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MOTOR UNITS IN CAT SOLEUS MUSCLE: PHYSIOLOGICAL, HISTOCHEMICAL AND MORPHOLOGICAL CHARACTERISTICS

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SUMMARY

1. Physiological properties of motor units in the soleus muscle were studied in anaesthetized cats using intracellular stimulation of motoneurones to ensure functional isolation of single units. The muscle fibres belonging to 6 units were identified by glycogen depletion following prolonged stimulation, permitting analysis of their histochemical profiles and anatomical organization.

2. The studied units in soleus were all classed as type S and were extremely resistant to fatigue during prolonged stimulation. Twitch contraction times ranged from 64 to 131 msec (mean 97·1 msec) and tetanic tensions ranged from 3·5 to 36 g (mean 10·5 g). Most units exhibited depression of twitch tension in the wake of a short high-frequency tetanus and few of the units showed any significant degree of post-tetanic twitch potentiation.

3. Muscle fibres belonging to single soleus motor units were found to be scattered through territorial volumes occupying a large fraction of the total muscle volume. The available data suggest that different soleus motoneurones may innervate from less than 50 to more than 400 muscle fibres, with an average innervation ratio between 140 and 190 muscle fibres per unit.

4. The results were compared with observations on type S motor units in the synergist gastrocnemius, obtained under similar conditions. The

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evidence suggests that soleus units are not equivalent to the type S units of the mixed gastrocnemius but rather constitute a unique population.

INTRODUCTION

The soleus muscle is closely related to the gastrocnemius in anatomical position and mechanical effect, and motoneurones innervating muscle units in these synergists share common synaptic connexions (Burke, 1967; Burke, Jankowska & ten Bruggencate, 1970; Eccles, Eccles & Lundberg, 1957). The nominally fast twitch 'white' gastrocnemius heads contain a mixed population of muscle units, including a set of slowly contracting S type units which exhibit some physiological properties resembling those of soleus units (Burke, 1967). The present work was done to investigate interrelations between physiological, histochemical and morphological characteristics of soleus muscle units in order to compare them with the type S units in the mixed gastrocnemius heads (Burke, Levine, Tsairis & Zajac, 1973; Burke & Tsairis, 1973*a*, *b*). The results demonstrate that soleus muscle units are significantly different from gastrocnemius type S units in several respects.

METHODS

The methods used in the present experiments have been described in detail in preceding papers (Burke, 1967; Burke *et al.* 1973; Burke & Tsairis, 1973*a*). Briefly, adult cats (2·0–3·3 kg), anaesthetized with pentobarbitone, were held in a frame immobilizing the vertebral column and the left hind leg. In some experiments, both hind legs were used. The soleus tendon was freed from that of the lateral gastrocnemius and the lateral gastrocnemius-soleus nerve was placed in continuity on a flexible stimulating electrode pair. Intracellular micropipette electrodes were used to record from and to stimulate single alpha motoneurones innervating soleus muscle units. Electrical activity of the muscle units was recorded with fine monopolar stainless steel wires hooked into the exposed surface of the muscle. Mechanical responses of single units were recorded with the muscle held at passive tension of about 100 g. Stimulation patterns used to evaluate the mechanical responses of single units were the same as described earlier in connexion with studies of gastrocnemius units (Burke *et al.* 1973).

With histochemical analysis, only one unit was studied per soleus muscle. After full assessment of physiological responses, the isolated unit was stimulated with repeated short tetani (thirteen stimuli at 40 pulses/sec, with trains repeated every one sec) for 50–60 min and then the whole muscle was quickly removed, cut into cross-section blocks, and frozen at -160° C. Further details and references for histochemical staining methods are given elsewhere (Burke *et al.* 1973).

RESULTS

This report is based on a study of 30 soleus muscle units obtained in thirteen cats. In three animals we attempted to study as many units as possible but each preparation yielded only seven units, perhaps reflecting the low total number of soleus motor units in the cat (about 150; Boyd & Davey, 1968) and the selectivity of the intracellular sampling technique used (cf. Burke, 1967).

