J. Physiol. (1984), **349**, *pp.* 397–410
With 1 plate and 7 text-figures
Printed in Great Britain

DIFFERENT PATTERN OF RECOVERY OF FAST AND SLOW MUSCLES FOLLOWING NERVE INJURY IN THE RAT

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(*Received 6 May 1983*)

SUMMARY

1. The sciatic nerve was crushed in 5–6-day-old rats and the time course of recovery and changes in physiological and morphological properties of reinnervated fast and slow muscles was compared.

2. The maximal tetanic tension developed by the reinnervated muscles was recorded at different times from about 18 days of age, when functional recovery was first seen, until 2 months.

3. The maximal indirectly elicited tetanic tension of the reinnervated slow soleus muscle gradually increased from 55% of normal at 18 days to 75% of normal at 2 months. In contrast, the tension of the reinnervated fast muscle extensor digitorum longus (e.d.l.) fell sharply from 70% of normal at 18 days to 40% at 21 days and remained at that level till the end of the study.

4. The total number of muscle fibres in control, reinnervated and denervated e.d.l. muscles was counted. At 18 days the number of fibres in the reinnervated e.d.l. was similar to normal but by 1 month it had fallen to one-third. This decrease did not take place in permanently denervated muscles until at least 35 days. Loss of fibres in the reinnervated soleus was small.

5. During the early stages of reinnervation the contraction and relaxation of the fast muscles was very prolonged. By 1 month the time taken to reach peak twitch tension had decreased to normal values but the relaxation was still slower and remained so for several months.

6. The study of fatigue resistance showed that at 18 days the reinnervated fast muscles were as fatigable as normal muscles from animals of the same age. The fatigability of normal muscles increased with age to adult levels, but the reinnervated muscles became more fatigue resistant and remained so.

7. Our findings suggest that fast muscles become selectively impaired after nerve injury at 6 days because they lose a large number of fibres after reinnervation.

INTRODUCTION

Innervation plays an important role in the normal development of mammalian skeletal muscle. If young muscles are permanently disconnected from their motor nerves, growth is seriously retarded, but the speed with which the muscle tissue

disintegrates depends upon the timing of denervation. Surgical or pharmacological disconnexion of the muscle early in embryonic life results in rapid atrophy and degeneration (Eastlick & Wortham, 1947; Drachman, 1968; Gordon, Perry, Tuffery & Vrbová, 1974), whereas muscles denervated at or soon after birth atrophy slowly and even continue to grow slightly for several weeks (Vrbová, 1952; Zelená, 1962; Stewart, 1968). Nevertheless, muscles temporarily denervated at birth and reinnervated a few days later, never recover their strength despite the apparent preservation of most of the muscle fibres during the denervation period (Romanes, 1946; Bueker & Meyers, 1951; Zelená & Hník, 1963; McArdle & Sansone, 1977). Several authors have attributed this permanent impairment mainly to the direct effect of nerve damage on the still immature motoneurones, causing a large reduction in their number (Romanes, 1946; Bueker & Meyers, 1951; Zelená & Hník, 1963).

In a previous study we temporarily denervated the leg muscles of rats 5 or 6 days after birth (Lowrie, Subramaniam Krishnan & Vrbová, 1982). At this later stage of development the motoneurones survive injury to their axons, and we found virtually no motoneurone loss. It was therefore surprising to find that, when the reinnervated muscles were examined several months after nerve crush, the fast muscles tibialis anterior and extensor digitorum longus were permanently impaired, only recovering about 50% of normal weight and tension, but the slow soleus had recovered almost completely. Histological examination of the impaired fast muscles revealed a substantial reduction in the number of fibres. These fast muscles contain virtually the full adult complement of muscle fibres at birth (Ontell, 1979), therefore, the reduction after nerve crush at 6 days represents a real loss rather than failure of post-natal increase, as happens in more distal muscles of the foot (Zelená, 1962; Betz, Caldwell & Ribchester, 1980).

Such a large loss of muscle fibres is unlikely to have occurred entirely within the 10 day period before reinnervation began, for even complete denervation at an earlier time, i.e. at birth, results in only slight fibre degeneration during the first few weeks (Zelená & Hník, 1957). Thus, if the muscle fibre loss occurred after reinnervation, was this because motoneurones to fast muscles are less able to reoccupy completely their original peripheral field, or because some muscle fibres of immature fast muscles are unable to respond appropriately to the incoming 'fast' nerve? According to the latter explanation, nerve crush at 6 days of age retards the differentiation of the muscles while that of the motoneurones continues, i.e. they increase their rate of firing (Navarrete & Vrbová, 1983*a*). Indeed, it has recently been found that motoneurones reinnervating fast muscles after nerve crush shortly after birth are if anything more active than normal (Navarrete & Vrbová, 1983*b*). Thus, upon reinnervation the mature activity of the regenerating axons of 'fast' motor units would adversely affect immature muscle fibres and induce them to degenerate.

