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TRANSMISSION AND CONTRACTION FATIGUE OF RAT MOTOR UNITS IN RELATION TO SUCCINATE DEHYDROGENASE ACTIVITY OF MOTOR UNIT FIBRES

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SUMMARY

1. The fatigue in rat anterior tibial (a.t.) motor units was studied and related to microphotometric determinations of succinate dehydrogenase (SDH) activity of the motor unit muscle fibres.

2. Anterior tibial contains fast-twitch type II fibre units with an average contraction time of 11 msec and about 5% slow-twitch type I fibre units with an average contraction time of 20 msec.

3. In type II fibres stained for SDH, absorbance varied continuously from 0·046 to 0·569 and inversely to fibre size, except for the largest fibres.

4. Resistance to fatigue of fast motor units to 100 Hz intermittent stimulation varied continuously within a wide range in near linear relations to absorbance for SDH of unit fibres and inversely to tetanic tension, except for motor units with the largest fibres and the largest tetanic tension.

5. Neither resistance to fatigue nor SDH activity lent itself to any categorization of motor units or fibres into well demarcated functional or histochemical types, since both parameters varied continuously in the unit and fibre population of the muscle.

6. The direct relation between resistance to fatigue of fast-twitch motor units and SDH activity of unit fibres appeared valid for fatigue resistance of: (*a*) neuromuscular transmission, tested with 100 Hz intermittent stimulation which gave concomitant failure of electrical and mechanical response, (*b*) excitation–contraction coupling, demonstrated by post-stimulatory depression of twitch tension with preserved maximum tetanus tension and action potential, and (*c*) contractile mechanism; excitation–contraction coupling?, tested with low frequency stimulation which gave decline of twitch and maximum tetanus tension with preserved action potential.

7. It is suggested that the endurance of each link in the chain of events leading to contraction, including neuromuscular junction and the excitation–contraction coupling system, is under aerobic conditions matched to the contractile capacity of the fibre expressed by its oxidative enzyme activity.

INTRODUCTION

Glycogen depletion as marker of previous muscle fibre contractions (Kugelberg & Edström, 1968) and repetitive stimulation of single motoneurones enabled demonstration of histochemical uniformity of muscle fibres in the motor unit, which

permitted direct examination of unit functional-histochemical correlations (Edström & Kugelberg, 1968; Burke, Levine, Tsairis & Zajac, 1973; Kugelberg, 1973, 1976).

In fast-twitch motor units, in a heterogeneous rat muscle, a direct relation was demonstrated between resistance to fatigue to repeated contractions and the intensity of fibre staining for an oxidative enzyme, succinate dehydrogenase (SDH) (Edström & Kugelberg, 1968). The same correlation was found in cat motor units (Burke *et al.* 1973). In both studies the intensity of enzyme staining was estimated merely by inspection of the preparation in the microscope or on photomicrographs.

The limited number of units studied on the rat formed an apparently continuous series with different resistance to fatigue and SDH activity of fibres. In the cat gastrocnemius the distribution of fatigue resistance was bimodal with two distinct types of units with few units of intermediate fatiguability (Burke *et al.* 1973; Hammarberg & Kellerth, 1975). However, in the same muscle a less clear grouping with many intermediate units was reported by Stephens, Gerlach, Reinking & Stuart (1973).

The low frequency continuous stimulation (2–10 Hz) used in the rat was supposed to differentiate the units on the basis of contractile fatigue (Edström & Kugelberg, 1968). Burke *et al.* (1973) usually found little changes in e.m.g. amplitude with fatigue caused by stimulation at 40 Hz for 330 msec each second, indicating contractile fatigue. Stephens *et al.* (1973) using the same stimulus sequences observed a reduction in e.m.g. amplitude and concluded that the relative importance of contractile versus a possible failure of neuromuscular transmission remained uncertain Olson & Swett (1966, 1971), observed in the cat on 100 Hz continuous stimulation decline of e.m.g., which was more rapid in large units assumed to be white than in small units assumed to be red. However, no units were histochemically identified in these studies.

In view of the uncertainty of the specificity of motor unit types based on fatiguability and of the nature of the fatigue that seems to be correlated to oxidative activity of the motor unit muscle fibres, fatigue in rat anterior tibial motor units has been examined and related to microphotometric determinations of SDH activity of the fibres.

METHODS

Physiological technique. Adult male rats of the Sprague-Dawley strain weighing 380–400 g were anaesthetized with sodium pentobarbital. The L4 was exposed by laminectomy. The skin over the lower part of the left hind limb was removed as well as the fascia overlying the anterior tibial muscle. The distal part of the muscle was freed. The left popliteal artery was exposed in some experiments and a fine thread was loosely placed round the artery to make it more easily accessible by a pull on the thread. In fatigue studies it was of paramount importance that circulation to the muscle was kept intact and therefore preparation of the hind limb was kept to a minimum.

The animal was placed in the prone position on a steel plate with the vertebral column fixed at two points. The dissected limb was put into a bath through a hole in the wall and the skin of the thigh stretched over a flange round the hole and tied in position making the bath leak proof. The limb was rigidly fixed with a steel drill through the tibia near the knee joint as well as a clamp on the foot. The bath was circulated with mineral oil maintained at 35–36 °C.

The tendon of the anterior tibial muscle was attached to a strain gauge (Statham UC 4) orientated along the natural direction of pull of the muscle. The e.m.g. was generally recorded with fine unlaquered steel needles (eye suture needle 8–0 Ethicon) with one needle between the proximal part of the muscle and the tibia and the other at the same level on the medial aspect

of the bone. Single motor units in the anterior tibial muscle were functionally isolated by dissection of the L4 root. The criterion was an all-or-none response of e.m.g. and contraction to finely graded short current pulses.

Mechanical and electrical responses were recorded on a double beam oscilloscope and photographed. Contractions were recorded with the muscle set at the optimal length as determined from isometric twitch contractions of single motor units and checked for each five to ten units examined. Isometric twitch contraction time was measured from beginning of contraction to peak.

The muscle fibres of the motor units examined physiologically were depleted of glycogen by stimulation with trains of 20 impulses at 100 Hz repeated once a second until tetanic tension had dropped to near zero. Stimulation was thereafter terminated and the unit stimulated with one impulse train each 10 sec until tension had almost recovered. The sequence was repeated 5 times. Fatigue resistant units were stimulated under ischaemia produced by clamping the popliteal artery with a small spring clip. The clip was removed under the recovery periods. Rapidly fatiguing units are easily depleted of glycogen without ischaemia.

Histological technique. The motor units were mapped as unstained fibres in perodic acid-Schiff (PAS), stained sections for glycogen and the following sections stained for SDH, using the Nitroblue Tetrazolium method of Nachlas, Tsou, De Sousa, Cheng & Seligman, 1957, and for myofibrillar ATPase after preincubation at pH 4·5 and 4·35 according to Dubowitz & Brooke (1973). Cross-sectional areas of single fibres were determined planimetrically on photomicrographs.

