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Disorders in the Structural and Metabolic Organization of Liver Acini in Systemic Endotoxemia

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Injection of *Escherichia coli* lipopolysaccharide to dogs caused pronounced structural and metabolic changes of liver acini, characterized by the development of compensatory reactions to the endotoxin followed by their failure. In addition to hypoxia, depressed activity of hepatocyte dehydrogenases and diaphorases with impairment of the intraacinar gradient of their activity, leading to the development of hepatocellular insufficiency, appears to underlie the involvement of the liver in systemic endotoxemia.

Key Words: *liver acinus; metabolism; endotoxin*

Polyorgan insufficiency develops in the course of shock, and systemic endotoxemia of enteric origin plays an important role in its pathogenesis [7,17]. The main factor reliably indicating the threat of systemic pathological effects of endotoxemia is not so much the quantitative level of lipopolysaccharide in the bloodflow, but the failure of the organism's antitoxic defense systems and, primarily, of the liver [10]. The development of liver insufficiency is to a large extent determined by disorders of hepatocyte metabolism, the interzonal gradient of enzymatic activity in the liver acinus being one of its characteristics [4,5].

This study was aimed at a histophotometric analysis of the activities of hepatocyte dehydrogenases in various acinus zones in the course of endotoxemia.

MATERIALS AND METHODS

The study was carried out using 97 liver biopsy specimens from 11 mongrel dogs weighing 13 to 18 kg, intravenously infused *Escherichia coli* lipopolysaccharide in a single dose of 2 mg/kg. Oxygen tension in liver tissue was measured and a liver biopsy carried out initially (control) and every hour for 8 h after the infusion of endotoxin. Cryostat slices 10 μ thick were prepared, in which the activities of the following dehydrogenases were measured: succinate, isocitrate, malate, glutamate, 3-hydroxybutyrate (BDH), glucose-6-phosphate (G-6-PD), lactate, α-glycerophosphate (GPDH), and NAD and NADP diaphorases [14]. For screening analysis, cryostat and paraffin slices were stained with hematoxylin and eosin. The activities of the enzymes in the first and third zones of the liver acinus were quantitatively assessed by a Wang 720c computer-operated television image analyzer Microvideomat (Opton) using software for photometric analysis of histological preparations [2]. The mean optical density proportionate to the enzyme activity in the tissue was assessed. Oxygen tension (Po_2) in the liver tissue was measured polarographically [1]. Digital material was computer-processed by methods of variational statistics.

RESULTS

The detected histoenzymatic profile was initially characterized by a peculiar ratio of the activities of

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redox enzymes in various zones of acini and between themselves (Table 1). This is in line with published data [3,14]. The activities of succinate, malate, and lactate dehydrogenases was 1.4-1.5 times higher in the hepatocytes of the first zone than in perivenular (centrolobular) cells. For the other enzymes the gradient of activity was inversely directed, the greatest difference (1.5 times) being observed for G-6-PD.

Developing systemic endotoxemia leads to pronounced structural and metabolic disorders of hepatocytes and to changes in the spatial organization of liver acini.

One hour after the infusion of lipopolysaccharide, variously directed changes in the activities of the dehydrogenases were observed. The activities of succinate dehydrogenase, NAD, NADP, and GPDH increased, whereas those of all the other dehydrogenases decreased. The degree of enzymatic changes in the first and third zones was different. In the first zone NAD activity surpassed the initial values by 94.8% ($p<0.01$), while in the third zone the increase was 79.7% ($p<0.01$). The activity of isocitrate dehydrogenase was lowered to the same extent in the periportal and perivenular cells (by 19.8 and 19.7%, respectively, $p<0.05$). GPDH values in the first zone were 9.1% higher and in the third zone 1.6% lower than initially.

Hematoxylin-eosin staining showed uneven filling of the portal vessels, especially the portal vein branches, and small hemorrhages in the portal tracts. Vacuolar dystrophies and monocellular necroses involving mainly the periportal cells were detected.

As the intoxication progressed, the activities of NAD and NADP diaphorases in the entire acinus progressively declined, reaching by the 8th hour 70.2 and 50.5% of the control values in the first zone and 54.1 and 39.9%, respectively, in the third zone ($p<0.05$). A reduction of BDH and G-6-PD activities was observed only in the perivenular cells. In the periportal hepatocytes their activities first increased and then progressively decreased; however, the activity of G-6-PD was even higher than initially after 3-5 h. The time course of the other enzymes was characterized by a rise of activity followed by a decline, but the degree to which enzymatic activity increased in the course of intoxication differed appreciably for the different dehydrogenases. The periportal and perivenular hepatocytes were nonuniformly involved in the reaction of stimulation of cellular energogenesis (Table 1). Eight hours after endotoxin infusion, the minimal enzyme activities were observed, the activity of GPDH in the perivenular cells being reduced most of all: by 69.6% ($p<0.05$) and that of lactate dehydrogenase in the same zone least of all (by 9.2%, $p<0.05$). An increased ratio of enzymatic activities in the first and third zones was characteristic of all enzymes except lactate dehydrogenase. However, whereas for succinate and malate dehydrogenases (enzymes having the highest activity in the first zone) this means an increase of the intraacinar gradient, for all other dehydrogenases (whose values are higher in the third zone) this implies a change of the sites of predominant localization.

