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SKELETAL MUSCLE FIBER REGENERATION FOLLOWING HETEROTOPIC AUTOTRANSPLANTATION IN CATS

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Summary

Whole 3 g extensor digitorum longus (EDL) muscles of cats were autotransplanted. The EDL muscles were either transplanted without denervation prior to transplantation (normal transplants) or denervated 3 to 4 weeks prior to transplantation (pre-denervated transplants). A few peripheral skeletal muscle fibers survived transplantation but most fibers degenerated and then regenerated as the transplant became revascularized. Both normal and pre-denervated muscles regenerated successfully and by 50 days after transplantation fibers which had reinnervated showed high and low myofibrillar ATPase activity. Compared to controls, the smaller mean fiber cross-sectional area of the transplants was due to the large number of small fibers, but some fibers in the transplant were larger than any fibers observed in the controls. Transplants regained 57 percent of the muscle mass of the controls. Contraction and half relaxation times of transplanted muscles were slower than controls, but peak isometric tetanus tension per cm^2 of muscle was nearly normal. Fifty to 170 days after transplantation, muscles showed low oxidative capacity and fatigued rapidly.

Since Studitsky and Bosova (13) demonstrated the successful free autologous (belonging to the same organism) transplantation of whole skeletal muscles, survival of transplants have been reported for rats (3, 4), cats (7), dogs (14), and man (6, 7, 14). Controversy still surrounds the degree of success and the reasons for the success of the skeletal muscle transplants. The degree of restoration of normal structure and function after transplantation, the mass of muscle that can be transplanted, and the relative percentages of surviving compared to regenerating muscle fibers are all unresolved questions. The resolution of these controversies is important because several hundred transplantations have been made for the clinical treatment of partial or complete facial paralysis (6, 14) or for anal incontinence (7). Our purpose was to investigate the controversial aspects of skeletal muscle transplantation.

Materials and Methods

Surgery was performed on 32 adult cats 4.2 ± 0.2 kg in weight that were free of disease and that had been maintained in the Animal Care Facilities for at least one month. Surgery was performed with the cats anesthetized by

ketamine (20 mg/kg) and pentobarbital (10 mg/kg). Supplemental doses were given as required. Free heterotopic auto-transplants were made of whole extensor digitorum longus (EDL) muscles (mean weight = 3.3 ± 0.2 g)[1]. The muscles were transplanted without denervation prior to transplantation (normal transplants), or with denervation 3 to 4 weeks prior to transplantation (pre-denervated transplants). The muscle was denervated by sectioning three branches of the peroneus profundus nerve at the point where each branch enters the muscle. No attempt was made to prevent re-innervation but at the time of transplantation stimulation of the nerve proximally failed to elicit contraction of the muscle. This in addition to the lengthened time to peak tension when the pre-denervated muscle was stimulated directly indicated that these muscles were denervated and had not functionally re-innervated.

Transplantation proceeded as follows: The skin was incised and the fascia was cut over the full length of the EDL. The muscle was isolated by severing all nerves and vascular connections, as well as both tendons. Each EDL was removed and weighed under sterile conditions. Each muscle was then placed into the bed of the contralateral muscle with the EDL oriented in the proximal-distal, and medial-lateral aspects as it was in its original site. Consequently, the muscle was reversed in its dorsal-ventral orientation. Both tendons of each transplanted muscle were sutured into place and fascia and skin were closed by sutures. No attempt was made to surgically reestablish nervous or vascular connections.

The operated cats were sacrificed between 4 and 170 days after the transplantation. From 4 to 50 days after transplantation (N = 9), our studies focused on the histological analysis of hematoxylin and eosin (H and E) stained sections, and from 50 to 170 days (N = 23), on histochemical, biochemical, and contractile property analyses.

At sacrifice, sections of each of the 64 transplanted muscles were quick frozen in acetone and dry ice. Ten µm sections were cut and incubated for the activities of succinic acid dehydrogenase (SDH) (10), myofibrillar ATPase (5), and capillary membrane phosphatase. Myofibrillar ATPase reactions were performed without fixation or preincubation. Capillary membrane phosphatase was performed in the same medium as myofibrillar ATPase except that 2.5 mM para-hydroxymercuribenzoate was added to inhibit the myofibrillar ATPase. Control and transplanted muscles were incubated in the same media. Fibers were classified as high or low oxidative based on SDH activity (7) and fast or slow-twitch[2] based on myofibrillar ATPase activity (7). Capillary membrane phosphatase activity was used to count the number of capillaries per mm^2. The division of the capillaries per mm^2 by the number of skeletal muscle fibers per mm^2 was defined as the capillary-fiber ratio.