Comparative microphotometric determinations of SDH in single fibres were performed using a Leitz M.P.V. microphotometer. Thickness of cryostat sections was 10 μm, incubation time 30 min at 36 °C and measuring field 200 μm^2 in the centre of the fibre. Transmittance of light was measured at 546 nm as percentage of transmission of slide, mounting medium and cover glass just outside the section and converted into figures representing absorbance. The muscles were stored and all motor units subjected to measurements were cut on the same occasion and incubated in the same baths.

The staining is particulate as most of the dye is located in the mitochondria which tend to accumulate at the periphery of the fibre under the sarcolemma. Measurements were therefore in the beginning taken from both centre and periphery. Absorbance in the centre was lower but in proportion to absorbance in the periphery which, however, in one and the same fibre showed larger variations than in the centre. We were therefore content with measurements only from the centre for comparative determinations of SDH activity of fibres.

RESULTS

Fibre and motor unit composition of the anterior tibial muscle

The muscle consists of a deep central part composed of a high proportion of fibres with intense SDH activity, and a superficial zone virtually lacking this fibre type (Pl. 1). Sections incubated for myofibrillar ATPase show that all type I fibres, that is fibres with low activity at pH 9·4 and high at pH 4·35, are restricted to the central part. This accounts also for the muscle spindles, which are anatomically associated with type I fibres and type II fibres with high oxidative activity. Type I fibres are, however, comparatively few, about 5% of the whole population (Pullen, 1977).

SDH activity of a representative population of fibres was determined microphotometrically in one muscle by examining all fibres on two perpendicular lines drawn through the longest superficial-deep and medial-lateral axes. In a second section stained for myofibrillar ATPase the same fibres were classified according to type I or II (Text-fig. 1). There is a continual gradation of fibres with different SDH activities. The type I fibres show in the rat hind-limb muscles intermediate activity as demonstrated by Stein & Padykula (1962), Padykula & Gauthier (1967) and others, but there are also many intermediate type II fibres.

The absorbance of every third fibre along the two axes plotted against the cross-

sectional area of the fibre indicates for fibres below 3000 μm^2 an inversed linear relationship between SDH activity and fibre size (Text-fig. 2) in accordance with the finding by Goldspink (1969) in the mouse and by Goldspink & Waterson (1971) in some other fast rat muscles.

The motor units in the anterior tibial muscles are highly intermingled (Edström & Kugelberg, 1968) but the fibres are not quite randomly distributed. As illustrated by the glycogen depleted unit in Pl. 1 the units in the superficial layer with large fibres and low SDH activity extend their territory further along the superficial layer than perpendicular to it avoiding penetration into the central high oxidative part.

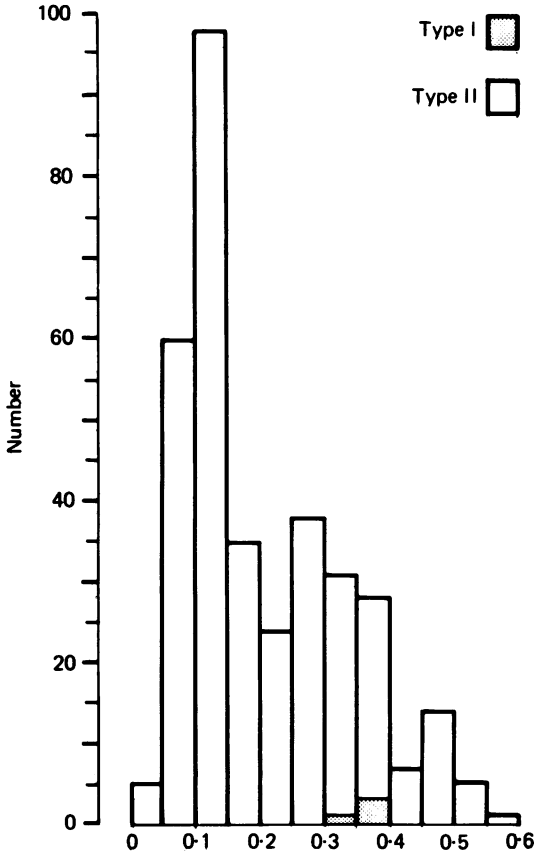


Fig. 1

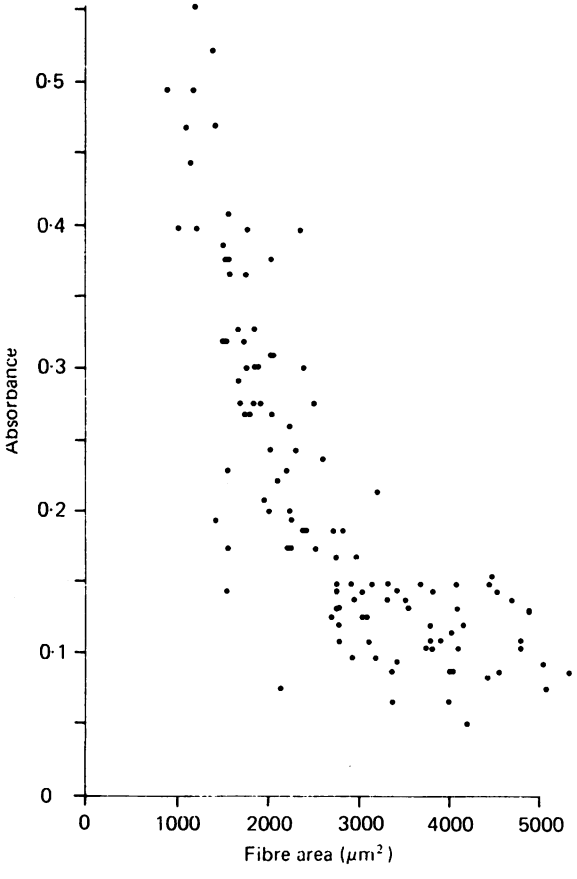


Fig. 2

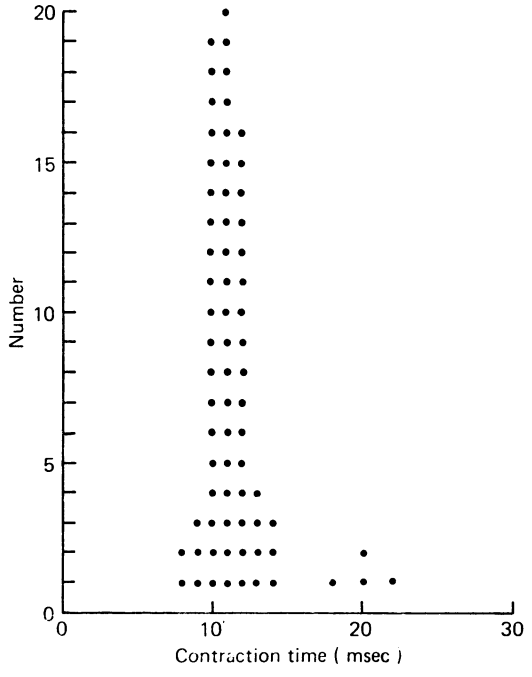
Text-fig. 1. Absorbance (λ = 546 nm) of rat anterior tibial muscle fibres stained for SDH.