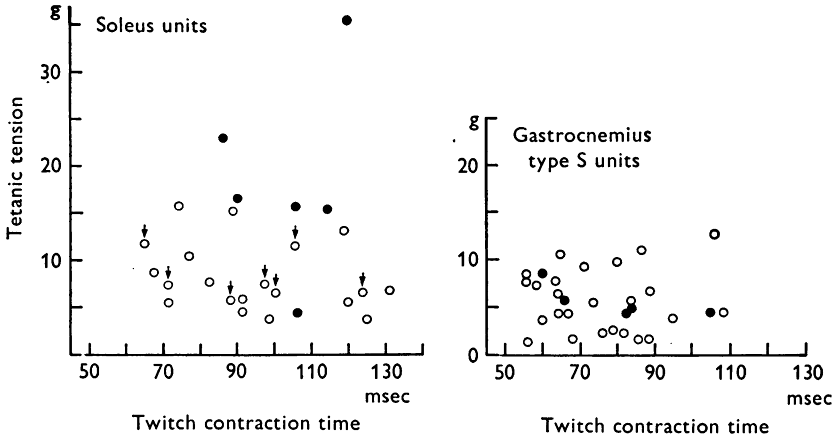
Physiological properties

All soleus units examined in the present material were type S on the basis of criteria developed for gastrocnemius muscle units (Burke *et al.* 1973). Units studied histochemically exhibited a very marked degree of fatigue resistance, even after one hour of repeated tetanization (approximately 47,000 stimuli) used to deplete glycogen in the unit fibres. In these respects, the soleus unit population resembled the type S units studied in the synergist gastrocnemius (Burke *et al.* 1973).

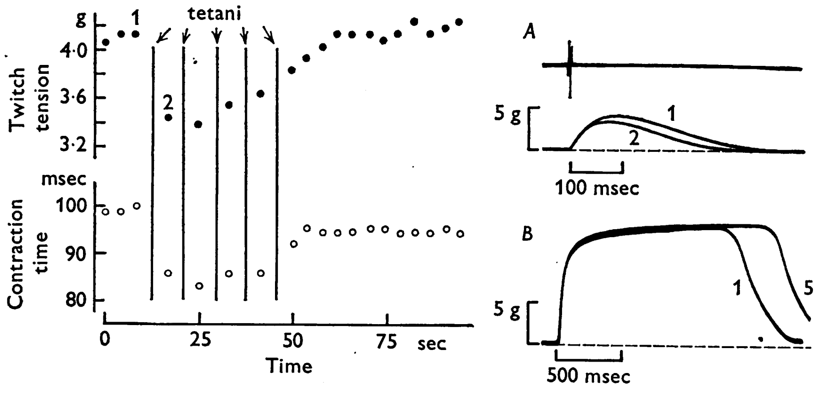
Comparison between some properties of soleus and gastrocnemius S units is shown in Text-fig. 1. The gastrocnemius sample represents material included in an earlier report (Burke *et al.* 1973). It is evident that soleus units, as a group, produced more tension during fused tetani and contracted more slowly than gastrocnemius S units (the respective means ± S.D. for each unit group are: 10·5 ± 6·9 g compared to 5·6 ± 3·0 g, $P < 0{\cdot}005$; and 97·1 ± 18·8 msec compared to 75·8 ± 14·9 msec, $P < 0{\cdot}001$).

The twitch contraction times for soleus units were measured from responses obtained before tetanization, in contrast to the procedure used for gastrocnemius S units in which measurements were made from twitch responses after maximum post-tetanic potentiation (PTP; see Burke *et al.* 1973). The difference in method was due to the anomalous response of most soleus muscle units to tetanization, an example of which is illustrated in Text-fig. 2. The graph shows the tension production (filled circles) and contraction times (open circles) of single twitch responses before and after the delivery of short tetani (1·5–2·0 sec duration, frequency 100 pulses/sec). The short tetani (vertical lines) were alternated with single twitches for five trials and twitch amplitudes and contraction times both decreased in the wake of the first several tetani. Subsequently, there was some return toward control values. Superimposed records of the twitch just before (*A*, 1) and just after (*A*,2) the first tetanus in the series illustrate the post-tetanic depression. There was no change in the electrical response of the unit (upper trace in *A*) or in the amplitude of any of the 5 tetani, of which the first and last are shown superimposed in *B*.

