

Celebration of Ernest Gutmann's life

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Ernest as a person

In my talk I will give several examples to illustrate the most endearing characteristics of Ernest's personality. Here I mention just one particular incidence. There was a meeting of heads of divisions of the Institute of Physiology to discuss some questions of interdepartmental organization of the Institute. This meeting coincided with one convened by the cleaners and maintenance workers of the Institute. For most of the heads of Divisions the decision as to which meeting to attend was not a problem and all apart from Ernest attended the meeting of the heads of divisions. Ernest's choice was quite unexpected; he attended the meeting of the cleaners and maintenance workers. At the time, this choice seemed quite inappropriate, but with hindsight it became clear that the meeting Ernest attended was much more important for the smooth running of the Institute, than that of the heads of Divisions. Such unconventional and original decisions were also reflected in Ernest Gutmann's work, where he was attracted to the study of problems that were not necessarily fashionable, but attracted his attention by their uniqueness. Since this meeting is concerned with the changes in denervated muscles I will discuss a special topic that is unique and interested Ernest, i.e. denervation hypertrophy.

Effects of denervation on different types of skeletal muscle fibers

The majority of vertebrate skeletal muscles are involved in locomotor functions and these are controlled by the CNS, where the motoneurone is 'the final common path' and connects the skeletal muscles to the nervous system. It is therefore not surprising that the motoneurone exerts the most important influence upon skeletal muscles and mediates the adjustment of skeletal muscle fibers to the functional requirements imposed upon them by the CNS. In most cases studied there appear to be two major mechanisms that control muscle properties: activity and load. Removing activity entirely, by disrupting the connection between the motoneurone and muscle, as is the case in denervation has a dramatic effect on muscle. Most muscles atrophy and suffer rapid structural deterioration. However, not all muscle fibers react to denervation by atrophy and structural deterioration. In mammals most muscle fibers that have one endplate, conduct action potentials and respond by a twitch contraction when stimulated, undergoes atrophy after denervation. The cause for these changes have been studied mainly in these muscle fibers. Indeed, the mechanism by which the rapid loss of muscle mass is brought about is becoming understood [7].

However, in addition to twitch fibers there are other muscle fibers present mainly in lower vertebrates, birds and in some specialized mammalian muscles referred to as tonic. Unlike twitch fibers which have a single site of innervation and conduct action potentials, tonic fibers have several endplates along a single muscle fiber, they do not conduct action potentials and are activated by currents that spread from the endplate along the surface of the muscle initiating a contraction of a segment of the muscle fiber (for review see Vrbová et al. 1978 [8]). This allows for very fine control of movement, for each section of the muscle can contract independently. The ALD muscle of the chick is a muscle composed entirely of such muscle fibers. It also contains 2 isoforms of myosin heavy chains SM1 and SM2 and the proportions of the SM1/SM2 isoforms of myosin change with development [4]. In young chicks the SM1 isoform is the predominant form and with age it shifts towards the SM2 isoform. The ALD

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muscle is a muscle which controls the movement of the wing during flight and on the ground keeps the wing in position alongside the trunk.

It came as a surprise when Te Pei Feng and his colleagues [2] published in 1962 that the tonic fibers of the chick ALD muscle hypertrophy after denervation. It was later found that the transition of the SM1 to SM2 myosin heavy chains is accelerated after denervation. Similar changes in ALD muscle fibers could be induced by increasing the load of the muscle. Like denervation, this too caused hypertrophy and shifts of the SM1 towards SM2 isoforms of myosin heavy chains [5]. These results were puzzling, for inactivity that follows denervation had the same effect as increased activity induced by loading the muscle. It was therefore possible that innervation has a special function in this muscle that controls its growth, which is independent of muscle activity.

Control of muscle mass and myosin isoforms of ALD by activity

When considering the mechanism by which the innervation of the ALD muscle inhibits hypertrophy and controls the expression of the 2 myosin isoforms two possibilities have to be considered: a) is the restriction of growth mediated by muscle activity, and b) does the nerve exert a special trophic influence that controls muscle mass. In the early work of Feng's and Gutmann's laboratory the second possibility was favored. This was due to the finding that unlike in mammalian twitch fibers cross-innervation experiments did not produce any changes in contractile properties of ALD muscles [3]. Nevertheless, direct evidence was lacking to resolve the question of activity vs. trophic influence.

To continue the 'Gutmann' tradition another of Ernest Gutmann's students, the late Radovan Zak and I together with colleagues from our laboratories, carried out experiments to establish whether activity was important for denervation changes in the ALD muscle [1]. Instead of denervation we paralyzed the ALD muscle by blocking the AChR by local application of α -bungarotoxin. Fig. 1 shows that the paralyzed muscles hypertrophied. Moreover, like in denervated muscle, there was a shift of the SM1 myosin isoform towards SM2. Thus inactivity had the same effect as denervation.