Text-fig. 2. Absorbance of anterior tibial muscle fibres stained for SDH in relation to fibre cross-sectional area.

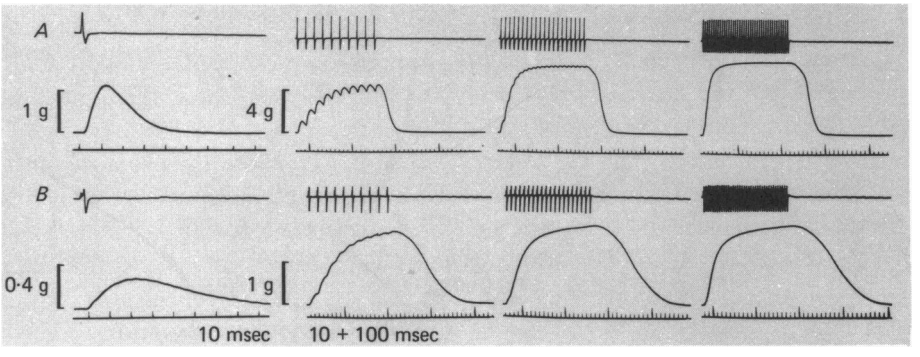
Text-fig. 3 shows the distribution of isometric contraction times for seventy-one motor units from three animals. Sixty-seven units were fast-twitch with a mean contraction time of $11{\cdot}2 \pm \psi 1{\cdot}27$ msec, a value in agreement with that found by Close (1967) in the rat extensor digitorum muscle. Four units (5·6 %) were slow-twitch with a mean contraction time of 20 msec. Of about 50 fast units histo-

chemically classified by the glycogen depletion method all were type II fibre units, and six examined slow units all were type I.

In the fast units there was a considerable summation of contractions to repetitive stimulation at 100 Hz and relatively little further gain in tension at 200 Hz, when fusion appears complete. For the slow units comparative frequencies are 50 and 100 Hz (Text-fig. 4). Both fast and slow motor units show post-tetanic potentiation of twitch tension in variance with slow units in the rat soleus which do not (Kugelberg, 1973).



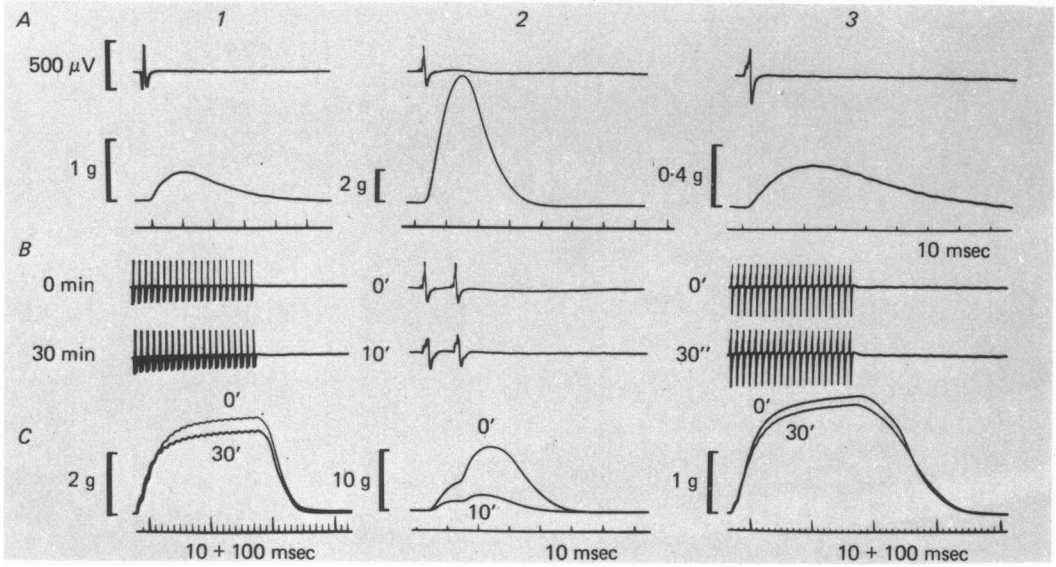
Text-fig. 3. Distribution of isometric twitch contraction times for anterior tibial motor units.



Text-fig. 4. Isometric twitch and response to stimulation at 50, 100 and 200 Hz. *A*, fast-twitch type II fibre motor unit. *B*, slow-twitch type I fibre motor unit.

Fatigue: high frequency, intermittent stimulation

100 Hz was chosen as a reasonable stimulation frequency to match the fusion frequency of fast contracting units. Fast units with the largest endurance could sustain stimulation *in situ* of trains of 20 pulses at 100 Hz delivered twice a second for 30 min or more (Text-fig. 5, *1*). Some decline in tetanic tension occurred during the first 10 sec of stimulation and may well have a purely mechanical origin, a further fraction of decline is caused by shortening of twitch contraction time and decreased fusion.



Text-fig. 5. Variations of fatigue resistance among anterior tibial motor units to prolonged intermittent stimulation. *A*, isometric twitch. *B*, electrical and *C*, mechanical response to trains of pulses at 100 Hz. *1*, fast-twitch fatigue resistant unit. *B*, *C*, initial response (time 0) and response after 30 min stimulation with 20 pulses twice a second. *2*, fat-twitch fatigue sensitive unit. *B*, *C*, initial response (time 0) and response after 10 min stimulation with 2 pulses twice a second. *3*, slow-twitch unit. Same stimulation as in *1*.

In contrast, the unit in Text-fig. 5 (*2*) from the same muscle could not sustain tension even when the number of pulses was reduced by a factor of 10, i.e. from 40 to 4 each second, delivered in pairs twice a second.

The slow-twitch motor unit (Text-fig. 5, *3*) from the same muscle sustained well the same stimuli as unit *1*. In as much as 100 Hz produced maximum tetanus tension in the slow unit, it was superior to the fatigue-resistant fast unit, which did not sustain a stimulus frequency of 150 Hz necessary for maximum tetanus tension, without reduction of the number of impulses in the trains. Even in absolute terms, fatigue resistance of the slow units was probably somewhat superior to the most fatigue-resistant fast-twitch units in spite of lower SDH activity of fibres as was the case in soleus (Kugelberg, 1973). Slow-twitch units in the anterior tibial muscle were too scarce for more extensive comparisons.

Owing to the large differences in endurance among the units it was not possible to

find a simulus sequence which permitted good differentiation of fatigue resistance over the whole range. As a compromise, stimulation for 4 min with trains of 20 impulses at 100 Hz twice a second was used. The area within the envelope of the recorded tetanic contraction after 4 min stimulation expressed in fraction of initial tetanus area, here called 'fatigue ratio', served as an index. The area which represents the total work of tetanus was measured planimetrically.