In an attempt to distinguish between these alternative explanations we have followed the early stages of recovery of fast and slow muscles, after temporary denervation, from the onset of reinnervation up to 2 months of age, at which point the difference between the recovery of slow and fast muscles is clearly established (Lowrie *et al.* 1982). We have also assessed the effects of permanent denervation upon muscles of the same age for comparison with the newly reinnervated muscles.

METHODS

Surgery

All surgery was performed on 5–6-day-old rats of the Wistar strain, using ether anaesthesia and aseptic precautions. In fifty-two rats the sciatic nerve of one leg was exposed in the popliteal fossa and crushed with watchmakers' forceps about 8 mm away from the leg muscles to be studied. The skin was then sutured. In a further fourteen rats the sciatic nerve of one leg was similarly exposed, but a 2–3 mm section was completely removed.

TABLE 1. Comparison of the tension developed by normal muscles (control) and those contralateral to operated muscles (contralateral) at different ages during the first post-natal month. Each value is the mean ± S.E. of the mean of four to eleven animals

Age during expt. (days)	Maximal tetanic tension (g)			
	T.a.		E.d.l.	
	Control	Contralateral	Control	Contralateral
17–18	78·7 ± 6·0	88·9 ± 4·3	18·5 ± 3·5	19·8 ± 1·3
21–23	147 ± 22	154 ± 15	41·0 ± 8·1	36·2 ± 3·6
25–26	228 ± 23	201 ± 14	52·6 ± 5·2	51·7 ± 4·5
29–38	298 ± 50	293 ± 31	71·9 ± 4·8	71·1 ± 7·2

Tension recording

At ages varying from 18 to 62 days, the animals which had undergone nerve crush, were anaesthetized with chloral hydrate (4·5%; 1 ml/100 g body weight) and the fast muscles tibialis anterior (t.a.) and extensor digitorum longus (e.d.l.) and the slow muscle soleus of both the reinnervated and contralateral control leg were prepared for tension recording. The muscles of the contralateral leg were considered suitable controls because their tension increased with age in a similar way to that of muscles of normal, unoperated animals (Table 1). The distal tendons were dissected free and attached to strain gauges, and the exposed parts of the muscles were kept moist with Krebs saline solution. Isometric contractions were then elicited from the muscles, either directly, or indirectly by stimulating the cut end of the motor nerve. In both cases supramaximal square-wave pulses were delivered via bipolar silver electrodes. The length of each muscle was adjusted so as to produce maximal twitch tension. Single twitch and tetanic (40–200 Hz) contractions were displayed on, and photographed from, an oscilloscope screen. Maximal tetanic tension was achieved by the fast muscles at a stimulation frequency of about 100 Hz and by the soleus muscle at about 60 Hz. At the end of recording, each muscle was stimulated at 40 Hz, for 250 ms every second, for a total period of 3 min. The contractions were displayed on a Devices pen recorder and the decrease in tension was measured. A fatigue index was then calculated:

$$\text{F.i.} = \frac{\text{initial tension} - \text{tension after 3 min stimulation}}{\text{initial tension}}.$$

Muscle histology

At the end of each tension recording experiment, the t.a., e.d.l. and soleus muscles from both legs were removed, weighed and frozen in isopentane cooled with liquid nitrogen. Each pair of corresponding operated and control muscles were frozen together and subsequently processed as a single block of tissue. Transverse sections of 10 μm thickness were cut from the middle third of each pair of muscles using a cryostat at -20 °C. The staining techniques used were Haematoxylin and Van Gieson for general morphology, NADH tetrazolium reductase (Dubowitz & Brooke, 1973) for oxidative enzymes, and the myosin ATPase methods of Dubowitz & Brooke (1973) and Guth & Samaha (1969).

Muscle fibre counting

In order to count the total number of fibres in a muscle, an entire transverse section from the middle of the muscle was photographed through the microscope field by field. The enlarged prints

were then fitted together to form a complete photo-montage. This was done for six reinnervated e.d.l. and four reinnervated soleus muscles ranging from 18 to 29 days of age, three denervated e.d.l. 18–22 days old, and one denervated soleus 18 days old. Sections stained with the myosin ATPase method of either Guth & Samaha (1969) or Dubowitz & Brooke (1973) were used. The former method differentiated best between fibre types but the latter method gave the clearest picture for counting fibres in the denervated and youngest reinnervated muscles.