Post-tetanic depression of twitch amplitude and contraction time was found in thirteen of the twenty soleus units systematically studied for this phenomenon. The curves of twitch tension during intermittent tetanization were complex and in some units, twitch tension reached somewhat higher levels than control after repeated short tetani, although such potentiation never exceeded 10%. In the other seven units, an initial decline after the first tetanus did not occur but there was instead a gradual increase in twitch amplitude after successive short tetani, usually to a level



Text-fig. 1. Graphs showing the distribution of twitch contraction times (abscissae) and maximum tetanic tensions (ordinates) produced by individual soleus muscle units (left-hand graph) as compared to data from type S units in the gastrocnemius (right-hand graph; data from Burke *et al.* 1973). Units studied histochemically are indicated by filled symbols, and soleus units from a single animal are denoted by arrows, showing that that data scatter was not an artifact of pooling results from different animals.



Text-fig. 2. The effect of high-frequency (100 pulses/sec) tetani on twitch responses of a soleus muscle unit. See text.

of some 10–20 % above control twitches. In only two of the units without post-tetanic depression twitch amplitudes reached levels above 20 % potentiation. Post-tetanic repetitive firing (Standaert, 1964) was not observed in any unit (cf. Burke *et al.* 1973). The complexity of post-tetanic behaviour among soleus units, particularly the presence of post-tetanic depression in a majority of them, was in marked contrast to observations among gastrocnemius type S units in which post-tetanic potentiation was the rule (Burke *et al.* 1973, Text-figs. 3 and 4) and depression was not seen.

Twitch/tetanus ratios for soleus muscle units ranged between 0·16 and 0·4, as compared to a range of 0·125–0·5 for gastrocnemius S units (Burke *et al.* 1973). The motoneurones of soleus units tended as a group to have more slowly conducting axones (mean = 71·5 ± 7·5 m/sec) than the motoneurones of gastrocnemius S units (mean = 85·8 ± 7·4 m/sec; $P < 0{\cdot}001$; cf. Burke, 1967).

Histochemical characteristics

Pl. 1 illustrates the histochemical profile of two fibres belonging to the soleus unit which produced the responses shown in Text-fig. 2. Unit fibres were unstained (i.e. glycogen-depleted) in the section stained for glycogen by the PAS method (arrows; Burke *et al.* 1973). Unstained fibres of otherwise normal appearance were not found in unstimulated control muscles and it is thus assumed that PAS-negative fibres observed after prolonged repetitive stimulation of a single motor unit indeed belong to that unit (Burke *et al.* 1973; Edström & Kugelberg, 1968). The same two unit fibres could be identified in serial sections stained for other reactions (arrows; see Burke *et al.* 1973 for discussion and references), where their appearance is identical to that of the surrounding fibres, providing strong support for the crucial assumption (Burke *et al.* 1973) that the stimulation sequence used to deplete fibre glycogen does not cause alteration in the histochemical profile of muscle unit fibres, except for glycogen and phosphorylase activity. The latter reactions diminished in parallel in stimulated fibres (Burke *et al.* 1973; Edström & Kugelberg, 1968).

In order to compare the staining of soleus and gastrocnemius muscle fibres, it was necessary to process portions of the two muscles as a single unit (Burke & Tsairis, 1973*b*; Romanul & Van Der Meulen, 1967). In sections of adjacent portions of soleus and lateral gastrocnemius stained for myofibrillar ATPase activity, soleus fibres were stained less intensely than fibres presumed by histochemical profile to belong to FF and FR units, but they were somewhat darker than presumed S unit fibres in the gastrocnemius (see also Nyström, 1968). The distinction was more pronounced in Ac ATPase sections (Pl. 2), in which soleus fibres stained with an intensity approximately equal to that in presumed FF unit fibres but quite different from the darkly stained fibres presumed to belong to gastrocnemius type S units. Both groups of S unit fibres stain lightly in preparations demonstrating enzymes involved in anaerobic glycolysis (Burke *et al.* 1973; Romanul & Van Der Meulen, 1967).

