

Mapping of motor units in experimentally reinnervated rat muscle

Interpretation of histochemical and atrophic fibre patterns in neurogenic lesions

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SUMMARY The distribution of fibres in the motor units of reinnervated muscle is demonstrated. As shown in some experimental models, the histochemical and atrophic fibre patterns in neurogenic lesions are determined by the morphology of the specific motor units involved. This also helps to explain the alterations of motor unit action currents in neurogenic lesions.

It has been shown that contractions of the anterior tibial muscle of the albino rat, induced by repetitive stimulation of a single motor nerve fibre in the ventral root, produce striking changes in the phosphorylase activity and glycogen content of the muscle fibres, which permits histochemical mapping of the motor unit (Kugelberg and Edström, 1968a, and b). The motor unit was found to be largely uniform as regards histochemical fibre type. The fibres lie scattered singly or a few together and the different units are highly intermingled (Edström and Kugelberg, 1968).

Histochemical mapping has now been extended to the motor unit in experimentally reinnervated rat muscles after (1) transection and reunion of the motor nerve and (2) transection of the ventral root. The latter leads to reinnervation of muscle fibres normally supplied by the resected root by motor nerve fibres in the intact root or roots through terminal collateral branching (for pertinent literature see Edds, 1953; Coërs and Woolf, 1959).

The demonstration of normal and pathological motor units provides a rational basis for interpretation of histochemical and atrophic fibre patterns encountered in neurogenic lesions. The patterns, which are used as diagnostic criteria in muscle biopsies, may be considered as an image of the motor units involved. In order to elucidate this relationship we have produced experimental models of histochemical and atrophic fibre patterns by (1)

partial denervation of normal muscle, (2) partial reinnervation of denervated muscle, and (3) partial denervation of reinnervated muscle. The latter produced the classical group atrophy found in motoneurone diseases.

The architecture of the motor units is discussed in relation to the characteristic changes of motor unit action currents in neurogenic lesions. A brief account of our results has been published (Edström and Kugelberg, 1969).

MATERIAL AND METHODS

OPERATIVE PROCEDURES Young albino rats weighing 150 g were anaesthetized with sodium pentobarbital. Four operative procedures were performed. In procedure 1 (seven rats) the bifurcation of the sciatic nerve of one hind limb was exposed in the popliteal fossa. The common peroneal nerve was completely transected and the cut ends resutured with silk. In procedure 2 the nerve root was exposed by laminectomy and about 4 mm resected. The proximal cut end was reflected 180° on itself and fixed with sutures to prevent reinnervation through the same root. L₄ resection was done in five rats and L₅ resection in six rats. In preliminary experiments the distribution of the L₄ and L₅ roots in the anterior tibial muscle had been histochemically mapped with the PAS technique after repetitive stimulation of the root. It was found that the main innervation comes from L₄, which innervates fibres located over the entire cross-sectional area of the muscle. L₅, although subject to individual variations, innervates a considerably smaller number of fibres situated predominantly in the deeper, red portion of the muscle.

In procedure 3, performed five to six months after

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procedure 1, the reinnervated muscle was partially denervated by section of L₄ or L₅ root. In procedure 4, performed five to six months after procedure 2, the reinnervated muscle was partially denervated by section of part of the peroneal nerve.

MAPPING OF MOTOR UNITS Five to six months after procedures 1 and 2 the animals were anaesthetized and the lumbar roots exposed by laminectomy. The proximal stumps of the transected L₄ and L₅ roots were electrically stimulated and found unresponsive, showing that reinnervation had not occurred through the transected roots. The L₄ and L₅ motor roots were teased apart under a dissection microscope until one motor fibre to the anterior tibial muscle was found. The criterion was an all-or-nothing response in the muscle to finely graded stimuli from sub-threshold up to supramaximal current strength recorded with a Grass Ft 03 force-displacement transducer. In two additional experiments a filament containing two fibres to the muscle was stimulated in order to determine the position of two units supplied by two closely situated nerve fibres.

The motor nerve fibres were stimulated at a frequency of 10/sec for 10 minutes. During stimulation the contractions were continuously recorded isometrically. The fatigability of the unit served as a physiological check on the histochemical classification, since type A fibres fatigue rapidly, the B fibres less rapidly, and the C fibres not at all under the conditions of stimulation. For further details see Edström and Kugelberg (1968).

The histochemical technique was the same as that described earlier (Kugelberg and Edström, 1968b). The succinic dehydrogenase, phosphorylase, and glycogen (PAS) reactions were determined in serial sections of the muscles. Some sections were also incubated for the myofibrillar adenosine triphosphatase (ATPase) reaction according to the method described by Padykula and Herman (1955). Some of the PAS-stained sections were retained with aniline blue for connective tissue.

The cross-sectional areas of muscle and motor units were measured planimetrically on photomicrographs. Single muscle fibres were measured according to the method described by Edström and Torlegård (1969).

