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Effect of neonatal denervation on the distribution of fiber types in a mouse fast-twitch skeletal muscle

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Summary. This study was designed to assess the changes in fiber-type distribution of the extensor digitorum longus (EDL) muscle of the mouse during the first 21 days of age following neonatal sciatic neurectomy. Denervated and normal muscles were compared at 7, 14, and 21 days of age and the normal EDL was also studied at 1 day of age. Frozen sections of the EDL were treated histochemically to detect NADH-tetrazolium reductase and myosin ATPase reactions. Quantitative assessment included measurements of cross-sectional areas and fiber counting. Denervation resulted in muscle atrophy which was due primarily to a decrease in individual fiber area as opposed to fiber loss. Histochemical maturation of the EDL was severely affected by neonatal denervation during the first three postnatal weeks. By 21 days, two extrafusal fiber types which were both oxidative could be distinguished. One type was highly atrophied and resembled an immature fiber exhibiting myosin ATPase staining at both acid and alkaline preincubation conditions, whereas another type was less atrophied and showed myosin ATPase staining resembling fast-twitch (type IIA) fibers. These findings emphasize the importance of an intact nerve supply in determining the phenotypic expression of skeletal muscle, and point to the early postnatal period as a critical stage in fiber type differentiation.

Introduction

Immature skeletal muscles are more dependent on continued innervation for maintenance of their structural integrity than mature muscles (Kumar and Talesara 1977). When deprived of neural influence at an early stage in differentiation into either fast or slow twitch muscle, there is an impairment of morphological (Engel and Karpati 1968), ultrastructural (Shafiq et al. 1972; Schiaffino and Settembrini 1970; Hanzlikova and Schiaffino 1973) and histochemical (Shafiq et al. 1972; Dhoot and Perry 1983) maturation. In fast-twitch muscle, this impairment has been found predominantly in the fast-twitch extrafusal fibers. This correlates with alterations of contractile properties of fast-twitch muscle in which speeding of contraction time with development does not occur following neonatal denervation (Brown 1973). Even though the expression of mature contractile and regulatory protein isozyme patterns occurs following neonatal denervation (Rubinstein et al. 1985; Butler-Browne et al. 1982), the time course of the development of these parameters has been reported to be expressed more slowly than in normal muscle (Butler-Browne et al. 1982).

In order to ascertain whether neural influence is essential to normal fiber type expression of fast-twitch skeletal muscle, we have examined the fiber type distribution following neonatal denervation of fast-twitch extensor digitorum longus muscles (EDL) during the first 3 weeks of postnatal development. The distribution of fiber type profiles in these muscles compared to age-matched normal mice suggests that early denervation results in a predominance of immature fibers and a failure of the normal fiber type differentiation. A preliminary report has been published (Redenbach and Bressler 1985).

Materials and methods

All experiments were carried out on the fast-twitch extensor digitorum longus muscle (EDL) of the C57BL/6J strain of mouse. Animals were bred and raised in our own colony from breeding pairs originally obtained from Jackson Laboratories, Bar Harbor, Maine. For each denervated group, pups from time-mated breeding pairs underwent right sciatic neurectomy at 32 ± 8 h of age. Four denervated and 4 control EDL muscles were examined at each of 7, 14 and 21 days of age. In addition, one group of four normal EDL muscles was examined from unoperated animals at 1 day of age to characterize the muscle at a time coinciding with the time of denervation.

Denervation. Pups were anaesthetized with ether and placed on their left side with their right leg supported in a plasticine splint. A skin incision was made along the lateral compartment of the thigh, the underlying fascia divided, and the biceps femoris was retracted to expose the sciatic nerve. The nerve was then carefully retracted with fine forceps and excised from the level of the greater trochanter to beyond its bifurcation into the posterior tibial and common peroneal nerves at the knee. The animal was returned to its mother and allowed to recover. All animals used in the denervated group demonstrated positive signs of denervation including paralysis, dragging of the right foot, and complete loss of active movement at the ankle and toes. Preliminary experiments were undertaken, in which normal and denervated EDL muscles were dually stained with silver to identify nerve and with a method to detect acetycholinesterase activity at myoneural junctions (Goshgarian 1977). Lack of nerve-endplate complexes in all denervated samples at 7, 14 and 21 days of age indicated absence of reinnervation up to 21 days following this denervation protocol.

In spite of the flaccid paralysis of the right leg, pups developed an efficient gait, consisting of passive extension of the hip and knee followed by an abduction-adduction maneuver to bring the

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denervated limb beside the contralateral limb. The denervated limb was therefore held in the same posture as the normal limb except during ambulation. Compensatory muscle hypertrophy of the contralateral limb was likely and therefore, muscle from this side was not used as a control.

