PHYSIOLOGICAL TYPES AND HISTOCHEMICAL PROFILES IN MOTOR UNITS OF THE CAT GASTROCNEMIUS

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SUMMARY

1. A variety of physiological properties of single motor units have been studied in the gastrocnemius muscle (primarily in the medial head) of pentobarbitone-anaesthetized cats. Intracellular stimulation of individual motoneurones ensured functional isolation of the muscle units innervated by them.

2. A system for muscle unit classification was developed using a combination of two physiological properties. Almost all of the units studied could be classified into one of three major types, including two groups with relatively short twitch contraction times (types FF and FR, which were differentiable from one another on the basis of sensitivity to fatigue) and one group with relatively long contraction times (type S, which were extremely resistant to fatigue and were differentiable from FF and FR units on the basis of the shape of unfused tetani). Post-tetanic potentiation of twitch responses was observed in all three muscle unit types. The distributions of axonal conduction velocities for motoneurones innervating FF and FR muscle units were essentially the same, while conduction velocities for motoneurones innervating type S units were, in general, slower.

3. Histochemical profiles of muscle units representative of each of the physiological classes present in the gastrocnemius pool were determined using a method of glycogen depletion for muscle unit identification. Each of the physiological categories of muscle units exhibited a corresponding

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unique set of muscle fibre staining reactions, or histochemical profile. Within each physiological type, all of the units examined had the same histochemical profile. The results generally support the hypothesis that the histochemical characteristics of muscle fibres are meaningfully related to the physiological properties of the same fibres. However, certain limitations in the detailed application of the hypothesis were also apparent.

4. Systematic assessment of the histochemical profiles of relatively large numbers of fibres belonging to single muscle units provided strong support for the hypothesis that all of the muscle fibres innervated by a single α-motoneurone are histochemically identical.

INTRODUCTION

Since Ranvier's observation that the colour and histological features of whole muscles are in many cases correlated with their speed of contraction (Ranvier, 1874), it has become generally accepted that the increasingly well-studied morphological and histochemical characteristics of individual muscle fibres must be related in some way to the physiological properties of the same fibres (e.g. Henneman & Olson, 1965; Olson & Swett, 1971; see review by Close, 1972). However, experimental evidence supporting the above hypothesis, while considerable and intuitively satisfying, has until recently been indirect and in some respects contradictory (Denny-Brown, 1929; Gauthier, 1969; Hall-Craggs, 1968; Nyström, 1968).

Two recent experimental approaches have been used to test directly whether the structural and histochemical characteristics of muscle fibres are indeed correlated with physiological properties. Lännergen & Smith (1966) investigated the contractile responses, morphology and histo-chemistry in dissected single fibres of toad muscle. In mammalian muscle, Edström & Kugelberg (1968) studied twitch contractions and fatigue characteristics in a small sample of motor units in the rat tibialis anterior muscle. They were able to identify the muscle fibres innervated by a single motoneurone axon (called hereafter the 'muscle unit' portion of the motor unit; cf. Burke, 1967), using a method for depletion of muscle fibre glycogen following prolonged repetitive stimulation. The technique permitted histochemical examination of the stimulated fibres in some, but not all, of the motor units studied physiologically. Edström & Kugelberg found a correlation between relative resistance to fatigue and the apparent activity of oxidative enzyme systems in the same fibres, but no apparent relation between the twitch contraction times and histochemical appearance.

The present study was designed to assess the possible interrelations between physiological properties exhibited by single muscle units in the

medial and lateral gastrocnemius muscle of the cat and the histochemical and morphological features characteristic of muscle fibres making up the same units. A glycogen depletion method similar in principle to the one described by Edström & Kugelberg (1968) was used to 'mark' the muscle fibres in physiologically studied units for histochemical analysis. Units representative of the entire population of motor units in the cat gastrocnemius pool were successfully analysed and a system for classification of motor units, based on physiological properties, was developed which permitted exact matching with the histochemical categories of their muscle fibres. A preliminary report of some of this material has appeared elsewhere (Burke, Levine, Zajac, Tsairis & Engel, 1971).

METHODS

Adult cats (2·0–3·3 kg) were anaesthetized with Halothane in 98 % O_2–CO_2 during surgical preparation. Details of preparation and mounting have been given elsewhere (Burke, 1967). Briefly, the medial and lateral gastrocnemius muscles were carefully separated, preserving their blood supplies. The tendons were arranged for separate attachment to a strain gauge through short lengths of no. 2 braided silk suture (compliance 0·08 mm/kg.cm.) The nerves to both medial and lateral muscles were freed of surrounding tissue and placed intact on flexible stimulating electrodes. The lumbosacral spinal cord was exposed and the animals were mounted in a rigid frame immobilizing the spinal column and left and right hind limbs. Exposed tissues were covered with mineral oil kept at 36–38° C with DC heating coils. Body temperature was regulated with heating pads. Blood pressure and in many experiments expired CO_2 were monitored continuously.

Single motor units were studied in functional isolation by intracellular stimulation (depolarizing pulses lasting 0·5 msec) of the innervating motoneurones through micropipette electrodes filled with 3 M-KCl (Burke, 1967). Following penetration of a medial or lateral gastrocnemius motoneurone, the antidromic conduction time was recorded. The appropriate muscle was then attached to the strain gauge and stretched to a passive tension of about 100 g. Muscle unit responses were thus recorded under quasi-isometric conditions. Passive initial tensions of 80–100 g give nearly maximal twitch tensions with most gastrocnemius muscle units (cf. Burke, 1967) but there was no assessment in the present work whether the optimal initial tension might be different for twitch as opposed to tetanus responses (cf. Lewis & Luck, 1968). The range of passive tensions used in the present experiments correspond to ankle angles (dorsiflexion) of 60–80°.

In searching for motoneurones and obtaining antidromic spike records, it was necessary to stimulate the muscle nerves of the medial and lateral soleus. During such stimulation, both medial and lateral muscles were loaded with a spring so that contraction without load did not occur (cf. Burke, Jankowska & ten Bruggencate, 1970a). Every effort was made to limit the number of whole muscle contractions during the course of individual experiments. These points represent significant differences in technique from earlier motor unit work from this laboratory (Burke, 1967, 1968a, b) and such differences may partially explain variations in the present results from the earlier observations. Twitch tensions produced by the whole medial and lateral gastrocnemius muscles were recorded at the beginning of recording sessions and checked periodically thereafter. Experiments were terminated when fall-off in twitch tensions suggested possible deterioration in the preparation.

The electrical activity of the active muscle units was recorded with fine (AWG 36) flexible stainless-steel wires bared of insulation at the tip and hooked into the fascia over the localized dimpling produced by contraction of the unit.

Experimental procedure

Mechanical and electrical responses of muscle units were recorded photographically during the following sequence of stimulation paradigms: (1) single pulse activation producing what will be referred to as 'initial' twitch responses (cf. Figs. 1*A*, 2*A*, 3*A*); (2) pairs of stimuli at varying intervals (cf. Burke, Rudomin & Zajac, 1970*b*); (3) short (0·8–2·0 sec) trains of pulses at various relatively low frequencies producing unfused tetani (Figs. 1*C*, 2*C*, 3*C*, *D*); (4) alternation between single pulses and short (0·5–2 sec) trains of pulses at 200 pulses/sec (100 pulses/sec with slowly contracting units). Either single pulses or trains were delivered every 3–4 sec in alternation, causing post-tetanic potentiation of the twitch response, until the twitch no longer continued to increase in amplitude. Following completion of the above, the unit under study was subjected to repeated tetanization using trains of pulses composed of 13 pulses recurring at 40 pulses/sec, each train lasting 330 msec. Trains were repeated every 1 sec for at least 5 min in order to assess the sensitivity of the unit to fatigue (Figs. 1*D–F*, 2*D–F*, 3*E–G*).

Histochemical analysis

In cases selected for histochemical analysis, only one muscle unit was examined in each muscle head. The selected unit was studied as above but the repeated tetanization used for fatigue sensitivity study was continued for periods lasting between 15 and 60 min, depending on unit type (cf. below). At the end of the stimulation period, which was designed to deplete glycogen in muscle fibres of the studied unit, the muscle head containing the unit was quickly excised and cut into cross-section blocks 8–10 mm in thickness. The blocks were placed on cryostat chucks in gum acacia and frozen in 2-methyl-butane cooled to $-160°$ C in liquid N_2. Frozen blocks were temporarily stored in dry ice and transferred within a few hours to a freezer maintained at $-80°$ C. The time lag between muscle removal and completion of freezing all blocks was between 2 and 5 min. In a few cases, larger pieces of muscle were directly frozen in liquid N_2.

