Muscle Fiber Regeneration After Transplantation: Prediction of Structure and Physiology from Electromyograms

Abstract. *Digitized electromyographic activity of transplanted extensor digitorum longus (EDL) muscles in cats differs from that of control EDL and anterior tibialis muscles lying adjacent to transplanted EDL muscles. In autotransplanted muscles, the cross-sectional area of the fibers shows a negative correlation with mean spike frequency and a positive correlation with mean amplitude. The mean frequency-amplitude products correlate with isometric tetanic tensions.*

There is substantial evidence that muscles transplanted within the same organism (autografts) revascularize, reinnervate, regenerate, and recover some modicum of function (*1, 2*). In cats, autografts continue to show considerable changes for up to 280 days after transplantation (i) in the area of the muscle that appears to be revascularized and reinnervated, (ii) in the percentage of connective tissue mass, (iii) in the cross-sectional area and length of muscle fibers, (iv) in the myofibrillar adenosinetriphosphatase and succinate dehydrogenase activity, and (v) in contractile properties such as time to peak tension, half relaxation time, maximum isometric twitch and tetanus tensions, and fatigability (*2-5*). However, autografts show marked variability in their morphological and physiological characteristics for reasons that are unclear. The variability may relate to the operative procedures as well as to pre- or postoperative conditions.

Most characteristics of autografts have to be determined at autopsy. This requires the sacrifice of numerous specimens, particularly if one is to monitor the time course of changes when one is assaying the effect of various procedures on the success of autografts. Furthermore, simple motion recordings do not permit accurate predictions regarding postoperative recovery. When only a single muscle, such as the extensor digitorum longus (EDL) of the cat, is involved the animals locomote using a nearly normal step cycle. Electromyography (EMG), which provides an absolute indicator of muscular activity and does not require that the animal be killed, represents a potentially promising technique for continuous or intermittent monitoring of recovery after transplantation (*6*). It has been shown that EMG electrodes yield no signals from freshly denervated muscle nor from freshly transplanted muscle; they do provide records once autografts show contractile responses and may be used to determine this stage of functional recovery in small autografts.

Records from standardized EMG electrodes can provide information about the onset, cessation, and possibly the magnitude of activity. Recordings confirm that by some 11 to 12 months after transplantation the start and stop of activity of stabilized (*7*) unilateral EDL autografts of conscious locomoting cats show remarkably little difference from those of control EDL muscles. However, the frequency and amplitude of the individual EMG events noted in EDL's show much greater intrinsic variability among autografts in various cats than do equivalent records taken from intact limb muscles in cats and other animals. These results made it desirable to test whether this variability could be correlated with the observed morphological and physiological variability of the autografts.

Control electromyograms were taken bilaterally from anterior tibialis (ATB) and EDL muscles of five normal cats. In 14 other cats, both EDL muscles were transplanted orthotopically. We recorded from these EDL autografts, as well as from the ATB muscles lying adjacent to them (*8*). The animals were allowed to move freely and the EMG activities were telemetered to the recording system (*9*); thus the records analyzed represent total activity generated rather than that associated with a particular step cycle or running velocity. Although cats with EDL autografts were less willing to move for prolonged periods, there were no other obvious behavioral differences between control and experimental cats.

Control EDL muscles did not differ significantly from the control ATB muscles in the number of spikes nor in mean spike amplitude per 200-msec sampling interval (*10*). However, three of the ten ATB muscles lying adjacent to a transplanted EDL muscle showed significantly different activity patterns from control muscles, with the former having a significantly greater mean number of spikes (but no significant difference in spike amplitude) (Fig. 1). Of the 16 EDL autografts studied, eight were not significantly different from control muscles, three autografts showed a significantly greater number of spikes of a significantly lower amplitude, and five autografts showed a significantly lower number of spikes of a significantly higher mean amplitude than did the controls (*11*) (Fig. 2).

The product of EMG mean spike frequency and mean amplitude (number of spikes times their mean amplitude for a sampling interval) did not differ significantly among the control EDL muscles, the control ATB muscles, and those ATB muscles lying adjacent to autografts; also, it did not differ from pooled data from all EDL autografts.