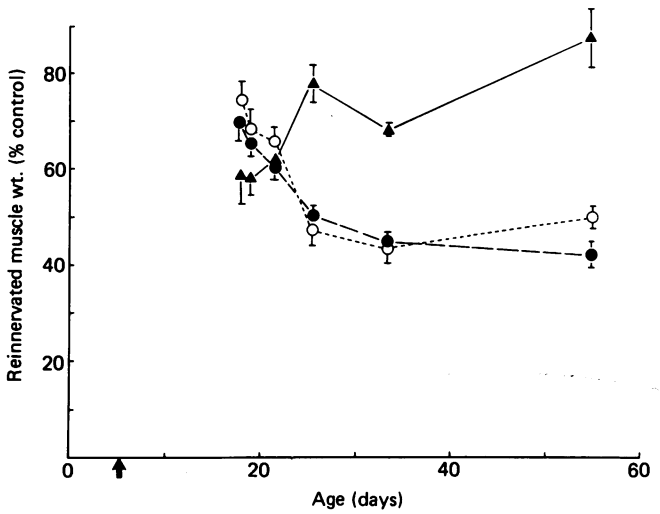

Fig. 1. The recovery of muscle weight following nerve crush. The weight of each reinnervated muscle was expressed as a percentage of the contralateral control value. Each point represents the mean ± S.E. of the mean of six to ten muscles. The animals were grouped according to age as follows: 18, 19, 21–22, 25–26, 29–38 and 48–62 days. ●, t.a.; ○, e.d.l.; ▲, soleus. Arrow shows time of nerve crush.

RESULTS

Recovery of weight in reinnervated fast and slow muscles

The first sign of recovery of the affected leg muscles was seen 10–12 days after the operation, when the animals were 15–18 days old. This was detected by the presence of the toe-spreading reflex when the rat was lifted with its hind legs clear of the ground (Gutmann, 1943). At ages ranging from 18 to 62 days the rats were prepared, under chloral hydrate anaesthesia, for physiological and histological examination of the muscles. At all ages the operated leg appeared wasted when compared to the contralateral leg. In the anterior compartment the wasting of t.a. and e.d.l. seemed more severe in the 8-week-old rats than in the younger ones, while soleus was affected more in the younger animals and appeared almost normal by 2 months of age. Comparison of the muscle weights confirm these gross observations (Fig. 1). At 18 days of age the reinnervated fast muscles t.a. and e.d.l. weighed about 72% of control values but in the following week increase in weight was so small, compared to that of the normal muscle, that the value fell to 45% and remained at this level till the end of the study. It is known from our previous work that subsequent improvement was slight even at 1 year. In contrast, the soleus muscles weighed only 58% of normal at 18 days but recovered quickly to reach 90% by 8 weeks. The greater degree of wasting seen initially in the soleus muscle could be due either to more degeneration

during the denervation period or to a slower rate of reinnervation. To distinguish between these possibilities e.d.l. and soleus muscles were completely denervated and their weights recorded. At 18 days the relative weight loss of both muscles was comparable to that of the newly 'reinnervated' muscles of the same age (Fig. 2). This suggests that the soleus muscles had indeed atrophied more rapidly after denervation

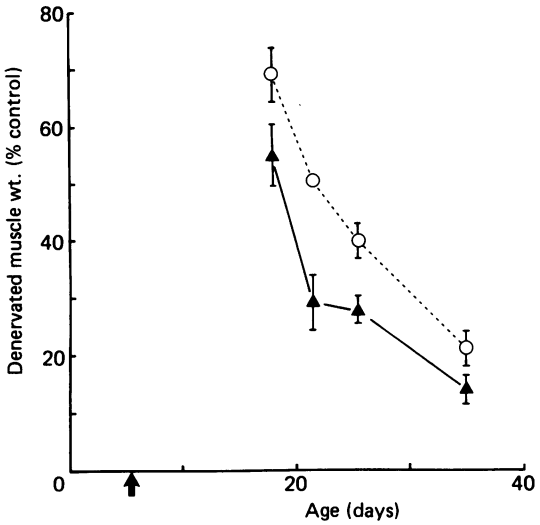

Fig. 2. The change in weight of muscles following permanent denervation. The weight of each denervated muscle was expressed as a percentage of the contralateral control value. Each point represents the mean ± S.E. of the mean of three to nine muscles. The animals were grouped according to age as follows: 18, 21–22, 25–26 and 34–37 days. ○, e.d.l.; ▲, soleus. Arrow shows time of denervation.

than the e.d.l., an observation made by other authors (Zelaná & Hník, 1957, Stewart, 1968). Comparison of Figs. 1 and 2 shows that whereas the wasting of the soleus muscles is immediately halted by reinnervation, that of the reinnervated fast muscles continues at a similar rate to that of completely denervated muscles until after 25 days of age.

Recovery of tension in reinnervated fast and slow muscles

At intervals during the first 8 weeks after reinnervation the tension developed by the reinnervated fast and slow muscles was recorded and compared to that of the corresponding muscles in the unoperated leg. The maximal tetanic tension achieved by each reinnervated muscle in response to stimulation of its nerve, was expressed as a percentage of the contralateral control value (Fig 3). The soleus showed 55% of tension at 18 days, and, after an initial delay improved to 75% by 8 weeks. However, the fast muscle t.a. and e.d.l. had recovered only by about 40% at this time, and it is known from the previous study that little further improvement occurs subsequently, while soleus reaches an optimum recovery of about 85% by between 40 and 60 days.

Although from 1 month onwards both t.a. and e.d.l. muscles showed a similar

degree of recovery, it is clear from Fig. 3 that at earlier stages the recovery of the two muscles was different. At 18 days t.a. had recovered only 28% of tension and thereafter gradually improved, while the tension of e.d.l. initially sharply rose to about 66% of normal and subsequently dropped to 40% within 4 days. The reason for this can be seen when the increase in tension with age of the operated and control muscles is displayed separately (Fig. 4). While the tension of the reinnervated t.a. gradually increased from 18 days at a steady rate, that of e.d.l. remained virtually unchanged for the first 4 days.