Histochemical and quantitative procedures. Each animal was killed with chloroform, and its right leg was removed and pinned to a cork board. The leg was skinned, the crural fascia excised and the overlying tibialis anterior muscle removed, exposing the EDL. The proximal and distal tendons were cut and the EDL was embedded in a block of mouse liver. The block was then mounted in gum tragacanth on a cork chuck with the muscle orientated perpendicular to the chuck in order to obtain cross-sections. The sample was frozen in isopentane, which had been cooled to −160° C in liquid nitrogen, and immediately placed in a cryostat cabinet at −20° C for 1 h. Serial sections of 10 μm thickness were taken from the midbelly of the muscle, collected on glass coverslips and air dried for one hour. Adjacent sections were either stained with Haematoxylin and Eosin, treated to detect NADH Tetrazolium Reductase (NADH-TR) or treated to detect myosin ATPase reactions, using the method of Dubowitz and Brooke (1973). Age and species differences require adjustments of time, temperature and pH for optimal identification of fiber types (Gollnick et al. 1983). Preliminary work in our laboratory indicated that while type IIA, IIB and I fibers could be distinguished by preincubations of pH 4.35, pH 4.6 and pH 9.4 on adult mouse EDL, type IIA and IIB fibers both showed moderate staining at pH 4.6 and a preincubation of pH 4.2 was required to distinguish between type IIA and IIB in the immature muscle. For this reason, myosin ATPase reaction times were modified from those of Dubowitz and Brooke, as follows: pH 4.2, 2.5 min and 40 min, pH 4.6, 5 min and 40 min, and pH 9.4, 15 min and 5 min pre-incubation and incubation times, respectively. For the myosin ATPase reaction in the normal EDL, extrafusal fiber typing was done according to the nomenclature of Brooke and Kaiser (1970).

Using a Zeiss photomicroscope, overlapping fields of a whole muscle cross section that had been stained for myosin ATPase (pH 4.2), from 4 muscles of each group ($n=28$), were photographed at a magnification of 160 X. Montages were assembled from the prints at a final magnification of 800 X, and direct counts were made of each extrafusal fiber type. Fiber typing was therefore done from the pH 4.2 Myosin ATPase staining after preliminary work showed that the three types could be distinguished at that pH. Myosin ATPase staining of serially sectioned fibers at pH 9.4 was compared with that at pH 4.2 to check for reversibility of staining. The population of each fiber type was expressed as a percent of the total fiber number. In addition, the total number of extrafusal fibers in each muscle was calculated. The unpaired Student's *t*-test was used for all groups compared. The difference between group means was considered significant at $p \leq 0.05$.

To assess the extent of atrophy following denervation, a group of 4 normal and 4 denervated EDL was examined at 21 days of age. Quantitative comparisons were made of whole muscle cross-sectional areas and individual fiber cross-sectional areas in these muscles.

Whole muscle cross-sections were traced onto the digitizing board of a Zeiss Mop 3 Image Analyzer. Using a Leitz bright field microscope, fitted with a camera lucida drawing attachment, calibrated by means of a micrometer slide and checked against a known area, a whole muscle section was projected onto the digital pad of the image analyzer. Whole muscle cross-sectional areas were measured using the average of three readings for each muscle. From the same samples, individual myofiber cross-sectional areas were similarly measured using systematic sampling (Weibel 1979) of all cells that fell on a line predefined as that which passed at right angles to and through the mid point of the greatest diameter of the muscle. The magnification for tracing whole muscles was X 100 and for individual fiber cross-sections was X 400. Group means of whole muscle cross-sectional areas of normal and denervated muscles at 21 days were compared using unpaired Student's

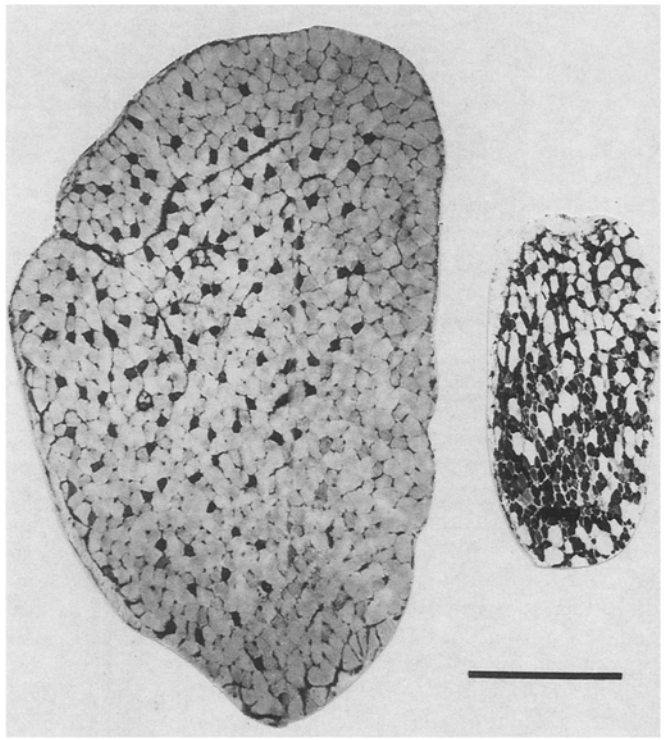

Fig. 1. Whole muscle cross-sections of normal (*left*) and denervated (*right*) EDL at 21 days of age, stained for myosin ATPase at pH 4.2. *Bar* = 250 μm

