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Histochemical characteristics and contractile properties of the spinotrapezius muscle in the rat and the mouse

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INTRODUCTION

There are large variations in the mechanical and circulatory requirements of skeletal muscle between the extremes of exercise and rest. The wide range of mechanical demands are usually met by the provision of a heterogeneous mixture of muscle fibre types, variously classified on structural, dynamic and cytochemical grounds. The histological properties of each fibre type are thought to be correlated with their probable functions (Close, 1972). It is widely accepted that three main types are present in mammalian skeletal muscle. The large diameter white (*A*) fibres are apparently concerned with evanescent phasic anaerobic contractions; in contrast, small diameter red (*C*) fibres have a rich capillary vascular supply and are better adapted to sustained phasic aerobic activity. In addition to these phasic fibres, there are low speed contractile units of intermediate (*B*) fibres more suited to prolonged aerobic contractions. Whole muscles are rarely homogeneous: their overall contractile characteristics must be governed, therefore, by the proportions of each of the types present.

In recent years the microcirculation of the spinotrapezius muscle of the rat has been investigated (Gray, 1973). The spinotrapezius is a thin strap-like muscle extending along the spine in the thoracic and upper lumbar region of rodents. The muscle fibres arise from the spines of the fourth thoracic to the third lumbar vertebrae and converge to their insertion on the scapular spine. In the mouse the characteristics of the muscle allow great resolution of its innervation and of the terminal vascular bed. This paper is concerned with the histochemical and dynamic classification of fibres in the spinotrapezius muscle of both rat and mouse, and with a comparison of this muscle with the 'classical' skeletal muscles commonly studied (viz. soleus and tibialis anterior).

MATERIALS AND METHODS

Histological studies

Adult Wistar rats and albino mice were anaesthetized with urethane (1·4 g/kg) prior to dissection. The spinotrapezius muscles were removed, stretched to their resting lengths over blocks of liver, and rapidly frozen in a mixture of solid carbon dioxide and 2-methylbutane. After 2–5 minutes, the muscle was transferred to a cryostat and allowed to warm to −20 °C. The tissue was sectioned (10–20 μm) and mounted serially on clean dry coverslips.

Succinic dehydrogenase

Succinic dehydrogenase (SDH) was demonstrated by the incubation (37 °C for 20–40 minutes) of sections in a medium containing substrate (sodium succinate; 0·1 M) and an electron acceptor (tetranitro blue tetrazolium chloride, 0·5 g/litre) in phosphate buffer (0·1 M, pH = 7·6) (Nachlas *et al.* 1957). After incubation, the sections were washed in distilled water, dehydrated in alcohol, cleared in xylene, and mounted in Uvinert (G. T. Gurr Ltd).

Actomyosin adenosine triphosphatase

Acid and alkali stable adenosine triphosphatase (ATPase) was demonstrated by incubation with substrate at pH 9·4 after preincubation at room temperature in acid (pH 4·35) or alkali (pH 10·4) (Guth & Samaha, 1969).

Phosphorylase

Phosphorylase activity was demonstrated by the method of Meijer (1968). Dextran (average molecular weight = 40,000) and disodium adenosine triphosphate (0·4 g/litre) were used in the incubation medium and the reaction product was developed with Grams iodine (1:9, v/v in distilled water).

Nicotinamide adenine dinucleotide dehydrogenase

Nicotinamide adenine dinucleotide dehydrogenase (NADD; reduced diphosphopyridine nucleotide dehydrogenase) was demonstrated in frozen sections (Sternberg, Farber & Dunlap, 1956). Cold incubation medium, containing blue tetrazolium as an electron acceptor, was placed in a flask which was flushed with nitrogen. The frozen sections were transferred to the medium within 3 minutes and incubated for 0·4–2 hours at 37 °C.

Measurement of fibre diameter

The diameters of individual muscle fibres were determined from photomicrographs of representative transverse sections. The maximum diameter and the diameter at right angles to it were both measured. Fibre diameter was expressed as the mean of these two measurements.

