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RECOVERY OF SLOW AND FAST MUSCLES FOLLOWING NERVE INJURY DURING EARLY POST-NATAL DEVELOPMENT IN THE RAT

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SUMMARY

1. The sciatic nerve was crushed in 5–6-day-old rats and the recovery of function of slow and fast muscles was studied. The first signs of recovery of function were seen 10–12 days after the operation.

2. Maximal tetanic tension developed by the reinnervated muscles was recorded and taken as an indication of their recovery. Two months after nerve crush, slow soleus muscles developed only slightly less tension than the control unoperated soleus muscles. The reinnervated fast muscles tibialis anterior (t.a.) and extensor digitorum longus (e.d.l.) developed only about 50% of the tension of the unoperated controls.

3. The fast muscles never recovered, remaining weaker and smaller throughout the animals' life.

4. The number of muscle fibres in the reinnervated fast muscles was substantially reduced and their fibre composition altered in that they contained mainly muscle fibres with high levels of oxidative enzymes.

5. The reinnervated fast muscles became much more fatigue resistant than the unoperated controls.

6. The possibility that these changes are due to motoneurone death was examined. The motoneurones innervating the fast muscles were labelled by retrograde transport of HRP. No significant reduction in the number of motoneurones innervating the operated muscles was found.

7. These results show that nerve injury during early post-natal life causes permanent changes in fast muscles that are not caused by motoneurone death.

INTRODUCTION

The normal post-natal development of skeletal muscle depends upon interaction between the muscle and its motor nerve. Muscles disconnected from their motoneurones early in embryonic development continue to develop for a short time only to disintegrate later (Eastlick & Wortham, 1947). Even when innervation is preserved, but transmission from nerve to muscle is prevented, degenerative changes are seen in the muscle fibres (Drachman, 1968; Gordon, Perry, Tuffery & Vrbová, 1974; Srihari & Vrbová, 1978). At later stages of development, however, the effects of permanent denervation are less severe. Some fibres degenerate but others remain, and even continue to grow very slowly (Vrbová, 1952; Zelená, 1962; Stewart, 1968).

Although it is clear that permanent denervation modifies the development of skeletal muscle, it is not known whether continuous contact of the muscle with the nerve throughout the post-natal period is essential for normal development. Adult mammalian muscles recover virtually completely from temporary denervation provided that the reinnervation is allowed to proceed unhindered (Gutmann & Young, 1944; Beránek, Hník & Vrbová, 1957). Previous studies, involving temporary denervation in young animals, have been limited to the effects of nerve-crush injury immediately after birth. They show that the reinnervated muscles are affected to a much greater extent than after similar injury in adult animals. The effects include gross loss of weight (Romanes, 1946; Bueker & Meyers, 1951; Zelená & Hník, 1963; McArdle & Sansone, 1977), and are observed in both fast and slow muscles. These changes are thought to be due to incomplete reinnervation: the nerve fibres are smaller and greatly reduced in number (Bueker & Meyers, 1951; Zelená & Hník, 1963), and far fewer ventral horn cells are counted in the spinal cord, suggesting substantial loss of motoneurones (Romanes, 1946). There is evidence that immature motoneurones are more likely to die as a result of axonal damage than mature ones (Lieberman, 1971). Even the surviving motoneurones are unable to recover the original size of their peripheral field, for the size of motor units in the soleus muscle after nerve crush at birth was found to be much reduced (Zelená & Hník, 1963). This reduction in size of the surviving motor units was attributed to the inability of the 'injured' motoneurone to support all its branches. It is however possible to explain this result differently. Due to the temporary denervation, the development of the muscle fibres is arrested, while that of the motoneurones continues. Upon reinnervation the still immature muscle fibres may not be able to match the functional demands imposed upon them by the now mature nervous system. Motor reflexes in rats change during the second and third week of development. At first all motor units fire at low rates and for brief periods of time. With age motor units to soleus increase the duration of their firing but not the frequency, whereas motor units to fast muscles like extensor digitorum longus (e.d.l.) fire at much faster rates for a short time (Bursian & Sviderskaya, 1981; preliminary communication by Navarrete & Vrbová, 1980). Normally muscle fibres supplied by these motoneurones acquire the appropriate characteristics to match these activity patterns; the soleus muscle becomes slow contracting and fatigue resistant, while fast muscles increase their contraction speed (Buller, Eccles & Eccles, 1960; Close, 1964; Brown, 1973). The possibility that the development of muscles may be permanently impaired if they are not activated by their motor nerves during the transition from the immature to the mature stage is studied here.

METHODS

Surgery

In the main experimental group of twenty-three rats the sciatic nerve in one leg was crushed at 5 or 6 days of age, under ether anaesthesia and aseptic conditions. The nerve was crushed with fine watchmaker's forceps in the popliteal fossa about 8 mm away from the leg muscles to be studied. The skin was then sutured. The same operation was performed on four adult (2 months old) rats. Another control group consisted of six sham-operated rats in which the sciatic nerve of one leg had been exposed but not crushed at 5 or 6 days.

Tension recording

At intervals varying from one to eleven months after operation nineteen animals were prepared for recording of muscle tension under chloralhydrate anaesthesia (3·5 %; 1 ml./100 g body weight). The distal tendons of the fast muscles tibialis anterior (t.a.) and extensor digitorum longus (e.d.l.) and the slow muscle soleus of both legs were dissected free, and attached to strain gauges (Dynamometer UFI, Devices). Isometric contractions were elicited by stimulating the cut end of the motor nerve via bipolar silver electrodes using supramaximal square wave pulses. The length of the muscles was adjusted so as to produce maximum twitch tension. Single twitch and tetanic contractions were displayed on, and photographed from, an oscilloscope screen. At the end of recording the muscles were stimulated at 40 Hz, for 250 msec every second, and these contractions were displayed on a Devices pen recorder. The decrease in tension after 3 min of such stimulation was measured. A fatigue index was then calculated as follows:

$$\frac{\text{Initial tetanic tension} - \text{tetanic tension after 3 min stimulation}}{\text{Initial tetanic tension}}$$

Histology of muscles

Following each tension recording experiment, t.a., e.d.l. and soleus muscles were removed from both the operated and contralateral control leg. Corresponding control and experimental muscles were then mounted side by side on the same block, at the same length, and frozen in isopentane cooled with liquid nitrogen. Each pair of muscles was subsequentially handled as a single block of tissue. Transverse sections from the middle third of each muscle pair were cut at a thickness of 10 μm in a cryostat. Sections from all muscles were stained routinely by haemotoxylin and Van Gieson, and either for succinic dehydrogenase (SDH) by the method of Nachlas, Tsou, De Souza, Cheng & Seligman (1957) or for NADH tetrazolium reductase (Dubowitz & Brooke, 1973). In addition some sections were stained for myosin ATPase at pH 9·4 (Round, Matthews & Jones, 1980).