RESULTS

MOTOR UNITS AND HISTOCHEMICAL 'TYPE-GROUPING'

We have used the histochemical classification of fibre types suggested by Stein and Padykula (1962), which is based on mitochondrial enzymic activity such as succinic dehydrogenase. The A fibres show very weak reactions, the B fibres intermediate, and the C fibres strong reactions.

Classification based on the myofibrillar ATPase reaction—that is, type I with weak, and type II with strong reaction, was less suitable for our purpose. Of interest is that the type I fibres are composed solely of B fibres, whereas the type II fibres contain some B as well as A and C fibres

(Edgerton, Gerchman, and Carrow, 1969). Thus, in the anterior tibial muscle type I fibres are scarce as compared with type II fibres. Moreover, the succinic dehydrogenase reaction shows many grades of intensities. The ATPase reaction is much less graded. Since the motor units are largely homogeneous even as regards the finer grades of succinic dehydrogenase activity, this provides more detailed information of the fibre distribution in the units.

SELF-REINNERVATED MUSCLE Five to six months after transection and reunion of the common peroneal nerve, recovery of the anterior tibial muscle was clinically good. The cross-sectional area of the muscle was 10 to 30% smaller than in the control muscle. Microscopically the great majority of the muscle fibres were of normal size, a few fibres were extremely atrophic and obviously not reinnervated.

'Type groups' (Engel, 1965) were prominent—that is, larger aggregations of enzymatically similar muscle fibres than normal. This is in agreement with the observations of Dubowitz (1967) and Karpati and Engel (1968a) in similarly self-reinnervated muscle. Type-grouping was very distinct in some areas (Fig. 1a). Figure 1b shows a motor unit mapped on the basis of PAS negativity located within the same area. It displays a very compact arrangement of its 280 fibres. The unit is identifiable by the large almost homogeneous field of type A fibres in Fig. 1a. The histochemical homogeneity of the unit and the compact distribution of its fibres are responsible for the type-grouping in the reinnervated muscle.

Figure 1c shows, for comparison, the checker-board pattern of the normal muscle and Fig. 1d part of a motor unit located in the same area. Although the normal unit contains a smaller number of fibres (150) it occupies an area three times as large as the reinnervated unit, for which reason only part of the unit could be accommodated within a photomicrograph of the same magnification. The normal motor unit is homogeneous as regards histochemical fibre type but the scattered distribution of its fibres results in a mosaic pattern and prevents type-grouping.

A motor unit located in an area of the reinnervated muscle with less distinct grouping (Fig. 2a) showed a less compact unit (Fig. 2b). In other areas it was not possible, even with the succinic dehydrogenase reaction, to determine whether type-grouping was present or not. Units in such areas showed still greater overlapping. However, in no instance did a reinnervation unit display the characteristic single fibre distribution of normal units; this was

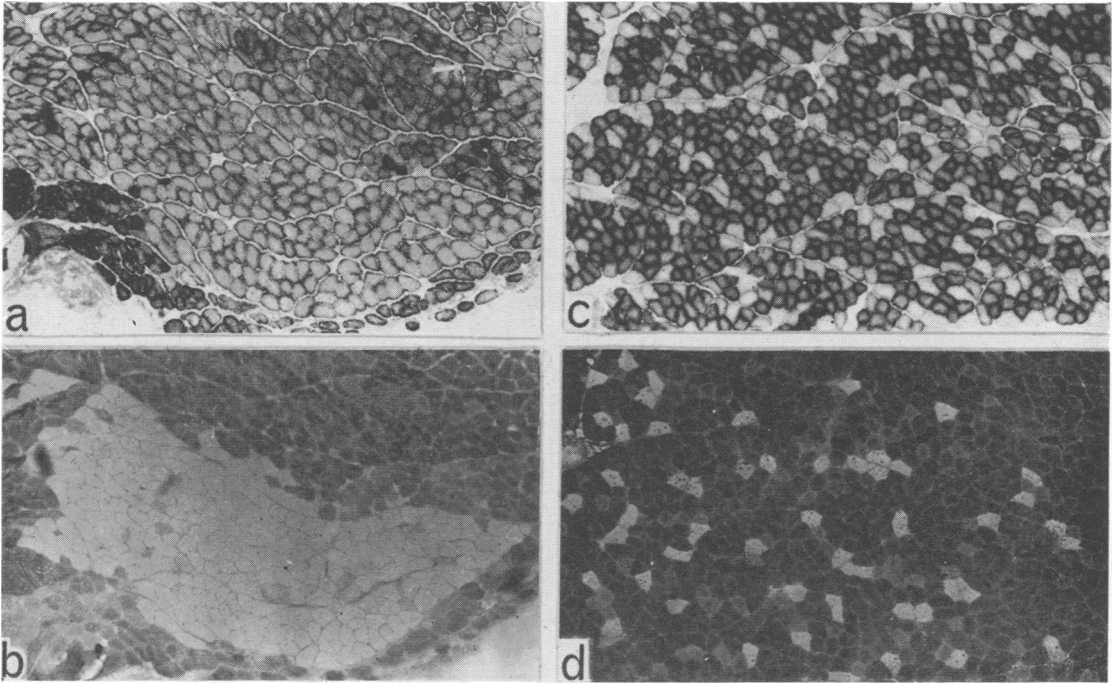


FIG. 1. Rat 4 a and b. Cross-sections of reinnervated anterior tibial muscle after transection and union of nerve. a: incubated for succinic dehydrogenase, showing histochemical type-grouping. b: PAS. Same area, showing one compact motor unit of 280 fibres identical with the large type A fibre group in a. c and d: cross-sections of control muscle—c, incubated for succinic dehydrogenase, showing the normal mosaic pattern; d, PAS. Same area showing one-third of a normal motor unit of about 150 widely scattered fibres, most of them occurring as single fibres. $\times 40$.

replaced by groups containing a varied number of fibres.