Measurement of contractile responses of muscle

Adult Wistar rats and albino mice were anaesthetized with urethane (1·4 g/kg), and isometric contractions at optimal length were recorded from their spinotrapezius, soleus and tibialis anterior muscles. The muscles were dissected free from surrounding tissues, leaving the origin and all vascular and nervous connexions intact. In all instances skin flaps were raised and the muscles were covered with liquid paraffin (34·5 °C–36·0 °C). Supramaximal stimuli were delivered to the whole muscle by two partially insulated silver wires which were mounted on fine springs that encircled the muscle belly. Stimulation was carried out at three times maximal intensity at six different frequencies (10 Hz, 20 Hz, 40 Hz, 75 Hz, 100 Hz and 200 Hz); the duration of each stimulus was 0·5 msec. Single twitches were also recorded at a higher paper speed to give a clearer indication of the temporal characteristics of

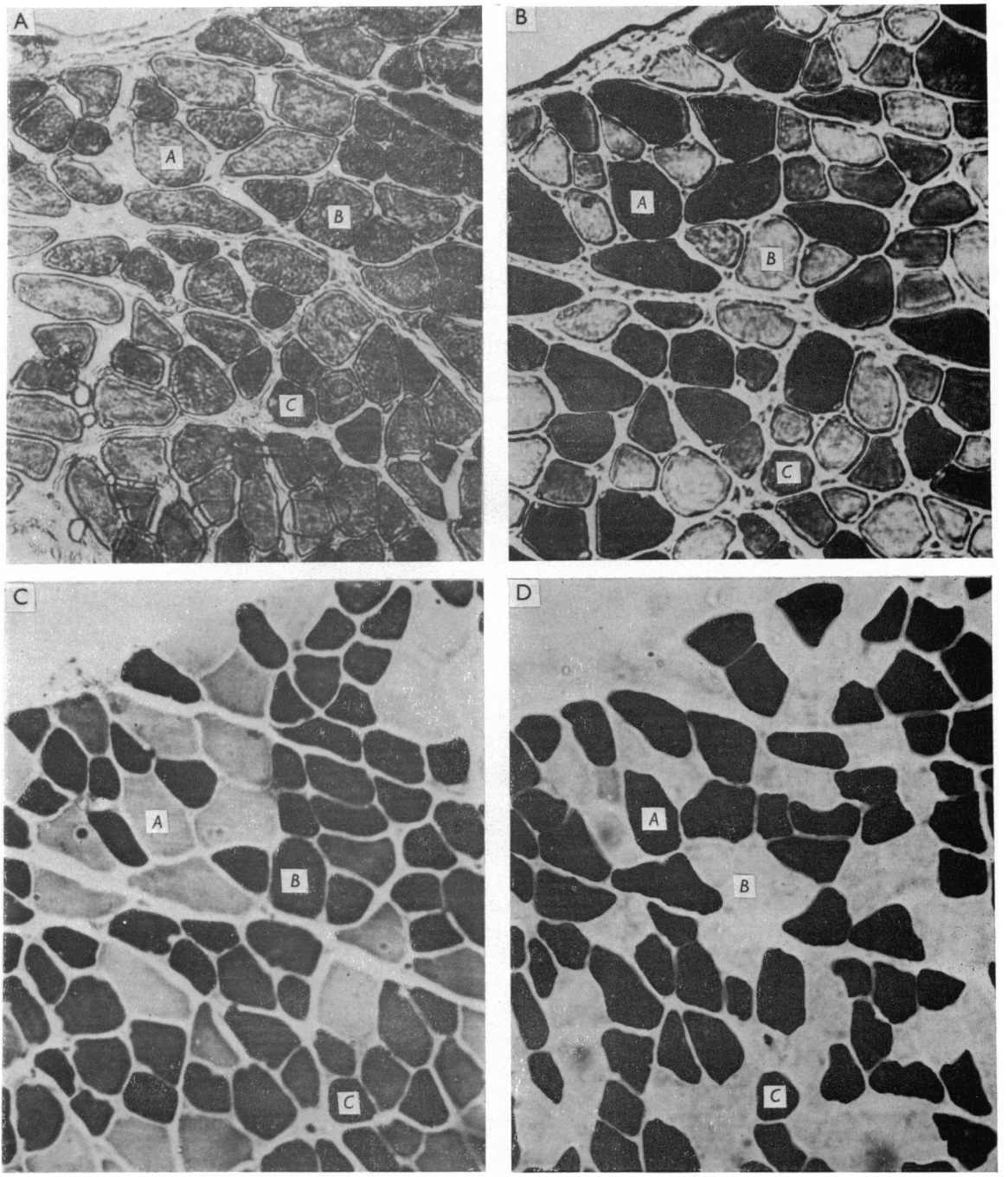

Fig. 1. Serial sections of the spinotrapezius muscle of the mouse, demonstrating SDH (A), phosphorylase (B), actomyosin ATPase after acid preincubation (C), and actomyosin ATPase after alkaline preincubation (D). Characteristic *A*, *B* and *C* fibres are shown.

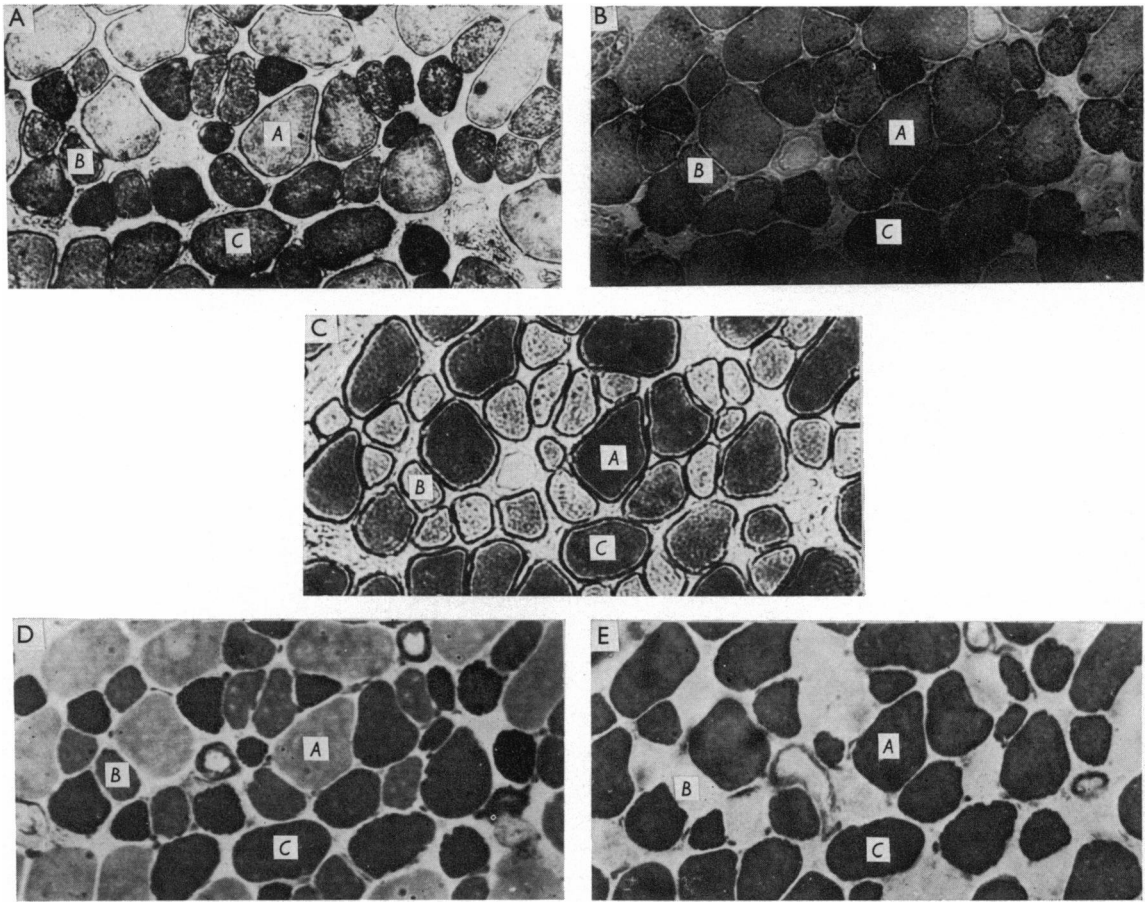

Fig. 2. Serial sections of the spinotrapezius muscle of the rat, demonstrating SDH (A), NADD (B), phosphorylase (C), actomyosin ATPase after acid preincubation (D), and actomyosin ATPase after alkaline preincubation (E). Typical *A*, *B* and *C* fibres are shown.