Counting and measurement of muscle fibres

In six animals, operated at 6 days of age, the total number of fibres in each e.d.l. muscle was counted from a photomontage of an entire transverse section. The cross-sectional area of muscle fibres in e.d.l. muscles from five of these animals was also measured. Muscle fibre profiles were traced from a section stained for SDH onto paper, using a camera lucida attached to the microscope. The profiles were then retraced onto a Tektronix 4956 digitizing tablet, and the area within each profile calculated by a Tektronix 4052 graphic computer. In order that sampling should take account of the variable distribution of different types of fibres within the muscles, all the fibres in successive fields in three parallel rows, extending down the central $\frac{2}{3}$ of the muscle from the deep to the superficial regions, were measured (Pullen, 1977). In this way between 10 and 18 % of the total population of fibres was measured. The fibres sampled were distinguished according to the intensity of staining as either non-oxidative (pale-staining) or oxidative (dark-staining). This simple categorization was relatively clear, but no attempt was made to subdivide the fibres further.

Labelling of motoneurones by retrograde axonal transport of horseradish peroxidase

In four animals, the sciatic nerve was crushed on one side at 5 or 6 days of age. Twenty-eight to thirty-two days after the operation 10 % horseradish peroxidase (HRP: Sigma type VI) in sterile saline was injected into the t.a. and e.d.l. muscles of both legs under aseptic conditions and chloralhydrate anaesthesia. The HRP was injected very slowly into the body of each muscle through a fine micro-syringe, the total amount injected (4–10 μl.) depending on the size of the muscle. Any leakage was immediately mopped up with cotton wool and the skin incision was carefully sutured over the muscles. HRP was injected in the same way into muscles of three control animals.

Twenty-four hours later the animals were anaesthetized and perfused with 2·5 % glutaraldehyde in phosphate buffer, pH 7·3. The spinal cord was then removed and processed for the demonstration of HRP according to the method of Hanker, Yates, Metz & Rustioni (1977). Once mounted onto slides, the sections were lightly counter-stained with gallocyanin.

The number of HRP-labelled motoneurones in each ventral horn of the spinal cord was counted. In order to avoid counting the same cell twice in adjacent sections, only cells containing a nucleolus were included.

RESULTS

Effect of age on recovery of slow and fast muscles from nerve injury

A nerve crush injury in adult rats leads to temporary denervation, and muscle wasting, but on reinnervation these changes are completely reversed. The present results confirm this observation. (See Figs. 1 and 3.)

When the sciatic nerve is crushed in 5- or 6-day-old rats, about 8 mm from the muscles, the first signs of recovery of function can be seen 11 days later. As functional recovery was followed, clinical examination revealed that while the animals could

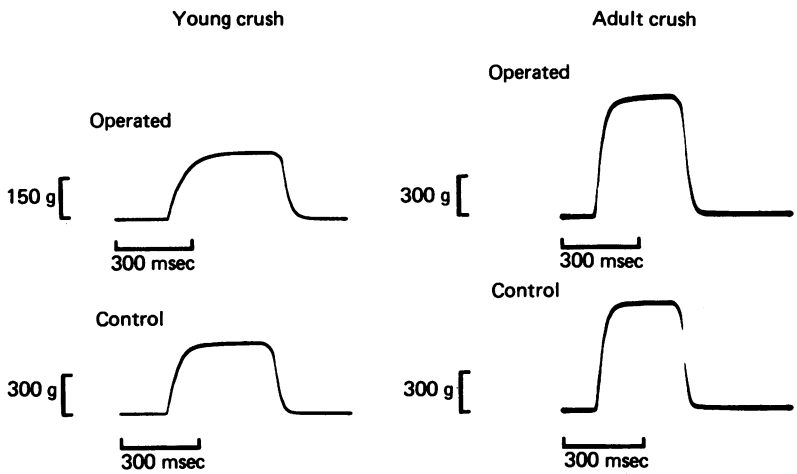

Fig. 1. Records of isometric tetanic (80 Hz) contractions of control and reinnervated t.a. muscles 60 days after nerve crush.

contract the muscles of the operated leg, the function was very different from that of the unoperated control. The animals appeared to have a weakness of those muscles that performed dorsiflexion of the ankle joint, so that on lifting the animal off the ground the operated leg assumed an extended position. This change in function persisted even 1 year after the operation. On inspection the calf muscles appeared to be wasted.

The sham-operated animals showed neither impaired function, nor wasting of their muscles.

At different intervals after the initial operation the rats were anaesthetized and the tension developed by the control and operated t.a. and e.d.l. muscles was compared. Fig. 1 shows an example of such an experiment. It is clear that, in contrast to animals in which the nerve is crushed later in life, the reinnervated t.a. muscles developed much less maximal tetanic tension than the control muscles. This effect of early nerve crush on the development of these muscles was permanent. Fig. 2 shows that although the maximal tetanic tension of the operated as well as the control muscles increased with age, the difference between them remained. The weight of the operated muscles was also less than that of the control muscles, and the difference in weight was similar to that in tension. The results are summarized in Fig. 3 which shows the extent of the change in animals that had their nerves crushed during early

development and the insignificant change in animals that were operated during later life.

