

DYNAMICS OF LACTATE DEHYDROGENASE ACTIVITY OF SKELETAL AND HEART  
MUSCLES AFTER A SINGLE EXPOSURE TO AN ALTERNATING MAGNETIC FIELD

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After a single exposure for 24 h to an alternating magnetic field (200 Oe, 50 Hz) increased lactate dehydrogenase activity was observed in the cardiac and skeletal muscles of albino rats for the first 48 h, together with a change in the distribution of the enzyme between the cell structures and a shift of the isozymes toward the M type. A complete return to normal occurred only in the third to fourth week.

**KEY WORDS:** alternating magnetic field; lactate dehydrogenase; isozymes; skeletal and heart muscles.

A pulsed magnetic field has been shown [3, 4] to activate hydrolysis. The writers have shown that the action of an alternating magnetic field (AMF) is similar in character [6].

Because of the important role of the isozyme ratio and intracellular distribution of lactate dehydrogenase (LD) in the regulation of carbohydrate metabolism [2, 5, 11], an investigation was carried out to study the dynamics of these indices in muscle tissue after a single exposure to an AMF.

#### EXPERIMENTAL METHOD

Experiments were carried out on male rats kept for 24 h in the interpolar gap of a magnet with a magnetic field of intensity 200 Oe and a frequency of 50 Hz. The animals were decapitated after 1, 4, 12, and 24 h and 2, 7, 14, and 28 days.

Total LD activity [7] and the activity of its isozymes in the skeletal and heart muscles were determined by disk electrophoresis in 6% polyacrylamide gel [8, 12], followed by densitography [1] and estimation of proteins [10]. A histological survey examination was made with sections stained with hematoxylin and eosin. The activity and distribution of LD in the intracellular structures were studied by a histochemical method [9].

#### EXPERIMENTAL RESULTS

The total LD activity in skeletal muscle 1 h after exposure to the AMF was increased from  $489 \pm 24$  to  $612 \pm 27$  mmoles/min/g wet weight of tissue ( $P < 0.01$ ); it remained at this level for 2 days, and then fell gradually for 7 days and reached the control level after 28 days. Investigation of the isozyme ratio in the cytoplasm showed that in intact animals H subunits accounted for 66.4% and M subunits for 33.6% of the total activity. The ratio was altered 2 days after exposure to the AMF: Activity of the H subunits fell to 38.3% while that of the M subunits rose to 61.7%. By the seventh day these indices had returned to their initial levels. Five isozymes were found in the mitochondria. In the control series LD<sub>5</sub> accounted for 51.7% of the total activity, but by the second day this had fallen to 32.6%. The ratio between the other isozymes showed less substantial changes.

On histological examination at times until 24 h marked dilatation of the capillaries, venules, and lymphatics, perivascular edema, swelling of the tissues, and vacuolation of the

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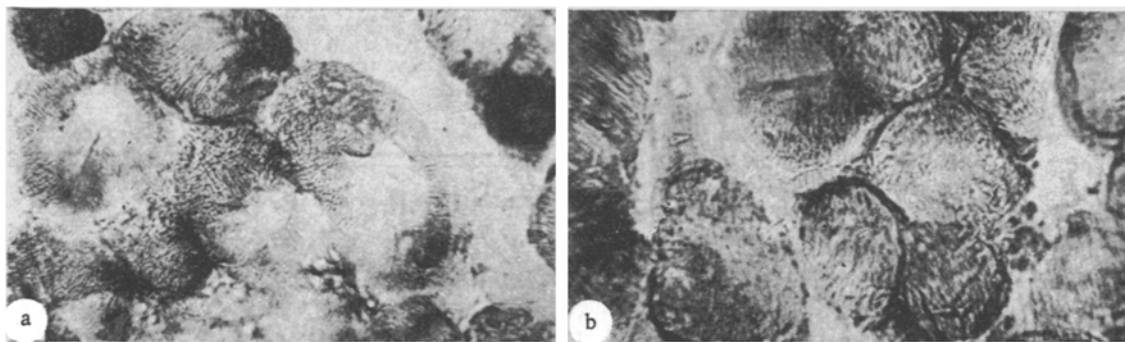


Fig. 1. Dynamics of lactate dehydrogenase activity of the rat soleus muscle after a single exposure to an AMF. Here and in Table 2: a) control; b) 2 days after action of AMF, 500  $\times$ .

