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MOTOR UNIT ORGANIZATION OF HUMAN MEDIAL GASTROCNEMIUS

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SUMMARY

1. The properties of fifty-seven motor units in human medial gastrocnemius have been studied using controlled intramuscular microstimulation, glycogen depletion and muscle biopsy.

2. Motor units could be divided into three classes on the basis of their mechanical properties. Type S units were slow, small and fatigue resistant. Type FR units were fast, intermediate in size, and fatigue resistant. Type FF units were fast, large and fatigable.

3. Glycogen depletion of a number of type S and FF units revealed them to be composed of type 1 and type 2b muscle fibres respectively.

4. The results suggest that during slowly increasing voluntary contractions where units are recruited in order of size, type 1 and 2a muscle fibres would be employed at low force levels followed by type 2b muscle fibres in stronger contractions.

INTRODUCTION

Motor units in the medial gastrocnemius muscle of the cat may be grouped into three major classes on the basis of their contractile properties. Slowly contracting units form a homogeneous group developing low tetanic tensions and showing considerable resistance to fatigue. Histochemically such units are composed of type 1 (or B) muscle fibres that stain weakly for myosin ATPase (pH 9·4), and strongly for oxidative enzymes such as succinic dehydrogenase. The faster units are less uniform than the slow group, developing a wide range of tetanic tension and possessing a range of fatigue resistance. However, they can be divided into two groups, one of which contains units, composed of type 2a (or C) muscle fibres, which are resistant to fatigue while the other consists of highly fatigable units composed of type 2b (or A) muscle fibres. (Burke, Levine, Zajak, Tsairis & Engel, 1971).

The purpose of the present investigation was to determine whether a tripartite scheme of motor unit classification also applies to human as well as cat gastrocnemius and additionally to determine the histochemical constitution of the muscle fibres belonging to the various physiologically defined unit types. The value of such information concerning the histochemistry of human motor units, apart from the question of the generality of motor unit organization across different species, is that it provides some insight into the recruitment of muscle fibres during *voluntary* movements. Since it is known that motor units are recruited in order of increasing size and fatigability

(Milner-Brown, Stein & Yem, 1973; Stephens & Usherwood, 1977), a knowledge of the histochemical composition of motor units enables inferences to be made concerning the likely recruitment pattern of the muscle fibre types when such units are ranked by these parameters.

The introduction of controlled intramuscular microstimulation for stimulating single motor units in human muscle (Taylor & Stephens, 1976), together with glycogen depletion and a muscle biopsy (Bosley, O'Donovan, Stephens, Taylor & Usherwood, 1976) has made it possible for the first time to examine motor unit properties in man in similar detail to that achieved in animals.

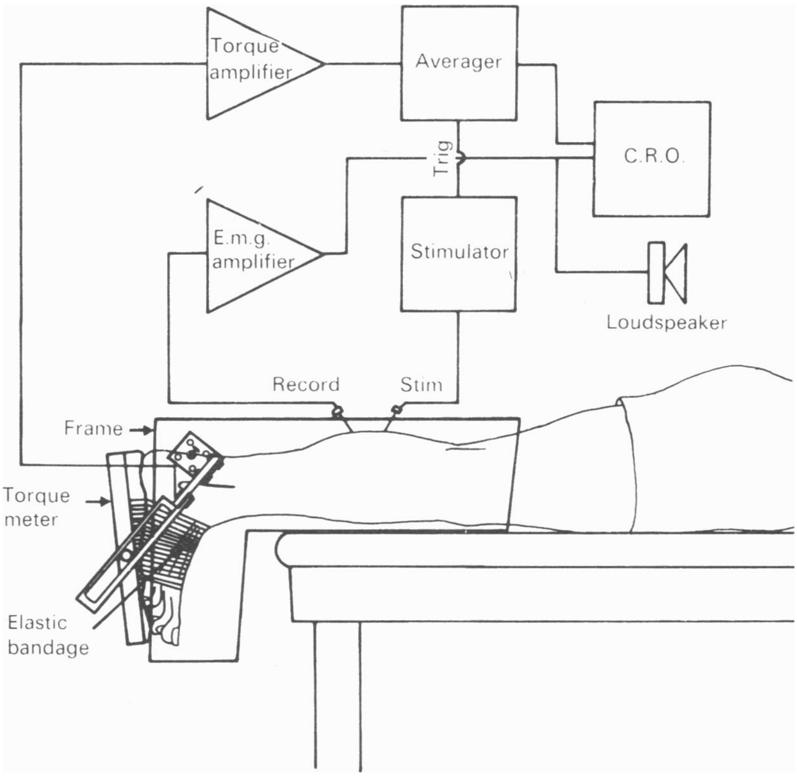

Fig. 1. The experimental arrangement for the recording of motor unit contraction characteristics.

