Histochemical composition, distribution of fibres and fatiguability of single motor units

Anterior tibial muscle of the rat

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Histochemically, the anterior tibial muscle of the albino rat presents a mosaic pattern of fibres with different enzymatic activity. From the standpoint of esterase and oxidative enzymes, such as succinic dehydrogenase, three different types of fibres have been distinguished: type A fibres, which are the classical white muscle fibres, and B and C fibres, which are two kinds of red fibres (Stein and Padykula, 1962). The A fibres are large with low enzymatic activity and the C fibres small with high enzymatic activity. The B fibres form a more heterogeneous group with various intermediate sizes and intensities of activity.

It has been shown that muscle contractions induced by shock stimulation to the sciatic nerve produce striking changes in the phosphorylase activity and glycogen content, which vary in the different types of fibres (Kugelberg and Edström, 1968a, b). The changes were sufficiently distinct and constant to serve as a histochemical index of previous muscle activity. In a few experiments the effect of stimulation of single motor nerve fibres and contractions of single motor units were studied instead of the synchronized contractions of the whole muscle. This seemed to provide a picture of the histochemical composition and distribution of the fibres within a single motor unit and permitted correlation between its histochemical and physiological properties. From the clinical point of view this is of interest for the interpretation of the electromyogram and the findings in muscle biopsies.

Some of these aspects of the motor unit have been investigated earlier by less direct methods (see Discussion) and therefore require further study in a heterogeneous muscle. In the present study the effects of stimulation of single motor nerve fibres and contractions of single motor units have been studied in greater detail. A brief account of our findings has been reported (Edström and Kugelberg, 1968).

METHODS

The investigations were carried out on 20 adult albino rats weighing 400 g, anaesthetized with sodium pentobarbital. The body temperature was maintained between 37 and 39°C.

The animal was placed in the prone position on a steel plate with the vertebral column fixed at two points. The left hind limb was fixed with steel pins drilled through the tibia near the knee joint and through the calcaneus and clamped so that the leg was held rigidly with the thigh and lower leg at right angles. The foot was fixed in a holder. The left hind limb was denervated by transecting the nerves to all the muscles with the exception of the anterior tibial muscle and the extensor digitorum longus and extensor hallucis longus. The latter muscles were not denervated because it was found that this procedure was apt to impair the circulation to the anterior tibial. The tendon of the anterior tibial muscle was dissected free, cut and attached with a thin steel wire to a Grass Ft 03 force-displacement transducer for recording from single units, and to an Ft 10 for recording whole muscle contractions. The transducer was fastened to a rack and pinion device which permitted easy adjustment of muscle length and tension. The transducer was connected to a BUDD model P-350 portable digital strain indicator and its output displayed on a Tektronix 502A oscilloscope and photographed. Isometric contractions of the muscle were recorded with the initial tension of the muscle adjusted to 12 g, which is the lower range of optimal tension.

The L_4 and L_5 ventral roots were exposed by laminectomy and transected proximally near the spinal cord. The skin around the incision over the vertebral column was drawn up to form a pool for mineral oil, which bathed the roots. The temperature of the fluid was maintained at 37 to 38°C. The filaments of the L_4 or L_5 motor roots were dissected under a microscope until it was ensured that it contained only one motor fibre to the muscle. The criterion was an all-or-nothing response in the muscle to finely graded stimuli from sub-threshold up to supra-maximal current strength.

In some experiments the sciatic nerve was cut and stimulated with supra-maximal shocks.

In the main experiments the following procedure was

used. First, it was ensured that the criterion of single motor nerve fibre stimulation to the muscle was fulfilled. The initial tension of the muscle was then checked and the filament stimulated with 5-10 stimuli for recording the twitch tension and twitch time to peak. The initial tension was again checked and the filament then stimulated at a frequency of 10/sec for 10 min. At the end of stimulation the twitch tension was measured on the oscilloscope. The whole event was photographed on a Grass camera with the film moving at a speed of 0.25 mm or 1 mm/sec. After stimulation both the stimulated and non-stimulated muscles were removed and rapidly frozen by immersion in propane cooled to -160°C with liquid nitrogen.

The histochemical technique was the same as that described in an earlier paper (Kugelberg and Edström, 1968b). The succinic dehydrogenase, esterase, phosphorylase, and glycogen (PAS) reactions were determined in serial cross sections of the muscles.

