ANSC/FSTC 607
Biochemistry and Physiology of Muscle as a Food
INNERVATION AND DIFFERENTIATION OF MUSCLE

I. Organization of the motor neuron and myofibers

A. Motoneuron bifurcates into many branches (terminal axons)

B. Motor end plates tend to line up in register.

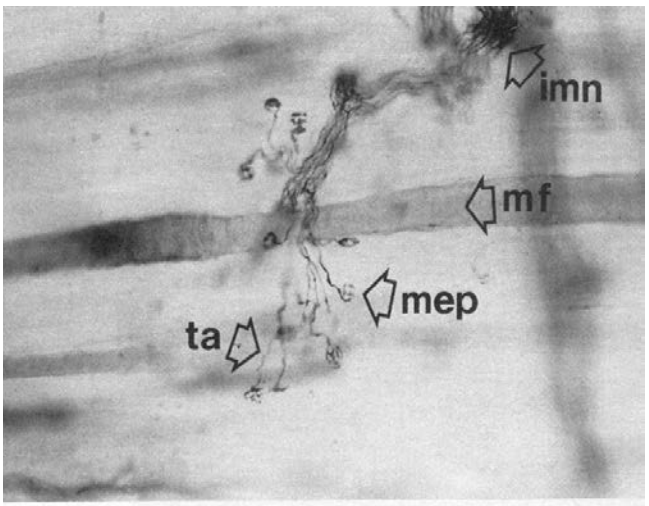
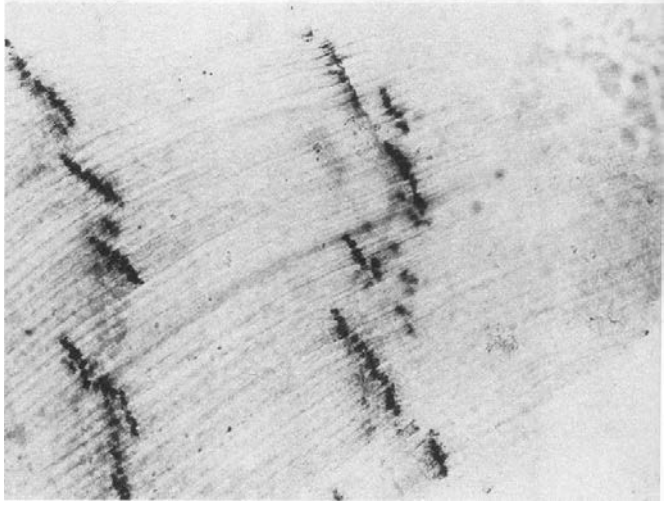


Plate 11 Thick frozen section of beef stained with methylene blue to show the innervation of muscle fibers: (imn) intramuscular nerve; (mf) a muscle fiber; (mep) motor end plate; (ta) terminal axon. Most of the muscle fibers are unstained.

Plate 12 Acetylcholinesterase activity in the innervation zones across a muscle fasciculus. Each small round area of reaction product indicates the position of a neuromuscular junction.



II. Polyneural innervation

A. General features

1. Only in very young
2. Increases size of motor units
   a. Several axons converge on the same motor end plate
   b. Causes compound end-plate potentials
   c. No electrical coupling
3. In rats, all muscle fibers innervated by single motor axon

B. Surgical removal of axons in neonates

1. Delays decline in motor unit size
2. Leads to atrophy of muscle
   a. Polyneural innervation disappears
   b. Some myofibers lose all innervation

III. Innervation and myofiber development

A. Denervation

1. Causes atrophy of myofibers (trophic substances or loss of contractions?)
2. Some regeneration possible

   a. After crush of soleus nerve in neonates, there is a return of axons to original motor end-plates.

   b. Axons migrate along original pathways between muscle fibers.

B. Nerve transection and cross-innervation

1. Cross-innervated fast muscles convert to slow muscles completely.
2. Cross-innervated slow muscles converted *mostly* to fast muscles.