METHODS

Fifty-seven motor units were studied in the medial gastrocnemius muscle of thirteen healthy male subjects who, with one exception, were not currently involved in sports requiring excessive exercise. Subjects were five medical students, seven members of the academic staff and one technician. The age range was 22–46 years. The agreement of this Institution's ethical committee was obtained for these experiments.

The experimental arrangement is shown in Fig. 1. Motor units were isolated by the method of controlled intramuscular microstimulation (Taylor & Stephens, 1976). A bipolar stimulating electrode (Medelec type E/ND1) was inserted into the proximal one third of the muscle near its motor point, and a unipolar concentric e.m.g. needle was situated a few centimetres distally. The stimulating current was delivered from a constant current device (Merrill, 1972) which allowed fine adjustment of the current in steps of less than 1 μA (pulse width 50 or 100 μsec). This was arranged to trigger an oscilloscope time base at 2 msec/cm at 2 or 5 pulses/sec, which displayed e.m.g. activity recorded by the unipolar electrode, and in addition an averaging computer to which was fed the torque signal recorded about the ankle in response to the unit contractions. The bandwidth of the e.m.g. amplifier (Medelec type AA6, Mk. II) was generally set to be flat between 32 Hz and 32 kHz. The criteria for single unit activation were those described by Taylor & Stephens (1976).

Torque developed at the ankle joint in response to a unit contraction was measured as described by Bosley *et al.* (1976). In general thirty-two or sixty-four successive contractions were averaged, and occasionally 256. Tension in the tendon was estimated by dividing the measured torque by the distance between the axis of rotation of the ankle joint and the Achilles tendon, for which a mean value of 6 cm was used (Haxton, 1944).

Muscle length was held constant during the experiments with the knee extended and the ankle dorsiflexed to 85°. It is known that gastrocnemius produces its maximum force with the knee extended, and with the ankle dorsiflexed to 85° the muscle length was probably close to the optimum for whole muscle twitch tension. Because some units could only be successfully isolated for a relatively short period of time, measurements of contractile parameters for all units in the study were performed on the unpotentiated twitch at the same muscle length for each unit.

Although it is known from animal work that the contractile properties of individual units are dependent both on the muscle length and on the prior activity of the unit, and that these effects vary between individual units (Bagust, Knott, Lewis, Luck & Westerman, 1973; Bagust, 1974; Stephens & Stuart, 1975), it is unlikely that this potential source of variation was of great significance in interpretation of the present motor unit data as these effects do not appear significantly to distort the qualitative relations between unit classes in animal studies (Burke, Levine, Tsairis & Zajac, 1973; Reinking, Stephens & Stuart, 1975).

In order to deplete the muscle fibres of a particular unit of glycogen, the unit was stimulated repetitively for up to 2·5 hr at a frequency compatible with maintained unit isolation. A muscle biopsy was then performed (Bosley *et al.* 1976) and the sample frozen into a chuck and stored in liquid nitrogen at − 170 °C before the cutting of serial sections. Sections were stained for myosin ATPase pH 9·4 (Padykula & Herman, 1955), succinic dehydrogenase (Pearse, 1960), and glycogen (PAS, McManus, 1946). Because of the limited glycogen depletion data on normals, reference will also be made to one unit studied by glycogen depletion in isolated perfused human peroneus longus muscle. A description of the techniques involved has already been published (O'Donovan, Rowlerson & Taylor, 1976*a*, *b*).

