Pflügers Arch. 353, 227–239 (1975)

Contractile and Histochemical Properties of Regenerating Cross-Transplanted Fast and Slow Muscles in the Rat*

Ernest Gutmann and Bruce M. Carlson

Institute of Physiology, Czechoslovak Academy of Sciences, Praha, Czechoslovakia and Department of Anatomy, University of Michigan, Ann Arbor, Michigan, USA

Received September 4, 1973

Summary. The soleus (SOL) or extensor digitorum longus (EDL) muscles of month-old rats were denervated for 14 days and then cross-transplanted so that the fast muscle was placed into the bed of the slow muscle and vice versa. At 17, 30, 60, and 90 days the transplants were tested for certain contractile and histochemical properties. By 90 days the cross-transplanted SOL showed complete conversion of the full contraction time and nearly complete conversion of the half relaxation time to those of the normal EDL. In contrast, the contraction and relaxation times of the cross-transplanted EDL became considerably slowed, but did not attain the values of the normal SOL. Histochemical staining for ATPase and SDH activity demonstrated similar transformations of fiber types. The degree of transformation of twitch and histochemical characteristics in cross-transplanted muscles was greater than the values reported after cross-innervation of the same muscles. The cross-transplantation model has certain advantages over nerve cross-union experiments because the cross-transplanted muscle is placed in the normal functional environment of the other muscle.

Key words: Muscle Transplantation — Muscle Regeneration — Histochemistry of Muscle — Contractile Properties of Muscle Transplants — Denervation of Muscle.

It is well known that skeletal muscle not only depends upon nerve for maintenance and normal functional ability but that many of the properties which characterize and distinguish one type of muscle from another are a reflection of a nervous influence [13, 19, 24, 25]. The most cogent evidence for the latter assertion was provided by the work of Buller *et al.* [4], who found that by crossing the nerves from a fast to a slow muscle and vice versa in cats, the contractile properties of these muscles become modified to resemble those of a muscle normally innervated by the transposed nerve. Work conducted since that time has confirmed this phenomenon in rats [1, 11, 12]. It has been demonstrated by now that not

* Supported by grants from the Muscular Dystrophy Associations of America and a scientific exchange between the Academies of Sciences of Czechoslovakia and the United States.

only the contractile properties but also the histochemical [14,22,23,29, 35,37] and biochemical [13,18,23,30,34] profiles of the muscles are correspondingly modified. More recently changes in specific properties of the myosin molecules themselves have been demonstrated in cross-innervated muscles [1,5,40].

Almost all studies on the cross-innervation of muscles have been conducted upon normal growing or mature muscles. In these studies the muscles have been allowed to remain in their normal location and the nerves were anatomically translocated. Another dimension to the study of cross-innervation effects can be added by translocating fast and slow muscles instead of their motor nerves. Effective autotransplantation of mammalian muscles can be accomplished by employing the minced muscle system of regeneration [6,7,41] or the free grafting of previously denervated muscles (Carlson and Gutmann: unpublished data; see also [42,43]). In both of these models extensive regeneration of new muscle fibers accounts for the success of the transplantation procedure. Therefore "cross-innervation" studies using the model of muscle transplantation necessarily involve mechanisms effecting changes in fundamental properties of regenerating muscle cells under the influence of foreign nerves. Salafsky *et al.* [39] have recently reported the results of muscle cross-transplantation with the minced muscle system in rats, and in this report we shall consider the effects of cross-transplantation upon certain contractile and histochemical properties of the soleus (SOL) and extensor digitorum longus (EDL) muscles in the rat.

Materials and Methods

The experiments were conducted upon 34 1 month-old male Wistar rats from the colony at the Institute of Physiology in Prague. Previous experiments had suggested and shown that the regeneration process in transplants is more successful after prior denervation of the graft [8]. Therefore we denervated the left limbs of all rats 2 weeks before muscle transplantation. This experiment consisted of two cross-transplantation series. In one, the 2 week denervated left EDL was freely grafted in place of the normal right SOL, and in the other series the denervated left SOL was grafted in place of the right EDL. The grafted muscles were held in place by suturing their tendons of origin and insertion to the corresponding tendons of the removed muscles. The stumps of the nerves leading to the muscles were positioned near the grafts. Previous work [8] had shown that suturing of the nerves to the grafts was not necessary to obtain innervation of the transplanted muscles.

