THE CONCENTRATION OF RIBONUCLEIC ACID IN A DENERVATED MUSCLE

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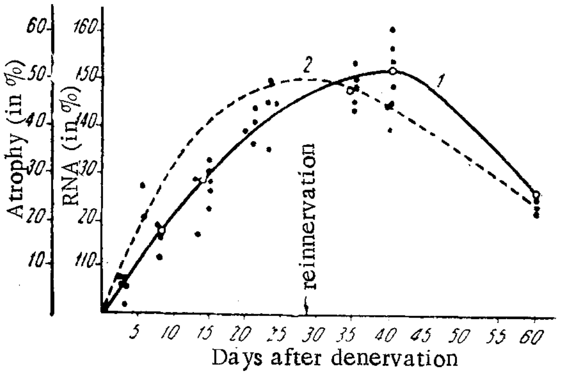
A number of biochemical, functional, and morphological changes occur in the muscle during the first few days after denervation. The question of the various destructive processes in the denervated muscle is clarified very well in the literature [1,2,16,30,36]. However, there is still great interest in the data on the positive changes in denervated muscles during the first 30-40 days after transection of the nerve.

According to the concepts developed by A. N. Studitskii, after any tissue injury, including denervation, a plastic state develops within it, characterized by high regeneration activity and the ability to withstand insufficiencies in the supply of oxygen and nutrient materials [10,11,13,14]. There are data available on the rapid regeneration of atrophied muscle following regrowth of the nerve [4,6] and incomplete transection [9]. However, the most convincing proof of the high regenerative capacity encountered in the denervated muscle is its successful autoplasticism, accomplished by A. N. Studitskii and N. N. Bosova [12]. The question is, what are the properties that are characteristic for the plastic state of the denervated muscle?

Morphological data indicate that the myofibril apparatus of the muscle is not disturbed for a long time after denervation. Only some of the fibers undergo intense breakdown and degeneration; others, however, although becoming thinner, exist in a reactive state and preserve their cross striation for a long time [26]. The number of nuclei in them increases considerably. Quite often one sees the picture of amitotic nuclear division, which is evidence of a real increase in the number of nuclei, and not of the multinucleation that occurs as a result of muscle fiber wasting. As was noted by Z. P. Ignat'eva [4], only 2 weeks after denervation it is possible to observe a significant increase in both the size and number of nucleoli in the nuclei. It is interesting that, along with the clear signs of atrophy-wasting of the muscle fibers, fatty infiltration, connective tissue proliferation, etc. – the formation of new muscle fibers by splitting may also be observed in the denervated muscle [1].

Certain changes in the metabolism of the denervated muscle indicate that it exists in a reactive state, similar to the state of regeneration. Use of the isotope method has made it possible to establish that many metabolic processes are markedly accelerated in the denervated muscle. It was shown that the intensity of renewal of myosine and adenosine-triphosphoric acid in the atrophied muscle is significantly higher than in the norm [7-20,27]. The intensified synthesis of the contractile muscle protein after denervation also explains why the complicated structure of the myosine molecule is preserved intact in the denervated muscle for a long period of time [29,34]. Despite the fact that the amount of actomyosine in the muscle decreases after denervation, the remainder possesses normal ATP-ase activity and contractile properties [7]. S. E. Severin [8] even noted an increase in the ATP-ase activity of myosine within the deefferentized muscle of a cat. The activity of many enzymes in the denervated muscle increases appreciably [3,5,23]. There is an elevation in the concentration of free SH-groups and glutathione [6,7]. Up to the present, the question of the character of respiration in the denervated muscle has remained unclarified. The majority of investigators have concluded that, in the atrophic state, the muscle absorbs more oxygen than in the norm [15,17,21,28]; others believe that the intensity of respiration remains unchanged [22,32,33]. There are also data indicating that respiration of the red muscles is reduced after denervation, while in the white it is elevated [24]. During the first month after denervation, the very important process of oxidative phosphorylation is not disturbed [8,16].