The EDL autografts on which this report is based were studied independently for their histological and histochemical profiles and contractile properties and

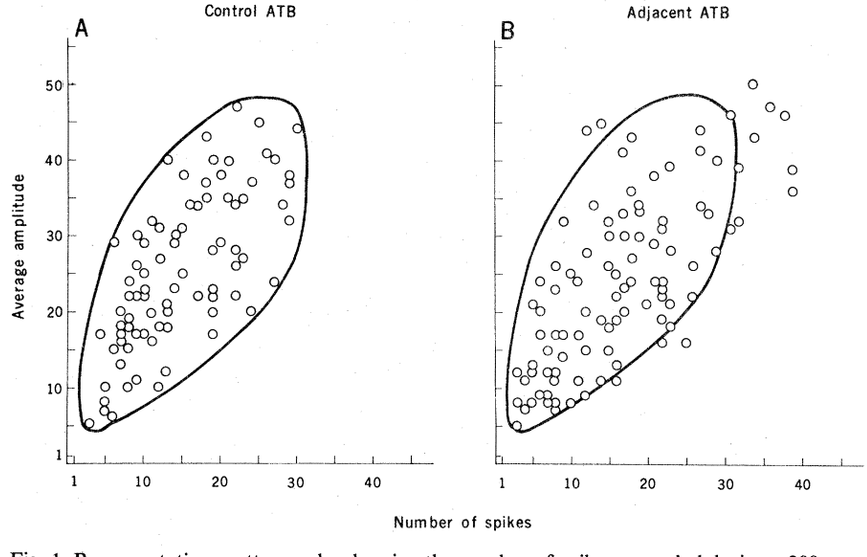

Fig. 1. Representative scattergraphs showing the number of spikes recorded during a 200-msec interval plotted against the mean amplitude of control and adjacent ATB muscles during the particular interval. (A) The oval which encompasses the range of points for control ATB activity (B) is superimposed on a scattergraph from three ATB muscles lying adjacent to autographs. Most of the points lie within the same region as those for control ATB muscles.

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were part of a larger study of transplanted muscles in cats (*2*). In autografts with fiber diameters (range 2426 to 3016 μm) not different from those of controls (2300 ± 220 μm), the mean number of spikes and mean amplitude of EMG activity did not differ significantly from those of controls. However, those autografts exhibiting a marked increase in fiber diameter (3425 to 4637 μm) over that seen in controls showed both a significant decrease in the mean number of spikes and a significant increase in their mean amplitude. Autografts with significantly lower fiber diameters (range 1100 to 1300 μm) showed a significantly higher mean number of spikes and a significantly lower mean amplitude than control muscles.

Autografts of EDL muscles always show significantly lower isometric tetanic tensions than those of control EDL muscles (*2*). However, autografts which showed relatively high isometric tetanic tensions (44.21 ± 12.68 percent of controls) have mean frequency-amplitude products that do not differ from those products of control EDL and ATB muscles. These autografts showed significant differences in both the mean number of spikes and mean amplitude as well as autografts in which separate means were not significantly different from those of control muscles. Autografts with relatively low isometric tetanic tensions (13.78 ± 2.96 percent of controls) showed significantly low frequency-amplitude products. These autografts showed both lower (but not significantly different from controls) mean numbers of spikes and mean amplitudes than those of control muscles.

Table 1. Correlation coefficients of EDL autografts relating EMG activity with morphological and physiological characteristics (*12*).

Muscle characteristics	Spikes (No.)	Average amplitude	Mean products
Fiber area	−.867	.829	.370
Percentage oxidative	.618	.128	.482
Twitch tension	.013	.252	.406
Tetanus tension	.117	.276	.824
TW/TE ratio	.419	.125	.012
Time	.618	.176	.163

Table 1 indicates that the fiber diameters of autografts show a negative correlation of .87 with the mean number of spikes and a positive correlation of .83 with the mean spike amplitude. The mean frequency-amplitude products of these muscles show a positive correlation of .82 with isometric tetanic tensions, providing a reason why "integrated" electromyograms show some correlation with the force involved. However, EMG activity shows no significant correlation (below $r = .63$) (*12*) with isometric twitch tensions, time to peak twitch tensions, and twitch to tetanus tension ratios, although a weak correlation may exist between the number of spikes, the percentage of oxidative fibers ($r = .61$) and the number of days after transplantation ($r = .61$).