After postoperative intervals of 17, 30, 60, and 90 days, grafts were subjected to physiological and histochemical analysis. The cross-transplanted muscles were removed from etherized animals and placed into an oxygenated tissue culture medium. Contractile responses were elicited in vitro by direct stimulation with rectangular pulses of 1.0 msec. Recordings were made with an automatic analyzer described earlier [7,36]. The following contractile properties were measured: twitch and tetanic tension, latency period (LP), full contraction time (FCT), half relaxation time (HRT) and time parameters of contraction (TPC) of the twitch and tetanus.

Following the determination of contractile properties, the regenerates were frozen in liquid nitrogen and tested histochemically for succinic dehydrogenase (SDH) [32] and myosin adenosine triphosphatase (ATPase) [21,33] as in our previous study [8].

Results

Contractile Properties. Both the slow SOL muscles transplanted into the bed of the fast EDL and the EDL muscles transplanted in place of the SOL muscles demonstrated conversions of their contractile properties in the direction of those possessed by the muscles in whose beds they were grafted and by whose nerves they were innervated.

Table 1 shows the changes in contractile behavior of the 14 day denervated SOL muscle grafted in place of the contralateral EDL. The originally slow SOL muscle is progressively converted into a fast muscle. By 90 days the FCT and TPC of the twitch are practically the same as those in the normal EDL 90 days after birth. Both the LP and HRT of 90 day transplants were prolonged in comparison with the normal EDL of a comparable age. The tetanic TPC was somewhat longer than that of the normal EDL.

The changes in contractile properties of the EDL muscle cross-transplanted into the bed of the SOL are illustrated in Table 2. The contraction times start out slow, as would be expected for a denervated fast muscle, and after an intermediate period of slightly increased speed (30 and 60 days), they remain slower than those of the normal EDL, but not so slow as those of the normal SOL. The temporary slight speeding up of the contraction times of 30 and 60 day EDL cross-transplants resembles the similar phenomenon observed in the normal SOL muscle during the postnatal period [3,10,26] and is due to a progressive loss of muscle fibers with high ATPase activity [27]. Although the LP of the 90 day EDL cross-transplants was slower than that of either the normal EDL or SOL, the other contractile parameters (TPC, FCT and HRT) are clearly intermediate between those of the normal SOL and EDL. The recovery of twitch tension and particularly tetanic tension in the cross-transplanted EDL is considerably less successful than that of the cross-transplanted SOL.

Histochemical Properties. The histochemical observations are in agreement with the contractile behavior of the cross-transplanted muscles. Despite the histochemical inhomogeneity of fiber types in both the EDL and SOL muscles of the rat, differences between these muscles are pronounced in terms of overall enzyme activity and fiber pattern. This is particularly true with respect to ATPase and SDH activity of the muscle fibers. In the present experiments, conversion of fiber type patterns after cross-transplantation was readily apparent in both the EDL and SOL.

Table 1. Contractile properties of the 14-day denervated

Age (days)	TwT (g)	LP (msec)	TPC (msec)	FCT (msec)
17 ($n = 3$)	1.62 ± 0.59	4.77 ± 0.19	7.63 ± 0.09	24.13 ± 0.76
30 ($n = 4$)	1.56 ± 0.22	4.43 ± 0.12	6.08 ± 0.36	20.43 ± 0.90
60 ($n = 5$)	2.72 ± 0.59	3.90 ± 0.21	3.28 ± 0.27	13.90 ± 0.66
90 ($n = 6$)	5.11 ± 1.52	3.63 ± 0.13	3.68 ± 0.29	12.95 ± 0.26
	Contractile properties of normal EDL 90 days after birth			
90 ($n = 3$)	23.60 ± 2.40	3.00 ± 0.12	4.47 ± 0.32	12.67 ± 0.75