individual twitches. Stimulus spread to adjacent deflected muscles was minimal and did not contribute significantly to the recorded responses.

RESULTS

The three types of mammalian muscle fibre were readily identified in serial sections of the spinotrapezius muscle of both rat and mouse (Figs. 1, 2). The recognition and identification of these fibres was dependent on staining for actomyosin ATPase, phosphorylase, and the oxidative enzymes SDH and NADD. In other mammalian muscles these cytochemical techniques permit the unequivocal differentation of *A*, *B* and *C* fibres (Close, 1972), and similar criteria were used in the present study. In the rat and mouse spinotrapezius muscle, *A* fibres were characterized by a low intensity staining reaction for the oxidative enzymes SDH and NADD, and the formation of an even network of fine formazan crystals throughout the muscle fibres

Table 1. *Relative proportions of A, B and C fibres in the spinotrapezius muscle of the mouse and rat*

Species	Exp.	No. of fibres	Muscle fibre type *A*	*B*	*C*
Mouse	1	129	41	49	39
	2	66	24	17	25
	3	128	37	45	46
	Total	323	102 (32%)	111 (34%)	110 (34%)
Rat	1	151	51	41	59
	2	130	38	59	33
	3	82	27	20	35
	Total	363	116 (32%)	120 (32%)	127 (36%)

Table 2. *Diameters of A, B and C fibres in the spinotrapezius muscle of the mouse and the rat*

Species	Diameters (μm + S.E.M.) *A*	*B*	*C*
Mouse	47·9 ± 0·8 (102)	30·5 ± 0·4 (111)	31·3 ± 0·7 (110)
Rat	43·0 ± 1·1 (116)	31·4 ± 0·9 (120)	30·3 ± 0·9 (126)

The number of fibres of each muscle type are shown in parentheses.

was observed (Fig. 1A). In contrast, phosphorylase activity was high in these fibres (Fig. 1B). Actomyosin ATPase activity was acid-labile: the intense staining of *A* fibres in alkaline conditions (Fig. 1D) was abolished by preincubation for 10 minutes at pH 4·35 (Fig. 1C).

A different staining pattern was observed in *B* and *C* fibres. In *B* fibres the formazan crystals formed when staining for oxidative enzymes were larger and more intense than in *A* fibres (Fig. 1A). In contrast, phosphorylase activity was comparatively low (Fig. 1B). After pretreatment at pH 4·35, *B* fibres produced a more intense ATPase reaction than *A* fibres, although enzymic staining was largely abolished by preincubation in alkaline conditions (Fig. 1C, 1D).

C fibres were associated with high levels of the oxidative enzymes SDH and NADD (Fig. 1A), and contained large formazan crystals that were occasionally situated in a sub-sarcolemmal position in the cytoplasm. Their phosphorylase activity was less than that of the *A* fibres, but greater than that of the *B* fibres (Fig. 1B). The actomyosin ATPase reaction of *C* fibres was not diminished by alkaline preincubation at pH 10·4 for 20 minutes. After preincubation in acid conditions for 10 minutes, an intense reaction for actomyosin ATPase was observed, although this reaction was not present with longer periods of preincubation.

