“The Denervated Muscle” – 45 Years Later

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Abstract

Forty five years after its publication, Ernest Gutmann’s book, “The Denervated Muscle,” still stands as a landmark publication. It summarized the state of knowledge of the time and introduced much new research that was ongoing at the Institute of Physiology in Prague. At the time, the response of a muscle to denervation was viewed primarily through the lens of the neurotrophic theory. Advances in our understanding of neurotrophic effects and mechanisms would now call into question some of the hypotheses and interpretations presented in the book, but many of the research findings have stood the test of time. This review will cover some of the questions asked and data presented in this book and will place them into the context of contemporary muscle biology.

Key Words: Skeletal muscle, denervation, neurotrophic theory, nerves.

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“The Denervated Muscle” [12] was written from the Institute of Physiology in Prague as a collective publication by a group of talented investigators who had been assembled as a multidisciplinary research collaborative by Ernest Gutmann. At the time of its writing, the neurotrophic theory was in full sway, and studies of denervated muscle were part of a large effort to study trophic effects of nerves on their end organs. Because at the time this group was working behind the Iron Curtain, neither conducting research nor writing a book was an easy proposition. A major problem for East European scientists in this era was access to contemporary international scientific literature, as well as maintaining the normal interpersonal scientific contacts that are typically taken for granted. Nevertheless this group prepared a remarkable synthesis of the contemporary state of knowledge of denervation effects on skeletal muscle and in addition provided a comprehensive review of their own research findings in this field.

While in England during World War II, Gutmann and his collaborators conducted research on long-term denervation of human muscle and on reinnervation of muscle that is still widely cited in the medical and scientific literature [3, 14]. This largely descriptive research was the starting point for the major effort in the Institute of Physiology to understand mechanisms underlying denervation atrophy.

Neurotrophism and the Denervated Muscle

The late 1950s and early 1960s was a period of intense interest in neurotrophism in a wide variety of systems [11, 27]. A principal question was whether there is a

trophic function of the neuron that is distinct from its role in impulse conduction and transmission. This

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concept was suggested by the centuries-old question of Prochaska (1784, cited in Gutmann, 1962, p. 26 [12]) about whether or not nerves serve a nutritive function and Cajal's later formulation of the trophic concept [5]. Research on several widely different systems provided evidence suggestive of a non-impulse role for nerves in maintaining end organs.

Singer [26] had demonstrated that regeneration of the limb in urodele amphibians depends upon the quantity, not the type of nerve fibers at the amputation surface, and an intense search for the nature of the trophic agent in this system had begun, because by 1960 it was widely assumed that adequate innervation was the basis for successful limb regeneration. Attempts were underway to try to stimulate limb regeneration in mammals by increasing the local nerve supply. Similarly, a number of experiments had indicated that some special property of gustatory nerve fibers was necessary for the maintenance of taste buds [23].

Another significant influence was the demonstration of neurosecretion within the hypothalamus [24]. The discovery of axoplasmic flow [30] was still relatively new, and research on axoplasmic streaming [20], provided data that suggested a mechanism for transporting trophic substances from the neuronal cell body to axon terminals. An experimental model that was very influential to the Czech group was that of long- and short-stump denervation, in which denervation effects in several systems occurred more slowly when the nerve was cut farther away from the end organ [13]. A common interpretation was that a long stump contained more trophic substance and therefore delayed the onset of denervation atrophy.

Research by Tower [28] on denervation atrophy in skeletal muscle was widely viewed at the time as supportive of a trophic function for nerves.

The influential study of cross-innervation effects between fast and slow muscles by Buller et al. [4] had just been published in abstract form as of the time *The Denervated Muscle* went to press. Analyses of the mechanisms by which the cross-innervation effect took place had not yet been done.

Overall, research findings as of 1960 were highly suggestive of the existence of neurotrophic effects in a variety of systems, but it was not possible to rule such effects in or out on the basis of existing data.