Serial sections were cut from the muscle blocks at 10–15 μm thickness in a cryostat kept at $-20°$ C. Sections were air dried on cover-slips before staining. Sections were obtained from several blocks along the length of each muscle containing a studied muscle unit. Serial sections from each block were stained for the following substrates: glycogen by the periodic acid–Schiff (PAS) method (McManus & Mowry, 1960); phosphorylase (Barka & Anderson, 1963); reduced diphosphopyridine nucleotide dehydrogenase (DPNHD; Farber, Sternberg & Dunlap, 1956; synonymous with nicotinamide nucleotide dehydrogenase, or NADH diaphorase, or tetrazolium reductase, cf. Pearse, 1968); succinic dehydrogenase (SDH; Nachlas, Tsou, DeSousa, Cheng & Seligman, 1957); and myofibrillar adenosine triphosphatase (ATPase; Padykula & Herman, 1955). Further serial sections were stained for ATPase activity after incubation in one of two acidic buffers, either edetic acid buffer at pH 4·35 (denoted EDTA-ATPase; Drews & Engel, 1966) or veronal acetate buffer at pH 4·65 (Ac-ATPase; Brooke & Kaiser, 1969). The above series of preparations were made with each unit in the present series. In addition, a number of representative unit sections were also examined using the following substrate stains: esterase (Barka & Anderson, 1963); menadione-linked α-glycerophosphate dehydrogenase (M-α-GPD; Pearse, 1968), lactate dehydrogenase (LDH; Brody, 1964); and for neutral fat with Oil Red O (Armed Forces Institute of Pathology, 1949).

<div align="center">RESULTS</div>

The results to be reported are based on study of 117 motor units obtained from fifteen cats. Of this total 105 units were studied in the medial and the remaining twelve in the lateral gastrocnemius. Histochemical analysis was completed successfully on twenty-eight units, sixteen medial and twelve lateral. There were no evident differences between medial and lateral gastrocnemuis units with respect to the material to be discussed.

<div align="center">PART I</div>

Physiological classification of muscle units

The first objective of the present work was to investigate a variety of mechanical response characteristics of muscle units in the gastrocnemius in order to assess the distributions of such properties within the population of units making up this heterogeneous muscle. A corollary aim was to develop a system for classification of muscle units into clearly definable groups using combinations of physiological properties. Single twitch responses before and after post-tetanic potentiation (PTP), tetanic responses to stimulation at various frequencies, and fatigue during prolonged repetitive stimulation were systematically examined. Representative records from three units are shown in Text-figs. 1–3. We found that two physiological parameters – (1) measurement of the sensitivity to fatigue and (2) the shape of the tension envelope during unfused tetani – were best suited to separate the gastrocnemius unit population into unambiguous and non-overlapping groups.

Fatigue sensitivity

Any measurement of muscle fatigue depends on the stimulation paradigm utilized. In selecting a paradigm for the present studies, we attempted to find a stimulation sequence which permitted some differentiation between effects of repeated activation on neuromuscular transmission and the effects on muscle fibres themselves. The stimulation sequence finally chosen (see Methods) was used because it appeared to minimize the possibility of significant degrees of failure of fibre activation during repeated stimulation (cf. below).

The effect of the standard sequence of pulse trains on a muscle unit highly susceptible to fatigue is shown in Text-fig. 1*D* and *E*. During the 30th tetanus in the sequence (*E*, 30 sec; a total of 390 stimuli), the tension produced by the unit began to decline and showed marked slowing, particularly of the falling phase as compared to the initial tetanus response

(*E*, unlabelled). Tension decline was quite profound during the 60th tetanus (*E*, 1 min; a total of 780 stimuli), while the electrical response of the unit (*D*, 1 min) exhibited some widening of individual spikes but no diminution of amplitude. The complete course of maximum tension output during sequential tetanization is shown in Text-fig. 1*F*; after 2 min of stimulation (120 tetani) the tension produced was less than 10 % of that produced by the first tetanus in the sequence.

Greater resistance to fatigue was exhibited by the unit illustrated in
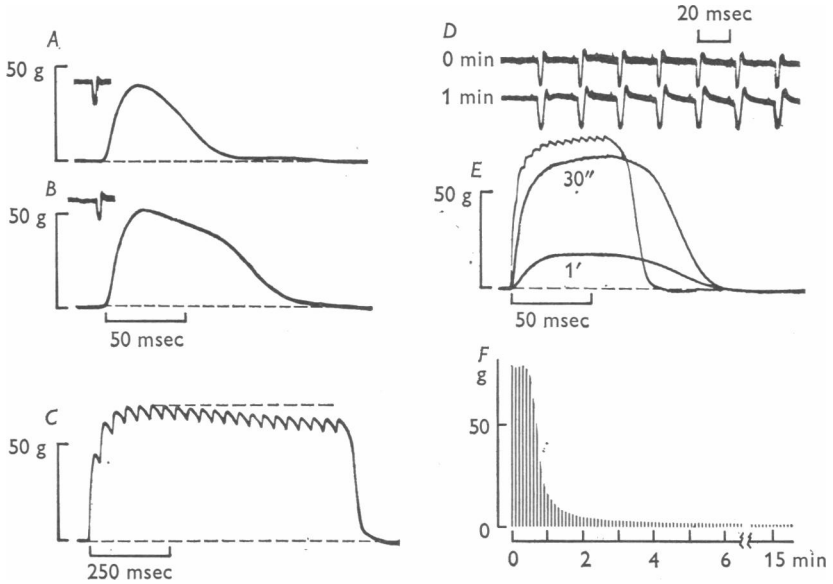
Text-fig. 1. Mechanical and electrical responses of a type FF muscle unit in a lateral gastrocnemius muscle. The histochemical profile of muscle fibres belonging to the same unit is illustrated in Pl. 1. *A*: initial twitch response, with electrical activity (EMG) of the muscle unit above the mechanical record. *B*: twitch response after maximum post-tetanic potentiation (PTP). Note increase in both amplitude and duration of the twitch, without change in the EMG. *C*: unfused tetanus to stimulus train of 25 pulses/sec, showing early peak in tension (about the 6th response) and subsequent decline, or 'sag', in tension toward a lower plateau level. *D* and *E*: records selected from a sequence of responses to short duration (330 msec) 40 pulses/sec tetani recurring every 1 sec. *D*: EMG responses during the first tetanus in the sequence (0 min) and during the 60th tetanus (1 min). *E*: photographic-ally superimposed records of unit mechanical responses during the first (un-marked), the 30th (30 sec) and the 60th (1 min) tetani in the repetitive sequence. Note slowing of the rising and falling phases with onset of fatigue. *F*: graph of maximum tension produced by individual tetani such as shown in *E* for the entire sequence of recurrent tetani. The fatigue index at 2 min was less than 0·1. Interrupted abscissa indicates that the unit was stimu-lated for 15 min with repeated tetani, after which the LG muscle was removed for histochemical analysis (see text).

Text-fig. 2 (*D–F*). Maximum tension production during the 120th tetanus (*E*, 2 min) was nearly the same as that during the initial tetanus (un-labelled) although diminution of the early components of the response was evident, as was slowing of the falling phase. Progression of fatigue was again evident in the 300th tetanus (*E*, 5 min) but, as shown in the graph (Text-fig. 2, *F*) the decline in maximum tension with prolonged stimulation occurred relatively slowly. The unit still produced measurable tension after 50 min of repeated tetanization (a total of about 39,000 stimuli). Electrical
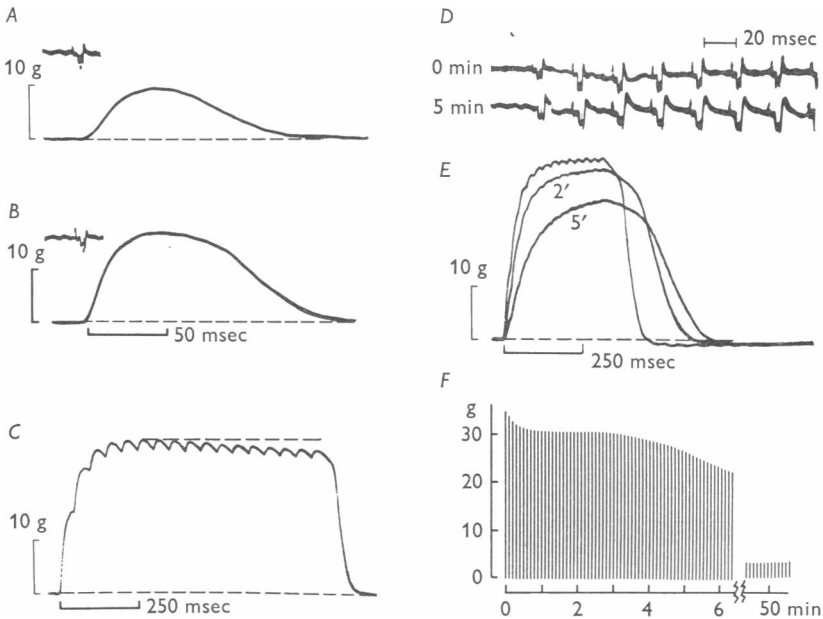
Text-fig. 2. Mechanical and electrical responses of a type FR muscle unit in a medial gastrocnemius muscle. The histochemical profile of unit fibres is shown in Pl. 2. Format of the figure as in Text-fig. 1. This FR unit, like the FF unit in Text-fig. 1, exhibited 'sag' in unfused tetani (*C*), but showed considerably more resistance to fatigue during repeated tetanization (*E*, *F*). The fatigue index at 2 min was > 0.9. After 50 min of repeated tetanization (graph in *F*) the FR unit still produced some tension and at this point the medial muscle containing the unit was removed for histochemical processing.

responses of the unit showed only some broadening of spikes after 5 min of stimulation. In contrast to the above, the muscle unit shown in Text-fig. 3 exhibited no fatigue during prolonged tetanization. Mechanical and electrical responses obtained after 60 min of stimulation (3600 tetani, or about 47,000 stimuli) were essentially superimposable on the initial responses (*E*, *F*).

