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# QUANTITATIVE HISTOPHOTOMETRIC STUDY OF LIVER DEHYDROGENASE ACTIVITY DURING TEMPORARY LIMB ISCHEMIA

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One of the most serious complications of certain cardiovascular disease is arterial occlusion caused by thrombosis or embolism of the main arteries. The increase in the number of patients treated surgically for this condition has shown [6, 14, 17] that mortality after reconstructive vascular operations lies between 15 and 35% [10, 19]. Restoration of the circulation in ischemic limbs is known to lead to a systemic stress reaction, characterized by the development of a complex group of morphological and biochemical changes both in the affected tissue and in the body as a whole, and which in the recent literature has been called ischemic shock or the postischemic syndrome (PIS) [7]. The mortality from development of PIS is 25-40% [14, 17], and one cause of this is acute hepatorenal failure.

The liver occupies a central position in the regulation of metabolism, in the binding and neutralization of toxic substances of exogenous and endogenous origin. The morphological study of changes in hepatocyte metabolism in PIS is thus essential for the understanding of the key components of the pathogenesis of early postischemic disturbances. The important role of the liver in occlusion of the aorta and the main limb arteries and in states with similar pathogenesis has been stated repeatedly [1, 16, 18]. However, data in the literature on this problem are fragmentary [8] and the research has been done mainly at the biochemical level [11]. No reports of the quantitative enzyme histochemical study of changes in the liver in acute arterial occlusion of the limbs could be found in the accessible literature. Yet such an evaluation is necessary in order to elucidate the mechanisms of disturbance of hepatocyte activity under experimental and clinical conditions.

On the basis of previous experience of the use of a television image analyzer [4, 15] it was decided to undertake a quantitative study of the enzyme histochemical characteristics of changes in the liver during temporary limb ischemia followed by revascularization, and also during pharmacological correction in the ischemic and postischemic periods.

## EXPERIMENTAL METHOD

Experiments were carried out on 31 mongrel dogs of both sexes (including five control dogs) weighing 13-18 kg. Occlusion of the trifurcation of the aorta was produced by the method of Zatevakhin et al. [5]. The duration of ischemia of the limbs was 3, 6, and 12 h, and the subsequent period of revascularization lasted 2 h (the surgical part of the experiment was performed by Candidate of Medical Sciences N. P. Istomin.). Correction was carried

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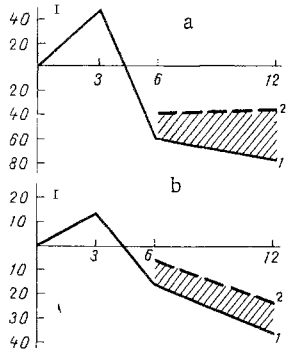


Fig. 1

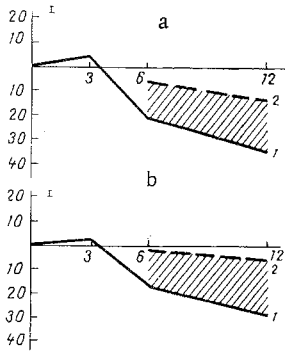


Fig. 2

Fig. 1. Time course of changes in G6PDH (a) and SDH (b) activity in the liver during temporary limb ischemia, revascularization (1), and pharmacological correction (2). Here and in Fig. 2: abscissa, time of limb ischemia (in h); ordinate, dehydrogenase activity.

Fig. 2. Time course of changes in GDH (a) and LDH (b) activity in liver during temporary limb ischemia, revascularization (1), and pharmacological correction (2).

out by a special scheme developed in the Professorial Surgical Department of the Faculty of Medicine, N. I. Pirogov Second Moscow Medical Institute, N. P. Istomin. To evaluate the time course of the changes in the liver a group of four enzymes concerned with different metabolic pathways was chosen: glutamate dehydrogenase (GDH), linked with amino acid and protein metabolism, succinate dehydrogenase (SDH), reflecting the intensity of oxidative processes in the Krebs' cycle, glucose-6-phosphate dehydrogenase (G6PHD), taking part in the pentose shunt of glucose oxidation, and lactate dehydrogenase (LDH), a marker of anaerobic glycolysis. Enzyme histochemical reactions were carried out on frozen sections 10 μ thick by the usual methods [12]. For quantitative analysis a "Microvideomat" television apparatus (from Opton, West Germany), controlled by a Wang 720c computer (USA) by a specially drawn up program for photometric analysis of histological preparations [9], was used. The parameter to be monitored was the mean optical density which is proportional to enzyme activity in the tissues. The significance of the values was estimated by student's t test.