It should be emphasized that a type group does not permit any conclusions regarding the exact distribution and number of fibres in the units. Units of similar histochemical type may intermingle or lie in contact with one another, in which case the type group is larger than one unit. Moreover, the unit may often have less distinct borders, being mixed with fibres from foreign units, resulting in a type group smaller than the unit.

All units in the reinnervated muscle occupied a considerably smaller area than normal, despite the fact that they contained a similar mean number of fibres (Table 1). The number of fibres, however, showed considerable variation. The fact that the mean number of fibres in the units was only slightly greater than in normal muscle shows that most of the motor fibres had succeeded in taking part in reinnervation.

In the normal motor unit the distribution of

TABLE 1

MOTOR UNITS IN REINNERVATION AFTER SECTION AND REUNION OF NERVE

	Percentage muscle atrophy	Fibres in unit (no.)	Unit area (mm ²)	Unit area in % of muscle area
R 1	27.2	161	4.23	11.0
R 2	25.9	197	1.13	3.0
R 3	28.8	160	1.97	5.1
R 4	36.5			
unit I		280	2.26	5.7
unit II		112	2.26	5.7
R 5	13.2	177	1.27	3.5
R 6	9.5	115	1.41	3.0
R 7	12.8			
unit I		83	0.85	1.9
unit II		16	0.28	0.6
Mean	22.0	145	1.82	4.4
Normal values ¹		127 (80-178)	10 (7-15)	1.7 (12-26)

¹cf. Edström and Kugelberg (1968).

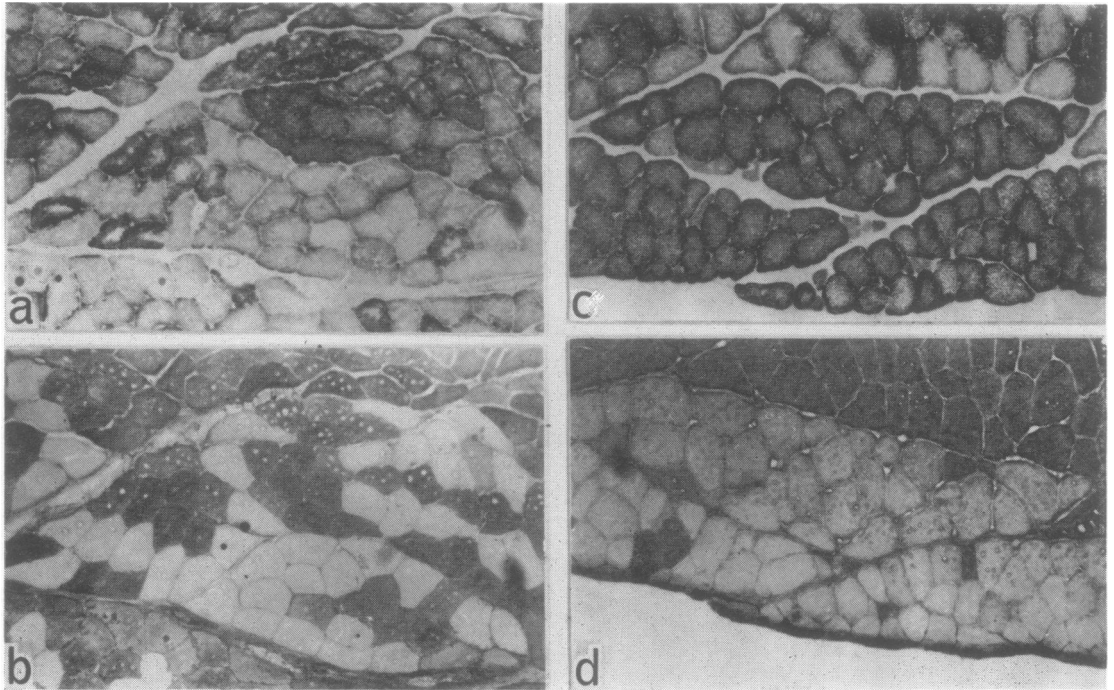


FIG. 2. Cross-sections of reinnervated anterior tibial muscle after transection and reunion of nerve. Rat 6 a and b a: incubated for succinic dehydrogenase, showing indistinct type-grouping; b: PAS. Shows one moderately compact unit of 115 fibres in the same area. Rat 7 c and d: c, incubated for succinic dehydrogenase, showing a large type B fibre group delineated by perimysium; d, PAS. Same area showing one motor unit, identical with the B fibre group and delineated by perimysium. $\times 90$.

fibres has no clear relationship to the fascicles of the muscle. In reinnervated muscle the motor units are generally located in several fascicles. However, the boundaries of the units coincide to such a large extent with the perimysium that this cannot be mere chance. Figure 2c shows three fascicles with type B fibre groups which are identified as a single motor unit on the basis of PAS negativity (Fig. 2d). The upper borders of the unit area clearly follow the fascicular boundaries. This applied also to the units shown in Figs. 1b and 2b, but was better demonstrated with larger magnification.