Considering the fatigue curves (Text-figs. 1*F*, 2*F*, 3*G*) from the entire sample of gastrocnemius units studied, it was striking that there were few units with curves intermediate between the rapid decline group (Text-fig. 1*F*) and the more slowly declining group (Text-fig. 2*F*). As a single measure of the difference between such curves, we have taken the ratio of
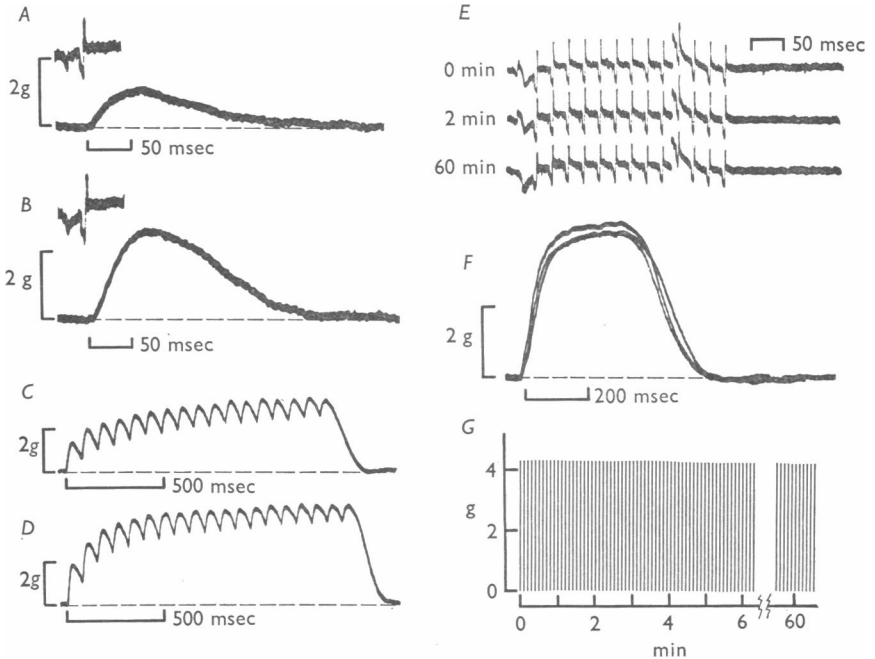
Text-fig. 3. Mechanical and electrical records of a type S muscle unit in lateral gastrocnemius muscle. Format as in Text-figs. 1 and 2, except that unfused tetani are shown for two slightly different stimulation frequencies (*C* and *D*). Note absences of 'sag' in these responses. Twitch response before (*A*) and after (*B*) tetanization showed significant PTP, without any change in the unit EMG. The extreme resistance of this unit to fatigue is illustrated by the records and graph in *E*, *F* and *G*. Mechanical records during the first (0 min), 60th (2 min) and 3600th (60 min) tetanus are shown photographically superimposed in *F*. EMG records during the same tetani showed no changes (*E*). The histochemical profile of the unit fibres is illustrated in Pl. 3.

maximum tension produced during the 120th tetanus (i.e. after 2 min of stimulation) to the tension output during the first tetanus in the standard sequence, calling this ratio the 'fatigue index'. The fatigue index was obviously much smaller (< 0.25) for the unit shown in Text-fig. 1 than for the other two units illustrated (> 0.75).

Diminution in tension output from an indirectly stimulated muscle unit may be due to fatigue of the contractile mechanism within the muscle fibres themselves (cf. Eberstein & Sandow, 1963), to failure of activation of some unit fibres due to presynaptic factors such as diminution of neuromuscular transmitter output (cf. Brooks & Thies, 1962) or failure of action potential invasion into motoneurone telo-dendria (Krnjević & Miledi, 1958a), or indeed to a combination of the above. The possible existence of failure of fibre activation could not be directly assessed in the present work. However, during the first 2 min of sequential tetanization there was in most units little or no decline in the amplitude of muscle unit action potentials recorded from the muscle surface, irrespective of the changes in mechanical output (e.g. Text-fig. 1*D* and *E*). The broadening of electrical unit spikes early in the course of fatigue development may be explainable on the basis of some depression and slowing of muscle fibre action potentials (Bergmans & Maréchal, 1960; Eberstein & Sandow, 1963) and does not necessarily indicate fibre drop-out. The available evidence suggests that the early course of muscle unit fatigue under the present conditions (i.e. up to 2 min of stimulation) probably reflected fatigue intrinsic to the muscle fibres.

The shape of unfused tetani

Unfused mechanical tetani in gastrocnemius muscle units exhibited, with stimulus trains of appropriate frequency, one of two general shapes. The responses illustrated in Text-figs. 1*C* and 2*C*, for example, were characterized by an early tension maximum produced by the first 6 to 7 stimuli, with a subsequent slight decline, or 'sag', in tension to a lower plateau. In contrast, the responses in Text-fig. 3*C* and *D* showed no such 'sag'; rather the tension envelope described by the peaks of individual components rose monotonically to reach a stable plateau at long tetanus durations (cf. Burke *et al.* 1970*b*, Fig. 2).

Assessment of the presence or absence of the 'sag' phenomenon in unfused tetani proved to be a useful criterion in muscle unit classification. In applying this test, however, it was necessary to specify the stimulus frequencies used. Some units, for example those shown in Text-figs. 1 and 2, exhibited 'sag' in unfused tetani produced by a relatively wide range in stimulus frequencies (up to 30–40 pulses/sec) while others (e.g. Text-fig. 3) showed 'sag' only in unfused tetani produced by very low frequency stimulation (usually less than 5 pulses/sec). The 'best' frequencies appeared to be correlated with muscle unit twitch contraction times. In order to avoid possible ambiguity, the 'sag' phenomenon was assessed as present or absent in tetanic responses produced by stimulus trains in which the interpulse intervals were approximately 1·25 times the muscle unit contraction time.

The presence of 'sag' has been noted in unfused tetani of nominally 'fast' muscles such as cat gastrocnemius but not in 'slow' muscles such as the soleus (Cooper & Eccles, 1930). It has also been observed in unfused tetani of fast contracting single muscle units (e.g. Steg, 1964, Fig. 7; Wuerker, McPhedran & Henneman, 1965, Fig. 7). The 'sag' was not due to fibre drop-out in the later stages of tetani, since

the unit action potentials were identical before and after its development. It was also not due to muscle fibre fatigue in the usual sense, since muscle units developed the same maximum tension during fused tetani, whether or not such tetani were superimposed on an unfused tetanus after development of 'sag' (unpublished observations). The phenomenon may be due to a subtle change in the fibre active state (R. E. Burke, P. Rudomin & F. E. Zajac, in preparation), but whatever the mechanism, it appeared to be present in all muscle unit types in the cat gastrocnemius, although expressed in different units at quite different frequencies. For this reason the testing frequencies must be specified.

TABLE 1. Classification of gastrocnemius muscle units using two physiological parameters. Sample population includes all of the units studied in the present series of experiments

| | Fatigue index evaluated at 2 min | | | |
	< 0·25	0·25 < FI < 0·75	> 0·75	Totals
'Sag' present	Type FF $n = 51$	Unclassified $n = 3$	Type FR $n = 32$	86
'Sag' absent	— $n = 0$	— $n = 0$	Type S $n = 31$	31
Totals	51	3	63	117

Unit classification

We have divided the gastrocnemius muscle unit population into three major types using as criteria the fatigue index and presence or absence of 'sag' in standardized tetani. As shown in Table 1, only three of the 117 units studied in the present sample fell outside the major type categories. The letter designations used for each category are meant to suggest key physiological parameters characteristic of the group: 'type FF' – fatigue sensitive units with relatively fast twitch contraction (see below); 'type FR' – fatigue resistant units with fast twitch contraction; 'type S' – very fatigue resistant units with relatively slow twitch contraction. The category designations include specification of twitch contraction speed to indicate continuity of the present results with previous work from this laboratory, in which motor units were designated as 'type F' or 'type S' on the basis of contraction speed (Burke, 1967, 1968a, b). Type S units as presently defined appear to be identical with units of similar designation from the earlier work, since review of the original records from the previous experiments showed that 'type S' units did not show 'sag' while 'type F' units did (for example, see Burke, 1967, Fig. 4). Thus it is assumed that the original 'type F' group must have included rapidly contracting units with varying resistance to fatigue.