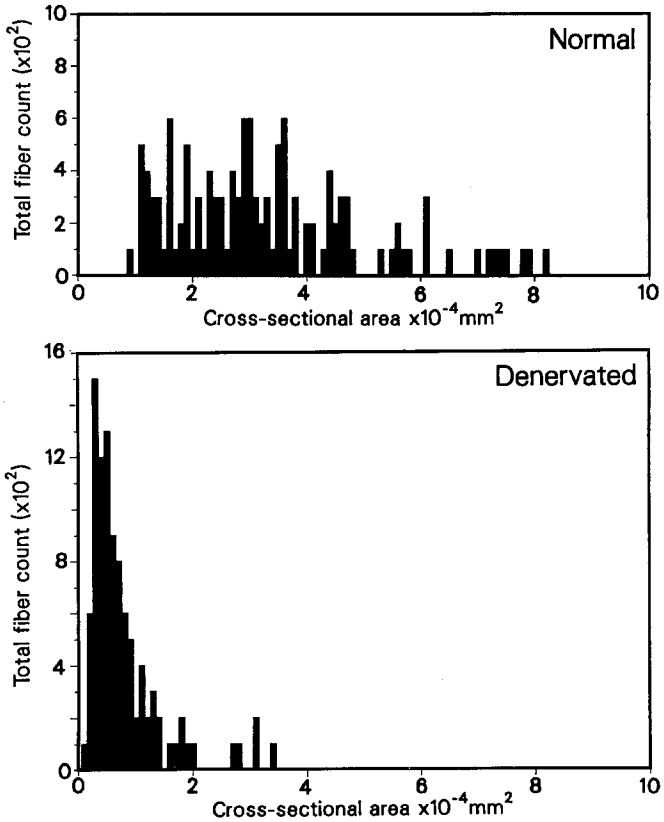

Fig. 2. Cross-sectional area of individual muscle fibers from normal ($n=4$) and denervated ($n=4$) EDL at 21 days of age

t-test. Results from measurements of individual fibers from 4 normal and 4 denervated EDL at 21 days of age were collapsed to groups of 10 μm^2 and the frequency distribution was plotted for each group.

Results

Cross-sectional areas

In Fig. 1 it may be seen that the denervated EDL exhibited a marked degree of atrophy compared with its normal counterpart by 21 days of age. In a comparison of eight muscles at this age (4 normal and 4 denervated) there was a significant difference in the mean whole muscle cross-sectional area which were $0.55 \pm 0.017\ mm^2$ and $0.173 \pm 0.063\ mm^2$ for the normal and denervated muscles, respectively. In addition, the cross-sectional areas of individual fibers from these muscles demonstrated a clustering of fibers in the denervated muscle with a cross-sectional area range of 0.1 to $1.0 \times 10^{-4}\ mm^2$ (Fig. 2). The control muscle, however, exhibited a wide distribution of fibers whose cross-sectional area ranged from 1.0 to $8.0 \times 10^{-4}\ mm^2$.

Total fiber number

In unoperated animals, mean values of the total fiber number in the EDL did not change significantly between 1 day and 21 days of age (Fig. 3). Moreover, denervation did not result in a significant decrease in fiber number (by 7, 14, or 21 days) compared with controls, at 7, 14 or 21 days. However, the denervated EDL at 7 and 21 days of age showed a small but significant decrease in fiber number compared with those at 1 of age.

Fiber type distribution

The histochemical profile from serial sections of the normal EDL at 1 day of age is shown in Fig. 4. In the normal EDL there was moderate and uniform staining of all fibers for oxidative enzyme activity using the NADH-TR reaction (Fig. 4B). All extrafusal fibers showed moderate staining with the myosin ATPase reaction after both alkaline (Fig. 4C) and acid (Fig. 4D) preincubation. At pH 9.4, all fibers showed moderate staining and no pale fibers characteristic of those seen in mature muscle were seen. However, at pH 4.2, two fiber types were discernible: medium staining fibers or presumptive type II (IIp) and dark staining fibers or presumptive type I (Ip).

NADH-TR and myosin ATPase (pH 4.2) staining profiles in the normal EDL at 7 days of age are shown in Fig. 5A and C. At this age all extrafusal fibers remained moderately oxidative and could not be distinguished into fiber types with NADH-TR. However, at this age it was possible to distinguish two groups of type II fibers at pH 4.2, pale staining IIA_{ox} and medium staining IIB. The dark-staining fibers (type Ip) were again seen at pH 4.2 and did not show a reversal of staining at pH 9.4. Type I and Type II fibers all stained alike at pH 9.4 at 7 days of age.

By 14 days of age, the distribution of fiber types was the same as that seen at 21 days. Serial sections of muscle stained for NADH-TR at 21 days and for myosin ATPase at pH 4.2 and 9.4 are shown in Fig. 6. In the normal EDL, three fiber types could be seen at acid preincubation

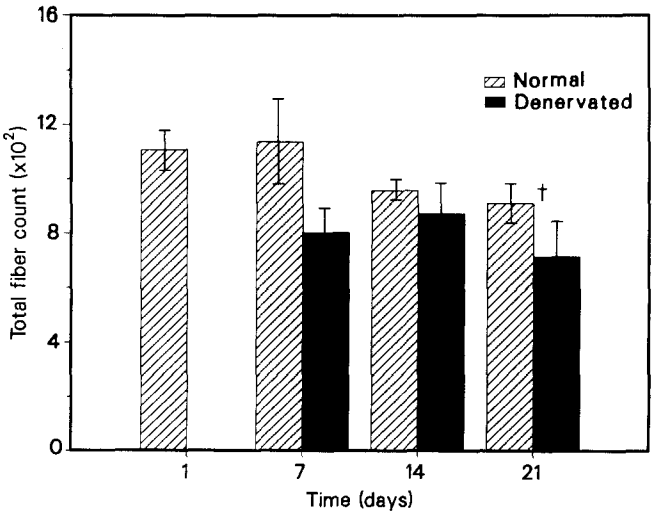

Fig. 3. Total fiber number of normal (*hatched bars*) and denervated (*solid bars*) EDL during the first 21 days postnatal. The dagger indicates a significant difference at $p \leq 0.05$ between 1 day normal and 21 days of age

(pH 4.2): small, dark-staining fibers (I), medium to pale-staining fibers (IIB) and pale-staining fibers (IIA). The type I fibers could be further subdivided into those which continued to stain after alkaline preincubation (type Ip), as seen at 1 and 7 days, and those which were now pale-staining (type I) due to alkaline instability (Fig. 6A). The pale staining fibers at pH 4.2 (type IIA) now showed reduced oxidative enzyme reaction with NADH-TR lacking a subsarcolemmal accumulation of formazan particles while the medium to pale-staining fibers (type IIB) were more intensely oxidative (Fig. 6C).

