Anat. Embryol. 153, 9–21 (1978)

Anatomy and Embryology



# Course of Denervation Atrophy in Type I and Type II Fibres of Rat Extensor Digitorum Longus Muscle*

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**Summary.** In the denervated extensor digitorum longus muscle of the rat type I and type II muscle fibres were differentiated histochemically and their course of atrophy was studied. Until 42 days after denervation type I and type II fibres could be identified by means of the myofibrillar ATPase reaction. Up to that time an exclusive atrophy of type II fibres was found. Type I fibres, the smallest of the normal muscle, did not change their diameters and therefore represented the largest fibres 42 days after denervation. Type II fibres of the "white" muscle portion, in which the larger IIB fibres are predominant, showed a higher rate of atrophy than those of the "red" muscle portion, in which the smaller IIA fibres are predominant: by 42 days the diameters of all type II fibres had gone down to equal values. Combined with a further progress of atrophy at later stages, there was a dedifferentiation of the histochemical properties, and the type I fibres exhibited atrophy as well. 120 days after denervation all muscle fibres were found to be highly atrophied.

**Key words:** Rat EDL – Denervation atrophy – Muscle fibre types.

## Introduction

Since the observation of Bajusz (1964) that the agranular "white" muscle fibres of denervated rat and mouse triceps surae muscles atrophy more heavily than the granular "red" muscle fibres, numerous papers dealing with the behaviour of different muscle fibre types after denervation have been published (Hogenhuis and Engel, 1965; Romanul and Hogan, 1965; Engel et al., 1966; Karpati and Engel, 1968a, 1968b; Guth et al., 1971; Gutmann et al., 1972; Tomanek and Lund, 1973; Gauthier and Dunn, 1973; Wuerker and Bodley, 1973; Riley and Allin, 1973;

* Dedicated to Prof. Dr. A. Faller on the occasion of his 65th birthday.

** Supported by the "Fonds zur Förderung der wissenschaftlichen Forschung in Österreich".

*** Miss F. Schramm provided skilful technical assistance.

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0340-2061/78/0153/0009/$02.60

Melichna and Gutmann, 1974; Jaweed et al., 1975; Hikida and Bock, 1976). There is good agreement in the literature concerning the observation that the (ATPase positive) type II fibres show a distinct atrophy after denervation. However, the findings concerning the behaviour of the (ATPase negative) type I fibres after denervation differ widely: On the one hand Bajusz (1964) stated "that the diameters of the 'red' muscle fibres", which probably correspond to the type I fibres, "remain approximately the same for a long time". On the other hand we have the statement of Romanul and Hogan (1965) that all fibre types atrophy to the same extent. Several authors confirm the finding of a "preferential type II fibre atrophy" (Engel et al., 1966) after denervation, at which the type I fibres exhibit a lower atrophy rate than the type II fibres (Wuerker and Bodley, 1973; Melichna and Gutmann, 1974). Type I fibres of the slow soleus muscle are said to be more atrophied 2 to 3 weeks after denervation than type I fibres of fast muscles (Tomanek and Lund, 1973, guinea pig; Jaweed et al., 1975, rat). In contrast to this, the muscle fibres of cat soleus muscle (exclusively type I fibres) showed only moderate atrophy 8 weeks after denervation. In the same experiment, the type I fibres of the deep portion of the fast gastrocnemius muscle exhibited almost no atrophy, whereas those of the superficial portion of the same muscle were atrophied to the same extent as the type II fibres (Guth et al., 1971).

These differences in the literature on the behaviour of type I muscle fibres after denervation gave rise to the present work in which a new study of their course of denervation during a longer period of time was performed.

## Materials and Methods

The denervation atrophy of type I and type II muscle fibres was investigated in the extensor digitorum longus muscles of 50 male Wistar rats (body weight 300–350 g). The denervation was performed under Nembutal anaesthesia (4 mg per 100 g of body weight i.p.) by section of the right common peroneal nerve in the distal part of the thigh. To avoid reinnervation, the proximal nerve stump was led through a slit of the biceps femoris muscle and fixed subcutaneously by Histoacryl. The following table shows the number of animals examined at the different stages of denervation:

| Days after denervation: | 4 | 6 | 7 | 10 | 14 | 21 | 28 | 42 | 80 | 120 |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of animals: | 3 | 3 | 8 | 3 | 5 | 10 | 3 | 7 | 5 | 3 |

From each animal the denervated extensor digitorum longus muscle of the right side and, as a control, the innervated contralateral muscle were excised. The muscles were immediately frozen in isopentane cooled to −60° C by dry ice. Cross-sections of 10 $\mu$m from the muscle belly (middle third of the muscle) were cut in a cryostat (Cryocut AO). In order to differentiate muscle fibre types, the following histochemical methods were applied: 1) Myofibrillar ATPase in the modification of Guth and Samaha (1970), 2) NADH tetrazolium reductase (Dubowitz and Brooke, 1973).