Twitch responses and post-tetanic potentiation

Twitch contraction time has been a parameter of interest in most studies of motor unit properties (e.g. Burke, 1967; Olson & Swett, 1971; Wuerker *et al.* 1965). Early in the course of the present work it became apparent that muscle unit twitch characteristics showed marked dependence on the stimulus history, in particular exhibiting considerable post-tetanic potentiation of twitch tension in many units (cf. Close, 1972). Comparing 'initial' twitches (Text-figs. 1*A*, 2*A*, 3*A*) with maximally potentiated twitches (Text-figs. 1*B*, 2*B*, 3*B*; cf. Method), it is evident that PTP prolonged the twitch contraction times and half-relaxation times in the illustrated units as well as causing increased tension output.

The distributions of twitch tension augmentation produced by PTP in muscle units of different types is shown in Text-fig. 4. The graph illustrates the relation found between the amplitude of 'initial' twitches (abscissa) and those of maximally potentiated twitches (ordinate), each point representing a different unit with the unit type denoted by the symbols. It is evident from the graph that there was no significant difference between the various muscle unit types in the amount of PTP exhibited, comparing the data scatter of one type group with another. Units of each type with relatively large 'initial' twitches (for that type) tended to exhibit less increase in twitch tension after PTP than the same type units with smaller 'initial' twitches (cf. Olson & Swett, 1971).

Twitch contraction times measured after PTP tended to be longer in duration than in 'initial' twitches, although a few units in each physiological type group showed decreases in contraction time after PTP. As with the amplitude data in Text-fig. 4, there was no difference between the muscle unit groups in the direction or degree of change in contraction times before and after PTP. It appears reasonable to conclude that all gastrocnemius muscle units in the cat exhibit qualitatively similar responses to repeated tetanization, irrespective of unit type.

Despite the sometimes dramatic changes in mechanical twitch responses with PTP, the muscle unit action potentials recorded from the muscle surface were identical throughout the development of PTP (Text-figs. 1–3), suggesting that presynaptic factors play little or no role in the effect (cf. discussion in Close, 1972). This point is of particular importance with respect to the type S muscle units (e.g. Text-fig. 3). There is evidence that slowly contracting muscle units in the gastrocnemius and soleus muscles of the cat may exhibit repetitive firing to single volleys in the motoneurone axon in the aftermath of a prolonged high-frequency tetanus, with consequent tension augmentation (Olson & Swett, 1971; Standeart, 1964). Such repetitive firing was not observed in any of the type S units in the present experiments, presumably because intermittent and relatively short tetani were used to produce PTP. Thus, the tension and time course changes in unit twitch responses in the present results have been interpreted as 'true' PTP, even among the type S

units. This point is stressed because the response of type S muscle units in the cat soleus to repeated tetanization was found to be qualitatively different (R. E. Burke, D. N. Levine, M. Salcman & P. Tsairis, to be published).

Twitch to tetanus ratios were measured for the present unit sample, using the amplitudes of maximally potentiated twitches, and the maximum tension produced by fused tetani (stimulus frequency 200 pulses/sec for FF and FR units and 100 pulses/sec for type S units). Grouping muscle units together by type, the twitch/tetanus ratios were higher (0·3–0·75; mean 0·58) for type FF units than for type FR (0·25–0·6; mean 0·43) or type S units (0·125–0·5; mean 0·31). These ratios would be smaller and the ranges wider had the values for 'initial' twitches been used in the computation (cf. earlier results, Burke, 1967; Wuerker et al. 1965).
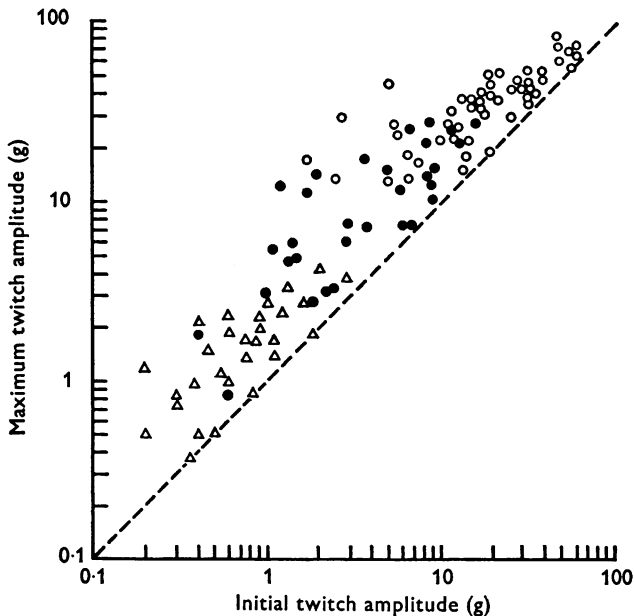

Text-fig. 4. Graph of initial twitch amplitudes (abscissa) and maximum twitch amplitudes after PTP (ordinate) for the entire population of studied gastrocnemius muscle units. Each point represents a different unit, with unit types denoted by the symbols (FF, ○; FR, ●; S, △). Both axes of the graph are logarithmic, permitting direct estimation of the relative change in twitch amplitude with PTP, irrespective of absolute tensions of the individual units. The unit slope line (dashed line) denotes the locus of points showing no change in twitch amplitude. The vertical distance between points for individual units and the dashed line is equivalent to the percentage of tension augmentation.

Physiological profiles of MG muscle units

The distributions of physiological properties of gastrocnemius muscle units were interrelated. The three-dimensional diagram in Text-fig. 5 is intended to convey the interrelations found among four physiological

parameters, limiting the sample population to eighty-one units studied in the medial muscle of three cats. The distributions of unit properties in samples from individual animals were in good agreement and pooling the results seemed reasonable. The experiments were designed to obtain systematic physiological data on as many medial muscle units as possible, without conscious bias in selection of units studied and with as few failures as possible. In fact, only a few medial units encountered by the
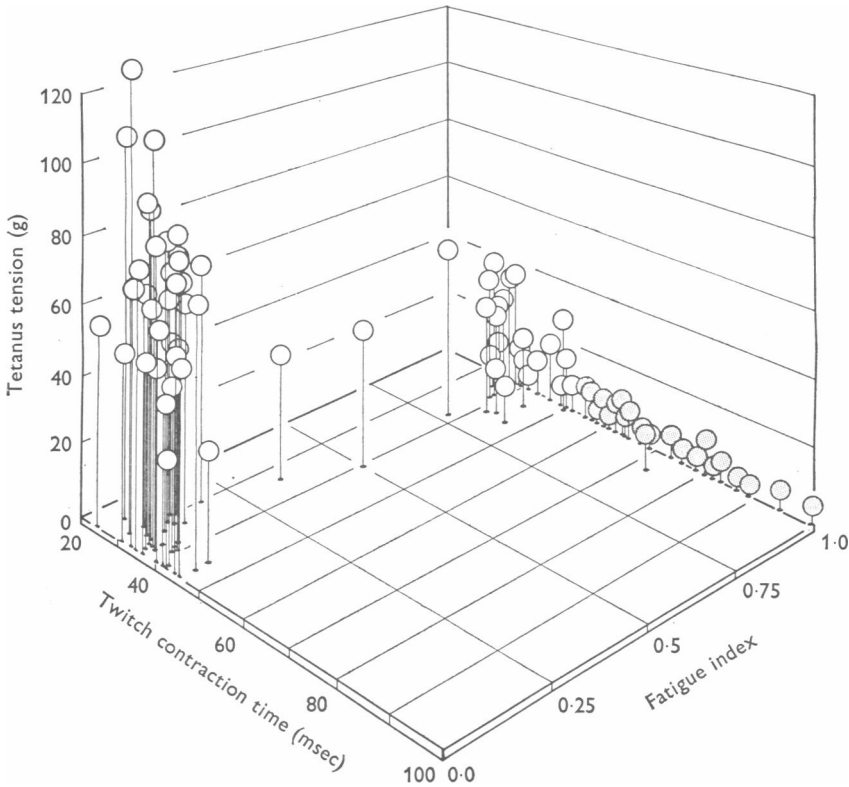
Text-fig. 5. Three-dimensional diagram summarizing the physiological profiles found in a sample of eighty-one medial gastrocnemius muscle units studied in three cats. Units with 'sag' shown as open circles (types FF and FR units); muscle units without 'sag' denoted by stippled circles (type S units). Note two 'unclassified' units with fatigue index values between 0·25 and 0·75.

micro-electrode were not fully studied and we conclude that this population represented as accurate a sample of the medial gastrocnemius unit pool as we could achieve using the present techniques.

