR UNITS

BY S. CHAMBERLAIN AND D. M. LEWIS

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

(*Received 4 November 1988*)

SUMMARY

1. Physiological properties of motor units in the soleus muscle were studied in anaesthetized rats using ventral root splitting to isolate single units.

2. Motor unit types were classified by the same criteria used to classify cat hindlimb motor units into types FR (fast-twitch, fatigue-resistant) and type S (slow-twitch, fatigue-resistant). Type FR units were estimated to generate 10% of whole-muscle tension and type S 90%. All FR units showed sag in the unfused tetanus at frequencies with interpulse intervals greater than 175% of twitch time to peak, but not at 125% (Burke, Levine, Tsairis & Zajac, 1973).

3. The muscle fibres belonging to twelve single motor units were depleted of glycogen by prolonged stimulation, permitting analysis of their histochemical profiles. Type FR units were found to consist of type IIA muscle fibres and type S units of type I muscle fibres.

4. Direct determinations were made of fibre area, innervation ratio (number of muscle fibres supplied by one motoneurone) and hence specific tension (tetanic tension generated per unit cross-sectional area) of individually identified motor units. Motoneurones were found to innervate between 84 and 178 muscle fibres (mean 110) in type S units and between 126 and 161 fibres in type FR units (mean 142). Fibre areas were larger for type FR units and there was a significant difference in specific tension of the two unit types (type S lower).

5. Indirect estimates of innervation ratio and specific tension were obtained from counts of muscle fibre types, and relative frequencies of motor unit types in the soleus unit pool. Observations agreed well with results of direct measurements.

6. The evidence provided suggests that differences in tension generated by type FR and S units in rat soleus muscle are primarily due to differences in innervation ratio and fibre area, with a small contribution from differences in specific tension.

INTRODUCTION

The motor units comprising an individual muscle can produce a range of tensions. It is, however, a subject of controversy as to whether this is due to differences in the number of muscle fibres per motor unit, or to differences in the intrinsic strength of the contractile material (Close, 1972; Burke, 1981). Estimates of the number of fibres

contained within a single motor unit (innervation ratio), and tension per unit area (specific tension), have been previously determined by indirect methods (Burke & Tsairis, 1973; Edjtehadi & Lewis, 1979; McDonagh, Binder, Reinking & Stuart, 1980*a*, *b*), but their results are conflicting.

The development of histochemical methods for 'marking' the component fibres of individual motor units, by depleting them of glycogen, make it theoretically possible to study innervation ratio directly. Such direct estimates have been attempted in cat gastrocnemius and soleus (Burke & Tsairis, 1973; Burke, Levine, Salcman & Tsairis, 1974). In both these muscles, in particular gastrocnemius, counts of fibre number are complicated by the geometrical arrangement of muscle fibres, and a single cross-section does not necessarily reflect the total number of fibres. Burke & Tsairis (1973) reconstructed the unit from serial sections taken at various levels of the muscle in an attempt to overcome this problem. The large number of muscle fibres in cat hindlimb muscle, however, makes precision difficult. It is also possible that glycogen depletion of units was incomplete.

The aims of the present study were to classify the motor unit types of rat soleus muscle, and to elucidate if the differences in tension developed by fast- and slow-type units are due to an increased number of fibres per unit, a greater specific tension of the component fibres or larger fibre cross-sectional area. The soleus muscle of the rat has an advantage over most other muscles for this type of investigation in that its fibres are arranged approximately parallel to the long axis of the muscle and extend for more than 55% of the muscle length. Theoretically, therefore, a mid-belly section should contain all of the fibres in the muscle, enabling a more direct count of the number of fibres in a glycogen-depleted motor unit. The distribution of motor unit fibres is also fairly uniform throughout the rat soleus (Kugelberg, 1973), and so the proportion of fibres missing from a single cross-section should be approximately the same for all units. This is not true of cat soleus where there is a considerable localization of fibres of a single motor unit (Burke *et al.* 1974).

Unit types were identified by physiological criteria, based on those used for classification of cat motor units by Burke *et al.* (1973). The fibres of a single motor unit per muscle were then depleted of glycogen by repetitive stimulation, allowing histochemical identification of the unit, and estimation of fibre counts and fibre areas.

The results suggest that the greater tensions developed by fast compared with slow motor units in rat soleus muscle are largely because of a greater innervation ratio and larger fibre cross-sectional area. Large-tension units had higher specific tension but this factor was relatively small.

METHODS

Adult male Wistar rats (450 g; S.D. = 50 g) were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (Sagatal; May & Baker): 60 mg/kg initially, supplemented as necessary.

A lumbar laminectomy was performed and ventral roots L4 and L5 cut at entry to the spinal cord. The soleus muscle was exposed and its nerve supply freed from surrounding tissue. All other hindlimb muscles were denervated. The animal was mounted in a rigid frame immobilizing the spinal column and hindlimb. Exposed tissues were covered with liquid paraffin BPC maintained at

37 °C. A steel hook was firmly attached to the distal tendon of the muscle for connection to a tension transducer.

The muscle nerve was stimulated via a bipolar silver wire electrode with a 0·05 ms duration stimulus. The anode of a second pair of electrodes was used to stimulate the ventral root filaments and to record the latency of the antidromic action potential produced on stimulation of the muscle nerve. Electromyographical (EMG) activity was monitored using silver wire electrodes tipped with cotton wool soaked in 0·9 % saline.

Muscle length was adjusted to give maximal twitch tension, and held constant for all subsequent tension measurements. Whole-muscle twitch tension was monitored at frequent intervals, and the experiment was discontinued if tension fell by 10 % from the initial value. Whole-muscle tetanic tension was measured intermittently by replacing the motor unit tension transducer with a less-sensitive one. The muscle nerve was stimulated with a voltage some 2–3 times the value for a just-maximal response.

Tension was recorded by one of two dynanometers constructed by mounting four semiconductor strain gauges on a titanium beam. The whole-muscle transducer had a sensitivity of 25 mV/N, a stiffness of 0.02 mm/N, a resonant frequency of 1·8 kHz and a half decay time of about 15 ms. The motor unit transducer had a sensitivity of 114 mV/N, a stiffness of 0·2 mm/N, a resonant frequency of 0·5 kHz and a half decay time of about 10 ms. Both transducers had a low oscillatory response to a step change of tension. Motor units were functionally isolated by splitting ventral root filaments, and testing for the presence of all-or-none motor unit tension, EMG and latency of antidromic action potential.

For each isolated unit, measurements were made of the unpotentiated peak twitch tension and time to reach peak tension (average of more than ten sweeps). Analysis was made 'on-line' by a digital computer (Bagust, 1971). Maximum tetanic tension was recorded at a stimulation frequency of 200 Hz for 400 ms duration. This was found to be sufficient to produce a fused tetanus in all the units studied.

Trains of twenty-five pulses with interstimulus intervals from 50 to 300 % of the twitch time to peak of the unit under study were used to test for the presence of 'sag' in the unfused tetanus (after Burke *et al.* 1973).

Fatigue resistance of the units was tested with bursts of pulses at 40 Hz for 500 ms, repeated once a second. A fatigue index was calculated as the ratio of the tension developed after 2 min to the initial tension or to the greatest tension (in those cases where the first tetanus was not the largest).

The fibres of a selected unit were visualized histochemically by depleting them of their glycogen stores by repetitive stimulation (after Kugelberg & Edström, 1968). A single motor unit was stimulated with short tetani (40 Hz for 500 ms) repeated every second until tension dropped to half the initial value. During this procedure the popliteal artery was clamped to prevent blood glucose being used as an energy source, forcing fibre glycogen supplies to be mobilized. This was achieved by means of a cotton thread which was looped around the popliteal artery and could be put under tension to bend the vessel and so occlude the flow of blood. Once unit tension dropped to half the initial value, the thread was released allowing blood to flow back to the muscle and the unit tension was allowed to recover to a steady level. This cycle was repeated until tension fell to zero and failed to recover. The procedure generally took between 20 min and 1 h depending on the unit type. The EMG was monitored on an oscilloscope throughout the depletion period.