Measurements of muscle fibre diameters were performed in a light microscope equipped with a drawing device. By this means muscle fibre cross-sections were projected upon a graded series of circular profiles with diameter differences corresponding to steps of 5 $\mu$m at the given magnification. In this way muscle fibres could be classified in diameter classes of 5, 10, 15, 20, 25 . . . to 100 $\mu$m. Diameter histograms are based on measurements of sections stained for myofibrillar ATPase after alkaline preincubation. All ATPase negative fibres (= type I fibres) found on one and the same cross-section have been measured. Concerning the ATPase positive fibres (= type II fibres), two samples of 100 fibres taken from opposite regions of the muscle cross-section, which we will call the "red" and "white" muscle portion because of their muscle fibre compositions (see results), were used for diameter measurements. The data were expressed as means and standard error of the means. For both fibre types the correlation between muscle fibre diameter and denervation time up to 42 days after denervation was examined by a regression analysis.

The distal stumps of the N. peroneus communis of all operated legs were excised together with the

denervated muscles. They were fixed in 5% buffered glutaraldehyde, post-fixed in 1% $OsO_4$, and embedded in Epon. Sections of 1 $\mu$m were cut on a Reichert OmU2 ultramicrotome, stained with toluidine blue, and examined in the light microscope for signs of reinnervation. One animal had to be excluded from evaluation because of reinnervation.

## Results

### *The Innervated Extensor Digitorum Longus Muscle of the Contralateral Side*

The extensor digitorum longus muscle of the adult rat is, with respect to its histochemical muscle fibre composition, a mixed (Schiaffino et al., 1970; Pullen, 1970), physiologically (Close, 1967, 1972; Edgerton and Simpson, 1972) a fast twitch muscle.

In sections stained for NADH tetrazolium reductase we find, according to the classification of Stein and Padykula (1962), A fibres with large diameters and low enzyme activity, B fibres with small diameters and high enzyme activity, and C fibres with intermediate diameters and high enzyme activity of primarily subsarcolemmal localization (Fig. 1a). After incubation for myofibrillar ATPase (Fig. 1b) fibres with high enzyme activity (type II fibres, Engel, 1962) predominate over a small amount of fibres with low activity (type I). Formaldehyde fixation prior to incubation leads to a differentiation of the type II fibres into darkly stained "formaldehyde resistant" and less intensely stained "formaldehyde sensitive" muscle fibres. Kaiser and Brooke (1970) subdivided type II fibres, according to the pH lability of their myofibrillar ATPase after acid preincubation, into IIA, IIB (and IIC) fibres. As the formaldehyde resistant fibres correspond to the IIA fibres and the

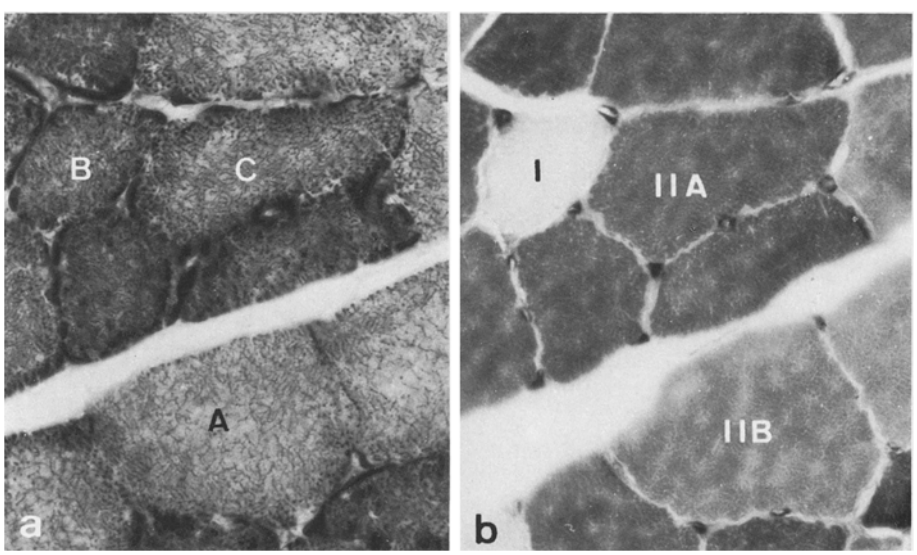



**Fig. 1a and b.** The three fibre types of rat extensor digitorum longus muscle. Serial sections, ×500. **a)** NADH tetrazolium reductase, **b)** myofibrillar ATPase after formaldehyde fixation and alkaline preincubation

formaldehyde sensitive fibres to the IIB fibres (Close, 1972; own unpublished observations), the well-known classification of Kaiser and Brooke will be used in this paper. Serial sections show the homology of the type A, B, and C fibres according to Stein and Padykula with the IIB, I, and IIA fibres of Brooke and Kaiser (Fig. 1). The formaldehyde sensitivity of their type A fibres in the reaction for myofibrillar ATPase was already mentioned by Stein and Padykula (1962).

