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Comparative Histophotometric Characteristics of the Structural-Metabolic Heterogeneity of Hepatocytes in Peritonitis and Limb Gangrene

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Comparative histophotometric and correlation analysis was carried out to study dehydrogenase activity in hepatocytes of different acinar zones in peritonitis and gangrene of the lower extremities. Structural-metabolic disturbances of hepatocytes were found to be responsible for the weakened detoxicating function in patients with endotoxicoses. Special features of metabolic disturbances in the liver acini were demonstrated in peritonitis and limb gangrene.

Key Words: *liver acinus; metabolism; peritonitis; gangrene*

Signs of endotoxicosis are found in surgical patients under a wide variety of pathological conditions and are characterized by disturbances of homeostasis associated primarily with intoxication [1,5,8]. Since the liver is the central organ where detoxication occurs, disturbances in its structure and metabolism result in the development of liver and then multiple organ failure [10]. For a more reliable assessment of the morphofunctional changes and an explanation of the pathogenesis of the damage, the zonal structural-metabolic heterogeneity of hepatocytes in the acinus must be taken into consideration and correlation analysis must be used to study the interactions between the different metabolic pathways [7,9].

The goal of the present study was to perform a comparative histophotometric and correlative analysis of dehydrogenase activity of hepatocytes in different zones of the acinus in peritonitis and gangrene of the lower extremities.

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MATERIALS AND METHODS

Twenty-seven early autopsies were studied. The immediate cause of death in 21 patients was multiple organ failure due to peritonitis (11 cases) or gangrene of the leg as a result of an acute thromboembolism (10 cases). The control group consisted of six early autopsies for medicolegal purposes: sudden cardiac death was the diagnosis in four of them, and fatal trauma in two cases. The patients' ages ranged from 52 to 78 years. Autopsies were carried out 45-90 min after certifying death. Pieces from the right lobe of the liver were frozen and kept in liquid nitrogen, this being followed by simultaneous histoenzyme assay on cryostat sections in order to detect the activity of the following dehydrogenases: succinate (SDH), malate (MDH), glutamate (GDH), 3-hydroxybutyrate (BDH), glucose-6-phosphate (G6PDH), and lactate (LDH), and of NAD- and NADP-diaphorases [13]. The activity of the enzymes in zones I and III of the liver acini was estimated quantitatively by means of a Mikrovideomat television image analyzer (Opton, Germany) controlled by a Wang 720c computer, using specially devised software for photometric analysis of histological preparations [2]. The numerical results were computer-processed using methods of parametric and nonparametric statistics. The correlation between parameters was studied by correlation analysis with calculation of correlation coefficients. The strength of correlation was estimated in degrees: strong - $r=0.7$-1.0, significant - $r=0.5$-0.7, moderate - $r=0.3$-0.5, and weak - $r<0.3$ [3].

RESULTS

Microscopic examination of the liver in the control group revealed slight nonuniform congestion of the sinusoidal vessels, moderately expressed granular and vacuolar dystrophy, and monocellular necrosis of hepatocytes in the perivenular zone of the acini. Together with the above-mentioned changes, dilation and congestion of the periportal vessels and sinusoids, leukostasis, aggregation of erythrocytes with microthrombosis, widening of the perisinusoidal Disse's spaces, and more marked dystrophic changes and injuries of the hepatocytes in the

TABLE 1. Dehydrogenase Activity of Hepatocytes in Zones I and III of Acini in Peritonitis and Limb Gangrene ($M \pm m$)

Enzyme	Zone of acinus	Dehydrogenase activity, arb. units		
		Control	Peritonitis	Gangrene
SDH	I	547±15	245±11	264±12
	III	462±13	190±10	210±12
	K	1.18	1.29	1.26
MDH	I	606±20	241±13	234±12
	III	470±16	170±11	190±12
	K	1.29	1.41	1.23
GDH	I	511±13	222±12	188±11
	III	607±23	177±12	151±12
	K	0.84	1.25	1.25
BDH	I	497±13	375±13	338±13
	III	604±17	305±16	236±14
	K	0.82	1.23	1.43
G6PDH	I	430±18	210±12	172±13
	III	516±13	166±13	148±12
	K	0.83	1.27	1.16
NAD	I	678±18	458±18	476±19
	III	779±18	398±19	360±16
	K	0.87	1.15	1.32
NADP	I	678±15	501±14	557±14
	III	803±23	438±17	433±16
	K	0.84	1.14	1.29
LDH	I	770±10	632±18	733±19*
	III	669±19	533±21	582±20
	K	1.15	1.19	1.26

Note. *K* = periportal–perivenular gradient of enzyme activity; all values except those marked by * differ significantly from the control ($p<0.05$).