These results suggest that quantification of the frequency and amplitude of EMG activity may provide a method for correlating muscle activity with certain histological and physiological characteristics. The results correlate well with those from earlier studies in which the frequency and amplitude of the muscle action potentials are compared between diseased and normal muscles (*13, 14*). Depending upon the muscle disease, the differences in muscle action potentials vary from (i) a reduction in frequency and amplitude, (ii) an increase in the amplitude and a decrease in the duration of action potentials, to (iii) a reduction in amplitude and a frequency range similar to that of "normal" muscle. Muscle fibers of larger diameter exhibit action potentials of higher amplitude (*15*).

Thus, quantitative EMG analysis might be a feasible nondestructive method for assaying certain morphological and physiological changes of muscles which may occur during growth and development and after different regimes of exercise and as a result of disease and surgical manipulations. Quantitative EMG analysis may ultimately permit further predictions and is, of course, potentially useful in reducing the number of animals required to determine success of different transplant techniques. This report also suggests that standardized electrodes and electrode placements and automated analysis may permit reliable predictions from extracellular electromyography.

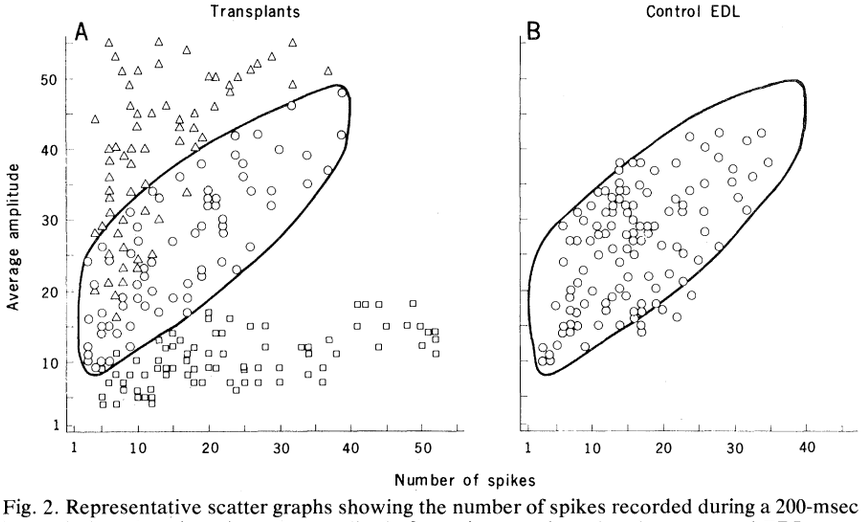

Fig. 2. Representative scatter graphs showing the number of spikes recorded during a 200-msec interval plotted against the mean amplitude from six transplanted and two control EDL muscles. (A) Three populations of points representing muscle activity are shown: (i) those muscles (triangles) with a low number of spikes, high mean amplitude, and large mean fiber diameter, (ii) those muscles (squares) with a high number of spikes, low mean amplitudes, and small mean fiber diameter, and (iii) those muscles (circles) with means and fiber diameters similar to those of control EDL muscles. (B) The loop encompasses the circles of the third population and is superimposed for comparison on those points obtained for control EDL muscle activity.

