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# PROPERTIES OF MOTOR UNITS OF THE FROG SARTORIUS MUSCLE

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## SUMMARY

1. The mechanical properties of single motor units in the sartorius muscle of the frog *Litoria aurea* were examined during single shock and repetitive stimulation of motor axons.

2. The tetanic tension developed by motor units lay in the range 1–40 % of whole muscle tension with two peaks in the distribution, in the range 5–10 % and 25–30 %. The large units had briefer times-to-peak for the twitch than the small units and were more readily fatigued during prolonged repetitive stimulation.

3. Histological examination of the muscle gave a count of 620 muscle fibres with a diameter range of 28–128 $\mu$m. Cholinesterase stained preparations showed that the majority of muscle fibres had several nerve terminals (mean 3, range 1–5).

4. Muscle fibres received their multiple innervation from different axons (polyneuronal) or branches of the same axon (multiterminal). The presence of polyneuronal innervation of muscle fibres was confirmed by a comparison of the tensions when each of a pair of motor units was stimulated alone and when they were stimulated together. The tension excess, or overlap, was up to 60 % when expressed in terms of the tension developed by either unit alone. Motor units developing similar amounts of tension tended to show more overlap in their innervation than units with very different tensions.

5. An estimate of the amount of multiterminal innervation gave variable results but could account for up to 60 % of a motor unit's tension. No correlation could be detected between the values for multiterminal innervation and any other measured parameter. However, it is argued that because of the limitations of the measurements the existence of a relationship between the extent of multiterminal or polyneuronal innervation and the mechanical properties of the motor unit cannot be excluded.

## INTRODUCTION

The classification of amphibian skeletal muscle into two types, twitch and slow, is no longer adequate. Recent data from experiments using histochemical staining techniques and measurements of mechanical properties of motor units have suggested the existence of up to five different types of muscle fibres (Smith, Blinston & Ovalle, 1973). Until now no measurements have been made of the properties of motor units of a twitch muscle such as sartorius, although in this muscle two types of fibres have been demonstrated histochemically by Smith *et al.* The histochemical data suggest that sartorius contains two types of motor units: large rapidly contracting units which are readily fatigued during prolonged stimulation and smaller units, also with brief contraction times but which are less susceptible to fatigue.

It has been known for many years that the frog sartorius muscle contains muscle fibres with more than one nerve terminal per fibre (Katz & Kuffler, 1941). We decided to study the properties of motor units in sartorius looking for the sorts of differences described by Smith *et al.* and to seek evidence for multiple innervation at the level of single motor units.

## METHODS

The experiments were carried out on the sartorius muscle of the frog *Hyla aurea* (recently renamed *Litoria aurea*). The muscle was dissected free, together with its nerve supply, and placed in a bath through which an oxygenated Ringer solution flowed. The composition of the Ringer solution was NaCl 111·1 mM, KCl 2·5 mM, $CaCl_2$ 1·08 mM, $NaH_2PO_4$ 0·5 mM, $Na_2HPO_4$ 2·5 mM, and glucose 10 mM.

The muscle nerve was placed in a paraffin filled cavity adjacent to the main perfusion chamber. Single functional motor units were prepared in the following way: a pair of platinum stimulating electrodes was placed on the nerve, close to the muscle. Antidromic action potentials evoked by single stimuli were recorded with a second pair of electrodes in portions of ventral root, teased into filaments. Finely graded stimulation of the nerve, at threshold for motor axons, readily revealed whether or not a filament contained a single functional axon. If several axons could be detected, the filament was further subdivided until a portion remained containing only one axon. On several occasions filaments were obtained which were too fine to subdivide further, but in which two or occasionally three axons could still be detected. These filaments were discarded after the number of axons contained by them had been noted. It was quite easy to estimate the number of axons in such strands using graded stimulation since the relative differences in potential amplitude were large, and because the filament never contained more than three functional axons. Each ventral root of the three segmental nerves (7, 8 and 9) was examined in this way and provided an estimate of the total number of motor axons supplying sartorius.

Axonal conduction velocities were calculated from the latency of a unitary antidromic action potential recorded in a ventral root filament and from the length of nerve between stimulating cathode and first recording lead (commonly 40–50 mm). The latency represented the time interval between the stimulus artifact (using twice threshold stimulus strength) and the positivity at the foot of the action potential. This positivity was easily recognized unless the axon had been damaged during the splitting procedure, in which case the filament was discarded.