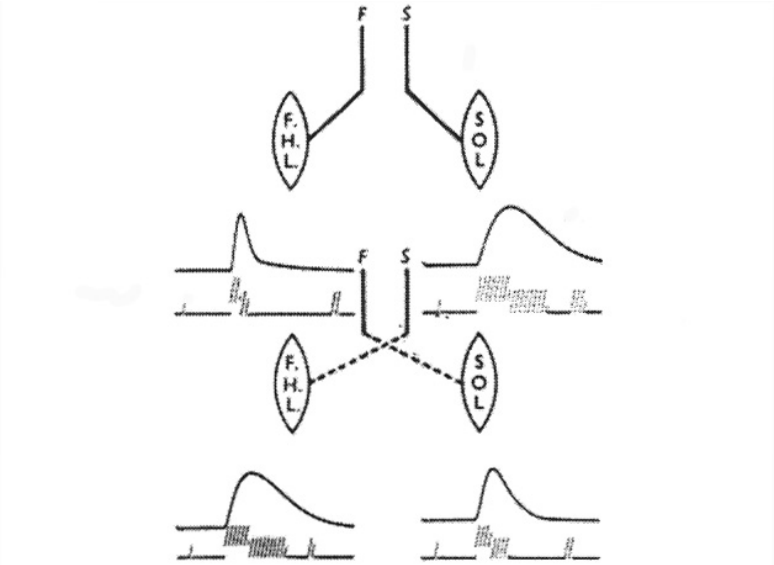


Fig. 1. Upper diagram and pair of records: isometric twitch responses from normal F.H.L. and soleus muscles of the cat. Lower diagram and pair of records: isometric twitches from cross-innervated F.H.L. and soleus muscle. *F* and *S* indicate the faster and slower conducting alpha motor axons respectively. The four groups of dots displayed under the isometric records in this and succeeding illustrations indicate the initial tension on the muscle, the time to peak tension (raised on a pedestal), the time to half relaxation and the twitch tension, respectively. For all four twitches each dot of the first group indicates 5 g of initial tension, each dot of the second and third groups indicate 1 msec, and each dot of the fourth group 5 g. It will be noted that the twitch of the cross-innervated F.H.L. muscle more closely resembles the normal soleus than the cross-innervated soleus twitch resembles the normal F.H.L.

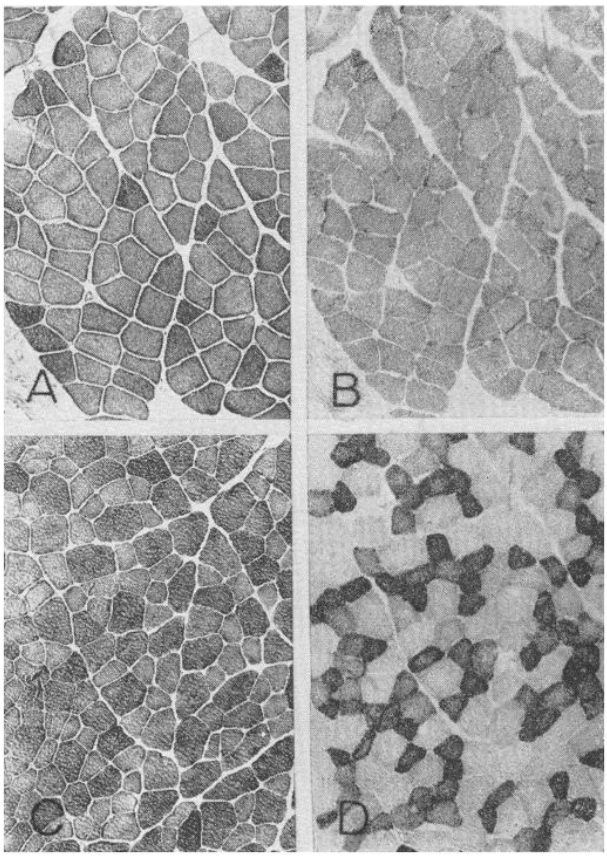
C. Changes in muscle fiber types

1. Complete conversion of type II myofibers to type I myofibers in fast → slow cross-innervation.
2. Partial conversion of type I myofibers to type II myofibers in slow → fast cross-innervation.
3. All conversions are complete within myofibers.