Table 2. Contractile properties of the 14-day denervated

Age (days)	TwT (g)	LP (msec)	TPC (msec)	FCT (msec)
17 ($n = 3$)	3.29 ± 0.27	4.15 ± 0.35	7.7 ± 0.1	23.30 ± 0.30
30 ($n = 5$)	1.44 ± 0.54	4.64 ± 0.27	6.44 ± 0.07	21.60 ± 0.82
60 ($n = 4$)	1.40 ± 0.31	4.28 ± 0.12	6.68 ± 0.28	20.97 ± 0.92
90 ($n = 4$)	2.43 ± 4.37	4.52 ± 0.15	7.75 ± 0.16	23.55 ± 1.20
	Contractile properties of normal SOL 90 days after birth			
90 ($n = 3$)	16.93 ± 1.56	4.03 ± 0.20	14.13 ± 0.38	37.00 ± 1.79

The normal EDL muscle is characterized by relatively high ATPase activity, with a predominance of type II fibers whereas the normal SOL possesses low ATPase activity and a majority of type I along with a few type II fibers [17]. The recovery of fiber pattern in cross-transplanted muscles differs somewhat from that observed after the free orthotopic grafting of these muscles [8]. In transplants of the EDL into the bed of the contralateral EDL, a predominantly uniform pattern of muscle fibers (ATPase) is found at 30 days. By 60 days after transplantation a mosaic of fiber types, consisting of a few type I fibers interspersed among a majority of type II fibers, has been established. Thirty day transplants

SOL muscle transplanted in the place of the EDL

HRT (msec)	TetT (g)	Tet TPC (msec)	Wt (mg)
38.80 ± 2.0	9.07 ± 8.95	56.05 ± 2.20	
33.13 ± 2.46	6.05 ± 1.71	38.80 ± 2.76	
17.76 ± 1.06	26.27 ± 9.37	64.16 ± 9.37	85.20 ± 14.73
18.00 ± 0.69	94.75 ± 9.18	39.02 ± 6.24	88.75 ± 8.75
11.37 ± 1.53	175.7 ± 7.00	30.00 ± 3.79	131.30 ± 8.97

EDL muscle transplanted in place of the SOL

HRT (msec)	TetT (g)	Tet TPC (msec)	Wt (mg)
23.00 ± 0.10	18.08	35.7	
26.96 ± 3.53	2.10 ± 0.96	78.6 ± 5.9	39.67 ± 5.49
31.85 ± 1.95	4.27 ± 1.20	78.83 ± 4.93	44.67 ± 4.37
31.50 ± 2.16	17.82 ± 8.12	86.93 ± 20.06	55.00 ± 20.01
54.30 ± 3.45	81.07 ± 7.51	96.6 ± 2.98	109.00 ± 12.00

of the SOL in place of the contralateral one are characterized by fibers with low ATPase activity and only occasional fibers with higher activity.

The SOL muscle cross-transplanted into the bed of the EDL already shows by 30 days considerable histochemical differences from both the normal SOL muscle and orthotopic SOL grafts. The majority of fibers are type II, with high ATPase activity (Fig. 1). Some type I fibers possessing less ATPase activity are, however, interspersed among these. SDH activity of the 30 day SOL cross-transplants is low. By 90 days the muscle fiber diameters have increased to near normal values and the conversion of histochemical pattern is almost complete. Fig. 2 illustrates the increased

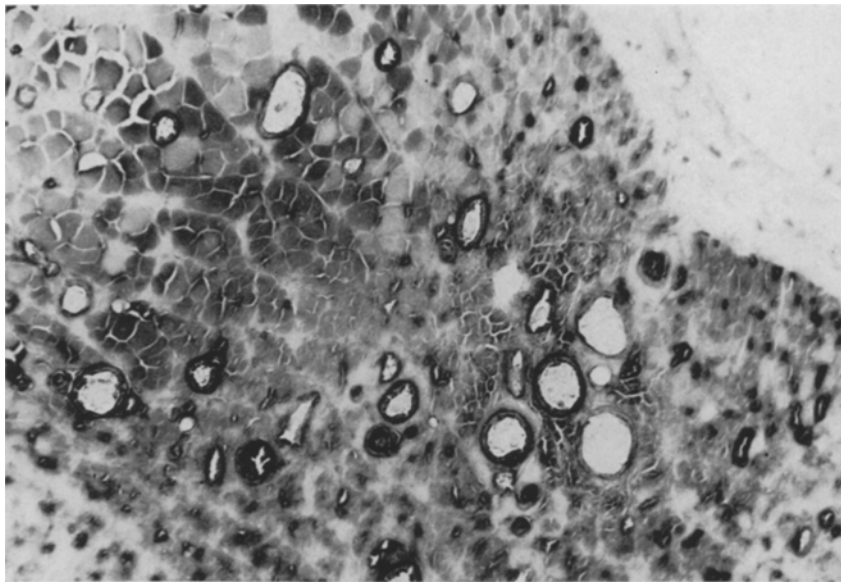

Fig. 1. Thirty day graft of the denervated (14 days) SOL muscle transplanted in place of the contralateral EDL. ATPase. 155×