A modified Fales muscle lever (9) was used to measure the contractile properties of 10 control muscles and 22 transplanted muscles. EDL muscles were maintained at 30° C and stimulated by direct supra-maximal stimuli. The

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1. All statistics include mean ± one standard error of the mean.
2. In normal skeletal muscle motor units, histochemical evidence of high and low myofibrillar ATPase has been correlated with the contractile characteristics of fast and slow twitch respectively (2). Therefore, we infer the twitch characteristics from the myofibrillar ATPase and use the same terminology as Peter et al. (11). Such correlations have not been made for transplanted muscle but the transplants show predominantly high myofibrillar ATPase and the time to peak tension is in the range of fast-twitch fibers so we have chosen to use the same terms as are used for fibers from control muscles.

current flow during stimulation and the resting muscle length were adjusted to obtain maximum isometric twitch tension. Maximum isometric tetanus tension was obtained by increasing the frequency of 2 msec pulses delivered during a 90 msec period. This brief stimulation period was used because of the rapid onset of fatigue in the transplanted muscles. The fatigability of the control and transplanted muscles was estimated by stimulating the muscle with 2 msec pulses, 70 pulses per sec, a 200 msec duration, with one stimulation burst each sec. The time in secs required for the maximum isometric tetanus tension to decrease to 50% of the initial value was used as an estimate of fatigability since longer periods of stimulation resulted in a considerable increase in muscle mass due to fluid shifts (9).

Results

In the histological sections of early transplants of normal and pre-denervated muscle, a small number of skeletal muscle fibers just under the muscle sheath survived the transplantation process, did not degenerate, and were ultimately re-innervated. The majority of the muscle fibers degenerated during the first 40 days and were gradually replaced by regenerating fibers. The process of degeneration and regeneration began just under the external surface of the muscle (Figure 1) and proceeded gradually inward toward the central core of the muscle. The initiation of degeneration (sarcolysis), the appearance of macrophages, and revascularization correlated with respect to both time and location within the muscle cross section.

FIG. 1

Cross section through 8 day transplant of a cat EDL muscle that had been pre-denervated for 24 days. Throughout most of the graft the original muscle fibers are in a state of ischemic necrosis, but along one side (darkly stained area around top) the degeneration of old and the regeneration of new muscle fibers is beginning. (H and E)

Following transplantation, original skeletal muscle fibers lost both SDH and myofibrillar ATPase activity as they degenerated, and capillary phosphatase activity disappeared. Between 50 and 170 days following transplantation both pre-denervated and normal transplants were revascularized and re-innervated. Data on all transplanted muscles obtained during this period were grouped. Figure 2 shows a typical section of control and transplanted muscle. In addition to undifferentiated regenerating fibers, differentiated slow-twitch and fast-twitch fibers were observed (Figure 2E). The slow-twitch fibers, which were broadly dispersed in control muscle, were often clustered together in groups in the transplanted muscle (Figure 2E). Mean skeletal muscle fiber cross-sectional areas were 2300 ± 220 μm^2 for control muscles and 1750 ± 180 um^2 for transplanted muscles. Some large, as well as numerous small, skeletal muscle fibers were seen in the transplanted muscles (Figure 2E). These small fibers may be newly regenerating fibers or non-innervated fibers that will remain small or atrophy. The transplanted skeletal muscle fibers showed varying intensities of SDH activity (Figure 2D) but the intensity was always less than the SDH activity usually associated with highly oxidative fibers in control muscles (Figure 2A). A biochemical assay of succinate oxidase activity supports the histochemical observations of reduced oxidative capacity. The succinate oxidase activity of transplanted muscle was 30% of the control value of 55 µl/g·min at 37° C. The capillary-fiber ratio (Figure 2C, F) of the transplants (0.89 ± 0.08) was significantly less than that of the controls (1.75 ± 0.18).