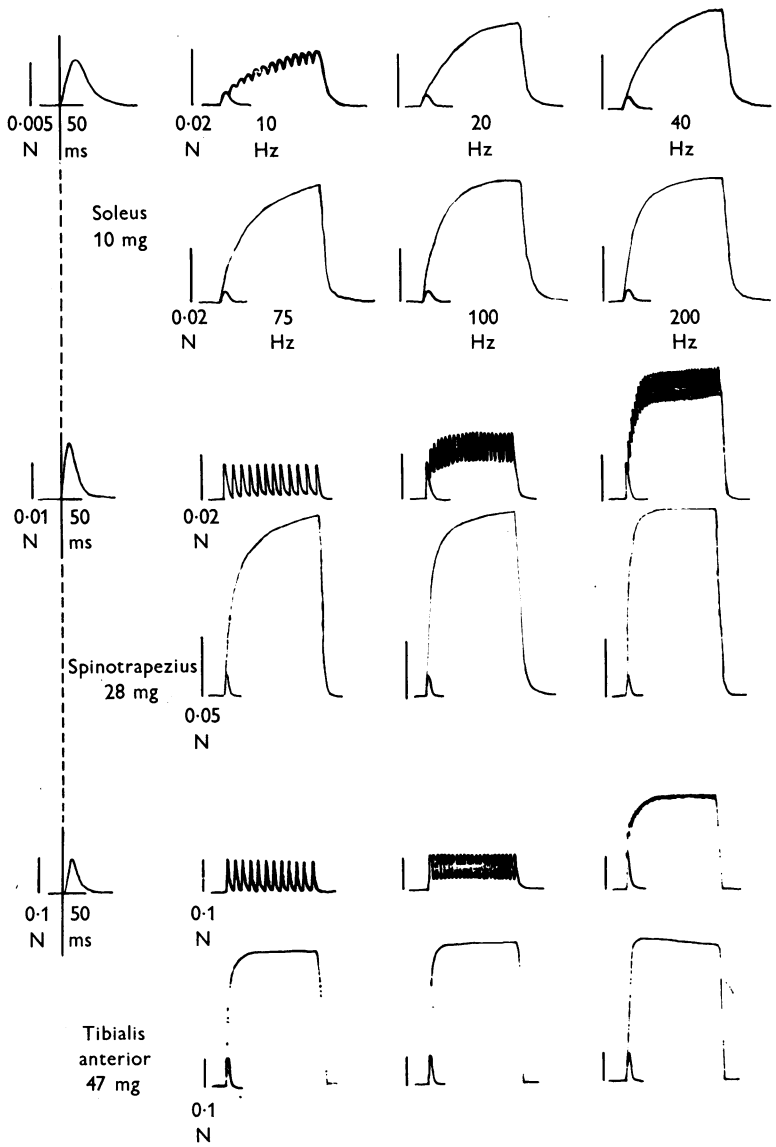

Fig. 3. Isometric responses of soleus (upper two rows), spinotrapezius (middle two rows) and tibialis anterior (lower two rows) in the mouse. Responses were recorded at optimal lengths and three times maximal intensity at six different frequencies (10, 20, 40, 75, 100 and 200 Hz).

The relative proportions of *A*, *B* and *C* fibres in the rat and mouse spinotrapezius muscles were investigated in three animals of each species. In both rat and mouse approximately equal proportions of the three types of fibre were present (Table 1); differences in the percentages of *A*, *B* and *C* fibres in the two species were not statistically significant ($P > 0{\cdot}05$). In the mouse, the mean diameter of *A* fibres ($47{\cdot}9 \pm 0{\cdot}8$ μm; mean $\pm$S.E.M.) was significantly greater than that of *B* fibres ($t = 19{\cdot}431$;