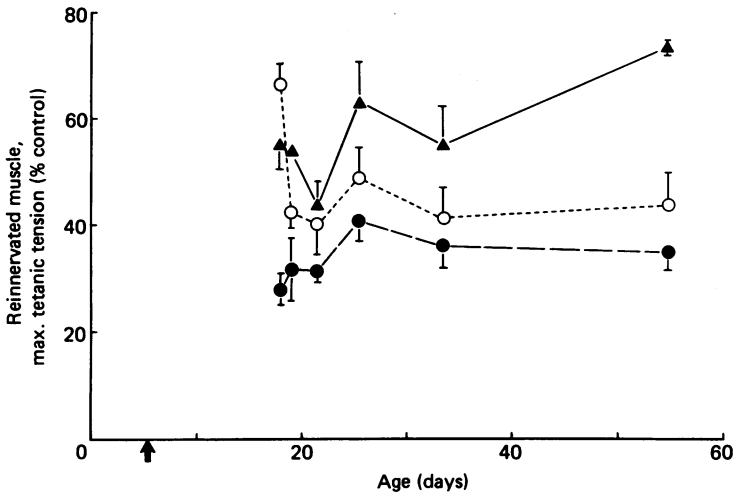

Fig. 3. The recovery of muscle tension following nerve crush. The maximal tetanic tension of each reinnervated muscle was expressed as a percentage of the contralateral control value. Each point represents the mean ± S.E. of the mean of six to ten muscles. The animals were grouped according to age as in Fig. 1. ●, t.a.; ○, e.d.l.; ▲, soleus. Arrow shows time of nerve crush.

At 18 days the recovery of tension (28%) of t.a. was much less than the recovery of weight (70%), while for soleus and e.d.l. the values were closer. This is probably a consequence of the larger size of t.a. Complete penetration by the regenerating nerve would be expected to take rather longer in t.a. than in the smaller muscles e.d.l and soleus. This explanation is supported by the results obtained from the ratio of tensions developed in response to direct and indirect stimulation (Table 2). In six animals studied shortly after reinnervation, t.a. yielded about 51% more tension when directly stimulated than when indirectly stimulated through the motor nerve. In contrast, direct stimulation evoked only 19% more tension in the reinnervated e.d.l. This suggests that in the operated t.a. there was at this time a relatively large number of muscle fibres that could not be stimulated through the nerve. As discussed more fully below, histological examination also indicates that a considerable part of t.a. was still denervated (Pl. 1).

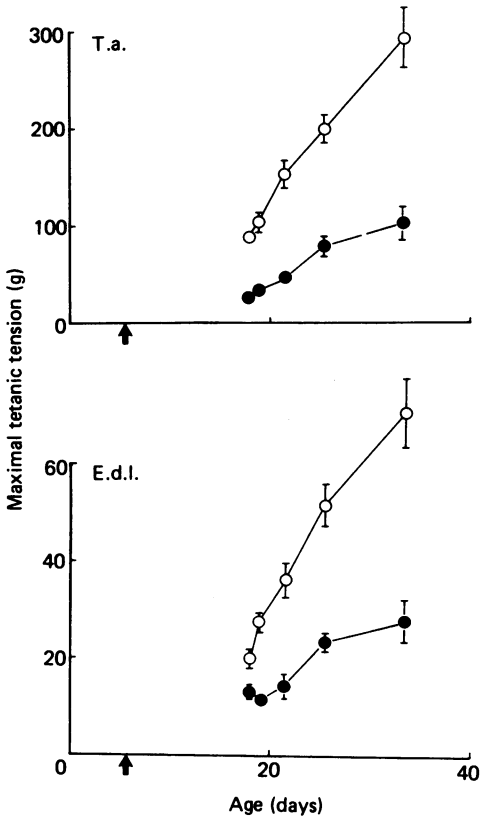

Fig. 4. The change in maximal tetanic tension of reinnervated and control fast muscles following nerve crush. Each point represents the mean ± S.E. of the mean of six to ten muscles. In some cases the S.E. was too small to be marked. The animals were grouped according to age as in Fig. 1. ●, reinnervated; ○, control. Arrow marks the time of nerve crush.