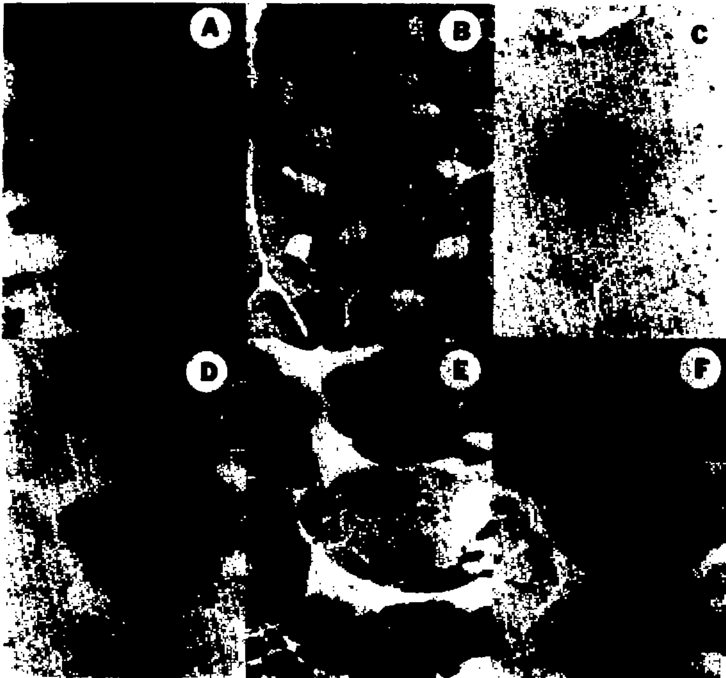

FIG. 2
Typical serial cross-sections of cat control EDL muscle (A, B, C) and EDL muscle 50-170 days after transplantation (D, E, F) incubated for SDH, myofibrillar ATPase, and capillary membrane phosphatase activities respectively.

In the control EDL muscles, 40% of the fibers were fast-twitch glycolytic, 44% fast-twitch oxidative, and 16% slow-twitch oxidative (Figure 2). This is in reasonable agreement with previous estimates (1). Transplants averaged 98% fast-twitch fibers and the SDH activity of all fibers was low (Figure 2). The percentage of fast twitch fibers in the transplants was significantly different ($P < 0.05$) from controls even though the transplants showed considerable variability in composition. Some transplants had a percentage composition similar to controls (87% fast-twitch), while others had 100% fast-twitch fibers.

The control muscles had a maximum isometric tetanus tension of 1.4 ± 0.1 kg absolute and the transplanted muscles averaged $24 \pm 9\%$ of that of control muscles. The maximum isometric tetanus tension of both control and transplanted muscles (Figure 3B, D) normalized per cm^2 of the functional cross-sectional area of the skeletal muscle fibers was approximately 1.5 $dynes/cm^2 \times 10^6$. The value for the transplanted muscles was corrected for their four-fold increase in noncontractile tissue. Compared to control muscles, the transplants took longer to achieve peak isometric twitch tension and had a longer half relaxation time (Figure 3A, C). The capacity of the transplant to shorten (9.6% of the resting length of 9.5 cm), and to exert tension per cm^2 of fiber cross-sectional area was only slightly impaired. The transplanted muscles fatigued much more rapidly than the control muscles. With repeated stimuli, the time required for the maximal isometric tetanus tension to decline to half the initial value was 162 secs for the controls and 75 secs for the transplants.

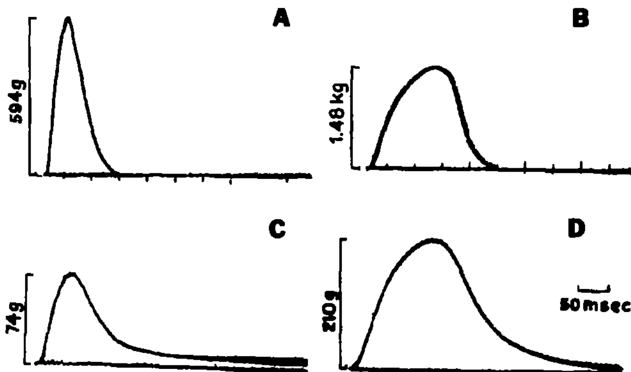

FIG. 3
Responses of a cat control EDL muscle (A, B) and a 133 day transplanted muscle (C, D) during maximum isometric twitch and tetanus respectively. The muscle weights of 4.7 g for the control and 1.2 g for the transplant were corrected for weight gain during stimulation through multiplication by 0.83. The 50 msec marker record is for all 4 records.

Although the time course of the histological changes following transplantation was more rapid in the pre-denervated transplants than in the normal transplants, a comparison of the muscles 50 to 170 days after transplantation showed few significant differences. The means for normal transplants and the pre-denervated transplants respectively were 1.8 and 1.9 g for the mass of the transplant, 98 and 98 for percentage of fast-twitch fibers, 200 and 251 g for absolute maximal isometric tetanus tension, and 10.5 and 9.2 for percentage shortening. None of these differences were significant ($P < 0.05$).