RESULTS

Synchronized contractions of the whole muscle elicited by 10/sec stimuli for 10 min produced negative PAS and phosphorylase reactions in 100% of the A fibres, a negative PAS reaction in 90% of the B fibres, and a very weak reaction in 8%. About 60% of the B fibres were phosphorylase negative. C fibres showed a slight increase in PAS reaction and a strong increase in phosphorylase activity. The combined changes permitted the demonstration of previous contractions in about 95% of the fibres (Kugelberg and Edström, 1968b).

Thus the histochemical technique may be considered satisfactory for the demonstration of previous contractions in the A fibres of a single motor unit, but less so for some B fibres. The 10% of B fibres which did not become PAS negative showed intense succinic dehydrogenase activity. The histochemical index of previous activity is less valid in these fibres.

The method is not suitable for the detection of previous C fibre activity. After contractions of the whole muscle the increased phosphorylase activity in C fibres is very clearly seen against a background of inactive A and B fibres. Against a background of non-stimulated and, accordingly, phosphorylase active A and B fibres, however, the C fibres with increased activity are easily masked and tedious to localize. No attempt has been made to do so in this investigation and therefore we are not certain that changes were present under the experimental conditions.

The prolonged preparation of the animal, lasting 5-6 hr, gave rise to certain histochemical changes in the muscles. The PAS reaction tended to diminish in the A fibres and increase in the B and C fibres. The changes are the same as those obtained with stimulation of the muscle at a frequency of 5/sec for 1-2 min and indicate a moderate load on the fibres. Since the

A fibres did not become PAS negative and the histochemical effect of 10/sec stimulation for 10 min was unchanged, it does not affect the results.

It was observed that in non-stimulated muscle a few fibres (less than one in 1,000) were often PAS negative. They did not exhibit the usual rounded form but appeared atrophic and compressed. In contrast to stimulated A fibres, which are both PAS and phosphorylase negative, these fibres exhibited phosphorylase activity. Such fibres were not considered as taking part in the contractions evoked by single nerve fibre stimulation.

HISTOCHEMICAL COMPOSITION Fifteen motor nerve fibres were stimulated for analysis of histochemical changes in the muscle. No selection was used in the dissection procedure and consequently there is reason to believe that the sample investigated was representative of units comprising the whole muscle. Table I shows that of the 15 units, seven were composed of A fibres with few or no B fibres and six units were composed of B fibres with few or no A fibres. In two of the motor units there were no histochemical changes either in the A or B fibres and consequently it was concluded that the unit was composed entirely of C fibres. This was confirmed by the type of response to prolonged repetitive stimulation described in the following.

TABLE I
HISTOCHEMICAL COMPOSITION AND DISTRIBUTION OF FIBRES
IN SINGLE MOTOR UNITS

	Fibres in unit (no.)	Fibres typed in part of unit A fibres (no.)	B fibres (no.)	Area of unit in % of muscle area
R.2	138	96	4	15
R.8	111	88	0	17
R.10	178	100	0	20
R.12	170	154	3	12
R.13	137	67	1	16
R.17	112	54	0	18
R.19	80	80	0	18
R.3	140	1	78	12
R.5	135	0	81	19
R.6	130	1	116	25
R.11	93	0	80	16
R.16	160	1	100	26
R.18	131	0	100	12
R.14	—	0	0	—
R.15	—	0	0	—

Each unit was fairly uniform not only as regards fibre type but also as regards the finer variations in the intensities of succinic dehydrogenase and esterase activity. Otherwise these intensities vary considerably in B fibres and to a lesser extent in A fibres. Figure 1 shows six representative fibres of a B unit incubated for succinic dehydrogenase (1a) and esterase (1b) and localized by the presence of both

PAS (1c) and phosphorylase (1d) negativity. All fibres showed the same intensity of succinic dehydrogenase and esterase activity. Eighteen representative fibres of an A unit, incubated for succinic dehydrogenase (Fig. 2a) and localized by PAS (Fig. 2b) exemplify the same remarkable histochemical uniformity of the motor unit.

COMPOSITION ACCORDING TO FIBRE SIZE The cross sectional areas of the different types of fibres varied in size, A fibres being largest and C fibres smallest. The size of the fibre areas varied in any one group.

