alongside hepatocytes, and also alongside connective-tissue cells (Fig. 1d). During regression of the cirrhosis a much larger quantity of reaction product than normally was observed in the extracellular space (Fig. 1e). The control preparations contained no reaction product (Fig. 1f).

The extracellular activity of the two proteinases, cathepsin B and cathepsin H, which we discovered in vivo in normal and cirrhotic liver tissue is evidence that besides the role in the intracellular degradation of various proteins, thiol proteinases also are secreted by the hepatocytes and connective-tissue cells of the liver into the intercellular space and they can participate in extracellular collagen resoration.

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BIOCHEMICAL AND MORPHOLOGICAL CHANGES IN THE MYOCARDIUM OF ACUTE VASCULAR SURGICAL PATIENTS (EARLY AUTOPSY RESULTS)

O. A. Trusov, V. V. Avilov, L. A. Tsareva, and A. A. Mozhina

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Acute surgical states in angiology constitute extremal situations in which the risk of complications and a lethal outcome increases with age. A most suitable method which allows objective evaluation of the state of the myocardium from the morphological and functional standpoints is an enzyme-histochemical investigation [6]. In this connection a combined biochemical and enzyme-histochemical study of enzyme activity and its comparison with the results of histologic investigation, conducted on material from patients dying in a vascular surgical department is particularly interesting.

Department of Pathological Anatomy, Faculty of Internal Medicine, Pirigov Second Moscow Medical Institute. Laboratory of Pathological Anatomy and Autopsy Department, A. N. Bakulev Institute of Cardiovascular Surgery, Moscow.

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TABLE 1. G6PD Content in Left Ventricular Myocardium

Method of expression of enzyme content	Samples of myocardium			
	control myocardium, n = 9	myocardium in AOLA, thrombosis of femoral arteries, n = 3	myocardium in rupture of atherosclerotic aortic aneurysm, n = 3	myocardium in TEPA and thrombosis of deep veins of legs, n = 3
Activity, U/g tissue	0,310±0,032	0,108±0,031	0,051±0	0,104±0,03
% of control	100	34,5	16,5	33,5
Activity, U/mg creatine	0,186±0,026	0,178±0,154	0,034±0,007	0,051±0,006
% of control	100	95,7	18,3	27,4

TABLE 2. G6PD Content in Left Papillary Muscle

Method of expression of enzyme content	Samples of myocardium			
	controll papillary muscle, n = 9	papillary muscle in AOLA and thrombosis of femoral arterties, n =3	papillary muscle in rupture of atherosclerotic aortic aneurysm, n = 3	papillary muscle in TEPA and thrombosis of deep veins of legs, n = 3
Activity, U/g tissue	0,336±0,076	0,175±0,1	0,073±0,037	0,063±0,008
% of control	100	52,1	21,7	15,8
Activity, U/mg creatine	0,581±0,169	0,108±0,065	0,049±0,03	0,031±0,004
% of control	100	18,6	8,4	5,3

The aim of the investigation described below was to study changes in activity of glucose-6-phosphate dehydrogenase (G6PD), a key enzyme of energy metabolism, in patients with acute occlusion of a limb artery (AOLA) as a result of thrombosis of the femoral arteries, thromboembolism of the pulmonary artery (TEPA), and thrombosis of the deep veins of the leg.