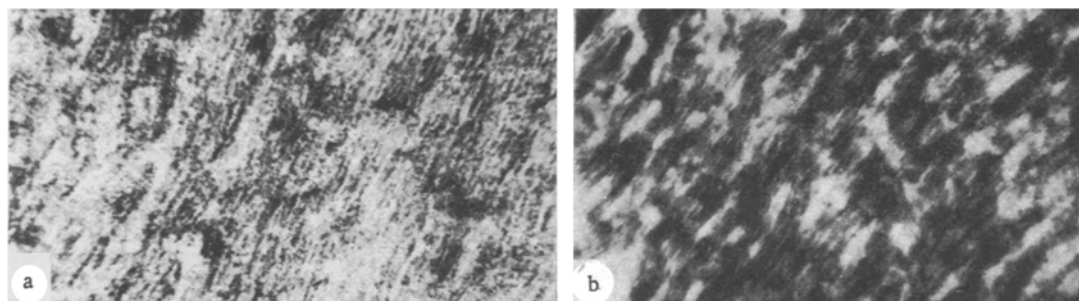


Fig. 2. Dynamics of lactate dehydrogenase activity in rat myocardium after a single exposure to the AMF.

cytoplasm were observed. After 2 days the cross striation could not be detected in some areas of the muscle fibers. After 28 days the muscle tissue was very little different from the control. On histochemical investigation, LD was revealed in the control as a cytoplasmic component and as tiny, clearly defined, irregularly shaped granules, uniformly distributed (Fig. 1a). After 2 days the cytoplasmic component was more intensely stained and the granules were denser and localized beneath the sarcolemma (Fig. 1b); after 7 days they appeared in the center of the fibers and the sarcoplasm was more uniformly stained.

Total LD activity in the heart muscle was increased only after 2 days ( $P < 0.052$ ) and returned to near the control level after 7 days. In the cytoplasm H subunits accounted for 76.4% of the total activity. The ratio was altered 1 h after exposure to the AMF: The activity of the H subunits fell to 40.7%, at which level it remained for 2 days. Only three isozymes were found in the mitochondria. After 2 days the content of LD<sub>1</sub> and LD<sub>2</sub> showed a tendency to fall.

Histologically, after 48 h marked vasodilatation was observed, with changes in the staining properties of the cardiomyocytes and their increased affinity for acid dyes. The nuclei were displaced toward the sarcolemma, they lost their regular shape, they became pale or hyperchromic, and sometimes the chromatin was concentrated at the margin. Individual foci of destruction were encountered beneath the endocardium. Histochemical investigation showed that LD again was represented by cytoplasmic and granular components (Fig. 2a). Activity of cytoplasmic LD in the swollen cardiomyocytes was increased after 2 days (Fig. 2b) and after 7 days many edematous areas could still be seen beneath the endocardium, but the cytoplasmic reticulum and thin granules were already visible, although the enzyme was still located at the periphery of these fibers. The changes disappeared after 28 days.

These experiments thus showed that after a single exposure to an AMF for 24 h the following changes were observed: some increase in LD activity in the skeletal and heart muscles, more especially 1-2 days after exposure, changes in the intracellular distribution of the enzymes, and a shift of the LD isozyme spectrum of the cytoplasm of these tissues toward an increase in the M type. In the writers' opinion these changes are evidence of activation of glycolytic processes as the result of tissue hypoxia. The cause of the developing hypoxia could be the well-marked edema of the tissues resulting from a disturbance of the circulation of blood and lymph.

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## CHANGES IN LYMPHOCYTE CHROMATIN IN DOWN'S SYNDROME REVEALED BY THE THERMAL DENATURATION METHOD

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A luminescence-microscopic study using acridine orange with a short-term culture of human cells showed that DNA melting profiles of the chromatin of intact lymphocytes of healthy donors are curves with maxima (F 530) in the temperature regions of 45, 65, 78, 85, 88, and 92°C ( $P < 0.01$ ). The melting profiles of lymphocytes of patients with Down's syndrome are curves with maxima in the temperature regions of 65, 85, 88, and 92°C ( $P < 0.01$ ). The absence of a decrease in the intensity of fluorescence between 78 and 85°C is evidently due to the greater degree of condensation of certain regions of the chromatin complex of the trisomic cells. The possible mechanisms of the structural changes in the interphase chromatin of human lymphocytes under the influence of temperature are discussed.

KEY WORDS: Down's syndrome; lymphocytes; interphase chromatin.

By luminescence microscopy with acridine orange the writers previously showed changes in the structure and function of chromatin of interphase nuclei of peripheral blood lymphocytes from patients with Down's syndrome [5]. It was postulated that changes in the functional activity of the cell chromatin were connected with an increased degree of its condensation. Differences in the degree of condensation of the chromatin of normal and trisomic cells, whatever the changes inducing them, must ultimately, it is considered, be determined by changes in the relations between DNA and protein. This change must be expressed as a change in the degree of dissociation of the protein of the chromatin complex. Unfortunately, no methods are yet available for determining the quantity of dissociated protein directly in interphase nuclei. However, the number of phosphate groups of DNA accessible to binding with test agents can be determined. Acridine orange [7] was used as the test agent, and the temperature factor [6, 8] as the procedure leading to dissociation of the protein component of the cell chromatin in a medium of physiological ionic strength.

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