Motor unit tension was recorded using a Devices Dynamometer (2 or 4 oz) following stimulation of a ventral root filament. Since the nerve was kept in paraffin, spread of the stimulus to adjacent filaments did not occur unless very high stimulus strengths (10 times threshold) were used. Nevertheless as an additional check, graded stimulation of a filament, at threshold, was used at the beginning of a set of tension recordings to ensure that tension appeared in an all-or-none manner. Muscle length was set at the optimum for the whole muscle twitch.

Motor units were characterized according to the isometric tetanic tension they developed, their isometric twitch time-to-peak and susceptibility to fatigue. The fatigue test consisted of tetanic stimulation at 40/sec for 330 msec, repeated once a second for 2 min. The size of the tetanic contraction at the end of the fatigue test was expressed as a percentage of the control value. Motor unit tetanic tensions have been expressed as a percentage of their respective whole muscle tetanic tension, thus reducing the variation due to differences between animals.

Since the test for polyneuronal innervation compared the tetanic tension of pairs of motor units, it was essential that tension measurements were accurately reproducible. This could only be achieved with a stimulation rate of 100/sec or less as with higher rates the tension began to fall in subsequent trials. Therefore in all comparisons the rate of 100/sec was used, even though for some motor units the tension developed was less than the maximum. The tension produced by a unit could be reproduced in successive trials with an accuracy of 1 % or better. However some deterioration did occur during the course of the experiment and when this exceeded 5 % of the initial value, no further comparisons were attempted.

All experiments were carried out at room temperature, 22–24° C.

Cross-sections of muscle were prepared from material snap frozen in a mixture of liquid nitrogen and isopentane. 5 $\mu$m thick sections were cut in a cryostat at $-16$° C and then stained using van Gieson's method. Nerve terminals on muscle fibres were stained for cholinesterase according to the method of Koelle & Friedenwald (1949). The stained muscle was cleared in glycerine. To enable precise counting of the numbers of nerve terminals, single fibres were dissected out under a microscope.

## RESULTS

### *Mechanical properties of motor units*

Typical twitch and tetanic contractions of two units are shown in Fig. 1. There is a striking difference between the twitches. The unit on the left shows a considerable amount of fluctuation in amplitude (20 %) in comparison with that on the right (5 %). Most of the motor units examined showed some measurable fluctuation (up to 30 %). Another difference between the two units of Fig. 1 is the response to tetanic stimulation at different frequencies. Units with large twitch fluctuations tended to increase in tension with stimulation rates of up to 200/sec. At higher rates the tension dropped dramatically, suggesting the occurrence of neuromuscular failure at some of the motor terminals. Units with small fluctuations in twitch amplitude, on the other hand, reached their peak tension with tetani of 100/sec and showed no further increase at higher rates.

Fig. 2 shows the distribution of conduction velocities of motor axons (*A*) and the isometric tetanic tension developed by motor units (*B*), for a total of eighty-one units from eighteen muscles. The relatively narrow range of conduction velocities (24–37 m.sec$^{-1}$) and their distribution suggests a single population of large, rapidly conducting motor axons. The tetanic tensions however are spread over a wide range (Fig. 2*B*), from 1 to 40 % of whole muscle tension ($P_0$), with two peaks in the distribution, in the range 5–10 % and 25–30 %.

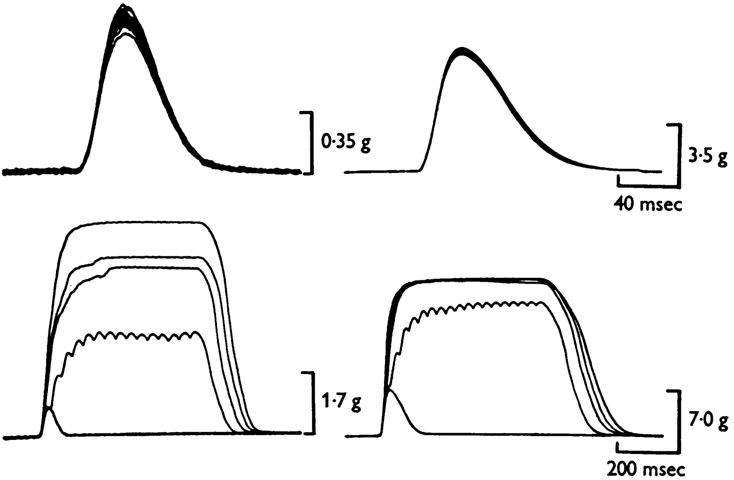

Fig. 1. Isometric twitch and tetanic tensions for two motor units from a sartorius muscle. The twitch consists of ten superimposed records to show the variation in amplitude. Tetanic tension obtained using stimulus frequencies of 30, 80, 100 and 150/sec for 500 msec duration. Temperature 22–24° C.