EXPERIMENTAL METHOD

This paper describes a study of material from 18 early autopsies devoted to the investigation of samples of myocardium of the left ventricle and the left papillary muscle. Material was obtained from the clinic of the S. I. Spasokukotskii Department of Surgery, at the Pirogov Second Moscow Medical Institute. The immediate cause of death was acute cardiovascular failure. Autopsy was carried out on the cadavers during 1 h after death [4] and samples of myocardium were taken. Depending on the underlying disease, three groups were distinguished: 1) AOLA due to thrombosis of the femoral arteries – three cases (aged 61-79 years); 2) rupture of an atherosclerotic aneurysm of the abdominal aorta – three cases (69-77 years); 3) TEPA and thrombosis of the deep veins of the leg – three cases (44-63 years). The control consisted of nine cases of accidental death. Autopsy material from visually intact zones of the regions of the myocardium chosen for study were quickly frozen during autopsy in petroleum benzin cooled with dry ice and kept in liquid nitrogen (−170°C), after which all the material was treated simultaneously. Myocardial homogenates, after centrifugation, were diluted before the measurements with dilution medium consisting of 0.1 M Na phosphate, pH 7.4, with 0.1% Triton X-100. Enzyme activity was measured by a kinetic spectrophotometric technique on an LKB-2086 reaction velocity analyzer (Sweden) at 37°C for 1-2 min [7]. When G6PD activity was measured in the "initial homogenate" the total dilution of the sample was 30 times compared with the initial myocardium. By multiplying measured activity, expressed in units (U)/ml by 30, the concentration of G6PD in the myocardium was obtained in U/g tissue. To reduce the effect of different ratios between muscular and connective tissue in the myocardial samples studied on the results, the enzyme content was calculated relative to the creatine concentration. The enzyme-histochemical study of G6PD activity was carried out on frozen sections [5]. Paraffin sections of parts of the myocardium were stained with hematoxylin and eosin, and myocardial damage was demonstrated by Regaud's method.

EXPERIMENTAL RESULTS

Investigation of homogenates of the left ventricular myocardium (Table 1) revealed a sharp decrease in G6PD activity in all pathological states studied. The greatest decrease of activity took place in patients with rupture of an atherosclerotic aneurysm of the abdominal aorta. Investigation of the G6PD content in the left papillary muscle (Table 2) also showed a decrease of activity, which was most marked in patients with TEPA and thrombosis of the deep veins of the leg. Enzyme-histologic study of G6PD activity revealed a decrease, reflected in the appearance of foci of coarsely granular diformazan in the sarcoplasm of the cardiomyocytes.

Morphologic investigation of the heart muscle of the control groups revealed interstitial edema and moderate congestion of the vessels of the microcirculatory system both of the left ventricle and of the papillary muscle. Individual lesions of cardiomyocytes, characterized by increased eosinophilia of the sarcoplasm and intensive staining by Regaud's method, were observed in the same parts of the heart. In all groups of patients studied interstitial edema also was found, but lesions of the cardiomyocytes were found more frequently and their volume was larger. Foci of cardiosclerosis were found with moderate hypertrophy of the cardiomyocytes. This picture was most marked in patients with AOLA, due to thrombosis of the femoral arteries. In all groups of patients, abundant lesions of cardiomyocytes and foci of cardioslcerosis were observed in the papillary muscle, the structure bearing a greater functional load than the left ventricular myocardium. In all parts studied focal concentrations of macrophages, lymphocytes, and granulocytes also were observed.

Focal cardiosclerosis and hypertrophy of cardiomyocytes in the walls of the left ventricle and in the papillary muscles are characteristic features of the combination of myocardial changes taking place in old age [3]. The decrease in G6PD activity revealed by the biochemical tests is in agreement with the results of the enzyme-histologic study and also with data showing that this feature is evidence of wear and tear of the myocardium [2]. The greatest decrease in G6PD activity was found in patients with rupture of an atherosclerotic aortic aneurysm, possibly due to marked inhibition of enzyme activity in all parts of the heart in hemorrhagic shock. This is accompanied by a significant decrease in the blood flow in the inner layers of the myocardium [1].

Focal zonal lesions of the cardiomyocytes, combined with a sharp decrease in activity of G6PD, a key enzyme of the pentose shunt, were thus found. The appearance of focal lesions of the myocardium in old age indicates considerable vulnerability of the heart. The use of quantitative biochemical methods combined with enzyme-histologic and histologic studies on early autopsy material offers real prospects for a solution to problems in thanatology and for the further study of morphological and functional changes in the myocardium.

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