3. Z. D. Ismailova, Ya. D. Mamedov, D. Ya. Guseinov, *et al.*, *Farmakol. Toksikol.*, № 4, 26 (1991).
4. Yu. M. Levin, *Fundamentals of Therapeutic Lymphology* [in Russian], Moscow (1986).
5. Yu. B. Lishmanov and I. G. Avten'eva, *Byull. Sibirsk. Otdel. Akad. Med. Nauk SSSR.*, № 4, 17 (1984).
6. Ya. D. Mamedov, *Myocardial Infarction* [in Russian], Moscow (1989).
7. V. K. Khugaeva and V. V. Suchkov, *Byull. Vsesoyuz. Kardiol. Nauch. Tsentra*, № 1, 95 (1980).
8. V. K. Khugaeva, *Byull. Eks. Biol. Med.*, **105**, № 3, 300 (1988).
9. V. K. Khugaeva, *Fiziol. Zh. SSSR.*, № 1, 92 (1990).
10. V. K. Khugaeva, *Byull. Eksp. Biol. Med.*, **112**, № 8, 117 (1991).
11. V. K. Khugaeva, *Fiziol. Zh. SSSR.*, № 6, 138 (1991).
12. W. M. Armstead, R. Mirro, D. W. Busija, *et al.*, *Circ. Res.*, **68**, № 4, 922 (1991).
13. J. H. Hanko and J. E. Hardebo, *Europ. J. Pharmacol.*, **51**, 295 (1978).
14. M. Kobari, F. Gotoh, N. Suzuki, *et al.*, *J. Cereb. Blood Flow Metab.*, **5**, 34 (1985).
15. E. Okada, *Heart Vessels*, **4**, № 4, 215 (1988).

A Quantitative Study of Dehydrogenase Activity of Hepatocytes in Systemic Endotoxinemia

O. D. Mishnev, A. P. Ettinger, A. I. Shchegolev, M. V. Anurov, and S. P. Yavolov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, No. 1, pp. 105-108, January, 1995
Original article submitted February 28, 1994

A histophotometric study of the liver dehydrogenase activity reveals the nature of changes of enzymatic homeostasis and its periodicity in the dynamics of endotoxinemia in dogs. A compensatory reaction to lipopolysaccharide administration develops during the first two hours. A decrease of dehydrogenase and diaphorase activity and the development of structural damage to hepatocytes appear later. It is shown that the activation of free-radical oxidation as well as an increase of the level of medium-sized molecules in the blood plasma play a key role in the pathogenesis of liver damage.

Key Words: *metabolism; liver; endotoxin*

Recent experimental studies have confirmed the importance of endotoxin from Gram-negative bacteria as a triggering element in the total reaction of the organism, which is accompanied by typical clinical-functional changes leading to the development of endotoxin shock and of multiple organ failure [1,13]. Being responsible for the development of the somatogenic stage of intoxication, the liver is the chief organ fulfilling a barrier and detoxication function. At the same time, the initial period of endotoxicosis may proceed asymptomatically due to pronounced adaptive-compensatory reactions of the hepatic cells [9]. The above considerations call for a study of the alterations in hepatocyte metabolism which determine the level of compensatory resources and which are an important pathogenic element in hepatic insufficiency.

The aim of the present study was a quantitative histoenzyme assay of hepatocytes in the dynamics of systemic endotoxinemia.

MATERIALS AND METHODS

The study was carried out on the 97 liver biopsies from 11 mongrel dogs weighing 13-18 kg which were injected i. v. simultaneously with *E. coli* lipopolysaccharide in a dose of 2 mg/kg. Bi-

Department of Pathological Anatomy, Medical Faculty, Department of Digestive Pathophysiology, Russian State Medical University, Moscow. (Presented by V. V. Kupriyanov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0001-0101$12.50