TABLE 2. Comparison of the tension developed by reinnervated fast muscles (18–21 days old) in response to direct and indirect stimulation

Maximal tetanic tension (g)					
T.a.			E.d.l.		
Indirect	Direct	Increase (%)	Indirect	Direct	Increase (%)
29·5	37·5	27	11·0	12·3	12
24·3	45·7	88	8·9	13·1	48
24·3	44·3	82	9·2	12·2	33
52·8	80·6	53	21·1	21·1	0
61·1	75·0	23	23·9	26·7	12
65·3	86·1	32	20·6	22·2	8
Mean ± S.E. of the mean		50·8 ± 11·7			18·8 ± 7·3

Physiological changes in reinnervated fast muscles

Our previous study found no change in the time to peak twitch tension of the reinnervated fast muscles, when measured at intervals later than 1 month after nerve crush. In the present work, we have measured both time to peak and half-relaxation in the early stages of recovery. Fig. 5 shows the change in time to peak and

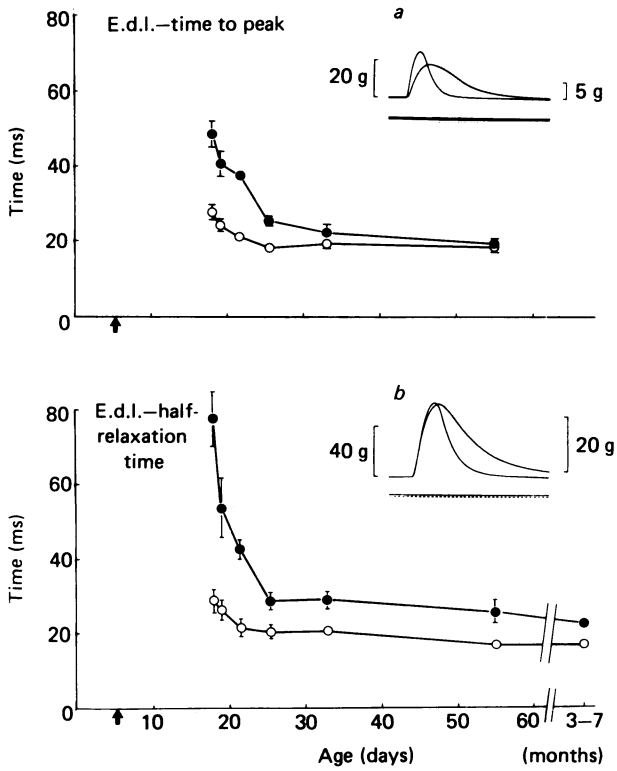

Fig. 5. The time course of contraction of reinnervated and control fast muscles following nerve crush. The upper graph shows the change in time to peak of e.d.l. with age; the lower graph shows the change in half-relaxation time of e.d.l. with age. Each point represents the mean ±S.E. of the mean of five to nine muscles. The animals were grouped according to age as in Fig. 1. In addition, half-relaxation time data were used from animals aged 3–7 months which were used in a previous study (Lowrie *et al.* 1982). ●, reinnervated; ○, control. Arrow marks the time of nerve crush. Insets *a* and *b* compare the single contractions of a reinnervated and control t.a. muscle. *a* is at 18 days when the contraction of the reinnervated muscle is much slower both in time to peak and half-relaxation time. *b* is at 32 days by which time the time to peak of the reinnervated muscle is almost normal but the half-relaxation time is still prolonged. In both insets the calibration bar on the left refers to the control muscle, the bar on the right refers to the slower reinnervated muscle and the time-base pulses are 10 ms apart.

half-relaxation of both normal and reinnervated e.d.l. muscles from 18 days to 7 months of age. Reinnervated t.a. muscles gave similar values. At 18 days time to peak and half-relaxation were still slightly high in the normally developing muscles but had reached adult values by 21 days. Inset *a*, in Fig. 5, illustrates the much slower

contraction speed of the reinnervated muscles at 18 days. It can be seen from the graphs that while both time to peak and half-relaxation were prolonged at this point, the large change was in the latter. By about 1 month of age time to peak in the reinnervated muscles was already similar to normal values but the half-relaxation time was still significantly higher, and even after several months had still not reached normal levels.

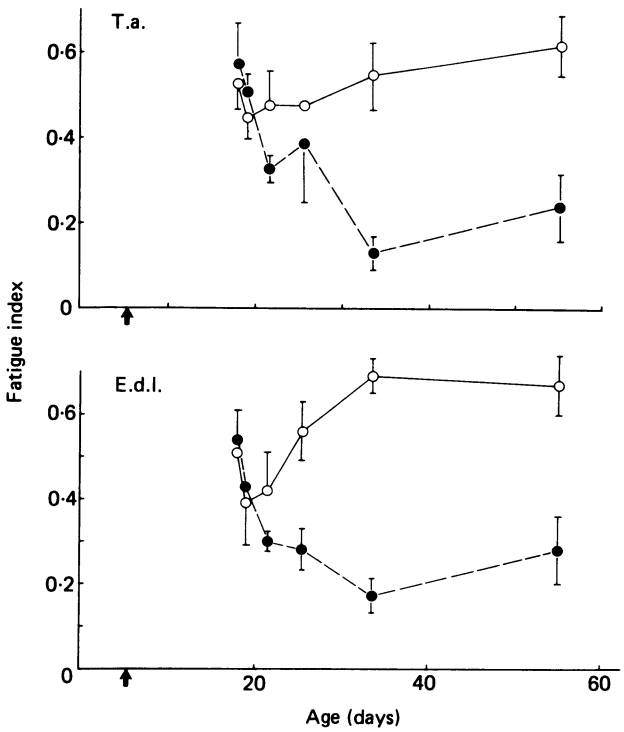

Fig. 6. Fatigability of reinnervated and control fast muscles following nerve crush. Each point represents the mean ± S.E. of the mean fatigue index of four to seven muscles. The fatigue index was calculated as the reduction in tension following 3 min of stimulation, therefore the higher the fatigue index, the more fatigable the muscle. The animals were grouped according to age as in Fig. 1. ●, reinnervated; ○, control. Arrow indicates time of nerve crush.