The areas of 100 fibres of two different A motor units were measured according to the method of Edström and Torlegård (1968). The intensity of

FIG. 1. Rat 16. Serial sections showing homogeneity of a B fibre motor unit, (a) succinic dehydrogenase, (b) esterase, (c) PAS and (d) phosphorylase. $\times 200$. The six PAS and phosphorylase negative fibres (open circles) represent part of a single motor unit of 160 fibres. All fibres are of the B type and show the same intensity of succinic dehydrogenase and esterase activity.

succinic dehydrogenase activity differed in the fibres in respective units. The difference was small but distinct in all fibres. The unit with less activity had a slightly larger mean area, but the fibre areas overlapped to a considerable extent (Fig. 3). Thus intensity of succinic dehydrogenase activity was a much better index than fibre size for the characterization of fibres belonging to a certain motor unit. Since it may be assumed that all fibres in a motor unit are subject to the same amount of neuronal influence it

indicates that fibre size is not entirely neurone regulated.

DISTRIBUTION OF FIBRES The fibres of the anterior tibial muscle do not extend over the entire length of the muscle, since they originate from the tibia and the interosseus membrane and insert at different levels in the intramuscular part of the tendon. It is assumed, however, that the cross section of the thickest part of the muscle contains most or all of the fibres (Hag-

FIG. 2. Rat 8. Serial sections showing histochemical homogeneity of an A fibre motor unit, (a) succinic dehydrogenase and (b) PAS $\times 150$. The 18 PAS negative fibres represent part of a unit of 111 fibres. All fibres are of the A type and with the same intensity of succinic dehydrogenase activity.

FIG. 3. Distribution of the areas of fibres belonging to two different type A motor units. R8 (open circles), R10 (crosses), the latter with slightly higher intensity of succinic dehydrogenase activity than R8.

barth and Wohlfart, 1952). In one muscle in this region we counted 9,325 fibres. This area also contains the largest number of fibres per motor unit (Table I). The A and B motor units contained an average of 127 fibres. Thus the number of motor units in the muscle would be 73, assuming that the C units are the same size.

In cross sections the fibres of a single motor unit were scattered, usually isolated but sometimes two to four together and occasionally up to six in a straight or curved row (Figs. 1, 2, 4). The fibres were irregularly spaced with no signs of grouping. The density of fibres was somewhat greater in the centre of the unit than in the periphery (Fig. 4).

The large scale overlapping of the motor units is evident from the fact that the fibres of single units are distributed over 12-26% (mean 17%) of the entire cross section of the muscle (Table I, Fig. 5). Theoretically the whole section can be covered by the areas of only six of the 73 units present in the muscle.

The motor units did not necessarily extend over the entire length of the muscle, a few units were found to insert a short distance up in the intramuscular part of the tendon.

To check the accuracy of the histochemical mapping the number of units was also calculated from

the isometric tetanic tension of the whole muscle divided by the average isometric tetanic tension of 31 unbiased motor units. The stimulus frequency was 100/sec.

The tetanic tension was 600 g for the whole muscle and for the motor units 7, 8 g, which makes 77 motor units. This corresponds reasonably well with the 73 units found with histochemical mapping.

FATIGUABILITY Observations from 'synchronized contractions of the whole muscle suggested that the tension produced by A fibres declines towards zero already after 1,500 to 2,000 contractions (5 min) at a stimulus frequency of 5/sec, that some B fibres fatigue within 10 min at 10/sec, while others function for longer periods of time. Furthermore, C fibres probably responded at 10/sec for several hours with little loss of tension (Kugelberg and Edström, 1968b). The striking relationship between fatiguability and histochemical type of fibre was nicely confirmed by single unit stimulation (Fig. 6).

In all seven A units the twitch tension declined 90% or more after 10 min stimulation at 10/sec. Most of the decline was obtained after about 3 min—that is, after about 1,800 contractions (Fig. 6a). The response of the B units was much more variable.

FIG. 4. Rat 12. Cross-section showing the distribution of the fibres of a single motor unit, which are PAS negative. Section from the distal part of the muscle where the unit is smaller, to allow accommodation in sufficient magnification on the microphotograph. $\times$ about 40.

FIG. 5. Cross-section of the thickest parts of the anterior tibial muscle, incubated for succinic dehydrogenase, showing the outline of three different motor units. Left to right R17, R8, R12. $\times$ about 14. The outlines of the units were drawn from sections $\times$ 50 and superimposed on their precise location on a microphotograph of the whole muscle $\times$ 50.