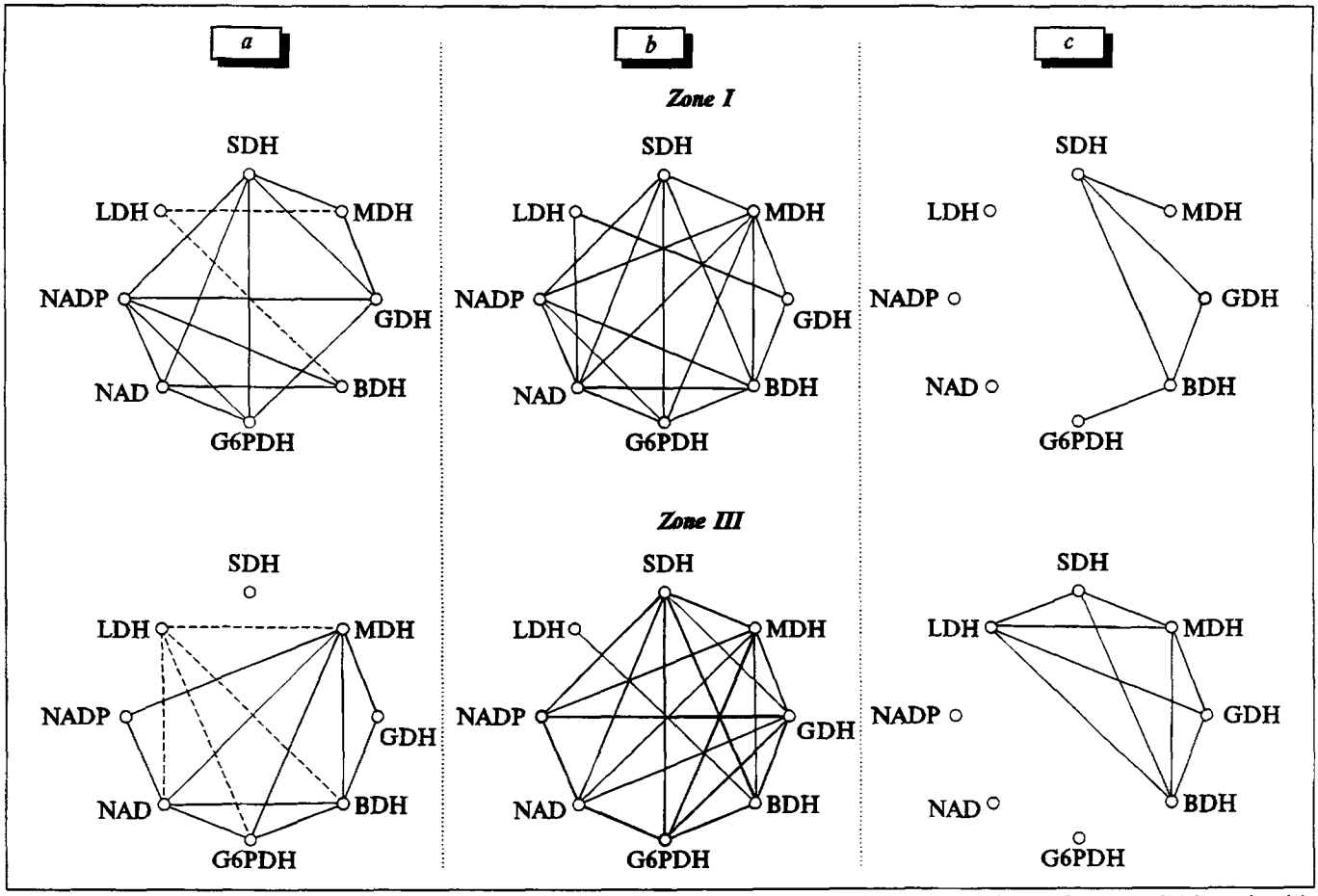

Fig. 1. Scheme of correlations of dehydrogenase activities of hepatocytes in different zones of acini in the control (*a*), peritonitis (*b*), and gangrene of the leg (*c*). Continuous line: strong positive correlation; broken line: negative correlation.

perivenular regions, amounting in some cases to triangular and periacinar necroses, were observed in the liver of patients who had died as a result of multiple organ failure due to endotoxicoses of different genesis. However, in comparing the micropreparations of the liver, we did not find any pathognomonic features or significant differences between the groups of patients with peritonitis and limb gangrene.

The histoenzyme spectrum of the human liver acini was determined in the histophotometric study of dehydrogenase activity in the control group and was characterized chiefly by SDH, MDH, and LDH activity in zone I and BDH, GDH, G6PDH, NAD, and NADP in zone III, as is consistent with data in the literature [6,11]. The primary localization of dehydrogenase activity in one zone or another is reflected in the values of the periportal-perivenular gradient of activity (Table 1).