Whether slow muscles of the leg were equally affected by the sciatic crush was tested. The tension of the soleus muscle from the operated and control leg was compared and as Fig. 3 shows the difference between the control and operated soleus muscles was very small. Indeed in view of the impaired function of the leg, it was

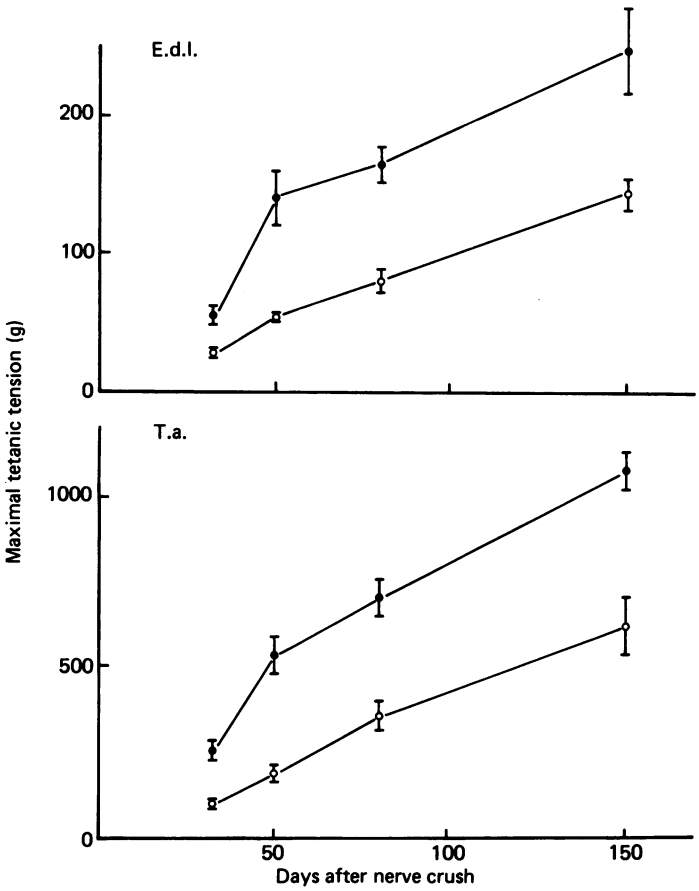

Fig. 2. Maximal tetanic tensions developed by control and reinnervated muscles are plotted against time after nerve crush at 5–6 days. The animals were grouped as follows: 25–40 days, 40–60 days, 60–100 days, 100–200 days. n = 5–8 for each group. Vertical bars represent S.E. of the means. ●, control; ○, operated.

surprising that the operated soleus was nearly normal. Thus, the operation affected fast muscles to a much greater extent.

The sham operation had no effect either on the tension or weight of the muscles studied.

Physiological and histochemical properties of reinnervated muscles

In adult muscles following reinnervation after nerve crush most of the physiological characteristics return to normal. The time course of contraction is similar to normal

and individual motor units resume their previous size and characteristics (Gordon & Stein, 1980). This applies for fast as well as slow muscles, and our present results confirmed these findings, the only difference being that the reinnervated e.d.l. muscles were slightly more fatigue resistant than the unoperated controls (see Figs. 4 and 5). It may be that axons originally supplying oxidative, fatigue-resistant muscle fibres occupy on reinnervation a relatively larger territory than before, or motoneurones may become more excitable after injury so that the over-all activity of reinnervated

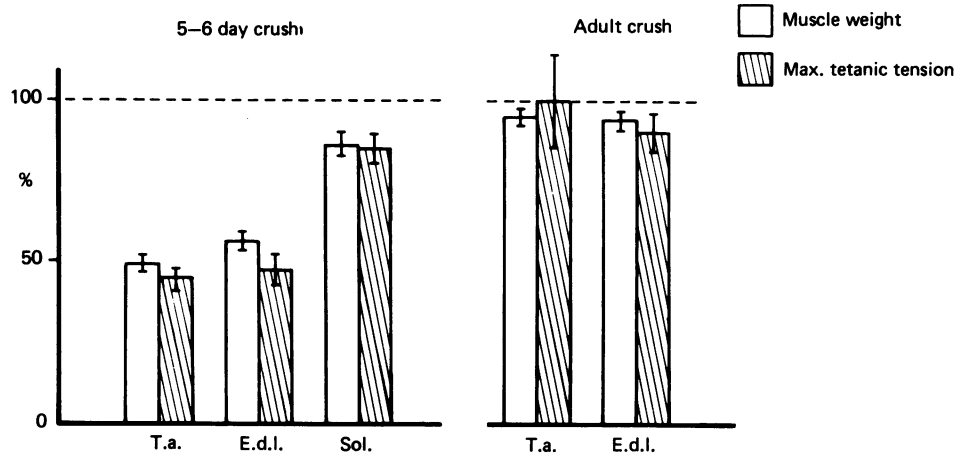

Fig. 3. Muscle weights and tetanic tensions of reinnervated t.a., e.d.l. and sol. muscles are expressed as a percentage of control unoperated values. Note that the recovery of the fast muscles in the adults is nearly complete, while that in animals which received nerve crush at 5–6 days is very poor. n for adults nerve crush = 4; 2 months after nerve crush, n for 5–6 day crush = 10–19; 1–11 months after nerve crush. Vertical bars represent S.E. of the means.

muscles is greater. Consistent with the increased resistance to fatigue was the appearance of the muscle fibres when stained for SDH. This staining revealed that the reinnervated muscles contained a higher proportion of dark staining fibres.

When the nerve was crushed during early post-natal life the time course of contraction and relaxation of e.d.l. and t.a. was not different from that of normal muscles. This is apparent from Table 1. Consistent with this, the ATPase histochemistry revealed that the reinnervated muscles like the unoperated controls contained mainly fibres staining densely for alkaline stable ATPase. The reinnervated muscles only differed in the pattern of distribution of their pale-staining fibres, which tended to be grouped and to show some variability in staining (Pl. 1*A*).

The most conspicuous difference was in the fatigue resistance of the muscles. Fig. 4 shows an example from an experiment in which the fatigue resistance of the control and reinnervated muscles was compared. From results like these a fatigue index was calculated. The diagrams in Fig. 5 summarize the results from all experiments and confirm the results illustrated in Fig. 4.