Discussion

Early transplants of cat muscles, both normal and pre-denervated, followed a sequence of histological changes similar to those observed in the rat (3, 4) but the time course was different. Events characteristic of the first 5-6 days in the transplanted EDL muscle of the rat were extended over 6-7 weeks in the cat.

A small number of surviving skeletal muscle fibers in the periphery of the transplant was typical of transplants of rats (2) and cats. These peripheral fibers appear to obtain sufficient oxygen and nutrients by diffusion to survive until revascularization occurred. Contrary to the assumption that survival of skeletal muscle fibers contributes to the success of transplantation in cats (8), dogs (14), and man (14), the majority of the muscle fibers in the transplant degenerated and then regenerated. Most variables showed a similar time course of degeneration and regeneration. This consists of approximately 35 days of degeneration followed by a gradual restoration of structure and function. None of the variables reached non-denervated, non-transplanted control values by 170 days. Although the rate was slower, the histological reactions in the EDL muscle of the cat were similar to those reported for the cat peroneous longus muscle (12). The process of degeneration and regeneration seemed to depend on revascularization since sections of transplants that did not revascularize remained as a mass of connective and necrotic muscle tissue.

Previous reports suggest that only pre-denervated muscles transplant successfully (6, 8, 14), however, in our material, skeletal muscle fibers regenerated successfully in both pre-denervated and normal transplants. The degenerative and regenerative processes occur more rapidly in pre-denervated transplants but by 50 days there were few differences between the two types of transplants. We are unable to explain the emphasis (6, 8, 14) that has been placed on the need to predenervate muscles prior to the time of transplantation.

The slower time to peak tension and ½ relaxation time of the transplants compared to the controls are not consistent with the higher percentage of fast-twitch fibers or with the contractile properties of long term transplants in rats. In rats (4), the time to peak tension returns to normal, but the half relaxation time remains somewhat slower. This may be a function of the increased amounts of connective tissue in the grafts. Since motor units are composed of only one fiber type (2) the grouping together of slow-twitch fibers (type grouping) in some transplants suggests a clustering of the fibers of some motor units. Such clustering is not observed in control muscles.

The gradual increase in SDH activity and the hypertrophy of some skeletal muscle fibers indicates that the transplanted muscle is used. Both SDH activity and the resistance to fatigue usually correlate directly with the frequency of recruitment (2, 11). Thus the low SDH activity and rapid onset of fatigue of the transplant indicate less than normal recruitment of what appears to be a viable transplant.

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References

1. M.A. ARIANO, R.B. ARMSTRONG and V.R. EDGERTON, J. Histochem. Cytochem. 21 51-55 (1973).
2. R.E. BURKE, D.N. LEVINE, F.E. ZAJAC,III, P. TSAIRIS and W.K. ENGEL, Science 174 709-712 (1971).
3. B.M. CARLSON and E. GUTMANN, Anat. Rec. 183 47-62 (1975).
4. B.M. CARLSON and E. GUTMANN, Pflügers Arch. 353 215-255 (1975).
5. J. CHAYEN, L. BITENSKY, R.G. BUTCHER and L.W. POULTER, editors, A GUIDE TO PRACTICAL HISTOCHEMISTRY, P. 129-132) J.B. Lippincott & Co., Philadelphia (1972).
6. L. HAKELIUS, Scan. J. Plast. Reconstr. Surg. 8 220-230 (1974).
7. L. HAKELIUS, Acta Chir. Scand. 141 69-75 (1975).
8. L. HAKELIUS, B. NYSTROM, and E. STALBERG, Scand. J. Plast. Reconstr. Surg. 9 15-24 (1975).
9. R.A. MURPHY and A.C. BEARDSLEY, Am. J. Physiol. 227 1008-1018 (1974).
10. M. NACHLAS, M.K. TSOU, E. DE SOUZA, C. CHENG and A.M. SELIGMAN, J. Histochem. Cytochem. 12 740-743 (1964).
11. J.B. PETER, R.J. BARNARD, V.R. EDGERTON, C.A. GILLESPIE, and K.E. STEMPEL, Biochem. 11 2627-2633 (1972).
12. S. SCHIAFFINO, M. SJOSTROM, L.E. THORNELL, B. NYSTROM, and L. HACKELIUS Experientia 15 1328-1330 (1975).
13. A.N. STUDITSKY, and N.N. BOSOVA, Arkh. Anat. Gist. Embriol. 396 18-32 (1960).
14. N. THOMPSON, Plastic and Reconstr. Surg. 48 11-27 (1971).