

THE STUDY BY THE METHOD OF VITAL STAINING OF THE SORPTIVE
PROPERTIES OF CERTAIN ORGANS IN EXPERIMENTAL TUBERCULOSIS
COMMUNICATION II. INVESTIGATION OF THE SORPTIVE PROPERTIES OF SKELETAL MUSCLE

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Analysis of the physiological condition of muscle tissue cells in tuberculosis is of definite interest on account of the fact that a number of workers believe that muscle possesses particular resistance to tuberculous infection. In a small number of papers devoted to the study of the state of the striated musculature in tuberculosis there are indications of gross pathohistological changes discovered at necropsy in advanced cases of tuberculosis with extensive spread in the internal organs [1, 5, 6, 8, 14]. Only a few isolated papers [10, 12] have been devoted to the experimental study of the early changes in muscle tissue. This is presumably due to the fact that the pathohistological method alone is inadequate for the study of this problem. The purpose of the present investigation was to study the functional state of the muscle tissue in experimental tuberculosis in guinea pigs. In this work we used the method of vital staining devised by D. N. Nasonov.

This method was used by several workers to study the functional state of the skeletal muscles after various forms of trauma [2, 3, 4, 7, 9, 11, 13, 15].

EXPERIMENTAL METHOD

The study of the sorptive properties of the skeletal muscle cells of guinea pigs was made in three series of experiments: after infection of the animals with a virulent culture of bovine tuberculosis, after infection of the animals with an attenuated culture of tuberculosis — BCG, and after infection of the animals with a virulent culture of bovine tuberculosis following preliminary two-stage immunization. In this way three gradations of tuberculosis were produced in the experimental animals, which facilitated the solution of the problem.

The experimental conditions, the method of investigation and also the principle used in drawing up the curves of sorption were described in the first report of an investigation of the sorptive power of brain tissue in infection with tuberculosis.

The experiments were performed on the soleus muscles of both hind limbs. After careful preparation of the muscles the tendons were divided on both sides and the muscles transferred in a resting state to Ringer solution for 10–15 min. Before staining the muscles from both sides were attached by the ends of their tendons to specially curved glass rods, separately, and suspended in a solution of neutral red (0.01%) at a temperature of 18–20°C. After 30 min the muscles were taken out and rinsed in Ringer solution, and the tendons, remains of fatty tissue and fascia removed. After this, the muscles were placed for 24 hrs in a tube with a known volume of 70% alcohol, acidified with a 2% solution of sulfuric acid in order to extract the dye.

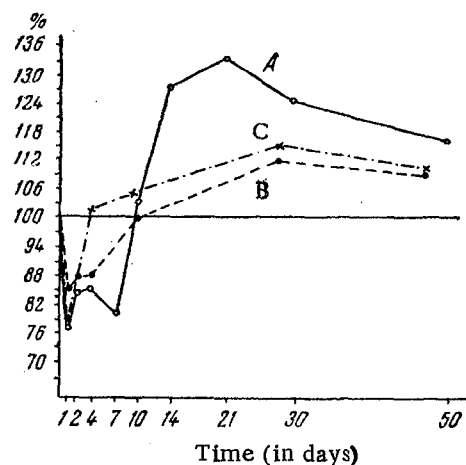
A study of the special features of vital staining of muscle tissue was carried out at the same intervals of time after infection as in the investigation of the tissue of the cerebral cortex. In the first series of experiments the animals were killed after 1, 2, 4, 7, 10, 14, 21, 30, 50 days, and in the second and third groups of experiments after 1, 2, 4, 10, 30, 50 days.

* Deceased.

Combination of the Dye with the Soleus Muscles of Healthy and Tuberculous Guinea Pigs

Conditions of infection	No. of expts.	Period of infection								
		1 day	2 days	4 days	7 days	10 days	14 days	21 days	30 days	50 days
TB	10-30	-23.8±4.6	-16.2±2.8	-15.3±2.5	-20±4	+3.4±1.4	+27±1.8	+33.2±4.7	+24.2±5.5	+14.7±3.1
BCG	3	-16±0.58	-13.3±3.7	-13±1	—	+0.7±0.33	—	—	+12±2.67	+8.7±3.35
BCG + TB	3	-22.3±1.85	-16±2.51	+1.3±4.05	—	+5.3±1.2	—	—	+14.7±5.04	+10±4.51

Note. The signs (+) and (-) indicate an increase and decrease in the quantity of dye combined with the soleus muscles of the experimental animals in percentages of the quantity of dye combined with the soleus muscles of control animals. The results are shown in the form of the arithmetic mean. of the total number of experiments.



Curves showing the changes in the sorptive power of the soleus muscles of a guinea pig in experimental tuberculosis. Along the axis of the abscissas - time after infection (in days); along the ordinate axis - amount of combined neutral red, expressed as a percentage of the amount of neutral red combined with the muscles of the control animals; A) in animals infected with a virulent culture of bovine tuberculosis; B) in animals infected with an attenuated culture of tuberculosis, BCG; C) in doubly immunized animals infected with a virulent culture of bovine tuberculosis.