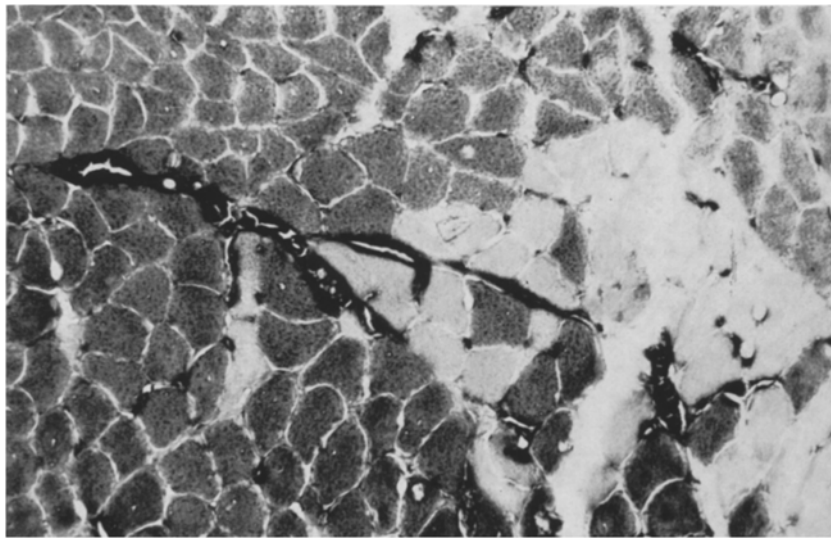

Fig. 2. Ninety day graft of the denervated (14 days) SOL muscle transplanted in place of the contralateral EDL. ATPase. 155×

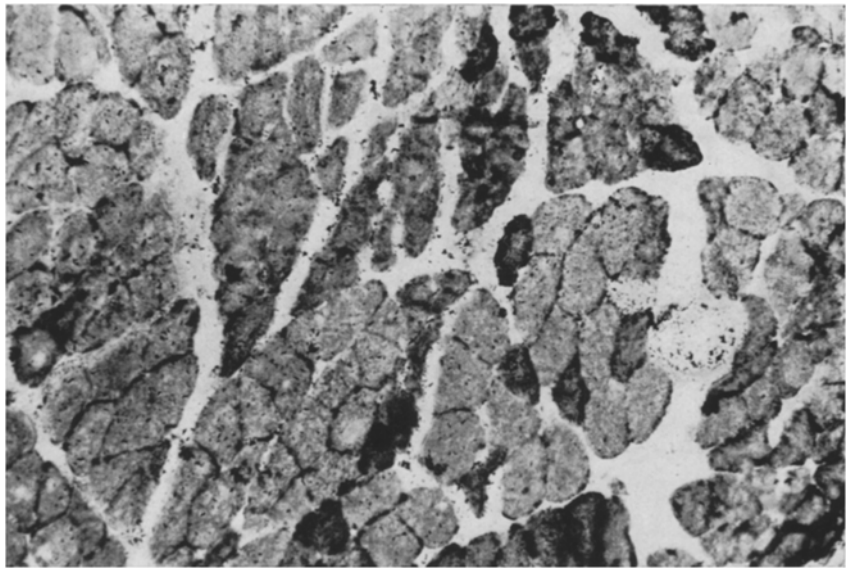

Fig. 3. Ninety day graft of the denervated (14 days) SOL muscle transplanted in the place of the contralateral EDL. SDH. 155×

fiber diameter and the well-demarcated populations of type I and II fibers, with the prevalence of type II fibers in ATPase preparations. A mixed fiber pattern is also observed with respect to SDH activity in 90 day SOL cross-transplants (Fig. 3), and in this case fibers of low oxidative activity predominate.