Staining for oxidative enzymes revealed that most muscle fibres in the reinnervated e.d.l. and t.a. muscles were highly oxidative. While the staining intensity is probably

TABLE 1. Time-to-peak twitch tension (msec) of control and reinnervated muscles is compared

	T.a.				E.d.l.				Sol.			
	Operated	Control	*n*	Significance	Operated	Control	*n*	Significance	Operated	Control	*n*	Significance
5–6 day crush	23±1·0	22±0·9	17	n.s.	22±0·8	20±0·7	18	n.s.	48±2·6	55±3·2	11	< 0·05
Sham-operated	23±1·2	23±1·2	6	n.s.	21±0·4	22±1·5	6	n.s.	54±7·2	53±9·0	3	n.s.
Adult crush	27±3·0	26±2·5	4	n.s.	26±0·6	26±2·2	4	n.s.	—	—	—	—

n.s. = not significant.

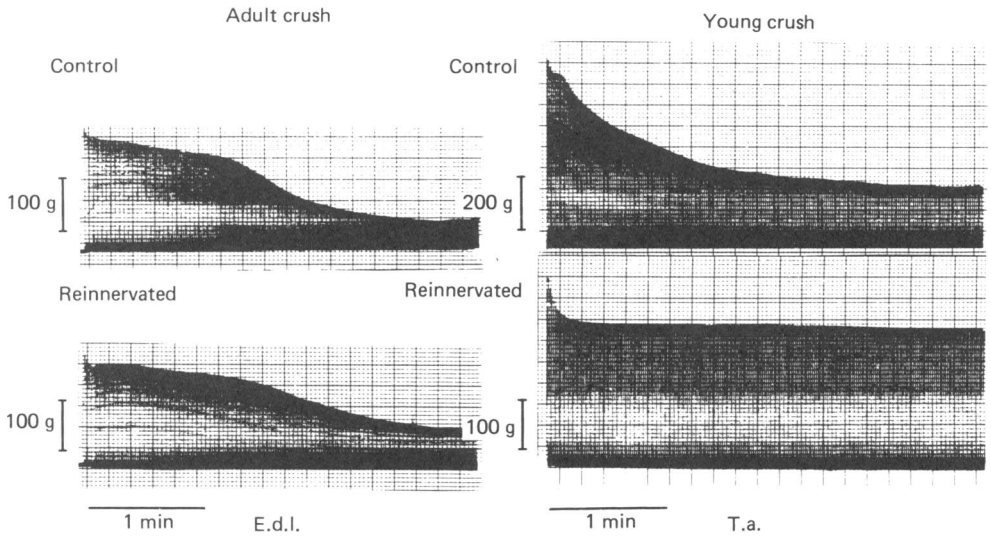

Fig. 4. Records of contractions elicited by stimulating the lateral popliteal nerve at 40 Hz for 250 msec every second are shown. Note the resistence to fatigue of the reinnervated t.a.

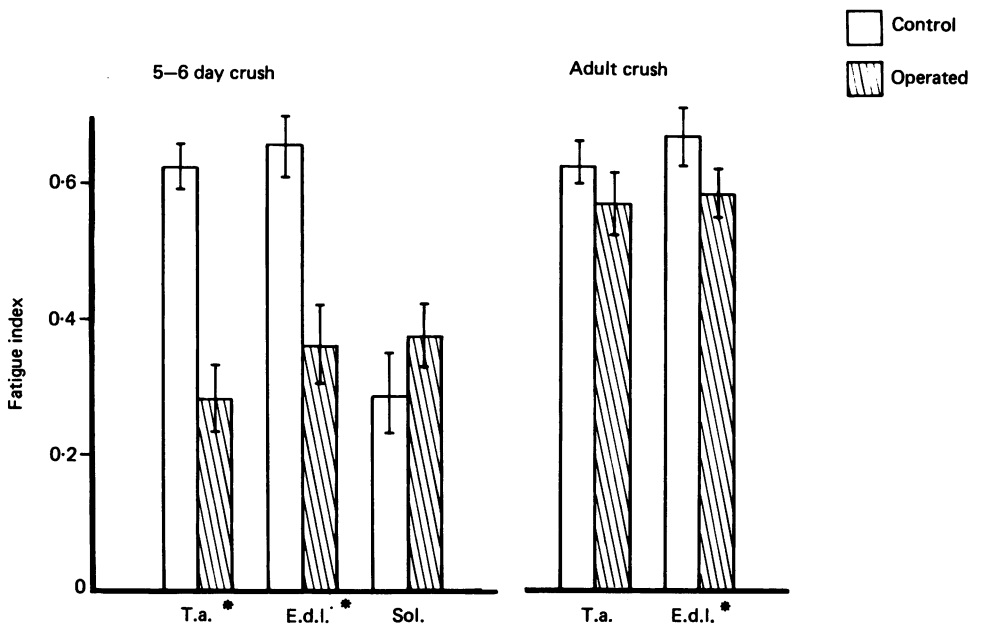

Fig. 5. Fatigue index calculated as the reduction of tension following 3 min of stimulation. Note that the reduction of tension is less in the reinnervated t.a. and e.d.l. muscles when their nerves were crushed at 5–6 days after birth ($P < 0{\cdot}001$) than after nerve-crush later in life (e.d.l.: $P < 0{\cdot}05$) n for adult crush = 4; n for 5–6 day crush = 10. Bars represent S.E. of the means.

not homogeneous, there is no doubt that in these muscles pale-staining fibres are rarely seen. *B* and *C* in Pl. 1 compare a reinnervated e.d.l. and t.a. muscle with homologous muscles of the contralateral side and illustrate the predominance of fibres with high content of oxidative enzymes. Since oxidative enzymes are directly related to fatigue resistance this result is in keeping with the exceptionally high resistance to fatigue of these muscles.

The resistance to fatigue of normal soleus muscles is known to be greater than that of fast muscles, and fatigue resistance of the reinnervated soleus muscles was not different from that of the control muscles (Fig. 5). Similarly the reinnervated soleus muscles showed no alteration in intensity or pattern of staining for oxidative enzymes. A slight but significant increase in contraction speed was found in the operated soleus muscles (Table 1).