Each symbol in the diagram of Text-fig. 5 denotes a different unit. The cluster of units in the left-hand corner of the diagram, with low values of

fatigue index and short contraction times, represents the type FF unit group and it is evident that these produced, on the average, the largest tetanic tensions. The values for twitch contraction time plotted in the diagram are those for maximally potentiated twitches; these were used in order to minimize inter-unit variability due to differences in stimulus history (cf. above). The units with fatigue indices greater than 0·75 formed two groups distinguishable on the basis of presence (open circles) or absence (stippled circles) of 'sag' in unfused tetani (cf. Table 1). Thus the type FR units (open circles) as a group produced intermediate tension outputs while the type S units (stippled circles) produced uniformly small tetanic tensions. It can be noted that the distribution of twitch contraction times in the FR and S unit groups was apparently continuous, although there was no overlap between the two groups. Type FR units can be regarded as 'fast twitch' units, and type S as 'slow', only in a statistical sense. For this reason, twitch contraction time was discarded as a criterion for unit classification.

There were two 'unclassified' muscle units in the sample population, both obtained from the same animal, with fatigue indices between 0·25 and 0·75 (cf. Table 1). With respect to the physiological parameters in the diagram, these 'unclassified' units were intermediate between the FF and FR unit groups. The relative frequencies of the different muscle unit types in this sample of medial units, obtained by simply averaging the results from the three animals surveyed, were: type FF, 41·5 %; 'unclassified', 2·5 %; type FR, 28 %; and type S, 28 %.

In the three animals surveyed above, the relative frequencies of the various muscle unit types encountered in the individual animals were different. For example, type FF units made up about 29 % of one sample (six of twenty-one units studied), 60 % of another (eighteen of thirty-two units) and 32 % of the third (ten of thirty-one units). In the absence of contrary evidence, we have assumed that the actual relative frequencies of the different unit types is about the same in all normal adult cats, and this assumption is implicit in giving the average frequencies above. The relative frequency of slowly contracting type S units in the present sample population was not much different from that found in previous samples drawn from the medial gastrocnemius muscle unit pool, making allowances for differences in experimental techniques (Burke, 1967; Olson & Swett, 1971; Wuerker *et al.* 1965).

As an additional check on the reliability of the above relative frequency estimates, we calculated the amount of tension which would be contributed, on the average, by each of the muscle unit types in the cat medial gastrocnemius, including 'unclassified' units with type FF. The total number of motor units in the medial gastrocnemius has been estimated at about 280 by counting α-motoneurone axons (Boyd & Davey, 1968). Using the relative frequencies of the various unit types from the present data, there should be a total of about 124 type FF units, 78 FR units and 78 type S units in the medial muscle unit pool. The mean tetanic tensions for each unit group were: type FF, 69·3 g; type FR, 21·9 g; and type S, 4·9 g (cf. Text-fig. 5). Given simultaneous activation of the entire unit pool, and assuming linear addition of tensions of individual muscle units, the total tension

contribution of each unit group would be as follows: type FF, 8600 g; type FR, 1700 g; and type S, 380 g. This gives a total average tension of about 10,680 g, which compares favourably with our own measurements of total tetanic tension of cat medial gastrocnemius and with those of Eccles & Sherrington (1930), who found that this muscle of cats weighing about 2 kg produced between 7200 and 9080 g while heavier animals (3·3–3·6 kg) gave values between 12,700 and 15,800 g. The survey animals in the present experiments weighed between 2·2 and 2·5 kg.

The only property intrinsic to the motoneurone portion of the motor units which has been measured in the present series was the conduction velocity of the motoneurone axon. As in previous experiments (Burke,
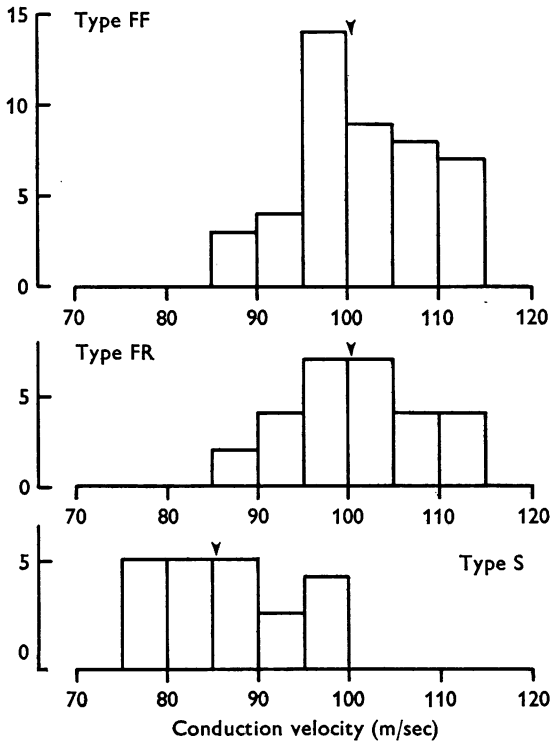
Text-fig. 6. Histograms of motoneurone axonal conduction velocities in the three major types of gastrocnemius motor units. Median value of each distribution indicated by arrows.

1967), this parameter was measured from records of the antidromic conduction time after peripheral nerve stimulation (see Methods) and from estimation of the conduction distance obtained after sacrifice of the individual animals. The histogram in Text-fig. 6 shows that the distributions of conduction velocities for FF and FR units were almost identical while

motoneurones innervating type S units had more slowly conducting axons, confirming earlier observations (Burke, 1967; Olson & Swett, 1971; Wuerker *et al.* 1965).

PART II

Histochemical identification of single muscle units

Some time ago, Krnjević & Miledi (1958*b*) suggested that the muscle fibres innervated by a single motoneurone might be histologically identified by depletion of their glycogen content on prolonged stimulation. More recent work has demonstrated the feasibility of this method, in that glycogen-depleted muscle fibres (i.e. unstained fibres in PAS sections, see Methods) have been found in muscles following prolonged stimulation of a single motor unit (Edström & Kugelberg, 1968). PAS-negative fibres also were unstained in sections processed to demonstrate phosphorylase activity (Kugelberg & Edström, 1968; confirmed in the present work). Since PAS- and phosphorylase-negative muscle fibres of otherwise normal appearance (i.e. excluding section areas with trauma or processing arti-facts) were not found in normal muscle of control cats, it is assumed that such fibres which were observed following prolonged stimulation of a single motor unit indeed belonged to the stimulated unit.

In the present work, selected motor units were stimulated for prolonged periods after initial study of their physiological responses (cf. Methods). Repetitive tetanization was continued for about 15 min (for a total of about 12,000 stimuli) with type FF units but was more prolonged (30–60 min, or 23,000–47,000 stimuli) for type FR units and type S units (the latter stimu-lated for at least 50 min in each case). The long durations of stimulation were used with fatigue resistant FR and S units since previous work has shown that the susceptibility to fibre glycogen depletion varies directly with the susceptibility to mechanical fatigue among mammalian muscle units (Edström & Kugelberg, 1968; Kugelberg & Edström, 1968; cf. also Edger-ton, Barnard, Peter, Simpson & Gillespie, 1970). Muscle fibres were regarded as glycogen-depleted only when there was no detectable pink-staining reticulum (presumably glycogen) anywhere within the fibre with examination of technically acceptable PAS-stained sections under high magnification (cf. Pls. 1–3).

Histochemical profiles of physiologically characterized muscle units

The histochemical preparations illustrated in Pl. 1 were obtained in a lateral gastrocnemius muscle in which a single type FF muscle unit had been stimulated. Mechanical and electrical responses produced by the same unit are shown in Text-fig. 1. The PAS-stained sections contained a large number of unstained muscle fibres, two of which are shown in Pl. 1

(PAS, arrows). Note that the same muscle area also contained some small-diameter fibres which were relatively lightly stained; since these clearly contained a visible reticulum they were not regarded as glycogen-depleted.

The same FF unit fibres were identifiable in serial sections stained for other substrates (DPNHD, myofibrillar ATPase, Ac-ATPase; cf. Methods for abbreviations). It is important to note that, in preparations other than PAS (or phosphorylase, which is not illustrated), the stimulated muscle fibres were similar in appearance to many neighbouring fibres not depleted of glycogen (therefore presumably unstimulated, belonging to other muscle units). This observation, which was consistent in all units studied (cf. Pls. 2 and 3), strongly suggests that the repetitive stimulation used to produce glycogen depletion did not alter fibre staining characteristics in the other histochemical reactions employed (see also Edström & Kugelberg, 1968). This conclusion is critical to any interpretation of the histochemical profiles of identified muscle units.

