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THE POSSIBILITY OF STUDYING LIPID PEROXIDATION IN SURVIVING TISSUES

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Considerable disturbances of metabolism of lipid peroxides take place in experimental hypercholesteremia in animals [3, 4] and in coronary atherosclerosis in man [5, 7, 8]. According to existing views, lipid peroxides and radical intermediates of lipid peroxidation may have a harmful action on biopolymer molecules and on supramolecular structures of the cell [3] and may thereby facilitate the primary lesion in the blood vessel wall [4]. It is thus very important to study disturbances of lipid peroxide metabolism in the human aorta and other tissues in atherosclerosis. Cadaver material obtained at early autopsy (after 1-3 h) in cases of sudden death can be used for such investigations. However, it is difficult to determine the time elapsing after death during which the test material remains in its native state.

The object of this investigation was to study the effect of postmortem changes arising in the liver and aorta of mammals on the content of lipid peroxidation (LPO) products and on activity of the antioxidant enzymes — superoxide dismutase and glutathione peroxidase II — in these tissues.

EXPERIMENTAL METHOD

Experiments were carried out on 24 male chinchilla rabbits weighing 2-2.5 kg and on 16 male August rats weighing 110-120 g. All the rabbits were killed simultaneously by a blow on the head and the cadavers were kept at 4-8°C for 9 h. Ischemia of the liver was produced in the rats by compression of the vascular pedicle (maximal duration of ischemization 2).* The liver and aorta were perfused with cold isotonic KCl solution, cut into small pieces (the aorta after preliminary separation of intima and media), and homogenized in a glass homogenizer with Teflon pestle or in a glass Potter's homogenizer. The tissue homogenates were centrifuged (750g, 10 min) on a K-70 centrifuge (from Janetzky, East Germany); microsomes and

*The experiments on ischemization of the rat liver were carried out jointly with M. N. Bilenko and L. B. Dudnik, of the Laboratory for Transplantation of Organs and Tissues, Academy of Medical Sciences of the USSR.

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TABLE 1. Content of LPO Products in Surviving Rabbit Tissues (M ± m)

Time after sacrifice of animals, h	Aorta		Liver			
			microsomes		cytosol	
	LPO products					
	primary, ΔD_{233}/mg protein	secondary, relative fluorescence units/mg protein	primary, ΔD_{233}/mg protein	secondary, relative fluorescence units/mg protein	primary, ΔD_{233}/mg protein	secondary, relative fluorescence units/mg protein
0	0,36±0,095	69±15,9	0,16±0,021	7,1±1,43	0,04±0,002	3,6±0,20
1½	0,20±0,028	70±14,3	0,39±0,053	24,1±0,70	0,05±0,004	11,6±2,59
3	0,30±0,117	52±15,7	0,38±0,088	22,5±6,18	0,06±0,003	11,3±2,97
4½	0,29±0,080	51±10,0	0,35±0,108	36,1±4,45	0,09±0,017	13,4±4,04
6	0,31±0,037	79±17,1	0,35±0,117	15,0±0,55	0,07±0,013	9,0±0,50
9	0,44±0,162	89±30,6	0,31±0,028	16,4±1,94	0,10±0,045	16,0±2,80

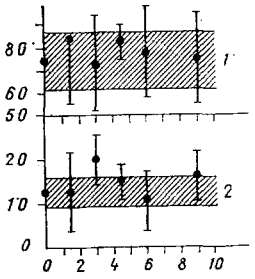

Fig. 1

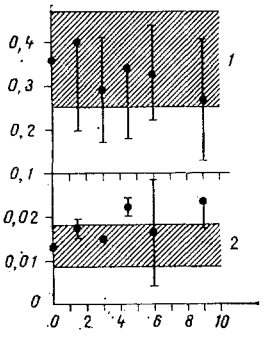

Fig. 2

Fig. 1. Activity of superoxide dismutase in surviving liver (1) and aorta (2) of rabbits. Abscissa, time (in h); ordinate, activity (in units/mg protein).

Fig. 2. Activity of glutathione peroxidase II in surviving liver (1) and aorta (2) of rabbits. Legend as to Fig. 1.

cytosol were obtained from the liver (105,000g, 60 min) on an L-50 centrifuge (from Beckman, USA), as described previously [9]. The content of primary LPO products (conjugated dienes) was determined from the absorption at 233 nm on an Aminco DW-2A spectrophotometer (USA) and the content of secondary LPO products (intermolecular cross-linkages) was determined on an Aminco SPS-500 spectrofluorometer (USA), from the intensity of fluorescence of 1-amino-3-iminopropenes [1]. Superoxide dismutase activity in the supernatants of the homogenates of aorta (750g, 10 min) and liver (105,000g, 60 min) was determined from kinetic curves of inhibition of the reaction of reduction of nitro-BT by the superoxide radical generated by a xanthine oxidase system at 25°C on the Aminco DW-2A spectrophotometer (USA) [6]. Activity of glutathione peroxidase II in the supernatants of the tissue homogenates was determined from the kinetics of oxidation of NADPH in a conjugated glutathione reductase system, using tert-butyl hydroperoxide as substrate, at 30°C on the same spectrophotometer [6].

EXPERIMENTAL RESULTS

In the course of 9 h after sacrifice of the animals practically no change took place in the content of primary or secondary LPO products (Table 1). Conversely, the content of secondary LPO products in the microsomes and cytosol of the liver and the content of both secondary and primary products in the microsomes rose sharply as early as 1.5 h after sacrifice of the animals, after which it remained at a higher than normal level (Table 1). Unlike in the aorta, LPO processes are thus probably activated in the rabbit liver immediately after sacrifice.

Since antioxidant enzymes play an important role in maintenance of a steady level of lipid peroxides in the body [3, 4], superoxide dismutase and glutathione peroxidase II activity was studied in the rabbit aorta and liver during 9 h after sacrifice. It was found that

TABLE 2. Changes in Activity of Antioxidant Enzymes in Rats with Experimental Ischemia of the Liver (M ± m; n = 4)

Experimental conditions	Activity, units/mg protein	
	glutathione peroxidase II	superoxide dismutase
Control	0,26±0,03	25±0,7
Ischemia for 2 h	0,16±0,01	12±0,9

superoxide dismutase activity remained unchanged during the period of observation in both the liver and aorta of the experimental animals (Fig. 1). Similarly, glutathione peroxidase II activity was unchanged during 9 h after sacrifice of the rabbits in the tissues tested (Fig. 2). Intensification of LPO in the surviving rabbit liver may be due to intensification of nonenzymic lipid oxidation. Since in hypoxia a significant increase is observed in the content of LPO products in the tissues because of inhibition of antioxidant enzymes [1], it can be tentatively suggested that the intensification of LPO in the liver of the animals after sacrifice was largely attributable to a sharp decrease in the partial pressure of oxygen. It was therefore decided to study changes in activity of antioxidant enzymes after hypoxic damage caused by experimental ischemia of the liver.

Ischemia was shown to cause a sharp decrease in activity of the antioxidant enzymes (Table 2). Consequently, stability of the level of enzyme activity in the surviving tissues, found in these experiments, probably was not directly connected with the onset of hypoxia. Preservation of a stable level of activity of "antioxidant" enzymes in the surving tissues confirmed their native state and enabled the activity of these enzymes in the tissues (aorta and liver) and LPO products in the aorta of the cadavers to be investigated correctly, at least for 3-6 h after death.

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