TABLE 2. Total number and mean cross-sectional area of muscle fibres in control and reinnervated e.d.l. muscles

Weeks after operation	Total number of fibres in muscle			% non-oxidative fibres		Mean fibre area (μm^2)		
	Control	Operated	% decrement	Control	Operated	Control non-oxidative	Control oxidative	Operated oxidative
4	4288	1308	70	36	0	1311	774	628
4	3411	1268	63	37	0	1677	905	821
11	2926	1948	33	33	0	3015	1471	1186
13	2603	1818	30	31	0	3092	1396	1739
17	2711	2222	18	—	—	—	—	—
33	3295	2584	22	41	0	4122	2154	2456

Changes in the number and size of muscle fibres in reinnervated fast muscles

Counts were made of the total number of fibres in e.d.l. muscles from six animals at intervals varying from 29 to 228 days after nerve crush at 6 days. The results are summarized in Table 2. It is apparent that there is considerable variation in the number of muscle fibres in the control muscles. For this reason the difference between the control and operated muscle in each animal was also calculated. Table 2 shows that in all cases the operated e.d.l. muscle contained fewer fibres, and that this loss tended to be more severe at the earlier recovery times. It is not clear why, with longer recovery intervals, the number of fibres in the reinnervated muscles increased. One contributory factor may be fibre-splitting, as clear signs of this were seen in a proportion of fibres in these older muscles. Our fibre counts for the control e.d.l. muscles agree with those of other authors (McArdle & Sansone, 1977). The area of fibres in e.d.l. muscles of five animals was also measured. Mean fibre areas for all the muscles are given in Table 2. In addition, the full distribution of fibre areas for three animals, representing the range of recovery periods examined, is illustrated in Fig. 6. In each case the control muscle consisted of two groups of fibres: one group of small oxidative fibres, and another group of larger non-oxidative fibres representing 31–41 % of the total population. These values agree closely with those described by other authors for the e.d.l. muscle of the rat (Pullen, 1977; Kelly, 1978). Fig. 6 also shows that the mean fibre area and range of both types of fibre increases with age.

In contrast to the control muscles, the reinnervated e.d.l. muscles contained predominantly oxidative fibres and Fig. 6 shows clearly that the size distribution of these fibres matches that of the oxidative fibres in contralateral control muscles. Although less numerous, the larger non-oxidative fibres accounted for 48–57 % (mean 51 %) of the total cross-sectional area of the control muscles. Thus the 50 % reduction in tension found in the reinnervated muscles can be entirely explained by the absence of these fibres.

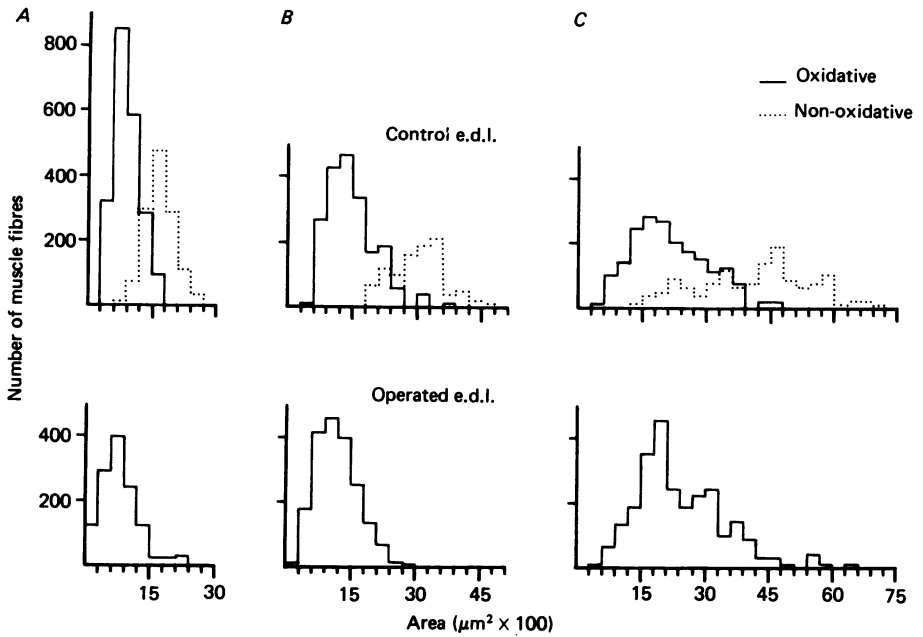

Fig. 6. Histograms show the distribution of areas of muscle fibres in control and reinnervated e.d.l. muscles from animals (*A*) 4 weeks, (*B*) 11 weeks and (*C*) 33 weeks after nerve crush at 5–6 days.

The reduction of the number of muscle fibres in muscles of animals that had their motor nerves injured during early post-natal life could be due to loss of motoneurones. To examine this possibility the number of motoneurones innervating t.a. and e.d.l. muscles was assessed.

Effect of post-natal nerve crush on motoneurone number

All the HRP-labelled motoneurones were found in spinal cord segments L3, L4 and L5, the majority being in L4. Within these segments they were restricted to the dorsolateral column of the ventral horns (Pl. 2*A*). All the animals showed this discrete localization, which agrees well with that described by Brushart & Mesulam (1980) for the rat, and Romanes (1946) and McHanwell & Biscoe (1981) for the mouse.

Table 3 shows the number of labelled motoneurones counted in each ventral horn of both the experimental and control animals. The greatest variation in counts between left and right ventral horns in the control animals was 11 %, and the mean count for both ventral horns of the three control animals was 171 (±4·7 S.E.M.). The

mean count for the control horns of the experimental animals was $151 \pm 9{\cdot}3$, and did not differ significantly (by the unpaired t test) from the mean for the control animals. The mean number of motoneurones innervating the operated leg of the experimental animals ($144 \pm 5{\cdot}0$) was not significantly different, by the paired t test, from those innervating the contralateral unoperated leg. Although it is possible that the operation might have resulted in a reduced motoneurone pool in both experimental and contralateral control horns, such a decrease was clearly too slight to significantly affect subsequent muscle development since the control muscles did not differ from sham-operated or normal muscles in any of the properties measured.

