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# BIOCHEMISTRY OF RAT SINGLE MUSCLE FIBRES IN NEWLY ASSEMBLED MOTOR UNITS FOLLOWING NERVE CRUSH

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## SUMMARY

1. A partial crush was applied surgically to the common peroneal nerves of rats, producing motor deficits lasting 4 weeks; the tibialis anterior muscles supplied by the crushed nerves were removed 5 weeks after recovery along with the contralateral control muscles.

2. Myosin ATPase staining following pre-incubation at pH 4·5 was used to determine fibre types and to demonstrate areas of fibre-type grouping in the reinnervated areas of the muscles. Enzyme activities of lactate dehydrogenase (LDH), adenylokinase (AK) and malate dehydrogenase (MDH) were measured using micro-analytical techniques on the individual fibres within the histochemically identical groups and on fibres of the same types selected from areas of the test muscle or the contralateral control which appeared normal.

3. The results show that the degree of enzymatic variation among single fibres reinnervated by a common axon is very small when compared to the general fibre population and, moreover, to fibres of the same histochemical type.

4. Enzyme variability within the newly formed motor units was only slightly greater than the variability reported for normal motor units (Nemeth, Pette & Vrbová, 1981).

5. The results indicate that skeletal muscle fibres originally having great differences in levels of enzyme activity, as demonstrated in the general fibre population, acquire considerable enzymatic similarity following reinnervation by a common motor neurone.

## INTRODUCTION

The muscle fibres belonging to individual mammalian motor units have been shown to have similar histochemical staining characteristics for energy-related enzymes (Edström & Kugelberg, 1968; Kugelberg & Edström, 1968; Burke, Levine, Tsairis & Zajac, 1973). More recently, quantitative microchemistry of single muscle fibres has shown the motor unit to be quantitatively identical with respect to the enzymes malate dehydrogenase and fructose-1,6-bisphosphate dehydrogenase (Nemeth, Pette & Vrbová, 1981). It is not known to what extent the muscle fibres themselves determine metabolic specialization or the extent to which a common influence of the

motoneurone accounts for the uniform biochemical character of the motor unit. The present study was designed to test whether enzymatic uniformity can be acquired in dissimilar fibres under conditions in which they become innervated by branches of a common axon.

It has been demonstrated previously that on crushing the motor nerve a transient denervation occurs followed by axonal regeneration (Edds, 1950; Karpati & Engel, 1968). The new axon branches subsequently establish functional contacts with contiguous fibres in the muscle, some of which may have belonged to the original motor unit and some which certainly did not. Thus, fibres of the same histochemical type grouped together within a muscle fascicle are taken to be newly innervated by a single nerve axon. The distribution of the fibres of these new motor units do not have the mosaic arrangement seen in normal motor units (Kugelberg, Edström & Abbruzzese, 1970). The aggregated pattern of the fibre types, however, provides a means to readily identify motor units. By taking advantage of an *in situ* method of fibre procurement (described in this report), the constituent muscle fibres were analysed quantitatively for lactate dehydrogenase, malate dehydrogenase and adenylokinase. The results show that muscle fibres of newly formed motor units exhibit substantial biochemical uniformity. The uniformity is acquired in motor units of all major histochemically defined fibre types. This suggests an essentially complete metabolic transformation from any original fibre type by a common motoneurone.

## METHODS

Two adult male rats (Sprague–Dawley, 250 g in weight) were anaesthetized by intraperitoneal injections of sodium pentobarbitone (40 mg/kg). The common peroneal nerve was surgically exposed and repeatedly crushed with sterilized long-nosed forceps at a 45° angle to the long axis of the nerve. An attempt was made not to completely crush the nerve, in order to aid in rapid recovery of innervation to the muscle. A sham operation was performed on the contralateral limb. The surgery produced a deficit in hind-foot dorsiflexion that gradually resolved within 4 weeks; otherwise the animals were in excellent health. After an additional 5-week-recovery period the rats were killed.