The phenomenon of perimysial boundaries is also observed in the demarcation of type groups (Fig. 2c), although generally less clearly.

MOTOR ROOT TRANSECTION Five to six months after transection of the L_5 root the anterior tibial muscle showed no visible changes in five of the six rats. In these rats the L_5 nerve fibres to the

muscle were apparently too few to produce atrophy or type groups. One motor unit examined in each animal showed normal distribution and number of fibres and normal size of territory. In the remaining rat the muscle showed slight atrophy and type-grouping. One motor unit examined in the muscle showed an altered fibre distribution as compared with normal units (Fig. 3a). There was a considerable decrease in single fibres. Many of these were replaced by small groups of five to 15 closely packed, histochemically uniform fibres (Fig. 3a), showing that the collateral sprouts preferably innervated neighbouring denervated muscle fibres. This indicates that the effective collaterals were of terminal origin and of short length.

L_4 root transection produced considerably more severe denervation. Only five to 15 motor nerve fibres to the muscle were found in the L_5 root when it was teased into fine filaments during the mapping procedure. Thus, 80 to 90% of the approximately 75 units in the muscle were denervated.

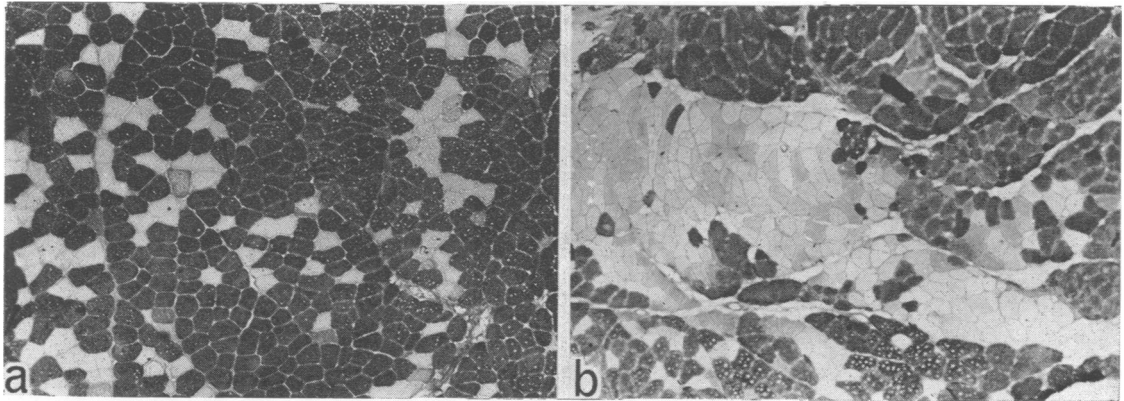


FIG. 3. Anterior tibial muscle reinnervated, following root section, by collaterals from intact nerve fibres. Rat 13. a: moderate denervation after L_5 motor root section, showing part of one motor unit of 454 fibres with some grouping. Rat 16. b: severe denervation after L_4 motor root section, showing part of one motor unit of 551 fibres, for the most part with compact distribution.

This gave the remaining motor units ample opportunity to develop their potential capacity for collateral reinnervation. The smaller groups of fibres in the less severely denervated muscle had aggregated to much larger groups (Fig. 3b). Some of these groups were very large and contained hundreds of fibres, but smaller groups and some single fibres were also present. The larger groups were often partly delineated by perimysium, as in the case of reinnervated muscle after section and reunion of the nerve. The large histochemically homogeneous groups resulted in prominent type-grouping.

Measurements of the fibres showed that the majority were of normal size but there was an increased number of both smaller and larger fibres. The latter were especially prominent in extremely atrophic muscles.

The number of fibres in the motor units was increased (Table 2). The highest value observed was seven times greater than the normal mean number. The absolute value of the territory of the unit, however, was within normal limits or smaller than normal, but the relative value in relation to the cross-sectional area of the atrophic muscle was within normal limits.

The decrease in the territory of the unit was due to atrophy of muscle fibres of both indigenous and foreign types within the territory. Since the atrophy was not severe, it is apparent that although the unit could not expand its outer border to any large extent, it was, on the other hand capable of reinnervating most of the denervated fibres within its territory. The

TABLE 2
MOTOR UNITS AFTER ROOT SECTION

	Percentage muscle atrophy	Fibres in unit (no.)	Unit area (mm^2)	Unit area in % of muscle area
R 13 (L 5)	4.7	454	9.98	30.7
R 14 (L 4)	30.0	650	6.23	19.2
R 15 (L 4)	74.6	334	1.75	14.8
R 16 (L 4)	54.5	551	2.48	11.8
R 17 (L 4)	67.2	847	3.95	26.0
R 18 (L 4)	58.0	190	2.75	14.1

fact that the different units to a large extent overlap increases the possibility of successful reinnervation by collateral sprouting.