The muscle was then quickly removed, together with the contralateral soleus and extensor digitorum longus (control for staining reactions). The mid-belly portion of each muscle was mounted in Tissue Tek embedding medium on cork blocks, with the fibres orientated vertically. The blocks were frozen in isopentane super-cooled in liquid nitrogen. Serial sections (10 μm) were cut on a cryostat microtome at −20 °C. Sections were air-dried for 20 min and serial sections stained for glycogen by the Periodic acid-Schiff method (PAS: Pearse, 1968), myosin ATPase after acid (pH 3·9–4·8, in steps of 0·1) and alkaline (pH 10·1–10·6) pre-incubations (Guth & Samaha, 1970), and for succinic dehydrogenase (SDH: Nachlas, Tsou, Desousa, Cheng & Seligman, 1957).

Depleted fibres were counted in PAS sections and fibre areas measured from camera lucida images of depleted fibres with a digitizer linked to a microcomputer. Repeated measurements of a sample of fibres showed that area measurements were consistent to within ±5 %

Throughout this paper, arithmetic means are given with standard deviation. Means of two

normally distributed samples were compared using Student's two-tailed t test. P values less than 0·05 were considered significant. Where linear regression analysis was performed, slopes of the relationship were considered significantly different to zero at the 5% level of probability.

RESULTS

Physiological classification

We examined 128 units in nineteen muscles, which is an average of 6·7 units/muscle, or about one-quarter of the total number of units in each muscle. Many experiments were ended after a small number of units had been isolated in order that glycogen depletion could be undertaken in a healthy muscle, and the choice of unit for depletion was normally made without reference to its physiological characteristics to avoid bias. Thus, the lowest number of units examined in any one muscle was three, but there were three muscles in which at least fifteen units (56% of the total) were studied. We have not found any systematic time-related changes in these three more extensive experiments, in that the size of motor units isolated early were not different to those recorded at the end. Units were assessed physiologically according to twitch time to peak tension, resistance to fatigue and the presence or absence of 'sag' in the unfused tetanus after the work of Burke *et al.* (1973). We used only the presence of sag to classify motor units as fast, although we modified the original criteria of Burke. Modification was necessary perhaps because Burke *et al.* (1973) always potentiated their motor units before recording, by stimulating at 2 Hz over long periods. Our muscles (and those from most other laboratories) were subjected to minimal stimulation (at 0·1 Hz). Potentiated twitches have tensions some two to three times larger than rested ones and times to peak at least 50% longer. All of our units were resistant to fatigue so all the fast ones will be referred to as FR following the classification of Burke *et al.* (1973). Similarly the slow units (showing no sag at any frequency: Fig 1*Ba*) will be termed type S.

Type S units are shown as circles in Figs 1, 3 and 4 and type FR as squares. In these illustrations units that were also classified histochemically are indicated by filled symbols – in all cases S units consisted of type I fibres and FR were all type IIA (see below).

Contractile speed was assessed on the basis of measurements of the time taken from the initial development of tension to the peak tension, which will be referred to as twitch time to peak.

Figure 1*A* shows the distribution of twitch times to peak of those units isolated. The shaded areas represent units which were classified by histochemical criteria. Values were normally distributed around 32·6 ms (S.D. = 6·07 ms, n = 128). There was no evident bimodal division into fast- and slow-type units on the basis of time to peak alone (contrast Close, 1967).

In an attempt to differentiate unit types physiologically motor units were assessed for the presence or absence of 'sag' in the unfused tetanus (see Burke *et al.* 1973). Motor units either developed sag at interpulse intervals between 125 and 175% of time to peak, or completely failed to show sag over the range tested (50–300%). On this basis all units showing sag at any interval were classified as fast and those units that failed to develop sag were classified as slow. The responses of two rat soleus units to various stimulation frequencies are shown in Fig. 1*B*. All units that showed sag

had contraction times equal to or less than 21·6 ms. There was some overlap since one unit with this time to peak had no sag.

Motor units were also tested for fatigue resistance. Figure 1*C* shows the relationship between twitch time to peak and fatigue resistance of rat soleus motor units. Histochemical type and the presence or absence of sag are shown by different symbols. All units had fatigue indices greater than 0·7. Units showing sag (squares in Fig. 1*C*) were generally more fatigue-resistant and had indices greater than 0·88.

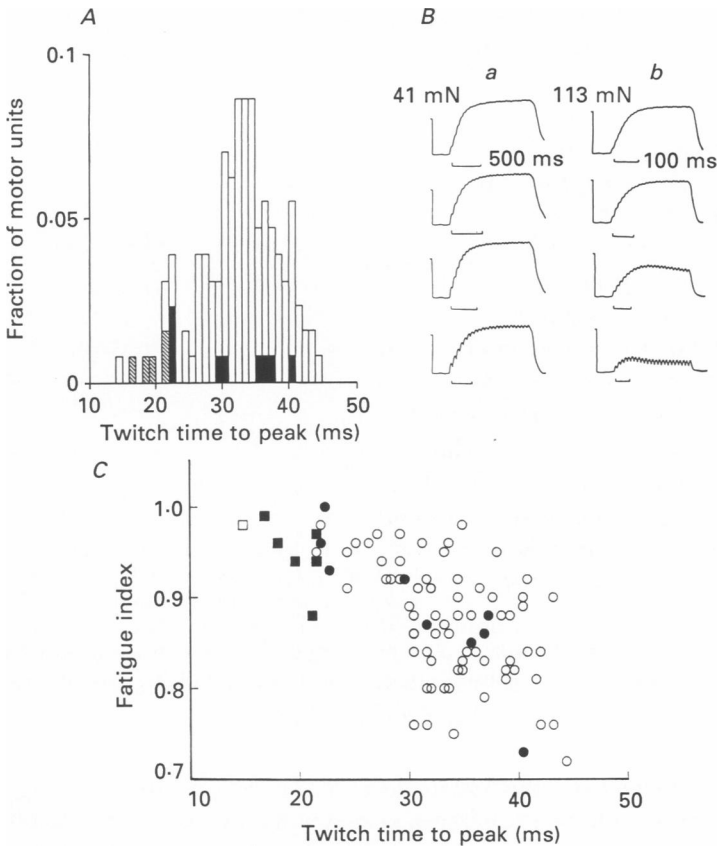

Fig. 1. *A*, distribution of 128 motor unit twitch times to peak tension. Shaded columns represent units in which the component fibres were identified by histochemical methods as type I (filled) or type IIA (hatched). *B*, isometric tetanic myograms from: *a*, a type S motor unit (twitch time to peak 44 ms). There is no sag in the tetanic envelope at any of the frequencies of stimulation shown. *b*, a type FR motor unit (twitch time to peak 15·6 ms) showing sag at all interstimulus intervals of greater than 150% of the twitch time to peak. Reading from top to bottom the interpulse interval was 100, 125, 150 and 200% of the twitch time to peak for the particular unit under investigation. *C*, fatigue index of eighty-nine soleus motor units plotted as a function of their twitch time to peak. Motor units were classified as fast or slow by the presence (squares) or absence (circles) of sag in the unfused tetanus. Filled symbols represent units identified histochemically as type IIA (filled squares) and type I (filled circles). Note these units were also FR and S respectively. All motor units had a fatigue index above 0·7, and were considered to be fatigue-resistant. The regression of the slope of the relationship is significantly different from zero ($r = -0{\cdot}63$, $P < 0{\cdot}001$).