FIG. 6. Records showing the effect of a stimulus frequency of 10/sec for 10 min. on A (a), B (b), and C (c) fibre motor units. The A unit (R17) shows a 90% decline, B (R20), 50% decline, and C (R15) about 100% increase of twitch tension. Initial twitch tensions were 1.25, 1, and 0.5 g respectively.

one unit, which histochemically showed low intensities of succinic dehydrogenase and esterase activity, the maximal tension decline was 80%. In three the tension declined 30 to 50% (Fig. 6b). In two units there was no decline in relation to the initial tension. In fact, owing to a slight tetanic fusion, the tension was somewhat higher after 10 min stimulation than before. However, there was a considerable decrease in tension at the end of stimulation compared with the high values obtained during the first few minutes. In the two C motor units there was about a 100% rise in twitch tension at the beginning of stimulation, which was maintained with little fatigue for 10 min. This gave the repetitive response curve a flat appearance (Fig. 6c). All the A, B, and C units showed slight tetanic fusion, but only C units kept up the fused tension for 10 min.

The mean twitch tension was higher in A units (1.8 g) than B units (0.75 g) and lowest in the two C units (0.4 g). Since, however, some B units produced a higher twitch tension than some A units, there was no strict correlation between twitch tension and type of unit. Factors other than the number of fibres in the A and B motor units—for example, myofibrillar area—must play a role in the difference in twitch tension, since the number of fibres in the A and B motor units do not appear to differ.

No definite difference was observed in the contraction times of the A, B, and C units; all were fast with an average contraction time of 12.5 msec. This value is in good agreement with that of Close (1967), who found an average of 11 msec in 48 units of the extensor digitorum longus of the rat, which is histochemically similar to the anterior tibial muscle. Only one additional unit had a longer contraction time of 23.5 msec.

Contraction time was considered a criterion for the differentiation of histochemically different groups of fibres in cat triceps surae (Wuerker, McPhedran, and Henneman, 1965; Henneman and Olson, 1965) and flexor digitorum longus (Olson and Swett, 1966). This does not seem to be valid generally.

DISCUSSION

It may be argued that the criterion we used for single motor nerve fibre stimulation does not exclude stimulation of several motor nerve fibres with nearby thresholds. Since there seemed to be about the same number of A and B motor units, stimulation of two fibres instead of one should have resulted in mixed activation of A and B units in about every second experiment. The fact that the motor units were homogeneous as regards fibre type shows that stimulation of several fibres was not a common event. If it occurred in a few instances it does not,

however, invalidate the principal results, which concern the homogeneity and fatiguability of the units and the distribution of the fibres in the muscle. It does affect the number of fibres and the territory of the units.

HOMOGENEITY OF THE UNITS The results of cross-union of the nerves to fast (white) and slow (red) muscles, initiated by Buller, Eccles, and Eccles (1960), suggested that the fibres in a motor unit are homogeneous. Cross innervation results in reversal of contraction speed (Buller *et al.*, 1960; Close, 1965), lessened difference in the myoglobin concentration of fast and slow muscles (McPherson and Tokunga, 1967), and partial reversal of the histochemical enzyme profile of the fibres (Romanul and Van Der Meulen, 1966, 1967; Dubowitz, 1967; Yellin, 1967). Thus the contractile and metabolic characteristics of the muscle fibres are subject to alteration with change in innervation and, apparently, neurone regulated. Our results are in agreement with this view.

The A fibre units contained about 2% B fibres. C fibres, if present, cannot be numerous as shown by the great fatiguability of the A units. The B units contained less than 1% A fibres. The percentage of C fibres, if any, could not be estimated. However, only about 20% of the fibres in the anterior tibial muscle are C fibres, the majority of which are very likely organized within C fibre units. Thus the two fibre units examined contained neither A nor C fibres. We believe this proves that A and C motor units are largely homogeneous as regard fibre type and that the B units are very likely so. This is the more probable since the A and B units were uniform even to finer variations in the enzymatic intensities of the fibres.

The few histochemically foreign fibres, sometimes found in the units, are not an artefact, at least not as regards B fibres in an A unit. Their presence may be explained as a result of innervation of the fibre by two different motoneurons. Hunt and Kuffler (1954) considered that polyneuronal innervation of individual muscle fibres occurs on a rather large scale, but repeated experiments by Brown and Matthews (1960) failed to confirm this. On the other hand the possibility of its occurrence on a small scale was not excluded. Mårtensson's (1966) physiological studies gave no indication of double innervation in the intrinsic laryngeal muscles of the dog. If B fibres in an A unit are an expression of double innervation, it occurs only on a small scale.