## EXPERIMENTAL RESULTS

The enzyme histochemical profile of the hepatocytes established in the control group was taken as 100%. In experiments in which ischemia of the hind limbs lasting 3 h was followed by revascularization for 2 h, a significant increase in G6PDH activity by 46% ($P < 0.05$) and in SDH activity by 14% ($P < 0.01$) was found in the animals, but the increases in LDH activity (by 2%) and GDH activity by 4% were not significant ($P > 0.05$). The sharp increase in activity of the dehydrogenases during 3 h of ischemia followed by revascularization may be connected with swelling of the intracellular organelles and increased membranes permeability, evidence of an increase in the functional load on the cell [4], and it was interpreted as a compensatory response to stress with activation of one of the most energy-yielding pathways of glucose oxidation (the Krebs' cycle), and also of the apotomic pathway (Fig. 1). In this situation activation of the hexose phosphate shunt — the shortest and most economic energy-yielding mechanism, is more rational because stimulation of glycolysis during the course of shock would be accompanied by accumulation of incompletely oxidized products, which detracts from the value of glycolysis as a source of energy [2], as shown by the fact that the level of LDH activity did not differ significantly from the control (Fig. 2b). After revascularization following ischemia of the limbs for 6 and 12 h, a sharp decrease in the level of G6PDH activity corresponding to the periods of ischemia by 60 and 77% from the initial level, of SDH activity by 16 and 36%, of GDH activity by 21 and 36%, and of LDH activity by 17 and 29% ($P < 0.05$) was observed in the liver; this was interpreted as increasing exhaustion of the enzyme systems as a result of the collapse of adaptation. Changes in activity of the enzymes of glycolysis and the tricarboxylic acid cycle indicate disturbance of the connection between

them and the ornithine cycle of urea synthesis, for which they provide intermediate substrates and ATP [13]. The increase found in the plasma concentration of free amino acids, urea, and ammonia after 12 h of aortic occlusion [11] indicates definite functional changes in the liver and, in particular, inhibition of some pathways of amino-acid metabolism, as was confirmed by the decrease in GDH activity (Fig. 2a), and it is evidence of insufficiency of liver function.

During the study of the effect of pharmacological correction on the development of changes in the liver during ischemia and the postischmic syndrome higher values of enzyme activity were found after 6 h of ischemia than were obtained without correction: 21% greater for G6PDH, 10% for SDH, 15% for LDH, and 15% for GDH. Even greater changes were found when the results with and without correction were compared after revascularization of the limb preceded by 12 h of ischemia, when the corresponding increase in enzyme activity was 43% for G6PDH, 14% for SDH, 24% for LDH, and 22% for GDH. The values obtained for GDH and SDH activity after ischemia for 6 h and with correction did not differ statistically significantly from the control ($P > 0.05$).

Consequently, the PIS arising after occlusion of the trifurcation of the aorta in dogs leads to changes in activity of the dehydrogenases studied in the liver, as manifested by different rates of shift of the amplitudes of enzyme activity. Data obtained by television histophotometry suggest that changes in enzyme histochemical homeostasis of the liver in PIS follow a definite time course: a compensatory response, followed by a period of extinction of compensation and a period of exhaustion. It is a very interesting fact that the quantitative study of the enzyme histochemical spectrum of the liver following correction of the PIS confirmed the rational character of the measures adopted, leading to an improvement of the metabolic situation in the liver; correction of the early stages of development of PIS, moreover, was most effective. The special computer program compiled for processing of the results of histological analysis enables the method of processing to be quickly adapted for various histophotometric studies involving the use of a television image analyzer, thus giving it a wider range of possible application than analogous existing methods.

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