The tibialis anterior muscles were removed, trimmed of their tendons and mounted in Tragacanth embedding media (Fisher Scientific, Pittsburgh, PA) on blocks, with the fibres oriented vertically. The blocks were quickly frozen in liquid nitrogen which had been cooled to near its freezing point (−210 °C). Serial cross-sections of 10–20 $\mu$m thickness were cut from the tissue block with a cryostat microtome at −20 °C. Alternate sections were prepared either for histochemistry or for micro-analytical biochemistry.

### *Histochemistry*

Sections for histochemistry were mounted on cover-slips, air dried and stained for myosin ATPase following the original method of Brooke & Kaiser (1970) for determining fibre types by differential staining. Three pre-incubation reagents were used: (1) pH 10·2 containing 20 mM-sodium barbitone and 18 mM-$CaCl_2$; (2) pH 4·5 containing 50 mM-sodium acetate and 30 mM-sodium barbitone; and (3) pH 4·25 containing 50 mM-sodium acetate and 30 mM-sodium barbitone. Following 5 min pre-incubations, all sections were identically treated for 30 min in an assay medium containing 20 mM-sodium barbitone, 9 mM-$CaCl_2$, 2·7 mM-ATP at pH 9·4. Sections were then washed three times in 1 % $CaCl_2$, 2 % $CoCl_2$ (w/v) and water, each for 3 min. Thereafter the sections were incubated 1 min in 1 % $(NH_4)_2S$. After rinsing with distilled water, sections were dehydrated in ethanol and mounted in DPX (K and K Rare and Fine Chemicals, Plainview, NY). The pH was adjusted at room temperature using a glass electrode calibrated with fresh buffer standards.

Pre-incubations 1 and 3 were used to differentiate fibres into types I and II; at pH 10·2, type I fibres stain lightly and type II stain darkly whereas at pH 4·3, type I fibres stain darkly and type

II stain lightly. Pre-incubation 2, pH 4·6, was used to distinguish type I (dark-staining), type IIA (light-staining) and type IIB (intermediate-staining) fibres. The multiple pre-incubations also distinguished type IIC fibres which have intermediate staining with both pH 4·3 and pH 4·5.

*Microchemistry*

Sections for microchemistry of 20 $\mu$m in thickness were maintained at −20 °C while being collected in aluminium holders and immediately thereafter lyophilized at −38 °C at a pressure less than $10^{-2}$ Torr for 24 h. The sections were stored under vacuum at −70 °C until further processing. Tests show that frozen-dried tissue can be stored in this way for years without the loss of activity of the enzymes measured in this study (Lowry, Kimmey, Felder, Chi, Kaiser, Passonneau, Kirk & Lowry, 1978). The dried tissue can be handled outside vacuum for several hours in a controlled environment (20 °C, not more than 50 % relative humidity) without loss of enzyme activity.

Photomicrographs of the histochemically stained sections were used to select fibres for microchemistry and to determine their myosin ATPase type. The fibres were selected from newly assembled motor unit aggregates and from mosaic areas in the same cross-section or in the contralateral control muscle. The corresponding areas were identified in the unstained lyophilized sections and cross-sectional pieces of the individual fibres were isolated by microdissection. The pieces, 8–30 ng, were weighed on a quartz fibre balance. Quantitative enzyme determinations were carried out at 20 °C in volumes of 5–10 $\mu$l using the methods of Lowry & Passonneau (1972). All assay reactions yielded a pyridine nucleotide product which was measured fluorometrically. Details of the assays have been described for adenylokinase (AK) and lactate dehydrogenase (LDH) (Lowry *et al.* 1978) and for malate dehydrogenase (MDH) (Hintz, Lowry, Kaiser, McKee & Lowry, 1980).

Nine motor units representing fibre types I, IIA and IIB were analysed. Enzymes were also measured in non-aggregated fibres of all types including type IIC. The enzyme activities (units: mol/kg dry weight.h) are expressed as the means of duplicate or triplicate determinations. Multiple samples of the same fibre were obtained from sequential serial sections.