The EDL muscles transplanted in place of the SOL are also characterized by changes in histochemical pattern. Thirty day EDL cross-transplants possess primarily fibers with low ATPase activity (type I) as well as fibers with an intermediate type of ATPase activity. The SDH activity of these 30 day cross-transplants is high. In 90 day EDL cross-transplants, ATPase staining reveals a predominance of type I fibers along with a few interspersed intermediate or type II fibers (Fig. 4).

Although the cross-transplanted EDL and SOL muscles do demonstrate conversions in the patterns of ATPase and SDH activity, it is not so clear-cut as in the case of cross-innervation studies. This may be due to several possible causes—1. changes in the fiber pattern associated with the preliminary denervation period, 2. "type grouping" of similar muscle fibers into uniform fields due to the pattern of reinnervation [29] or 3. topographical differences due to the pattern of revascularization of the grafts. Often groups of fibers with higher activity are found around capillaries.

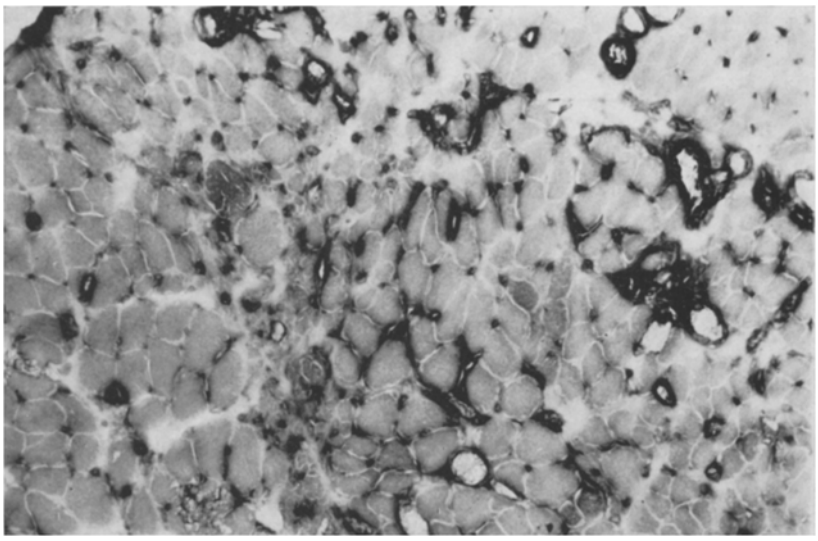

Fig. 4. Ninety day graft of the denervated (14 days) EDL muscle transplanted in place of the contralateral SOL. ATPase. 155×

Discussion

The results of this experiment are in agreement with the findings of others [4, 12, 15] that the motor nerve exerts a profound influence upon the contractile properties of a muscle. Most of the results in the literature to date have been obtained by leaving the muscles intact and crossing the nerves supplying the respective fast and slow muscles. In this procedure the muscles go through a temporary period of denervation before the foreign nerve can exert its effect. The physical relationships of the cross-innervated muscles to the surrounding tissues remain unchanged except that in some instances the nerve-crossing experiments may leave neighboring groups of muscles denervated, thus altering the functional pattern of the limb as a whole. The experimental design of the present work as well as that of Salafsky *et al.* [39] differs from the classical cross-innervation design in that it is the muscle itself which is translocated, not the nerve. This experimental model has the advantage of not disturbing the functional relationships of the other muscles of the limb. Some caution must be exercised in making exact comparisons between properties of cross-transplanted and cross-innervated muscles because of peripheral mechanical factors, especially stretching or shortening of the muscle by changes in antagonistic muscles. In view of the recent report by Guth *et al.* [20], this consideration appears to be more important in cross-innervation experiments than has been hitherto suspected.

Both these results and those of Salafsky *et al.* [39] demonstrate that regenerating fast and slow muscle is equally subject to conversion into the opposite type by a foreign nerve as is intact mature muscle. Salafsky *et al.* [39] employed the minced muscle system of regeneration for their experiments. This system has the advantage of providing a pure population of regenerating muscle fibers without any residual mature muscle. The disadvantage of mincing, for the SOL and EDL or tibialis anterior muscles in rats, is the inconsistency of the results. Particularly in the case of minced muscle transplanted into the bed of the SOL, the results are erratic, with a large percentage of cases producing non-functional regenerates or no regenerates at all. Because of poor to inconsistent results in almost 150 orthotopic and cross-transplantations of the SOL and the EDL muscles in rats (Carlson and Gutmann: unpublished data), the free grafting of denervated but otherwise intact muscles was employed in the work reported here. A major advantage of freely grafted muscles as opposed to minced muscles as a source of regenerating material is the greater proportion of functional regenerates (100 %) and their consistently larger size. A disadvantage of free grafts over minced muscle regenerates, although not so significant in long term experiments (such as cross-transplantations) as in short term ones, is the survival of a small number of original denervated muscle fibers along the periphery of the grafts [8].