In any one experiment not more than four or five motor units could be isolated successfully. Axons tended to 'bunch' together in small filaments of ventral root, making the isolation of single units difficult. Nevertheless it was possible, using graded stimulation, to estimate the total number of motor axons supplying sartorius. Typically eleven to fourteen axons could be detected. In all but two experiments these were located exclusively in ventral root 8. In the two exceptions axons to sartorius occurred in root 7 as well as root 8. Thus, in this species of frog, multisegmental innervation appears to be relatively rare (cf. Sandmann, quoted by Katz & Kuffler, 1941; cf. Cattell, 1928).

In one experiment nine motor units of an estimated total of thirteen were successfully isolated. Each unit was found to develop a tetanic tension proportional to the conduction velocity of its axon (Fig. 2*C*). A similar proportionality has been shown for motor units in mammalian muscle

(Bessou, Emonet-Dénand & Laporte, 1963; Proske & Waite, 1974). In other experiments a similar relation was suggested, although fewer units were available for comparison. However, pooling the data from more than one experiment obscured the relation, presumably because of large differences between animals.

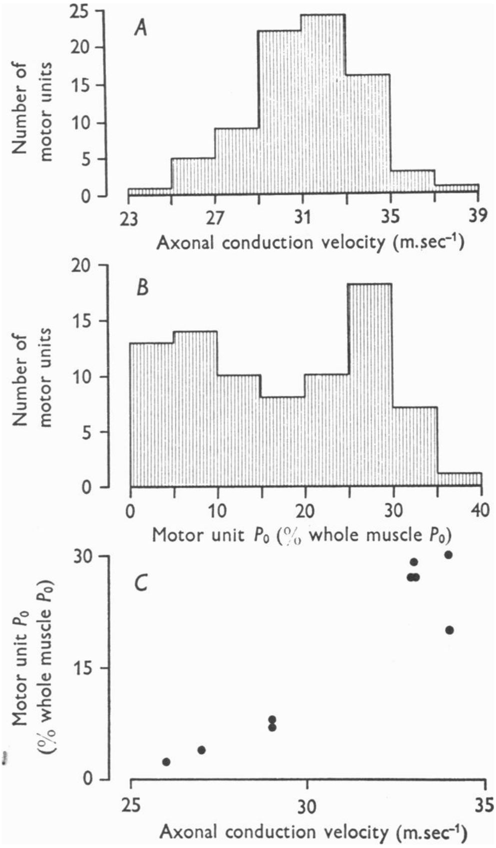

Fig. 2. *A*, frequency distribution histogram of the axonal conduction velocity of motor units. *B*, frequency distribution of the tetanic tension of motor units. Unit tension has been expressed as a percentage of respective whole muscle tension. *C*, tetanic tension plotted against axonal conduction velocity for nine motor units from one muscle. Temperature 22–24° C.

The mechanical properties of fifty motor units were examined in more detail. Isometric twitch times to peak were distributed over a relatively narrow range, from 21 to 36 msec. The relationship between twitch time to peak and motor unit size is shown in Fig. 3*A*. The susceptibility to fatigue showed that single motor units varied in their resistance to fatigue. The tension after fatigue when expressed as a percentage of the control value, varied between 1 and 100% (Fig. 3*B*). Small motor units had relatively long twitch times to peak and were fatigue-resistant, while large units had brief twitch times to peak and were readily fatigued.