RESULTS

The distribution of various mechanical properties

The distributions of unit contraction time (time from motor unit action potential to peak twitch force), size and fatigue index are displayed in Fig. 2. The fatigue index is the ratio of the isometric tension after 3000 successive stimuli to the starting isometric tension. A unit which is very susceptible to fatigue therefore has a low fatigue index and an index greater than unity indicates a potentiation. Stimuli were usually given at 10 or 20 pulses/sec for 0·5 sec repeated once per second or once per 2 seconds. The isometric tensions compared were generally the tetanic tensions at 10 or 20 pulses/sec, but were occasionally the twitch tensions. Contraction times were distributed over a wide range (40–110 msec), mean 75·5 ± S.D. 21·4 msec, with a major peak in the distribution between 60 and 80 msec, and a smaller peak between 100 and 120 msec. The distribution of motor unit twitch tensions was positively skewed as has been reported previously for fast twitch muscles in animals (Wuerker, McPhedran & Henneman, 1965; Bagust *et al.* 1973) with the majority of units developing twitch forces between 4 and 20 g wt. Twitch tensions estimated at the tendon covered a wide

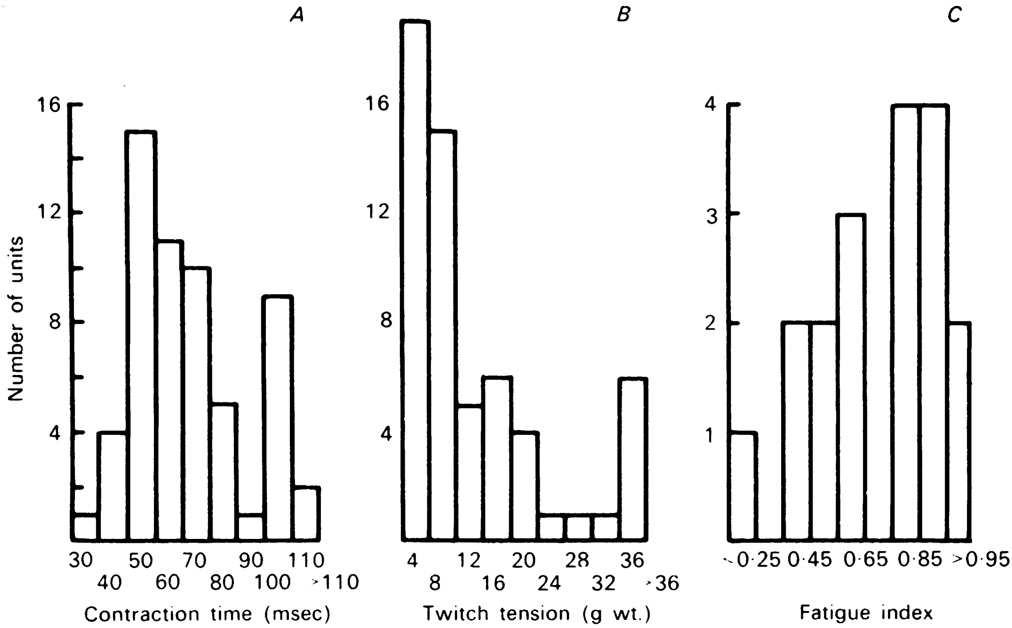

Fig. 2. The distribution of contraction times (*A*) and twitch tensions (*B*) for fifty-eight medial gastrocnemius motor units, and fatigue indices (*C*) for eighteen of these units.