Homogeneity of the motor units is a prerequisite for the organization of different types of muscle fibres in various types of movements, according to their functional capacity.

DISTRIBUTION OF FIBRES No definite view of the arrangement of the muscle fibres in a motor unit has so far been accepted. The first observations came from the study of muscles in motoneurone diseases, which are characterized by the atrophy of fibres in groups. The fibres in each group exhibit the same degree of atrophy, but differ in the different groups. Slauck (1921) suggested that the groups represented denervated motor units. Wohlfart (1949) pointed out that the number of fibres in an atrophic group was much smaller than the calculated number of fibres belonging to a motor unit. Therefore the motor unit possibly corresponded to several atrophic groups. Buchthal (1961) adopted this view and suggested that the fibres of each motor unit are assembled in several tightly packed groups of fibres, so called subunits, each containing an average of ten fibres. Ekstedt (1964), using a special type of multielectrode, found no indication of subunits.

It is generally agreed, however, that the fibres of different motor units intermingle and the territories of the units overlap. This was likely from the picture of atrophy in mononeurone diseases (Wohlfart and Wohlfart, 1935) and in experimental partial motor nerve lesions (Van Harreveld, 1947). Moreover, Feindel, Hinshaw, and Weddell (1952) and Feindel (1954), in rabbit and monkey, clearly showed intensive overlapping of motor units with intravital methylene blue staining of axons. Meanwhile the same conclusions can be drawn from the study of the distribution of action potentials (for example, Jasper and Ballem, 1949; Krnjević and Miledi, 1958; Buchthal, 1961). The finer details of fibre distribution were, however, difficult to observe with these methods.

Our results certainly show very extensive overlapping of motor units. Only a fraction of the units in the cross section of the muscle was sufficient to cover the whole area. This facilitates widespread contractions even on weak efforts, when only a few motor units are recruited. The discharge frequency of the motor unit on weak effort allows for little or no fusion of its tetanic contractions. The assembly of widely-spread intermingled units in a muscle would seem to compensate for the intermittent operation of its parts and facilitate the smooth summation of the asynchronously discharging units.

No subunits were observed. The synchronous atrophy of fibres in motoneurone diseases is apparently not directly related to the anatomy of the motor unit, but is possibly the result of sprouting. If, as seems likely, nerve fibre sprouts preferably reinnervate adjacent muscle fibres, this will cause increasing rearrangement of the innervation pattern. When the disease process continues, sprouting nerve fibres will eventually degenerate and produce typical groups of

atrophic fibres. According to this view, in motoneurone diseases one would expect to find histochemically uniform groups of non-atrophic fibres as a result of sprouting. Indeed such groups have been observed and attributed by Engel (1965) to sprouting.

FATIGUABILITY It is generally acknowledged that white muscles, which depend on anaerobic glycolysis for their energy supply, fatigue more rapidly than red muscles which depend on oxidation (for example, Szent-Györgyi, 1953). Thus the pale anterior tibial muscle of the cat fatigues more rapidly than the red soleus (del Pozo, 1942). In the cat gastrocnemius (Wuerker *et al.*, 1965) and flexor digitorum longus (Olson and Swett, 1966) it was found that the EMG of large motor units assumed to be white declined more rapidly than the EMG of small motor units assumed to be red. The cause of the EMG decline was presumed to be due to progressive failure of neuromuscular transmission, since high frequency nerve stimulation of 100/sec was used.

The low frequency stimulation of 2-10/sec, used in this and our earlier work, causes exhaustion of the contractile mechanism of the muscle fibre rather than failure of transmission at the neuromuscular junction (del Pozo, 1942; Kugelberg and Edström, 1968b). The fatiguability of the units to low frequency repetitive stimuli was closely correlated with the histochemical type of unit. This correlation was in fact greater than the correlation between histochemical type of unit and any other factor examined such as area of the fibres, twitch tension, and contraction time.

In the anterior tibial muscle the intensities of succinic dehydrogenase and esterase activity in the different types of fibres vary in parallel, but have been reported to vary to some extent independently in the triceps surae of the rat (Romanul, 1964). In such muscles one enzyme should show a closer correlation to fatiguability than the other. Any enzyme utilized in oxidative metabolism of carbohydrate or lipids might with advantage be tested from this point of view.