Glycogen depletion in soleus muscle units

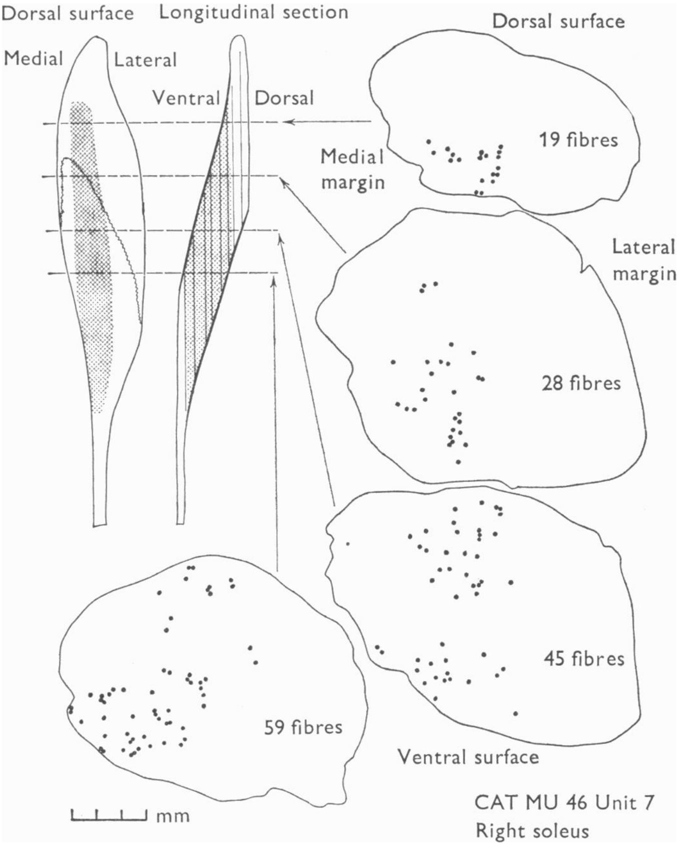
The question of completeness of glycogen depletion in all unit fibres is important in interpreting results bearing on the question of anatomical muscle unit organization (Burke & Tsairis, 1973*a*). Because of its histochemical uniformity (Ariano, Armstrong & Edgerton, 1973; Guth & Samaha, 1969; Henneman & Olson, 1965; Nyström, 1968), the cat soleus presented a favourable situation in which to examine glycogen-depletion in muscle fibres which depend mainly on oxidative metabolic pathways.

In each of the six soleus units studied histochemically, unit fibres completely depleted of glycogen were found as judged by complete absence of a detectable pink-staining reticulum under high magnification (x 450; Burke *et al.* 1973). Other fibres in the same sections contained a faint PAS-positive reticulum in parts of the fibre cross-section, probably representing residual glycogen. We have interpreted such fibres as belonging to the stimulated units, since control muscles did not contain fibres of similar appearance. In addition, each muscle also had five to twenty fibres which were more lightly stained than normal but which showed a definite PAS-positive reticulum throughout the fibre cross-section. Such 'questionable' fibres may or may not have belonged to the stimulated muscle unit but their presence suggested that some unit fibres may have gone undetected under the present conditions.

Muscle unit anatomy

The fusiform cat soleus consists of parallel fibre fascicles originating from an extensive deep aponeurosis and inserting on a similarly extensive superficial aponeurosis which ultimately joins the Achilles tendon (Text-fig. 3, longitudinal section). Based on measurements of formalin-fixed control muscles, it was estimated that cross-sections through the mid-belly should contain about 80 % of the total number of fibres in the muscle (which may be compared with the more complicated fibre arrangement in the synergist medial gastrocnemius; see Burke & Tsairis, 1973*a*).

We attempted to reconstruct the anatomical arrangement of muscle fibres belonging to identified soleus units using PAS-stained sections containing depleted unit fibres (see Burke & Tsairis, 1973*a* for method). All units studied exhibited the same basic pattern of fibre distribution and the diagrams in Text-fig. 3 illustrate an example. The positions of glycogen-depleted unit fibres (including about ten 'questionable' fibres) were plotted on cross-sections taken through various levels along the muscle. The approximate levels of sectioning and the apparent extent of the unit territory (cross-hatching) are noted on the whole muscle diagrams to the left (cf. Burke & Tsairis, 1973*a*). Unit muscle fibres were scattered through a remarkably extensive territory, within which the density of unit fibres appeared to be relatively low, often less than one unit fibre per 100 total fibres. Although such estimates of unit fibre density may be too low due to the presence of fibres not detectably depleted of glycogen, it appears,