TABLE 3. Total number of HRP-labelled motoneurones found in the spinal cords of control and experimental animals

Control animals		Experimental animals	
Left horn	Right horn	Control horn	Operated horn
153	166	179	133
180	175	139	154
166	185	145	137
		143	150

With regard to the efficiency of labelling of the motoneurones, a comparison can be made between the present counts and those expected on the basis of other work. Edström & Kugelberg (1968) estimated that the t.a. muscle of the rat contains seventy-seven or seventy-three motor units according to tension recording or histochemical mapping respectively. Close (1967), also recording the tension of single motor units, estimated that the rat e.d.l. muscle contained about forty units. Thus one might expect the total number of α-motoneurones innervating these two muscles would be 117. Several workers have estimated the number of γ-motoneurones labelled when HRP is applied to muscles in the leg. The results, expressed as a percentage of the total motoneurone pool, are variable: 16–22 % in rats (Brushart & Mesulam, 1980), 20 % in mice (McHanwell & Biscoe, 1981) and 25–30 % in cats (Burke, Strick, Kanda, Kim & Walmsley, 1977). Assuming an average value of 22 %, the estimated number of γ-motoneurones innervating the t.a. and e.d.l. muscles would be thirty-three. Therefore the total number of motoneurones, both α and γ, innervating these two muscles would be 150. The results of the present study accord well with this expected value.

Furthermore, attempts to increase the efficiency of HRP labelling by increasing either the survival time or injection volume of HRP was unsuccessful. A survival time of 48 hr resulted in very faint labelling, and higher doses of HRP either showed no difference in labelling, or labelled additional motoneurones outside the normal motoneurone pool.

Since it seems unlikely that the number of motoneurones innervating the control t.a. and e.d.l. was underestimated in this study, it can be concluded that nerve crush 5–6 days after birth did not result in significant loss of motoneurones.

DISCUSSION

Our present results show that the extent to which skeletal muscles recover following injury to their motor nerves depends on the age at which the injury occurred, and upon the muscle type. In adults after a similar injury slow and fast muscles recover almost completely (Beránek *et al.* 1957). Contrary to general belief that repair is more complete in young animals, when the motor nerves are crushed at 5–6 days of age subsequent recovery of fast muscles is permanently impaired.

In the present experiments the reinnervated fast muscles developed less than half normal tension and their weights were reduced to a similar extent. This incomplete recovery can be explained entirely by the absence of large non-oxidative muscle fibres. The consequent relative increase in oxidative capacity explains the increase in fatigue resistance found in the reinnervated muscles.

Inflicting an injury to the motor nerve during the early post-natal period can influence the developing motor unit by affecting the motoneurone, or the muscle fibres. The relative contribution of these two possible influences to the permanent changes found in muscles following nerve injury will be discussed.

Immature motoneurones are more likely to die after axotomy than mature ones. Romanes (1946) studied the pattern of loss of anterior horn cells innervating leg muscles during post-natal development in mice and rats. He found that although axotomy at birth led to a severe reduction in the number of anterior horn cells, axotomy during the first week after birth resulted in far less motoneurone loss. Our present study, using HRP to label motoneurones, suggests that there is little if any motoneurone loss after nerve crush at 5 or 6 days. Thus it is unlikely that the more than 50 % reduction in tension found in the reinnervated fast muscles is due to loss of motoneurones.

The possibility that injured immature neurones are unable to reoccupy their original peripheral field was favoured by Zelená & Hník (1963). They studied the effects of sciatic nerve crush at birth on the development of the soleus muscles in rats. They not only observed that the number of motor axons in the regenerated nerve was reduced by half, but that the motor units contained far fewer muscle fibres. This reduction in the size of the motor units was thought to result from the inability of the regenerating axons to reoccupy their original peripheral field and not from the lack of available muscle fibres, for while the muscle was denervated the number of muscle fibres did not decrease significantly, and the loss of fibres took place only after reinnervation had taken place. Different results were reported by Brown, Jansen & Van Essen (1976), who found complete reinnervation and recovery of the soleus muscle after nerve crush at birth. The discrepancy between the results of Zelená & Hník (1963) and Brown *et al.* (1976) could be due to the site of the lesion, and thus the time it took the axons to reach their target. Brown *et al.* (1976) crushed the nerve near to the muscle, whereas Zelená & Hník (1963) placed their lesion some distance away from the muscles and thus disconnected the nerve from the muscle for a considerably longer period of time. Thus these results suggest that injury to the nerve *per se* does not preclude total recovery of the neurone's peripheral field but that the time delay seems to be an important factor.

Whatever the limiting effects of axotomy at birth may be on subsequent reoccu-

pation of the original peripheral field, it is unlikely that this factor will have the same importance at later stages of development. Bueker & Meyers (1951) have shown that the reduction in diameter of the regenerated nerves after axotomy is less the later after birth it is performed. From the present study it is clear that the recovery of the soleus muscle is only slightly impaired when the nerve is injured at 5–6 days after birth, while it is seriously affected when the damage occurs immediately after birth at a similar distance from the muscle (Zelená & Hník, 1963; McArdle & Sansone, 1977). Thus it is possible that the impaired recovery of fast muscles after nerve injury at 5–6 days after birth is not caused only by the inability of the motoneurone to regenerate and recover its peripheral field but to another factor associated with the disturbed connexion between nerve and muscle at this crucial period of development.

The reinnervated fast muscles, when examined 1 month after operation at 6 days, contained 60–70 % fewer muscle fibres than normal muscles. Betz, Caldwell & Ribchester (1980), in a study of the effects of denervation at birth on the fourth lumbrical muscle of the rat foot, also found a decrease in the number of muscle fibres. They considered that it resulted from inhibition of the growth of new fibres which apparently occurs in this muscle during normal post-natal development. Such an explanation cannot, however, be applied to the present findings. Zelená (1962) compared the relative maturity of tibialis anterior (t.a.) in the leg, and flexor digitorum brevis (f.d.b.) in the foot, of the rat 3 days after birth. T.a. contained very few myotubes and had more or less completed its maturation, while f.d.b. contained more myotubes and their transition into muscle fibres proceeded more slowly. When both muscles were denervated 2 days after birth, f.d.b. was more affected than t.a. Further evidence of the relative maturity of the leg muscles comes from a comprehensive study of the e.d.l. muscle by Ontell (1979). She concludes that no new formation of fibres occurs to any significant degree post-natally in the e.d.l. muscle of the rat. In the present study, therefore, it seems very unlikely that the decrease in muscle fibre number is due to the suppression of new fibre formation.