Fatigue ratio plotted against isometric tetanic tension for forty-five motor units isolated in one muscle indicates a reversed near linear relationship between fatigue resistance and tetanic tension for units with tetanus tension of up to 15 g (Text-fig. 6). A similar result was obtained in all five muscles examined.

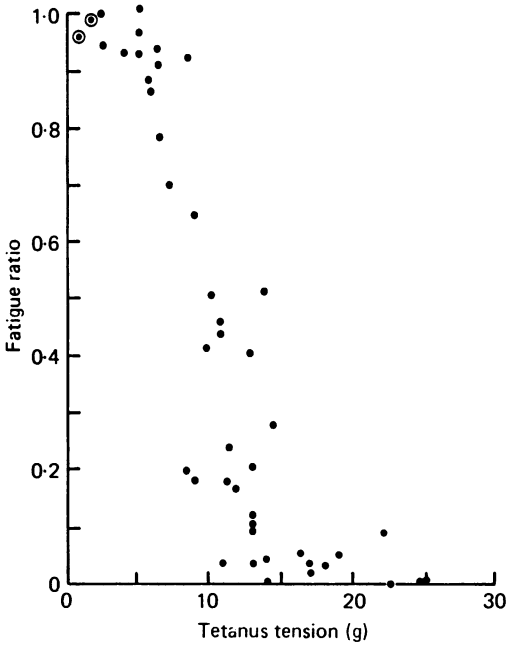


Fig. 6

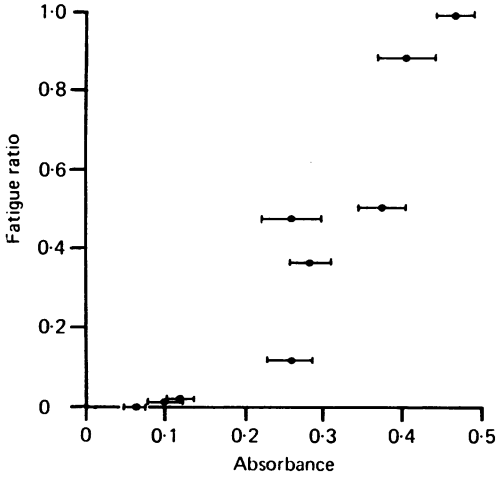


Fig. 7

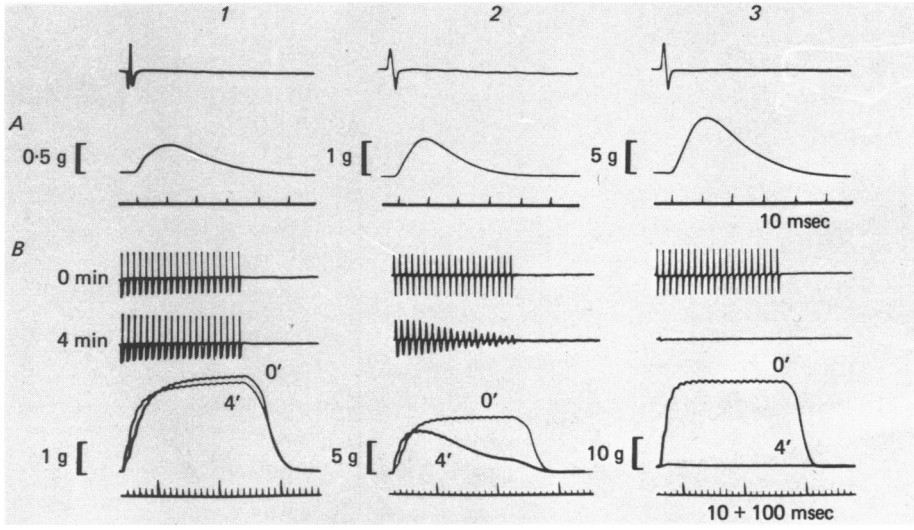
Text-fig. 6. Resistance to fatigue in relation to tetanic tension of forty-three fast-twitch • and two slow-twitch (⊙) motor units from one anterior tibial muscle. Fatigue ratio = the area within the envelope of the recorded tetanic contraction after 4 min stimulation to area of initial tetanus. Stimulus: trains of 20 pulses at 100 Hz, twice a second.

Text-fig. 7. Resistance to fatigue of fast-twitch motor units in nine muscles in relation to absorbance of motor unit fibres stained for SDH. One motor unit from each muscle. Absorbance: mean ± S.D. of ten fibres in the unit. Fatigue ratio see Text-fig. 6.

It is also evident that the different units exhibit a continuous spectrum of resistance to fatigue and that few units show an identical specification. The variability among the units is in fact larger than is evident from the Figure. For instance, the four units with fatigue ratios 0·92–0·93 could be separated by extending the stimulus from 4 to 8 min. Conversely shorter stimulus time improved discrimination among the most fatigue-sensitive units.

The inverse relationship between SDH activity and fibre size for fibres with a

cross-sectional area below 3000 μm^2 (Text-fig. 2) and between resistance to fatigue and tetanic tension for units below 15 g (Text-fig. 6) indicate that SDH activity and resistance to fatigue are proportional except for units with large fibres and large tension output. A more direct proof of the relationship between fatigue ratio and absorbance is presented in Text-fig. 7. Fibres of one unit from each animal were examined microphotometrically (Pl. 2). In spite of possible individual variations in the energy pool of the fibres and in experimental conditions there is an indication of a linear relationship between fatigue ratio of units and absorbance of unit fibres except for the three units with absorbance below 0·15. Tetanus tensions of these units were 20·25 and 18·5 g and their fibre size was over 3000 μm^2.



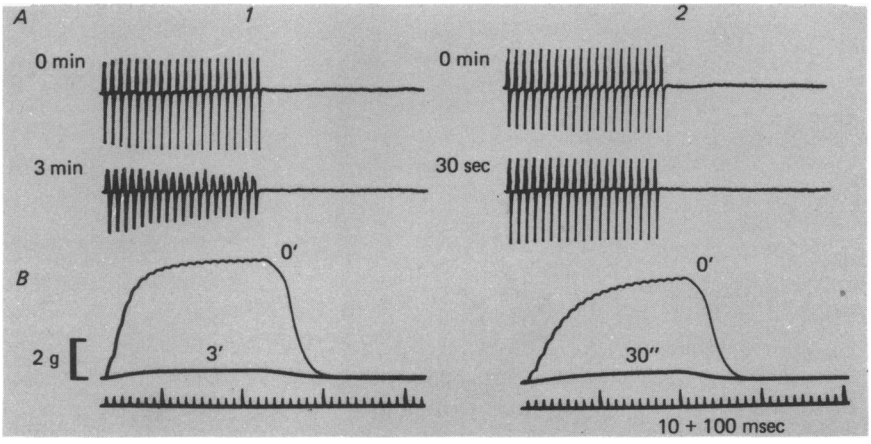
Text-fig. 8. Responses of fast-twitch motor units to intermittent stimulation demonstrating fatigue resistance directly related to SDH absorbance of unit fibres and a close coupling of electrical and mechanical responses. *A*, e.m.g. and isometric twitch. *B*, electrical and mechanical responses to trains of 20 pulses at 100 Hz twice a second for 4 min. *1*, unit absorbance 0·457. *2*, unit absorbance 0·38. *3*, unit absorbance 0·067 (histochemical profile of unit *1* and *3* is shown in Pl. 2).