Only three units fell below an index of 0·75 (Burke's lower limit for classification as fatigue resistant) and these had very slow contraction times. The present data cannot be compared directly with those of Burke *et al.* (1973) obtained from cat motor units, as at a stimulation frequency of 40 Hz the degree of mechanical fusion is less in rat than in cat motor units. If stimulation frequency is increased neuromuscular failure is more likely to occur (Krnjević & Miledi, 1958; Kugelberg & Lindegren, 1979). In the present study, the duration of the stimulus bursts was

TABLE 1. Summary of the properties of rat soleus motor unit and fibre types: either slow motor units/type I fibres (left column) or fast units/type IIA fibres (right column)

	Type S/I	Type FR/IIA
(1) Proportion of each fibre type (%)	93·3	6·7
(2) Number of fibres	2706	194
(3) Proportion of unit types (%)	94·5	5·46
(4) Number of each unit type	24·6	1·4
(5) Mean tension (% whole muscle)	3·7	7·1
(6) Contribution to whole-muscle tension (%)	90	10
(7) Innervation ratio (direct)	98 ± 14	142 ± 16
(8) Innervation ratio (indirect)	110	138
(9) Mean motor unit tension (mN)	90 ± 25·6	176 ± 37·7
(10) Mean cross-sectional area (μm^2)	3985	4550
(11) Specific tension, direct (N/mm^2)	0·24 ± 0·030	0·29 ± 0·052
(12) Specific tension, indirect (N/mm^2)	0·21	0·28

Explanations in text or as indicated as follows:

(1) Mean percentage of each fibre type in soleus; (2) calculated numbers of each fibre type per muscle, using a calculated mean of 2900 (±205, $n = 11$) fibres per muscle; (3) percentage contribution to whole-muscle tetanic tension of each unit type; (4) numbers of each unit type in muscle (assuming the total number of motor units is twenty six; see text); (5) mean tension of unit type as a percentage of whole-muscle tension; (6) percentage contribution of each unit type to whole muscle = (4) × (5); (7) innervation ratio measured from fibre count of depleted units; (8) indirect estimate of innervation ratio = (2)/(4); (9) and (10) are direct measurements on depleted units; (11) mean specific tension from innervation ratio and fibre areas of individual units; (12) specific tension calculated indirectly = (9)/((8) × (10)).

increased to 500 ms from the 330 ms used in the cat by Burke *et al.* (1973). This gives a total of 2400 stimuli over the 2 min period, which is equivalent to 60 Hz for 330 ms. This regime was sufficient to cause a fall of 90% from peak tension in a gastrocnemius unit over 2 min (S. Chamberlain, unpublished observation). Bearing this in mind, all units isolated were classified as fatigue resistant.

Contractile properties

The values for several variables are set out in Table 1 but some general comments are presented below.

The distribution of motor unit tetanic tension is shown in Fig. 2, expressed in terms of absolute tension (Fig. 2*A*) and as a percentage of parent muscle tetanic tension (Fig. 2*B*) to normalize data obtained from different animals. We have found differences between the FR and S units (classified on physiological characteristics alone, as described above). The mean tetanic tension of type S units (3·7% of whole-muscle tension) was approximately half that of type FR (7·1%). The mean tension

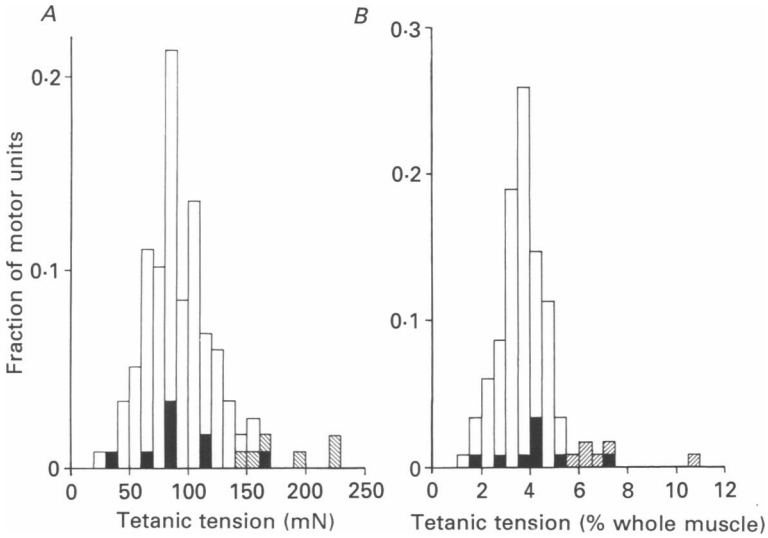

Fig. 2. Distribution of 115 motor unit tetanic tensions expressed in *A* as absolute tension and in *B* as a percentage of whole-muscle tension. Shaded symbols represent units identified histochemically as type I (filled) and type II A (hatched).

of all units was 3·9% of whole-muscle tension, which leads to an estimate of twenty-six units per muscle, (cf. Close, 1967; Brown, Jansen & Van Essen, 1976). The relative proportions of the two types of unit isolated were 94·5% (type S) and 5·46% (type FR). The values can be used to make an estimate of 24·6 type S motor units and 1·4 type FR motor units per muscle. The relative contribution of each unit type to whole-muscle tension can, therefore, be calculated for FR units as 10% (1·4 units × 7·1% of whole muscle) and for type S as 90% (24·6 units × 3·7% whole muscle).

The relationship between twitch time to peak and tetanic tensions of the motor units is shown in Fig. 3*A*. There appears to be a division of the population into two groups, one of large-tension, fast-contracting units and the other of smaller, slow-contracting units. There is a significant correlation between these two parameters both for type S units considered separately and for the whole population (see legend to Fig. 3 for statistics). There are too few data points to comment conclusively on the relationship for the type FR units.

Figure 3*B* shows the relationship between motor unit twitch time to peak and the ratio of twitch to tetanic tension. For type S units the twitch:tetanus ratio increased with increasing twitch time to peak. Type FR units form a separate group. The sample is small and it is not possible to confirm if a significant relationship exists for FR units. The range of twitch:tetanus ratios displayed by type S units (0·07–0·29) was greater than that of type FR units (0·15–0·23). In the overlap zone of twitch time to peak of the two unit types the twitch:tetanus ratio of FR units was about twice that of type S.

The motoneurones of type S units tended as a group to have more slowly

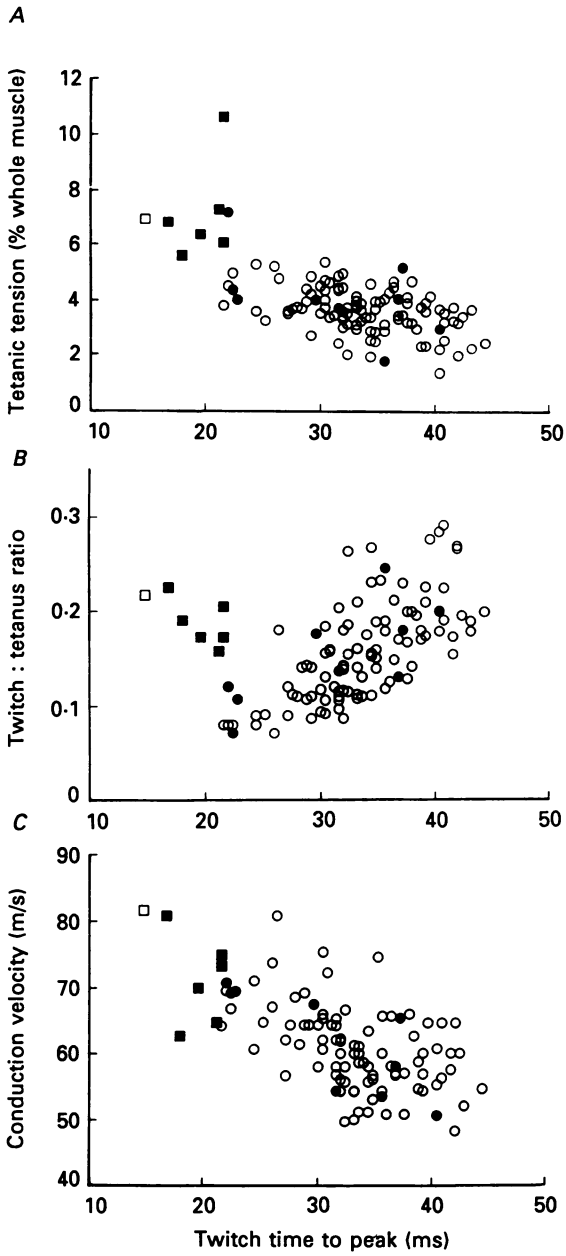

Fig. 3. Units classified as type S are shown as circles and type FR as squares; filled symbols represent units that were identified histochemically (type I = circles, type II A = squares). *A*, tetanic tension plotted against twitch time to peak. Regression for slopes of all the data ($r = -0{\cdot}65$, $P < 0{\cdot}001$, $n = 117$) and for type S units ($r = -0{\cdot}46$, $P < 0{\cdot}001$, $n = 110$) are significant. The relationship for type FR units was not significant ($r = -0{\cdot}35$, $P < 0{\cdot}2$, $n = 7$). *B*, twitch : tetanus ratio plotted as a function of twitch time to peak. Slope of the regression for all data ($r = +0{\cdot}45$, $P < 0{\cdot}001$, $n = 116$) and for type S units ($r = +0{\cdot}68$, $P < 0{\cdot}001$, $n = 109$) are significant. For type FR units there are too few data to confirm if a different relationship exists ($r = -0{\cdot}69$, $P < 0{\cdot}1$, $n = 7$, not significant). *C*, conduction velocity plotted against twitch time to peak. Regression is significant for all data ($r = -0{\cdot}59$, $P < 0{\cdot}001$, $n = 109$) and for type S ($r = -0{\cdot}49$, $P < 0{\cdot}001$, $n = 102$). That for type FR units is not significant ($r = -0{\cdot}48$, $P < 0{\cdot}2$, $n = 7$).