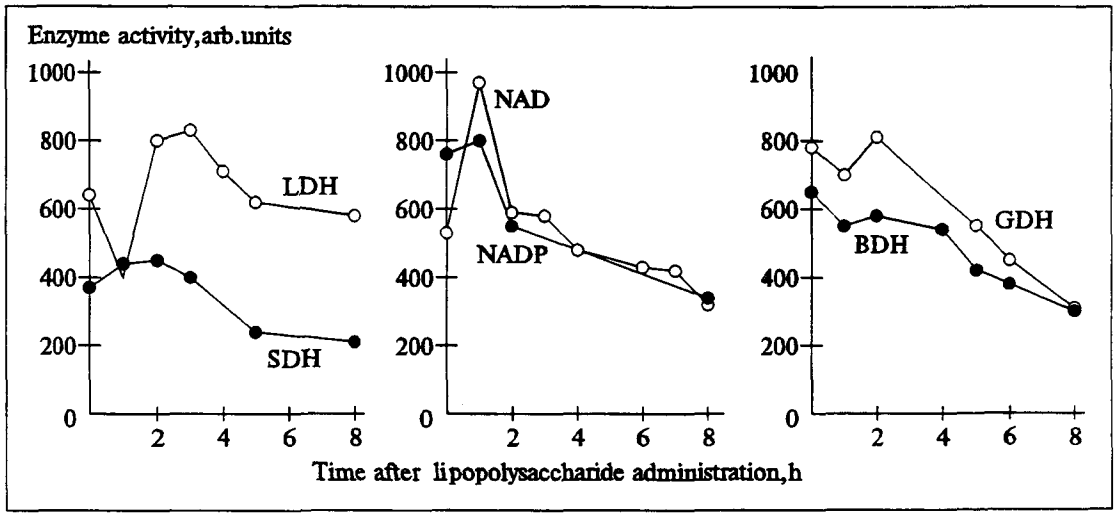

Fig. 1. Alterations of hepatocyte dehydrogenase and diaphorase activity in endotoxinemia.

opsy of the liver tissue and sampling of the blood from the portal vein were performed initially and during 8 h after endotoxin injection. For the assessment of the dynamics of hepatocyte enzyme activity we tested a complex of 6 enzymes involved in different metabolic pathways: succinate dehydrogenase (SDH) as the index of the intensity of oxidative processes in the Krebs cycle; lactate dehydrogenase (LDH), characterizing glycolysis; glutamate (GDH) and β-hydroxybutyrate dehydrogenase (BDH), related to protein and lipid metabolism. The total index of the energy potential was studied with NAD-diaphorase. NADP-diaphorase was used for assessing the state of the energy provision of synthetic processes. Histoenzyme reactions were performed on 10-μ cryostat sections as described elsewhere [15]. Quantitative evaluation of enzyme activity was carried out 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 [5]. Microtome sections were stained with hematoxylin-eosin. The intensity of lipid peroxidation was assessed by the level of malonic dialdehyde in the portal vein [2]. The degree of intoxication was evaluated according to the concentration of medium-weight molecules in the blood plasma [3]. The numerical results were computer-processed using variational statistics analysis.

RESULTS

Microscopic examination of the liver tissue in the time course of endotoxinemia reveals progressive microcirculatory failure and damage to hepatocytes. An hour after lipopolysaccharide administration nonuniform congestion of the portal vessels is observed, particularly of the portal vein branches. In isolated portal tracts minor hemorrhages are noted, whereas large foci of hemorrhages are found after 2-3 h, and after 5-8 h they even spread to individual periportal regions. Nonuniform widening and congestion of the sinusoidal vessels and hepatic venules, erythrocyte aggregation and leukocyte stases, and widening of perisinusoidal Disse's spaces are noted 2-3 h later. As early as 2-3 h after lipopolysaccharide administration stellate reticuloendotheliocytes and endothelial cells increase in size and their nuclei become hyperchromic. Focal clusters of polymorphnonuclear leukocytes are found after 5-8 h in certain portal tracts and regions of hemorrhages. Hepatocyte destruction predominantly manifests itself in vacuolar dystrophy and monocellular necrosis, which encompasses mostly periportal cells 1 h after intoxication. Focal necroses are revealed 3 h later and occasional periacinar necroses are found by 5-8 h.