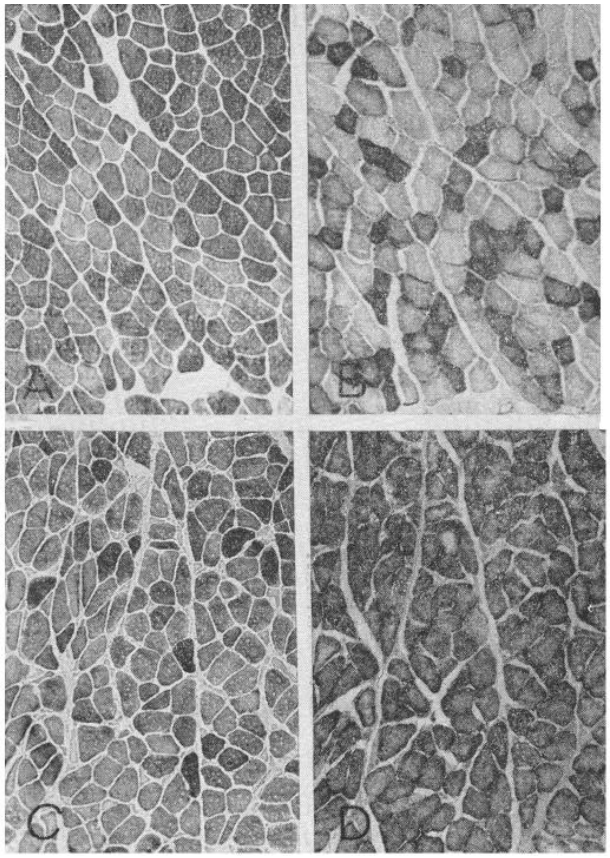
TABLE 1. *Percentage distribution of fiber types in normal and self- and cross-innervated muscles at 120 days*

	No. of Muscles	Fiber Type			
		1	*2*	*3*	*4*
		Soleus			
Normal	4	89.3 (6.2)	0	10.7 (6.3)	0
Self-innervated	4	98.8 (1.2)	0	1.2 (1.2)	0
Cross-innervated	4	43.5 (17.8)	0	56.5 (17.8)	0
		Flexordigitorum longus			
Normal	4	23.0 (9.8)	32.0 (4.6)	39.4 (11.1)	5.6 (4.9)
Self-innervated	4	15.3 (6.2)	50.1 (6.4)	26.8 (4.5)	7.8 (6.3)
Cross-innervated*	4	83.3 (6.2)	4.4 (3.5)	12.3 (3.8)	

Values are means with SE given in parentheses. *Type 1:* light PL and dark SD. *Type 2:* dark PL and light SD. *Type 3:* dark PL and dark SD. *Type 4:* light PL and light SD. * Denervated fibers were not included.



Examples of normal soleus and FDL muscles stained for PL and SD. *A:* normal soleus stained for PL. *B:* serial section to *A* but stained for SD. *C:* normal FDL stained for PL. *D:* serial section to C but stained for SD. X75.



Examples of 120 day self- and cross-innervated FDL stained for PL and SD. *A:* self-innervated FDL stained for PL. *B:* serial section to *A* but stained for SD. *C:* cross-innervated FDL muscle from same cat stained for PL. *D:* serial section to *C* but stained for SD. X75.

C. Changes in metabolic enzyme activities

1. Malate dehydrogenase and isocitrate dehydrogenase (mitochondrial enzymes) increase and pyruvate kinase and aldolase (glycolytic enzymes) decrease in fast → slow cross-innervation.
2. The reverse is seen in slow → fast cross-innervation.

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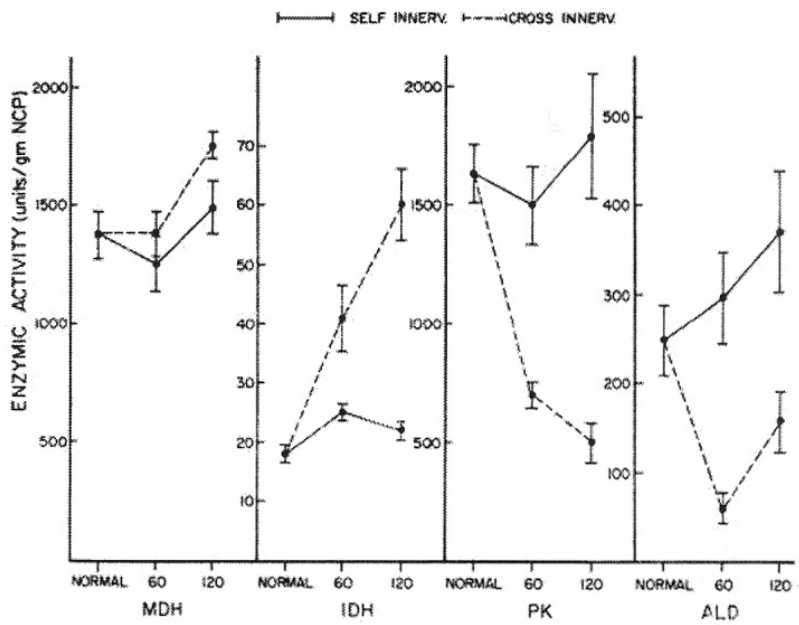