conducting axons (60·8, S.D. = 6·40 m/s, $n = 120$) than those of type FR units (72·6, S.D. = 7·32 m/s, $n = 7$). There was a significant correlation between conduction velocity of axons of type S units and the twitch time to peak of the muscle fibres illustrated in Fig. 3*C* but correlation was not significant for type FR units. The two groups, however, appear by eye to be adequately represented by a single regression.

In addition to the relationships illustrated in Fig. 3, there was also a significant

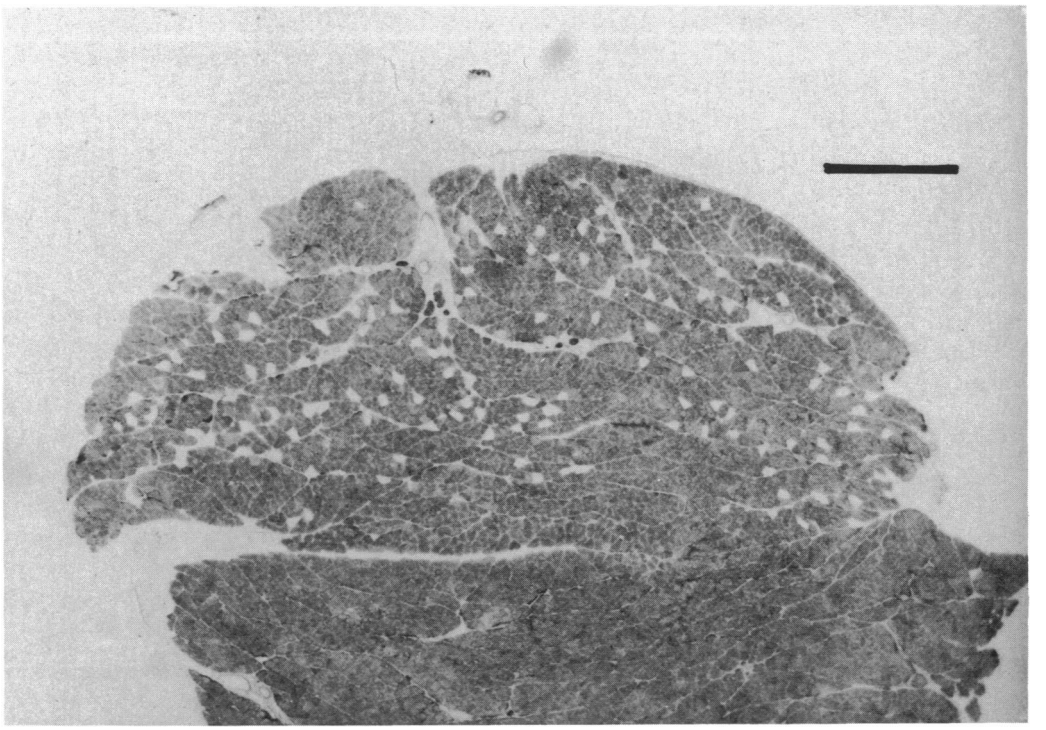

Fig. 4. Section (10 μm thickness) of a whole soleus muscle stained for glycogen (PAS) to show the distribution of fibres belonging to a single depleted motor unit. Physiological characteristics were: time to peak = 18 ms, fatigue index 0·90, sag present. (The bottom part of the section is part of the contralateral soleus muscle cut together with soleus for control staining.) Calibration bar 1 mm.

linear increase of motor unit tetanic tension with axonal conduction velocity ($r = 0{\cdot}35$, $n = 103$, $P < 0{\cdot}001$). The data were fitted no better by a semilogarithmic regression, and in this resembles cat slow muscle rather than fast despite the presence of fast motor units found in rat but not cat soleus.

Histochemical characteristics

In most experiments one unit was stimulated repetitively to deplete its component fibres of glycogen. Fibres of that unit therefore appeared unstained in sections treated to reveal the presence of glycogen. It is very difficult to be sure of the completeness of the glycogen-depletion routine, but we attempted to verify its

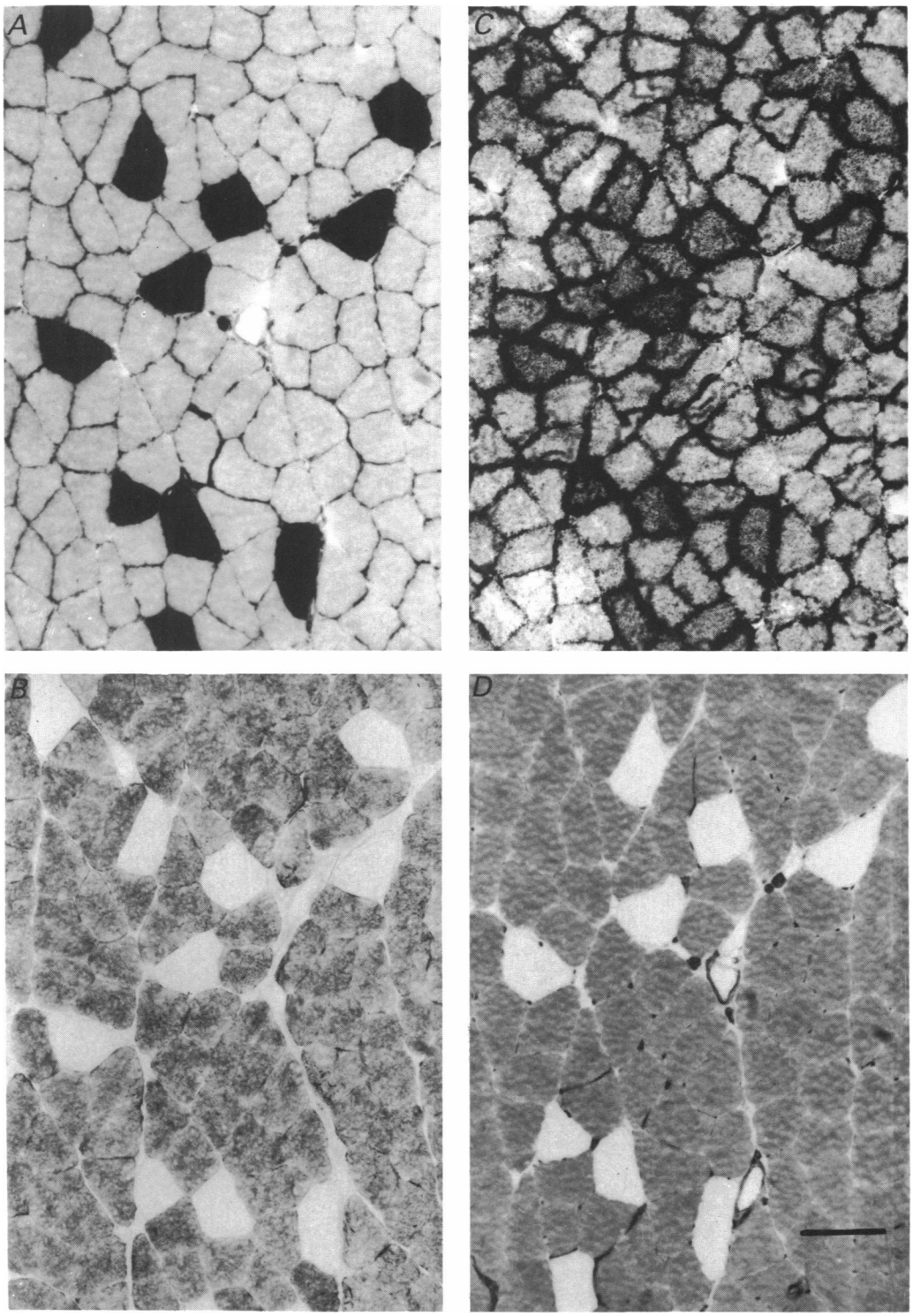

Fig. 5. For legend see facing page.