The correspondence of fatigue resistance to oxidative activity of fibres would suggest that a purely muscular type of fatigue was involved in the test. However, the decline in tetanic tension is closely coupled to decline in the e.m.g., illustrated by Text-fig. 8. In the fatigue-resistant unit *1* with absorbance 0·457 of fibres, neither action potentials nor tetanus area decreased after 4 min stimulation. The diminished peak tetanic tension was compensated for by a steeper rise of tension due to post-tetanic potentiation of the first two contractions in the train.

In unit *2* with a fatigue ratio of 0·5 and absorbance 0·380 the progressive decline of tetanic tension parallelled decline in e.m.g. (Text-fig. 8, *2B*). The e.m.g. decline was associated with increased duration of the action potentials and particularly in the later part of the train by a step-wise irregular drop in potential amplitude. The latter was better demonstrated by a more selective electrode placed within the muscle and indicated elimination of activity from single muscle fibres or groups of them.

In unit *3* with fatigue ratio near 0 and absorbance 0·067, tension development and e.m.g. went through the end state of unit *2* within 30 sec. After 4 min the potentials were almost completely blocked (Text-fig. 8, *3B*).

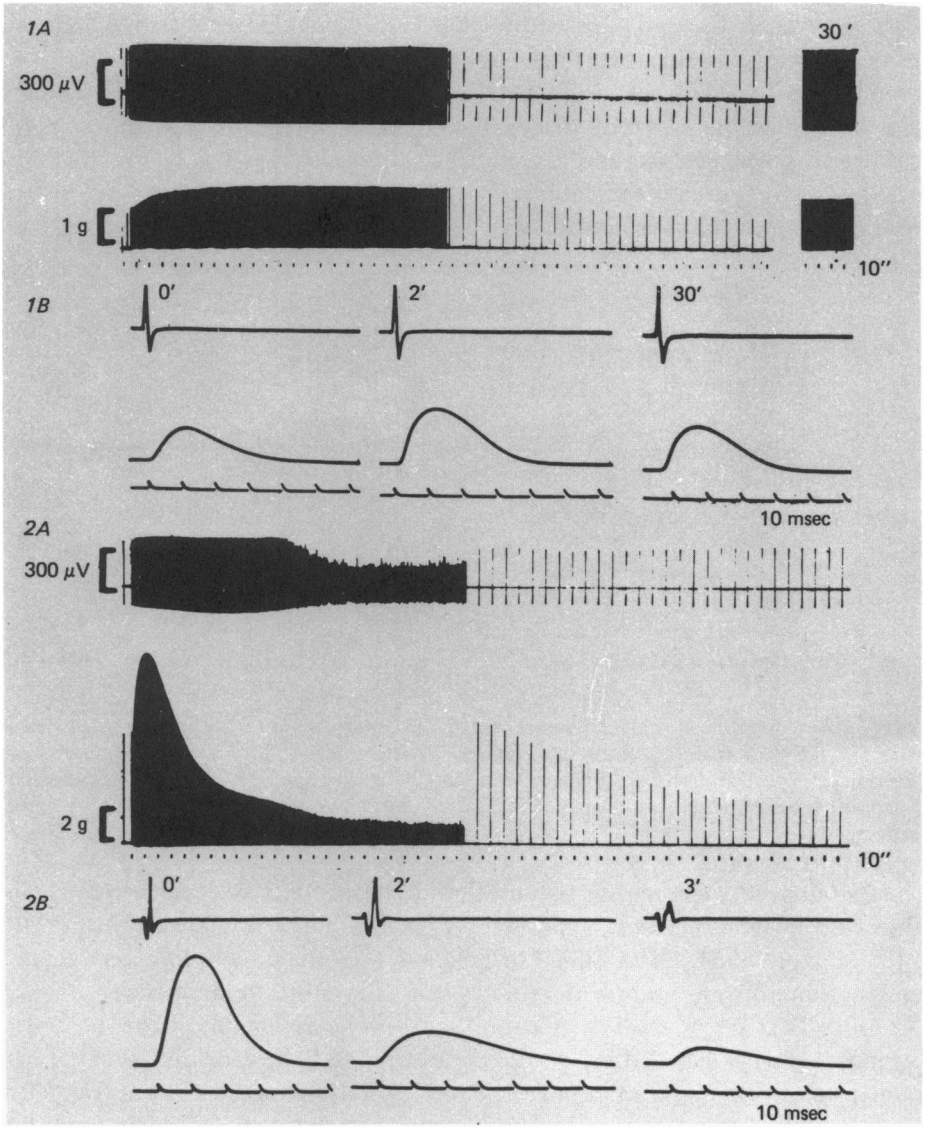
A progressive block of action potentials of the type shown by units *2* and *3* in Text-fig. 8 is generally attributed to failure of neuromuscular transmission rather than failure to propagate the impulse along the muscle fibre (cf. Krnjević & Miledi, 1958). This was further substantiated by the fact that an almost complete contractile fatigue did not prevent propagation of the action potential over the muscle fibres.



Text-fig. 9. Uncoupling of electrical and mechanical responses of fatigue-resistant fast-twitch motor unit stimulated during ischaemia with trains of 20 pulses at 100 Hz twice a second. *A*, electrical and *B*, mechanical response. *1*, beginning and end of first period of stimulation. *2*, beginning and end of second period of stimulation performed after 5 min recovery.

Thus uncoupling between action potential and contraction was transiently achieved by subjecting fatigue-resistant type II or type I units to ischaemia during stimulation (cf. Merton, 1954), exemplified in Text-fig. 9. Some uncoupling between e.m.g. and contraction appeared under the first period of stimultion of the fatigue-resistant type II unit (Text-fig. 9, *1*). When stimulation under ischaemia was repeated after 5 min recovery with circulation restored, a rapid fall in tension occurred within 30 sec in spite of preserved e.m.g. (Text-fig. 9, *2*). Ischaemia accelerates depletion of the energy pool of the fibres, which affected contraction to a larger extent than neuromuscular transmission or propagation of the action potential over the fibres. The same degree of uncoupling was not observed in rapidly fatiguing units with their very low safety margin for neuromuscular transmission.

The capacity of neuromuscular transmission in fast units appeared to match the oxidative activity of its fibres and to be a major factor in resistance to fatigue under the conditions studied. However, contraction fatigue was also present even if failure of transmission tended to mask it. It was most easily demonstrated 10–20 min after the end of stimulation when the action potential had recovered, but twitch tension remained decreased for several hours (see next section). In fatigue-sensitive units depression of twitch tension was profound and lasted 5–6 hr or more. Glycogen was depleted histochemically in the fibres of such units already after 2 min stimulation.