Altogether 400 experiments were made in the first series, and 40 each in the other two series.

Meanwhile at the same times histological examinations were made of the internal organs and skeletal muscles (2 animals for each period of time). For this purpose the hematoxylin-eosin and picrofuchsin methods were used to stain the organs.

In order to estimate correctly the changes in the sorptive properties of the muscles in the experimental animals it was essential first of all to find out how the dry weight of the muscles altered during the development of the disease. Investigations showed that in all 3 series of experiments the dry weight of the muscles varied only insignificantly, particularly with infection by BCG and by a virulent culture when the animals were immunized, which leads us to consider that our results obtained from the study of the changes in the sorptive properties are reliable in all stages of the infection.

EXPERIMENTAL RESULTS

On histological examination we were unable to find any specific morphological changes in the muscle tissue of the animals in all three series of experiments at all stages of development of tuberculosis.

In studying the sorptive properties of the muscles in the three series of experiments we observed a change in the degree of combination of dye, which was biphasic in character. The first phase was characterized by a fall and the second by a rise in the sorptive power. We observed an analogous phenomenon when studying the changes in the sorptive power of the cerebral cortex.

The duration of the first phase in the three series of experiments was not the same. The longest duration of this phase was observed in the first series of experiments with infection of the animals by a virulent culture of Koch's bacilli (see figure and table).

The degree of lowering of the sorptive power in the first phase after infection with a virulent culture of unimmunized animals and also of those previously immunized was the same, while after infection with a culture of BCG there was a less pronounced fall in the sorptive power.

Variations in the sorptive level were most clearly expressed after infection of the animals with a virulent culture of Koch's bacilli.

The second phase of the reaction — a rise in the sorptive power of the muscles — was also unequal in all three series of experiments. The quantity of combined neutral red in all the groups of experimental animals in this phase was from 12-33% greater than that found in the control group, and moreover the greatest degree of change in the functional state of the muscle tissue was observed after infection of guinea pigs with a virulent culture of Koch's bacilli. In this case these changes reached their maximum much sooner (on the 21st day) than in the other two series of experiments (on the 30th day after infection).

In all the series of experiments, after the second phase of the reaction a fall in the sorptive power of the muscles was observed, although it was still higher than in the control animals and remained so until the terminal period.

In this period, when the second phase of change in the sorptive power of the muscles was observed, the tuberculous process was progressing unrestrainedly in the internal organs.

The experiments which we performed showed that even within a few hours of infection with tuberculosis, the guinea pigs showed changes in the functional state of the muscle tissue.

As the disease progresses, with development of severe specific morphological changes in the internal organs, the sorptive power of the muscle cells rises intensively. This indicates the intensification of paranecrosis and alterative processes in the sarcoplasm of the cells. The muscle tissue at this period undergoes profound changes, as shown by the alteration in its sorptive properties. With the development of the disease irreversible degenerative changes arise in the cell structures of all the organs, including the muscles. This is reflected in the character of the sorptive power of the cells.

Thus our investigation of the functional state of the muscle cells by the method of vital staining showed that these cells in experimental tuberculosis are actively involved in the reaction of the body within a few hours of infection. Analogous findings were observed after vaccination with a BCG culture.

SUMMARY

The method of vital staining was used in experimentally-induced tuberculosis for the study of the physiological condition of the cells of the cross-striated muscle.

It was established that at the early stages of this infection when no morphological changes are, as yet, present in internal organs, substantial changes take place in the cells of the skeletal muscles. These changes are reflected in the change of the sorptive properties of the cellular sarcoplasm.

The dynamics of the sorptive power is not the same in all cases but has a regular wave-like biphasic character. The sorptive power of the cells is intensively increased with increase of the morphological changes in internal organs.

The method of vital staining demonstrated that the cells of the skeletal muscle do not remain intact and are actively involved in the reaction of the organism to tuberculous infection.

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THE SELECTIVE EFFECT OF SOME CHEMICAL STIMULANTS ON THE REFLEXES FROM CHEMORECEPTORS

COMMUNICATION VIII. THE EFFECT OF STREPTOMYCIN ON REFLEXES FROM THE CHEMORECEPTORS AND MECHANORECEPTORS OF THE PERICARDIUM

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In previous reports [1, 3, 4, 5] it has been shown that the parenteral injection of streptomycin depresses reflexes from the chemoreceptors of the small intestine, spleen, kidneys and the tissues of the hind limb.

According to reports in the literature, the conducting pathways from the chemoreceptors of the zones mentioned above pass through the spinal cord [2, 8, 9, 11]. In this connection it is important to ascertain the character of the effect of streptomycin on the reflexes from those reflexogenic zones from which the conducting path-

* In Russian.