These fibre types of rat extensor digitorum longus muscle can be correlated to other nomenclatures as shown in Table 1.

In normal extensor digitorum longus muscle type I fibres have the smallest diameters, type IIA fibres are of medium size, and type IIB fibres are the largest.

In cross-sections a checkerboard pattern of these three muscle fibre types is seen; however a certain polarity of the fibre distribution can be observed: In the deep medial region of the muscle cross-section there is a predominance of large A(=IIB) fibres with low mitochondrial content; in the direction towards the distal end of the muscle this region occupies an increasingly larger part of the cross-section. The superficial lateral region of the cross-section prevailing at the proximal muscle end, contains primarily smaller muscle fibres with high mitochondrial content, i.e. type C(=IIA) and type B(=I) fibres. Therefore they shall be called "white" (predominantly IIB fibres) and "red" (predominantly IIA and I fibres) muscle portion in this paper. The small amount of type I fibres of the extensor digitorum longus muscle is exclusively restricted to the red muscle portion. The percentual distribution of the muscle fibre types within the white and red muscle portions from opposite cross-sectional areas in the middle of the muscle belly are given in Table 2. Between the two extremes there are continuous transitions in respect of the relative amounts of IIB and IIA fibres. The mean diameters of IIA and IIB fibres in the white muscle portion are distinctly larger than those in the red muscle portion (Table 2).

As a clear separation of IIA and IIB fibres was not possible in later stages of denervation, these two fibre types have been measured together also in the control muscles of the contralateral side. In this way the results could be compared. In the diameter histogram of the control muscle of the contralateral side (Fig. 2a), a peak of the small type I fibres is present only in the red muscle portion (above abscissa). As to the type II fibres, the histograms show two peaks in the red as well as in the white

**Table 1.** Correlation of some important muscle fibre classifications

| | | | |
|---|---|---|---|
| Brooke and Kaiser (1970) | I | IIA | IIB |
| Stein and Padykula (1962) | B | C | A |
| Gauthier (1969) | intermediate | red | white |
| Yellin and Guth (1970) | $\beta$ | $\alpha$ | $\alpha\beta$ |
| Barnard et al. (1971), Peter et al. (1972) | slow-twitch oxidative (=SO) | fast-twitch oxidative glycolytic (=FOG) | fast-twitch glycolytic (=FG) |
| Burke et al. (1971) | type S (=slow contracting) | type FR (=fast contracting, fatigue resistant) | type FF (=fast contracting, fast fatigue) |

**Table 2.** Percentage (%) and mean diameters (in $\mu$m) of the three types of muscle fibres in the white and red portion of five control extensor digitorum longus muscles of the contralateral side. Measurements were made in cross-sections from the middle third of the muscle length. Samples of 100 fibres were taken from both the white and red portion of each muscle

| Fibre type | White muscle portion | | Red muscle portion | |
|---|---|---|---|---|
| | % | $\mu$m | % | $\mu$m |
| I (B) | 0 | – | 4–7 | 37.2 ± 0.4 |
| IIA (C) | 36–43 | 52.0 ± 1.9 | 61–73 | 44.9 ± 1.5 |
| IIB (A) | 57–64 | 76.7 ± 2.5 | 22–34 | 66.1 ± 2.3 |

muscle portion representing the two subtypes of type II fibres. In the white muscle portion (below abscissa), according to their higher amount the peak of the larger IIB fibres is higher, in the red muscle portion the peak of the smaller IIA fibres is more accentuated. Further it should be noticed that the diameter histogram of the type II fibres in the white muscle portion is displaced to the right with respect to that of the red portion, this means that the type II fibres of the white muscle portion have larger diameters than those of the red one.

### *The Denervated Extensor Digitorum Longus Muscle*

In accordance with earlier authors (Bajusz, 1964; Smith, 1965; Romanul and Hogan, 1965) denervation was quickly followed by changes of the oxidative enzyme pattern. Therefore the NADH tetrazolium reductase reaction could not be employed for fibre typing in experimental muscles. However, the histochemical reaction to myofibrillar ATPase allowed a clear separation of type I and type II fibres up to 42 days after denervation.

Up to 42 days after section of the common peroneal nerve we found exclusively a type II fibre atrophy. The type I fibres did not show any significant change of their diameters (Fig. 3). In later stages (80 and 120 days after denervation), the ATPase activity of the type II fibres was decreased as compared to the contralateral control muscle, it was however increased in the type I fibres. For this reason, 80 to 120 days after denervation a clear separation of two fibre types was no longer possible by means of the histochemical demonstration of myofibrillar ATPase. But in these stages we still found fibres, although in small number, which were conspicuous by a larger size and a slighly lower ATPase activity (Fig. 5).

