HISTOPHOTOMETRIC CHARACTERISTICS OF STRUCTURAL—METABOLIC HETEROGENEITY OF HEPATOCYTES IN ACUTE BLOOD LOSS AND PULMONARY ARTERIAL THROMBOEMBOLISM

O. D. Mishnev and A. I. Shchegolev

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Changes in the systemic circulation are regularly reflected in the total hepatic blood flow, on which mainly depend the disturbances of structure and metabolism of hepatocytes and the subsequent development of hepatic failure [2, 10, 11]. For a more reliable estimate of changes and an explanation of the pathogenesis of the lesions, the zonal structural—metabolic heterogeneity of the hepatocytes in the acinus must be taken into consideration and correlation analysis must be used to study relationships between the different metabolic pathways [6, 8].

The aim of this investigation was a comparative histophotometric and correlation analysis of dehydrogenase activity of hepatocytes in different zones of the acini in acute blood loss and pulmonary arterial thromboembolism.

METHODS

Material from 15 early autopsies was investigated. The immediate cause of death in five patients was acute posthemorrhagic anemia as a result of rupture of an aneurysm of the abdominal aorta, and in another five it was pulmonary arterial thromboembolism (PATE). The duration of survival after the appearance of clinical signs in patients with acute blood loss for 7-15 h and in patients with PATE it was 9-30 h. The control group consisted of five early autopsies for medicolegal purposes: sudden cardiac death was the diagnosis in three of them, and fatal trauma in two cases. The patients' ages varied from 52 to 73 years. Autopsy was carried out 45-90 min after death. Pieces from the right lobe of the liver were frozen and kept in liquid nitrogen, followed by simultaneous performance of enzyme histochemical reactions on frozen sections in order to detect activity of the following dehydrogenases: succinate (SDH), malate (MDH), glutamate (GDH), β-hydroxybutyrate (BDH), glucose-6-phosphate (G6PDH), and lactate (LDH), and of NAD- and NADP-diaphorase [7]. Activity of the enzymes was estimated quantitatively by means of a "Microvideomat" television image analyzer (Opton, Germany), controlled by a Wang 720c computer, using a specially prepared program for photometric analysis of histological preparations [3]. The results were subjected to statistical analysis by computer. Correlation between parameters was studied by correlation analysis with calculation of coefficients of correlation. The strength of correlation was estimated in 4°: strong ($r = 0.7$-1.0), significant ($r = 0.5$-0.7), moderate ($r = 0.3$-0.5), and weak ($r < 0.3$) [4].

RESULTS

Microscopic investigation of the liver in the control group revealed unequal congestion of the sinusoidal vessels, moderately severe vacuolar and granular degeneration, and monocellular necrosis of hepatocytes in zone 3 of the acini. Perivenular foci of necrosis also were found in the liver of patients dying as a result of massive PATE, together with

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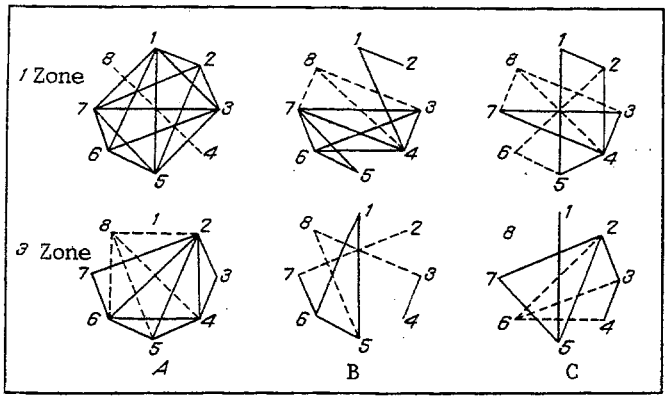

Fig. 1. Diagram showing correlations of dehydrogenase activities of hepatocytes in different zones of acini during pulmonary arterial thromboembolism and acute blood loss. A) Control; B) PATE; C) blood loss. 1) SHD; 2) MDH; 3) GDH; 4) BDH; 5) G6PDH; 6) NAD; 7) NADP; 8) LDH; continuous line) strong positive correlation; broken line) negative correlation.

TABLE 1. Dehydrogenase Activity of Hepatocytes in Different Zones of Acini in Acute Blood Loss and Pulmonary Arterial Thromboembolism (M ± m, conventional units)

Group	Zone	Enzyme							
		SDH	MDH	GDH	BDH	G6PDH	NAD	NADP	LDH
Control	1	556±15	623±14	517±15	508±18	440±19	690±17	688±14	763±24
	3	468±12	480±16	617±26	615±17	520±15	789±19	816±23	659±20
	K	1,19	1,30	0,84	0,83	0,85	0,88	0,84	1,16
PATE	1	470±16	527±27	487±14*	359±24	354±17	607±15	613±19	738±14*
	3	382±11	387±13	541±17	500±16	432±17	700±14	699±18	650±16*
	K	1,23	1,36	0,90	0,72	0,82	0,87	0,88	1,14
Blood loss	1	316±18	280±13	292±14	477±12	282±12	486±7	525±17	695±16
	3	246±20	226±11	232±13	390±14	240±18	438±8	462±14	600±13
	K	1,29	1,30	1,26	1,22	1,18	1,11	1,14	1,20

Notes. K) Periportal—perivenular gradient of enzyme activity; all values except those marked by an asterisk differ significantly from the control ($p < 0.05$).

the changes already mentioned above. Acute blood loss was characterized by anemia of the organ, vacuolar degeneration, and perivenular foci of necrosis of the hepatocytes.