MOTOR UNITS, HISTOCHEMICAL AND ATROPHIC FIBRE PATTERNS IN NEUROGENIC LESIONS

The experimental models described in the following serve to demonstrate the clear relationship between a specific type of motor unit architecture and the atrophic fibre patterns resulting from denervation of these specific units. This can be done with some precision, since the distribution and histochemical composition of the fibres in the motor unit have now been visualized both in normal and in reinnervated muscles.

PATTERN I PARTIAL DENERVATION OF NORMAL MUSCLE The atrophic fibres appeared against a background of the normal histochemical mosaic

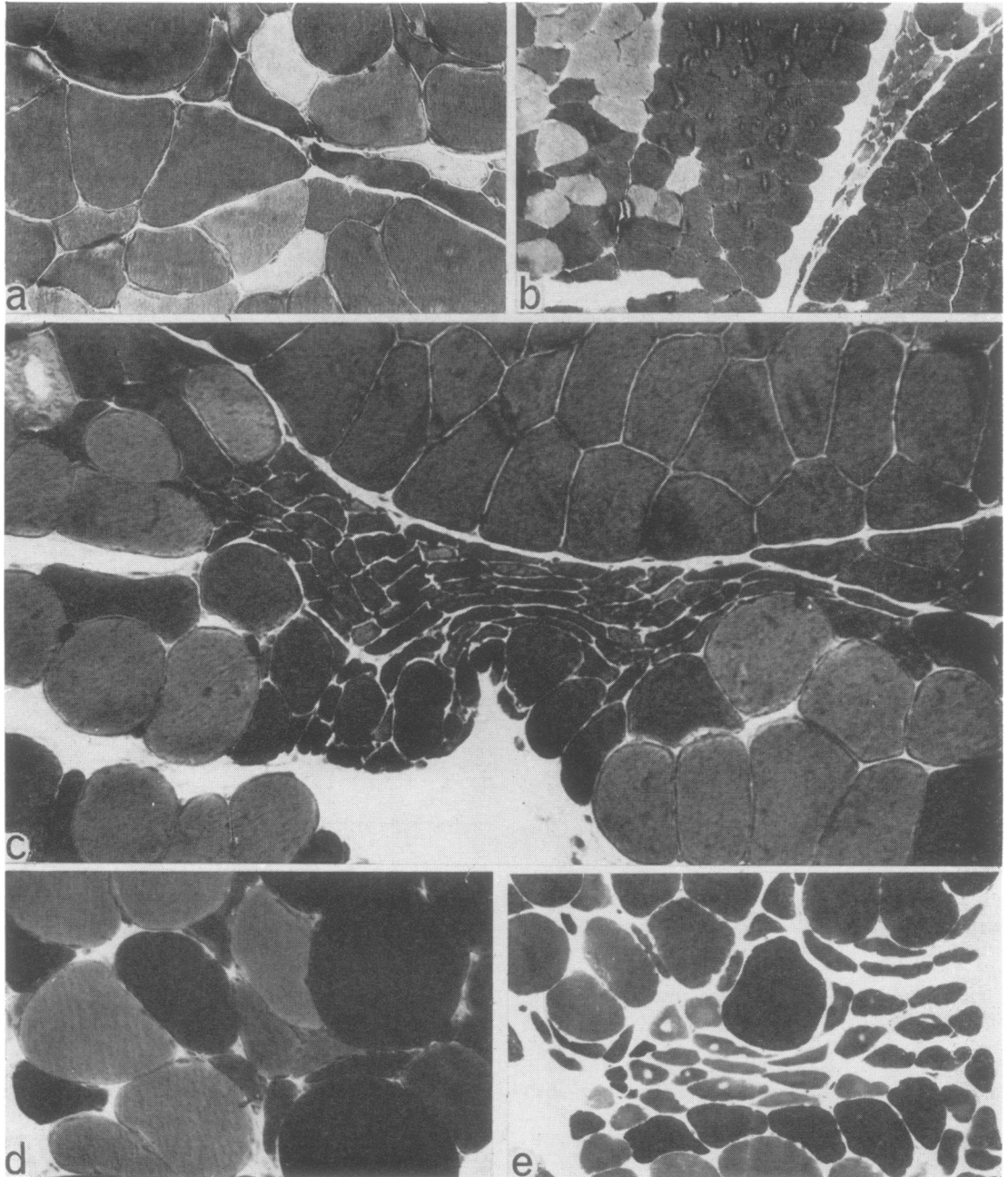


FIG. 4. Histochemical and atrophic fibre patterns in neurogenic lesions. Cross-sections incubated for myofibrillar ATPase. a: pattern I. Rat gastrocnemius muscle moderately denervated by L_5 root section, showing scattered atrophic fibres of different histochemical types. $\times 225$. b: pattern II. Anterior tibial muscle of the rat, partially reinnervated after section and reunion of nerve. Histochemically heterogeneous atrophic fibres with fascicular delineation. Background shows type grouping. $\times 90$. c: pattern III. Anterior tibial muscle of the rat reinnervated after section and reunion of nerve and later partially denervated by L_5 motor root section. Atrophic fibres histochemically homogeneous in a partly perimysium delineated group. Non-atrophic background fibres show type grouping. $\times 225$. d. For comparison, biceps muscle biopsy, from patient with early amyotrophic lateral sclerosis showing pattern I. $\times 190$. e: biopsy from patient with amyotrophic lateral sclerosis in a later stage showing pattern III. $\times 90$.

pattern of the muscle. In moderate denervation of the gastrocnemius muscle, produced by section of the L₅ motor root, atrophic fibres were visible three to four weeks after the operation. They were spread over the entire muscle and occurred as single fibres (Fig. 4a) or in discrete groups. The scattered distribution of atrophic fibres after root section was considered by Van Harreveld (1947) to indicate that fibres of different motor units are intermingled. Indeed, the atrophic pattern is the result of denervation of motor units with scattered distribution of fibres—that is, the normal unit (exemplified in Fig. 1d). Since several units were denervated, the atrophic fibres were of different histochemical types but any one unit was histochemically uniform.

In the more massive denervation of the anterior tibial muscle produced by section of a large part of the peroneal nerve, the atrophic fibres were aggregated in larger histochemically heterogeneous groups. Such aggregations lacked sharp boundaries, due to the scattered distribution of fibres in the single motor units which compose them. This was examined 14 days after denervation before signs of reinnervation had appeared.

All histochemical types of fibres show atrophy when denervated, but the white muscle fibres or A fibres do so more rapidly and to a larger degree than the red fibres (Bajusz, 1964) and type II fibres more than type I fibres (Engel, Brooke, and Nelson, 1966).

PATTERN II PARTIAL REINNERVATION The background for the atrophic fibres is the more or less distinct histochemical 'type grouping' of reinnervated muscle.

