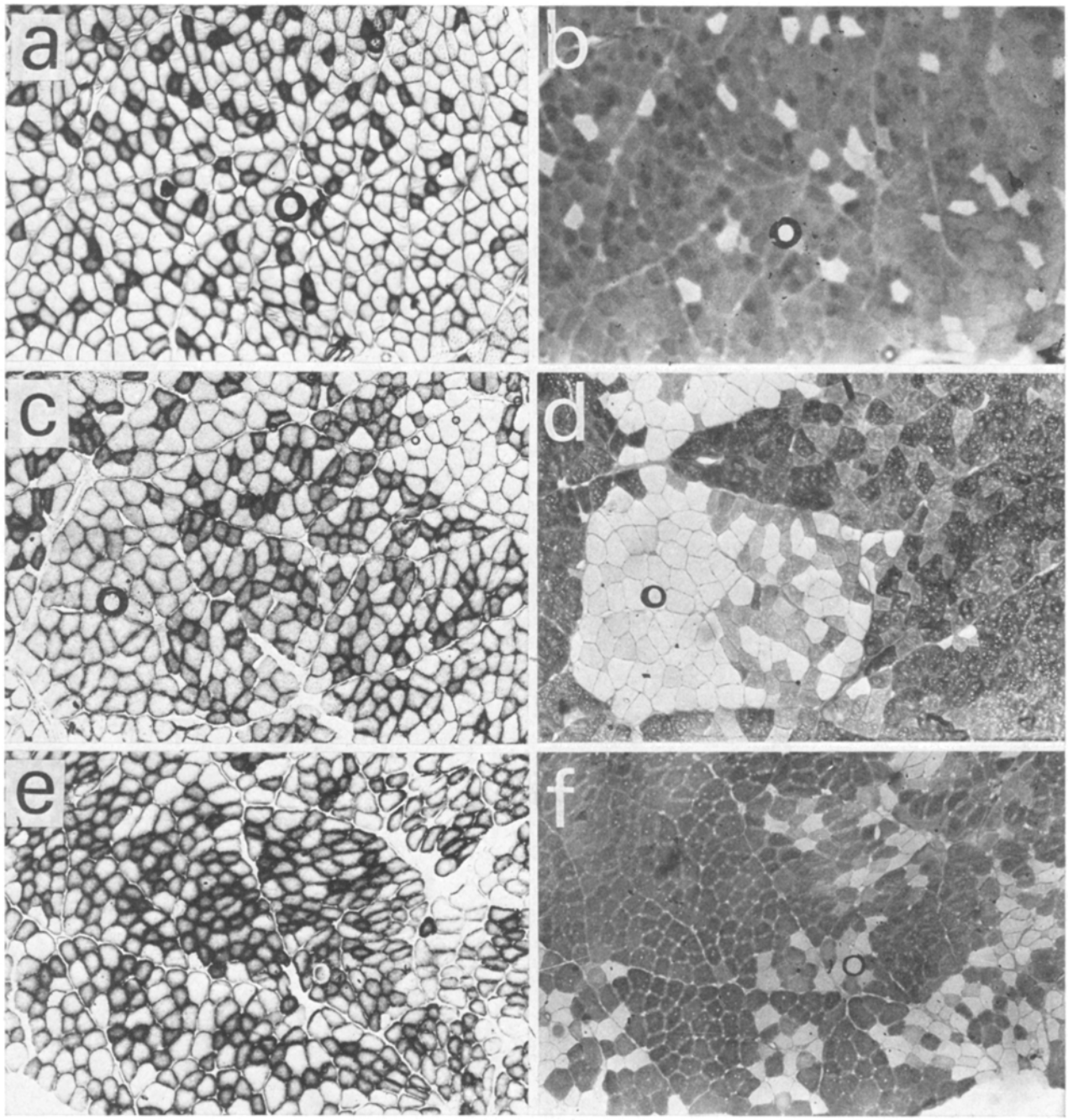
Histochemical Mapping of Motor Units in Experimentally Re-Innervated Skeletal Muscle

The heterogeneous mammalian skeletal muscle presents histochemically a mosaic pattern of fibres with different enzymatic activity. 3 main types of fibres have been distinguished: (a) fibres with low activity for oxidative enzymes of the Krebs cycle, (c) fibres with high enzymatic activity and (b) fibres with intermediate intensities of activity[1].

It was shown in the anterior tibial muscle of the albino rat that muscle contractions elicited by repetitive stimulation of single motor nerve fibres in the ventral root produced histochemical changes in phosphorylase and glycogen, which permitted mapping of the motor unit[2]. The motor unit was largely uniform as regards histochemical fibre type. The fibres lay scattered, isolated or a few together and the different units were highly intermingled[3].

The method of histochemical mapping has now been applied to the motor unit in reinnervation. Knowledge



Serial sections of normal anterior tibial muscle (a, b), after nerve transection and reunion (c, d), muscle reinnervated by collateral sprouting after L_4 section (e, f). a, c, e, incubated for succinic dehydrogenase; b, d, f, stained for glycogen by the PAS method. × 130. (a) Normal mosaic pattern; (b) part of a normal type A motor unit with scattered fibres. Only $^1/_5$ of the unit is accommodated in the photomicrograph; (c) shows type-grouping; (d) one of the groups represented by the whole motor unit of compact fibre distribution; (e) showing some group-typing; (f) part of a multiple cluster type of unit. Open circles indicate identical fibres in paired serial sections.

of the anatomical organization of the motor unit is essential for an understanding of certain features characteristic of the fully or partially reinnervated muscle. These include (a) formation of type-groups[4–8], i.e. replacement of the normal mosaic pattern by clusters or larger islands of histochemically uniform muscle fibres, (b) the classical picture of group atrophy, i.e. groups of fibres in a uniform state of atrophy interspaced with isolated fibres or groups of fibres of normal or enlarged size, (c) increased size and duration of motor unit potentials in the EMG.