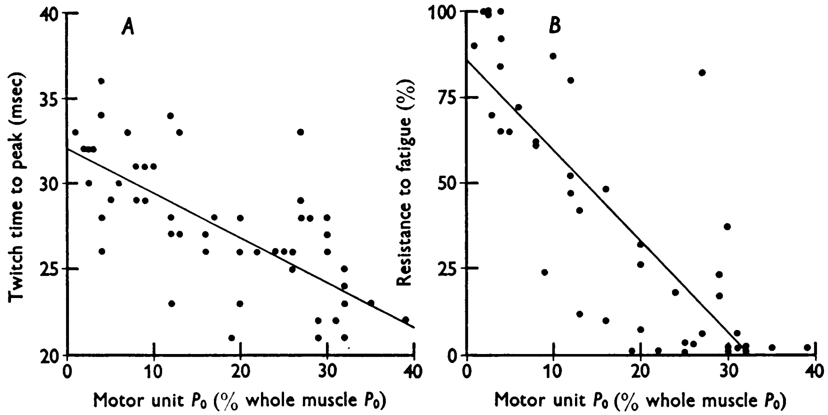

Fig. 3. *A*, isometric twitch time to peak plotted against motor unit size; (correlation coefficient 0·53, $P < 0·001$). *B*, resistance to fatigue plotted against motor unit size (correlation coefficient 0·69, $P < 0·001$). The fatigue test consisted of tetanic stimulation at 40/sec for 330 msec, repeated once a second for 2 min. The size of the tetanic contraction at the end of the fatigue test was expressed as a percentage of the control value.

### *Multiple innervation: polyneuronal*

An interesting aspect of the motor unit organization of sartorius is the presence of several nerve terminals on most of the muscle fibres. Before tests for multiple innervation were carried out it was necessary to know more of the structure and pattern of innervation of sartorius. First, in one muscle, the number of muscle fibres was counted and their diameters measured from cross-sections. The muscle chosen contained 620 fibres and the diameter distribution for a sample of 200 fibres is shown in Fig. 4*A*. A second muscle was stained for cholinesterase and the number of motor terminals was counted on each of 100 single muscle fibres teased out under a dissecting microscope. The frequency distribution of terminal numbers per fibre is shown in Fig. 4*B*. This gave an average of three motor terminals per fibre.

Assuming a total of twelve motor axons in the nerve, each axon should on average supply fifty-two muscle fibres. If the tension developed by a motor unit is proportional to the number of muscle fibres innervated, then the smallest unit would have only six fibres (1% $P_0$) and the largest 250 fibres (40% $P_0$). It is unlikely, however, that such a simple proportionality can be assumed (Burke & Tsairis, 1973).

The test chosen for measurement of polyneuronal innervation compared the tetanic tensions of pairs of motor units when each unit in the pair was stimulated separately and when the two were stimulated together. Any difference in tension between that predicted from the arithmetic sum and recorded on combined stimulation would indicate the existence of innervation-overlap between the two units.

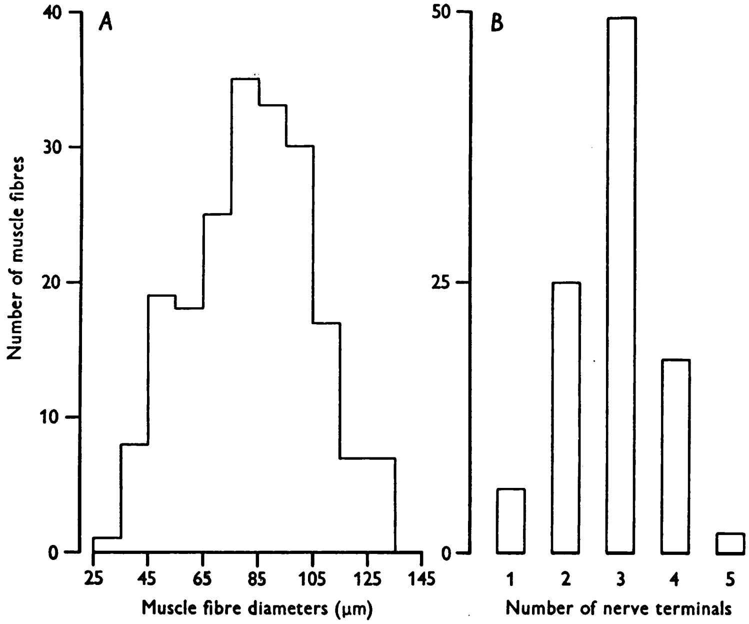

Fig. 4. *A*, frequency distribution of fibre diameters for a sample of 200 muscle fibres from a muscle containing a total of 620 fibres. *B*, frequency distribution of the number of nerve terminals on each of 100 muscle fibres in one sartorius.

