during the first 60 min of the postischemic period may be evidence that activation of LPO still persists during this period, and ultimately this may cause damage to the membranes.

LITERATURE CITED

1. S. V. Gasteva, O. N. Zamuruev, and L. M. Sharagina, Fiziol. Zh. SSSR, No. 10, 1499 (1979).
2. O. N. Zamuruev, Byull. Éksp. Biol. Med., No. 10, 419 (1978).
3. O. N. Zamuruev, Byull. Éksp. Biol. Med., No. 2, 139 (1979).
4. H. Demopoulos, E. Flamm, M. Seligman, et al., in: Oxygen and Physiological Function, ed., F. F. Jöbsis, Dallas, Texas (1977), pp. 491-508.
5. J. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
6. M. Kobayashi, W. D. Lust, and J. V. Passonneau, J. Neurochem., 29, 53 (1977).
7. M. D. Majewska, J. Strosznajder, and J. Lazarewicz, Brain Res., 158, 423 (1978).
8. G. E. De Medio, G. Trovarelli, G. Goracci, et al., in: Proceedings of the European Society for Neurochemistry, Weinheim (1978), p. 311.
9. R. N. Michell, Life Sci., 32, 2083 (1983).
10. S. J. Mittnacht and J. Z. Farber, J. Biol. Chem., 256, 3199 (1981).
11. C. H. Nordström, S. Rehncrona, and B. K. Siesjö, Stroke, 9, 335 (1978).
12. G. Porcellati, G. E. De Medio, G. Fini, et al., in: Proceedings of the European Society for Neurochemistry, ed., V. Neuhoff, Weinheim (1978), pp. 285-302.
13. S. Rehncrona, L. Mela, and B. K. Siesjö, Stroke, 10, 437 (1979).
14. P. Sobotka and D. H. Henzen, Activ. Nerv. Super., 15, 28 (1973).
15. S. Yoshida, S. Inoh, T. Assano, et al., J. Neurosurg., 53, 323 (1980).

EARLY EFFECTS OF SALMONELLA ENDOTOXIN ON THE ANTIOXIDANT ENZYME SYSTEM OF THE RAT LIVER AND INTESTINE

N. I. Popkova, B. S. Kernitskii, E. P. Sorochinskaya, V. A. Yurkiv, N. E. Kucherenko, and A. M. Gerasimov

UDC 612.351.11+612.33.015.11]:577.152.1]. 014.46:615.919:579.842.14

KEY WORDS: salmonella endotoxin; glutathione transferase; glutathione peroxidase; superoxide dismutase; liver; jejunum.

Interconnection between peroxidation and free-radical oxidation with the formation of the immune response of the organism to bacterial toxins, on the one hand, or with the development of tissue and cellular manifestations of toxicity at the host level, on the other hand, is nowadays being confirmed in many different aspects. The most convincing proof of this interconnection is given by: 1) the increase in generation of hydrogen peroxide and the superoxide anion (O_2^-) by phagocytes on contact with microorganisms or their toxins or antibodies; 2) changes in activity of enzymes regulating the formation or breakdown of peroxide or free-radical intermediates, under the influence of bacterial toxins, in the tissues; 3) accumulation of peroxidation products in the tissues during bacterial intoxication; 4) the ability of antioxidants to induce a protective, antitoxic effect when bacterial cells or their toxins are injected into animals [3, 4].

The writers showed previously that cholera toxin causes marked changes in levels of enzymes detoxicating activated forms of oxygen in the liver and intestine of rats [5]. The aim of the present investigation was to study the effect of salmonella endotoxin (ST) of lipopolysaccharide nature on the basic components of the antioxidant enzyme system of the cells of these tissues.

Laboratory of Molecular Bases of Pathogenesis of Infectious Diseases, Central Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Pokrovskii.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 98, No. 11, pp. 525-527, November, 1984. Original article submitted January 25, 1983.

0007-4888/84/9811-1468$08.50

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 120-150 g. The animals were deprived of food for 24 h before the experiment. Each experimental rat received an intraperitoneal injection of 700 μg ST/100 g body weight in 1 ml of physiological saline, and each control rat received physiological saline alone. The animals (six rats in each group) were decapitated under light ether anesthesia 30 min and 1, 2, and 4 h after injection of ST. The liver was perfused, and the jejunum was irrigated with cold 0.15 M Tris-HCl buffer, pH 8.0, containing 0.3 mM phenylmethylsulfonyl fluoride. Cytosols were isolated from the jejunal mucosa and liver by the method described previously [5].

Activity of the following enzymes was determined [5]: glutathione transferase — GT (EC 2.5.1.18), glutathione peroxidase for hydrogen peroxide (GP-H_2O_2) and for *tert*-butyl hydroperoxide — GP-TBH (EC 1.11.1.9), and superoxide dismutase — SOD (EC 1.15.1.1). Activity of glutathione reductase — GR (EC 1.6.4.2) was studied at 37°C [1].

Protein was determined by Lowry's method [6].

The significance of differences between experimental and control parameters was determined by Student's test [2].

EXPERIMENTAL RESULTS

As the writers showed previously [5], the main reserves for GT activity are concentrated normally in the liver, and the GP-TBH level there also exceeds that in the jejunum. SOD activity in the liver was twice as high as in the jejunum.

The present investigation showed that GP-H_2O_2 activity in the liver was three or four times higher than in the jejunal mucosa. The GR level, however, was 2-3 times higher in the jejunum than in the liver. This distribution of GP-H_2O_2 among the organs may indicate a higher intensity of peroxidation in the liver than in the mucosa, whereas predominance of GR in the mucosa is in good agreement with the biological transport function of the intestinal wall in connection with the important role of glutathione in the mechanisms of transport and permeability.