The findings of Zelená (1962) described above, also suggest it to be unlikely that the muscle fibre loss occurred during the interval between nerve crush and reinnervation. Even after permanent denervation at birth, relatively few fibres are seen to degenerate and the rate of growth of leg muscles during the first 2 weeks is almost as high as that of innervated muscles (Vrbová, 1952; Zelená, 1962; Stewart, 1968). If the effects of denervation on young muscles indeed depend on their degree of maturity, it would be expected that muscles which were denervated at 6 days and reinnervated by 18 days of age would show even less degeneration during the intervening period. We must therefore consider the possibility that most of the muscle fibre loss occurred after reinnervation began. This may have been due either to the motor nerves failing to reach all of the muscle fibres, or alternatively the muscle fibres may have failed to respond appropriately to the incoming motor nerve.

The activity of the motoneurone changes considerably during the second and third weeks of the post-natal development of the rat in that the frequency of firing of motoneurones to fast muscles increases, while motoneurones to slow muscles continue to fire at low rates (Bursian & Sviderskaya, 1981; preliminary communication by Navarrete & Vrbová, 1980). Nerve crush at 5–6 days isolates the muscle from its nerve during most of this transition period. It is known that the development of the

denervated muscle fibres is retarded (Zelená & Hník, 1963; Engel & Karpati, 1963; Shafiq, Asiedu & Milhorat, 1972), while the central connexions to motoneurones disconnected from their targets probably continue to develop. Thus upon reinnervation immature muscle fibres in fast muscles would be exposed to high firing rates from their motoneurones, while muscle fibres in slow muscles would be activated at low frequencies. It is possible that the high firing rates imposed on immature muscle fibres could bring about their degeneration. Preliminary histological examination of these muscles during early stages of reinnervation supports this view, and this aspect of the study is now being investigated. Thus only muscle fibres supplied by fast firing neurones would be affected, while muscle fibres supplied by motoneurones firing at low rates would be spared. The finding that soleus muscles were much less affected by the operative procedure than fast muscles supports this explanation.

Professor T. J. Biscoe kindly allowed us to use his computer system for measurement of muscle fibre and motoneurone areas. We thank both him and Dr S. McHanwell for their help in this aspect of the work and for their advice on the HRP labelling technique.

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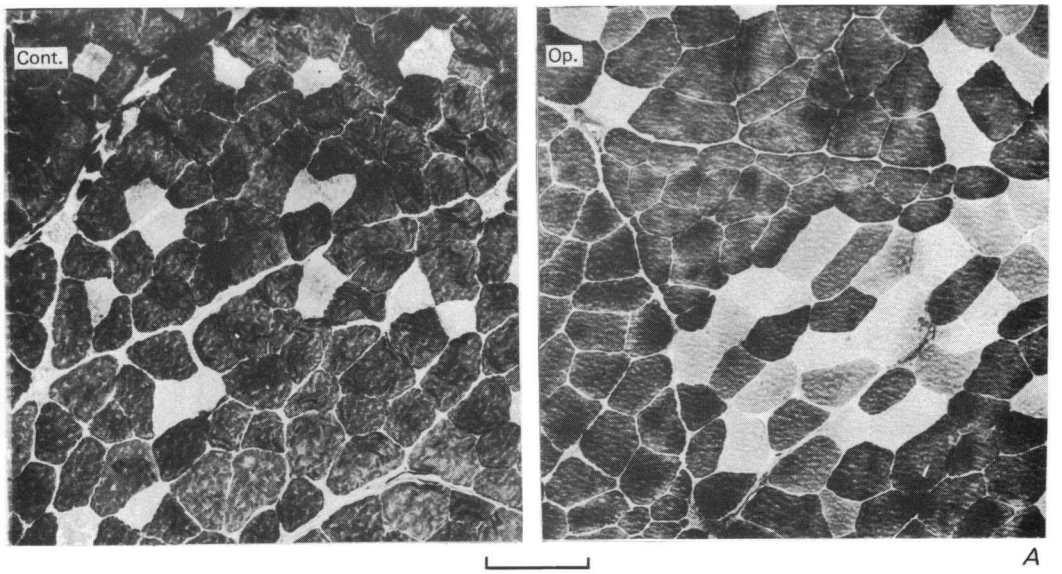
EXPLANATION OF PLATES

PLATE 1

A, transverse section from control and reinnervated e.d.l. muscles stained for ATPase (pre-incubated at pH 9·4). The muscles were taken 93 days after nerve-crush at 6 days.
B, transverse sections of control and reinnervated e.d.l. muscles stained for SDH. The muscles were taken 11 weeks after nerve crush at 6 days.
C, transverse sections of control and reinnervated t.a. muscles, from the same animal as in Fig. 2, stained for SDH.