An example of a pair of units with a large overlap (or 'tension excess', Bagust, Lewis & Westerman, 1973) is shown in Fig. 5*A*. The lowest trace is the tension developed by one unit, the middle trace that for the second unit and the top trace the tension on combined stimulation. The small unit developed 32g, the large unit 40g. On combined stimulation a total of 72g was expected; only 53g were observed so that muscle fibres developing 19g of tension must have been receiving an innervation from both axons. Thus, provided the tension per muscle fibre was similar for the two units, 59% of the fibres of the small unit or 48% of the fibres of the large unit were shared by the two axons.

If the two motor units being compared could both be fatigued, an additional test for the presence of innervation-overlap consisted of fatiguing one unit and looking for any effect on the second. Whenever the overlap measured prior to fatigue was substantial, fatiguing one unit severely reduced the tension developed by the second.

One interesting observation made during the course of these experiments was that rarely could any overlap be detected between units developing very different tensions. Small overlapped with small and large with large, but not large with small. This is shown in Fig. 5*B*. The overlap

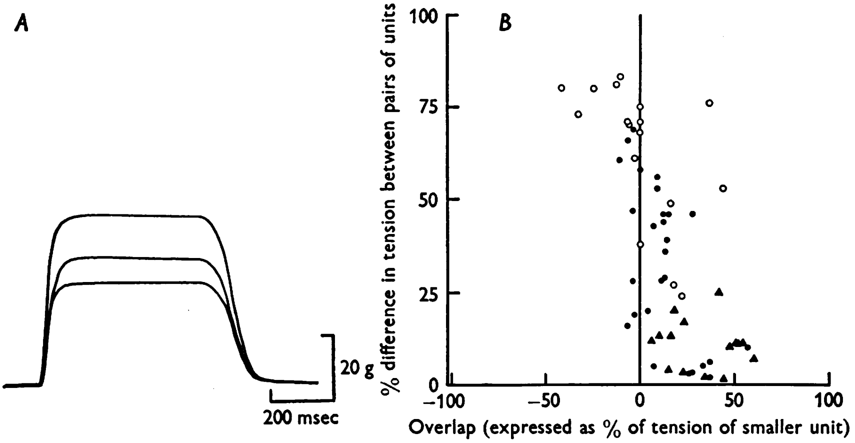

Fig. 5. *A*, the isometric tetanic tension developed by two motor units with some muscle fibres common to both. The two lower records represent the tension for each motor unit when stimulated alone, the uppermost record the tension when the two units contract together. Stimulus frequency 100/sec for 500 msec duration. *B*, a plot of the percentage difference in tension between pairs of motor units (the difference in tension when each unit was stimulated separately expressed as a percentage of their sum) against the amount of overlap. The overlap is expressed as a percentage of the tension of the smaller unit in the pair. ○, where the smaller unit of the pair developed less than 5% of its respective whole muscle tension; ▲, smaller unit of the pair greater than 20% whole muscle tension; ●, remaining pairs of units.

is expressed as a percent of the tension of the smaller unit in the pair under comparison. The percentage difference is the difference in tension when each unit was stimulated separately, expressed as a percentage of their sum. Thus an overlap of 50% or more was only shown by pairs of motor units having less than 30% difference in tension. Fig. 5*B* illustrates two further points. The triangles represent comparisons where the smaller unit in the pair developed 20% or more of whole muscle tension; open circles represent units developing 5% or less. Thus a majority of large units show considerable overlap although some units with intermediate tensions and a few small ones also overlap extensively.

It has been assumed in these comparisons that the tensions of units will sum arithmetically. This seems to hold provided tetanic stimulation is used (Brown & Matthews, 1960). However, one comparison which does lead to errors is for motor units developing very different tensions, especially if the smaller member of the pair is itself a small unit ( $< 5\% P_0$). The apparent negative overlap in Fig. 5*B* suggests that the small unit is developing less tension when stimulated alone than when stimulated together with a large unit. Perhaps frictional forces in the largely passive muscle are responsible. Whatever the explanation, such a result makes unreliable any comparison between motor units developing very different tensions. Further errors could arise when a comparison included motor units which developed their peak tension at a stimulation rate above 100/sec. Nevertheless Fig. 5 does suggest that some order exists in the pattern of polyneuronal innervation, large overlap being restricted to units developing similar tension.