efficiency in two experiments in which the entire muscle nerve was stimulated to fatigue. Over 90% of the total number of muscle fibres were found to be depleted. Two muscles were also examined after 8 h motor unit recording sessions in which no unit glycogen depletion was attempted. Neither of the muscles contained more than one or two unstained fibres (0·07%) and commonly these were hypertrophied fibres of unusual histological appearance. In twelve of the fifteen muscles where a unit was subjected to the depletion regime there was no ambiguity in identifying fibres of the unit, in that muscle fibres were either completely free of pink-staining reticulum or deeply stained, as in control muscles. In the remaining three cases, where EMG and tension declined more rapidly than usual, many fibres were depleted but there were also some pale-pink fibres suggesting that depletion was not complete. These muscles were not used in analysis of innervation ratio as some unit fibres may have gone undetected leading to inaccurate fibre counts.

Figure 4 shows the distribution of fibres of a single depleted unit. They are scattered throughout most of the muscle. A part of this section is shown at higher magnification in Fig. 5, together with the same field in serial sections stained to show the activities of myosin ATPase. This unit was classified by physiological criteria as type FR with a twitch time to peak of 18 ms, and displayed sag. The constituent fibres were type II A.

Innervation ratio

Fibres were estimated to run for approximately 55–60% of whole-muscle length, based on measurements of single fibres teased from relaxed muscles. The number of muscle fibres innervated by a single motoneurone was determined directly by counting glycogen-depleted fibres in mid-belly cross-sections. Innervation ratio ranged from 84 to 178 fibres per unit (see Table 2). The mean for type S units was 110 (S.D. = 32·6, $n = 7$) which is not significantly different to that for type FR units where the mean innervation ratio was 142 (S.D. = 15·9, $n = 5$).

One type S unit was unusual in that it was selected for depletion because its tetanic tension was far larger than other type S units. It was found to have a correspondingly high innervation ratio of 178. If this selected unit is excluded from mean calculations detailed above the innervation ratio for type S units was 98 (S.D. = 14·1, $n = 6$) which is significantly different to that of type FR units ($t = 5·68$, $P < 0·001$).

Figure 6*A* shows the relationship between the innervation ratio and tetanic tension for the depleted units. The regression between innervation ratio and tetanic tension generated by the unit was significantly different to zero. Figure 6*B* shows the relationship between twitch time to peak and innervation ratio of the units; there is apparently a negative slope but statistically the regression was not significant.

Fibre areas

Cross-sectional areas of fibres were measured from camera lucida images of the glycogen-depleted units, and the mean fibre area for each of these units is shown in

Fig. 5. Serial sections of the same soleus muscle shown in Fig. 4. Sections stained for glycogen (*B*), for ATPase with a pre-incubation at pH 10·4 (*A*) and at pH 4·35 (*D*) or for succinic dehydrogenase (*C*). Fibres belonging to the depleted unit were classified as type II A. Calibration bar (in *D*) 100 μm.

Table 2. The overall mean area for type I fibres in seven glycogen-depleted type S units was 3985 (S.D. 969) μm^2 ($n = 770$) and 4550 (S.D. 1646) μm^2 ($n = 711$) for type II A fibres in five glycogen-depleted type FR units. Type II A fibres were significantly larger ($t = 8{\cdot}2$, $P < 0{\cdot}001$, $n = 1481$) than type I fibres. Motor unit tetanic tension is plotted against total fibre area in Fig. 6*C*, and there was a significant regression between these two parameters, indicating that total fibre area plays the major part in determining motor unit tension.

TABLE 2. Summary of the properties of single depleted soleus motor units

(1) Type	(2) Type	(3) IR	(4) P_0 (mN)	(5) Area (μm^2)	(6) ST (N/mm^2)
S	I	84	88·1	4858	0·22
S	I	88	80·9	3362	0·27
S	I	178	166	4021	0·23
S	I	97	114	4138	0·28
S	I	92	81·7	4005	0·22
S	I	110	88·8	3891	0·21
S	I	121	115	3756	0·25
FR	II A	126	164	5513	0·24
FR	II A	149	226	4580	0·33
FR	II A	161	221	4302	0·32
FR	II A	150	158	4776	0·22
FR	II A	125	143	3596	0·32

Physiological and histochemical classifications are given in columns (1) and (2) respectively; (3) innervation ratio (IR) is a direct count of the number of fibres in a depleted unit; (4) P_0 is the tetanic tension of the unit in mN. (5) mean area of the fibres in the unit; (6) specific tension (ST) has been calculated from columns as (4)/((3) × (5)).

One source of error is that muscle length was not controlled during freezing, leading to a variable degree of contracture of muscles during the freezing process. We have made measurements of mean fibre area in right and left soleus muscles from four rats. One muscle of each pair was frozen as described in the Methods; the other was held at optimum length during freezing. The ratio of fibre areas of freely shortening to fixed-length muscle was 1·53 (S.D. = 0·137). Almost identical average ratios were obtained for type I (1·53) and type II A fibres (1·51) calculated separately. Areas of fibres frozen at fixed length may have been more liable to distortion by ice artifact which was more common than in muscles frozen by the method used for glycogen-depleted motor units. It is also known that sections shrink during histochemistry. We have estimates of 2% width reduction in PAS stains, corresponding to 4% loss of area.

Specific tension

In Fig. 6*C* there was an indication that the FR units (squares) developed more tension than S units (circles) of similar total area. This was investigated further by calculating the intrinsic strength of the fibres, or specific tension, for each unit from the cross-sectional area of its fibres, innervation ratio and tetanic tension. There was a difference between the specific tension of type S (0·24, S.D. = 0·03 N/mm^2, $n = 7$)

and type FR units (0·29, S.D. = 0·05 N/mm^2, $n = 5$) that was just significant statistically ($t = 1·88$, $P < 0·05$). These estimates of specific tension may be corrected to optimal length by allowing for the free shortening before freezing. We calculate that the true specific tension would have been 60% greater than the values quoted above, i.e. for S units 0·38 N/mm^2 and for the FR units 0·46 N/mm^2. The relative difference between the two types was not affected by these corrections.

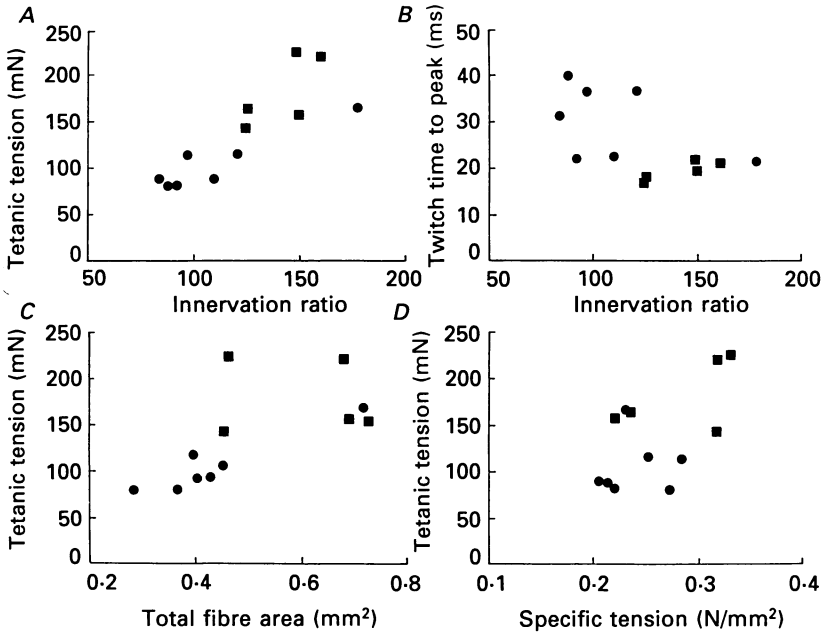

Fig. 6. *A*, relation between absolute tetanic tension and innervation ratio in single motor units. Regression for all data is significantly different from zero ($r = +0·84$, $P < 0·001$, $n = 12$). *B*, twitch time to peak *versus* innervation ratio ($r = -0·57$, $P < 0·05$, $n = 12$, not significant). *C*, tetanic tension as a function of total area of the unit fibres. Regression is significant ($r = +0·36$, $P < 0·02$, $n = 12$). *D*, tetanic tension plotted as a function of specific tension. There is a significant regression between these two parameters ($r = +0·6$, $P < 0·05$, $n = 12$). Symbols as in Fig. 3.