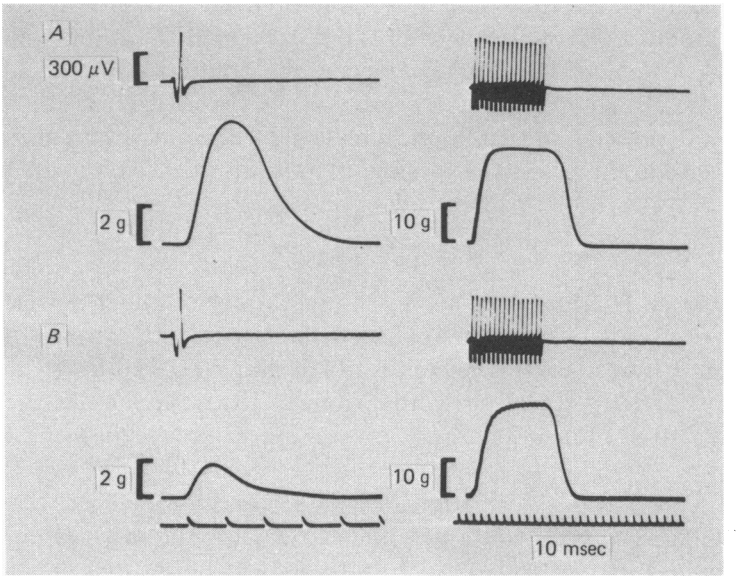
Text-fig. 10. Stimulatory and post-stimulatory effects of 10 Hz continuous stimulation on e.m.g. and isometric twitch of fast motor units. *1*, high SDH activity unit, absorbance of fibres 0·4 and *2*, low SDH activity unit, absorbance 0·13. *A*, compressed time scale. *B*, single sweeps from *A*. Upper records e.m.g., lower records mechanical response. Unit *1* responds with a staircase potentiated twitch maintained above intial level during 30 min stimulation after a break in stimulation (*1 A*) and with little change of twitch contraction and relaxation times (*1 B*). Twitch tension decreased during recovery after 4 min stimulation, 10% below initial value as tested every 10 sec (*1 A*). Unit *2* responds with a staircase potentiated twitch rapidly followed by a decline to 50% of initial twitch tension with increased amplitude of action potential, later followed by a further decline of twitch and decline of action potential. Twitch tension decreased during recovery after 4 min stimulation 70% below prestimulated value at a time the action potential had recovered (*2 A*). Twitch contraction and relaxation times increased during begin ning of stimulation (*2 B*).

In fatigue-resistant units, depression of twitch tension was slight, recovery much faster and the fibres not appreciably depleted of glycogen.

Contractile fatigue could also be observed during stimulation in some units where the e.m.g. amplitude was lowered in the later part of the impulse trains with the two or three first action potentials unaffected. The tension corresponding to the initial action potentials decreased, however, progressively during prolonged stimulation.

Fatigue, low frequency continuous stimulation

Fifty motor units were examined. In rapidly fatiguing units, twitch tension declined slowly at a stimulation frequency as low as 3–4 Hz. One representative fatigue-resistant unit and one fatigue-sensitive unit stimulated at 10 Hz is shown (Text-fig. 10, *1* and *2*). However, there was a continuum of transitional forms between the two.



Text-fig. 11. Post-stimulatory fatigue of twitch combined with recovery of action potential and of peak tetanic tension but with a slower rate of rise (same unit as in Text-fig. 10, *2*). *A*, isometric twitch and tetanus at 200 Hz before stimulation. *B*, 10 min after a 4 min period of stimulation at 10 Hz.

The high SDH active fast motor unit (absorbance 0·4) responded to stimulation at 10 Hz with staircase potentiation of twitches maintained for more than 30 min stimulation and with little change in duration of twitch (Text-fig. 10, *1A* and *B*). The action potential increased slightly in duration and 10% in amplitude. When stimulation was stopped after 4 min there was some further post-tetanic potentiation of the staircase potentiated twitch, followed by an exponential decline of twitch tension to 10% below pre-stimulation value.

The low SDH active unit (absorbance 0·13) responded with a staircase potentiation of twitches of similar magnitude, cut short however after some 30 sec by a rapidly progressing decline accompanied by prolongation of twitch contraction and relaxation times (Text-fig. 10, *2A* and *B*). Twitch tension was halved within 70 sec at a time

when the action-potential had increased about 15% in amplitude. Even after 2 min of stimulation when twitch tension had declined 70%, failure of neuromuscular transmission was not apparent in as much as action potential area had not diminished Peak tension of a 200 Hz tetanus interpolated in the 10 Hz stimulus sequence also decreased during these stages. A partial neuromuscular block developed thereafter rapidly with an irregular and stepwise elimination of action potential components (Text-fig. 10, *2A* and *B*). Twitch tension and e.m.g. amplitude eventually stabilized to some extent presumably due to intermittent recovery of transmission and rotation of contractions between muscle fibres or groups of them.

When stimulation was stopped after 4 min the post-tetanic potentiated twitch declined exponentially to 70% below pre-stimulation value in 10 min. At this time the action potential had recovered. Twitch contraction time was decreased, but relaxation time was still slightly prolonged. Recovery of twitch tension was very slow and not completed during 4 hr observation (cf. Kugelberg & Edström, 1968).

The post-stimulatory fatigue of twitch is not explained by an impaired contractile mechanism *per se*, since the unit developed full tetanic tension at 200 Hz (Text-fig. 11). The rate of rise of tetanus tension was, however, decreased and associated with the initial depression of twitch tension. The decline in twitch tension but not in peak tetanic tension increased the tetanus-twitch ratio from 3·9 to 14·1.

DISCUSSION

Type I and type II fibre units are in the fast anterior tibial muscle faster than respective type of unit in the slow soleus. In fact contraction times of 18–22 msec represent type I fibres in the anterior tibial, but type II fibres in the soleus (Kugelberg, 1973, 1976). In other words, even in the same species the contraction time of a slow twitch fibre in one limb muscle may represent a fast twitch fibre in another.

The earlier observations on fatiguability of rat motor units by Edström & Kugelberg (1968) have been confirmed and extended. The fast-twitch motor units exhibited a broad range of resistance to fatigue, which was proportionate to SDH activity of the fibres, except for units with fibres of low SDH activity and large fibre size. Few if any motor units show identical fatigue resistance. This is not surprising since few if any motoneurones subserving a muscle perform identical functional tasks, at least their recruitment threshold and minimal frequencies differ, e.g. Bigland & Lippold (1941), Hannerz (1974).