The presented morphological and biochemical properties of the denervated muscle characterize its plastic state. These changes are similar to the processes which occur in the regenerating muscle. From this point of view, it is



Change in the RNA concentration (1) and development of atrophy (2) of a muscle, following denervation. The points are related to the unbroken line.

interesting to study the question of the ribonucleic acid (RNA) concentration in the muscle after denervation. The scanty data present in the literature indicate that the concentration of RNA in the denervated muscle is elevated [25,35]. However, the hypothesis has been advanced that this increase is a passive process, occuring as a result of the decrease in muscle mass due to atrophy [31].

In this work, investigation of the RNA concentration in the muscle at various intervals after denervation was carried out by both the biochemical and histochemical methods, permitting an appraisal of the distribution of the acid in the denervated muscle.

EXPERIMENTAL METHOD

The work was carried out on 65 white rats, $2\frac{1}{2}$-3 months of age. In one of the posterior extremities, we transected the tibial nerve 2 cm above its entrance into the gastrocnemius muscle. The muscle in the other extremity served as the control. Spontaneous reinnervation of the muscle occurred after 28-30 days. The rats were sacrificed by decapitation after 3,7,14,20-26,34,40 and 60 days after the denervation.

The concentration of RNA was determined biochemically by the method of Meibaum, and histochemically by the method of Brashe with staining according to Unna. Atrophy of the denervated muscle was expressed in percents of the weight of the control muscle.

EXPERIMENTAL RESULTS

One week after denervation, the concentration of RNA in the experimental gastrocneimius muscle increased to an average of 115% (see the figure) as compared with the control muscle of the symmetrical extremity.

Subsequently, the amount of RNA in the denervated muscle gradually rose. Thus, after 2 weeks it reached 127%, and in the course of the 4th week, immediately before reinnervation – 142%. At 34 days after restoration of the neuromuscular connection, the concentration of RNA was 147%, while at 40 days after transection of the nerve, i.e., 10 days after reinnervation, it was 153% of the control level. Two months after denervation, the concentration of RNA in the muscle was reduced to 127%.

After denervation, the muscle gradually atrophied, and immediately after reinnervation it began to increase in weight. Thus, it is interesting to compare this process with the changes in RNA concentration within the muscle tissue. Both processes develop in parallel fashion (see the figure). On first glance, one gets the impression that the increase in RNA concentration in the muscle tissue is a passive process, occurring as a result of a decrease in the muscle mass; in other words, the wieght of the muscle decreases, while the total amount of RNA in it remains the same, and thus, one observes a relative increase in its concentration within the muscle. However, the histological data did not support this.

Our histological investigation of the RNA concentration in the denervated muscle showed that, as atrophy develops, RNA collects in many of the muscle fibers, mainly in the nucleoli, whose size and number increases notably in the nuclei, and also in the perinuclear cytoplasm. An active increase in RNA within the atrophied muscle, 20 days after denervation, is demonstrated by electron microscopy, performed by G. V. Elyakova. According to the data which she obtained, the nuclei in the fibers of a denervated muscle exist in a reactive state, enlarged, and possessing a large number of deep indentations and projections on their surface; the nucleoli are markedly enlarged, and a large number of ribosomes can be seen in the cytoplasm. Thus, one can say with certainty that the increase in RNA concentration within the denervated muscle is an active process. Beginning with the 28th-30th day, in connection with reinnervation, atrophy of the muscle decreases, and although the difference in the maximums of the two curves represented in the figure are not statistically significant, it may be postulated that the concentration of RNA in the regenerating muscle actually continues to increase for a certain amount of time, reaching 153% after 40 days.

Thus, an additional fact was obtained, showing that active synthetic metabolic processes develop in the denervated muscle characterizing its plastic state and also indicating that a unique process of regeneration begins in the atrophied muscle even before reinnervation, appearing as a compensatory reaction to the process of atrophy.