In the denervated muscles, minimal changes were seen at 7 days of age (Fig. 5). The fiber-type profile remained unchanged with the proportion of fibers in each group similar to that in the normal 7 day EDL. The type IIA_{ox} fibers stained less intensely for myosin ATPase at pH 4.2 in the denervated than in the normal muscles, however, this varied in degree between denervated samples. All fibers in the denervated muscles remained moderate to highly oxidative. There was a central clumping of formazan particles in some fibers, in the NADH-TR reaction which did not show preference for any particular fiber type. Using the myosin ATPase reaction (pH 4.2) at 14 and 21 days, it was no longer possible to distinguish fiber groups in denervated muscle using the normal criteria. A group of larger, pale-staining fibers could be distinguished from the remaining dark staining-fibers. These pale-staining fibers were equivalent in staining to the IIA fibers found in the normal EDL, however they were highly oxidative judged by NADH-TR reaction were therefore designated type IIA_{ox}. The remaining fibers (atypical) were heterogeneous with respect to size and stain, albeit variably, at both acid and alkaline preincubation for myosin ATPase. Up to 21 days, all fibers in the denervated muscle demonstrated intense staining with NADH-TR (Fig. 6F).

The mean distribution of fiber types expressed as a percent of total fiber number in normal EDL at 1, 7, 14 and 21 days of age is shown in Fig. 7 and in denervated EDL at 7, 14 and 21 days of age in Fig. 8. No significant difference was found in the distribution of fiber types between

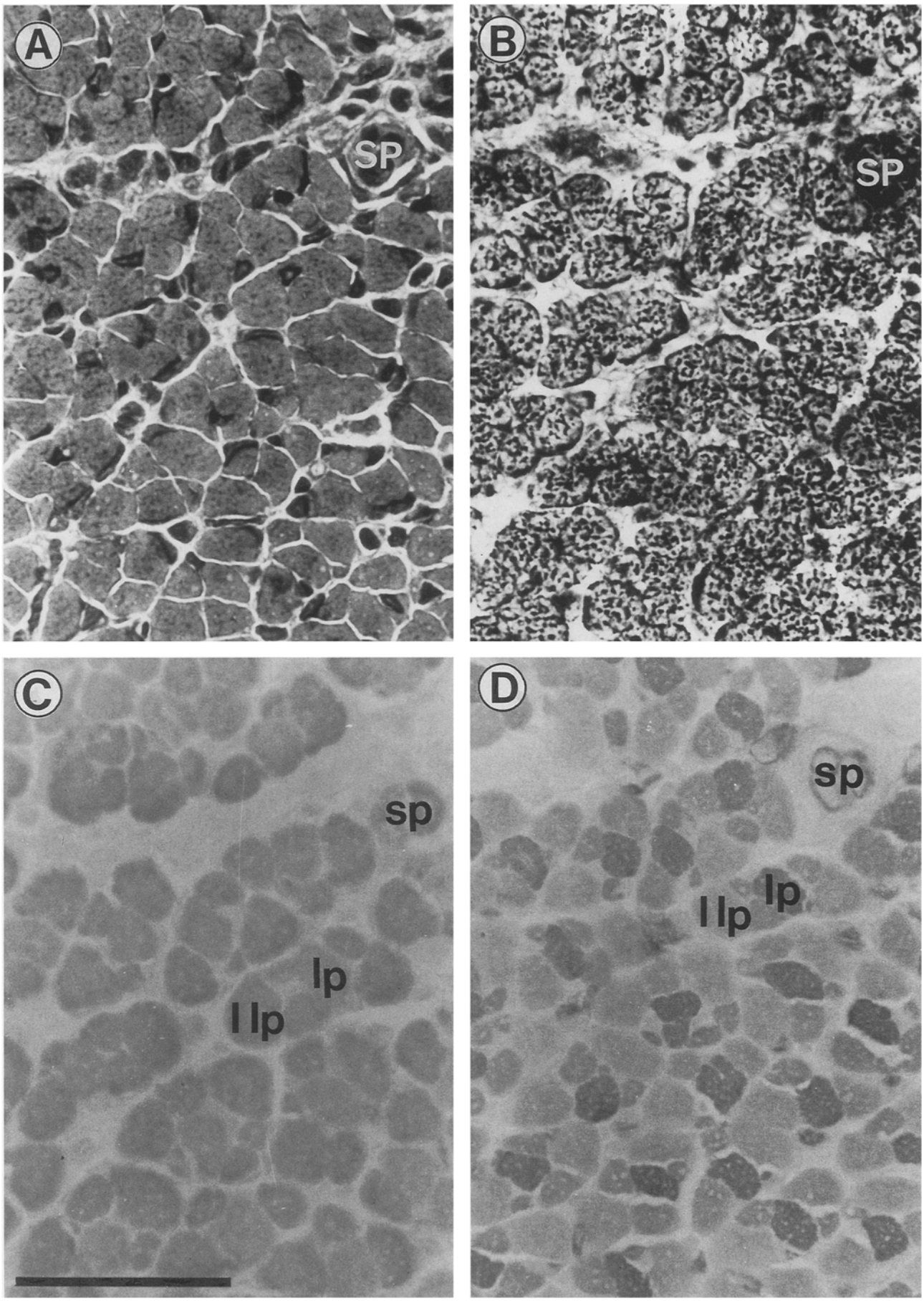

Fig. 4. Histochemical profile of normal EDL at one day of age. Serial sections stained for H & E (**A**), NADH-TR (**B**) and myosin ATPase at pH 9.4 (**C**), and 4.2 (**D**). *Bar* = 50 μm for all sections in this figure. A muscle spindle (*SP*) has been marked for orientation