Experimental reinnervation was produced in the anterior tibial muscle of 10 albino rats in 2 ways: (1) The bifurcation of the sciatic nerve of 1 hind limb was exposed in the popliteal fossa. The common peroneal nerve was completely transected and the cut ends resutured. (2) The L_4 root was exposed by laminectomy and resected. This will cause the motor nerve fibres in the L_5 to reinnervate muscle fibres normally supplied by the resected L_4 root through terminal collateral branching[9–12]. From 2–4 months after the operation one motor unit in the anterior tibial muscle of each rat was histochemically mapped by stimulation of a single motor nerve fibre in L_4 or L_5. The contralateral anterior tibial muscle served as control.

For comparison, the normal mosaic pattern of a control muscle incubated for succinic dehydrogenase is shown in Figure 1a, as well as part of a type A motor unit composed of 166 fibres within the same area (Figure 1b). The fibres of the unit were localized by PAS negativity after stimulation of its motor nerve fibre for 10 min at 10/sec. The muscle fibres belonging to the unit are widely scattered and only $^1/_5$ of the unit could be accommodated within the photomicrograph ($\times$ 130).

In contrast, the reinnervated muscle 4 months after transection and reunion of the nerve shows type-grouping (Figure 1c). A typical motor unit within the same area is shown in Figure 1d. It is histochemically homogeneous and composed of 160 A fibres. Their distribution is much more compact than in the normal unit, which permits display of the entire unit in the photomicrograph ($\times$ 130). In the section incubated for succinic dehydrogenase (Figure 1c) the most compact part appears as a histochemically uniform group of fibres. This obviously results in type-grouping. Furthermore, the solid cluster of fibres acts as a much stronger signal source than the scattered fibres in the normal unit. Consequently the action potential of the unit should be large as compared with that of a normal unit with an equal number of fibres.

A muscle whose L_4 root had been sectioned 2 months earlier also showed some type-grouping (Figure 1e). The B unit from the same area, part of which is shown in Figure 1f, is, as would be expected from sprouting, composed of a considerably increased number of fibres, 459. The territory of the unit was within the upper range of normal. The fibres appear in small clusters of 5–30 fibres. Each cluster is probably situated in the vicinity of a muscle fibre of the original unit. This view is supported by the fact that collateral sprouting is known to arise mainly within 200 μ of the end of the parental nerve fibre[11]. Since the clusters of fibres are histochemically fairly uniform this again results in type-grouping. The increase in the number of fibres and the density of the unit as well as the tendency for the territory of the unit to increase results in increased amplitude and duration of the action potential.

The distribution of fibres in the motor unit is more or less clearly shown in the distribution of atrophic fibres in peripheral motor neurone lesions. Denervation of a few motor units gives rise to scattered atrophic muscle fibres which reflects the distribution of fibres in the normal motor unit. In more massive denervation there is a confluence of the scattered atrophic fibres of many denervated motor units to irregular groups. Since several motor units are involved, the atrophic fibres show histochemical heterogeneity as long as histochemical specificity is retained after denervation. We may call this type of atrophy primary neurogenic atrophy.

Secondary neurogenic atrophy is characterized by group atrophy and occurs when the reinnervation types of motor units are denervated. Since the atrophic groups represent an image of a motor unit, they are histochemically homogeneous so long as histochemical specificity of the fibres is retained. Progressive motor neurone disease may exemplify this. Collateral sprouting of surviving motor neurones is here known to be prominent[13] and would form the multiple cluster type of motor unit described above. When these newly formed units become denervated as the disease progresses, secondary group atrophy ensues. In amyotrophic lateral sclerosis the fibres in the atrophic groups generally number 10–50[14], which corresponds well to the number of fibres in a cluster.

The two types of pathological motor units described here are typical examples. It is, however, clear that large variations in the number of fibres and the density and territory of the units occur, depending on the number of denervated muscle fibres available and the number of nerve fibres competing for them as well as the time factor. It is also probable that reinnervation does not necessarily causes changes in the motor unit morphology. Under favourable conditions the nerve fibre may grow out entirely in its old channels. For example, type-grouping was not observed in reinnervated muscles after nerve crushing[8].

Zusammenfassung. Die Verbreitung der Muskelfasern in den einzelnen Einheiten bei Reinnervation wurde bei Ratten durch histochemische Technik im M. tibialis anterior untersucht. Die Reinnervation nach Durchschneiden und Sutur des Nervus fibularis ergab eine motorische Einheit mit einer kompakten Verbreitung von histochemisch einheitlichen Muskelfasern. Das Durchschneiden der 4. Lumbalwurzel ergab nach Reinnervation der distalen intakten Nervenfasern durch terminale Kollaterale eine motorische Einheit mit einer vermehrten Anzahl von Muskelfasern, die vielfältige Gruppen bildeten.

L. Edström and E. Kugelberg

Department of Neurology, Karolinska sjukhuset, 10401 Stockholm 60 (Sweden), 13 May 1969.

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