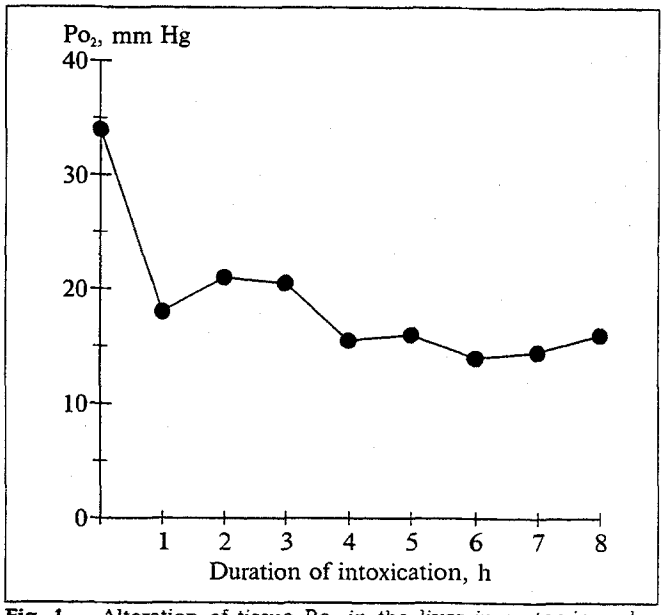

Fig. 1. Alteration of tissue Po_2 in the liver in systemic endotoxemia.

In parallel with the decline of enzyme activities, progressive disorders were observed in the liver microcirculatory bed and injuries to its structural elements. More extensive foci of hemorrhages were observed in the portal tracts, involving individual periportal areas by the 5th-8th hours of intoxication. Uneven dilatation and filling of the sinusoidal vessels was associated with red cell aggregation and leukocytic stasis. The perisinusoidal Disse's space was dilated, and the stellate reticuloendotheliocytes and endothelial cells were enlarged, with hyperchromatic nuclei. The hepatocyte lesions progressed and the areas of necrosis became enlarged to the size of foci after 3 hours of endotoxemia and to the size of individual periacinar foci after 5-8 hours. After 5-8 hours focal accumulations of polymorphonuclear leukocytes were seen in the portal tracts at the sites of hemorrhages.

Measurement of the tissue tension of oxygen (Fig. 1) revealed a 48.1% reduction ($p<0.01$) just 1 h after infusion of *E. coli*. Later it continued to fall, despite fluctuations, and was minimal 6 h postintoxication: 39.9% of the control value ($p<0.01$).

Hence, administration of lipopolysaccharide to dogs led to pronounced structural and metabolic disorders in liver acini. The increase of dehydrogenase

TABLE 1. Activities of Dehydrogenases in Dog Liver Acini in Systemic Endotoxemia ($M \pm m$, arb. U)