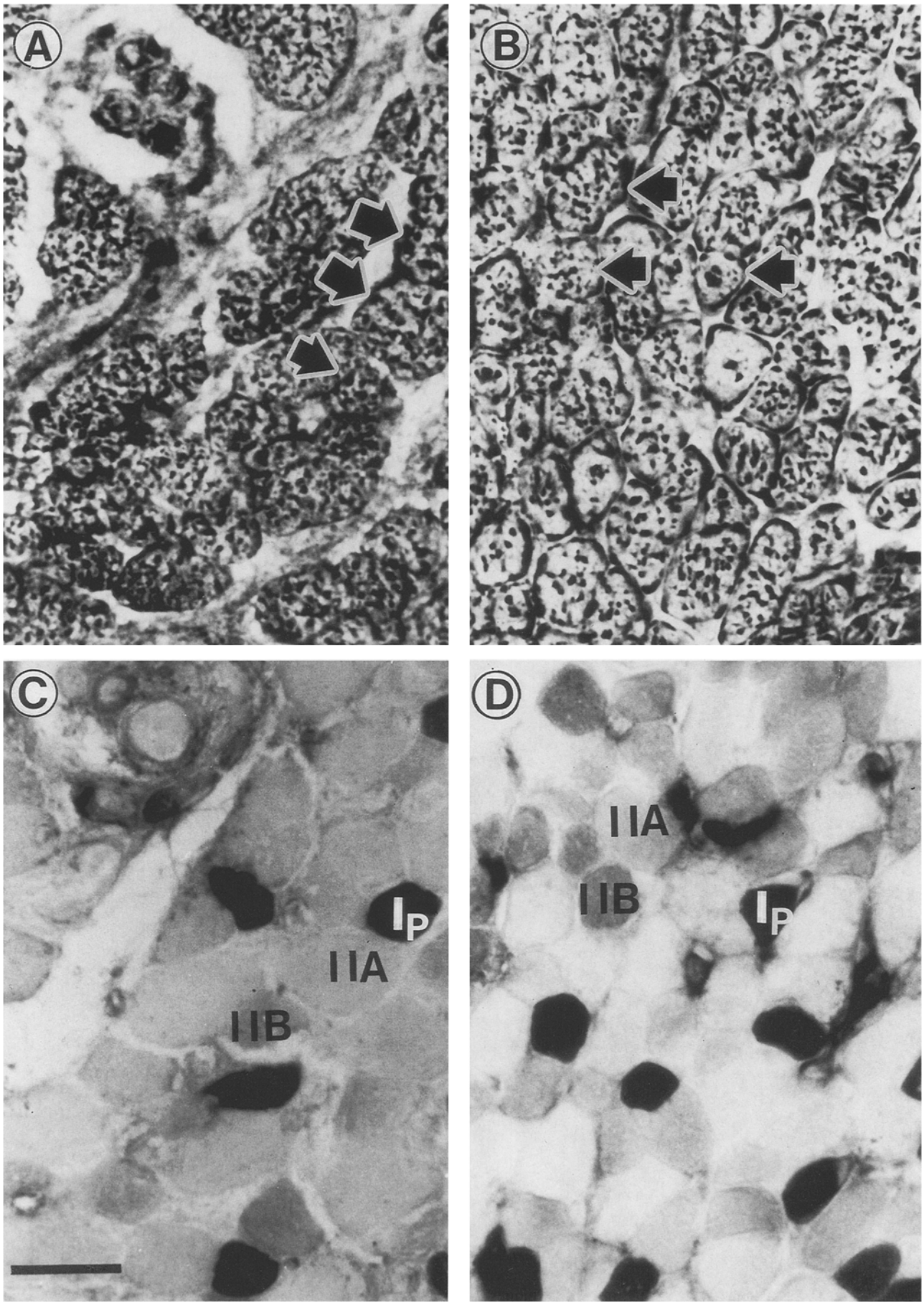

Fig. 5. Oxidative enzyme reaction (**A, B**) and myosin ATPase staining (**C, D**) of normal (**A, C**) and denervated (**B, D**) EDL at seven days of age. *Arrows* on **A** and **B** indicate corresponding fibers identified in **C** and **D**. *Bar* = 50 μm for all sections in this figure