There was a significant relationship between tension developed by a unit and the estimated specific tension of its fibres, illustrated in Fig. 6*D*. Total tension, however, was not significantly related to mean fibre area ($r = 0·30$, $P < 0·2$).

We have also looked for a relationship between the specific tension of motor units and the size of the component fibres, as measured by their mean cross-sectional area. The relationship was an inverse one, the smallest fibres having the highest specific tensions ($r = -0·56$, d.f. = 10, $P < 0·05$). Within this regression, the fast motor units appeared to have higher specific tension than the slow units at any given size of fibre.

Indirect estimates

In most previous studies innervation ratio and specific tension have been determined indirectly from the relative proportions of motor unit and fibre types within the muscle.

Such calculations have been performed on the present data to see how they compare to results obtained by the more direct methods employed in this study. The findings are summarized in Table 1. Innervation ratio was calculated from the number of each type of fibre within the muscle (direct total fibre counts), and the proportion of each unit type isolated. Only three of fifteen muscles contained type IIC fibres (mean 2, S.D. = 1·4%). These have been grouped together with type IIA fibres. The indirect estimates of 110 fibres for type S and 137 fibres for type FR units compare favourably with those obtained by direct counts. Specific tension was calculated from mean fibre areas, innervation ratio and the mean motor unit tension. The estimate for type S units was 0·21 N/mm^2 which was slightly lower than that observed directly for single type S units (0·24 N/mm^2). The estimate of 0·28 N/mm^2 for type FR compares well with the value of 0·29 N/mm^2 from direct estimates.

Conclusions

In summary, innervation ratio was higher in type FR than in type S units and increased with motor unit tension. Fibre area was also higher in FR units than S, but correlation with tension was not statistically significant. Specific tension was slightly larger in high-tension units, and was related to fibre type.

DISCUSSION

As is generally agreed, we confirmed that one factor accounting for the range of motor unit tensions was variation in muscle fibre area, the highest tension units having larger fibres. We have found two additional factors to explain differences between large and small motor units.

The most important of these two was the direct relationship between motor unit tension and innervation ratio; so units are large, in part, because they consist of more fibres. We are not sure whether this conclusion is true within subpopulations of units or whether it is just that the slow units have a smaller number of fibres than the fast units. This contrasts with the conclusion of Burke & Tsairis (1973) that there is no difference in the average number of fibres between fast and slow cat motor units. The conclusion of Burke & Tsairis (1973) was based on indirect measurements of innervation ratio, and they thought that there was error in their direct measurements in which they had counted small numbers of fibres in glycogen-depleted slow motor units. They guessed that there was substantial failure to deplete slow units completely. In the present study only muscles in which fibre glycogen was either high or completely absent were used in the measurements of innervation ratio and any muscles with pale-pink fibres were discarded on the basis that depletion might not be complete. Such pale fibres were not found in control muscles. Mid-belly sections were estimated to contain at least 90% of the total complement of muscle fibres. In rat soleus Finol, Lewis & Owens (1981) found a fibre length: muscle length ratio of 0·71 (S.E.M. = 0·035) and Elmubarak & Ranatunga (1988) reported a value of 0·64 (S.E.M. = 0·05). In some of our experiments we have observed that the ratio was greater than 55% by inspection of the ends of the aponeuroses into which muscle fibres are inserted. Unit fibres were quite evenly distributed throughout the cross-section of the muscle, unlike in cat soleus (Burke *et al.* 1974). We consider, therefore,

that our direct counts of innervation ratio are reasonably reliable, and it may be that those of Burke & Tsairis (1973) should also be accepted in preference to their indirect estimates.

In our experiments, innervation ratios measured directly were also consistant with those estimated from indirect estimates by dividing the total number of muscle fibres by the number of motor units.

It is known that slow motor units are innervated by axons with smaller diameters, and we deduce from innervation ratios that small axons branch less than large ones. There is some evidence for this from Eccles & Sherrington (1930) who dissected single axons, and found a lower incidence of branching in smaller axons. If the number of branches were directly proportional to axon diameter, the innervation ratio would increase exponentially with axon diameter. This would account for the logarithmic relationship between motor unit tension and axon conduction velocity found by Bagust, Knott, Lewis, Luck & Westerman (1974) in cat fast muscle. The relationship between tension and conduction velocity in our data was fitted equally well by linear as semilogarithmic regressions. Linear regressions also fit the data of cat soleus (Bagust, 1974). The difference between fast and slow muscle motor units may lie in the smaller range of tensions in the latter, which would conceal any non-linear relationship.

The third factor accounting for the differences in tension between units was a difference in specific tension between type S and type FR units. There has been considerable debate about such differences, partly because a number of experimental approaches have been made to the problem.

Our estimates of specific tensions were 0·29 N/mm^2 for seven fast (fatigue-resistant) units and 0·24 N/mm^2 for five slow. The difference was significant at the 0·05 level, although there was a considerable range within each group (0·22–0·32 and 0·21–0·28 N/mm^2, respectively). These values must be low because the muscle was allowed to shorten freely during freezing, so that measured fibre areas were too high by about 50%. Although this is a large error, the area changes were almost identical in type I and type II fibres, so the difference of specific tension between fast and slow units was not affected. The specific tensions corrected for length changes during freezing were 0·46 (mean, range 0·35–0·51) N/mm^2 for fast and 0·38 (0·33–0·44) N/mm^2 for slow units.

There are very few other estimates of motor unit specific tension made directly that can be compared with our data. Burke & Tsairis (1973) examined one fatigue-resistant fast unit in cat gastrocnemius muscle and obtained a value of 0·27 N/mm^2. This is within our range of directly estimated values because they did not control muscle length during freezing.

There are, however, a number of indirect estimates of specific tension, derived from the proportions of fast and slow units, their mean absolute tensions and the mean areas and the proportions of type I and II fibres. Most authors find that fast units have higher specific tensions than slow. Uniquely in this group Edjtehadi & Lewis (1979) held the muscle (cat extensor digitorum longus) at optimum length during freezing and calculated 0·39 and 0·29 N/mm^2 for fast (both types combined) and slow units, respectively. These are somewhat lower than our results corrected for shortening. It may be that our correction is too high, as the muscles frozen at

optimum length had ice artifact which might have caused some expansion of the fibre areas. Ice artifact was not a problem in the results from the larger cat muscle in the work of Edjtehadi and Lewis. Despite these differences the two papers agree in the ratio of specific tensions for fast and slow motor units. Gardiner & Olha (1987, rat plantaris) also found that fast units developed 1·4 times more tension than slow units.

A much larger difference has been reported in several papers (Burke & Tsairis, 1973: cat gastrocnemius; McDonagh *et al.* 1980*a*: cat tibialis anterior; Dum, Burke, O'Donovan, Toop & Hodgson, 1982: cat extensor digitorum longus). For example, Burke and Tsairis calculated values of 0·17 and 0·29 N/mm^2 for fast easily fatigued (FF) and fast fatigue-resistant (FR) units, but slow units gave a value as low as 0·06 N/mm^2. Experiments on single fibres from this muscle (Lucas, Ruff & Binder, 1987) indicate that type I and II fibres are not very different, so it is likely that the indirect method of measuring specific tension may be subject to additional errors. Clearly it is based on four rather than two measurements and random errors must be higher; however, there must be systematic errors to account for such a large difference in specific tensions. One likely source of such errors is in the unit selection procedure which may be biased, resulting in a false ratio of numbers of slow-to-fast units. Burke and his colleagues isolated units by penetration of motoneurones by microelectrodes, a technique that may be expected to select preferentially large somata connected to fast motor units. Certainly the present data show no major difference between specific tensions calculated directly and indirectly, indicating that ventral root splitting introduces much less bias.