The contraction times of the cross-transplanted muscles in these experiments demonstrated a greater degree of conversion than the results reported after cross-innervation of the same muscles [1]. The mean contraction time of the cross-transplanted SOL was 12.95 msec in comparison with the 15.6 msec reported by Bárány and Close [1] for the cross-innervated SOL. These values represent a considerable reduction from that of the normal SOL (37.0 msec in this experiment and 37.8 msec by Bárány and Close [1]) and approach very closely that of the normal EDL (12.57 msec [8] and 12.56 [1]). The cross-transplanted EDL has a mean contraction time of 23.55 msec and that of the cross-innervated EDL is 21.33 msec [1]. These latter values are almost midway between those of the normal EDL and SOL and do not differ greatly from the contraction times of the long term denervated EDL. The greater degree of conversion of the cross-transplanted muscles may be attributed to 1. the nerves' acting upon developing, rather than mature, muscle fibers, 2. the fact that the transplanted muscle rests in the functional environment of a muscle of the opposite functional type and 3. the avoidance of peripheral complications, such as denervation effects, to other muscles as occurs in cross-innervation experiments.

Our finding agree with those of Salafsky *et al.* [39] with respect to slow muscles transplanted into the bed of fast muscles, but they differ in the case of fast muscles transplanted into the bed of slow muscles. In minced

muscle regenerates they found almost complete conversion of the contraction times of cross-transplanted fast muscles (tibialis anterior). The basis for this difference in the two systems of cross-transplanted regenerating muscles is not readily apparent.

The conversion of half relaxation times follows a pattern similar to that of the full contraction times. As compared with HRT's reported as 11.37 msec [8] and 8.68 msec [1] for the normal EDL and 54.30 msec [8] and 54.75 msec [1] for the normal SOL, the HRT of the cross-transplanted EDL (31.50 msec) exceeds that of the cross-innervated EDL (25.74 msec [1]), and that of the cross-transplanted SOL (18.00 msec) is less than that of the cross-innervated SOL (19.31 msec [1]).

The reason for the incomplete conversion of contraction times of the cross-transplanted EDL remains unexplained. It is known from cross-union experiments in chickens that transformation is much less successful when performed at a later age [28] than when performed at an early age [44]. Nevertheless the muscle transplantations were performed when the rats were still in a rapid pre-pubertal phase of growth. It is possible that the contractile properties of fast muscles are more completely dictated by their motor nerve supply than are those of slow muscles and that the difference between the contraction times of fast muscles transplanted in place of slow ones and the contraction times of normal slow muscles is due to some inherent myogenic properties of slow muscles. On the other hand the same pattern of results could be explained by slow muscles' having no independent myogenic properties whereas fast muscles possess an intrinsic myogenic component which is partially resistant to the transforming effects of a slow nerve.

At the onset of the cross-transplantation experiment, the EDL contracts more slowly than normally and the SOL more quickly because of the preliminary 14 day period of denervation [26]. Nevertheless the conversion of contractile and histochemical properties of the muscle fibers begins by the end of the first month after cross-transplantation. According to Eccles *et al.* [16], the neural influence upon the speed of muscle contraction becomes effective within a few days of reinnervation in cross-innervated muscles. The time of establishment of motor end plates has not yet been determined in cross-transplanted muscles, but in regenerating minced muscles, motor end plates become established during the late third or early forth week of development (Mong, unpublished data; see also [2,45]). If the temporal pattern of reinnervation in the regenerating cross-transplanted muscles is similar to that of minced muscles, then the early phases of the conversion of contractile properties in the present experiment would agree with the findings of Eccles *et al.* [16].