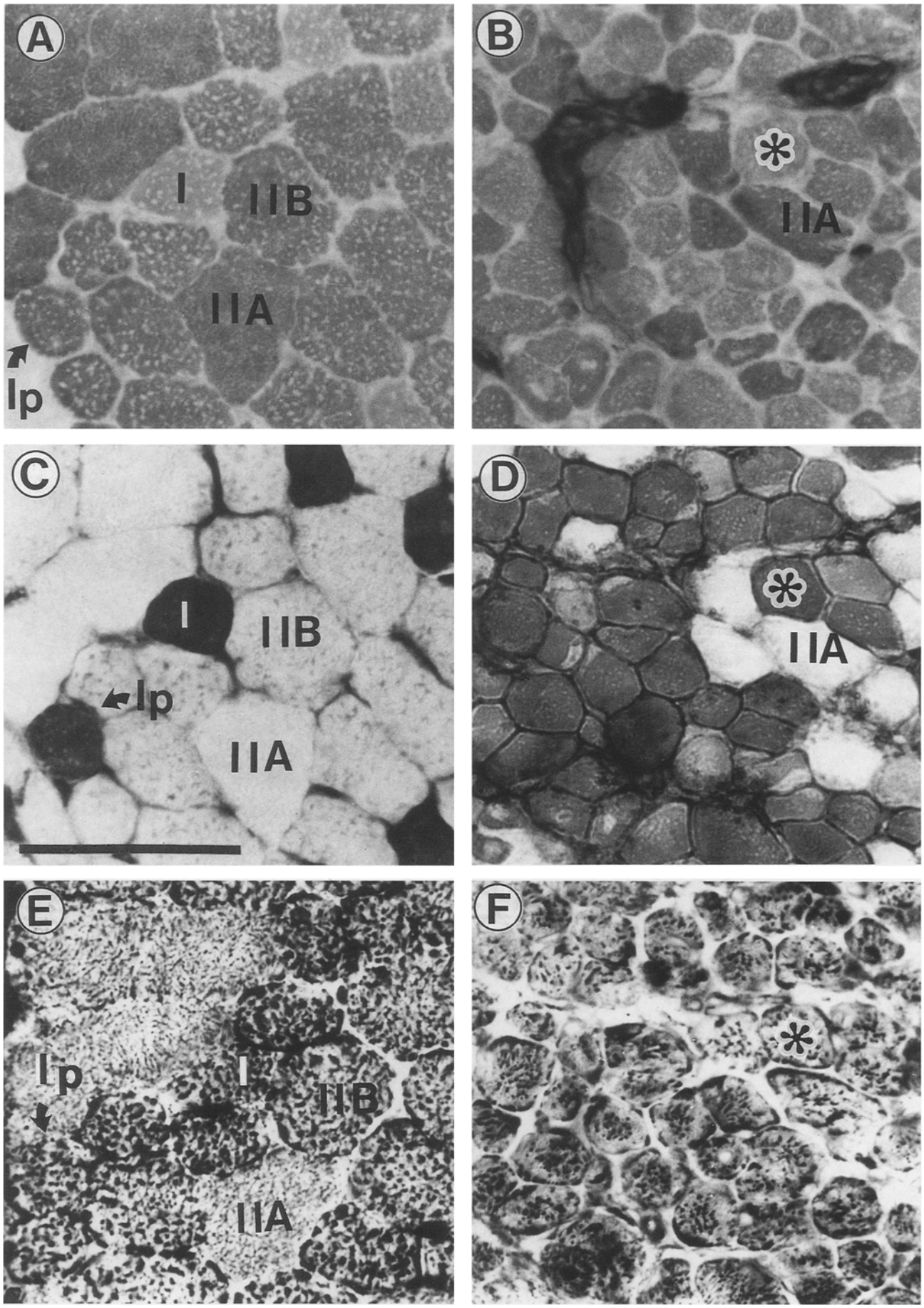

Fig. 6. Histochemical profile of normal and denervated EDL at 21 days of age. Normal EDL (*left panels*) and denervated EDL (*right panels*). Myosin ATPase pH 9.4 (**A, B**), myosin ATPase pH 4.2 (**C, D**), NADH-TR (**E, F**). *Asterisks* indicate atypical fiber. *Bar* = 50 μm for all sections in this figure

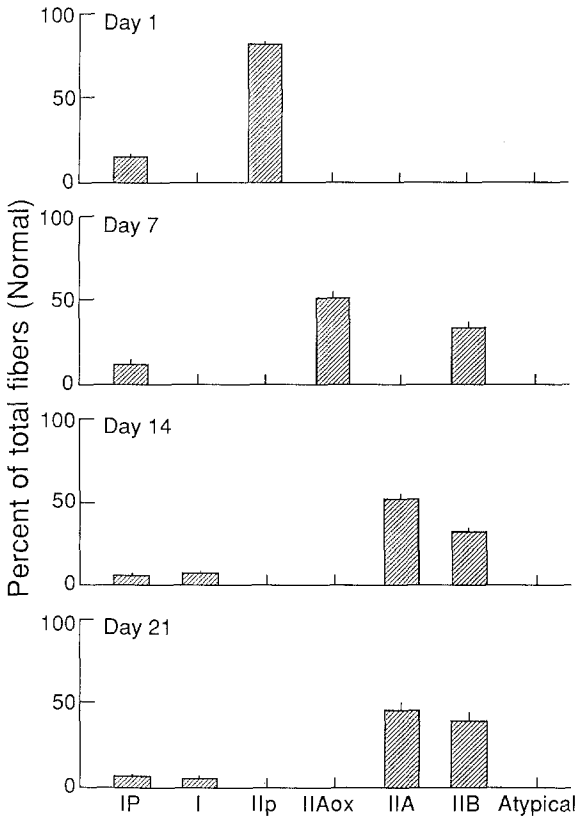

Fig. 7. Fiber type distribution of normal EDL at 1, 7, 14 and 21 days of age. All fibers were individually typed for the myosin ATPase reaction