At the beginning of the recovery period the reinnervated fast muscles fatigued at the same rate as the control muscles but thereafter they quickly diverged (Fig. 6). The normal muscles gradually became more fatigable, reaching fatigue indices by 2 months of age, similar to those found in adult muscles (Lowrie *et al.* 1982), whereas the reinnervated muscles sharply increased their resistance to fatigue. This higher fatigue resistance is known to be sustained for at least 1 year (Lowrie *et al.* 1982).

Morphological changes in reinnervated muscles

At the beginning of recovery cross-sections of reinnervated t.a. still contained large areas of very small fibres which stained weakly for NADH tetrazolium reductase (Pl. 1) indicating that most of the superficial part of the muscle was not yet reinner-

vated (Engel & Karpati, 1968; Shafiq, Asiedu & Milhorat, 1972; Tomanek & Lund, 1973). E.d.l. and soleus muscles stained more uniformly with only a few small patches of apparently denervated fibres. At no time during the recovery period studied, were the large pale-staining glycolytic fibres seen, which make up 30–40% of the fibre content of normal fast muscles. This type of fibre was similarly absent from reinnervated fast muscles up to at least 1 year (Lowrie *et al.* 1982) which accounts for their lower fatigability. These fibres have low levels of oxidative enzymes and their presence is directly correlated with susceptibility to fatigue (Kugelberg & Lindegren, 1979).

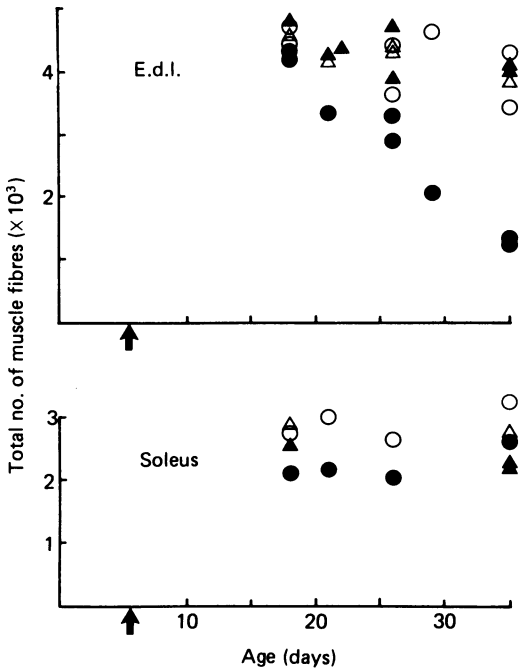

Fig. 7. Total fibre counts of e.d.l. and soleus muscles following either temporary or permanent denervation. Each point represents the total number of fibres in one muscle. ●, reinnervated; ○, control contralateral to reinnervated muscle; ▲, denervated; △, control contralateral to denervated muscle. Arrow indicates time of nerve crush or denervation.

Staining for myosin ATPase at pH 9·4 showed that the great majority of fibres in the reinnervated muscles were dark staining, whereas in soleus the larger proportion of fibres were pale; indicating that the relative proportion of fast and slow fibres in each muscle was not altered significantly from normal.

Since in the previous study, the permanent impairment of tension of the fast muscles was correlated with a reduced number of muscle fibres, counts of muscle fibres were made in the newly reinnervated muscles of the present study, and compared to those of muscles still completely denervated (Fig. 7). Control e.d.l. muscles contained between 3500 and 4500 fibres and there was no difference between those contralateral to newly reinnervated muscles and those contralateral to denervated muscles. These normal fibre counts are similar to those obtained previously by us (Lowrie *et al.* 1982) and other authors (McArdle & Sansone, 1977; Schiaffino, Pierobon

Bormioli & Aloisi, 1979; Ontell, 1979). The number of fibres in the reinnervated e.d.l. was near normal at 18 days but then declined sharply to about one-third at 35 days. In contrast, denervated e.d.l. muscles still contained the normal number of fibres at 35 days. Thus, fibre loss occurred after reinnervation and apparently as a consequence of it, rather than reflecting the gradual disappearance of fibres which had never been reinnervated.

The control soleus muscles contained about 3000 fibres. Similar numbers have been reported by other authors (Zelená & Hník, 1963; Frank, Jansen, Lomo & Westgaard, 1975; Kugelberg, 1976; McArdle & Sansone, 1977). Between 18 and 35 days the number of fibres in the denervated soleus was almost normal but the fibres seemed even smaller than those of the denervated e.d.l. This may account for the relatively greater loss of weight observed in the soleus during the early denervation period. The reinnervated soleus contained 70–80 % of the normal number of fibres at 18 days of age and in contrast to the e.d.l. did not decline subsequently.