The type II fibre atrophy commenced early after denervation and showed a progressive advance (Table 3, Figs. 2 and 3). In the white muscle portion, with its predominance of IIB fibres (see Table 2), a higher atrophy rate could be observed than in the red muscle portion containing predominantly IIA fibres: 42 days after denervation the diameters of all type II fibres of the white portion had decreased to 45% of the values of the contralateral control muscle, those of the red portion only to 58% (Table 2). In this way the type II fibre diameters of both muscle portions had gone down to a common value of about 30 $\mu$m at this stage. They were now appreciably smaller than the type I fibres whose diameter remained unchanged up to this stage (compare Fig. 4a–f).

For further elucidation of the relation between fibre diameter and denervation time of type I fibres as well as of the type II fibres of both muscle portions up to the stage of 42 days post denervationem, a regression analysis was performed (Fig. 3).

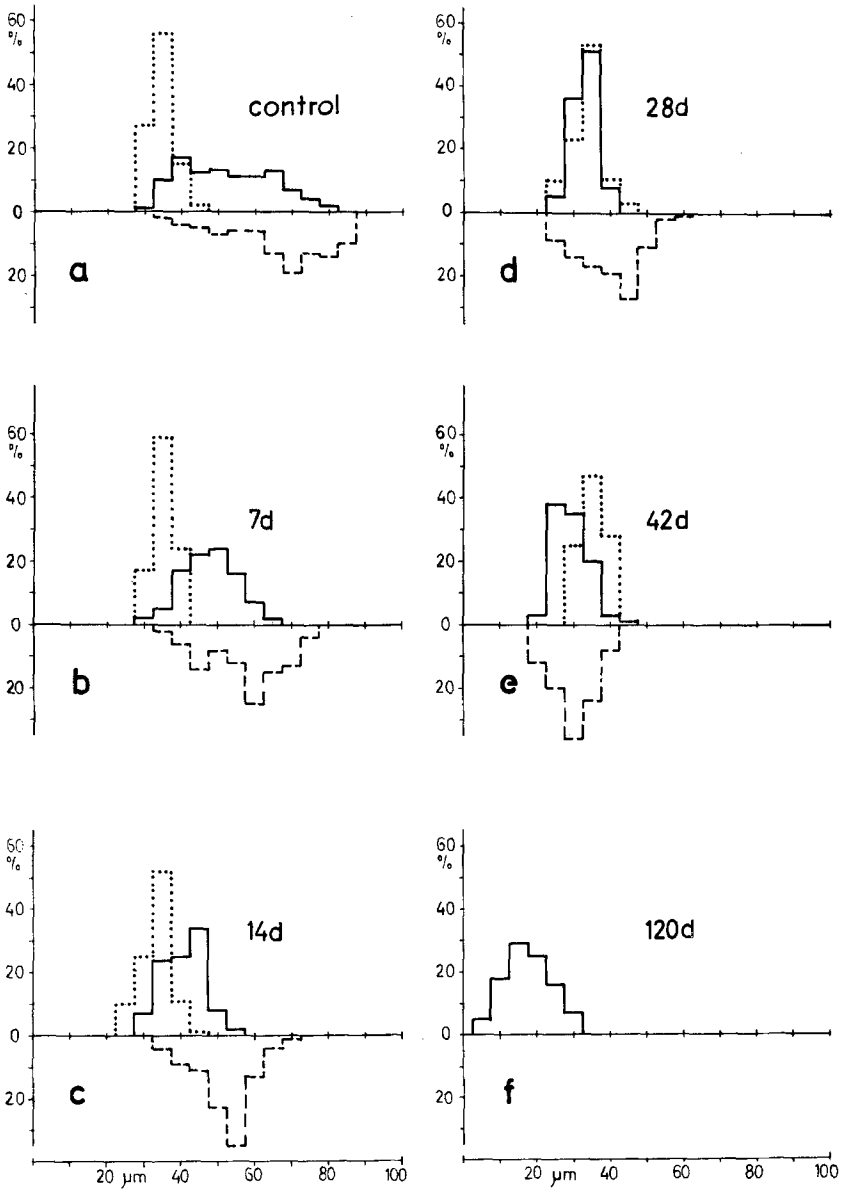



**Fig. 2a–f.** Diameter histograms of type I and type II fibres of rat extensor digitorum longus muscle after denervation. Values of red muscle portion above abscissa: type I fibres (dotted lines) and type II fibres (continuous lines); values of white muscle portion below abscissa: exclusively type II fibres (dashed lines). **a)** Contralateral control muscle, **b)** 7 days after denervation, **c)** 14 days after denervation, **d)** 28 days after denervation, **e)** 42 days after denervation, **f)** 120 days after denervation (fibre types and muscle portions no longer separated)