## RESULTS

### *Correlation of histochemistry and microchemistry*

In adult mammalian muscle, fibres constituting normal motor units are widely distributed throughout the muscle; therefore, differential stains for fibre types produce a mosaic staining pattern (Edström & Kugelberg, 1968). Following nerve crush, some areas of the test muscle appeared normal, suggesting an incomplete crush as desired. The fibre-type distribution within most areas of the muscle, however, was markedly altered by the crush, as illustrated in Pl. 1. Groups of fibres of the same histochemical type were present, typical of reinnervated muscle (Karpati & Engel, 1968) and presumably due to distal sprouting of the regenerating axon with consequent innervation of the most proximate denervated fibres. The fibre types were determined by myosin ATPase staining after pre-incubation at pH 4·5, and compared to AK activity in the individual fibres (Table 1). Histochemically determined types I, IIA and IIB fibres had low, intermediate and high AK activity, respectively. However, there was some overlap of AK activities across the ATPase histochemical types, as recently reported by Hintz, Coyle, Kaiser, Chi & Lowry (1984).

The important feature of AK is that it provides a range of activities within a fibre type, so that an enzyme involved in high-energy phosphate metabolism can be assessed quantitatively within and between fibre types, which is not possible with myosin ATPase staining. Furthermore, by plotting individual fibres on the basis of both AK and another enzyme of energy metabolism, fairly distinct fibre groups can be demonstrated, as seen in Fig. 1.

TABLE 1. Myosin ATPase staining and adenylokinase enzyme activities in individual fibres of rat tibialis anterior muscle

| No. of fibres | Myosin ATPase pH 4·5 (histochemistry) | Fibre type* | Adenylokinase (mol/kg dry weight.h) |
|---|---|---|---|
| 21 | Dark | I | 29·7–54·6 |
| 19 | Light | II A | 38·8–79·0 |
| 22 | Intermediate | II B | 54·9–119·0 |

* After Brooke & Kaiser (1970).

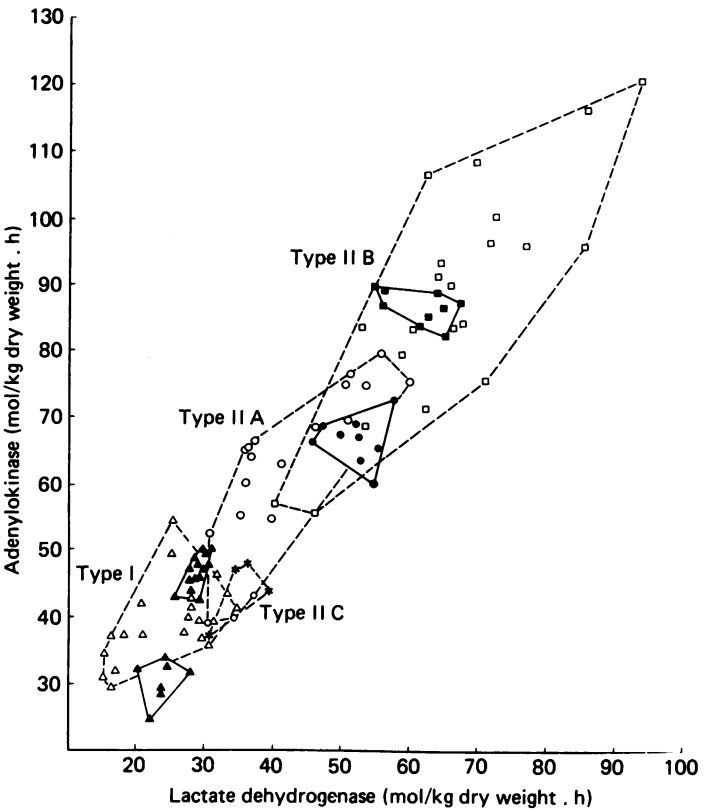


Fig. 1. Lactate dehydrogenase and adenylokinase activities plotted on the same individual fibres. Fibre types were determined by myosin ATPase staining as described in text and indicated as domains bound by dotted lines. Fibres comprising four reinnervated motor units (closed symbols) are superimposed on a sample of the whole population of fibres (open symbols) selected at random from the same or contralateral muscle. The type II C fibres (*) were obtained from an area of the muscle without reinnervation. Other symbols are △ for type I, ○ for type II A and □ for type II B fibres.