During these experiments very little, if any, overlap could be detected in a comparison of isometric twitches (cf. Cattell, 1928), despite a large overlap measured with tetani. Although twitches are known to be unreliable for measurements of overlap, the errors discussed by Brown & Matthews (1960) should, in a comparison of twitches, lead to an exaggeration of existing overlap, not a reduction. Thus an explanation based on compliance changes is unlikely to apply here.

If a muscle fibre has more than one nerve terminal then the other terminals may come from different axons (polyneuronal) or from branches of the same axon (multiterminal). The comparison between motor unit tensions described above tests for polyneuronal innervation. It was not possible to measure precisely the total amount of polyneuronal innervation. Any one motor unit could overlap with several others, and unless all motor units in the muscle were isolated, which was never achieved, the comparison remained incomplete.

### *Multiple innervation: multiterminal*

Since it was likely that on some occasions an axon supplied more than one nerve terminal to a particular muscle fibre (Iwasaki, 1957), experiments were set up to measure the amount of such multiterminal innervation. First, the earlier observations of Hunt & Kuffler (1954) were confirmed, that many axons branch and send collaterals into both tibial and pelvic portions of the muscle. This was done by comparing the tension produced on stimulating the intact nerve, with that from stimulating the central cut end of one of the branches. In three preparations in which the cut tibial branch of the nerve was stimulated, the tension developed, expressed as a percentage of the tension with the intact nerve, was 62, 90 and 95 %. Thus most of the axons branched. The branching occurred close to the point at which the nerve bifurcates, confirmed by progressively more distal crush of the nerve proximal to the branch point (Hunt & Kuffler, 1954). The tension remaining after nerve section to some extent reflects the size of the nerve branch cut and not just the degree of collateralization. Thus in the experiment yielding 62 %, the branch cut was visibly smaller than its intact partner.

After one of the branches of the nerve had been cut, both twitch and tetanic tensions of motor units were less than in the control, suggesting that some muscle fibres had been innervated exclusively by the one branch. In four of five experiments the branch cut was at least as large as its partner so that if similar numbers of muscle fibres were innervated by each branch and no fibres received terminals from both branches, then the expected drop in tension would have been about 50 %. For a total of thirteen units the observed drop ranged between 13 and 49 % with a mean of 33 %. This suggests that some of the muscle fibres were receiving an innervation from both branches of the nerve, i.e. these muscle fibres were receiving more than one terminal from the same axon.

In an attempt to obtain a more precise estimate of the extent of multiterminal innervation, the following experiment was set up. A number of single motor units were isolated in the usual way. The stimulating electrodes were then placed on either the tibial or pelvic branch of the nerve and the unitary antidromic action potential recorded in a ventral root filament. While monitoring the antidromic potential a local procaine block (0·5 % procaine in Ringer solution) was produced in the nerve branch, proximal to the site of stimulation. As soon as the antidromic potential could no longer be recorded, the filament of ventral root was stimulated and muscle tension recorded. The unblocked nerve branch was then cut, and the ventral root stimulated again. If no tension was recorded this confirmed that the procaine block was still operative. Then as the block wore off, some tension could again be recorded. A comparison of the tension before blocking with the sum of the tensions during the block and after the cut, gave a measure of the amount of multiterminal innervation contributed by the pelvic and tibial branches of the nerve.

In five experiments a total of twenty motor units were tested for multiterminal innervation. Five units had axons which did not branch into tibial and pelvic portions (four of these lay in the pelvic branch which in four out of five experiments was the larger branch). No multiterminal innervation could be detected for six of the fifteen units which branched. For the remainder, the sum of the tensions developed on separate stimulation of the pelvic and tibial branches of the axon exceeded the control value by 6–65 % (mean 28 ± 21 % S.D.). In a test for any correlation between multiterminal innervation and other measured parameters, the amount of multiterminal innervation was plotted in turn against $\% P_0$; twitch: tetanus ratio and twitch time-to-peak. No correlation could be detected in any of these comparisons.

The extent of multiterminal innervation, as expressed here by the percentage tension excess, is likely to be an underestimate. The procaine block could only be achieved successfully with the muscle suspended in paraffin oil. Under these conditions the tension of the whole muscle and of motor units fell by about 10% during the 15–20 min needed to effect the block. Since different motor units were not all affected to the same extent, a simple correction factor could not be applied. Furthermore, the above experiment assumes that all multiterminal innervation can be accounted for by a comparison of tension of the tibial and pelvic portions of the motor unit. It remains very likely however that some multiterminal innervation arises from further subdivisions of either the pelvic or tibial portions of the axon, and this would not have been included in the above measurements.