Thus the fast and slow muscles showed marked differences in their pattern of recovery following post-natal nerve injury. The newly reinnervated e.d.l. suffered a progressive loss of muscle fibres at the same time as the tension dropped steeply, relative to the normal developing muscle. Conversely, the newly reinnervated soleus which showed a smaller drop in tension and subsequently recovered almost completely, sustained a smaller and non-progressive loss of fibres during the early recovery period.

DISCUSSION

Response of fast and slow muscles to early post-natal nerve injury

Several studies have demonstrated that sciatic nerve crush in new-born rats severely impairs the development of both fast and slow leg muscles (Romanes, 1946; Bueker & Meyers, 1951; Zelená & Hník, 1963; McArdle & Sansone, 1977). However when the same operation is performed a little later, at 6 days of age, only the fast muscles are permanently affected: the slow muscle soleus recovers virtually to normal in a similar way to muscles which have been temporarily denervated in adulthood (Lowrie *et al.* 1982). The permanent weakness of the reinnervated fast muscle e.d.l. was correlated with the loss of a considerable number of muscle fibres. The present study has shown that these fibres were lost, not before, but after reinnervation, and with such speed that only one-third remained by 1 month of age. In contrast, the soleus muscle lost only a small number of fibres and its tension was already well improved by 2 months. This recovery happened in spite of the muscle having suffered a greater degree of atrophy during the period of denervation.

The recovery of tension in t.a. was initially different from that of e.d.l. although the eventual impairment was similar. As already suggested, this was probably a consequence of the large size of t.a. needing a prolonged period of reinnervation. The increased tension derived from the gradual reoccupation of the whole muscle would mask the decrease in tension due to loss of fibres.

Fibre loss in reinnervated fast muscles

One possible explanation of fibre loss in the reinnervated fast muscles is that the young regenerating motoneurones are unable to reinnervate all the available muscle fibres. The remaining denervated fibres would then be expected to eventually

degenerate. This was the way Zelená & Hník (1963) interpreted the severe reduction in motor unit size found in muscles temporarily denervated at birth. However, this explanation is less attractive in view of results of the present study where the rate of fibre loss in reinnervated e.d.l. muscles appeared to be far greater than that in permanently denervated fibres. Moreover, motoneurones injured a few days after birth apparently still attempt to regain the original size of their peripheral fields even when the number of available muscle fibres has been severely reduced. Silver–cholinesterase staining of reinnervated t.a. and e.d.l. muscles of the rat revealed a persisting high level of multiple innervation among the surviving muscle fibres (Domizio, Lowrie & Vrbová, 1981). Similar results were reported by Brown, Hopkins & Keynes (1982) for the tensor fasciae latae muscles of the mouse, which had lost a large number of fibres following paralysis just after birth. Thus it appears unlikely that the regenerative capacity of the motoneurones is seriously impaired by disconnexion at this time.

An alternative explanation for fibre loss is that some immature muscle fibres cannot accept reinnervation following nerve crush, but again our findings suggest that this is unlikely. The fibres which remained denervated should fade away gradually not suddenly as was found in this study. Furthermore, selective loss of fibres due to immaturity would be expected to affect soleus more than t.a. and e.d.l. The maturation of the spinal cord progresses in a cranio-caudal, medial–lateral direction, therefore the motoneurones and muscles of the calf, including soleus, would differentiate later than those of the anterior compartment. Indeed, we found in agreement with others (Zelená & Hník, 1957; Stewart, 1968) that denervation during development causes more atrophy in soleus than in t.a. or e.d.l. muscles, and yet, following reinnervation, soleus makes a better recovery.

The sharp decline in muscle fibre number following reinnervation suggests that the fibres may have been actively destroyed. When the nerve is crushed at 6 days, the muscle is isolated from all nervous influence and differentiation is retarded. Meanwhile the motoneurone, although disconnected from its target, probably continues to develop its central connexions and differentiates: the slow motoneurones firing for long periods at low frequency; the fast motoneurones firing phasically but at very high frequencies (Navarrete & Vrbová, 1983). Upon reinnervation 10 days later, the immature fibres of the fast muscle may not be able to withstand the high frequency activity imposed upon them by the fast motoneurones, whereas the soleus muscle would be better able to survive the lower frequency activity of the slow motoneurones which supply most of the innervation to this muscle. Thus, retarding the differentation of muscles in the period when the motoneurones are differentiating would have graver consequences for fast than slow muscles. Selective damage of fast muscles is a feature of muscular dystrophy (Dubowitz & Brooke, 1973). There is fibre loss, and many of the remaining fibres appear immature (Dubowitz, 1969). It is possible that a fundamental defect of dystrophic muscles is a slow rate of maturation resulting in irreversible damage when the fast motoneurones activating them develop mature high frequency firing patterns.