TABLE 2. Comparison of lactate dehydrogenase activities (mol/kg dry weight.h) in newly formed motor units and in fibres of the same histochemical type

| | Motor unit fibres | | | | Fibres representing the general population | | |
|---|---|---|---|---|---|---|---|
| Histochemical type | Motor unit | Activity (mean ± S.D.) | *f* ratio | No. of fibres sampled/ motor unit | Activity (mean ± S.D.) | *f* ratio | No. of fibres/ histochemical type |
| I | 1 | 29·1 ± 1·3 | 0·9 | 14 | 26·6 ± 7·1 | 31·8 | 20 |
| | 2 | 23·8 ± 1·2 | 2·2 | 6 | | | |
| IIA | 3 | 30·6 ± 1·9 | 2·0 | 13 | 40·4 ± 8·9 | 34·4 | 16 |
| | 4 | 46·4 ± 3·4 | 5·0 | 14 | | | |
| | 5 | 52·2 ± 3·8 | 5·2 | 8 | | | |
| | 6 | 48·3 ± 3·9 | 3·6 | 19 | | | |
| | 7 | 29·0 ± 3·0 | 3·5 | 11 | | | |
| IIB | 8 | 61·3 ± 5·1 | 5·9 | 6 | 68·3 ± 12·9 | 44·0 | 20 |
| | 9 | 66·4 ± 4·1 | 1·4 | 29 | | | |

TABLE 3. Comparison of adenylokinase activities (mol/kg dry weight.h) in newly formed motor units and in fibres of the same histochemical type

| | Motor unit fibres | | | | Fibres representing the general population | | |
|---|---|---|---|---|---|---|---|
| Histochemical type | Motor unit | Activity (mean ± S.D.) | *f* ratio | No. of fibres sampled/ motor unit | Activity (mean ± S.D.) | *f* ratio | No. of fibres/ histochemical type |
| I | 1 | 46·7 ± 2·5 | 1·8 | 14 | 39·4 ± 6·1 | 28·0 | 20 |
| | 2 | 30·6 ± 2·4 | 1·7 | 6 | | | |
| IIA | 3 | 53·2 ± 4·1 | 2·8 | 12 | 61·4 ± 13·9 | 33·2 | 16 |
| | 4 | 63·4 ± 6·9 | 6·5 | 12 | | | |
| | 5 | 66·0 ± 3·8 | 3·7 | 9 | | | |
| | 6 | 63·1 ± 4·5 | 4·1 | 18 | | | |
| | 7 | 35·7 ± 4·1 | 5·1 | 10 | | | |
| IIB | 8 | 85·6 ± 2·9 | 0·3 | 9 | 89·2 ± 15·6 | 30·5 | 20 |
| | 9 | 95·6 ± 7·2 | 3·5 | 27 | | | |

*Enzyme variations in the general fibre population*

Quantitative data for three enzymes on fibres selected from the test muscle or its contralateral control muscle having the normal fibre mosaic is given in Tables 2–4 for direct comparison with motor unit fibres. The efficacy of combining the data from unaffected regions of the test muscle with that from the contralateral control muscles was indicated by similar wide ranges of activities in both fibre groups. The variability of enzyme activities among the fibres of each myosin ATPase type was compared to the variation within a single fibre. The value for intracellular variation depends on both the biological variation of the particular enzyme along a fibre and the analytical error of the method; it was obtained from duplicate or triplicate enzyme determinations on the same individual fibre. Thus, intrafibre variation reflects a conservative estimate of possible analytical error. The relation of the variation among and within

fibres was obtained from a one-way analysis of variance to yield a ratio of mean-square errors, or $f$ ratio (Tables 2–4). A value of 1·0 indicates that the variations among and within fibres of the test group are identical; the larger the $f$ ratio, the more intercellular variation. The analysis indicated significant variation ($f > 1$; $P < 0{\cdot}0001$) within all three histochemical fibre types for each enzyme. The wide metabolic differences among fibres are illustrated for two of these enzymes together on the same individual fibres in Fig. 1. Type IIC fibres were included for completeness, although it is clear that there are insufficient numbers present to provide a definitive statement of interfibre variation.