Approaches to the Problem That Were Ahead of Their Time

Gutmann approached the problem of neurotrophism and specifically the study of denervated muscle in a number of ways that were clearly ahead of their time. First of all, he assembled a highly multidisciplinary research group, which included not only several varieties of physiologists, but morphologists, biochemists and individuals coming from clinical backgrounds. Throughout his career he emphasized a broad ontogenetic approach, in which phenomena were viewed as taking place over a developmental continuum that began with the embryo and continued into old age. In addition, physiological processes were often studied over time, rather than at a specific time point. He was unusual in relating physiology to embryology and morphogenesis. A classical example of this approach was the work of Zelená [32] on the influence of sensory innervation on the development of muscle spindles in the embryo. There was also considerable awareness of the influence of environment on the body and on physiological processes. This may have been related to the pervasive influence of Pavlov on the field of physiology in the USSR and Eastern Europe during that time.

Major Findings That have Stood the Test of Time

A surprising amount of the primary research presented in the book has stood the test of time and the data are still highly useful.

The curves showing relative loss of mass over time in the denervated rat soleus and extensor digitorum longus muscles (presented in Figure 46) mirror almost exactly those that were generated decades later in this author's laboratory.

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Changes in the frequency distributions of muscle fiber diameters over time were well documented for the denervated tibialis anterior muscle in the rabbit (see Figure 20), and differential atrophy of muscle fibers was illustrated, although the lack of histochemical and immunochemical techniques did not allow them to recognize that the atrophy was fiber type-specific, with type II muscle fibers undergoing much more rapid atrophy than type I fibers. Data were also presented showing a decrease in muscle fiber number over a long course of denervation atrophy (Table 2), but the available techniques did not allow an understanding of the complexity of post-denervation environment, in particular the fact that new muscle fibers form as old atrophic fibers degenerate [7]. The persistence of muscle spindles in long-term denervated muscle (Table 3) and later data on the persistence of spindles in regenerating transplanted muscles [8] provided the basis for understanding the importance of the capsule in the survival and regeneration of already formed muscle spindles. Muscle fiber degeneration after long-term denervation was well documented, but the prevailing sense at the time of the poor regenerative capacity of skeletal muscle inhibited further explorations into the cellular dynamics of denervated muscle.

The effects of denervation in muscle at various critical developmental stages received considerable attention. It was established that denervated developing muscle in perinatal rodents continues a certain degree of differentiation, but that it is retarded and incomplete. The lack of formation of muscle spindles in denervated fetal muscle has already been noted [32]. In addition, it was recognized that development of the subneural apparatus requires innervation.

Much attention was given to fibrillation activity in denervated muscle [15]. One of the reasons for this emphasis was the idea that denervation atrophy might be a byproduct of metabolic exhaustion resulting from fibrillation activity. The influence of denervation on overall energy metabolism of muscle was a prominent theme in this book. Blood flow is an essential modulator of the metabolic environment of an organ. Working mostly on dogs, Hudlická [16] provided striking data documenting the increase in blood flow in denervated muscles and concluded that insufficient blood flow cannot be a primary cause of denervation atrophy.

A final important conclusion based on findings reported in the book was that after a certain period of time (approximately 6 months in the rat), denervated muscle undergoes some irreversible changes that inhibit its full restoration even after reinnervation. Studies on a different experimental model [6] led to the same conclusion. In this model, muscles denervated for different periods of time were transplanted into the beds of fully innervated muscles in the rat, and the degree of regenerative restoration was assessed. Muscle denervated up to two months became restored as well as

grafts of control muscles, but between 2 and 7 months of denervation, the restorative capacity declined continuously before leveling out at a very low level at 7 months of denervation.

What We Now Know That Was Not Known in 1960

Many important scientific developments that have occurred since 1960 have considerably changed our view of denervation atrophy and the potential role of neurotrophism in the process. Much of this has been excellently reviewed by Midrio [22].