Histophotochemical analysis of dehydrogenase activity in the control group revealed a tissue enzyme spectrum of acini of the human liver (Table 1) which generally speaking is in agreement with the pattern of the liver in animals under physiological conditions [6, 9]. In zone 1 of the acini activity was chiefly of SDH, MDH, and LDH, whereas in zone 3 activity of all the other enzymes studied was higher, as is clearly shown by values of the periportal—perivenular activity gradient.

The study of the liver of patients dying as a result of PATE or acute blood loss revealed a decrease in dehydrogenase and diaphorase activity in the acini of the liver, but this decrease varied in degree. Activity of SDH and MDH in PATE was below the control values in zone 1 by 15.5 and 15.2%, respectively ($p < 0.05$), and 18.4 and 19.4% lower in zone 3 ($p < 0.05$). In acute blood loss SDH and MDH activity was considerably lower — by 43.2 and 55.1% ($p < 0.05$) in the periportal, and by 47.4 and 52.9% ($p < 0.05$) in the perivenular hepatocytes. Such a marked decrease in activity of enzymes of the citric acid cycle in all zones of the acinus in acute blood loss

points to a profound disturbance of energy homeostasis of the liver cells, which can be regarded as one mechanism of development of irreversible hemorrhagic shock [5]. Low values of G6PDH activity — a marker enzyme of the pentose phosphate shunt for glucose oxidation — were found, and the difference was particularly marked in cases with blood loss. The decrease in G7PDH activity was evidently connected with the marked inhibitory effect of lipid peroxidation products [1]. The fall in BDH and GDH activity observed in all zones of the acinus, and more marked in the presence of blood loss, is evidence of a severe disturbance of fatty acid and amino acid metabolism in the hepatocytes [13]. LDH activity showed the smallest change of all, evidence of better preserved metabolic pathways in anaerobic glycolysis in the states being investigated, but in cases of acute blood loss the degree of the fall in the level of LDH activity was greater.

Calculated coefficients of correlation are shown schematically in Fig. 1, only strong correlations being represented ($r > 0.7$). In the control group, in zone 1 of the acini strong positive correlations were found between activity of most enzymes studied, except LDH. The latter has strong negative correlation with BDH. In zone 3 of the acini, by contrast with zone 1, SDH has no strong correlations with the other dehydrogenases studied, evidently due to its predominant localization in the periportal cells. LDH in the perivenular zone has negative correlations with MDH, BDH, G6PDH, and NAD.

In the group of patients dying as a result of PATE strong positive correlation was observed in zone 1 between BDH, on the one hand, and SDH and GDH on the other, and negative correlation of LDH with GDH and NADP. The coefficients of correlation of SDH with GDH and G6PDH fell to 0.65 and 0.39, respectively. In zone 3 of the acini strong positive correlations of SDH and G6PDH and NAD and negative correlation between LDH and GDH appeared. Considerable changes were found in relations of MDH with other dehydrogenases, including the coefficient of correlation between MDH and NADH which was 0.78 in the control group and —0.85 in the PATE group, i.e., the direction of correlation was changed.

Marked changes of correlations between the different metabolic pathways also were observed in acute blood loss. In zone 1 of the acini strong positive correlations of BDH with MDH, GDH, G6PDH, and NADP appeared, evidence of the closer linking of the processes of fatty acid oxidation with energy and protein metabolism when marked hypoxia of the liver is present. In zone 3 strong positive correlation of G6PDH with SDH and NADP was observed. The negative correlations of NAD with MDH, GDH, and BDH which were observed indicate profound disorganization of metabolic processes, for these dehydrogenases are NAD-dependent and have strong positive correlations in the control cases.

Changes in strength of the correlations and also the appearance of new ones in case of blood loss and PATE are evidence of substantial structural-metabolic changes in the hepatocytes, as revealed by disturbance of the coordination of working of the enzyme systems. A greater decrease in dehydrogenase and diaphorase activity was observed in zone 3 of the acini, suggesting that the damage to the liver tissue is predominantly hypoxic in character [12, 14]. Under these circumstances, in studies of the liver in patients dying from PATE the zonal activity of the enzymes in the acinus was preserved. In acute blood loss a disturbance of the spatial organization of the liver acini was observed, one sign of it being an increase in the periportal—perivenular activity gradient of enzymes which, under physiological conditions, have greater activity in zone 3 (Table 1). The structural-metabolic disturbances which we found in hepatocytes n the acini evidently lie at the basis of the considerable weakening of the detoxicating function of the liver in patients with acute blood loss, for the main intoxicating systems are located in the perivenular hepatocytes.

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