FIG. 4. Enzymic activities in normal FDL muscles and FDL muscles cross- or self-innervated for 60 and 120 days. Small bars indicate SE.

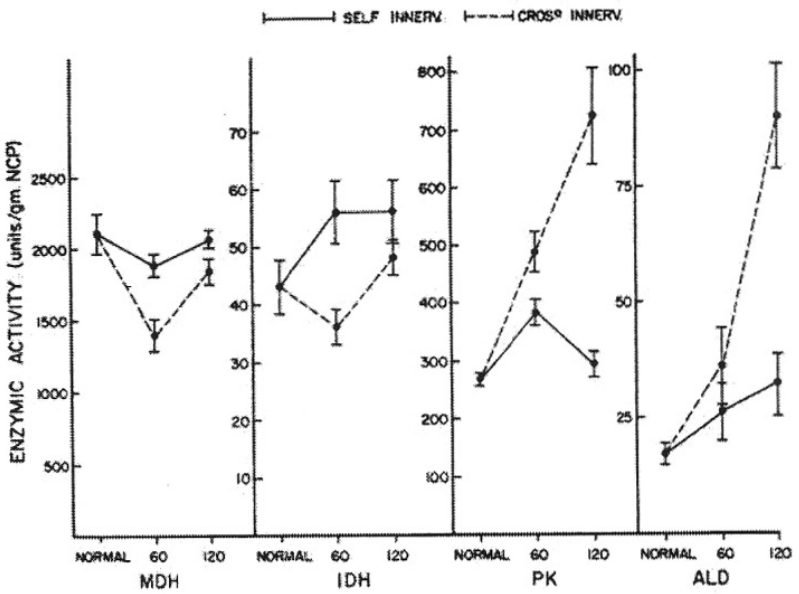


FIG. 5. Enzymic activities in normal SOL muscles and SOL muscles cross- or self-innervated for 60 and 120 days. Small bars indicate SE.

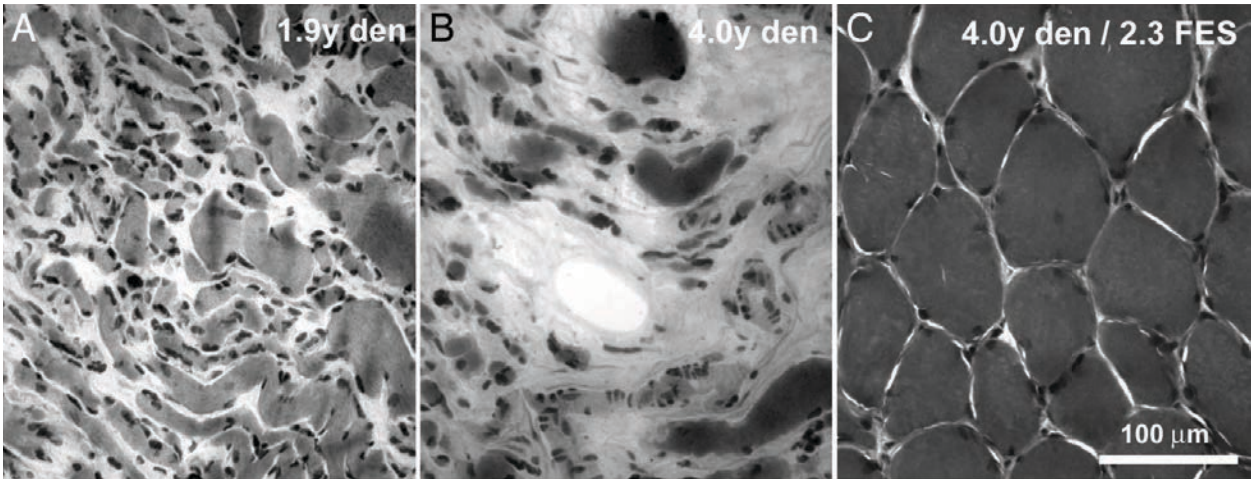
IV. Structural differentiation of skeletal muscle fibers in the absence of innervation in humans. PNAS 104:19339-19344. 2007.