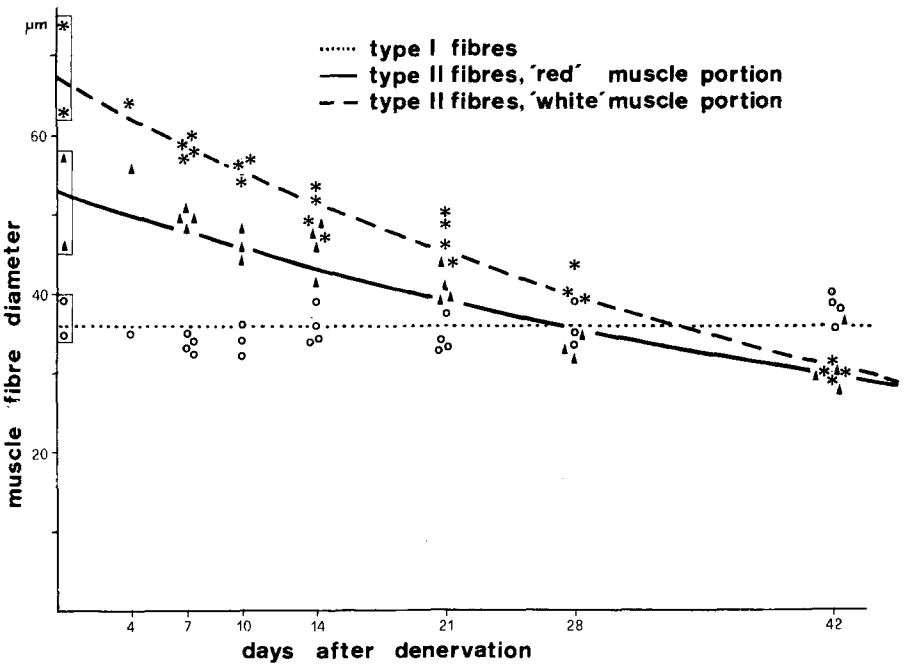
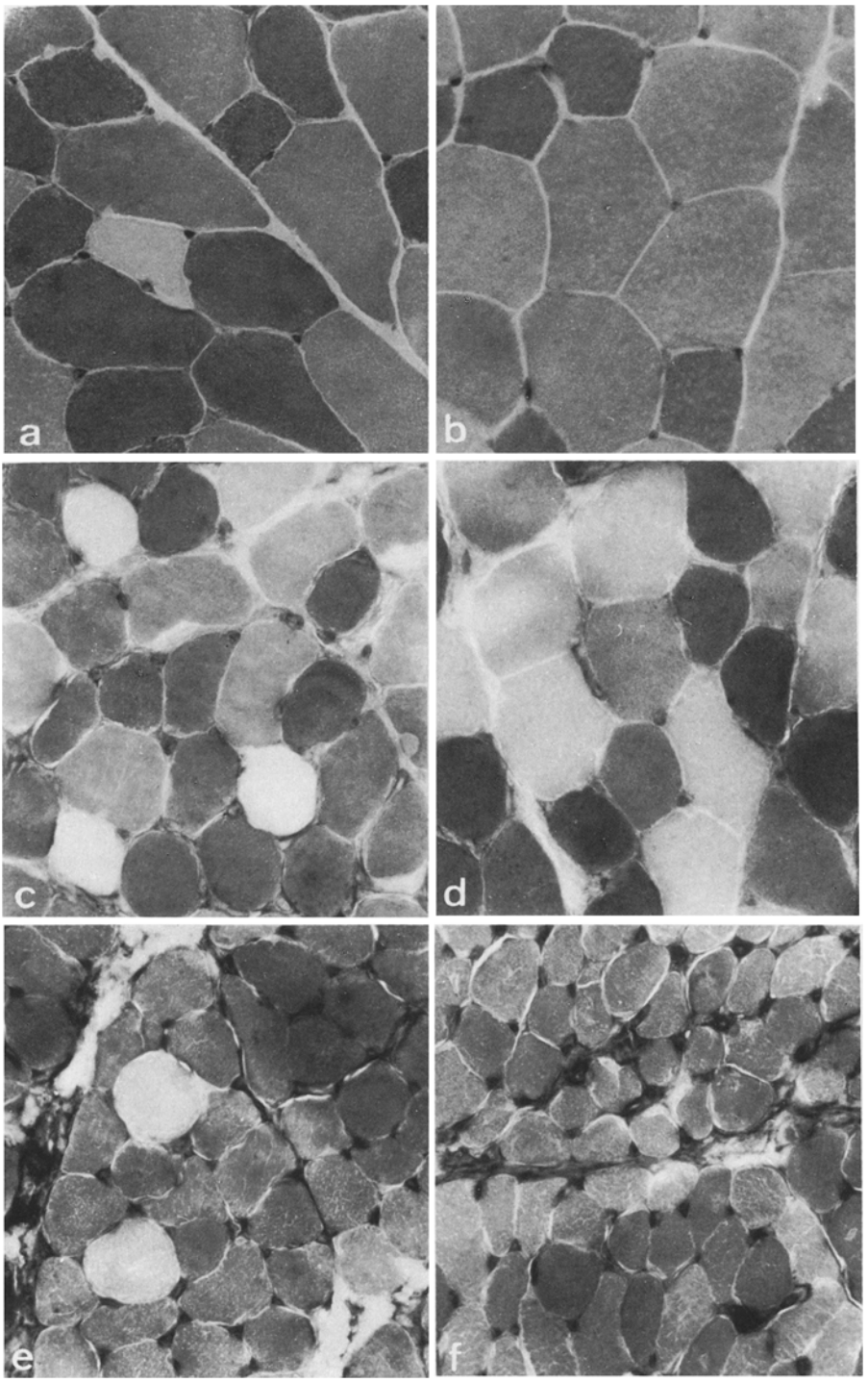