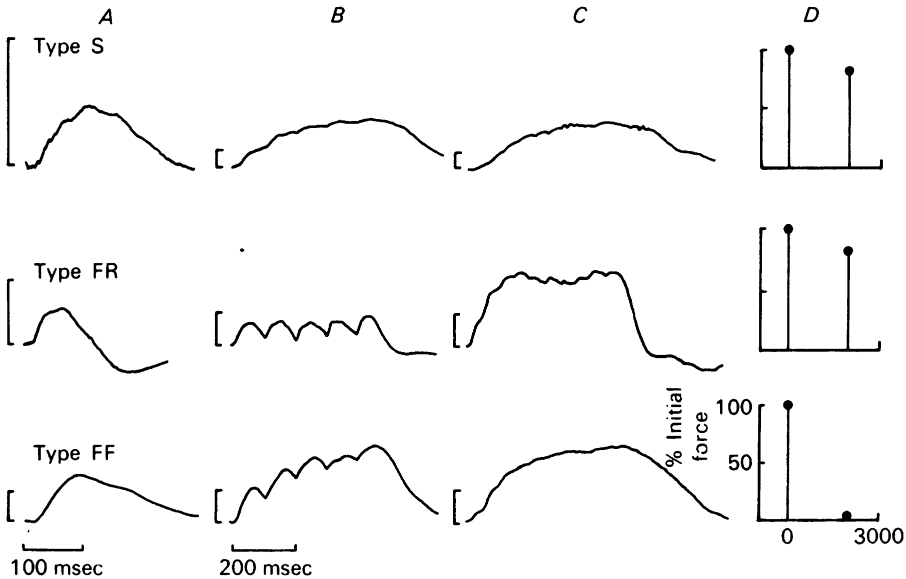

Fig. 3. Examples of the three motor units types found in human medial gastrocnemius. *A*, isometric twitch. *B*, isometric tetanus 10 pulses/sec. *C*, isometric tetanus 20 pulses/sec. *D*, fatigue test, control and after 3000 stimuli, expressed as a percentage of initial isometric tension.

range from 1·5 to 203·5 g wt. with an average value of 20·4 ± 31·1 g wt. The fatigue indices of eighteen of these units (Fig. 2*C*) ranged from 0 to 1·23 (mean 0·69 ± 0·28) with most units having a fatigue index between 0·4 and 1·0.

The division of motor units into sub-populations on the basis of mechanical properties

Motor units were divided into three groups, examples of which are illustrated in Fig. 3. Units whose contraction time was < 85 msec were designated type F, while those with contraction times > 99 msec were designated type S. The two populations separated in this way displayed characteristic differences with regard to other mechanical properties. Thus the type F units formed a heterogeneous group with

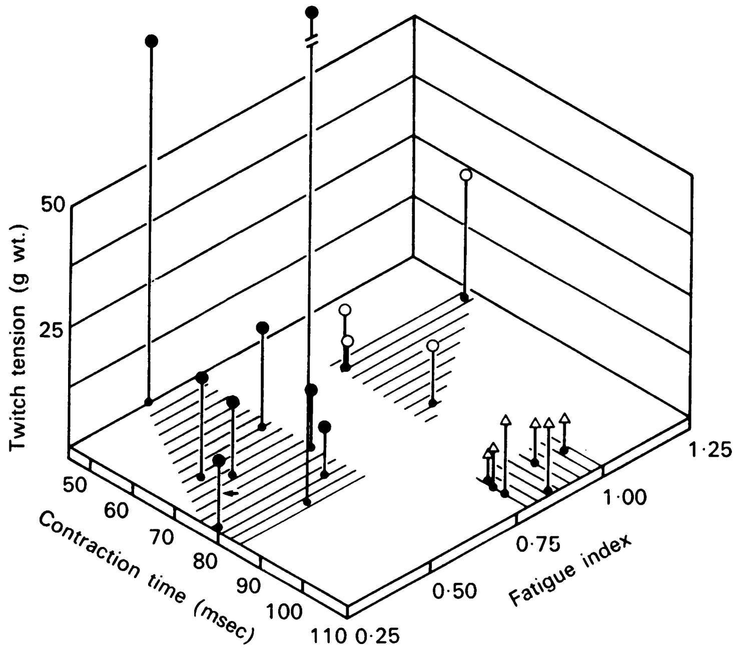

Fig. 4. Three-dimensional graph relating twitch tension, twitch contraction time and fatigue index for eighteen medial gastrocnemius motor units. Type S, 0 Type FR, 0 Type FF. The arrowed unit had a fatigue index of 0·0.

twitch tensions ranging from 4·6 to 203·5 g wt. (mean 23·3 ± 35·1 g wt.) and fatigue indices between 0 and 1·23 (mean 0·62 ± 0·31). The type S units on the other hand, were on average smaller (mean 11·5 ± 5·0 g wt., $P < 0.025$) and more fatigue resistant (mean 0·84 ± 0·08, $P < 0.025$) than their faster counterparts and displayed significantly less variability than the fast units.

Closer examination of the type F population revealed a discontinuity in the distribution of fatigue indices between 0·63 and 0·82. Units with fatigue indices < 0·63 (type FF) were significantly larger (mean twitch tension 46·3 ± 67·0 g wt., $P < 0.025$) than units with fatigue indices > 0·82 (type FR) whose average tension was 13·8 ± 8·9 g wt. ranging from 5·5 to 26·4 g wt. Mean contraction times for the FF and FR groups were similar at 69·6 ± 12·7 msec, and 64·5 ± 9·0 msec, respectively. These relations are conveniently summarized in the three-dimensional graph of Fig. 4.