Text-fig. 3. Diagrammatic reconstruction of the distribution of glycogen-depleted muscle fibres belonging to the soleus muscle unit shown in Text-fig. 2 and Pl. 1. The diagrams on the upper left represent the whole soleus muscle as seen from the dorsal surface and in longitudinal section. The extent of the ventral aponeurosis of origin and of the dorsal aponeurosis of insertion are indicated by heavier lines on the longitudinal section, and the arrangement of primary fibre fascicles is denoted by the parallel lines. Cross-hatched area represents the approximate territory of the muscle unit projected on to the muscle outline. Cross-sections taken through various levels along the longitudinal axis of the muscle are shown in outline tracings, on which the positions of glycogen-depleted muscle fibres belonging to the studied unit have been plotted as dots. The approximate levels of sectioning are indicated on the whole muscle diagrams. Fibre counts are given on each section (including 'questionable' fibres, see text).

nevertheless, that individual soleus muscle units are distributed through a larger fraction of the total muscle volume, perhaps with less density of unit fibres, than are units in the cat medial gastrocnemius (Burke & Tsairis, 1973*a*).

Direct determination of innervation ratios in histochemically marked muscle units is complicated by geometrical factors and by the possibility of incomplete glycogen depletion in an unknown fraction of unit fibres (Burke & Tsairis, 1973*a*). Assuming that the orientation of fibres found in fixed specimens was also characteristic of the soleii analysed histochemically, the glycogen-depleted fibres present in the most distal section in Text-fig. 3 (count = 59) were probably also represented in other sections, except for the 19 fibres found in the most proximal cross-section. The reconstruction suggests a minimal innervation ratio of about eighty fibres for this unit.

TABLE 1. Innervation ratios in six soleus motor units with known tetanic tension output (column 1) and average fibre cross-sectional area (column 2; mean of seven to thirteen unit fibres). Column 3 shows the maximum observed count of glycogen-depleted unit fibres in a single cross-section. Column 4 gives the calculated innervation ratio assuming a specific tension of 2 kg/cm^2 for all soleus muscle fibres

1 Tetanic tension	2 Mean fibre area	3 Observed fibre count	4 Calculated innervation ratio
4·2 g*	4200 μm^2	20	50
15·1 g*	4160 μm^2	59	181
16·6 g	4890 μm^2	170	170
21·7 g	5130 μm^2	155	211
23·0 g	3420 μm^2	60	336
35·2 g	4120 μm^2	78	427

* Units from left and right soleus in same animal.

Assessment of the validity of innervation ratio estimates based on glycogen depletion requires independent evidence (e.g. tension output) not available from purely anatomical studies (e.g. Doyle & Mayer, 1969). Tetanic tension in single muscle units is controlled by three major factors: the innervation ratio; the cross-sectional area of individual unit fibres; and the specific tension output of the fibres (cf. Burke & Tsairis, 1973*a*; Close, 1972). If the observed fibre counts (Table 1) were accurate, widely divergent values of specific tension (from 2 to 11·5 kg/cm^2) would be implied. Specific tension in individual muscle units cannot be directly observed and thus the validity of the fibre counts is impossible to demonstrate. However, specific tensions around 2 kg/cm^2 have been found in studies of whole mammalian muscle (Close, 1972), including rat soleus (Barany & Close, 1971). Since the cat soleus is histochemically homogeneous, it seems simplest to assume that 2 kg/cm^2 reflects more or less accurately the specific tension for individual soleus fibres, an assumption not necessarily valid for mixed muscles (see Burke & Tsairis, 1973*a*). With this assumption, an innervation ratio for each identified soleus unit was calculated (Table 1). Reasonable agreement with observed counts was found in two of the six units studied, implying that fibre counts in the other four were underestimated.

DISCUSSION

Correlations between some physiological properties and the histochemical profiles of soleus muscle units are evident when taking what might be termed a 'coarse-grain' view of the data. The marked resistance to fatigue appears to be closely related to a high capacity for oxidative glycolysis, as in gastrocnemius muscle units (Burke *et al.* 1973). The staining for myofibrillar ATPase activity in soleus muscle fibres is less intense than among the majority of fibres in nominally 'fast-twitch' muscles like gastrocnemius, which fits with evidence for a positive correlation between myosin ATPase activity and muscle contraction speed (Barany̆, 1967; cf. also Close, 1972). However, the availability of physiological and histochemical observations on single muscle units provides an opportunity for a further 'fine-grain' view of the same evidence.