The continuously variable resistance to fatigue and SDH activity did not lend itself to any categorization of units or fibres into distinct types. However, there are large histochemical and functional differences between fibres at both ends of the scale and a bimodal distribution as regards SDH activity of type II fibres has been reported in gastrocnemius of the rat (Schmalbruch & Kamieniecka, 1975) and as regards fatiguability of units in gastrocnemius of the cat (Burke *et al.* 1973). This has motivated a number of classification schemes, such as red and white fibres by Padykula & Gauthier (1967), fast-twitch fatigue-resistant and fast-twitch fast-fatigue by Burke *et al.* (1973) and fast-twitch-oxidative-glycolytic and fast-twitch-glycolytic by Ariano, Armstrong & Edgerton (1973). Such simplifications, though sometimes

useful, take no account of the many grades of intermediate fibres or units. Furthermore, what is fatigue resistant in one animal like the cat, need not be so in another animal such as the rat where fatigue-resistant units sustained much higher stimulation frequencies to judge from the figures reported by Burke *et al.* (1973). However, the reaction products of tetrazolium salts lend themselves to quantitative histochemistry of oxidative enzymes (Eadie, Tyrer, Kukums & Hooper, 1969; Nolte & Pette, 1972) and this is why oxidative activity of fibres may preferably be described in quantitative terms.

In the rat diaphragm Krnjević & Miledi (1958) found, by indirect stimulation and intracellular recording, that neuromuscular transmission could not sustain impulses for more than a few minutes at frequencies greater than about 50 Hz, but the likelihood for neuromuscular failure was small at a frequency of 10 Hz. A rise in muscle fibre threshold and diminution of the excitatory post-synaptic potential was responsible for the failure of neuromuscular propagation as well as presynaptic failure probably in the final ramification of the motor nerve.

For stimulation of single motor units and recording with gross electrodes in the anterior tibial muscle consistent frequencies for failure of the action potential were found. We therefore think it is justified to attribute the block of the action potentials to failure of neuromuscular transmission although its precise location was not examined as was the case in the rat diaphragm. The upper stimulation frequency the e.m.g. of motor units could sustain for 30 min was not much above 40 Hz. The likelihood for e.m.g. failure at 10 Hz was higher since low SDH active fibres are much more numerous in the anterior tibial than in the more 'oxidative' diaphragm (personal observation).

There appears thus to be large differences in the safety margin of neuromuscular transmission between different units. Tested with high frequency intermittent stimulation which produced fatigue predominantly through failure of neuromuscular transmission it was found that the safety factor in fast-twitch motor units was proportionate to oxidative activity of the unit fibres. In consequence, the question arises if transmission failure is not chiefly produced by decrease in post-synaptic responsiveness as the energy reserve of the fibre is strained. However, when depletion of the energy pool of fatigue-resistant fibres was accelerated by contractions under ischaemia the action potential was not blocked in spite of almost complete failure of contraction. Presynaptic factors seem therefore of importance such as conductivity of fine nerve terminals (Krnjević & Miledi, 1958) and transmitter output (cf. Brooks & Thiess, 1962).

Transmission and contraction fatigue appear at least to some extent independent of each other, but are nevertheless closely matched in any one motor unit. Fatigue-sensitive units demonstrate rapid failure of transmission as well as contractile fatigue though the actual type of fatigue, which predominated depended upon the frequency of excitation as proposed by Rosenblueth (1950). The rapid failure of transmission in fatigue-sensitive units did not protect the fibres from being histochemically depleted of glycogen.

Decline of twitch tension without decline in action potential occurred during low frequency stimulation of rapidly fatiguing units associated with decline in tetanus tension and with retained peak tetanic tension in the recovery period after stimulation

of any unit. Resistance to both types of fatigue was clearly directly correlated to intensity of SDH activity of the fibres, though no attempts were made to quantitate the relationship over the whole range of units.

The first mentioned type is probably similar to the fatigue studied in frog muscles where low frequency stimulation may cause almost complete loss of twitch tension in spite of little change in the propagated action potential and an unimpaired contractile mechanism as tested by reactivation of contraction by caffeine. This fatigue has been attributed to a failure in the coupling between excitation and contraction (Eberstein & Sandow, 1963; Mashina, Matsumura & Nakayma, 1962; Grabowsky, Lorsinger & Lüttgau, 1972).

The prolonged decline in twitch tension after previous stimulation was not caused by depletion of available energy for contraction or of an impaired contractile apparatus, since the units developed full tetanic tension on high frequency stimulation (Kugelberg, 1973; Edwards, Hill, Jones & Merton, 1977). It may be assumed that this form of fatigue is caused by deficiency in the system that activates the contractile apparatus.

The much more efficient release of energy by oxidation than by anaerobic glycolysis makes a key oxidative enzyme like SDH a natural marker for endurance of contraction under aerobic conditions. It is less evident why the over-all SDH activity of the fibre so well predicts the fatigue resistance of neuromuscular propagation and excitation–contraction coupling which precede contraction, even though some SDH activity is associated with the function of the latter.

However, it is essential that the energy pool of the fibre can be fully utilized without being completely exhausted to prevent rigor. To this end, it is appropriate that the links in the chain of events leading to contraction possess a predetermined capacity matched to available energy for contraction. Indeed, this does not exclude any further adjustment of transmission and excitation to the energy state of the fibre during sustained contractions. The rise in muscle fibre threshold to the postsynaptic excitatory potential in response to repetitive stimulation (Krnjević & Miledi, 1958) may exemplify this. The closely connected capacities of neuromuscular junction, the excitation–contraction coupling system and oxidative enzyme activity of the fibre are determined by the motoneurone, since the histochemical and related functional properties of all muscle fibres in the motor unit are almost identical (Edström & Kugelberg, 1968).