One of the most important was the discovery of the satellite cell by Mauro in 1961 [21]. This provided the basis for understanding the regenerative capacity of skeletal muscle. It also allowed a different view of the cellular environment following the denervation of a muscle. In *The Denervated Muscle*, the postdenervation environment was viewed as rather static, with atrophy and degeneration being the dominant reactions. Contemporary ultrastructural research has shown dynamic changes in the satellite population of a denervated muscle over time, with an early threefold increase in their incidence, followed by a steady decline before levelling off to lower than control values [29]. Subsequent studies [1, 2, 9, 25] have shown that the cellular environment of a long-term denervated muscle is very complex, with cellular atrophy, cell death and the formation of new muscle fibers all taking place at the same time.

At the time of writing the book, the Czech group did not have the capacity to measure contractile properties of muscles or to conduct histochemical analysis. Without these capabilities, they weren't yet able to probe the subtleties of some of the suspected denervation changes. Subsequent research showed that the decline of force in a denervated muscle is more precipitous than is the decline in overall mass of the muscle [6, 19]. The issue of maintenance of slowness, on which Gutmann focused much later attention, remained for the future.

Electrophoretic and histochemical investigations at the time could just provide a glimpse of the complexity of the protein and enzymatic changes brought about by denervation. The fact that at the time of the writing of *The Denervated Muscle* the genetic code was just becoming recognized offers a sense of perspective concerning the ability to make any interpretations at a level below the cell.

Similarly, attempts in the book to explain denervation atrophy on the basis of energy metabolism failed. At the time there was no inkling of the existence of molecular programs that control both hypertrophy and atrophy of muscle tissue [10, 18, 31].

For a number of years results obtained from long and short stump denervation experiments played a considerable role in the interpretation of the mechanisms underlying denervation atrophy [13]. Later research by Jones and Vrbová [17], however, provided

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an alternative explanation for these results when they found that products of nerve degeneration can elicit denervation hypersensitivity.

The effect of electrical stimulation on denervated muscle played an important role in Gutmann's view of the trophic influence of nerves on muscle. At the time, electrical stimulation protocols were unable to maintain the mass of a denervated muscle or to significantly increase the mass of an atrophic muscle. At present, a wide variety of stimulation techniques and protocols are almost fully capable of preventing denervation atrophy [22].

What Was the Value of "The Denervated Muscle?"

At the time of its publication *The Denervated Muscle* was a landmark that was the first major consolidation of a large body of literature on muscle denervation. It clearly laid out a number of important issues involving neurotrophic theory in a manner that allowed them to be experimentally tested. In addition, the book provided a significant body of original baseline data on the biology of denervated muscle that brought international recognition to the Czech group of physiologists.