The study of the liver of patients who had died of endotoxicoses reveals a decrease in dehydrogenase and diaphorase activity in the liver acini, but this decrease varies in degree. The activity of SDH and MDH in peritonitis is 55.2 and 60.2%, respectively below the control values in zone I ($p<0.05$), and 58.9 and 63.6% ($p<0.05$) lower in zone III. In gangrene their activity is 51.7 and 61.4% ($p<0.05$) below the control in the periportal, and 54.5 and 59.6% ($p<0.05$) lower in the perivenular hepatocytes. Such a marked decrease in the activity of enzymes of the citric acid cycle in all the zones of the acinus is in agreement with the data of electron microscopic examination of hepatocytes attesting to damage of mitochondria, and it is responsible for the development of irreversible hepatocellular failure [4]. A significant decrease of GDH activity, depending on amino acid metabolism, is found in both groups (by 69.2 and 63.7% on average over the acinus in peritonitis and gangrene, respectively) and may be associated with the synergic effect of hypoxia and intoxication on protein synthesis in hepatocytes. Inhibition of glycolysis, demonstrated by a reduction in LDH activity, may also be a result of toxin action. In both groups lower values versus the control were found for G6PDH (a marker enzyme of the pentose phosphate shunt for glucose oxidation) and BDH (an index of fatty acid metabolism) activity.

It is noteworthy, that in the investigated endotoxicoses a disturbance of the spatial organization of the liver acini was observed, one sign of this being an increase in the periportal-perivenular activity gradient of dehydrogenases, which under physiological conditions have greater activity in zone III (Table 1). In peritonitis, unlike gangrene, SDH and LDH activity falls markedly all over the acinus, whereas NAD and NADP activity decreases in zone I and MDH activity diminishes in zone III. Such differences may stem from the peculiarities of toxemia in the pathologies studied, namely the degree of development of intoxication and the nature of the toxic substances. In both groups the lowest values of dehydrogenase activity are noted in the perivenular (centrolobular) zones.

As is well known, structural-metabolic disturbances may be judged not so much according to changes in the activity of individual enzymes, as according to the disturbances of coordination in the work of the different enzyme complexes [14], which can be pointed up by correlation analysis. The calculated correlation coefficients of dehydrogenase activity are shown schematically in Fig. 1, only strong correlations being represented ($r>0.7$). In the control group in zone I of the acini strong positive correlations are found between the activity of almost all enzymes studied, except LDH. In zone III strong SDH correlations are absent, but LDH has inverse correlations with BDH, G6PDH, NAD, and NADP.

Changes in the strength and character of the correlations are observed in marked intoxications. In zone I the appearance of strong positive correlations of MDH with BDH, G6PDH, NAD, and NADP, GDH with BDH and LDH, as well as between BDH and G6PDH is noted in the group of peritonitis patients. Strong negative correlations of LDH with MDH and BDH disappear and the correlation coefficients between these enzymes are 0.54 and 0.67, respectively. The relationships of SDH and MDH with other enzymes change considerably in zone III of the acini. SDH demonstrates strong positive correlations with all enzymes studied except LDH. Negative correlations of LDH, which are characteristic for the control group, become positive in peritonitis, especially with BDH ($r=0.94$).

Marked changes of the correlations between the different metabolic pathways are also noted in gangrene of the lower extremity. Here, the total number of strong correlations is decreased, contrary to the case with peritonitis. In zone I of the acini, contrary to the control, positive correlations appear between BDH and SDH, and between GDH and G6PDH. In zone III of the acini, as in peritonitis, the correlation coefficients of LDH, which has strong positive correlations with SDH, MDH, GDH, and BDH, are markedly increased.

The results of our study are good evidence for the primary injury of perivenular hepatocytes in all observations of the liver in endotoxicoses. This is true both regarding the localization of the regions of dystrophy and necrosis and regarding the places of preeminent decrease of dehydrogenase and diaphorase activity. Such a localization of the damage may be attributed not only to the nature of the disturbances arising but also to the zonal heterogeneity of the liver acinus. This relates especially to the development of pronounced toxemia and hypoxia in endotoxicoses, which results in damage to the periphery of the liver acini, namely to the perivenular zones. This mechanism is aggravated by the added impact of microcirculatory disturbances in the liver, such as erythrocyte aggregation, leukostases, and microthromboses. The result is a functional reorganization of the liver acini, where the zones of predominant enzyme activity change. Similar changes in the indexes of different metabolic pathways as well as the appearance of strong positive correlations between all dehydrogenases studied in peritonitis are probably manifestations of the mechanism of hypoxia-coordinated regulation of metabolism [12]. In cases of leg gangrene, unlike peritonitis, the correlation coefficients between enzymes are lower, this being due to the different pathogenesis and duration of the diseases in question.

Thus, the structural-metabolic disturbances which we found in hepatocytes in the acini are evidently responsible for the considerable weakening of the detoxicating function in patients with endotoxicoses since it is in the perivenular hepatocytes that the main detoxicating systems are located.

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