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6. This approach was undertaken with some trepidation because numerous reports [N. A. de Vries, *Am. J. Phys. Med.* **47**, 10 (1968); S. Herring, *Am. Zool.* **17**, 954 (1977); J. C. Hickey, J. B. Woelfel, L. Rinear, *J. Prosthet. Dent.* **7**, 273 (1957); V. T. Inman, N. J. Ralston, J. B. De, C. M. Saunders, B. Feinstein, E. W. Wright, Jr., *Electroencephalogr. Clin. Neurophysiol.* **4**, 187 (1952); B. Jonsson and S. Reichman, *Acta Morphol. Neerl.-Scand.* **7**, 73 (1968); P. V. Komi and E. R. Buskirk, *Electromyography* **10**, 357 (1970); R. S. Person and U. M. Kushnarev, *Biophysics* **8**, 302 (1963); H. J. Ralston, *Am. J. Orthodont.* **47**, 521 (1961); E. N. Zuniga, X. T. Truong, D. G. Simons, *Arch. Phys. Med. Rehabil.* **51**, 264 (1970)] suggest that EMG activity compared among different electrodes (of very similar configuration), from different portions of a single muscle, and from placements in the same muscle of multiple individuals show sufficient differences to raise questions about the validity of the results. The present system appears to overcome some of these difficulties.
7. Autografts are referred to as stabilized when the fiber areas in the core of the muscle are similar to those of peripheral fibers. In cats, this stage is attained after approximately 280 days after transplantation [see J. A. Faulkner *et al.* (*2*)].
8. Electromyograms were recorded from the EDL autografts and adjacent ATB for periods ranging from 350 to 377 days after transplantation. The EMG activities were recorded 1 to 2 days before the animals were killed to permit analysis of the autografts for morphological and physiological characteristics. For transplant techniques, histological and histochemical profiles, and contractile properties of transplanted EDL muscles in cats, see J. A. Faulkner *et al.* (*3*); L. C. Maxwell *et al.* (*4*); S. A. Mufti *et al.* (*5*).
9. All electrodes were formed of 0.076-mm Teflon-insulated stainless steel wires (Medwire) spirally twisted about one another for approximately 2 to 3 cm. The free ends were bent backward into hooks after the insulation had been removed from the distal 2 mm of each wire [see G. C. Gorniak, *J. Morphol.* **157**, 427 (1977)]. The length of the bare ends was 2.16 ± 0.166 mm and the resistance, 42.6 ± 1.01 kohm (mean ± standard deviation, $N = 40$). Electrodes were inserted surgically into the muscles under Vetalar and local anesthesia with leads running subdermally to the hip and from there to a four-channel telemetry transmitter (Bio-sentry 7140). Outputs from the receivers were passed through Tektronix FM 122 preamplifers and Honeywell 117 DC Accudata amplifiers and stored on a Honeywell 5600 medium bandpass 1-inch tape recorder. Signals from four channels at a time were later analyzed on a modified Hewlett-Packard 2100A minicomputer, the number of spikes per unit time being counted as well as the mean amplitude (40 mv/unit) of spikes during each interval. A separate control permitted subtraction of noise from the zone scanned. The tape was marked electronically, permitting parallel analysis of additional records, and the digitized EMG data were stored on tape cassettes for further statistical analyses. The EMG activities were scanned in serial time sections, normally set at 200-msec intervals.
10. Control EDL muscles show a mean number of spikes of 15.92 ± 0.758 ($N = 82$) and a mean amplitude of 24.15 ± 1.67 ($N = 82$). For control ATB, the mean number of spikes is 15.37 ± 0.826 ($N = 82$) and the mean amplitude is 23.54 ± 1.85 ($N = 82$); both mean number of spikes and mean amplitude are not significantly different from those of control EDL muscle at a significance level of $P = .10$. However, three of the adjacent ATB muscles show significantly different ($P < .05$) mean numbers of spikes from those of control ATB muscles, ranging from 19.23 ± 1.36 ($N = 82$) to 20.16 ± 2.27 ($N = 38$). The ATB muscles were studied to determine whether transplantation procedures affect the EMG activity pattern of nontransplanted muscles adjacent to autografts, perhaps reflecting compensatory action of the synergistic ATB muscle for the loss of EDL function.
11. In those autografts with a significantly greater number of spikes and lower amplitude ($P < .01$), the mean number of spikes ranged from 19.37 ± 1.25 ($N = 53$) to 26.77 ± 0.73 ($N = 115$) and the mean amplitude ranged from 11.84 ± 0.73 ($N = 115$) to 14.75 ± 0.99 ($N = 53$). In autografts showing a significantly lower number of spikes and greater amplitude ($P < .01$), the mean number of spikes ranged from 11.72 ± 1.58 ($N = 37$) to 12.20 ± 0.98 ($N = 91$) and the mean amplitude ranged from 31.87 ± 1.16 ($N = 81$) to 38.97 ± 2.48 ($N = 37$).
12. Correlation coefficients (r) for two variables were calculated for all the EDL autografts studied. Correlation coefficients below 0.63 are not considered significant at a level of .01 [K. Diem and C. Lentner, *Scientific Tables* (Geigy, Basel, 1970)]. Fibers classified as oxidative showed distinct succinate dehydrogenase activity as well as subsarcolemmal aggregates of diformozon, especially near capillaries [L. C. Maxwell *et al.* (*4*)]. Time denotes the number of days after transplantation.
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Nuclear Transcripts of Mouse Heavy Chain Immunoglobulin Genes Contain Only the Expressed Class of C-Region Sequences