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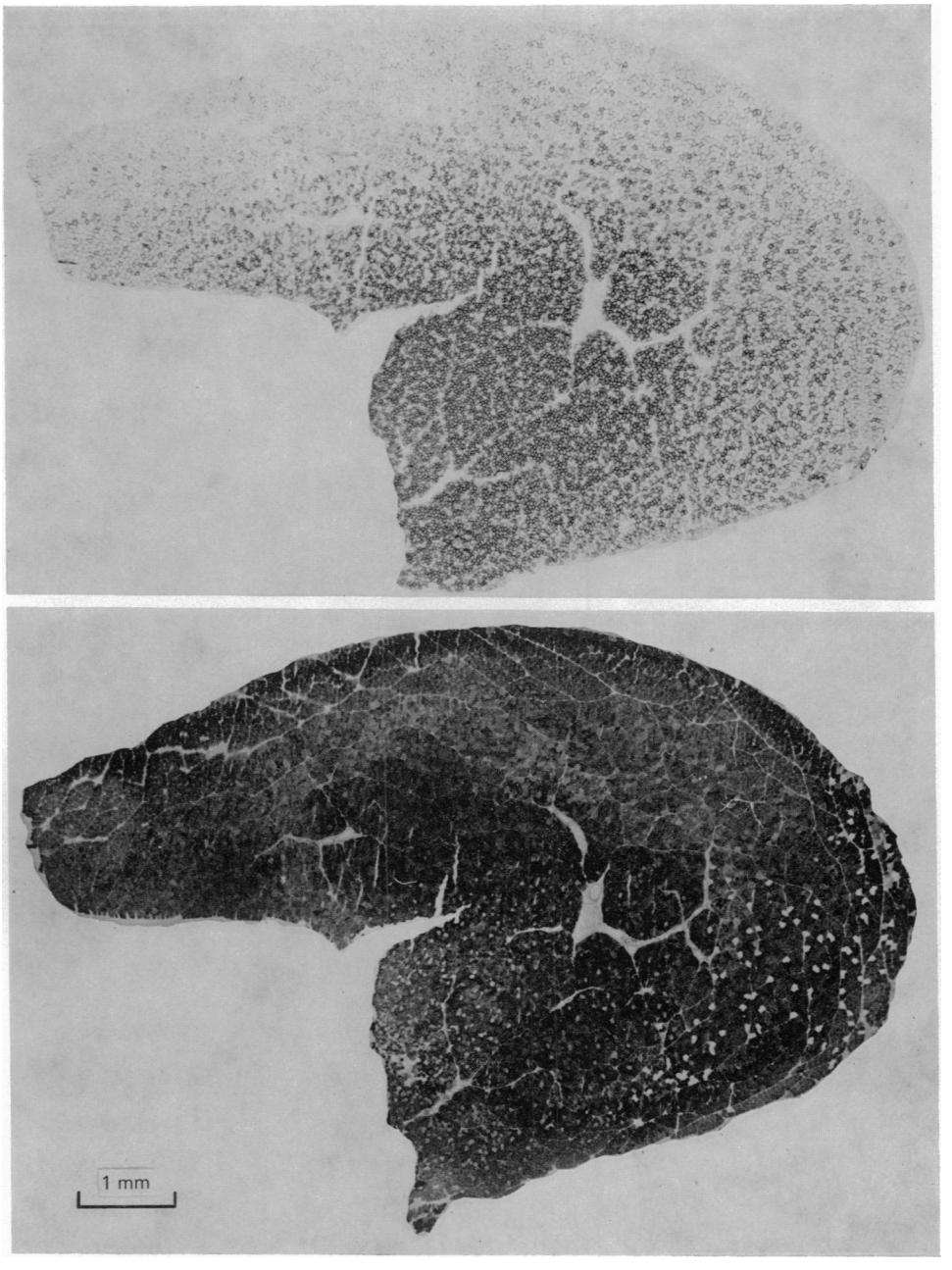
EXPLANATION OF PLATES

PLATE 1

Photomicrographs of serial sections of rat anterior tibial muscle. *Upper section*, stained for succinate dehydrogenase, shows the distribution of fibres with different SDH activity. *Lower section*, PAS preparation, demonstrates the distribution of the PAS negative (glycogen depleted) muscle fibres of one fast-twitch motor unit in the superficial layer of the muscle. The small lightly stained fibres in the deep central part are slow twitch type I fibres, which stain less intensely for glycogen in the rat. Right is medial, left lateral and up anterior aspect of the muscle.

PLATE 2

Photomicrographs of serial sections showing histochemical composition of three fast-twitch type II fibre motor units with different resistance to fatigue. *Upper* row, PAS, a few PAS-negative (glycogen depleted) fibres belonging to the motor unit. One fibre in each unit marked. *Middle* SDH and *bottom*, ATPase after preincubation at pH 4·5. *Left vertical* row, motor unit with fatigue ratio 1, SDH absorbance 0·457. *Middle*, unit with fatigue ratio 0·12 and absorbance 0·253. *Right*, unit with fatigue ratio 0, absorbance 0·967. Note the similiarity of enzyme activity and size of fibres of each motor unit.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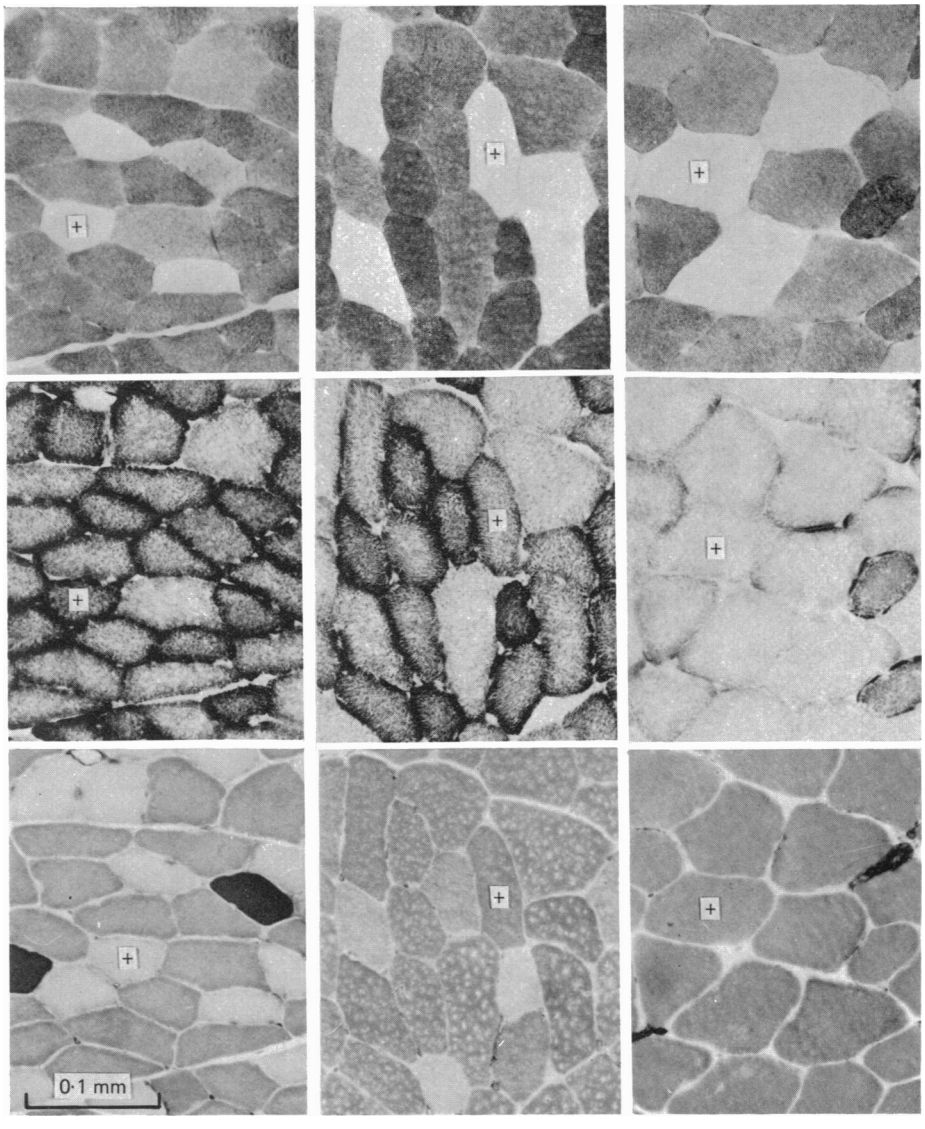
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