PLATE 2

Transverse sections of spinal cord processed to demonstrate HRP and counter-stained with gallocyanin. HRP had been injected into the t.a. and e.d.l. muscles at 5 weeks of age. *A* shows localization of the HRP-labelled motoneurones in the dorsolateral part of the anterior horn. The notch in the dorsal horn indicates the unoperated side contralateral to the nerve crush. *B* shows motoneurones from the same horn containing HRP granules in their cytoplasm, and their nucleoli stained with gallocyanin.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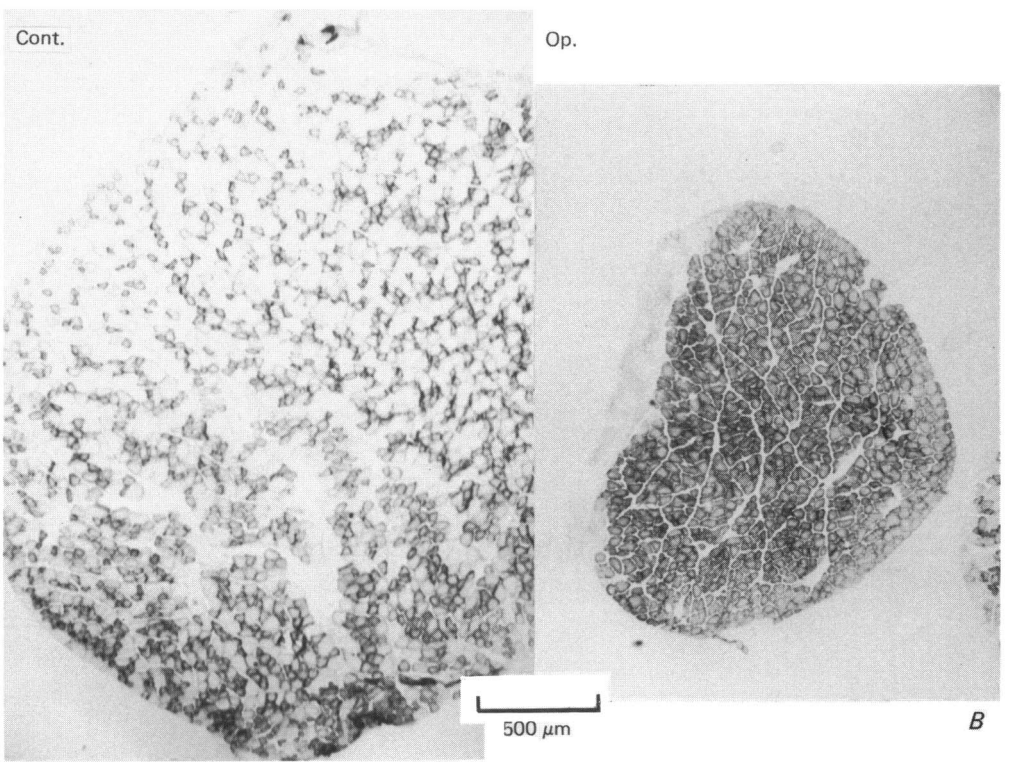
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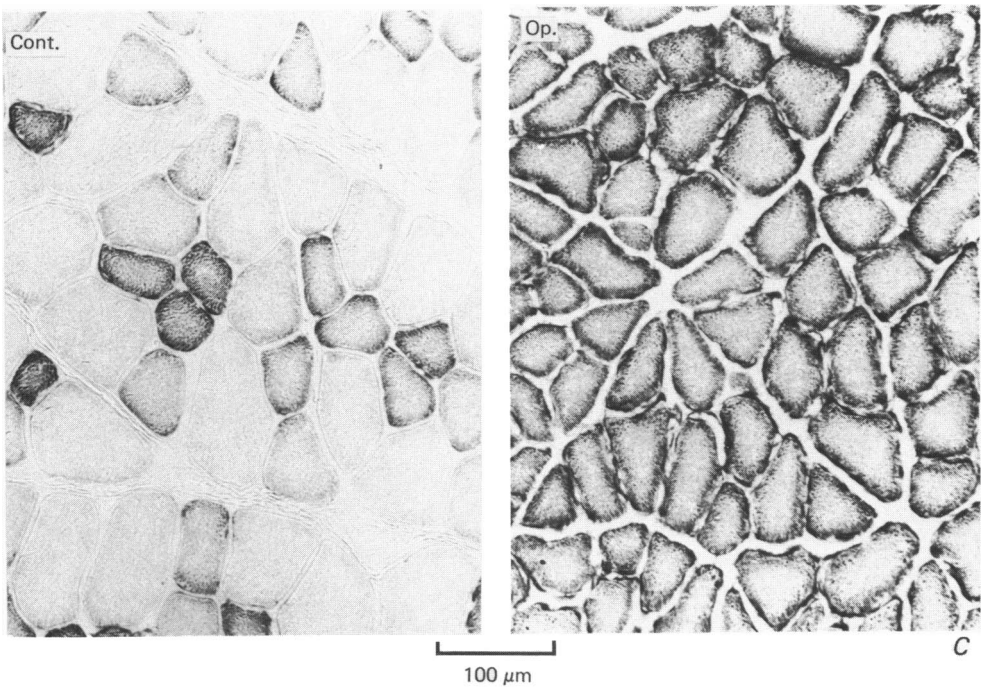
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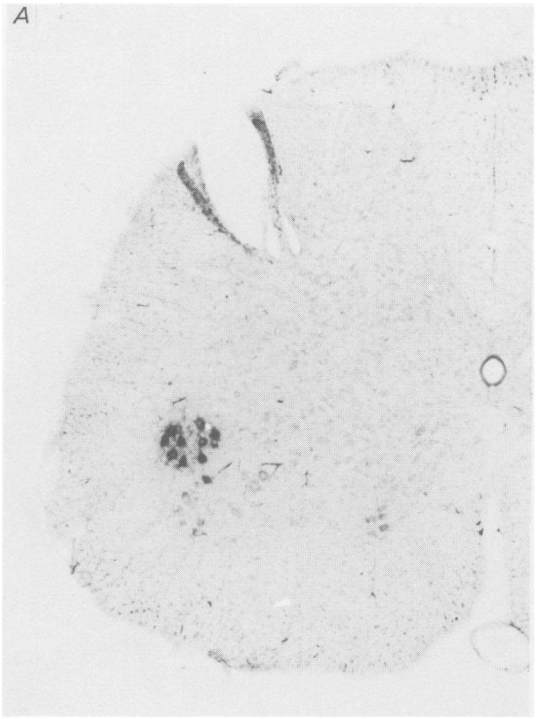
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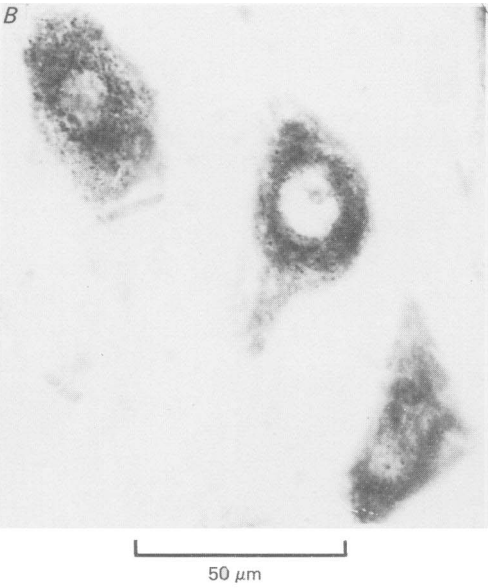
B
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