There was a twofold range in contraction times for individual soleus muscle units, even within subsets obtained from single animals (Text-fig. 1; see also McPhedran *et al.* 1965). Yet, in most cats virtually all fibres in the cat soleus stain with equal intensity for myofibrillar ATPase activity (Pl. 1; see also Guth & Samaha, 1969; Nyström, 1968). It seems unlikely that variations in anatomical fibre distribution alone could result in markedly different contraction times, since unit territories exhibit considerable overlap and interdigitation (Text-fig. 3). While variation in the mechanical conditions of twitch measurement may have caused some spread in contraction times, the evidence available suggests that the intensity of myofibrillar ATPase staining should not be overinterpreted in terms of the supposed speed of muscle fibre contraction (cf. Burke & Tsairis, 1973*b*; Guth & Samaha, 1972; Nyström, 1968). The latter conclusion is amplified by the observation that myofibrillar ATPase staining is somewhat more intense in cat soleus muscle fibres than in presumed type S unit fibres in gastrocnemius (Burke & Tsairis, 1973*b*; Nyström, 1968), even though soleus muscle units tend, on the average, to contract more slowly (Text-fig. 2). Interpretation of such results must take into account anatomical differences (e.g. fibre length and angulation) between gastrocnemius and soleus (cf. Burke & Tsairis, 1973*a*), which may play a role in determining the observed differences between soleus and gastrocnemius type S units.

Contrary to initial expectations, the present results' regarding unit innervation ratios proved to be ambiguous, largely because of possible variation in specific tension between individual muscle units. However, when the simplifying assumption is adopted that all soleus muscle fibres have a specific tension of 2 kg/cm^2, innervation ratios for individual units can be estimated simply from measurements of average fibre area and unit tetanic tension output as in Table 1. Because of the rather uniform dimensions of soleus muscle fibres (Table 1; see also Nyström, 1968), the range in tension output within the soleus unit population probably reflects an equivalent range in innervation ratios (from less than 50 to more than 400 fibres per unit).

The total number of muscle fibres in adult cat solei (between 22,000 and 30,000; Clark, 1931) divided by the total number of alpha motor axones in the soleus nerve (about 150; Boyd & Davey, 1968), yield average innervation ratios of 140–200 fibres per unit, considerably smaller than the estimate for an average medial gastrocnemius S unit (about 530 fibres/unit; Burke & Tsairis, 1973*a*). Assuming (*a*) an average innervation ratio of 150 fibres/unit, (*b*) that individual soleus muscle fibres have an average cross-sectional area of 3200 μm^2 (Henneman & Olson, 1965) to 4320 μm^2 (Table 1) and (*c*) that an average soleus unit produces about 10·5 g of tetanic tension (Text-fig. 1), specific tension values between 1·7 and 2·3 kg/cm^2 can be calculated, consistent with values derived from work with whole soleus muscle (cf. Close, 1972). Data from gastrocnemius S units indicate a specific tension for them of only 0·6 kg/cm^2 (Burke & Tsairis, 1973*a*) and this factor appears to represent an important intrinsic difference between the two groups of S unit muscle fibres.

The most striking difference between soleus and gastrocnemius S units was the presence of post-tetanic depression of twitch tension in many soleus units (Text-fig. 2), a phenomenon observed also in the whole soleus muscle (Brown & Euler, 1938; Bowmans, Goldberger & Raper, 1962; see also Close, 1972), but not among gastrocnemius S units (Burke *et al.* 1973). It may be that the differences in ATPase staining between the two groups of S unit muscle fibres, particularly after acidic pre-incubation (Ac ATPase; Pl. 2), may be in some way related to the difference in post-tetanic behaviour. The intrafibre structures stained in ATPase preparations after acidic treatment are largely located between the myofibrils (Guth & Yellin, 1971), including parts of the sarcoplasmic reticulum (Gauthier, 1967). Muscle fibres of the rat soleus, which also exhibits post-tetanic depression (cf. Close, 1972), contain sarcoplasmic reticulum with less rich development than found in fibres of the nominally fast-twitch extensor digitorum longus (Schiaffino, Hanzlíková & Pierobon, 1970). It would be of considerable interest to determine if cat soleus fibres differ in ultrastructure from the fibres in gastrocnemius which appear to belong to type S units. In any case, the existing evidence strongly suggests that the motor units of the cat soleus represent a unique group and are not equivalent to the type S units found in the synergist gastrocnemius.