The term 'histochemical profile' refers to the set of staining characteristics of a muscle fibre, or groups of similar fibres, evaluated in relation to surrounding fibres in the same section. Thus, when stained for the oxidative enzyme DPNHD the muscle fibres belonging to the FF unit shown in Pl. 1 (arrows) stained lightly relative to many surrounding fibres. The reaction product (formazan granules) distributed in a fine, interrupted network of strands and clumps tending to increase in density toward the periphery of the fibres. The pattern of staining with another oxidative enzyme, SDH, was essentially the same (not illustrated). When stained for ATPase activity of myofibrils in another serial section (Pl. 1, ATPase), the FF unit fibres were darkly stained relative to others in the region. Preincubation of sections in acidic buffers before ATPase staining leads to a reversal of the usual ATPase staining mosaic in mixed muscles (Drews & Engel, 1966). With preincubation in acetate buffer at pH 4·65 (Ac-ATPase; cf. Brooke & Kaister, 1969), the FF unit fibres stained with an intensity intermediate between the darkest and lightest fibres in the same region.

The histochemical profile of a typical type FR unit is illustrated in Pl. 2. Mechanical and electrical records from the same FR unit are in Text-fig. 2. The FR unit fibres stained darkly in the DPNHD preparation (arrows) but the qualitative nature of the intrafibre distribution of formazan granules was similar to that seen in FF unit fibres; that is, an interrupted network of strands and clumps rather unevenly distributed through the fibre cross-section. The unit fibres stained darkly in the ATPase preparation (the myofibrillar stain) but were among the lightest fibres in the Ac-ATPase preparation.

Pl. 3 illustrates the profile of the type S muscle unit which produced the

mechanical and electrical responses shown in Text-fig. 3. Note that the unit fibres were relatively lightly stained for myofibrillar ATPase activity but were extremely dark in the Ac-ATPase section. The unit fibres were darkly stained for oxidative enzyme activity (DPHND) and the distribution of formazan granules was qualitatively different from that observed in FF and FR unit fibres, in that there was a dense and uninterrupted network of granules evenly distributed throughout the cross-sectional area of the fibres (cf. Stein & Padykula, 1962).

A summary of the histochemical profiles of identified muscle units in the gastrocnemius is given in Table 2, which includes a number of pre-

TABLE 2. Histochemical profiles of gastrocnemius muscle units

	Muscle unit types			
	Type FF	Unclassified	Type FR	Type S
Myofibrillar ATPase	High	High	High	Low
Ac-ATPase	Intermediate	Intermediate	Low	High
EDTA-ATPase	Low	Low	Low	High
DPNHD*	Low	Intermediate	Int.-high	High
SDH*	Low	Intermediate	Int.-high	High
M-α-GPD†	High	—	High	Low
Esterase†	High	—	High	Low
Lactate dehydrogenase†	High	—	High	Low
Neutral fat (Oil Red O)†	Low	—	Low	High
Glycogen‡	High	—	High	Low
Phosphorylase‡	High	—	High	Low
Number of units studied	15	1	7	5

   * Pattern of reaction product also distinctive (see text).
   † Studied in 3 type FF, 2 type FR and 2 type S units.
   ‡ Presumed on the basis of appearance of fibres with similar histochemical profiles.

parations not illustrated in the Plates (cf. Methods). Each physiological unit type had a unique histochemical profile and no exceptions were found to the exact match between physiological and histochemical profiles. The profile of one 'unclassified' unit was studied (cf. also Text-fig. 7) and this was in a real sense intermediate between the profiles characteristic of FF and FR units. The three-dimensional diagram shown in Text-fig. 7 presents a summary of the physiological profiles of the histochemically studied muscle units. The diagram was constructed in the same way as Text-fig. 5 and the two sets of data illustrated were superimposable. We thus conclude that the present results include histochemical profiles representative of the entire population of muscle units which compose the gastrocnemius muscle in normal cats.

The cytochemical interpretation of the staining patterns observed in the various histochemical preparations of muscles is in a number of respects unclear. The DPNHD and SDH enzymes are involved in pathways of oxidative glycolysis and are localized in mitochondria and in the sarcoplasmic reticulum, both staining as linear elements (Brooke & Engel, 1966). The method used for demonstration of these enzymes also causes deposition of reaction product at aqueous-lipid interfaces (Novikoff, Shin & Drucker, 1961). Thus the distribution and density of formazan granules in muscle fibres reflects in part the density and distribution of mitochondria and to some extent differences in the distribution of lipid. The staining patterns in FF and FR units, and the difference between these patterns and that observed in
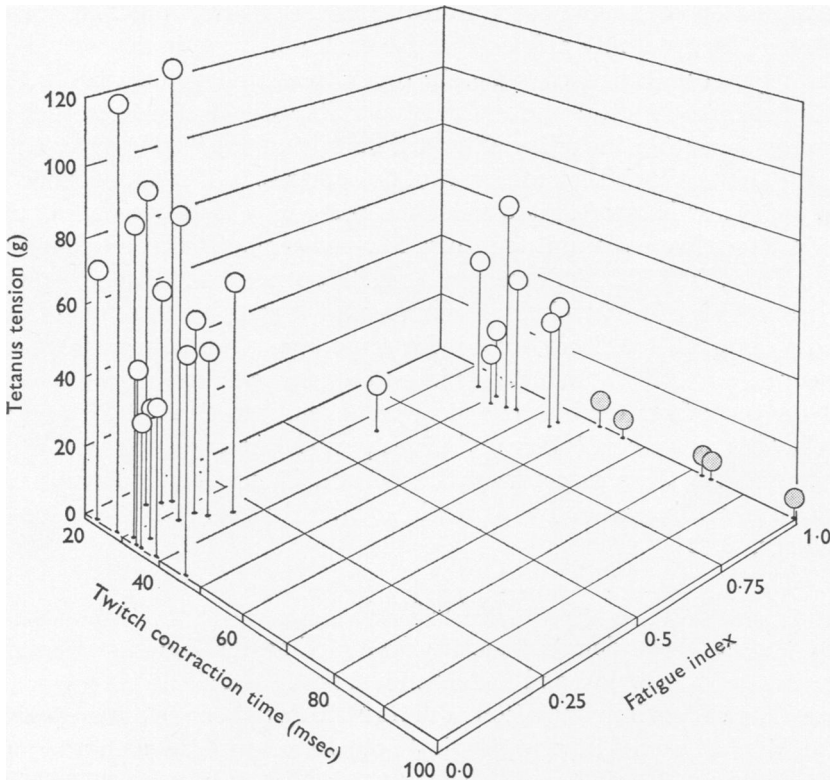
Text-fig. 7. Three-dimensional diagram summarizing the physiological profiles of those gastrocnemius muscle units which were also characterized histochemically. Format of the diagram as in Text-fig. 5.

type S units, presumably reflect variations in distribution of subcellular organelles observed in muscle fibres of presumed different type at the ultrastructural level (e.g Gauthier, 1969; Schiaffino, Hanzlikova & Pierobon, 1970).

In adult mammals, the histochemical reaction product deposited during ATPase staining at alkaline pH (referred to here as myofibrillar ATPase, see Methods) is largely localized in myofibrils (Guth & Yellin, 1971), and the intensity of staining, at least averaged for various whole muscles, is directly correlated with biochemical

assays for actomyosin ATPase activity (cf. Guth & Samaha, 1972). There is much less certainty about the cytochemical interpretation of ATPase staining after 'reversal' with acidic preincubation. The staining intensity of a given set of fibres depends on the pH lability of actomyosin, which varies with different 'types' of fibres, as well as on pH-dependent variations in ATPase staining of intermyofibrillar structures, possibly including sarcoplasmic reticulum (Gauthier, 1967; Guth & Yellin, 1971).

Histochemical uniformity within single muscle units

Assessment of the histochemical profile of each studied unit was based on examination of several dozen fibres belonging to that unit. Although the Plates illustrate only a few fibres for each unit, uniformity of profile within a given unit was the rule. In order to assess systematically the question of histochemical uniformity, five units were chosen from each of the type groups (thus including all type S units studied). The 'unclassified' unit was also examined for uniformity. Selecting sets of serial sections in which large numbers of unit fibres appeared and which were relatively free of artifacts over the unit territory, low-power photomicrographs were made of PAS, DPNHD, ATPase and Ac-ATPase sections. From these, the detailed profiles of relatively large numbers of unit fibres were examined (in most units 80–140 fibres, except for some type S units in which total fibre counts were low). In no case were any fibres found within a unit which deviated from the histochemical profile characteristic of the unit as a whole. The absence of 'aberrant' fibres provides strong support for the hypothesis that there is complete histochemical uniformity among the muscle fibres innervated by a single α-motoneurone (cf. Edström & Kugelberg, 1968).