TABLE 1. Summary of physiological and histochemical properties of glycogen depleted units in human muscle

Muscle	MATPase	SDH	Twitch tension (g wt.)	Contraction time (msec)	Fatigue index	Type		Number of depleted fibres
						Hist.	Phys.	
Peroneus longus*	High	Low	5·3	44	0·00	2b	FF	5
MG	Low	High	7·1	102	0·98	1	S	2
MG	Low	Int→High	5·5	60	0·86	1	FR	2
MG	Low	High	15·5	105	0·79	1	S	2
MG	Low	Intermediate	9·4	102	0·77	1	S	3
MG	High	Low	3·3	62	—	2b	F?	7

* Perfused.

Glycogen depletion of single motor units

A total of five examples of glycogen depletion will be considered – four in normal muscle and one in arterially diseased muscle. Table 1 displays the histochemical and physiological data for four units where glycogen depletion revealed a single fibre type, and for two depleted units within the same muscle. For the cases where a single histochemical muscle fibre type was glycogen depleted, three of the biopsies contained depleted type 1 fibres, and the remaining, in perfused peroneus longus, contained a depleted type 2b fibre. The contraction times of the motor units associated with the

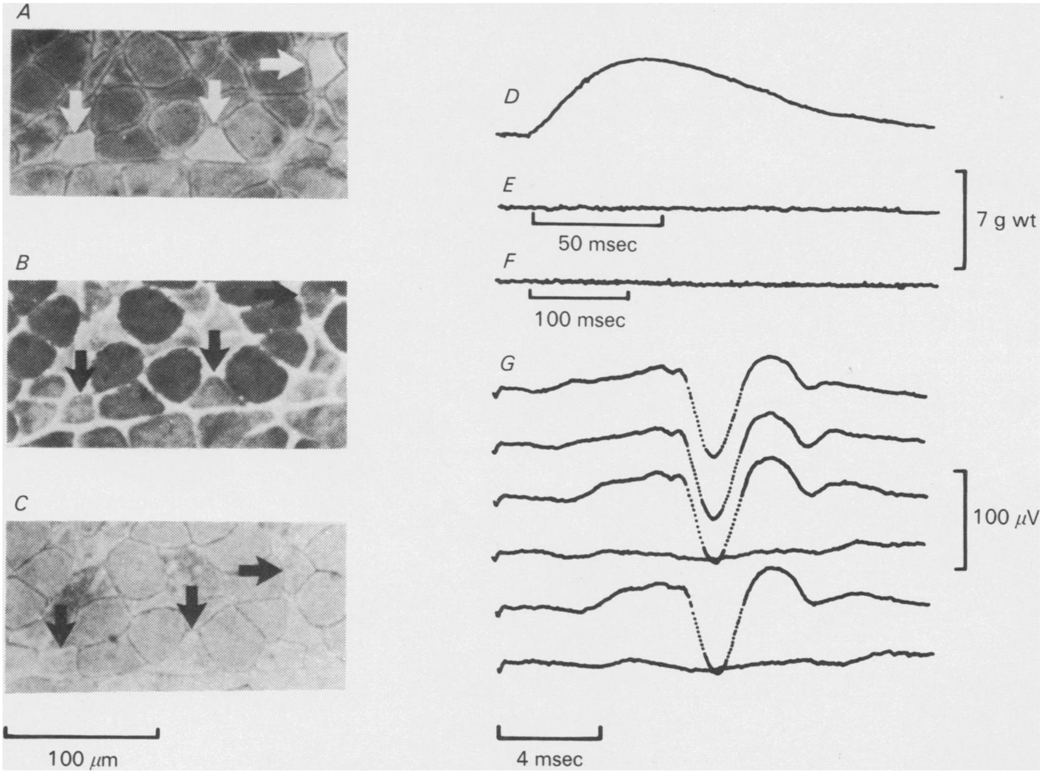

Fig. 5. Histochemical, mechanical and electrical records of a glycogen depleted unit in perfused human peroneus muscle. The photomicrographs are of serial sections through the muscle stained for glycogen (A), MATPase pH 4·2 (*B*), and succinic dehydrogenase (*C*). Three glycogen depleted fibres are arrowed which were high in MATPase (pH 9·4) and low in oxidative enzymes, type 2b muscle fibres. The photomicrograph in *B* of MATPase at pH 4·2 is shown because the section stained for this enzyme at pH 9·4 unsuitable for photography. At pH 4·2 fibres staining low would stain high at pH 9·4 In *D–F* the isometric myograms of the associated motor unit twitch (*D*), its control with subthreshold stimulation(*E*) and fatigue test after 3000 contractions of (*F*) are shown. This unit was type FF. (*G*) shows the all-or-nothing e.m.g. response to stimuli at threshold strength.