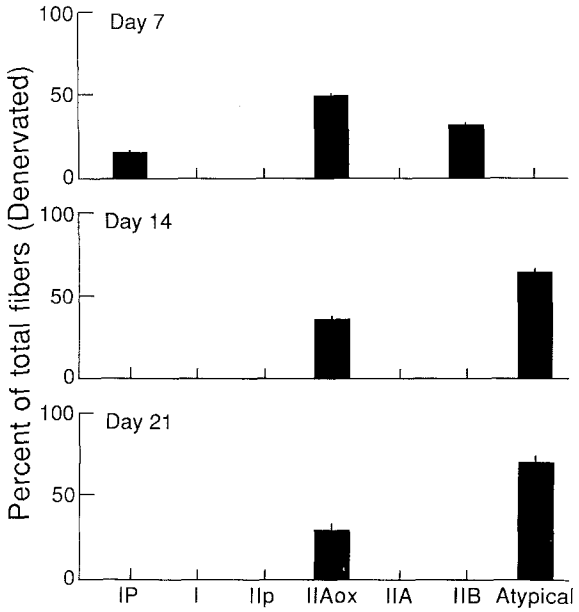

Fig. 8. Fiber type distribution of neonatally denervated EDL at 7, 14 and 21 days of age

normal and denervated muscles at 7 days of age. However, by 14 days of age a difference in pattern emerges between normal and denervated muscle which persists up to 21 days of age. In normal muscle at 7, 14 and 21 days and denervated muscle at 7 days, the proportion of combined fiber types I and Ip to total fiber does not change significantly from the one day values. The oxidative staining patterns of muscle fibers in the normal EDL coincided generally with the myosin ATPase fiber typing in the following way: I/SO IIA/FG IIB/FOG, using the nomenclature based on oxidative fiber typing of Peter et al. (1972). The type IIA fibers did show some variation in intensity in their oxidative staining, but they were less oxidative than type IIB and type I fibers. This was not the case with denervated muscles which showed no fiber type differentiation of oxidative enzyme staining throughout the time period studied.

Discussion

The maturational changes in histochemical and morphological parameters during the first 3 weeks postnatally in the mouse fast-twitch EDL, are severely affected by denervation at 1 day of age. In this study, no postnatal change in fiber number was noted during this early period of development in the normal mouse EDL. This is in agreement with the work of Goldspink (1980), in the normal mouse. Although there was no significant difference in fiber number between the normal and denervated EDL at 21 days of age, total fiber number in the denervated EDL was significantly decreased by 21 days when compared to that in the 1 day normal muscle. This reduction could be due to a real fiber loss or to difficulty in adequately distinguishing the small extrafusal fibers. However, in the present study, the reduction of fiber number was not adequate to account for the marked degree of atrophy seen in the denervated muscles.

The results of cross-sectional area measurements of both whole muscles and individual myofibers in this study are consistent with previous reports that denervation produced muscle atrophy (Engel and Stonnington 1974; Gauthier and Hobbs 1982; Webster and Bressler 1985). It has also been shown that muscle atrophy is greater following neonatal denervation than after adult denervation (Kumar and Talesara 1977). Furthermore, we have shown that this atrophy is primarily due to a decrease in individual fiber area as opposed to fiber loss.

Our study shows that the postnatal maturation of the normal mouse EDL occurs as a gradual transition of fiber types with differences betwen type I and type II fibers detectable at birth. There is a decrease in acid stable myosin ATPase activity of the type II fibers over the next 2 postnatal weeks. These fibers progressively stain more lightly in acid, with a decreased number of fibers exhibiting dual staining in both type I and type II groups with age. Muscle fibers of the type IIA and IIB variety can be discriminated at 7 days and by 14 days the difference in staining between the two types is even more distinct.

Ishuira et al. (1981) found that all extrafusal fibers in the EDL and soleus of the newborn rat exhibit uniform and non-reversible myosin-ATPase staining. They referred to them as type IIC fibers. In this study, all extrafusal fibers at 1 day of age showed a characteristic lack of reversibility, by staining at both pH 9.4 and pH 4.2 for myosin ATPase (Fig. 4). However, we designated them as either presumptive type I (Ip) or presumptive type II (IIp) fibers, because these fibers could be further subdivided into two groups at pH 4.2, those which were very dark (Ip) and those which were medium-staining (IIp). The group which was dark-staining with acid preincubation, did not change significant-

ly in percent of the total fiber population throughout the study (see Fig. 7), although an increasing number demonstrated reversibility of staining by 21 days of age. When we followed the myosin ATPase staining intensity throughout this three week period, we noted that the type II immature fibers progressively stained more lightly. Moreover, we found the distinction between IIA and IIb in the adult mouse EDL had to be undertaken at a higher pH. The type IIC fiber described by Brooke and Kaiser (1970) exhibited moderate staining in acid and is likely to correspond to the presumptive type II fibers described here. The oxidative nature of developing fibers was shown by their moderate staining for NADH-TR at 1 and 7 days postnatally.

In the oldest age group studied, the largest muscle fibers were classified as type IIA due to their total inhibition at pH 4.2 and stability at pH 9.4. In addition, these fibers showed minimal staining for NADH-TR, indicating they are anaerobic fibers that correspond to the fast-glycolytic (FG) fiber type (Peter et al. 1972). In our study, a second group of extrafusal fibers that was alkaline stable and acid labile and continued to react at pH 4.2 was classified as type IIB fibers. They stained moderately to intensely for NADH-TR and most likely correspond to the fast oxidative-glycolytic (FOG) fibers of Peter et al. (1972). These findings are consistent with the microphotometric results of Reichmann and Pette (1982) of SDH activity in mouse muscles. The third group of extrafusal fibers was acid stable for myosin ATPase, however, 50% retained alkaline stability (type Ip) with the remaining fibers alkaline labile for myosin ATPase (type I). These fibers were also oxidative in nature according to their intense NADH-TR reaction, similar to the slow oxidative fibers of Peter et al. (1972). Our study shows the histochemical staining pattern of the mouse EDL to be at variance with reported fiber typing profiles of fast-twitch fibers in the rat (Melichna and Gutmann 1974; Niederle and Mayr 1978) where the large diameter anaerobic fibers are type IIB and the small aerobic fibers are IIA.