DISCUSSION

The present results demonstrate for the first time detailed interrelations between the physiological and histochemical profiles of single muscle units representing the entire population of units making up a mixed mammalian muscle. While the present physiological data are compatible with previous results (Burke, 1967; Olson & Swett, 1971; Wuerker *et al.* 1965), the system of unit classification used here has two major advantages: (1) it provides unambiguous separation of distinct physiological groups of muscle units present in the gastrocnemius population; and (2) each physiological group consists of muscle units with common histochemical features. Since there is evidence (cf. above) that the type S units of the present system and of previous results (Burke, 1967) are essentially identical, a detailed view of motor unit organization is available for the cat gastrocnemius muscle in which muscle unit physiology, histochemistry and morphology (for the latter, see Burke & Tsairis, 1973 *a*) can be quite

precisely related to the intrinsic properties of the innervating alpha moto-neurones (Burke, 1967; Burke & Nelson, 1971; Burke & ten Bruggencate, 1971), to the organization of synaptic input to the unit motoneurones (Burke, 1968a, b; Burke et al. 1970a), and to the patterns of activity of different motor units in decerebrate rigidity (Burke, 1968b). A detailed discussion of these interrelations is available in a recent review (Burke, 1973).

Types FF and FR muscle units, which contracted relatively rapidly, stained rather darkly for myofibrillar ATPase activity in relation to some surrounding muscle fibres with histochemical profiles characteristic of slowly contracting type S muscle units (Text-fig. 7; Pls. 1, 2; Table 2). This finding is in agreement with the direct correlation of contraction speed with actomyosin ATPase activity apparent in a wide variety of muscles from different species (Barany, 1967), as well as with a similar correlation found in whole muscles, or portions of muscles, with different mixtures of histochemical muscle fibre types (Barnard, Edgerton, Furu-kawa & Peter, 1971). It should be noted, however, that there were wide ranges in twitch contraction times within each of the unit groups (Text-figs. 5, 7). Although it is possible that some of the variation in contraction times within a given unit type may have been due to mechanical factors of twitch measurement, including perhaps differences in the anatomical distribution of muscle units within the whole gastrocnemius, it seems unlikely that such factors can account for the entire variation observed in contraction times (cf. Burke & Tsairis, 1973a). With the evidence available, it appears important to suggest that the ATPase staining characteristics demonstrable in histochemical preparations should not be overinterpreted in terms of implied contraction speed of the muscle fibres (Burke & Tsairis, 1973b). It is particularly difficult to make such extrapolations between different muscles, even in the same species of animal (Burke & Tsairis, 1973b; Hall-Craggs, 1968; Nyström, 1968).

There was in the present data an apparently clear correlation between the resistance to fatigue exhibited by a muscle unit and the histochemical profile of the unit fibres related to major pathways of energy metabolism (see also Edström & Kugelberg, 1968). Both FF and FR muscle units had staining patterns indicating high capacity for anaerobic glycolysis, but there were clear differences in apparent capacities for oxidative glycolysis which were correlated with fatigue resistance (Text-fig. 7; Pls. 1, 2; Table 2). For convenience, the set of units which showed 'sag' in unfused tetani and relatively short twitch contraction times (i.e. FF, unclassified and FR units) can be referred to as the 'fast-twitch' group. Fatigue resistance was distributed in this fast-twitch group in a bimodal pattern, a fact used in defining the classification criteria (cf. above), but it can also

be pointed out that this distribution was essentially continuous, including several unclassified units between the larger FF and FR unit groups (Text-figs. 5, 7). Furthermore, prolongation of the repetitive tetanization up to 5 min in duration showed that there was a spectrum of fatigue resistance among the units classed as type FR (see Burke & Tsairis, 1973b, Fig. 1). In most gastrocnemii studied histochemically, there was a continuous spectrum in oxidative staining among muscle fibres which presumably belonged to units of the fast-twitch group on the basis of other histochemical reactions (e.g. myofibrillar ATPase; cf. Burke & Tsairis, 1973b, Fig. 3). Studies of whole muscle histochemistry before and after prolonged exercise suggest that there may be modulation of muscle fibre oxidative enzyme staining, and perhaps fatigue resistance, depending on conditions of varying output demand (Barnard, Edgerton & Peter, 1970). Such data suggest that the FF and FR unit categories may not be immutable; rather the relative frequencies of the unit types within the fast-twitch group may well vary from animal to animal, or within the same animal at different times, in response to stress. We suggest, therefore, that the fast-twitch group of gastrocnemius muscle units is in fact a single entity, containing muscle units which, while basically similar to one another, exhibit graded differences in oxidative enzyme capacity and fatigue resistance, in correlation.

Type S muscle units differ from units in the fast-twitch group in exhibiting much less apparent capacity for, and dependence on, anaerobic glycolysis (Table 2). In contrast, type S unit fibres have histochemical profiles suggesting primary dependence on oxidative glycolytic pathways. Their fibres are of small diameter (Pl. 3; cf. Burke & Tsairis, 1973a), possibly optimizing surface to volume ratio, and are liberally supplied with capillaries (visible in the Ac-ATPase preparations, e.g. Pl. 2; cf. Romanul, 1965). In accord with the pattern already evident within the fast-twitch group, most type S units studied were extremely resistant to fatigue even during very prolonged stimulation (Text-fig. 3). The available data provided no suggestion of identifiable subgroups within the population of type S units in the gastrocnemius. Furthermore, there was an apparent sharp transition between units of the fast-twitch group and type S units with regard to both physiological (e.g. 'sag') and histochemical characteristics (e.g. myofibrillar ATPase staining). Particular note should be taken of the difference between these groups in the pattern of intrafibre reaction product in DPNHD preparations (Pls. 1–3; cf. Stein & Padykula, 1962). It appears unlikely that the type FR units can be regarded as 'transitional' between FF and S muscle units. Rather, we have concluded that the type S group of muscle units is a distinct and essentially homogeneous set.

The present results provide direct confirmation of a number of points

regarding interrelations between physiological properties and histochemical profiles of muscle fibres which have been suggested using indirect evidence (see review by Close, 1972). Close (1972) has attempted to synthesize much of the available material dealing with the question, a task of considerable difficulty because of confused and in some cases contradictory usage of terminology with respect to classification of muscle fibres by histochemical profiles. The present results are compatible with Close's synthesis (see his Table 1) in many, but not in all, respects. Because of the current lack of a generally accepted nomenclature for codifying the histochemical 'types' of muscle fibres, we have used none in this discussion. It must be stated explicitly that we do not propose the use of the designations 'FF', 'FR' or 'S', which have been derived from physiological data, to denote any set of histochemical profiles for muscle fibres, for to do so would be essentially illogical despite the close correspondence demonstrated in the present work.

It seems likely that the general pattern of physiological–histochemical interrelations found in the present work may be broadly applicable to other motor unit populations, at least in large limb muscles of mammals. However, it is to be expected that details will vary with species, as is already evident comparing data from the cat with the rat (cf. Close, 1967; Edström & Kugelberg, 1968; Yellin & Guth, 1970). The results of direct matching of muscle unit physiological and histochemical profiles, when compared with some previous suggestions based only on histochemical results (see Close, 1972), illustrate the caution required in assigning physiological properties to particular histochemical profiles using only information derived from comparisons of different whole muscles.

REFERENCES

ARMED FORCES INSTITUTE OF PATHOLOGY (1949). *Manual of Histologic and Special Staining Techniques*, 2nd edn. New York: McGraw-Hill.

BARANÝ, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. gen. Physiol.* **50**, 197–216.

BARKA, T. & ANDERSON, P. J. (1963). *Histochemistry Theory, Practice, and Bibliography*. New York: Harper and Row.

BARNARD, R. J., EDGERTON, V. R., FURUKAWA, T. & PETER, J. B. (1971). Histochemical, biochemical, and contractile properties of red, white and intermediate fibers. *Am. J. Physiol.* **220**, 410–414.

BARNARD, R. J., EDGERTON, V. R. & PETER, J. B. (1970). Effect of exercise on skeletal muscle. I. Biochemical and histochemical properties. *J. appl. Physiol.* **28**, 762–766.

BERGMANS, J. & MARÉCHAL, G. (1960). Rapport entre potentiel d'action et tension du muscle strié. *Archs int. Physiol. Biochim.* **68**, 377–379.

BOYD, I. A. & DAVEY, M. R. (1968). *Composition of Peripheral Nerves.* London: E. and S. Livingstone.

BRODY, I. A. (1964). The significance of lactate dehydrogenase isoenzymes in abnormal human skeletal muscle. *Neurology* **14**, 1091–1100.

BROOKE, M. H. & ENGEL, W. K. (1966). Nitro blue tetrazolium: Selective binding within striated muscle fibers. *Neurology* **16**, 799–806.

BROOKE, M. H. & KAISER, K. K. (1969). Muscle fiber types: How many and what kind? *Neurology* **23**, 369–379.