A number of authors working with whole muscle also report lower specific tensions for slow compared with fast muscles. Early data (discussed by Close, 1972) indicated a small difference between fast and slow muscle. Some more recent measurements confirm a difference: Kean, Lewis & McGarrick (1974) gave values of 0·38 and 0·25 N/mm^2 for cat extensor digitorum longus and soleus; Finol *et al.* (1981) 0·26 and 0·22 N/mm^2 for rat extensor digitorum longus and soleus. In contrast measurements *in vitro* on small bundles from the same two rat muscles showed no significant differences (Ranatunga, 1984: 0·21 and 0·20 N/mm^2, respectively). The differences between fast and slow whole muscle might depend on factors other than intrinsic specific tension. Whole-muscle cross-sectional area is calculated as weight divided by fibre length, and this procedure assumes that fibres contract strictly as units in parallel. If this assumption were incorrect, errors would be introduced that might depend on the fibre length:muscle length ratio. In cat muscles these ratios are 0·26 and 0·41 for fast and the slow muscles (Al-Amood & Pope, 1972); in rat whole muscles they are 0·42 and 0·71 (Finol *et al.* 1981) and in rat bundles 0·76 and 0·81 Ranatunga (1984). The rank order of specific tensions is the inverse of the order of fibre:muscle length ratios. Perhaps fibre arrangement and cross-linkage are more complex in whole muscle than has been assumed in calculating specific tensions.

Another complication in whole-muscle data is that the cross-sectional areas include connective tissue, blood vessels etc. Close (1972) discussed several estimates of non-muscle tissue areas that range between 8 and 14% of the total area. Even this may be an underestimate since muscles swell during recording (H. J. Finol, personal communication, found a 22% weight increase in rat muscle after about 2 h *in vivo*

under liquid paraffin). This increase is presumably due to oedema consequent on increased blood flow, and even larger increases may be expected with *in vitro* preparations in saline solutions without proteins to maintain normal fluid equilibrium (Owens, 1978, found increases in weight in excess of 50% in a perfused preparation). If non-contractile tissue formed a larger component of slow muscle or if it swelled more during recording, specific tensions would be estimated lower for slow muscle even if there were no inherent differences. Another problem is that various fast muscles contain very different proportions of type II A and II B fibres and Burke & Tsairis (1973) found large differences for the specific tensions of fast fatigue-resistant motor units (0·29 N/mm^2) and fast easily fatigued ones (0·19 N/mm^2).

Although some of the uncertainties discussed above are absent in single-fibre measurements, there may be an additional problem of fibre swelling, particularly in skinned-fibre preparations (Godt & Maughan, 1977). Close (1972) discussed early data from myofibrillar bundles and concluded that there was no evidence for differences between preparations from fast and slow muscles. More recently Stephenson & Williams (1981) examined rat fibres that were skinned and activated with Ca^{2+}, and obtained specific tensions of about 0·4 N/mm^2 in extensor digitorum longus fibres and lower values from soleus (0·3 N/mm^2). Lännergren & Westerblad (1987) examined single, intact mouse fibres, which were probably fast, and found specific tensions averaging 0·39 (range 0·30–0·48) N/mm^2, which is close to our corrected value for fast motor units (0·46 N/mm^2). Lucas *et al.* (1987) have studied 5 mm lengths of single soleus and medial gastrocnemius muscle fibres of the cat, which had been skinned with EGTA and activated with Ca^{2+} at 22 °C. Lucas *et al.* (1987) identified the fibres histochemically, and found no differences between type I fibres and soleus (0·23 N/mm^2) and those of medial gastrocnemius (0·25 N/mm^2). Type II fibres also gave similar values (0·24 N/mm^2). They concluded that the specific tensions of all fibre types were similar although there was very wide variation within the subpopulations, perhaps because some fibres were more damaged by being cut and skinned. Their values ranged between 0·11 and 0·35 for soleus and between 0·12 and 0·46 N/mm^2 in medial gastrocnemius. If it is assumed that their lowest values were obtained from damaged fibres then the means would be less representative of the fibre types than the upper values which were similar to the data reported here.

A study by Rall, Wilson & Woledge (1986) in single frog fibres is relevant to this discussion. They showed that specific tension (0·17–0·34 N/mm^2) increased directly with fibre cross-sectional area. This relationship was not found in our results and, indeed, the reverse appeared to be more likely. However, we found that fast motor units had a higher specific tension than slow ones at any given fibre size, and this could also have been a factor in the results of Rall *et al.* (1986).

There seems to be sufficient evidence in our work and that discussed above to accept that there may be a real difference between the intrinsic ability of fast and slow muscle fibres to develop force. It is not known which factors account for this difference. Engel & Stonnington (1974) have measured the various intracellular components of rat fast and slow muscles. They found that myofibrils formed about the same proportion of fibre area in both types, because the additional mitochondria of slow muscle almost exactly balance the abundance of sarcoplasmic reticulum of

fast fibres. Unfortunately they did not attempt to subdivide the fast fibres, for the fatigue-resistant ones would be expected to have many mitochondria as well as abundant elements of the sarcoplasmic reticulum and so a lower proportion of myofibrils and lower specific tensions, opposite to our motor unit observations.

Another possible reason for the difference in specific tensions between fast and slow muscle is in the arrangement of thick and thin filaments in the fibres. H. J. Finol (personal communication) has found that the typical regular hexagonal filament array of cross-sections in the electron microscope is seen consistently in fast muscle, whereas slow muscle shows a number of irregularities with fewer or more than six thick filaments around each thin filament. An example of such irregularities is shown in Fig. 11a of Al-Amood, Finol & Lewis (1986). Unfortunately there is no estimate of the incidence of these irregularities, although they are not rare. If common in slow fibres they could reduce the number of force-generating sites and specific tension.

In summary, the evidence presented here for rat soleus muscle suggests that type S and FR units have different specific tensions, but most of the differences in total tension generated are due to differences in innervation ratio and fibre cross-sectional area.

The criteria for classification of motor units of cat muscle established by Burke *et al.* (1973) have been found adequate for the classification of rat soleus motor units. Physiological properties of rat motor units have been previously described in rat soleus by Close (1967) and Kugelberg (1973), who classified motor units as fast or slow on the basis of twitch time to peak. There was no clear-cut bimodal population apparent in the present study but units that demonstrated sag had twitch times to peak equal to or less than 21·6 ms and units failing to develop sag had twitch times to peak equal to or greater than 21·6 ms, but there was a small region of overlap. The sag criterion has been used to classify motor units into fast or slow types in rat plantaris muscle by Gardiner & Ohla (1987). We believe this test to be valid only if several interpulse intervals are tested.

Relationships between twitch time to peak and tension were comparable with those described for cat motor units by Bessou, Emonet-Dénand & Laporte (1963) with fast units developing greater tensions than slow ones. Close (1967) said there was no relationship between motor unit tetanic tension and twitch time to peak in the rat. We, however, found a significant inverse relationship similar to that described by many authors for the cat. The relationship held not only for the whole set of soleus units but for the subpopulation of slow units.

Relationships between time to peak and axonal conduction velocity were also similar to those of the cat (Bagust, 1974). Close (1967) did not measure conduction velocity in his investigation of rat motor units. Our results also differ from those of Close (1967) in that we found that the twitch:tetanus ratio increased with twitch time to peak for the slow soleus units. Other reports from this laboratory have described such a relationship in cat fast muscle (Bagust, *et al.* 1973) and soleus (Bagust, 1974). Further, if fast units and slow units with similar twitch times to peak are compared (Fig. 3*B*), it is seen that the former have larger twitch:tetanus ratios. Lewis (1983, his Fig. 12*A*) has commented on a similar observation in the cat but had to depend on comparisons between units in different muscles.