There is a lack of correlation between commonly used myosin ATPase fiber typing methods that is species dependent and is particularly pronounced in the rat (Green et al. 1982). There is also a lack of consistency between fiber type classifications using different criteria. In the mouse, Reichmann and Pette (1982, 1984) found a wide range of SDH activity measured microphotometrically in fiber types alike according to myosin ATPase. These workers also showed that while the IIA fiber is "oxidative" and the IIB fiber is "glycolytic" in the mouse tibialis anterior, the reverse is the case in rat tibialis anterior. This is in agreement with our findings in which the type IIA fiber exhibits a poorly oxidative staining pattern and most likely corresponds to a large anaerobic white fiber. In addition, differences in content of proteins such as parvalbumin have been reported in fibers from the same muscle which have been classified alike using myosin ATPase reaction (Muntner et al. 1985). We recognize that muscle fibers from the developing mouse cannot be assumed to have properties identical to those of mature animals based on myosin ATPase reaction alone. Whalen et al. (1979, 1981) have suggested that the difference between the dual staining of immature fibers and the reversible staining of mature fibers may correspond to factors influencing the sequential synthesis of fast and slow myosin isoforms during development. Indeed, Staron and Pette (1986) have shown a correlation between myosin ATPase staining and the heavy chain composition in rabbit soleus muscle. Therefore the changes reported here in developing mouse EDL probably reflect changes in the myosin heavy chain composition.

As has been found by others (Ishiura et al. 1981), the typing of denervated fibers in our study does not fit with any of the conventional descriptions of adult extrafusal fibers. One week after denervation, there was an enhanced staining contrast between the type IIA and IIB fibers compared to the normal group, yet muscle fibers from both groups had matured from the one day pattern. They remained uniformly oxidative. By 14 days, a heterogeneous population of fibers was seen that stained moderately to intensely at both acid and alkaline pH. They did not possess features consistent with any of the fiber types found in the normal mature fast-twitch muscle, as they stained after acid as well as alkaline preincubation, typical of immature fibers. In a histochemical study of the rat EDL that was denervated at birth, Shafiq et al. (1972) used the myosin ATPase reaction in both acid and alkaline preincubations and showed dual staining in the atrophied fibers. They concluded that these fibers had not differentiated into mature fibers. Their results correspond to the findings presented in this paper. Finally, in our study, a small population of fibers, generally of greater diameter than the others in the EDL, showed complete inhibition after acid preincubation yet stained darkly for NADH-TR. These fibers were light staining at pH 4.2, but differed from the IIA fibers in control muscles, in that they were also oxidative. However, there was no evidence to show that they were in fact the same fibers as those designated type IIA_{ox} in the normal EDL at 7 days of age. In mature rat diaphragm, Gauthier and Hobbs (1982) reported that antibodies to fast myosin heavy chains were localized only in muscle fibers shown to be alkaline stable to myosin ATPase whereas antibodies to slow myosin heavy chains were detected exclusively in fibers that were acid stable. However, following denervation, mature rat diaphragm posessed many fibers that stained concurrently with anti-fast and anti-slow myosin. They suggested that denervation alters the myosin heavy chain composition and may result in synthesis of a myosin specific to denervation. The changes they report in denervated mature muscle occur much more slowly than those in immature muscles reported here.

Following neonatal denervation, the fast-twitch EDL failed to convert to an anaerobic muscle. Fast-twitch muscle becomes increasingly oxidative with most experimental manipulations, including increased activity through exercise (Green et al. 1984), low frequency stimulation (Pette et al. 1973), denervation (Niederle and Mayr 1978) and immobilization (Melichna and Gutman 1974). These changes in activity of the muscle, be they an increase or decrease, all change the muscle in the same direction, that is, increased oxidative capacity. Jones (1981) has suggested that the common factor they all lack may be the presence of a specific fast-twitch motoneuron pattern. Rubinstein et al. (1985) found both the light chains and heavy chains unchanged after neonatal denervation in rat EDL. Butler-Browne et al. (1982) and Whalen et al. (1985) found that neonatally denervated rat EDL is eventually able to express mature fast myosin but, using antibodies to developmental and mature forms of myosin, they found the sequence to be delayed.

In contrast to the work of Rubinstein and Kelly (1978) and Rubinstein et al. (1985) in denervated muscles of developing rats, we have found that in the mouse the distribution of fiber types during fast-twitch muscle development is altered by denervation. Based on the histochemical evidence presented here, denervated fast-twitch fibers of the mouse fail to develop their normal fiber type characteristics by 3 weeks of age. Following neonatal denervation, the mouse EDL possesses two fiber types. One type is a highly atrophied fiber that resembles an immature fiber, many with central nuclei, dual myosin ATPase staining and an oxidative enzyme profile. The second type is less atrophied and corresponds to type II fibers histochemically. It is possible that these denervated fibers are unlike any found during normal development, resulting from loss of coordination of development of different facets of the cell and are therefore specific to denervation, rather than simply a result of the arrest of maturation. This is supported by the fact that, after denervation, there was some continued differentiation before the denervation changes were seen histochemically. These histochemical changes are delayed, occurring after 7 days of age compared with alterations in the contractile properties in which changes following denervation are more immediate (Finol et al. 1981). Moreover, the results of our contractile studies (Redenbach 1985; Redenbach and Bressler 1985; 1986) further confirm an early alteration in normal maturation sequences of neonatally denervated fast-twitch muscle in the mouse.

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