Enzyme	Zone	Control	Duration of intoxication, h							
			1	2	3	4	5	6	7	8
SDH	I	452±13	530±20	511±16	448±13*	380±15	306±11	299±9	285±8	279±8
	III	297±9	352±13	394±12	371±11	225±9	195±6	186±6	185±5	177±6
	K	1.52	1.50	1.29	1.20	1.68	1.56	1.60	1.55	1.57
IDH	I	182±5	146±5	151±4	161±5	168±7*	159±5	153±4	150±4	146±6
	III	233±7	187±9	161±6	163±5	157±5	147±5	132±4	119±4	110±3
	K	0.78	0.78	0.94	0.98	1.07	1.08	1.16	1.26	1.33
MDH	I	299±9	236±8	319±13*	309±9*	279±10*	262±6	240±7	221±7	199±6
	III	192±6	186±5*	180±7*	178±8*	155±5	148±4	137±4	122±5	99±5
	K	1.56	1.27	1.77	1.74	1.80	1.77	1.75	1.81	1.99
GPDH	I	425±13	464±8	503±18	523±16	451±17*	401±15*	390±8	352±12	329±10
	III	497±15	489±16*	509±17*	486±15*	418±14	362±14	308±11	275±9	237±8
	K	0.85	0.95	0.98	1.07	1.07	1.10	1.26	1.28	1.38
LDH	I	784±24	424±21	943±47	958±29	753±30*	718±22*	701±21	692±22	683±20
	III	546±16	393±16	682±31	695±21	666±21	534±20*	524±16*	503±15*	496±16
	K	1.43	1.08	1.38	1.38	1.13	1.34	1.33	1.37	1.37
GDH	I	756±23	600±24	807±38*	735±26*	698±23*	634±24	532±21	452±17	356±16
	III	830±25	794±29*	826±28*	704±27	508±23	459±14	366±12	311±10	276±10
	K	0.91	0.75	0.97	1.04	1.37	1.38	1.45	1.45	1.28
BDH	I	596±18	461±16*	565±23	541±16	532±16	441±18	406±14	355±11	328±11
	III	712±21	653±17	631±19	585±21	563±17	436±18	386±14	325±12	292±9
	K	0.83	0.70	0.89	0.93	0.94	1.01	1.05	1.09	1.12
G-6-PD	I	172±5	164±7*	162±5*	175±6*	187±5	182±4	152±7	139±5	119±4
	III	253±8	246±7*	225±7	222±9	203±6	180±7	138±5	105±3	77±4
	K	0.67	0.66	0.72	0.78	0.92	1.01	1.10	1.31	1.54
NAD	I	501±15	976±49	640±23	616±18	500±20*	472±16*	448±12	436±13	352±13
	III	563±17	1012±51	545±27*	534±26*	473±20	433±15	387±14	366±11	305±10
	K	0.88	0.96	1.17	1.15	1.05	1.08	1.15	1.18	1.15
NADP	I	702±21	765±20	566±23	557±17	538±16	503±16	449±17	422±13	355±14
	III	801±21	859±28*	545±25	468±18	448±13	421±13	378±13	353±11	320±10
	K	0.87	0.89	1.03	1.19	1.20	1.19	1.18	1.18	1.10

Note. *K*: periportal–perivenular gradient of activity in the acinus. All values except those marked with an asterisk reliably differ from the control ($p<0.05$).

and diaphorase activities and the minimal lesions of hepatocytes in the early periods of intoxication indicate a compensatory reaction of stimulation of the intracellular metabolic processes. This is particularly true for succinate dehydrogenase, the marker of the Krebs cycle, and NAD and NADP diaphorases, serving as the summary indicators of the energy potential of hepatocytes. On the other hand, the metabolic disturbances and reduced activities of a number of dehydrogenases after 1 h of intoxication may be related to the development of hypoxia (which is proven by the Po_2 values we obtained) and the direct toxic action of lipopolysaccharide on hepatocytes [12]. The latter is confirmed by the results of comparing the changes in enzymatic activities in various zones of the liver acinus: the activities of malate, glutamate, and lactate dehydrogenases and BDH are most depressed in the periportal cells. Hypoxia is known to promote an increase of the size and number of pores in stellate reticuloendotheliocytes and the number of fenestrae in endothelial cells, this increasing the permeability of the histohematic barrier in the liver and the availability of hepatocytes for lipopolysaccharide. In turn, the development of hypoxia during endotoxemia is related to a drop of arterial pressure, blood congestion in the celiac area [6,11], respiratory insufficiency, and hypoxemia [9,16].

The rise in the activities of a number of dehydrogenases after 2-4 hours of intoxication may be related to increased utilization of oxygen by liver tissue. On the other hand, impairment of the physiological intraacinar gradient of activity with a switch of the zones of predominant activity (of isocitrate and glutamate dehydrogenases, GPDH, NAD and NADP) indicates a breakdown of the spatial organization of the liver acini and may be regarded as an indicator of the failure of compensation of the hepatocyte metabolic systems [5]. Alteration of the zonal organization of the liver acini with the lowest activities of redox enzymes in the perivenular hepatocytes

is explained by the fact that these cells occupy a peripheral position in the microcirculatory functional unit and prove to be the most sensitive to hypoxia [15]. The development of hypoxia activates the process of free-radical oxidation of lipids, which causes structural damage in the third zone: perivenular necrosis after 3-4 h and periacinar necroses after 5-8 h of intoxication. The structural and metabolic disorders of liver acini severely compromise the detoxifying function of the liver in endotoxicosis, for it is in the hepatocytes that the main detoxifying systems are located. All this is confirmed by the data on the depression of the rate of the biotransformation reaction in the microsomal fraction of an animal liver after administration of lipopolysaccharide [8].

Hence, infusion of *E. coli* to dogs caused pronounced structural and metabolic changes of liver acini, characterized by the development of compensatory reactions to the endotoxin followed by their failure. Depression of the activities of hepatocyte dehydrogenases and diaphorases with impairment of the intraacinar gradient of their activity, involving the development of hepatocellular insufficiency, largely contributes, together with hypoxia, to the pathogenesis of liver damage in systemic endotoxemia.

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