Several mechanisms could account for these relationships, but we favour the one

that follows. It may be argued from similarities in histochemistry and immunocytology that all motor units within one subpopulation have similar contractile proteins and would, therefore, develop tension at the same rate in a fully fused tetanus. Differences in twitch contraction and relaxation times between units within the same subpopulation would, then, have to be the result of differences in the rate of release and re-uptake of calcium in response to a single stimulus. Thus some units would have fast twitches because the calcium transient is brief; these units would have a short time to develop tension and, therefore, show the smallest twitch : tetanus ratios. Fine control of motor unit properties (as distinct from major neurotrophic regulation of myosin isoforms) would be by modulation of the properties of the more labile sarcoplasmic reticulum.

Physiological properties were found to correlate with histochemical profile, type FR units being composed of type IIA muscle fibres and type S units of type I muscle fibres. Three of the nineteen muscles examined histochemically contained type IIC fibres ($2 \pm 1{\cdot}4\%$). Kugelberg (1973) found that IIC fibres in soleus occurred in heterogeneous motor units together with either type I fibres or type IIA fibres, and that units containing type IIC fibres had a narrow range of twitch contraction times intermediate to units composed of purely type I or IIA fibres. None of the units depleted in the present study was found to contain any type IIC fibres.

S.C. was in receipt of an MRC Research Studentship. We are grateful for technical help by S. Wernberg-Möller and advice from Dr A. Rowlerson. We also thank Mrs S. Maskell for her work on the typescript.

REFERENCES

Al-Amood, W. S., Finol, H. J. & Lewis, D. M. (1986). Chronic stimulation modifies the isotonic shortening velocity of denervated rat slow-twitch muscle. *Proceedings of the Royal Society* B **288**, 43–58.

Al-Amood, W. S. & Pope, R. (1972). A comparison of the structural features of muscle fibres from a fast- and a slow-twitch muscle of the pelvic limb of the cat. *Journal of Anatomy* **113**, 49–60.

Bagust, J. (1971). Motor unit studies in cat and rabbit solei. Ph.D. thesis, University of Bristol.

Bagust, J. (1974). Relationships between motor nerve conduction velocities and motor unit contraction characteristics in slow twitch muscle of the cat. *Journal of Physiology* **238**, 269–278.

Bagust, J., Knott, S., Lewis, D. M., Luck, J. C. & Westerman, R. A. (1973). Isometric contractions of motor units in a fast twitch muscle of the cat. *Journal of Physiology* **231**, 87–194.

Bessou, P., Emonet-Dénand, F. & Laporte, Y. (1963). Relation entre la vitesse de conduction des fibres nerveuses motorices et le temps de contraction de leurs unités motrices. *Comptes rendues hebdomadaire des Séances de l'Académie des Sciences* **256**, 5625–5627.

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, R. E. (1981). Motor units: anatomy, physiology, and functional organization. In *Handbook of Physiology*, section 1, *The Nervous System*, vol. 2, *Motor Control*, ed. Brooks, V. B., pp. 345–422. Bethesda, MD: American Physiological Society.

Burke, R. E., Levine, D. N., Salcman, M & Tsairis, P. (1974). Motor units in cat soleus muscle: physiological, histochemical and morphological characteristics. *Journal of Physiology* **238**, 503–514.

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

BURKE, R. E. & TSAIRIS, P. (1973). Anatomy and innervation ratios in motor units of cat gastrocnemius. *Journal of Physiology* **234**, 749–765.

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

CLOSE, R. (1972). Dynamic properties of mammalian skeletal muscles. *Physiological Reviews* **52**, 129–197.

DUM, R. P., BURKE, R. E., O'DONOVAN, M. J., TOOP, J. & HODGSON, J. A. (1982). Motor unit organization in flexor digitorum longus muscle of the cat. *Journal of Neurophysiology* **47**, 1108–1125.

ECCLES, J. C. & SHERRINGTON, O. M. (1930). Numbers and contraction values of individual motor-units examined in some muscles of the limb. *Proceedings of the Royal Society* B **106**, 326–357.

EDJTEHADI, G. & LEWIS, D. M. (1979). Histochemical reactions of fibres in a fast twitch muscle of the cat. *Journal of Physiology* **287**, 439–453.

ELMUBARAK, M. H. & RANATUNGA, K. W. (1988). Differentiation of fast and slow muscles in the rat after neonatal denervation: a physiological study. *Journal of Muscle Research and Cell Motility* **9**, 219–232.

ENGEL, A. G. & STONNINGTON, H. H. (1974). Morphological effects of denervation of muscle. A quantitative ultrastructural study. *Annals of the New York Academy of Sciences* **228**, 68–88.

FINOL, H. J., LEWIS, D. M. & OWENS, R. (1981). The effects of denervation on contractile properties of rat skeletal muscle. *Journal of Physiology* **319**, 81–92.

GARDINER, P. F. & OLHA, A. E. (1987). Contractile and electromyographic characteristics of rat plantaris motor unit types during fatigue *in situ*. *Journal of Physiology* **385**, 13–34.

GODT, R. E. & MAUGHAN, D. W. (1977). Swelling of skinned muscle fibres of the frog. *Biophysical Journal* **19**, 103–116.

GUTH, L. & SAMAHA, F. J. (1970). Procedure for the histochemical demonstration of actomyosin ATPase. *Experimental Neurology* **28**, 365–367.

KEAN, C. J. C., LEWIS, D. M. & MCGARRICK, J. D. (1974). Dynamic properties of denervated fast and slow twitch muscle of the cat. *Journal of Physiology* **237**, 103–113.

KRNJEVIĆ, K. & MILEDI, R. (1958). Failure of neuromuscular propagation in rats. *Journal of Physiology* **140**, 440–461.

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

KUGELBERG, E. & EDSTRÖM, L. (1968). Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibres: relation to fatigue. *Journal of Neurology, Neurosurgery and Psychiatry* **31**, 415–423.

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.

LÄNNERGREN, J. & WESTERBLAD, H. (1987). The temperature dependence of isometric contractions of single, intact fibres dissected from a mouse foot muscle. *Journal of Physiology* **390**, 285–293.

LEWIS, D. M. (1983). Mammalian motor units. In *Handbook of the Spinal Cord*, vols 2 and 3, *Anatomy and Physiology*, ed. DAVIDOFF, R. A., pp. 269–314. New York: Marcel Dekker.

LUCAS, S. M., RUFF, R. L. & BINDER, M. D. (1987). Specific tension measurements in single soleus and medial gastrocnemius muscle fibres of the cat. *Experimental Neurology* **95**, 142–154.

MCDONAGH, J. C., BINDER, M. D., REINKING, R. M. & STUART, D. G. (1980*a*). Tripatite classification of motor units of cat tibialis posterior. *Journal of Neurophysiology* **44**, 696–712.

MCDONAGH, J. C., BINDER, M. D., REINKING, R. M. & STUART, D. G. (1980*b*). A commentary on muscle properties in cat hindlimb muscles. *Journal of Morphology* **166**, 217–230.

NACHLAS, M. M., TSOU, K. C., DESOUSA, E., CHENG, C. S. & SELIGMAN, A. M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new *p*-nitrophenyl substituted ditetrazole. *Journal of Histochemistry and Cytochemistry* **5**, 420–436.

OWENS, R. (1978). An isolated perfused rat skeletal muscle preparation. *Journal of Physiology* **277**, 9*P*.

PEARSE, A. G. E. (1968). *Histochemistry: Theoretical and Applied*, vol. 1, 3rd edn, p. 660. London: J. and A. Churchill.

RALL, J. A., WILSON, M. G. A. & WOLEDGE, R. C. (1986). Variation in tetanus force production per cross-sectional area in single fibres from frog skeletal muscle. *Journal of Physiology* **371**, 169*P*.

RANATUNGA, K. W. (1984). The force–velocity relation of fast- and slow-twitch muscles examined at different temperatures. *Journal of Physiology* **351**, 517–529.

STEPHENSON, D. G. & WILLIAMS, D. A. (1981). Calcium-activated force responses in fast- and slow-twitch skinned muscle fibres of the rat at different temperatures. *Journal of Physiology* **317**, 281–302.