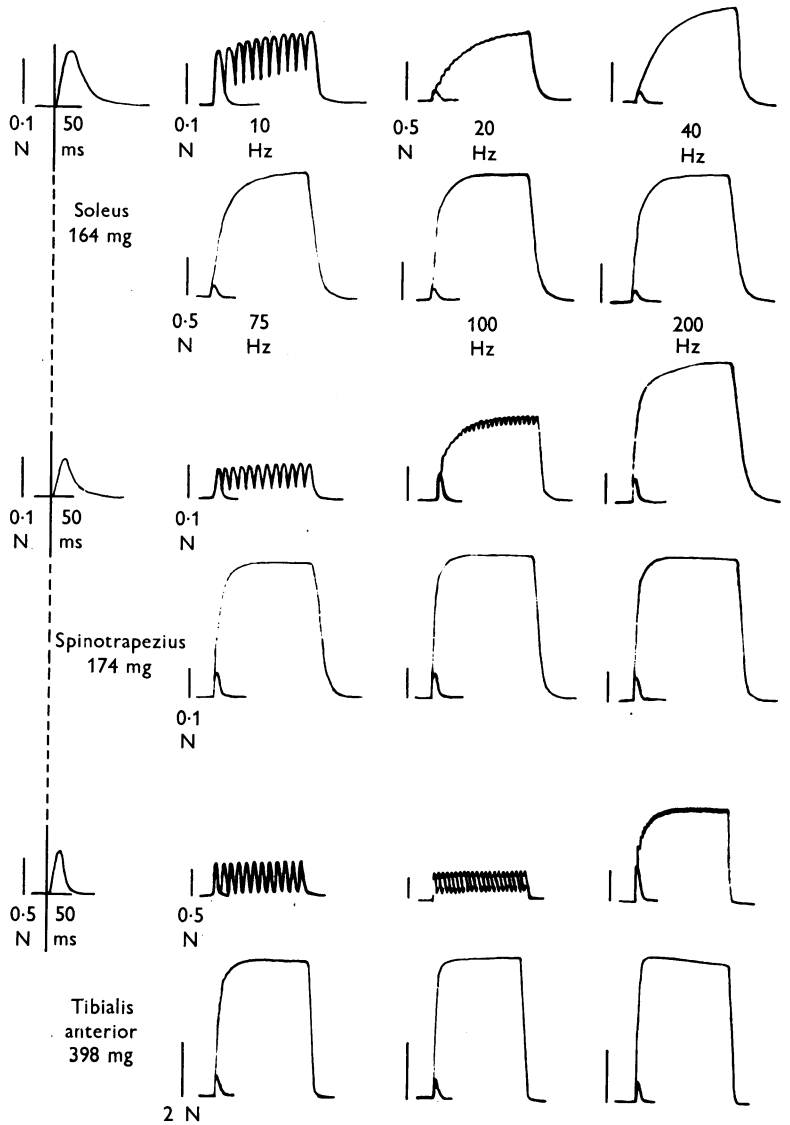

Fig. 4. Isometric responses of soleus (upper two rows), spinotrapezius (middle two rows) and tibialis anterior (lower two rows) in the rat. Responses were recorded at optimal lengths and three times maximal intensity at six different frequencies (10, 20, 40, 75, 100 and 200 Hz).

d.f. = 211; $P < 0{\cdot}001$) and *C* fibres ($t = 15{\cdot}656$; d.f. = 210; $P < 0{\cdot}001$). Similar differences were present in the rat (Table 2).

In both mouse (Fig. 3) and rat (Fig. 4) the isometric responses and the twitch summation of soleus, tibialis anterior and spinotrapezius were dissimilar. In both species the isometric twitch contraction time and the half relaxation time of soleus were longer than those of spinotrapezius, which in turn were longer than those of

tibialis anterior (Figs. 3, 4). Twitch summation occurred at a lower frequency of stimulation in soleus (less than 10 Hz) than in either spinotrapezius (greater than 10 Hz) or tibialis anterior (greater than 20 Hz). The spinotrapezius muscle did not show any 'sag' in tension during tetanic stimulation at 200 Hz, although this phenomenon was observed in the tibialis anterior of both species (Figs. 3, 4). Both spinotrapezius and soleus were much more resistant to fatigue during prolonged intermittent tetanic stimulation than was tibialis anterior.