## DISCUSSION

In reviewing the known structural and physiological differences between amphibian skeletal muscle fibres, Smith & Ovalle (1973) argued in favour of five distinct types. They attributed two types (types 1 and 2) to the sartorius muscle. Motor units composed of type 1 fibres developed large tensions, had brief contraction times and were readily fatigued. Units of type 2 also contracted rapidly but developed less tension and were fatigue-resistant. However, all of the available physiological data came from the iliofibularis muscle.

The results described here for the mechanical properties of motor units in sartorius are not easily reconciled with the classification proposed by Smith & Ovalle. The simplest interpretation of our data would be to consider sartorius as containing only one type of motor unit with a wide range of properties. It could perhaps be argued that the distribution of tetanic tensions represents two populations. If that were so then according to Smith & Ovalle's scheme, large units should be more susceptible to fatigue than smaller ones, which was indeed the case. However, the two groups in sartorius also showed differences in contraction time which is contrary to the predictions.

In their description of twitch motor units, Smith *et al.* (1973) included a third type which was characterized by large fluctuations in twitch amplitude and a small twitch : tetanus ratio. Our sample of motor units included only two units which showed no detectable fluctuation in twitch size, while many showed fluctuations of up to 30%. It may be then that some of the motor units in sartorius, especially those developing intermediate tensions and showing large fluctuations in twitch amplitude, were units called type 3 by Smith *et al.*

The most striking feature of the pattern of innervation of sartorius is the extensive multiple innervation. Although other muscles of frogs and toads contain twitch fibres with more than one nerve terminal (Kuffler, 1942; Orkand, 1963; Lännergren & Smith, 1966), multiple innervation is particularly prominent in sartorius. Perhaps this is because sartorius is a relatively long muscle with most fibres running from end to end.

It is not surprising that some muscle fibres in sartorius receive terminals from different axons while others are supplied by branches of the same axon. Such a difference in pattern of innervation does however provide the basis for an interesting interpretation of the recordings. The motor units with fluctuating twitch tension required high rates of tetanic stimulation to achieve peak tension and had low twitch : tetanus ratios. This suggests that some muscle fibres in these units had 'a low safety factor' for neuromuscular transmission. Low quantal content of end-plate potentials in some frog twitch fibres has been commented on previously (Fatt & Katz, 1951; Orkand, 1963). It is likely therefore that in any one motor unit neuromuscular transmission is not equally secure at all junctions. The possibility arises that the degree of security of transmission may be related to the pattern of innervation. This is suggested by the observation that overlap between a pair of motor units could not readily be detected in a comparison of twitches; those muscle fibres that consistently contracted during the twitch, i.e. fibres with relatively secure neuromuscular transmission, were not sharing their innervation with another unit.

If security of transmission can be associated with a low incidence of polyneuronal innervation, it might have been expected that motor units showing little fluctuation in twitch amplitude, and a large twitch : tetanus ratio would also give high values for the measured amount of multiterminal innervation. In the sample tested, no such correlation could be detected. However, in view of the probable underestimate of the extent of multiterminal innervation it was not possible to predict with confidence for any one motor unit which pattern of innervation, multiterminal or polyneuronal, was actually predominating. If a correlation between pattern of innervation and security of neuromuscular transmission does exist, this could suggest that motor axon terminals making contact on a muscle fibre already innervated, may be functionally less effective despite appearing morphologically normal (Cass, Sutton & Mark, 1973).

There does not seem to be any simple explanation for the extensive multiple innervation of sartorius. Katz & Kuffler (1941) considered that the reduced conduction time along the muscle fibre as a result of multiple innervation was of doubtful value in terms of increased speed of contraction. However, the time saved (quoted as 5 msec by Katz & Kuffler) does represent 25 % of the contraction time of the fastest motor units. It has been shown here that some order exists in the pattern of multiple innervation: motor units dissimilar in size are unlikely to 'share' many muscle fibres. Such an arrangement would permit independent reflex activation of large and small motor units. Because of extensive innervation-overlap, gradation of tension by recruitment of motor units within each of the two groups would be difficult to achieve. The muscle would then respond during recruitment as though it consisted of only two motor units, one large and one small.

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