Mechanism of fibre damage

The main change in the time course of contraction of the reinnervated fast muscles was the prolonged relaxation time. This was the only difference which persisted beyond 1 month of age and at 18 days, just after reinnervation, was almost 3 times longer than normal. Since relaxation largely depends upon the speed with which calcium is withdrawn into the sarcoplasmic reticulum, it may be that temporary denervation at this time prevents the full development of this system. In such circumstances, the high level of activity imposed upon fast muscle fibres by their motorneurones might lead to a permanently raised intracellular concentration of calcium. Such high levels of calcium are known to be harmful, perhaps through the activation of proteases (Salpeter, Kasprzak, Feng & Fertuck, 1979; Tóth, Karksú, Poberai & Sávay, 1981), and might therefore bring about the eventual disintegration of the muscle fibre. Free calcium has been observed in some muscle fibres from dystrophic patients (Bodensteiner & Engel, 1978) and several workers have shown that the ability of the sarcoplasmic reticulum to take up calcium is very much reduced in the muscles of these patients (Samaha & Gergely, 1969; Takagi, Schotland & Rowland, 1973; Peter, Worsfold & Fiehn, 1974).

We would like to thank Miss Angela Connold for her valuable technical assistance, and the Muscular Dystrophy Group of Great Britain for supporting this work.

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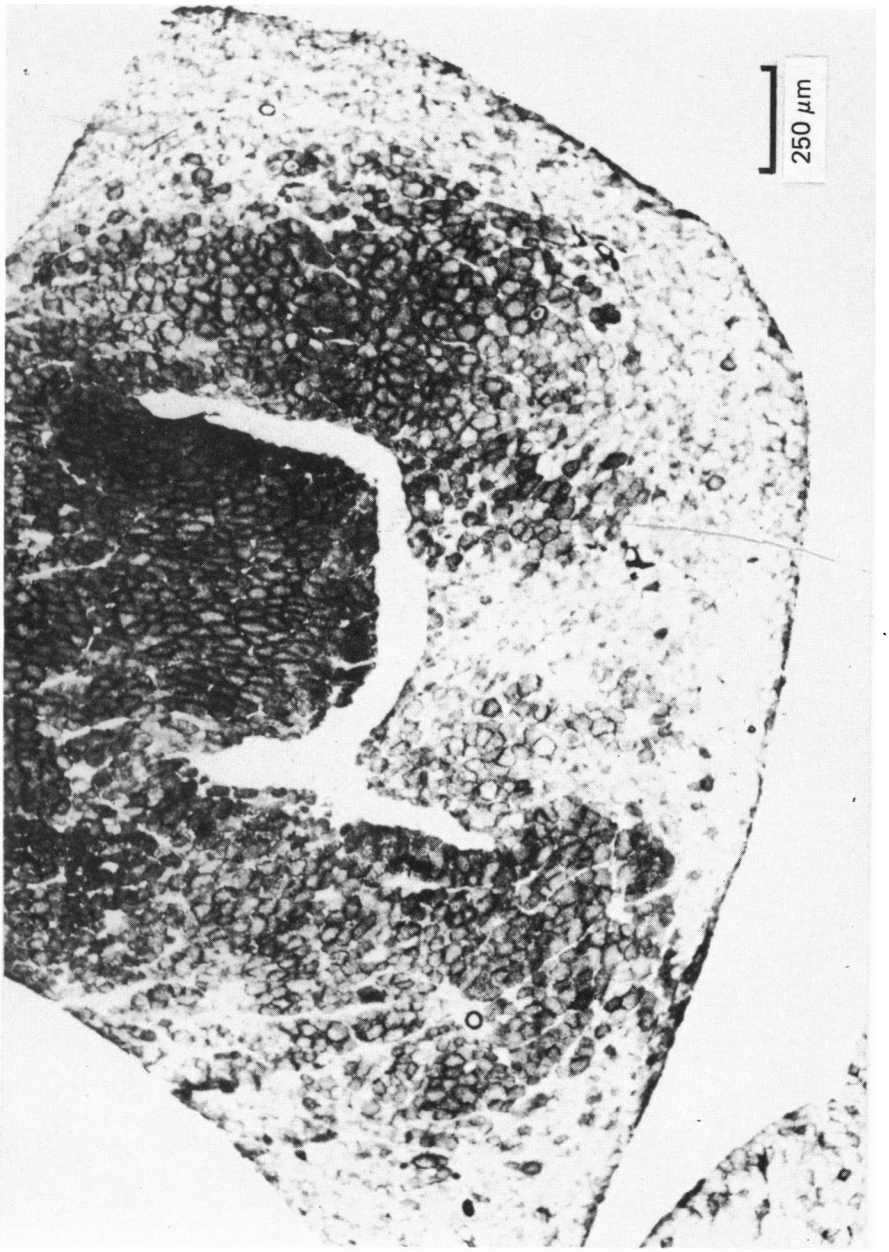
EXPLANATION OF PLATE

Transverse section from 21-day-old reinnervated t.a. muscle stained for NADH reductase showing large areas of the superficial part of the muscle remaining unstained.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