Five months after section and reunion of the nerve a number of atrophic fibres were still present, generally in singles or a few together. They represent residual fibres of the denervated motor units, which explains the scattered distribution. Since many units were involved they were histochemically heterogeneous. Larger, histochemically heterogeneous groups of atrophic fibres were also present (Fig. 4b). In contrast to the fibre groups in partially denervated muscle not reinnervated, these groups often had more distinctly delineated boundaries, formed by connective tissue. This is a counterpart to the perimysial boundaries often found in the reinnervated motor units. Whole fascicles outside the reinnervated areas may remain denervated.

PATTERN III PARTIAL DENERVATION OF REINNERVATED MUSCLE Five months after section and reunion of the peroneal nerve, the muscle was reinnervated by section of the L₄ or L₅ motor root and the

changes in the anterior tibial muscle studied three to four weeks later.

The background of the atrophic fibres was the histochemical type groups of the reinnervated muscle. The most characteristic change was the occurrence of small or large groups of atrophic fibres of uniform histochemical composition (Fig. 4c). Sometimes these groups were not homogeneous, in which case smaller homogeneous groups were observed within the larger aggregations. This was apparently the result of convergence of different denervated units. The boundary of the atrophic groups was often partly confined to the perimysium (Fig. 4c). This picture was to be expected from denervation of the type of units found in reinnervated muscle after section and reunion of the nerve. A similar picture was obtained by reinnervating the muscle five to six months after L₄ root section by section of one half of the peroneal nerve.

Muscle biopsies from two patients with amyotrophic lateral sclerosis are shown for comparison. The one patient in the early stage of the disease exhibits pattern I (Fig. 4d). The other, in a later stage, exhibits pattern III (Fig. 4e). See discussion.

DISCUSSION

Reinnervation produced histochemically uniform motor units with an altered distribution of fibres into smaller or larger groups. This presupposes a rearrangement of the muscle fibres into new constellations. The newly-formed units must therefore be assembled from muscle fibres of different histochemical types. Under the common influence of the motor neurone they are apparently transformed into histochemical uniformity. No attempt was made to follow the time course of the histochemical transformation but it was largely achieved two months after a root section. The phenomenon is similar to the partial reversal of the histochemical profiles of muscle fibres after cross union of nerves to white and red muscles (Romanul and Van Der Meulen, 1966, 1967; Dubowitz, 1967; Yellin, 1967; Robbins, Karpati, and Engel, 1969).

It is possible that the motor unit architecture is not always altered by reinnervation. Gutmann and Young (1944) found, under the favourable conditions of distal nerve crush, that one fibre returned down each Schwann tube leading to an end-plate. Karpati and Engel (1968a) observed no type grouping, which is another indication that units of normal fibre distribution were formed, provided the mild crushing which was used produced significant denervation.