TABLE 4. Comparison of malate dehydrogenase activities (mol/kg dry weight.h) in newly formed motor units and in fibres of the same histochemical type

| | Motor unit fibres | | | | Fibres representing the general population | | |
|---|---|---|---|---|---|---|---|
| Histochemical type | Motor unit | Activity (mean ± S.D.) | $f$ ratio | No. of fibres sampled/ motor unit | Activity (mean ± S.D.) | $f$ ratio | No. of fibres/ histochemical type |
| I | 1 | 15·5 ± 0·8 | 0·6 | 13 | 10·9 ± 3·1 | 37·2 | 20 |
| | 2 | 14·1 ± 0·5 | 0·9 | 7 | | | |
| | 3 | 13·4 ± 0·6 | 1·3 | 10 | | | |
| IIA | 4 | 20·4 ± 0·8 | 0·7 | 12 | 15·7 ± 2·6 | 15·6 | 5 |
| | 5 | 18·3 ± 1·0 | 2·0 | 12 | | | |
| | 6 | 18·4 ± 1·5 | 3·4 | 9 | | | |
| | 7 | 22·3 ± 1·9 | 2·2 | 18 | | | |
| IIB | 8 | 13·6 ± 0·5 | 2·2 | 7 | 9·4 ± 2·3 | 45·5 | 12 |

*Enzyme variations in motor units*

Superimposed on the general fibre population in Fig. 1 are LDH and AK activities in individual fibres from four motor units. There is a high degree of similarity in both enzymes among fibres of the motor units when they are compared to the broad range of enzyme activities present in fibres of each fibre type and of the whole muscle.

Enzyme activities within motor units representing all of the three major histochemical fibre types are given in Tables 2–4. As with the random fibres, the variability of enzyme activities among the fibres of a motor unit is expressed as the ratio of mean-square errors ($f$ ratio) by comparison of the variation within and among the single fibres. The results show much less enzymatic difference within a motor unit compared to randomly arranged fibres of the same type. The low $f$ ratios indicate considerable uniformity of enzyme levels within the limits of the analytical methods; the values below 1·0 theoretically mean that the variation is greater within than among fibres. While some degree of variability exists, the magnitude does not appear to be a function of fibre type or number of fibres analysed per motor unit. On average, the enzyme variations are 12 times greater among fibres of one histochemical type than among fibres of the newly formed motor unit of the same type.

## DISCUSSION

It has been known for some time that regeneration of a crushed motor nerve leads to the appearance of grouping of histochemically identical fibres (Edds, 1950; Karpati & Engel, 1968). Presumably, the enzymes are altered in the fibres of the original mosaic of mixed fibre types, since there is no evidence for complete fibre degeneration and regeneration in the time required in this study for reinnervation (Dubowitz, 1967). Glycogen-depletion studies have shown that the fibres of such histochemically identical aggregates are indeed innervated by a common axon (Kugelberg *et al.* 1970). Quantitative analysis of newly innervated fibres makes it possible to determine the extent of the influence of nerve on muscle biochemistry. This is important in light of the extreme biochemical heterogeneity of muscle fibres reported from laboratories engaged in micro-analytical biochemistry. Spamer & Pette (1978, 1979) reported variations of up to 3-fold in oxidative enzymes in type I fibres of rabbit soleus. Subsequently, Lowry's laboratory (Lowry *et al.* 1978; Hintz *et al.* 1980) and that of Pette (Nemeth & Pette, 1981; Buchegger, Nemeth, Pette & Reichmann, 1984) have reported wide ranges within fibre types for a variety of enzymes, including a 4-fold range of LDH in type II A fibres. In the present study, variations across all fibre types in the tibialis anterior muscle are 3·5-fold for MDH, 6·3-fold for LDH and 4·0-fold for AK. Since the fibre grouping spreads over areas large enough to encounter all major fibre types, it is clear that the metabolic transformation occurs in fibres which originally had differences in enzyme activities as great as these.

