

CHANGES IN CONTENT OF SULFHYDRYL GROUPS IN ORGANS OF ALBINO RATS AND MICE DURING REPARATIVE REGENERATION OF SKELETAL MUSCLES

V. S. Nikitin and G. A. Shafranskaya

UDC 616.74-003.93-092.9-07:616-008.932
691-074

The content of SH-groups falls significantly in regenerating skeletal muscles of albino rats and mice (21-60 days after injury). According to the results of histochemical investigations the reason for the decrease is excessive development of collagen fibers in the regenerating muscles. During the period of muscle regeneration, especially from the 21st to 30th days, a decrease in the content of SH-groups takes place in the liver and kidneys. No significant changes are observed in the blood serum and spleen.

* * *

Despite the large number of investigations of sulfhydryl groups which have been conducted, their role in repair has received little attention. Only isolated reports have been published on this subject [4].

The object of the present investigation was to study changes in SH-groups during regeneration of skeletal muscles.

EXPERIMENTAL METHOD

The gastrocnemius muscle was excised up to one-third of its thickness in experimental animals (albino rats and mice) under aseptic conditions. The animals were sacrificed 2, 6, 21, 30 and 60 days after the operation by exsanguination under light ether anesthesia. Samples of muscle weighing from 80 to 120 mg were taken from the region of the wound and ground in glass homogenizers on ice. The content of SH-groups in the blood serum, and in homogenates of liver, spleen, and kidney tissues was studied at the same time. The protein content in the serum and homogenates was determined by a colorimetric method using Gornall's reagent. The SH-groups were estimated by the method of Kolthoff and Harris [5], modified for tissue homogenates by several authors [1-3]. To 0.5 ml of homogenate of muscle (25 mg fresh tissue), spleen, liver, and kidneys (50 mg fresh tissue) or 0.5 ml of blood serum in a reaction vessel, 24.5 ml ammonium buffer (0.03 M ammonium hydroxide + 0.03 M ammonium nitrate in equal volumes) was added and the mixture titrated with 0.001 M silver nitrate solution. The readings of a type M 17/1 mirror galvanometer ($3.2 \cdot 10^{-3}$ A per division) were recorded at the end of each minute after addition of 0.05 ml silver nitrate solution. The control series consisted of homogenates of muscles, liver, spleen and kidneys and blood serum from animals not undergoing operation. Histochemical determination of SH-groups in the muscle were carried out by the method of Chèvremont and Frédéric.

EXPERIMENTAL RESULTS

Histochemical investigations showed that the small granular necrotic masses of muscle fibers gave very weak reaction for SH-groups. Only a few highly contracted fragments of injured fibers retained their ability to react with ferricyanide for a long time. The zone of the wound focus was clearly demarcated from the uninjured tissue (Fig. 1). The subsequent development of cells and myosyncytia in the wound led to some increase in intensity of the ferricyanide reaction. Young muscle fibers stained very intensively for SH-groups between the 21st and 30th days after injury, but because of the presence of a large number of con-

Department of Biology, Grodno Medical Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR S. E. Severin). Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 66, No. 8, pp. 103-105, August, 1968. Original article submitted June 15, 1967.



Fig. 1. Marked decrease in reaction for SH-groups in zone of granular degeneration of muscle 2 days after injury. Chèvremont and Frédéric's method. Magnification objective 8 times, ocular 10 times.

nective-tissue fibers the reaction of the regenerating muscle as a whole was less intensive than that of intact muscle.

Amperometric titration showed that at all stages of regeneration the content of SH groups in the injured muscle was reduced. However, in the first stages the content of reactive SH-groups showed considerable fluctuation and the decrease was not statistically significant. In later stages the decrease in content of SH-group become stable. During titration of 0.5 ml homogenate of regenerating muscle tissue, 0.001 M silver nitrate solution was used up in the following amounts: on the 21st day 0.12 ± 0.002 ml, on the 30th day 0.11 ± 0.002 ml, and after 2 months 0.12 ± 0.002 ml, compared with 0.14 ± 0.004 ml in the control. The content of free SH-groups thus showed a decrease of 14.3% after 21 days ($P < 0.001$), 21.5% after 30 days ($P < 0.001$), and 14.3% after 2 months ($P < 0.01$). The reason for the decrease in content of SH-groups was accumulation of collagen fibers in the regenerating muscle.

In the liver and kidneys a decrease in content of SH-groups was found in the stages from 2 to 30 days of development of regeneration in the muscle. The decrease was more marked on the 21st day (in the liver by 14.8%, $P < 0.01$; in the kidneys by 11.6%, $P < 0.001$), and on the 30th day (in the liver by 16.8%, $P < 0.02$; in the kidneys by 15.1%, $P < 0.001$). No significant changes were found in the spleen and blood serum. The changes in content of SH-groups in the internal organs are evidence of substantial biochemical changes throughout the body in the period of muscle regeneration. Quantitative estimation of protein in the homogenates and blood serum showed no significant differences from normal.

LITERATURE CITED

1. S. N. Nistratova, In: Thiol Compounds in Medicine [in Russian], Kiev (1959), p. 89.
2. K. V. Savich and V. A. Yakovlev, *Vopr. Med. Khimii*, No. 2, 121 (1957).
3. Yu. M. Torchinskii, *Byul. Éksperim. Biol. i Med.*, No. 12, 108 (1958).
4. M.P. Yavich, *Dokl. Akad. Nauk SSSR*, 139, No. 6, 1471 (1961).
5. J. M. Kolthoff and W. E. Harris, *Industr. Eng. Chem. Analyt. Ed.*, 18, 161 (1946).