**Fig. 3.** Correlation between fibre diameters and denervation time of type I and type II fibres of rat extensor digitorum longus muscle up to 42 days after denervation. The mean diameters of type I fibres (circles), of type II fibres in the red muscle portion (triangles), and of type II fibres in the white muscle portion (asterisks) have been mapped for each experimental animal. The three bars at the ordinate indicate the variation of the corresponding diameter means of all contralateral control muscles. Up to 42 days after denervation, type I fibres do not undergo any significant diameter changes; in contrast to this, highly significant regression lines were found for the relation between denervation time and diameter decrease of type II fibres (type II fibres of red muscle portion: continuous line $y = e^{3.9616-0.0133x}$, **p** of correlation coeff. $r < 0.01$; type II fibres of white muscle portion: dashed line $y = e^{4.2056-0.0185x}$, **p** of correlation coeff. $r < 0.01$)

In type I fibres no correlation between fibre size and denervation time could be determined, whereas the diameter decrease of the type II fibres of both the red and the white muscle portions has proved to be significantly correlated to denervation time ($P < 0.01$).

At stages of 80 and 120 days after denervation, at which period type I and type II fibres could no longer be readily distinguished, the mean fibre diameters had diminished to 26.9 and 18.1 $\mu$m resp. (Table 3, Figs. 2f, 5). These values are far lower than that of the smallest fibre population of the contralateral control muscles, the type I fibres (Table 3). Differences in fibre size between the red and white muscle portion, so clearly demonstrable in the control muscles, are no longer to be found 80 and 120 days after denervation. However, the occurrence of muscle fibre bundles exhibiting a lesser degree of atrophy than seen in the neighbouring bundles has to be mentioned.

In conformity with earlier observations (Bajusz, 1964; Riley and Allin, 1973; Wuerker and Bodley, 1973), muscle fibres lost their normal polygonal shape in the denervated muscles (Fig. 4). Although this tendency to rounded cross-sectional profiles was recognized in both fibre types, it was more prominent in the type I fibres.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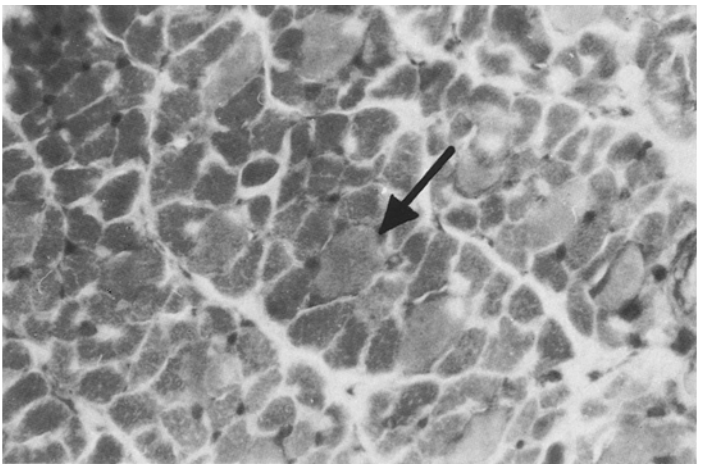

**Fig. 5.** Rat extensor digitorum longus muscle 120 days after denervation, myofibrillar ATPase after formaldehyde fixation and alkaline preincubation, ×300. Between the highly atrophic muscle fibres there still exist some fibres with slightly larger diameters (arrow)

**Table 3.** Diameters of type I and type II fibres of rat extensor digitorum longus muscles 4 to 120 days after denervation. Means and standard errors of the means (in μm) are given for denervated muscles (denerv.) and compared to those of innervated contralateral muscles (control); $n$ = number of experimental animals