LITERATURE CITED

1. N. N. Bosova, III Scientific Youth Conference [in Russian], p. 7, Moscow (1959).
2. I. I. Ivanov and N. I. Mirovich, Vopr. med. khimii, 4, 403 (1960).
3. A. V. Ignatovich, The Effect of Denervation on the Concentration of Glutathione, Catalase, and Ascorbic Acid in Muscle Tissue, Diss. kand. Kursk (1946).
4. Z. P. Ignat'eva, Dokl. AN SSSR, 105, 2, 360(1955).
5. R. Leibson, Byull. éksper. biol., 7, 6, 518 (1939).
6. M. F. Popova, Change in Certain Functional and Biochemical Properties of Striated Muscles Following Denervation and Tendonotomy, Avtoref. diss. kand., Moscow (1954).
7. M. F. Popova, Fiziol. zh. SSSR, 11, 977 (1956).
8. S. E. Severin, Biokhimiya, 1-2, 259 (1957).
9. A. R. Striganova, The Reactivity and Regenerative Capacity of Denervated Muscle at Various Stages of Atrophy [in Russian], Moscow (1961).
10. A. N. Studitskii, The Experimental Surgery of Muscles [in Russian], Moscow (1959).
11. A. N. Studitskii, in the book: Plastic and Regenerative Processes [in Russian], 243, Moscow (1959).
12. A. N. Studitskii and N. N. Bosova, Arkh. anat., 12, 18 (1960).
13. A. N. Studitskii, Theses from the Reports of the 2nd All-Union Conference on the Problem of Tissue Incompatibility, and the Conservation and Transplantation of Tissues and Organs [in Russian], p. 3, Odessa (1961).
14. A. N. Studitskii and Z. P. Ignat'eva, Regeneration of Muscles in Higher Mammals [in Russian], Moscow (1961).
15. V. I. Telepneva, Vopr. med. khimii, 6, 456 (1957).
16. V. I. Telepneva, Vopr. med. khimii, 3, 301 (1960).
17. D. L. Ferdman, The Biochemistry of Muscle Diseases [in Russian], Kiev (1953).
18. D. L. Ferdman, V. A. Grigor'eva, and E. N. Medovar, Ukr. biokhim. zh., 3, 278 (1956).
19. D. L. Ferdman, Vopr. med. khimii, 5, 351 (1957).
20. D. L. Ferdman, Ozv. AN SSSR. Seriya. biol., 3, 346 (1960).
21. O. P. Chepinoga, Biokhem zh., 10, 5, 823 (1937).
22. D. I. Shatenshtein and D. L. Tsyrlina, Arkh. biol. nauk, 40, 2, 93 (1935).
23. G. S. Sheves, Biokhimiya, 1, 63 (1953).
24. G. S. Sheves and V. I. Ryumina, Biokhimiya, 3, 385 (1956).
25. T. Yu. Shesno, Theses from the Reports of the 1st Conference on Nucleic Acids and Nucleoproteins [in Russian], p. 56, Moscow (1959).
26. P. I. Émdin, Changes in Skeletal Striated Muscle Following Transection of the Nerve [in Russian], Kazan (1914).
27. S. F. Épshtein, Ukr. biokhim. zh., 6, 826 (1959).
28. O. Gudlitska, in the book: Collection of the Works from the Conference on Neural Regulation of the Metabolism and of Active Transport of Ions [in Russian], p. 51, Prague(1958).
29. M. Aloisi, A. Ascenai, and E. Bonetti, Biochim. biophys. Acta, 10, 70 (1953).
30. E. Fischer and V. W. Ramsey, Am. J. Physiol, 145, 571 (1946).
31. E. Gutmann, The Denervated Muscle, Prague (1962).
32. G. C. Knowlton and H. M. Hines, Am. J. Physiol, 109, 200 (1934).
33. R. Levine, O. Hechter, and S. Soskin, Ibid., 132, 336 (1941).
34. G. Schapira, J. Dreyfus, and M. Joly, Nature, 170, 494 (1952).
35. C. Schmidt and H. Schlief, Z., ges. exp. Med., 127, 53 (1956).
36. S. S. Tower, Physiol. Rev., 19, 1 (1939).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.