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References

- [1] Borisov AB, Carlson BM: Cell death in denervated skeletal muscle is distinct from classical apoptosis. *Anat Rec* 2000; 258: 305-318.
- [2] Borisov AB, Dedkov EI, Carlson BM: Interrelations of myogenic response, progressive atrophy of muscle fibers, and cell death in denervated skeletal muscle. *Anat Rec* 2001; 264: 203-218.
- [3] Bowden REM, Gutmann E: Denervation and re-innervation of human voluntary muscle. *Brain* 1944; 67: 273-313.
- [4] Buller AJ, Eccles JC, Eccles RM: Interactions between motoneurons and muscles in respect of the characteristic speeds of their responses. *J Physiol (London)* 1960; 150: 417-434.
- [5] Cajal SR: Die Neuronenlehre, in Bumke O, Foerster O (eds.). *Handbuch der Neurologie*. Berlin, Springer, 1935, pp. 887-994.
- [6] Carlson BM, Billington L, Faulkner J: Studies on the regenerative recovery of long-term denervated muscle in rats. *Restor Neurol Neurosci* 1996; 10: 77-84.
- [7] Carlson, BM, Borisov AB, Dedkov EI, Dow D, Kostrominova TY: The biology and restorative capacity of long-term denervated skeletal muscle. *Basic Appl Myol* 2002; 121: 249-256.
- [8] Carlson BM, Gutmann E: Regeneration in free grafts of normal and denervated muscles in the rat: Morphology and histochemistry. *Anat Rec* 1975; 183: 47-62.
- [9] Dedkov EI, Kostrominova TY, Borisov AB, Carlson BM: Reparative myogenesis in long-term denervated skeletal muscles in adult rats results in a reduction of the satellite cell population. *Anat Rec* 2001; 263: 139-154.
- [10] Glass DJ: Skeletal muscle hypertrophy and atrophy signaling pathways. *Internat J Biochem Cell Biol* 2005; 37: 1974-1984.
- [11] Guth L: "Trophic" effects of vertebrate neurons *Neurosci Res Prog Bull* 1969; 7:1-73.
- [12] Gutmann E, ed: *The Denervated Muscle*. Publ. House of Czechoslovak Academy of Sciences, Prague, 1962, pp. 486.
- [13] Gutmann E, Vodička Z, Zelená J: Changes in cross striated muscle after the nerve interruption depending upon the length of the nerve stump (Russian). *Physiol bohemoslov* 1955; 4: 200-204.
- [14] Gutmann E, Young JZ: The re-innervation of muscle after various periods of atrophy. *J Anat* 1944; 78: 15-43.
- [15] Hník P, Škorpil V: Fibrillation activity in denervated muscle, in Gutmann E (ed.): *The Denervated Muscle*. Prague, Publ. House of Czechoslovak Academy of Sciences, 1962, pp. 136-150.
- [16] Hudlická O: Vasomotor mechanism in genesis of denervation atrophy, in Gutmann E (ed.): *The Denervated Muscle*. Prague, Publ. House of Czechoslovak Academy of Sciences, 1962, pp. 173-202.
- [17] Jones and Vrbová: Two factors responsible for the developments of denervation hypersensitivity *J Physiol*. 1974 ;236: 517-538.
- [18] Kandarian SC, Jackman RW: Intracellular signaling during skeletal muscle atrophy. *Muscle Nerve* 2006; 33: 155-165.
- [19] Lewis DM, Schmalbruch H: Contractile properties of aneurally regenerated compared with denervated muscles of rat. *J Muscle Res Cell Motil* 1994; 15: 267-277.
- [20] Lubińska L: Axoplasmic streaming in regenerating and in normal nerve fibers, in Singer M, Schädé JP(eds.): *Mechanisms of Neural Regeneration*. Amsterdam, Elsevier, 1964, pp. 1-71.
- [21] Mauro A: Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* 1961; 9: 493-495.

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- [22] Midrio M: The denervated muscle: facts and hypotheses. A historical review. Eur J Appl Physiol 2006; 98: 1-21.
- [23] Oakley B, Benjamin RM: Neural mechanisms of taste. Physiol Rev 1966; 46: 173-211.
- [24] Scharrer E, Scharrer B: Hormones produced by neurosecretory cells. Rec Progr Hormone Res 1954; 10: 183-240.
- [25] Schmalbruch H, Lewis DM: Dynamics of nuclei of muscle fibers and connective tissue cells in normal and denervated rat muscles. Muscle Nerve 2000; 23: 617-626.
- [26] Singer M: The influence of the nerve in regeneration of the amphibian extremity. Quart Rev Biol 1952; 27: 169-200.
- [27] Singer M, Schadé JP, eds: *Mechanisms of Neural Regeneration*. Elsevier, Amsterdam, 1964, pp. 241.
- [28] Tower SS: Atrophy and degeneration in skeletal muscle. Am J Anat 1935; 56: 1-34.
- [29] Viguie CA, Lu D-X, Huang S-K, Rengen H, Carlson BM: Quantitative study of the effects of long-term denervation on the extensor digitorum longus muscle of the rat. Anat Rec 1997; 248: 346-354.
- [30] Weiss P, Hiscoe HB: Experiments on the mechanism of nerve growth. J Exp Zool 1948; 207: 315-395.
- [31] Wing SS: Control of ubiquitination in skeletal muscle wasting. Internat J Biochem Cell Biol 2005; 37: 2075-2087.
- [32] Zelená J: The morphogenetic influence of innervation on the ontogenetic development of muscle spindles. J Embryol Exp Morphol 1957; 5: 283-292.