DISCUSSION

It is generally accepted that rat skeletal muscle contains at least three distinct types of fibre that can be readily distinguished by histochemical methods. In type *A* fibres oxidative enzyme activity is low and glycogen content is high (Stein & Padykula, 1962; Ogata & Mori, 1964). In such fibres actomyosin ATPase has moderate enzymic activity and is relatively stable after preincubation in either acid or alkaline conditions (Samaha, Guth & Albers, 1970; Yellin & Guth, 1970). In *B* type fibres oxidative enzyme activity is usually greater, although phosphorylase activity is more variable (Ogata & Mori, 1964; Eversole & Standish, 1970). Actomyosin ATPase activity is also low, and is readily inhibited by previous incubation in alkaline conditions (Guth & Samaha, 1969). In *C* type fibres high levels of oxidative enzymes are present; in the case of succinic dehydrogenase, enzyme localization is often subsarcolemmal (Stein & Padykula, 1962). Phosphorylase activity is usually low or absent (Eversole & Standish, 1970). In contrast, actomyosin ATPase activity is high and stable in alkaline conditions, but is readily inhibited by preincubation at pH 4·4 (Samaha, Guth & Albers, 1970).

In the spinotrapezius muscle of rat and mouse, *A*, *B* and *C* fibres could be readily recognized on the basis of these criteria, although the activity of oxidative and glycogenolytic enzymes was generally a more reliable guide than was actomyosin ATPase. Indeed, in some respects the staining reactions of this enzyme were at variance with the descriptions of the original authors (Guth & Samaha, 1969; Samaha *et al.* 1970): in *C* fibres, for instance, the enzyme was relatively acid-stable. These differences, however, did not as a rule obscure the recognition of the three types of muscle fibre. In the present study morphological differences between the fibres were also observed. In both species the mean diameter of *A* fibres was significantly greater than that of *B* or *C* fibres, although there was some overlap in the ranges of the diameters of the three types. Similar differences in fibre diameter in muscles of other mammalian species have been described (Stein & Padykula, 1962; Ogata & Mori, 1964; Gauthier & Padykula, 1966; Samaha *et al.* 1970).

In the rat there is considerable variation in the proportion of *A*, *B* and *C* fibres in different skeletal muscles. The diaphragm is mainly composed of *C* fibres, although a proportion of *A* and *B* fibres is also present (Gauthier & Padykula, 1966). Tibialis anterior contains approximately equal proportions (41 %) of *A* and *C* fibres, although *B* fibres (18 %) are also present (Kugelberg & Edström, 1968; Close, 1972). In contrast, the soleus muscle, as in other mammalian species, is predominantly composed of *B* fibres (Engel, 1962; Edgerton & Simpson, 1969; Guth & Samaha, 1969; Fex & Sonesson, 1970). In the rat, therefore, the spinotrapezius contains a lower

proportion of *B* fibres than soleus, but a higher proportion than tibialis anterior. There is considerable evidence that the proportion of *B* fibres to *A* and *C* fibres governs the overall functional characteristics of mammalian muscles (Edgerton & Simpson, 1969; Edgerton & Simpson, 1971; Barnard, Edgerton, Furukawa & Peter, 1971). Functionally these muscles can only be divided into two groups because *A* and *C* fibres are both 'fast-twitch' units. The present results are quite consistent with this concept: there was a clear distinction between the isometric responses and the twitch summation of soleus, spinotrapezius and tibialis anterior. In both rat and mouse spinotrapezius the isometric twitch contraction time, the half relaxation time, and the twitch summation frequency had values intermediate between those of soleus and tibialis anterior. The contractile characteristics of the spinotrapezius muscle, which are clearly different from those of a 'fast' muscle like tibialis anterior and those of a 'slow' muscle like soleus, are in agreement with the types of muscle fibre present. In this respect the low speed *B* fibre units, which are suited to prolonged aerobic contractions, appear to have an important role.

SUMMARY

In the spinotrapezius muscle of the rat and the mouse approximately equal proportions of *A*, *B* and *C* fibres are present. The spinotrapezius therefore contains a lower proportion of *B* fibres than are known to be present in soleus, but a higher proportion than in tibialis anterior. These results are consistent with the functional properties of the muscles, for the values for isometric twitch contraction time, the half relaxation time, and the twitch summation frequency of the spinotrapezius are also intermediate between those of soleus and tibialis anterior. The present experiments support the view that the proportion of *B* fibres to *A* and *C* fibres govern the overall functional characteristics of mammalian muscles.

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