BROOKS, V. B. & THIES, R. E. (1962). Reduction of quantum content during neuro-muscular transmission. *J. Physiol.* **162**, 298–310.

BURKE, R. E. (1967). Motor unit types of cat triceps surae muscle. *J. Physiol.* **193**, 141–160.

BURKE, R. E. (1968a). Group Ia synaptic input to fast and slow twitch motor units of cat triceps surae. *J. Physiol.* **196**, 605–630.

BURKE, R. E. (1968b). Firing patterns of gastrocnemius motor units in the de-cerebrate cat. *J. Physiol.* **196**, 631–654.

BURKE, R. E. (1973). On the central nervous system control of fast and slow twitch motor units. In *New Developments in Electromyography and Clinical Neuro-physiology*, vol. 3, ed. DESMEDT, J. E., pp. 69–94. Basel: Karger.

BURKE, R. E., JANKOWSKA, E. & TEN BRUGGENCATE, G. (1970a). A comparison of peripheral and rubrospinal synaptic input to slow and fast twitch motor units of triceps surae. *J. Physiol.* **207**, 709–732.

BURKE, R. E., LEVINE, D. N., ZAJAC, F. E., TSAIRIS, P. & ENGEL, W. K. (1971). Mammalian motor units: Physiological–histochemical correlation in three types in cat gastrocnemius. *Science, N.Y.* **174**, 709–712.

BURKE, R. E. & NELSON, P. G. (1971). Accommodation to current ramps in motoneurons of fast and slow twitch motor units. *Int. J. Neurosci.* **1**, 347–356.

BURKE, R. E., RUDOMIN, P. & ZAJAC, F. E. (1970b). Catch property in single mam-malian motor units. *Science, N.Y.* **168**, 122–124.

BURKE, R. E. & TEN BRUGGENCATE, G. (1971). Electrotonic characteristics of alpha motoneurones of varying size. *J. Physiol.* **212**, 1–20.

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

BURKE, R. E. & TSAIRIS, P. (1973b). The correlation of physiological properties with histochemical characteristics in single muscle units. *Ann. N.Y. Acad. Sci.* (in the Press).

CLOSE, R. (1967). Properties of motor units in fast and slow skeletal muscles of the rat. *J. Physiol.* **193**, 44–55.

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

COOPER, S. & ECCLES, J. C. (1930). The isometric responses of mammalian muscle. *J. Physiol.* **69**, 377–385.

DENNY-BROWN, D. (1929). The histological features of striped muscle in relation to its functional activity. *Proc. R. Soc. B* **104**, 371–411.

DREWS, G. & ENGEL, W. K. (1966) EDTA reversal of muscle fibre activity with the adenosine triphosphatase reaction. *Nature, Lond.* **212**, 1551–1553.

EBERSTEIN, A. & SANDOW, A. (1963). Fatigue mechanisms in muscle fibers. In *Effect of Use and Disuse on Neuromuscular Function*, ed. GUTTMANN, E. & HNIK, P. Prague: Czechoslovak Academy of Sciences.

ECCLES, J. C. & SHERRINGTON, C. S. (1930). Numbers and contraction values of individual motor-units examined in some muscles of the limb. *Proc. R. Soc.* B **106**, 326–357.

EDGERTON, V. R., BARNARD, R. J., PETER, J. B., SIMPSON, D. R. & GILLESPIE, C. A. (1970). Response of muscle glycogen and phosphorylase to electrical stimulation in trained and nontrained guinea pigs. *Expl Neurol.* **27**, 46–56.

EDSTRÖM, L. & KUGELBERG, E. (1968). Histochemical composition, distribution of fibres and fatiguability of single motor units. *J. Neurol. Neurosurg. Psychiat.* **31**, 424–433.

FARBER, E., STERNBERG, W. H. & DUNLAP, C. D. (1956). Histochemical localization of specific oxidative enzymes. *J. Histochem. Cytochem.* **4**, 254–263.

GAUTHIER, G. F. (1967). On the localization of sarcotubular ATPase activity in mammalian skeletal muscle. *Histochemie* **11**, 97–111.

GAUTHIER, G. F. (1969). On the relationship of ultrastructural and cytochemical features to color in mammalian skeletal muscle. *Z. Zellforsch. mikrosk. Anat.* **95**, 462–482.

GUTH, L. & SAMAHA, F. J. (1972). Erroneous interpretations which may result from application of the 'myofibrillar ATPase' histochemical procedure to developing muscle. *Expl Neurol.* **34**, 465–475.

GUTH, L. & YELLIN, H. (1971). The dynamic nature of the so-called 'fiber types' of mammalian skeletal muscle. *Expl Neurol.* **31**, 277–300.

HALL-CRAGGS, E. C. B. (1968). The contraction times and enzyme activity of two rabbit laryngeal muscles. *J. Anat.* **102**, 241–255.

HENNEMAN, E. & OLSON, C. B. (1965). Relations between structure and function in the design of skeletal muscles. *J. Neurophysiol.* **28**, 581–598.

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

KRNJEVIĆ, K. & MILEDI, R. (1958b). Motor units in the rat diaphragm. *J. Physiol.* **140**, 427–439.

KUGELBERG, E. & EDSTRÖM, L. (1968). Differential histochemical effects of muscle contractions of phosphorylase and glycogen in various types of fibres: relation to fatigue. *J. Neurol. Neurosurg. Psychiat.* **31**, 415–423.

LÄNNERGREN, J. & SMITH, R. S. (1966). Types of muscle fibres in toad skeletal muscle. *Acta physiol. scand.* **68**, 263–274.

LEWIS, D. M. & LUCK, J. C. (1968). Effect of initial length on the tension developed by motor units in flexor hallucis longus muscle of the cat. *J. Physiol.* **197**, 42–43P.

McMANUS, J. F. A. & MOWRY, R. W. (1960). *Staining Methods: Histological and Histochemical.* New York: Harper and Row.

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. *J. Histochem. Cytochem.* **5**, 420–436.

NOVIKOFF, B., SHIN, W. Y. & DRUCKER, J. (1961). Mitochondrial localization of oxidative enzymes: Staining results with two tetrazolium salts. *J. Biophys. biochem. Cytol.* **9**, 47–61.

NYSTRÖM, B. (1968). Histochemistry of developing cat muscles. *Acta neurol. scand.* **44**, 405–439.

OLSON, C. B. & SWETT, C. P. (1971). Effect of prior activity on properties of different types of motor units. *J. Neurophysiol.* **34**, 1–16.

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

PEARSE, A. G. E. (1968). *Histochemistry – Theoretical and Applied*, vol. 1, 3rd edn. Baltimore: Williams and Wilkins.

RANVIER, L. (1874). De quelques faits relatifs à l'histologie et à la physiologie des muscles striés. *Archs Physiol. norm. path.* **6**, 1–15.

ROMANUL, F. C. A. (1965). Capillary supply and metabolism of muscle fibers. *Archs Neurol. Psychiat., Chicago* **12**, 497–509.

SCHIAFFINO, S., HANZLIKOVA, V. & PIEROBON, S. (1970). Relations between structure and function in rat skeletal muscle fibers. *J. cell Biol.* **47**, 107–119.

STANDAERT, F. G. (1964). The mechanisms of post-tetanic potentiation in cat soleus and gastrocnemius muscles. *J. gen. Physiol.* **47**, 987–1001.

STEG, G. (1964). Efferent muscle innervation and rigidity. *Acta physiol. scand.* **61**, suppl. 225, 1–53.

STEIN, J. M. & PADYKULA, H. A. (1962). Histochemical classification of individual skeletal muscle fibers of the rat. *Am. J. Anat.* **110**, 103–116.

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.

YELLIN, H. & GUTH, L. (1970). The histochemical classification of muscle fibers. *Expl Neurol.* **26**, 424–432.

EXPLANATION OF PLATES

PLATE 1

Photomicrographs of serial sections of the lateral gastrocnemius muscle containing the type FF muscle unit which produced the responses shown in Text-fig. 1. Upper left panel (PAS) shows two PAS-negative (glycogen depleted) muscle fibres (arrows) presumed to have belonged to the studied muscle unit (see text). The same two fibres are also shown in serial sections stained for other histochemical reactions (arrows; DPNHD, reduced diphosphopyridine nucleotide dehydrogenase; ATPase, myofibrillar ATPase processed at pH 9·4; Ac-ATPase, ATPase staining following pre-incubation of section in acetate buffer at pH 4·65; cf. Methods). Note that the fibres of the stimulated muscle unit did not exhibit any unusual appearance in preparations other than PAS.

PLATE 2

Histochemical profile of the type FR muscle unit which produced the responses illustrated in Text-fig. 2 (arrows).

PLATE 3

Histochemical profile of the type S muscle unit which produced the responses shown in Text-fig. 3.

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