| Days after denerv. | $n$ | Type I fibres | | Type II fibres Red muscle portion | | Type II fibres White muscle portion | |
|---|---|---|---|---|---|---|---|
| | | denerv. μm | control μm | denerv. μm | control μm | denerv. μm | control μm |
| 4 | 1 | 34.8 | 34.2 | 55.8 | 45.0 | 64.1 | 68.2 |
| 7 | 4 | 34.3 ± 0.5 | 37.1 ± 0.9 | 49.2 ± 0.4 | 55.3 ± 1.0 | 58.7 ± 0.6 | 68.3 ± 0.8 |
| 10 | 3 | 33.9 ± 1.1 | 36.2 ± 1.3 | 46.1 ± 1.1 | 50.1 ± 1.6 | 55.8 ± 0.9 | 66.1 ± 1.6 |
| 14 | 4 | 35.5 ± 1.2 | 36.8 ± 0.5 | 55.4 ± 1.6 | 54.4 ± 0.1 | 50.3 ± 1.3 | 65.6 ± 1.4 |
| 21 | 4 | 34.6 ± 0.6 | 37.1 ± 1.2 | 40.6 ± 1.1 | 51.7 ± 1.3 | 47.4 ± 1.4 | 68.7 ± 2.9 |
| 28 | 3 | 35.8 ± 1.7 | 39.4 ± 0.6 | 33.4 ± 0.8 | 49.3 ± 1.6 | 41.1 ± 1.5 | 67.5 ± 4.2 |
| 42 | 4 | 37.7 ± 0.9 | 37.5 ± 1.8 | 30.5 ± 1.8 | 50.6 ± 0.4 | 30.1 ± 0.4 | 64.8 ± 0.7 |
| 80 | 3 | 26.9 ± 0.8[a] | 36.6 ± 0.6 | 26.9 ± 0.8[a] | 54.7 ± 0.4 | 26.9 ± 0.8[a] | 68.2 ± 1.5 |
| 120 | 3 | 18.1 ± 0.3[a] | 38.4 ± 1.5 | 18.1 ± 0.3[a] | 52.1 ± 0.7 | 18.1 ± 0.3[a] | 65.2 ± 1.8 |

[a] Fibre types and muscle portions no longer separable

**Fig. 4a–f.** Rat extensor digitorum longus muscle, myofibrillar ATPase after formaldehyde fixation and alkaline preincubation, ×300. **a)** Red and **b)** white portion of contralateral control muscle. **c)** Red and **d)** white muscle portion 21 days after denervation. **e)** Red and **f)** white muscle portion 42 days after denervation

## Discussion

### *The Normal Extensor Digitorum Longus Muscle*

The extensor digitorum longus muscle of the rat, like many other skeletal muscles, is composed of three histochemical muscle fibre types (Schiaffino et al., 1970; Edgerton and Simpson, 1971; Ariano et al., 1973; Pullen, 1977). For the denomination of these fibre types we used the widely applied classification of Brooke and Kaiser (1970) into type I, IIA, IIB (and IIC) fibres. Concerning the correlation of these fibre types with other classifications see Table 1. In this context it has to be emphasized that Brooke and Kaiser subdivided their type II fibres into IIA, IIB (and IIC) fibres on the basis of the pH lability of their ATPase after acid preincubation. Our observations are primarily based on sections stained for myofibrillar ATPase activity after formaldehyde fixation and alkaline preincubation, permitting the discrimination of type I fibres as well as of formaldehyde resistant and formaldehyde sensitive type II fibres in one and the same section. However, appropriate serial sections gave clear evidence that the IIA fibres as defined by Brooke and Kaiser are homologous to formaldehyde resistant type II fibres, and that IIB fibres correspond to formaldehyde sensitive type II fibres. Therefore the application of the Brooke and Kaiser nomenclature to our material seems to be justified. Lately also Tunell and Hart (1977) have reported that, after pretreatment with glycine–formaldehyde–calcium prior to a routine ATPase technique, type I, IIA, and IIB fibres can be readily differentiated in the same section.

The distribution of the three muscle fibre types within the normal extensor digitorum longus muscle of the rat and the diameters of these fibre types show considerable variations in different regions of the cross-section. For this reason, the standardization of the sampling areas was a prerequisite for reliable studies on the time course of denervation atrophy of type I and type II fibres. For comparison with the denervated muscle, the percentage of the three fibre types and their mean diameters in these sampling areas had first to be established for the control muscle of the contralateral side. Data on the proportions of the three fibre types of rat extensor digitorum longus muscle in the literature (Schiaffino et al., 1970; Edgerton and Simpson, 1971; Ariano et al., 1973; Pullen, 1977), except those of Pullen, do not pay regard to the regional variations, and are hardly comparable with each other because of different technical approaches. They proved to be unsatisfactory for comparison with our experimental data.