The fact that the ALD muscle fibers have endplates on several sites along their surface allowed us to paralyze/inactivate, only a segment of the muscle, and examine whether the paralyzed segment differed from the active part of the muscle. Accordingly, we placed an α -bungarotoxin containing silicone strip only on the central part of the muscle. Muscle fiber areas were then measured from hematoxylin eosin stained sections

taken from the central paralyzed segment and from the humeral and vertebral part of the ALD muscle which were not paralyzed. Fig. 2 shows that only muscle fibers from the central,

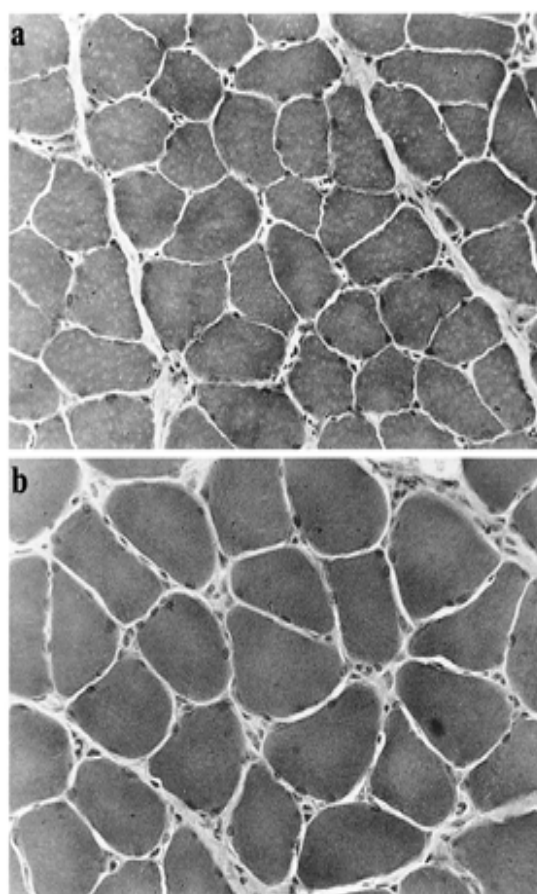


Fig. 1 a-b: The effect of application of α -bungarotoxin on muscle fiber diameters of ALD muscle. Microphotographs of hematoxylin eosin stained cross-sections of ALD muscle exposed to a saline (a) and α -bungarotoxin (b) containing silicone implant. Horizontal bar represents 50 μ m. From Connold et al, 1993 [1].

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paralyzed section of the muscle hypertrophied, while those either side of it retained their normal size. Moreover, only in the central segment did the ratios of SM1/SM2 isomyosins change so as to resemble denervated muscles.

In view of these results we concluded that denervation hypertrophy and shifts of SM1 to SM2 myosin isoforms of ALD muscle fibers after denervation are the result of inactivity, rather than the absence of the trophic influence of the nerve on the ALD muscle fibers. However, the fact that inactivity or denervation of twitch muscle fibers leads to completely different changes than the same intervention or denervation of tonic muscle fibers is surprising. It would be important to analyze the molecular mechanisms that cause these two different types of skeletal muscle fibers to react to inactivity in such opposite ways.

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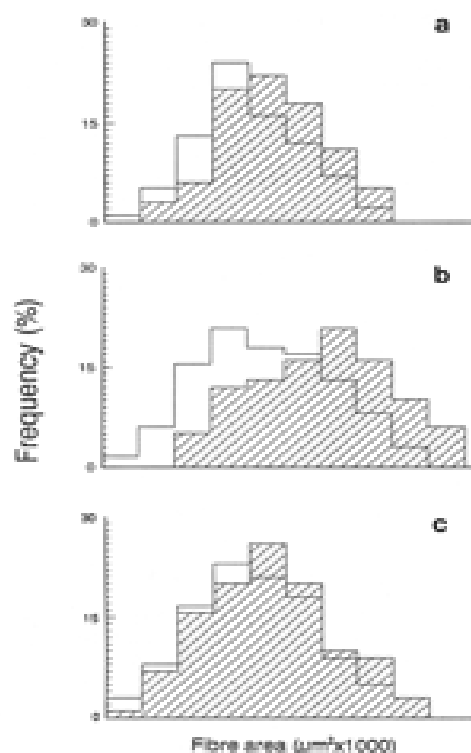


Fig. 2 a-c: the effect of α -bungarotoxin placed only over the central portion of the ALD muscle on muscle fiber areas from different parts of the muscle. Histograms of the frequency distribution of muscle fiber areas measured from hematoxylin -eosin stained cross-section taken from the vertebral (a), central (b), and humeral (c) part of ALD muscles. Only the central area had either a NaCl, or α -bungarotoxin containing silicone strip implant. The shaded histograms show results from α -bungarotoxin treated muscles and the open histograms from NaCl treated muscles. From Connold et al, 1993 [1].