Under the influence of ST (Fig. 1) SOD activity in the cytosol of the jejunal mucosa fell statistically significantly toward 30 min after injection, then returned toward the control level by 2 h, and fell again by 4 h. GR activity increased toward 30 min and reached the control level by 1 h, remaining at this level until 4 h. Activity of GP-TBH fell toward 1 h by 50% compared with the control, after which it was restored to the control values by 2 h, and remained unchanged thereafter until 4 h. GP-H_2O_2 activity fluctuated near the control level until 2 h without any statistically significant changes, then fell sharply until 4 h. GT activity in the jejunal cytosol was unchanged (data not shown).

The time course of GP-H_2O_2 activity in the liver (Fig. 2) was similar to that of GP-TBH activity in the jejunum. GP-TBH activity fell toward 2 h and returned to the control level toward 4 h. GR and SOD activity fell toward 30 min, as also did SOD activity in the jejunal mucosa, after which the GR level rose above the control by 1 h, returned to it again by 2 h, and thereafter remained unchanged until 4 h. SOD activity reached the control values by 1 h and did not differ from them until 4 h. The GT level was twice the control value by 1 h after injection of ST, it fell to that level by 2 h, and remained within these limits until 4 h.

The results thus indicate early and marked reorganization of the enzyme system in the liver and jejunum under the influence of ST. On the whole the character of the changes in enzyme levels in the organs studied can be described as phasic, with a more uniform pattern of interchange of phases of the dynamics of the level of each enzyme in the liver than in the intestine. In the latter, the antibathic nature of the curves reflecting the dynamics of enzyme activity in the first hours of action of ST will be noted in the latter case. Since changes in SOD and GP-H_2O_2 correlate with one another [1], the uniform trend of the time course of activity of these enzymes 30 min after injection of ST can be regarded not only as further biological confirmation of the theoretically necessary functional link between the most general cytosol generator of H_2O_2 (SOD) and the most important enzyme of its detoxication in this part of the cell (GP-H_2O_2), but also as proof of the common nature of the mechanisms controlling this interconnection. Changes in the levels of these enzymes in the liver in the late stages of salmonella poisoning also are similar in direction (Fig. 2).

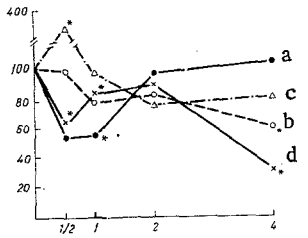

Fig. 1

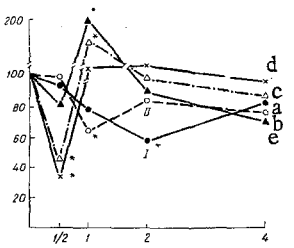

Fig. 2

Fig. 1. Time course of activity of enzymes of antioxidant system in cytosol of jejunal mucosa in endotoxin shock. Abscissa, time (in h); ordinate, activity (in % of control). a) GP-TBH, b) GP-H_2O_2, c) GR, d) SOD. *P < 0.05.

Fig. 2. Time course of activity of enzymes of antioxidant system in liver cytosol in endotoxin shock. e) GT activity. Remainder of legend as to Fig. 1.

Meanwhile the antibathic character of the time course of the GR and GP-H_2O_2 content during the first few hours of action of ST is evidence of the appearance of conditions in the jejunal mucosa favoring imbalance ofthe enzymic glutathione redox system for detoxication of H_2O_2, which, allowing for the fall in the GR level, may become the most important cause of the disturbance of H_2O_2 metabolism and, as a result, of oxidative destruction of tissue.

The mechanisms of such rapid changes in enzyme levels are not absolutely clear. Since addition of ST (0.2 mg/ml) to liver or mucosal homogenate did not affect the activity of the enzymes studied, any direct action of the toxin must be excluded. The rapidity of the manifestations 30 min after injection of ST also rules out changes in the heterogeneity of the cell population (migration of leukocytes, mitosis, and differentiation, and so on) as the cause of the dynamics of activity, at least for the liver. So far as the jejunum is concerned, a definite contribution is made by desquamation of the epithelium, but SOD and GP-TBH levels in the contents of the jejunal lumen were extremely low, and the other enzymes could not be demonstrated. Changes in the fraction of soluble protein in total tissue protein could affect the amplitude of the dynamics of activity of all enzymes simultaneously and in the same direction, but could not in any way be the cause of opposite changes. The role of intracellular factors of destruction, inhibition, induction, and activation of enzymes and a combination of them in the realization of the time course revealed in the activity of these enzymes requires special study.

LITERATURE CITED

1. A. M. Gerasimov, "The antioxidant enzyme system of the animal cytosol," Author's Abstract of Doctoral Dissertation, Moscow (1981).
2. G. F. Lakin, Biometrics [in Russian], Moscow (1980).
3. V. F. Lipkovskii, N. Yu. Mironov, and B. N. Pyasetskii, in: Alimentary Toxicoinfection [in Russian], Saratov (1979), pp. 89-91.
4. L. F. Panchenko, A. M. Gerasimov, and V. D. Antonenkov, The Role of Peroxisomes in Cell Pathology [in Russian], Moscow (1981).
5. V. A. Yurkiv and N. I. Popkova, Byull. Éksp. Biol. Med., No. 8, 190 (1984).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
7. R. F. Del Maestro, H. W. Thaw, J. Björk, et al., Acta Physiol. Scand., 110, Suppl. No. 492, 43 (1980).
8. R. Ogawa, T. Morita, F. Kunimoto, et al., Cir. Shock, 9, 369 (1982).