These experiments show that functional and histochemical conversion of heterotopically grafted muscles occurs according to the type of nerve

which innervates the graft and that the neural effect upon regenerating muscle is somewhat more successful than that of cross-united nerves upon muscles remaining in their normal location. Transplantation of muscle thus promises to become a potent tool in studies of neuronal specificity and in attempts to distinguish between neurogenic and myogenic factors affecting the differentiation of muscle.

References

1. Bárány, M., Close, R. I.: The transformation of myosin in cross-innervated rat muscles. J. Physiol. (Lond.) **213**, 455—474 (1971)
2. Bennett, M. R., Florin, T., Woog, R.: The formation of synapses in regenerating mammalian striated muscle. J. Physiol. (Lond.) **238**, 79—92 (1974)
3. Buller, A. J., Eccles, J. C., Eccles, R. M.: Differentiation of fast and slow muscles in the cat hind limb. J. Physiol. (Lond.) **150**, 399—416 (1960)
4. Buller, A. J., Eccles, J. C., Eccles, R. M.: Interaction between motoneurones and muscles in respect of the characteristic speeds of their responses. J. Physiol. (Lond.) **150**, 417—439 (1960)
5. Buller, A. J., Mommaerts, W. F. H. M., Seraydarian, K.: Enzymatic properties of myosin in fast and slow muscles of the cat following cross-innervation. J. Physiol. (Lond.) **205**, 581—597 (1969)
6. Carlson, B. M.: The regeneration of minced muscles. Basel: S. Karger AG 1972
7. Carlson, B. M., Gutmann, E.: Development of contractile properties of minced muscle regenerates in the rat. Exp. Neurol. **36**, 239—249 (1972)
8. Carlson, B. M., Gutmann, E.: Transplantation and "cross-transplantation" of free muscle grafts in the rat. Experientia (in press, (Basel) 1974)
9. Reference deleted.
10. Close, R.: Dynamic properties of fast and slow skeletal muscles of the rat during development. J. Physiol. (Lond.) **173**, 74—95 (1964)
11. Close, R.: Effects of cross-union of motor nerves to fast and slow skeletal muscles. Nature (Lond.) **206**, 831—832 (1965)
12. Close, R.: Dynamic properties of fast and slow skeletal muscles of the rat after nerve cross-union. J. Physiol. (Lond.) **204**, 331—346 (1969)
13. Drahota, Z., Gutmann, E.: Long-term regulatory influence of the nervous system on some metabolic differences in muscle of different function. Physiol. bohemoslov. **12**, 339—348 (1963)
14. Dubowitz, V.: Cross-innervated mammalian skeletal muscle: histochemical, physiological and biochemical observations. J. Physiol. (Lond.) **193**, 481—496 (1967)
15. Eccles, J. C.: The effects of nerve cross-union on muscle contraction, pp. 151 to 160, in Exploratory concepts in muscular dystrophy and related disorders. A. T. Milhorat (Ed.). Excerpta med. (Amst.) Internat. Congress Series, No. 147 (1967)
16. Eccles, J. C., Eccles, R. M., Kozak, W.: Further investigations on the influence of motoneurones on the speed of muscle contraction. J. Physiol. (Lond.) **163**, 324—339 (1962)
17. Engel, W. K.: The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. Neurology (Minneap.) **12**, 778—794 (1962)
18. Golish, G., Pette, D., Pichelmaier, H.: Metabolic differentiation of rabbit skeletal muscle as induced by specific innervation. Europ. J. Biochem. **16**, 110—116 (1970)