The fibres of motor units reinnervated following recovery from a nerve crush acquire metabolical similarity as indicated by their enzyme levels. Enzymes representing three energy pathways vary only slightly more than the maximum possible experimental error of the analytical method (based on intrafibre variation), and in some cases do not vary at all relative to this measure of experimental error. The MDH activities within newly formed motor units can be compared to published results of MDH activities in six motor units from normal rat extensor digitorum longus muscles that were typed by physiological parameters (Nemeth *et al.* 1981). The MDH activity levels were similar in each motor unit type in the two sets of experiments. However, the variation among normal motor unit fibres, taking into account their intrafibre variations, was much less than in the present results. Thus, it must be acknowledged that the high degree of uniformity within newly formed motor units is not as great as within those developed normally.

The enzyme levels in the reinnervated fibres are within the normal range for their respective histochemical fibre types. This is demonstrated by comparing values of motor unit fibres with those from areas of the test and contralateral control muscles which appeared normal (Tables 2–4). In addition, the values are in close agreement with the published data of Hintz *et al.* (1980) from single fibres selected from rat tibialis anterior and soleus muscles. This agreement in absolute enzyme activities is quite remarkable considering that the motor units were assembled from fibres having initially up to 6-fold differences in enzyme levels.

The present study provides new information on the extent to which nerve can induce metabolic plasticity in muscle. The original cross-innervation studies of Buller, Eccles & Eccles (1960) and the subsequent studies of its effect on enzyme activities

(Prewitt & Salafsky, 1967; Mommaerts, Buller & Seraydarian, 1969) consistently showed incomplete transformations from one type to another. Furthermore, there was divergence with respect to the direction of transformation. In all cases, the slow-to-fast transformation was less complete than the fast-to-slow transformation. In fact, Mommaerts *et al.* (1969) rarely found greater than a doubling of LDH after a slow-to-fast cross, and in some cases, no change at all. Recent studies of chronic stimulation (Buchegger *et al.* 1984) and endurance exercise (Chi, Hintz, Coyle, Martin, Ivy, Nemeth, Holloszy & Lowry, 1983) also suggest quantitatively incomplete fibre transformations. The degree of completeness of the transformation in the present study is thus greater than that induced by other means in previous studies, and moreover, is independent of fibre type. These findings suggest, at least with the particular reinnervation paradigm employed, that any neurone type is potentially capable of controlling the metabolic characteristics from any original type of muscle fibre.

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## REFERENCES

Brooke, M. H. & Kaiser, K. K. (1970). Three 'myosin ATPase' systems: The nature of their pH lability and sulfhydryl dependence. *Journal of Histochemistry and Cytochemistry* **18**, 670–672.

Buchegger, A., Nemeth, P. M., Pette, D. & Reichmann, H. (1984). Effects of chronic stimulation on the metabolic heterogeneity of the fibre population in rabbit tibialis anterior muscle. *Journal of Physiology* **350**, 109–119.

Buller, A. J., Eccles, J. C. & Eccles, R. M. (1960). Interactions between motoneurones and muscles in respect of the characteristic speeds of their responses. *Journal of Physiology* **150**, 417–439.

Burke, R. E., Levine, D. N., Tsairis, P. & Zajac III, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *Journal of Physiology* **234**, 723–748.

Chi, M. M.-Y., Hintz, C. S., Coyle, E. F., Martin II, W. H., Ivy, J. L., Nemeth, P. M., Holloszy, J. O. & Lowry, O. H. (1983). Effects of detraining on enzymes of energy metabolism in individual human muscle fibres. *American Journal of Physiology* **244** (*Cell Physiology* **13**) C276–287.