The large differences in fatiguability of the various types of motor units at stimulation frequencies as low as 5-10/sec have important physiological implications. The lower range of natural motor unit discharge lies within these frequencies and is found in tonic contractions for maintenance of posture (Denny-Brown, 1929). The A motor units, which fatigue almost completely after about 3 min and 1,800 contractions, cannot play any primary role in this respect. Even a proportion of the B units, which are red, cannot keep up tension for any length of time. The C motor units, which are composed of fibres which histochemically react to contractions in a manner similar to the soleus types of fibres

(Kugelberg and Edström, 1968b) are well adapted to prolonged activity.

The A and many B units must be protected from continuous use by a high threshold for this type of activity. Since their recovery is slow they otherwise cannot when needed take part in strong and rapid movements. In other words, any tonic contraction slowly increasing in strength would be expected to recruit the different units in the reverse order of their fatiguability—that is, in the order C, B, and A.

The difference in fatiguability of the motor units is very clearly seen in single nerve fibre stimulation. It is possible that this does not always occur under physiological conditions. Strong isometric contractions impede the circulation in the muscle, due to rise in intramuscular pressure. In these circumstances the A fibre units, which are mainly dependent upon anaerobic glycolysis for their energy metabolism are likely to be relatively less affected than the C and B units with higher oxidative metabolism.

SUMMARY

The properties of 15 single motor units of the anterior tibial muscle of the rat were examined by repetitive stimulation of single motor nerve fibres and mapping of the histochemical changes in phosphorylase and glycogen (PAS).

1. The motor units were largely uniform as regards histochemical type of fibre as well as the finer variations in intensities of succinic dehydrogenase and esterase activity. A few fibres with markedly different enzymatic activities were observed. Their presence in relation to double innervation of the fibres is discussed.

The cross sectional areas of the fibres of single motor units varied considerably.

2. The fibres lie scattered, isolated or a few together. There were no so-called subunits.

The number of fibres in A and B motor units varied between 80-178 (mean 127). The area of distribution of fibres was 12-26% (mean 17%) of the cross-sectional area of the muscle. Overlapping was very extensive since the calculated number of motor units was 73. This facilitates smoothness and strength of contraction due to fusion of the asynchronously discharging intermingled units, although each unit may discharge below its tetanic fusion frequency.

3. Fatiguability of the motor units was closely correlated to the histochemical type of its fibres. The initial twitch tension of the A motor units declined 90-100% after about 1,800 contractions at a stimulus frequency of 10/sec. C fibre units showed no fatigue and B fibre units showed a spectrum of intermediate reactions.

A units are thus designed for phasic, C units for tonic, and B units for intermediate types of activity. Tonic activity increasing in strength is assumed to recruit the units in the order of C, B, A.