Development of systemic endotoxinemia results in marked disturbances of the liver tissue metabolism. Quantitative study of the changes of dehydrogenase and diaphorase activity allows one not only to analyze the metabolic processes but also to determine the degree of involvement of membrane structures in cell function at a specific time point [17]. Diverse changes of dehydrogenase activity are noted 1 h after lipopolysaccharide administration (Fig. 1). The oxidation of metabolites in the Krebs cycle is boosted under conditions of cell loading as is evidenced by an increase of SDH activity by 17.9% as compared with the control ($p<0.05$). A higher activity of NAD-diaphorase (86.8%, $p<0.01$) over NADP-diaphorase (8.1%, $p<0.05$, Fig. 1, *b*) is observed and the bulk of energy is expended on cell functioning at the expense of plastic processes. LDH, BDH, and GDH

activity is reduced compared to the baseline level (Fig. 1, *a*, *c*). A progressive drop of NAD- and NADP-diaphorase activity to 62.6% and 44.9% of the control ($p<0.05$) is subsequently found toward 8 h of intoxication as endotoxinemia is prolonged. SDH activity rises somewhat by 2 h of endotoxicosis and then during the next 8 h also falls progressively by 39.0% of the baseline level ($p<0.05$). The indexes of protein and lipid metabolism are found to increase by 17.1% (GDH) and 7.4% (BDH) (Fig. 1, *c*) 2 h after lipopolysaccharide administration, as compared to 1 h of intoxication; GDH activity in this case is 2.9% higher, while BDH activity is 8.6% below the control values ($p<0.05$). Two to four hours later LDH activity exceeds the control level, but after 3 h of endotoxicosis the activity gradually decreases, to reach 88.6% of the control values after 8 h ($p<0.05$).

The study of malonic dialdehyde content in the portal vein revealed a 4.9% increase as early as 1 h after lipopolysaccharide administration (Fig. 2), followed by a steep rise of its level. An increase of the number of medium-sized molecules by 19.2% ($p<0.05$) was found just 1 h after endotoxin injection, whereas 8 h later their number exceeded the initial values by 81.3% ($p<0.05$).

The data obtained show a pattern of dehydrogenase variation rather similar to that of diaphorase in the dynamics of systemic edotoxinemia, evidently attesting to common pathological mechanisms of damage resulting from lipopolysaccharide administration. The increase of enzyme activity after 2 h may be interpreted as a compensatory response of the liver to the pathogenic action of the lipopolysaccharide. The subsequent decrease of oxidation-reduction enzyme activity probably stemmed from uncoupling of the respiration and oxidative phosphorylation in hepatocyte mitochondria and, together with the results of microscopic investigations, attests to a breakdown in the adaptation of metabolic systems. The changes of dehydrogenase activity confirm the reports [11] that the direct toxic effect of endotoxin on hepatocytes and sinusoidal cells together with progressive hypoxia [12,14] leads to the development of an initial phase of shock in the early period after endotoxin administration. These changes are achieved by free fatty acids blocking adenine nucleotide translocase [10], as is borne out by the data obtained on changes of BDH activity (Fig. 1, *c*). The disturbance of hepatocyte metabolism is mainly due to the destruction of cell and intracellular membranes in the course of lipid peroxidation. The activation of free-radical oxidation is noted at early stages of endotoxinemia [8,16]. In our assays the maximal level of malonic dialdehyde is noted within 5 h of intoxication and exceeds the control level by 70.6% ($p<0.05$). It is at this stage of endotoxicosis that periacinar necroses are observed, and LDH activity is below the baseline levels.

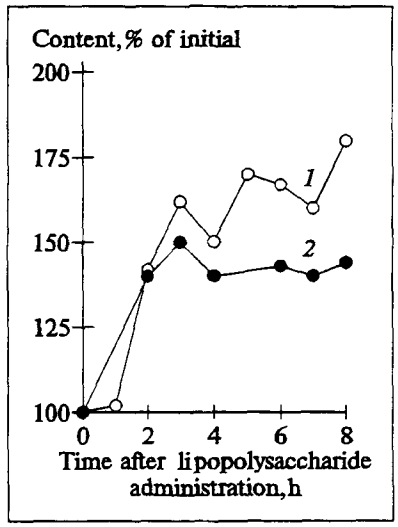

Fig. 2. Alterations of the number of medium-weight molecules (*1*) and malonic dialdehyde (*2*) in portal vein after lipopolysaccharide administration.