Chi, M. M.-Y., Lowry, C. V. & Lowry, O. H. (1978). An improved enzymatic cycle for nicotinamide-adenine dinucleotide phosphate. *Analytical Biochemistry* **89**, 119–129.

Dubowitz, V. (1967). Pathology of experimentally re-innervated skeletal muscle. *Journal of Neurology, Neurosurgery and Psychiatry* **30**, 99–110.

Edds Jr, M. V. (1950). Collateral regeneration of residual motor axons in partially denervated muscles. *Journal of Experimental Zoology* **113**, 517–551.

Edström, L. & Kugelberg, E. (1968). Histochemical composition, distribution of fibres and fatiguability of single motor units. *Journal of Neurology, Neurosurgery and Psychiatry* **31**, 424–433.

Hintz, C. S., Coyle, E. F., Kaiser, K. K., Chi, M. M.-Y. & Lowry, O. H. (1984). Comparison of muscle fiber typing by quantitative enzyme assays and by myosin ATPase staining. *Journal of Histochemistry and Cytochemistry* **32**, 655–660.

Hintz, C. S., Lowry, C. V., Kaiser, K. K., McKee, D. & Lowry, O. H. (1980). Enzyme levels in individual rat muscle fibres. *American Journal of Physiology* **239**, C58–65.

Karpati, G. & Engel, W. K. (1968). 'Type grouping' skeletal muscles after experimental reinnervation. *Neurology* **18**, 447–455.

Kugelberg, E. & Edström, L. (1968). Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibres: relation to fatigue. *Journal of Neurology, Neurosurgery and Psychiatry* **31**, 415–423.

KUGELBERG, E., EDSTRÖM, L. & ABBRUZZESE, M. (1970). Mapping of motor units in experimentally reinnervated rat muscle. *Journal of Neurology, Neurosurgery and Psychiatry* **33**, 319–329.

LOWRY, C. V., KIMMEY, J. S., FELDER, S., CHI, M. M.-Y., KAISER, K. K., PASSONNEAU, P. N., KIRK, K. A. & LOWRY, O. H. (1978). Enzyme pattern in single human muscle fibres. *Journal of Biological Chemistry* **253**, 8269–8277.

LOWRY, O. H. & PASSONNEAU, J. V. (1972). *A Flexible System of Enzymatic Analysis*. New York: Academic Press.

MOMMAERTS, W. F. H. M., BULLER, A. J. & SERAYDARIAN, K. (1969). The modification of some biochemical properties of muscle by cross-innervation. *Proceedings of the National Academy of Sciences of the U.S.A.* **64**, 128–133.

NEMETH, P. M. & PETTE, D. (1981). Succinate dehydrogenase activity in fibres classified by myosin ATPase in three hind limb muscles of rat. *Journal of Physiology* **320**, 73–80.

NEMETH, P. M., PETTE, D. & VRBOVÁ, G. (1981). Comparison of enzyme activities among single muscle fibres within defined motor units. *Journal of Physiology* **311**, 489–495.

PREWITT, M. & SALAFSKY, B. (1967). Effect of cross-innervation on biochemical characteristics of skeletal muscles. *American Journal of Physiology* **213**(1), 295–300.

SPAMER, C. & PETTE, D. (1977). Activity patterns of phosphofructokinase, glyceraldehyde-phosphate dehydrogenase, lactate dehydrogenase and malate dehydrogenase in microdissected fast and slow fibres from rabbit psoas and soleus muscle. *Histochemistry* **52**, 201–216.

SPAMER, C. & PETTE, D. (1979). Activities of malate dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase and fructose-1,6-diphosphate with regard to metabolic subpopulations of fast and slow twitch fibres in rabbit muscle. *Histochemistry* **60**, 9–19.

## EXPLANATION OF PLATE

Tibialis anterior muscle fibres stained with myosin ATPase pre-incubated at pH 4·5. The muscle was reinnervated for 9 weeks following surgical crush of common peroneal nerve and shows loss of mosaic pattern and areas of fibre-type grouping.