type 1 fibres were 60, 102 and 105 msec. All three of these units were relatively small with twitch tensions of 5·5, 7·1 and 15·5 g wt., and all units were fatigue resistant (fatigue indices 0·86, 0·98 and 0·79). The type 2b depleted fibres (Fig. 5) were associated with a fast, small and highly fatigable motor unit (contraction time 44 msec, twitch tension 5·3 g wt., fatigue index 0·0). In Fig. 6 a further example of the motor unit mechanical records and the histochemical sections showing the glycogen depleted fibre is illustrated. In this case the motor unit was type S, and was associated with type 1 depleted muscle fibres.

The remaining example of glycogen depletion was particularly interesting in that the biopsy contained two different types of depleted muscle fibres (type 1 and type 2b) which arose from the combined stimulation of two different motor units. At the start of this particular experiment an apparently 'single' unit was isolated with a contraction time of 102 msec, twitch tension 9·4 g wt. and fatigue index 0·77. During

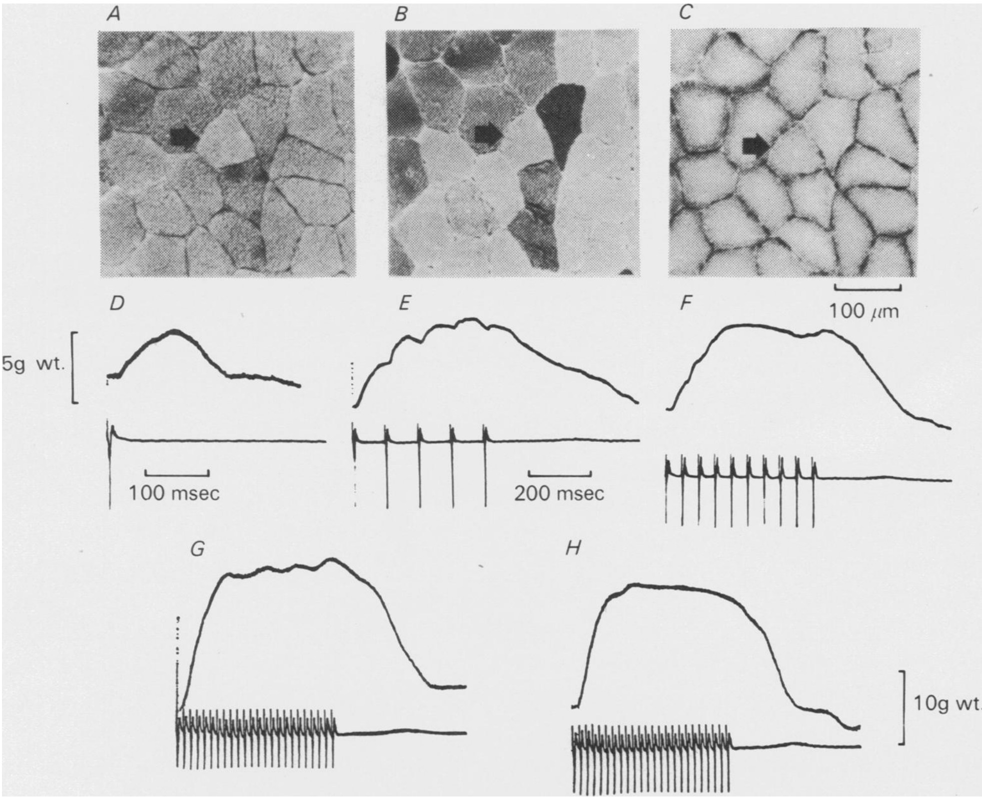

Fig. 6. Histochemical, mechanical and electrical records of a glycogen depleted type S motor unit in medial gastrocnemius. The photomicrographs are stained for glycogen (*A*), MATPase pH 9·4 (*B*) and succinic dehydrogenase (*C*). The depleted fibre which is arrowed is a type 1 muscle fibre. Records *D–H* display the mechanical properties of the stimulated motor unit. Single twitch (*D*), tetanus 10 pulses/sec (E), tetanus 20 pulses/sec (*F*), tetanus 50 pulses/sec (*G*), tetanus 50 pulses/sec after 3000 contractions (*H*).