19. Guth, L.: "Trophic" influences of nerve on muscle. Physiol. Rev. **48**, 645–687 (1968)
20. Guth, L., Jean, D. H., Wells, J. B., Albers, R. W.: Skeletal muscle plasticity (abstr.). Anat. Rec. **175**, 336 (1973)
21. Guth, L., Samaha, F. J.: Procedure for the histochemical demonstration of actomyosin ATPase. Exp. Neurol. **28**, 365–367 (1970)
22. Guth, L., Samaha, F. J., Albers, R. W.: The neural regulation of some phenotypic differences between the fiber types of mammalian skeletal muscle. Exp. Neurol. **26**, 126–135 (1970)
23. Guth, L., Watson, P. K., Brown, W. C.: Effects of cross-reinnervation on some chemical properties of red and white muscles of rat and cat. Exp. Neurol. **20**, 52–69 (1968)
24. Gutmann, E.: The denervated muscle. Prague: Publ. House Czechoslovak Acad. Sci. **1962**
25. Gutmann, E., Hník, P.: The effect of use and disuse on neuromuscular functions. Prague: Publ. House Czechoslovak Acad. Sci. 1963
26. Gutmann, E., Melichna, J., Syrový, I.: Contraction properties and ATPase activity in fast and slow muscle of the rat during denervation. Exp. Neurol. **36**, 488–497 (1972)
27. Gutmann, E., Melichna, J., Syrovy, I.: Developmental changes in contraction time, myosin properties and fibre pattern of fast and slow skeletal muscles. Physiol. bohemoslov. **23**, 19–30 (1974)
28. Hník, P., Jirmanová, I., Vyklický, L., Zelená, J.: Fast and slow muscles of the chick after nerve cross-union. J. Physiol. (Lond.) **193**, 309–325 (1967)
29. Karpati, G., Engel, W. K.: "Type grouping" of skeletal muscles after experimental reinnervation. Neurology (Minneap.) **18**, 447–455 (1968)
30. Mommaerts, W. F. H. M., Buller, A. J., Seraydarian, K.: The modification of some biochemical properties of muscle by cross-innervation. Proc. nat. Acad. Sci. (Wash.) **64**, 128–133 (1969)
31. Reference deleted.
32. Nachlas, M. M., Tsou, K. C., Souza, E., Cheng, C. S., Seligman, A. M.: Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. J. Histochem. Cytochem. **5**, 420–436 (1957)
33. Padykula, H. A., Herman, E.: The specificity of the histochemical method for adenosine triphosphate. J. Histochem. Cytochem. **3**, 170–195 (1955)
34. Prewitt, M. A., Salafsky, B.: Enzymic and histochemical changes in fast and slow muscles after cross-innervation. Amer. J. Physiol. **218**, 69–74 (1970)
35. Robbins, N. G., Karpati, G., Engel, W. K.: Histochemical and contractile properties in the cross-innervated guinea pig soleus muscle. Arch. Neurol. (Chic.) **20**, 318–329 (1969)
36. Rohlíček, V., Gutmann, E.: The contrast of contraction time, a new expression of maximal rate of development. Physiol. bohemoslov. **21**, 430–431 (1972)
37. Romanul, F. C. A., van der Meulen, J. P.: Reversal of the enzyme profiles of muscle fibers in fast and slow muscles by cross-innervation. Nature (Lond.) **212**, 1369–1370 (1966)
38. Romanul, F. C. A., van der Meulen, J. P.: Slow and fast muscles after cross innervation. Arch. Neurol. (Chic.) **17**, 387–402 (1967)
39. Salafsky, B., Akbarzadeh, P., Sahid-Sales, K.: Characteristics of orthotopic and heterotopic regenerated fast and slow skeletal muscle. In: Proceedings of the II International Congress on Muscle Diseases. B. Kakulas (Ed.). Excerpta med. (Amst.) Internat. Congress Series, No. 294-5 (1973).

40. Samaha, F. J., Guth, L., Albers, R. W.: Differences between slow and fast muscle myosin. J. biol. Chem. **245**, 219–224 (1970)
41. Studitsky, A. N.: Experimental surgery of muscles (Russian). Moscow: Izdatel. Akad. Nauk SSSR 1959
42. Studitsky, A. N., Bosova, N. N.: Development of atrophic muscular tissue in conditions of transplantation in place of mechanically damaged muscles (Russian). Arkh. Anat. Gistol. Embriol. **39**, 18–32 (1960)
43. Thompson, N.: Autogenous free grafts of skeletal muscle. Plast. reconstr. Surg. **48**, 11–27 (1971)
44. Zelená, J., Jirmanová, I.: Effect of cross innervation on the ultrastructure of avian muscle. Exp. Neurol. **38**, 272–285 (1973)
45. Zhenevskaya, R. P.: Development of innervation of muscle, restored by means of the transplantation of minced muscle tissue (Russian). Dokl. Akad. Nauk SSSR, **96**, 217–220 (1954)

Dr. Bruce M. Carlson
Department of Anatomy
4622 Medical Sciences II
University of Michigan
Ann Arbor, MI 48104/U.S.A.