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EXPLANATION OF PLATES

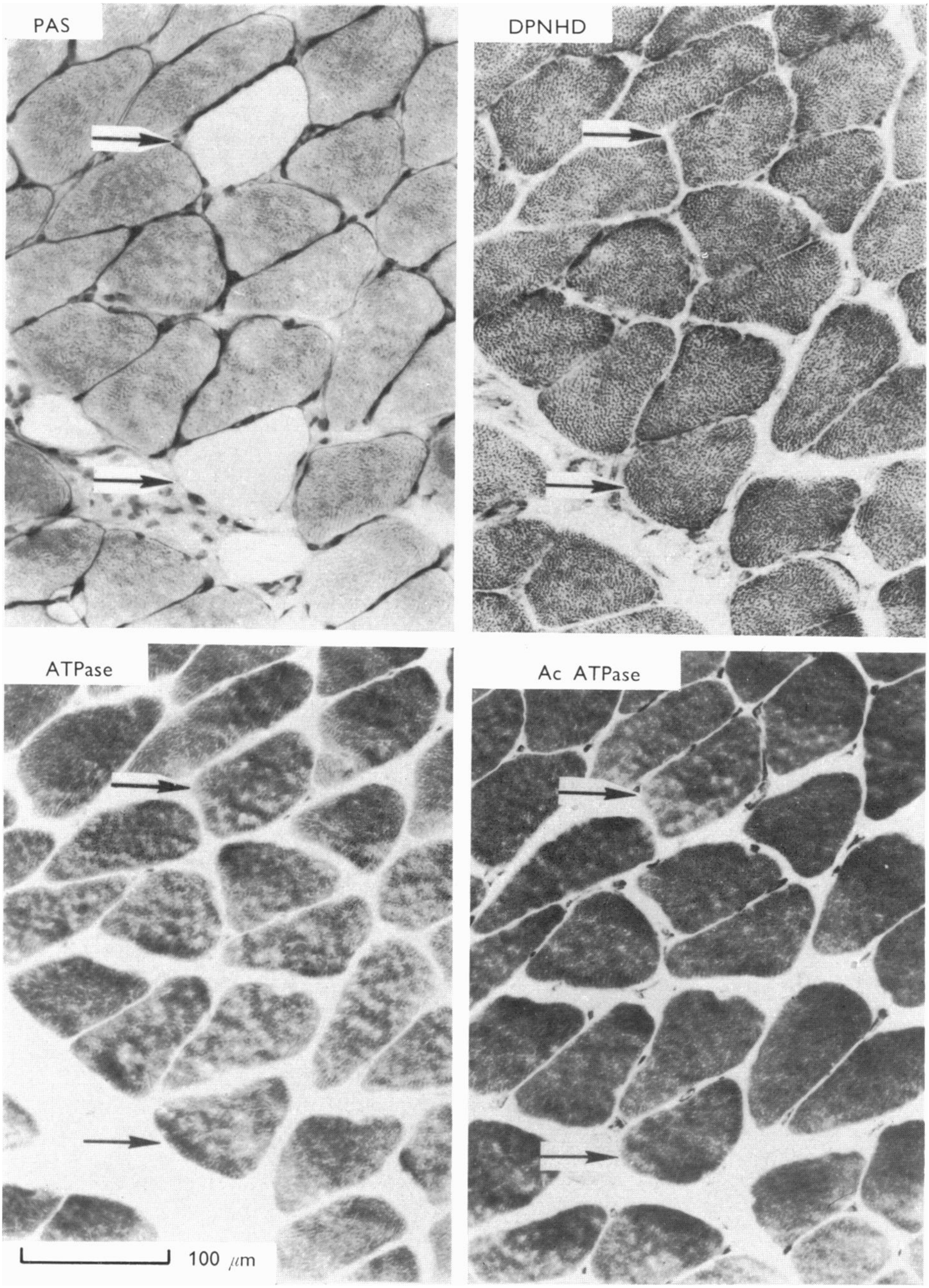
PLATE 1

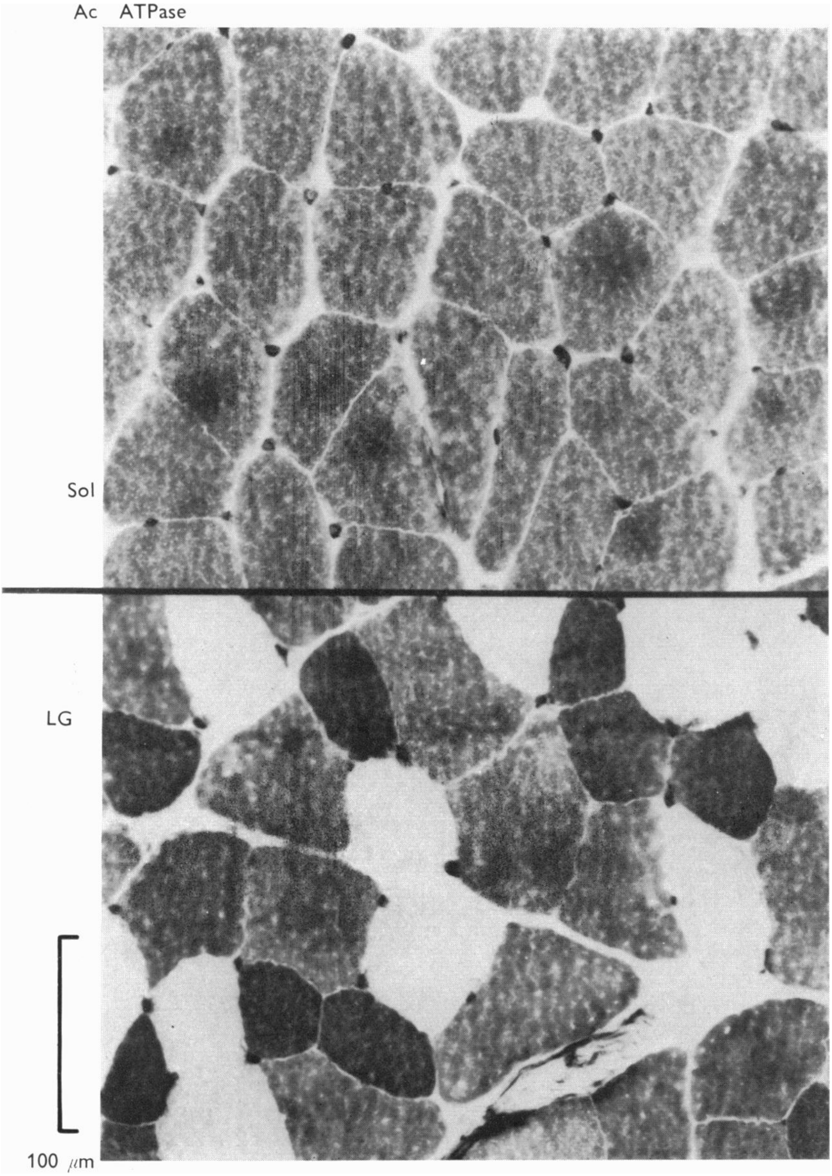
Histochemical profile of two fibres belonging to the soleus unit illustrated in Text-figs. 2 and 3, identified in the PAS-stained section as unstained fibres (arrows) among normally-stained fibres presumably belonging to other muscle units. The same unit fibres (arrows) can be identified in serial sections stained for reduced diphosphopyridine nucleotide dehydrogenase (DPNHD), for myofibrillar ATPase activity at pH 9·4 (ATPase), and for ATPase activity after pre-incubation in acetate buffer at pH 4·65 (Ac ATPase).

PLATE 2

Photomicrographs of a region in the soleus muscle (above) and a nearby region in the lateral gastrocnemius (LG; below) from a cross-key section block including both muscles stained with the Ac ATPase method. The sections were processed as a single block and photomicrographs were made using identical conditions. The intensity of staining in soleus fibres was almost identical to that in the largest diameter LG fibres, presumed to belong to type FF muscle units (Burke *et al.* 1973). In this stain, the lightest LG fibres probably belong to type FR units and the small, dark fibres to type S muscle units.

Plate 1

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