A. The relative importance of muscle activity versus neurotrophic factors in the maintenance of muscle differentiation has been greatly debated.

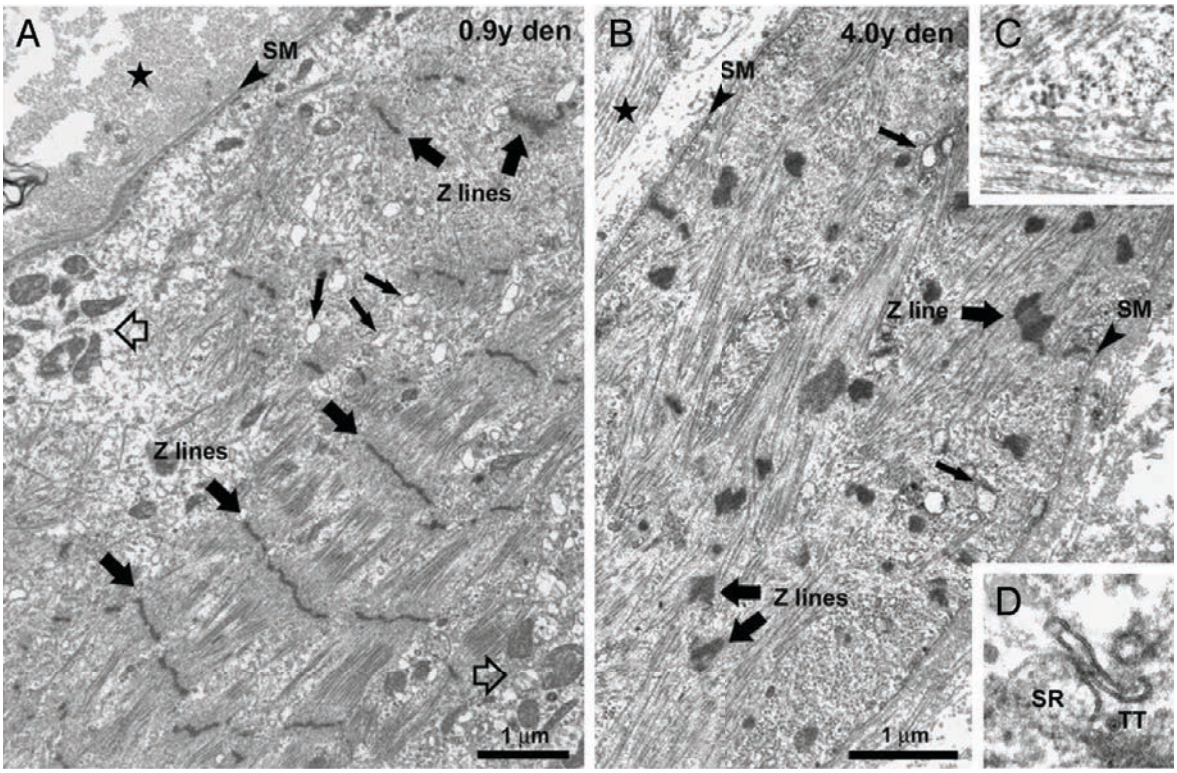
B. Muscle biopsies from spinal cord injury patients, who were trained with an innovative protocol of functional electrical stimulation (FES) for prolonged periods (2.4 –9.3 years), offered the unique opportunity of studying the structural recovery of denervated fibers from severe atrophy under the sole influence of muscle activity.

C. FES stimulation induced surprising recovery of muscle structure, mass, and force even in patients whose muscles had been denervated for prolonged periods before the beginning of FES training (up to 2 years) and had almost completely lost muscle-specific internal organization.