prolonged stimulation of this 'unit' it decomposed into two units, one with a much faster contraction time of 60 msec. Although the fatigability of the individual units was not determined their combined fatigue index was 0·77. It seems reasonable to suppose that the slower unit was composed of type 1 fibres and the faster unit of type 2b fibres.

Motor units with a contraction time > 100 msec and fatigue indices > 0·77 were all composed of type 1 muscle fibres. For the fast units two were associated with type 2b depleted fibres and one with type 1 depleted fibres. The finding of depleted type 1 muscle fibres associated with a type FR unit was somewhat surprising in view of the well established relations between motor unit mechanical properties and histochemistry in animal muscle. The most likely explanation for this apparently anomalous finding is the erroneous association of glycogen depleted fibres in the muscle biopsy with the particular motor unit being stimulated. Counts of glycogen depleted fibres made in biopsies from normal human muscle revealed the occasional occurrence of a fibre depleted of glycogen. Although the frequency of such fibres in the size of biopsy samples used in this study would be very low, there is clearly a small but definite chance that an unstimulated fibre which is low in glycogen could be mistakenly associated with the stimulated motor unit. In view of the consistency of the other results it is probable that this has occurred in the case of the type FR unit already discussed.

DISCUSSION

Data relating to the detailed organization and contraction characteristics of human muscle is still not plentiful. An early attempt to record contractile properties of whole human muscles was described by Buller, Dornhorst, Edwards, Kerr & Whelan in 1959. In 1970, Buchthal & Schmalbruch recorded the contraction times and forces of small fibre bundles in a number of human muscles. In this latter study a few motor units were also examined for the first time using spike-triggered force averaging. The first extensive report of human motor unit properties was that of Sica & McComas (1971) using graded percutaneous stimulation of motor nerves in extensor hallucis brevis. More recently spike-triggered force averaging has been employed to study motor unit contractions in human hand muscles (Milner-Brown *et al.* 1973; Stephens & Usherwood, 1977). The present work extends these observations to the medial gastrocnemius muscle, and for the first time combines the physiological characterization of motor units with the histochemical typing of their constituent muscle fibres. Furthermore, the present results strongly suggest that the three-way classification of motor units well established for cat medial gastrocnemius, also applies to human muscle.

The inherent difficulties of human experiments and the reluctance to impose upon subjects unduly has necessarily restricted the sample size, so that one cannot be confident of having explored the full range of unit properties. In addition, the methods of unit isolation employed in this study are inherently biased against both the largest and smallest units in the muscle. The largest unit contractions are incompatible with stable unit isolation, while the smallest are difficult to resolve in the mechanical noise. Nevertheless, the data as summarized in Fig. 3 can only be accepted as supporting the usefulness of a three-way division of human motor units according to mechanical properties. It may be that further work will reveal the discontinuities in the distributions to be relative rather than absolute.

The demonstration that type S, and type FF units are composed of type 1 and type 2b muscle fibres, with the probability that the type FR fibres are composed of type 2a muscle fibres allows some deductions to be made concerning the likely mode of muscle fibre type recruitment during a graded isometric voluntary contraction. It is now well established for a variety of human muscles that during a slowly increasing isometric voluntary contraction, motor units are recruited in order of increasing size (Milner-Brown *et al.* 1973; Stephens & Usherwood, 1977; Yemm, 1977; Desmedt & Godaux, 1977). Assuming this applies to medial gastrocnemius, the pattern of muscle fibre type recruitment can be deduced for the present sample of units by ranking the unit types in order of increasing size. The outcome of such an analysis on the present unit population reveals that at low force levels both type S and type FR unit would be recruited. Above about 30% of the total cumulative twitch tension only type FF units would be additionally recruited. Taken together with the glycogen depletion evidence this suggests that low forces in this muscle are sustained by the activity of *both* type 1 and type 2a fibres, while higher force levels are achieved by the additional recruitment of motor units composed of type 2b fibres.