The progression of secondary intoxication plays an important role in the pathogenesis of liver damage and in the development of multiple organ failure, as evidenced by the increase of the number of medium-sized molecules in the plasma obtained in the present study. An elevated level of these has been found in shock of various etiology, sepsis, and peritonitis [4,6]. This increase of the number of medium-sized molecules may be related to protein degradation when hepatic tissue is damaged, as well as to intestinal flora decay. These findings support the recommendations [7] for early artificial detoxication in endotoxicosis.

Thus, comparative quantitative studies of hepatocyte enzyme activity in the dynamics of endotoxinemia revealed a regular pattern of changes in enzymatic homeostasis and its periodicity. A compensatory reaction to lipopolysaccharide administration develops during the first 2 h. A decrease of dehydrogenase and diaphorase activity as well as progressive destruction take place at later times. The activation of free-radical lipid oxidation and an increase of the number of medium-weight molecules in the blood plasma play a key role in the pathogenesis of liver damage under conditions of bacterial endotoxicosis.

REFERENCES

1. V. B. Beloborodov and O. Sh. Dzheksenbaev, *Anest. Reanimatol.*, № 4, 41-43 (1991).
2. Yu. A. Vladimirov and A. I. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).

3. N. A. Gabrielyan, N. A. Dmitriev, O. A. Sevost'ytanov, *et al., Anest. Reanimatol.*, № 1, 36-38 (1985).
4. I. A. Eryukhin, V. Ya. Belyi, and V. K. Vagner, *Inflammation as a General Biological Reaction* [in Russian], Leningrad (1989).
5. A. V. Zhukotskii, L. E. Nemirovskii, and V. V. Kilikovskii, in: *Medical and Biological Cybernetics* [in Russian], Moscow (1978), pp. 123-126.
6. V. A. Kuznetsov, V. G. Chuprin, and A. Yu. Anisimov, *Khirurgiya*, № 9, 12-16 (1993).
7. E. A. Luzhnikov, Yu. S. Gol'dfarb, and S. G. Musselius, *Anest. Reanimatol.*, № 1, 56-65 (1993).
8. L. V. Natanzon and Yu. V. Galaev, *Vopr. Med. Khim.*, № 3, 58-59 (1989).
9. D. S. Sarkisov, *Essays on the Structural Basis of Homeostasis* [in Russian], Moscow (1977).
10. V. D. Uvarov, *Biochemical Mechanisms of the Development of Toxic Effects under Conditions of Experimental Endotoxinemia*, Abstract of Dissertation [in Russian], Rostov-on-Don (1991).
11. N. G. Kharlanova and E. A. Bardakhch'yan, *Anest. Reanimatol.*, № 4, 37-40 (1991).
12. G. E. Mazuski, G. L. Platt, M. A. West, *et al., Arch. Surg.*, **123**, 340-344 (1988).
13. F. Naess, O. Roeise, J. Pilgram-Larsen, *et al., Circulat. Shock*, **28**, 89-100 (1989).
14. J. M. Naylor and D. S. Kronfeld, *Amer. J. Physiol.*, **248**, E309-E316 (1985).
15. A. G. E.Pearse, *Histochemistry: Theoretical and Applied*, London (1972).
16. S. Rose, K. D. Tompson, and M. M. Sayeed, *Amer. J. Physiol.*, **263**, R553-R558 (1992).
17. H. G. Sasch, Verh. *Dtsch. Ges. Pathol.*, **62**, 2-10 (1978).

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

O. D. Mishnev, A. I. Shchegolev, and S. P. Yavolov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 1, pp. 108-112, January, 1995
Original article submitted February 15, 1994

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.

Department of Pathological Anatomy, Medical Faculty, Russian State Medical University, Moscow. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0001-0104$12.50