In the reinnervated muscle after transection and reunion of the nerve the territory of the unit was

much smaller and the fibre distribution much more compact than in normal units. This means that the arborizations of the motor nerve fibres were shorter than normal and thus were not directed into the channel of an original fibre right back to the end-plate.

The compact distribution of fibres in reinnervated muscle is probably due to the close link between growth and incentive to make contact with denervated muscle fibres. This would prevent branching fibres from bypassing a number of muscle fibres in order to attain the scattered distribution of the muscle fibres in the normal motor unit. According to Couteaux (1941), during embryonic life growth is a two stage process. In the first phase motor nerve fibres lie in close contact with the surface of immature myotubes. No connections are visible between the 'exploring' motor fibres and muscle. In the second phase, when the muscle fibres mature, adjacent nerve fibres sprout terminal collaterals which eventually make contact with muscle.

The shortness of arborizations is probably also the result of the restricting influence of connective tissue. The boundaries of the units clearly coincided to some extent with the fascicles. It is difficult to escape the impression that the perimysium presents a relative barrier, at least for terminal collaterals. It is also possible that it is difficult for the more proximal ramifications to penetrate into the fascicle, which would explain why it often contains fibres of only one motor unit and why whole fascicles sometimes escape reinnervation. During embryonic development connective tissue would not restrict the growth of the terminal fibres, since it becomes fully developed long after these are formed.

In the motor units examined, the muscle fibres were concentrated in one circumscribed area. It is likely that had more motor units been investigated some with two or perhaps more fibre aggregates would have been found, since a minority of fibres divide proximal to the point of entrance in the muscle (Eccles and Sherrington, 1930). A fibre which had dichotomized proximal to the site of the cross-section would potentially grow out to different parts of the muscle and form a motor unit of two circumscribed fibre aggregates.

After transection and reunion of nerve, the territory of the unit was diminished. It is true that the majority of the nerve fibres succeeded in reaching the muscle, and competition was great. Less competition increases the chances of larger unit territories. On the other hand, a smaller number of denervated fibres available for reinnervation, as in moderate partial denervation, would be expected to decrease

the territory still further. In this case, however, reinnervation through damaged motor nerve fibres would probably be overshadowed by collateral reinnervation from adjacent intact fibres.

After root section, the change in unit architecture starts from the level of the normal motor units. An increase in territory is then likely to occur with an increase in the number of muscle fibres but is masked by atrophy of some motor fibres within its territory. Even if most of the growth is due to terminal collaterals from nearby nerve fibres (Edds, 1950; Hoffman, 1950; Van Harreveld, 1952; Coërs and Woolf, 1959), more proximal collaterals may reinnervate fibres localized remote from the territory of the parent nerve fibre. This is likely to occur sometimes but was not observed with certainty in the limited number of units examined here. Increase in the number and density of fibres was much more prominent than increase in size of territory. The latter was probably restricted by the same factors restricting increase of territory in reinnervated muscle after transection and reunion of nerve. Thus the motor units were often delineated by the perimysium.

The compact distribution of fibres in the motor units results in decreased overlapping of different units. On weak effort the rate of contraction is below the tetanic fusion frequency of the units. Intermittent contractions are then more easily observed than in the normal unit, which contributes to the origin of 'contraction fasciculations' of Denny-Brown and Pennybacker (1938).

The interpretation of atrophic fibre patterns in muscle biopsies is complicated by the fact that in most chronic progressive lesions of the motor neurone and motor nerve fibres, denervation and reinnervation proceed simultaneously. For instance, in motor neurone disease, one motoneurone after the other is destroyed. This leads in the first phase to the picture of discrete denervation, described as pattern I (Fig. 4d). Reinnervation by terminal collaterals from intact fibres will ensue (Wohlfart, 1958; Coërs and Woolf, 1959) resulting in increased density of fibres in the intact motor units and consequently in type grouping with or without residual non-reinnervated atrophic fibres (pattern II). These motoneurons will eventually die, resulting in the classical group atrophy of motor neurone diseases—that is, groups of fibres in a uniform state of atrophy. So long as histochemical specificity is retained, such groups tend to be histochemically uniform (pattern III) (Fig. 4e). In rapidly progressive diseases pattern I dominates (Fig. 4d). Pattern III becomes more prominent in the slowly progressive diseases (Fig. 4e).

The denervated fibres in pattern III will eventually

be reinnervated for a second time and so forth. The dynamic relationship between motoneurons and muscles leads to a continuous rearrangement of the motor units. The atrophic groups in motor neurone diseases were considered by Slauck (1921) to represent motor units and by Wohlfart (1949) parts of units. These views are essentially correct if it is remembered that pathological units, remote from the normal, are concerned. Slauck also observed that fibres in a uniform state of atrophy could be confined to a fascicle, so-called fascicular atrophy. This again is a pathological phenomenon associated with the restricting influence of connective tissue on reinnervation.

