

# OXYGEN TENSION IN SKELETAL MUSCLE OF UNANESTHETIZED RATS DURING HYPOTHERMIA

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An amperometric method was used to investigate the oxygen tension in the gastrocnemius muscle of unanesthetized albino rats during hypothermia. A fall of body temperature was accompanied by a gradual decrease of  $pO_2$ : during cooling to  $34^\circ$  it fell on the average by 65% of its initial value, and to  $34-30^\circ$ , by 40%. The value of  $pO_2$  recovered during artificial reheating.

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The skeletal musculature plays an important role in cold thermogenesis [5, 8, 9, 11]. However, many of the characteristics and conditions of this thermogenesis remain unexplained.

In the present investigation the oxygen tension was studied in the muscles of hypothermic rats.

## EXPERIMENTAL METHOD

Experiments were carried out on 12 albino rats of both sexes weighing 150-350 g. The unanesthetized animal was fixed in the supine position, and cooled by covering its body with rubber bags containing crushed ice. When the rectal temperature had reached  $30^\circ$ , artificial reheating was begun by covering the body with bags containing water at  $45^\circ$ . At all stages of the experiment the oxygen tension was recorded in the gastrocnemius muscle. In 5 animals the tendo achillis was divided before the experiment began, while in the remaining 7 rats it was left intact.

The oxygen tension in the muscle was determined amperometrically [1, 2]. The polarizing electrode was a platinum wire 0.1 mm in diameter, soldered into a glass capillary tube, so that its free end did not exceed 1 mm in length. The nonpolarizing electrode was a calomel electrode connected to the object through 0.9% NaCl solution. A constant negative potential of 0.7 V was applied to the polarizing electrode (pushed into a muscle for 5-6 mm and left in that position until the end of the experiment). The current in the circuit was measured by a type M-21 mirror galvanometer with a sensitivity of  $0.62 \cdot 10^{-9}$  A/mm.

Because of inadequate stability of the open platinum electrodes, their sensitivity to oxygen was measured before each experiment. For this purpose, the current between the polarizing and nonpolarizing electrodes was measured in 0.9% NaCl solution, brought into equilibrium with the surrounding air. The strength of the residual current in 0.9% NaCl freed from oxygen by addition of sodium sulfite (0.1 g/100 ml solution) was then measured. A calibration curve was plotted from the results obtained.

In the course of the work, the surface of the polarizing electrode within the tissue changes its properties under the influence of surface-active substances and electroadsorption. To reduce the degree of electroadsorption, the measurements were made in an interrupted sequence: after every 3 min of work, the electrodes were short-circuited for 1 min - until their potentials were equalized. In this way, the next measurement could begin when the electrostatic properties of the active surface of the electrode were uniform. In addition, at the end of the experiment, the electrode calibration was repeated. Since the sensitivity of the electrode is a linear function of time, appropriate corrections were made to the figures obtained at the various periods of the experiment.

Because of changes in the rate of diffusion, which are proportional to the absolute temperature of the object, a second correction also was made. This was based on the temperature coefficient, and was determined for each polarizing electrode experimentally.

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TABLE 1. Effect of Hypothermia on Oxygen Tension in Gastrocnemius Muscle of Rat ( $M \pm m$ )

| Muscle                            | Initial  | Cooling to |          | Reheating |          |
|-----------------------------------|----------|------------|----------|-----------|----------|
|                                   |          | 34°        | 34—30°   | 30—34°    | over 34° |
| Intact                            | 27 ± 2,7 | 17 ± 1,7   | 11 ± 1,8 | 16 ± 3,0  | 23 ± 1,9 |
| With preliminarily divided tendon | 34 ± 2,6 | 24 ± 2,0   | 22 ± 1,8 | 21 ± 3,0  | 23 ± 2,5 |

## EXPERIMENTAL RESULTS

The results obtained showed that the development of hypothermia is accompanied by a decrease in the oxygen tension both in the intact muscle, and in the muscle whose tendon had previously been divided (see Table 1).

The marked decrease in oxygen tension discovered in these experiments in the muscle may be regarded as evidence that cold thermogenesis takes place under conditions of muscular hypoxia. This view is supported by indirect evidence of an increase in the relative importance of anaerobic processes in muscles during hypothermia induced by other means than by drugs: the appearance of a marked loss of glycogen in the muscles [3, 10, 13], and the accumulation of lactates both in the muscles and in the blood [6, 7, 12].

There are three possible reasons for the decrease in oxygen tension in the muscle: first, less oxygen reaching the tissue; second, increased utilization of oxygen by the cells; and third, the low temperature of the tissue. In hypothermia not due to drugs, all these factors evidently play a role. It has been shown experimentally that during shivering there is an increase in the oxygen demand [5]. On the other hand, the amount of oxygen reaching the muscle is inadequate: Our previous investigations [4] revealed a decrease of the blood supply, not only to the skin but also to the skeletal muscles of the cooled animal, by 33–50%.

Any mechanical vibration is known to increase the rate of diffusion of oxygen to an electrode, and thus, to give values for the oxygen tension in the tissue which are too high. To prevent such vibration from developing, the tendon of the gastrocnemius muscle was divided. Even if it did not completely abolish the contractile activity of the muscle, the division must have weakened it very considerably and reduced its oxygen demand. At the same time, the degree of compression of the blood vessels passing through the muscle must have been reduced after division of the tendon, and accordingly, the blood supply of the muscle must have been improved, and its oxygen supply increased. Nevertheless, even under these conditions, in response to hypothermia the oxygen tension clearly fell, although not by the same amount as in the intact muscle. The main reason for this decrease was evidently active vascular spasm.

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