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REFERENCES

Bagist, J. (1974). Relationships between motor nerve conduction velocities and motor unit contraction characteristics in a slow twitch muscle of the cat. *J. Physiol.* **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. *J. Physiol.* **231**, 87–104.

Bosley, M. A., O'Donovan, M. J., Stephens, J. A., Taylor, A. & Usherwood, T. P. (1976). Unit studies in normal human gastrocnemius by micro-stimulation, glycogen depletion and needle biopsy. *J. Physiol.* **260**, 11–12*P*.

Buchthal, F. & Schmalbruch, H. (1970). Contraction times and fibre types in intact human muscle. *Acta physiol. scand.* **79**, 435–452.

Buller, A. T., Dornhorst, A. C., Edwards, R., Kerr, D. & Whelan, R. F. (1959). Fast and slow muscles in mammals. *Nature, Lond.* **183**, 1516–1517.

Burke, R. E., Levine, D. N., Zajac, F. E., Tsairis, P. & Engel, W. K. (1971). Physiological-histochemical correlation in three fibre types in cat gastrocnemius. *Science, N.Y.* **174**, 709–712.

Burke, R. E., Levine, D. N., Tsairis, P. & Zajac, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J. Physiol.* **234**, 723–748.

Desmedt, J. E. & Godaux, E. (1977). Ballistic contractions in man. Characteristic recruitment pattern of single motor units of the tibialis anterior muscle. *J. Physiol.* **264**, 673–693.

Haxton, H. A. (1944). Absolute muscle force in the ankle extensors of man. *J. Physiol.* **103**, 267–273.

McManus, J. F. A. (1946). Histological demonstration of mucin after periodic acid. *Nature, Lond.* **158**, 202.

Merrill, E. G. (1972). A simple light-coupled stimulus isolator. *J. Physiol.* **222**, 120–121*P*.

Milner-Brown, H. S., Stein, R. B. & Yemm, R. (1973). The orderly recruitment of human motor units during voluntary isometric contractions. *J. Physiol.* **230**, 359–370.

O'Donovan, M. J., Rowlerson, A. & Taylor, A. (1976*a*). The perfused isolated human limb: an assessment of its viability. *J. Physiol.* **256**, 27–28*P*.

O'Donovan, M. J., Rowlerson, A. & Taylor, A. (1976*b*). Contraction characteristics and histochemistry of motor units studied in perfused human limb muscles. *J. Physiol.* **257**, 24–25*P*.

Padykula, H. A. & Herman, E. (1955). The specificity of the histochemical method for adenosine-triphosphatase. *J. Histochem. Cytochem.* **3**, 170–195.

Pearse, A. G. E. (1960). *Histochemistry. Theoretical and Applied*, 2nd edn. London: Churchill.

Proske, U. & Waite, P. M. E. (1974). Properties of type of motor units in the medial gastrocnemius muscle of the cat. *Brain Res.* **67**, 89–102.

REINKING R. M., STEPHENS, J. A. & STUART, D. G. (1975). The motor units of cat medial gastrocnemius: Problem of their categorization on the basis of mechanical properties. *Exp. Brain Res.* **23**, 1–13.

SICA, R. E. P. & MCCOMAS, A. J. (1971). Fast and slow twitch units in a human muscle. *J. Neurol. Neurosurg. Psychiat.* **34**, 113–120.

STEPHENS, J. A. & STUART, D. G. (1975). The motor units of cat medial gastrocnemius: speed-size relations and their significance for the recruitment order of motor units. *Brain Res.* **91**, 177–195.

STEPHENS, J. A. & USHERWOOD, T. P. (1977). The mechanical properties of human motor units with special reference to their fatigability and recruitment threshold. *Brain Res.* **125**, 91–97.

TAYLOR, A. & STEPHENS, J. A. (1976). Study of human motor unit contractions by controlled intramuscular microstimulation. *Brain Res.* **117**, 331–335.

WUERKER, R. B., MCPHEDRAN, A. M. & HENNEMAN, E. (1965). Properties of motor units in a heterogeneous pale muscle (m. gastrocnemius) of the cat. *J. Neurophysiol.* **28**, 85–99.

YEMM, R. (1977). The orderly recruitment of motor units of the masseter and temporalis muscles during voluntary isometric contraction in man. *J. Physiol.* **265**, 163–174.