Since the interpretation of the different atrophic patterns is to some extent dependent on the histochemical composition of the fibres it is necessary to know how long histochemical specificity is retained after denervation. Many of the fibres in a histochemically uniform group were originally of different histochemical type, transformed to uniformity by the common influence of the motor neurone they share. For that matter they may have changed histochemical type several times. It is not known if this affects the stability of histochemical reactions after denervation. However, in uncomplicated denervation the ATPase reaction seems to reflect the original fibre type for longer periods of time than most other histochemical reactions (Karpati and Engel, 1968b).

For the sake of completeness, it should be pointed out that the histochemical patterns in the experimental models described here are distorted by any process which affects mainly one histochemical type of fibre. Distortion may also occur if the remaining intact muscle fibres are enzymatically adapted to the altered functional situation. However, both these factors require further investigation.

Yahr and his collaborators (1950) investigated the motor unit action currents in the reinnervated muscles of patients three to five years after section and suture of a peripheral nerve. The major portion of the tracings showed the occurrence of spikes of very large amplitude, which they suggested was due to an increased number of fibres in the motor units. The occurrence of large amplitude spikes is in good agreement with the anatomical features of the motor units in reinnervated muscles after section and reunion of the nerve described in this study. However, the cause of the high amplitude is the characteristic increased fibre and current density in the units rather than increased number of fibres.

In longstanding neurogenic lesions, the motor unit action currents in the electromyogram show an increased amplitude and duration until secondary

myopathic changes take place (Kugelberg, 1949). These conditions favour collateral reinnervation, which gives rise to units of high fibre density. This again explains the high spike amplitudes. This is in agreement with the conclusions of Erminio, Buchthal, and Rosenfalck (1959) based on the finding of large amplitude motor units spikes in similar cases.

Erminio *et al.* (1959) also concluded, on the basis of electrophysiological evidence, that the territory of the motor units is considerably increased in many of these patients. In this respect it is not altogether inappropriate to generalize from rat to man, since the regenerative capacity of the rat is remarkably good. It seems unlikely that the motor units in man are capable of expanding their territories to a much larger extent than in the rat, in which it was insignificant.

The discrepancy may be due to technical causes. The authors' electrophysiological mapping of the territory of the unit was based on the assumption that the presence of action currents giving rise to a 50 μ V spike indicated that their rather unselective recording electrode was less than 1 mm from the outer boundary of the unit. This may hold true for the normal motor unit but does not necessarily hold true for the pathological unit. In the normal motor unit the territory is bordered mainly by single fibres. The pathological unit, which contained up to seven times as many fibres within a similar area, was bordered by closely packed fibre aggregates containing sometimes hundreds of fibres. A fall in the amplitude of the spike to the 50 μ V level would therefore be expected to occur at a longer distance from the border than in the normal unit. This may lead to an over-estimation of the size of the spike generator. Furthermore, the compact arrangement of the fibres in the reinnervated motor unit will increase the interaction between the muscle fibres. This may lead to an increased synchronization of their discharges and an increased strength of the signal source.

SUMMARY

Motor units have been histochemically visualized in the experimentally reinnervated anterior tibial muscle of the albino rat. Knowledge of the architecture of normal and reinnervated motor units enabled interpretation of the histochemical and atrophic fibre patterns encountered in neurogenic lesions in terms of the specific type of motor units involved.

1 In reinnervated muscle the motor units were composed of histochemically uniform fibres occur-

ring in small or large groups, depending on the experimental conditions. This grouping is responsible for the histochemical type-groups seen in reinnervated muscle. Although the units were distributed within several fascicles, the boundaries of the larger groups often coincided partly with the perimysium, which restricts reinnervation.

After transection and reunion of nerve, the territory of the units was much smaller than normal, although the mean number of fibres was within normal limits, resulting in a greatly increased density of the fibres in the unit.

After root section and reinnervation by collaterals from intact nerve fibres, the number of fibres in the units was up to seven times greater than in the normal unit. Although the relative size of the territory of the motor unit may be increased, its absolute value was within normal limits or smaller. The effective collaterals appeared to be mainly of terminal origin and short length, chiefly reinnervating denervated muscle fibres within the territory of the parent unit.

2 NEUROGENIC LESIONS *Pattern I* Partially denervated muscle showing histochemically heterogeneous atrophic fibres occurring as single fibres or discrete groups with irregular boundaries appearing against a background of the histochemically normal mosaic pattern. Unit architecture is normal in atrophy and background.

Pattern II Partially reinnervated muscle showing histochemically heterogeneous atrophic muscle fibres occurring as single fibres or in groups, often partly with fascicular delineation. Background shows some type grouping. Unit architecture is normal in atrophy and grouped in background.

Pattern III Partially denervated reinnervated muscle showing histochemically homogeneous atrophic groups of fibres, often with partly fascicular delineation, appearing against a background of histochemical type groups. Unit architecture is grouped in atrophy and in background.

The patterns occur mixed—for example, all three in progressive motoneurone disease.

3 The high density multifibre motor units of normal or somewhat diminished territory in collateral reinnervation act as a much stronger signal source than the normal unit. This would explain not only the large amplitude and long duration of the motor unit action currents but also their wider cross-sectional distribution in chronic neurogenic lesions.

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