In rat extensor digitorum longus muscle two muscle portions can be distinguished by differences in muscle fibre composition, a "white" muscle portion with a predominance of type A resp. IIB fibres exhibiting low mitochondria content, and a "red" muscle portion characterized by the predominance of the C resp. IIA fibres with their high mitochondria content and by the presence of the rather small number of type B resp. I fibres, the latter being also rich in mitochondria and exclusively restricted to the red muscle portion. The red muscle portion as defined by the presence of type I fibres, comprises the whole cross-sectional area in the most proximal parts of the muscle; however, at more distal levels it is confined to more and more superficial and lateral areas of the cross-section. The reverse is true for the white muscle portion: near the proximal end of the muscle it begins in the deep medial region of the muscle and, gaining continuously in width at more distal levels, comprises the whole cross-sectional area near the distal muscle end. Emphasizing the superficial and lateral position of the red muscle portion, we consider the

**Table 4.** Percentage of the three fibre types of extensor digitorum longus muscle on total cross-section. Data based on ATPase incubated sections

| | I | IIA | IIB |
|---|---|---|---|
| Ariano et al., 1973 | 3% | 59% | 38% |
| Pullen, 1977 | 9.61% | 51.18% | 39.34% |
| Own observations (middle of muscle length) | 2–4% | 50–59% | 35–48% |

contradictory statement of Schiaffino et al. (1970) to be erroneous. Pullen (1977) reported that fibres with low ATPase activity are scattered throughout the whole muscle cross-section. According to our observations this is only true for sections at rather proximal levels within the muscle, at which the red muscle portion still occupies the complete cross-sectional area.

Concerning the proportions of the three fibre types in the whole cross-section, there is faily good correspondence between the data of Ariano et al. (1973), of Pullen (1977), and our own observations (Table 4). Pullen's remarkably higher percentage of type I fibres could again be explained by the evaluation of sections from more proximal levels.

### *The Denervated Extensor Digitorum Longus Muscle*

The present study of the course of denervation atrophy in rat extensor digitorum longus muscle showed that the size of type I fibres remained the same under the given experimental conditions at least up to 42 days after denervation. In contrast to this, the type II fibres exhibited an early beginning and progressively advancing atrophy. As a result of this, the type I fibres, originally the smallest fibres of the muscle, had became the largest at 42 days after denervation. Only at later stages were type I fibres also affected by atrophy.

Data in the literature on the denervation behaviour of type I fibres or the corresponding fibres of other classifications differ widely. Our findings are in good agreement with those of Bajusz (1964) who stated, that the diameters of the "red" granular muscle fibres of rat and mouse triceps surae muscles remain approximately the same for a long time after denervation. Several authors (Engel et al., 1966; Wuerker and Bodley, 1973; Melichna and Gutmann, 1974) described a so called "preferential type II fibre atrophy" after denervation; this implies that type I fibres are also affected by atrophy, although to a much lower degree than type II fibres. Romanul and Hogan (1965) even stated that all fibre types of denervated rat gastrocnemius and soleus muscles atrophied to the same extent. According to our own observations type I fibres of rat extensor digitorum longus muscle are affected by denervation atrophy only at rather late stages, at which they could no longer be distinguished from other fibres. Thus different results may be due to evaluation at different times after denervation.

Moreover, type I fibres need not necessarily show the same denervation behaviour in different muscles. This is supported by observations of Tomanek and Lund (1973) and of Jaweed et al. (1975), who found type I fibre atrophy to be less pronounced in fast muscles than in slow muscles. According to Guth et al. (1971) type I fibres even in different portions of one and the same muscle can exhibit

different behaviour after denervation: they found an equal extent of atrophy in all muscle fibres of the superficial portion of cat gastrocnemius muscle, whereas in the deep portion of the same muscle type I fibres were less atrophied than type II fibres. The reasons for these differences of behaviour are unclear.

The more or less pronounced resistance of type I fibres to denervation atrophy may be due to a minor degree of neurotrophic dependence of these fibres, as first suggested by Bajusz (1964). This suggestion, among other things, is also supported by the observation that during development type I fibres, in contrast to type II fibres, are able to attain a high degree of differentiation in the absence of nerve fibres (Engel and Karpati, 1968).

However, the course and extent of denervation changes could also be influenced by other circumstances. In this context, the age of the experimental animals has to be taken into consideration (Kumar and Talesara, 1977), and, above all, attention has to be paid to the passive stretch of denervated muscles by their innervated antagonists, which tends to reduce the denervation rate. The latter factor can be eliminated by tenotomy of the denervated muscles or by simultaneous denervation of the antagonists. It has to be emphasized that neither tenotomy nor denervation of the antagonists have been performed during the present study; the denervated muscles have therefore been under passive stretch. Further experiments are needed to answer the question of whether elimination of this stretch may lead to an earlier onset of type I fibre atrophy.

Diameter measurements have not been performed separately for IIA and IIB fibres after denervation, nevertheless our data present indirect evidence that IIB fibres show a higher rate of atrophy than IIA fibres. The diameters of both the originally larger type II fibres of the white muscle portion with their predominance of the thicker IIB fibres and the originally smaller type II fibres of the red muscle portion with IIA fibres predominating attained equal final values 42 days or later after denervation (Fig. 3). This is in agreement with observations of Riley and Allin (1973), who found a more pronounced atrophy of the white muscle fibres (corresponding to IIB fibres) in denervated cat tail muscle.

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*Received November 21, 1977*