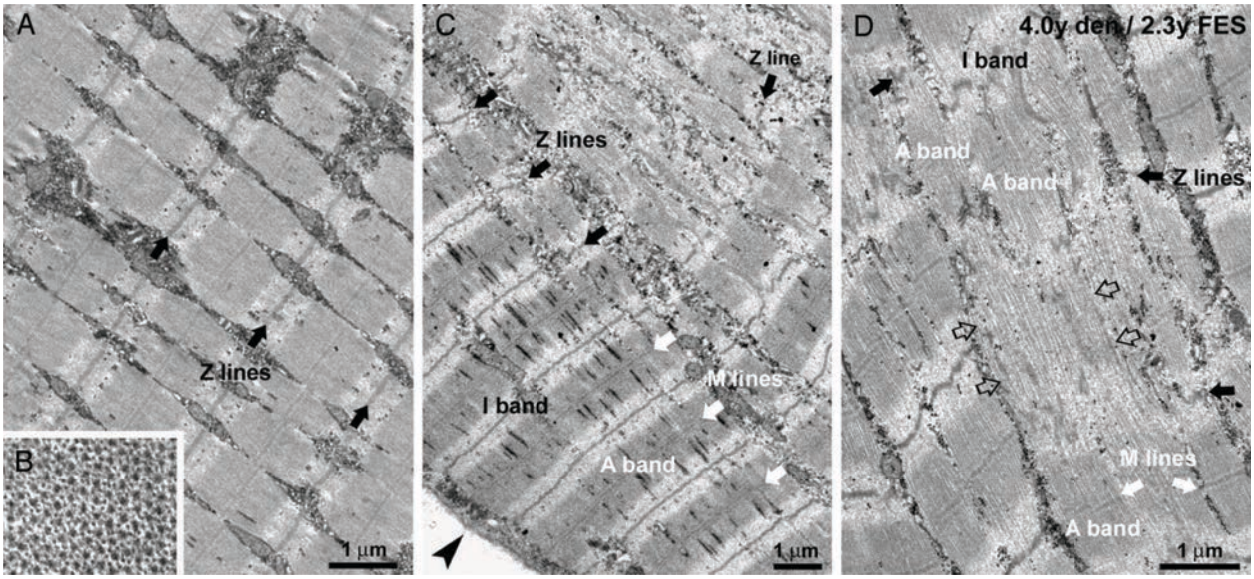
1. Ninety percent (or more) of the fibers analyzed by electron microscopy showed a striking recovery of the ultrastructural organization of myofibrils and Ca^{2+}-handling membrane systems.
2. This functional/structural restoration follows a pattern that mimics some aspects of normal muscle differentiation.
3. Most importantly, the recovery occurs in the complete absence of motor and sensory innervation and of nerve-derived trophic factors, that is, solely under the influence of muscle activity induced by electrical stimulation.



Denervation-induced atrophy of muscle fibers is reversed by FES. (A and B) Denervation (den) causes progressive atrophy of muscle fibers and a relative increase of connective and adipose tissues. (C) FES treatment greatly increases average diameter of muscle fibers and significantly reduces the relative content of collagen and adipocyte accumulation.



Effects of long-term denervation on skeletal fibers ultrastructure. (A and B) Disarrangement of the internal structure of fibers starts from the periphery and results in complete disruption of the internal organization. (C) Shown is an area with misoriented contractile filaments. (D) Shown is an abnormal SR/T tubule junction. Filled stars, extracellular space; open arrows, mitochondria grouping; small filled arrows, fragmented SR; large filled arrows, Z lines; SM, surface membrane.



FES-induced ultrastructural restoration of myofibrils. (A and B) Rescued fibers present a transversal dark–pale striation (A) and a regular hexagonal pattern of thick and thin filaments (B). (C) In partially recovered fibers, myofibrils are better organized at the fiber periphery (arrowhead). (D) The formation of new myofibrils resembles myofibrillogenesis in normal embryonal differentiation: alignment of filaments (open arrows), preassembly of A bands, appearance of Mlines, and formation of Z lines (see Results for more details). Black arrows, Z lines; white arrows,M lines; arrowhead, surface membrane; open arrows, aligned myofilaments, which are not yet assembled into sarcomeres.