REFERENCES

- Brown, M. C., and Matthews, P. B. C. (1960). An investigation into the possible existence of polyneuronal innervation of individual skeletal muscle fibres in certain hind-limb muscles of the cat. *J. Physiol. (Lond.)*, 151, 436-457.
- Buchthal, F. (1961). The general concept of the motor unit. *Res. Publ. Ass. nerv. ment. Dis.*, 38, 1-30.
- Buller, A. J., Eccles, J. C., and Eccles, R. M. (1960). Interactions between motoneurons and muscles in respect of the characteristic speeds of their responses. *J. Physiol. (Lond.)*, 150, 417-439.
- Close, R. (1965). Effects of cross-union of motor nerves to fast and slow skeletal muscles. *Nature (Lond.)*, 206, 831-832.
- (1967). Properties of motor units in fast and slow skeletal muscles of the rat. *J. Physiol. (Lond.)*, 193, 45-55.
- Denny-Brown, D. (1929). On the nature of postural reflexes. *Proc. roy. Soc. B.*, 104, 252-301.
- Dubowitz, V. (1967). Cross-innervated mammalian skeletal muscle: histochemical, physiological and biochemical observations. *J. Physiol. (Lond.)*, 193, 481-496.
- Edström, L., and Kugelberg, E. (1968). Properties of motor units in the rat anterior tibial muscle. *Acta physiol. scand.*, 73, In press.
- , and Torlegård, K. (1968). To be published.
- Ekstedt, J. (1964). Human single muscle fiber action potentials. *Acta physiol. scand.*, 61, (Suppl. No. 226), 1-96.
- Engel, W. K. (1965). Histochemistry of neuromuscular disease—significance of muscle fiber types, in *8th International Congress of Neurology (Neuromuscular Diseases)*. Egermann, Vienna.
- Feindel, W., Hinshaw, J. R., and Weddell, G. (1952). The pattern of motor innervation in mammalian striated muscle. *J. Anat. (Lond.)*, 86, 35-48.
- (1954). Anatomical overlap of motor units. *J. comp. Neurol.*, 101, 1-18.
- Hagbarth, K.-E., and Wohlfart, G. (1952). The number of muscle spindles in certain muscles in cat in relation to the composition of the muscle nerves. *Acta Anat.*, 15, 85-104.
- Henneman, E., and Olson, C. B. (1965). Relations between structure and function in the design of skeletal muscles. *J. Neurophysiol.*, 28, 581-598.
- Hunt, C. C., and Kuffler, S. W. (1954). Motor innervation of skeletal muscle: multiple innervation of individual muscle fibres and motor unit function. *J. Physiol. (Lond.)*, 126, 293-303.
- Jasper, H., and Ballem, G. (1949). Unipolar electromyograms of normal and denervated human muscle. *J. Neurophysiol.*, 12, 231-244.
- Krnjević, K., and Miledi, R. (1958). Motor units in the rat diaphragm. *J. Physiol. (Lond.)*, 140, 427-439.
- Kugelberg, E., and Edström, L. (1968a). Histokemiska förändringar vid muskelkontraktion. Transactions of the Swedish Neurological Society, 1967. *Nord. Med.*, in publication.
- (1968b). Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibres. Relation to fatigue. *J. Neurol. Neurosurg. Psychiat.*, 31, 415-423.
- McPherson, A., and Tokunga, J. (1967). The effects of cross-innervation on the myoglobin concentration of tonic and phasic muscles. *J. Physiol. (Lond.)*, 188, 121-129.
- Martensson, A. (1966). Testing for doubly innervated fibres in the intrinsic laryngeal muscles of the dog. *Acta physiol. scand.*, 67, 152-164.
- Olson, C. B., and Swett, C. P. Jr. (1966). A functional and histochemical characterization of motor units in a heterogeneous muscle (flexor digitorum longus) of the cat. *J. comp. Neurol.*, 128, 475-498.
- del Pozo, E. C. (1942). Transmission fatigue and contraction fatigue. *Amer. J. Physiol.*, 135, 763-771.
- Romanul, F. C. A. (1964). Enzymes in muscle. I. Histochemical studies of enzymes in individual muscle fibers. *Arch. Neurol. (Chic.)*, 11, 355-368.
- , and Van Der Meulen, J. P. (1966). Reversal of the enzyme profiles of muscle fibres in fast and slow muscles by cross-innervation. *Nature (Lond.)*, 212, 1369-1370.

- , —, (1967). Slow and fast muscles after cross innervation. Enzymatic and physiological change. *Arch. Neurol. (Chic.)*, **17**, 387-402.
- Slauck, A. (1921). Beiträge zur Kenntniss der Muskelpathologie. *Ztschr. ges. Neurol. Psychiat.*, **71**, 352-356.
- Stein, J. M., and Padykula, H. A. (1962). Histochemical classification of individual skeletal muscle fibres of the rat. *Amer. J. Anat.* **110**, 103-124.
- Szent-Györgyi, A. (1953). *Chemical Physiology of Contraction in Body and Heart Muscle*. Academic Press, New York.
- Van Harreveld, A. (1947). The structure of the motor unit in the rabbit's m. sartorius. *Arch. néerl. Physiol.*, **28**, 408-412.
- Wohlfart, G. (1949). Muscular atrophy in diseases of the lower motor neuron. Contribution to the anatomy of the motor units. *Arch. Neurol. Psychiat. (Chic.)*, **61**, 599-620.
- Wohlfahrt, S., and Wohlfart, G. (1935). Mikroskopische Untersuchungen und progressiven Muskelatrophien. *Acta med. scand.*, (Suppl. No. 63), 1-137.
- Wuerker, R. B., McPhedran, A. M., and Henneman, E. (1965). Properties of motor units in a heterogeneous pale muscle (m. gastrocnemius) of the cat. *J. Neurophysiol.*, **28**, 85-99.
- Yellin, H. (1967). Neural regulation of enzymes in muscle fibers of red and white muscle. *Exp. Neurol.*, **19**, 92-103.