Abstract. *In plasmacytoma cells producing IgG, IgA, or IgM immunoglobulin heavy chains, the large precursors of the heavy chain messenger RNA's contain nucleotide sequences that specify only the expressed class of constant region. This indicates that the switch from one class of heavy chain to another during B cell ontogeny does not occur by altered processing of a complex gene transcript.*

During the course of their ontogeny B lymphocytes produce heavy (H) chain immunoglobulins in which variable region (V_H) sequences of a characteristic idiotype can be associated with a variety of constant region (C_H) sequences (*1*). Thus, the commitment to produce the M, G, or A class of immunoglobulins is presumed to reflect a switch between V_HC_μ and V_HC_γ or V_HC_α (*2*) expression. Two types of molecular models have been proposed to account for this switch: one involving structural rearrangements of the V_H and C_H genes (*3*) and the other, variable processing of a complex messenger RNA (mRNA) precursor containing information for all of the C_H sequences (*4*). Some doubt has been cast on the RNA processing model by the observation that a hybridoma line formed by fusing $V_{H_1}C_\mu$- and $V_{H_2}C_\gamma$-producing cells does not produce a $V_{H_2}C_\mu$ hybrid immunoglobulin (*5*). However, this negative evidence is only inferential because it presumes capabilities of processing enzymes about which essentially nothing is known.

We have made a more direct test of the processing model by examining the nuclear transcripts in cells producing immunoglobulins of one particular class for the presence of sequences specifying other classes. This test is based on our ability to detect the nuclear precursors of H-chain mRNA's by their specific hybridization to cloned complementary DNA (cDNA) sequences (*6*). We have observed that the precursors of γ-, α-, and μ-chain mRNA's contain only the sequences of that particular class, a result that is inconsistent with the RNA processing model.

The myeloma cell line MPC-11, which produces γ_{2b} chains, and plasmacytoma tumors J558 and PC3741, which produce α and μ heavy chains, were used as the source of nuclear RNA and cytoplasmic mRNA (*7, 8*). Nuclear RNA's were extracted by the hot phenol method of Scherrer (*9*) from MPC-11 nuclei purified with detergent (*6*) and from tumor nuclei purified with citric acid (*10*). Cytoplasmic mRNA's were extracted from membrane-bound polyribosomes and further purified as indicated (*8*). Polyadenylate [poly(A)]-containing nuclear RNA was isolated by chromatography on oligodeoxythymidylate cellulose and size-fractionated on methylmercury hydroxide–agarose gels (*6*). The RNA molecules containing sequences coding for this H-chain constant region were identified by the RNA transfer technique of Alwine *et al.* (*11*) with the use of nick-translated cloned cDNA probes of ≥ 25 count/min per picogram (*8*).

The construction and characterization of the chimeric plasmid $p\gamma_{2b}(11)^7$ containing a 1-kilobase-pair (kbp) segment of γ_{2b} C-region nucleotide sequences derived from the H-mRNA of MPC-11 cells has been described (*6*). The plasmids

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