effect of hyperoxygenation is exhibited. Oxygen poisoning then develops: the ultrastructure and function of the mitochondrial and cytoplasmic membranes are damaged, free and phosphorylating oxygenation processes are uncoupled, and the efficiency of biological oxidation is reduced [6]. An oxygen concentration of up to 100% under a pressure of not more than 1 kgf/cm^2 with an exposure of up to a few tens of minutes may have a beneficial effect on the course of oxidative processes in the tissues in the presence of moderate degrees of hypoxia. Hyperbaric conditions of oxygenation (3-5 kgf/cm^2) with an exposure of 15-20 min ought to be maximally effective for producing urgent recovery from a state of deep hypoxia. To prevent the development of oxygen poisoning, fractional schedules of oxygenation are preferable.

LITERATURE CITED

1. V. Y. Berezovskii, The Tissure Oxygen Partial Pressure in Animals and Man [in Russian], Kiev (1973).
2. M. Dixon and E. Webb, Enzymes, Academic Press (1964).
3. E. A. Kovalenko and I. N. Chernyakov, in: Problems in Space Biology [in Russian], Vol. 21, Moscow (1972).
4. M. N. Kondrashova and A. A. Ananenko, in: A Manual for the Study of Biological Oxidation by the Polarographic Method [in Russian], Moscow (1973), pp. 106-128.
5. V. V. Matsynin, in: Polarographic Determination of Oxygen in Biological Objects [in Russian], Kiev (1968), pp. 64-69.
6. V. V. Matsynin, Dokl. Akad. Nauk Ukr. SSR, Ser. B. No. 12, 1117 (1978).
7. I. F. Sokolyanskii, in: Polarographic Determination of Oxygen in Biological Objects [in Russian], Kiev (1974), pp. 232-237.
8. I. F. Sokolyanskii, in: Mechanisms of Injury, Resistance, Adaptation, and Compensation [in Russian], Vol. 1, Tashkent (1976), pp. 450-451.
9. A. A. Sharaf, "A study of the mechanism of action of uncouplers of oxidative phosphorylation," Author's Abstract of Candidate's Dissertation, Moscow (1968).
10. C. J. Lambertsen, in: Treatment with Hyperbaric Oxygen [Russian translation], Moscow (1968), pp. 9-11.

LIPID PEROXIDATION IN MYOCARDIAL MEMBRANES AND ITS CONTROL DURING AGING

V. V. Lemeshko and Yu. V. Nikitchenko

UDC 612.173.1:612.397]:612.67

KEY WORDS: aging; lipofuscin; myocardium; mitochondria; glutathione transferase.

Accumulation of lipofuscin in cells, mainly in hypertrophied muscle fibers, is one of the characteristic changes in the myocardium during aging [1, 6, 8, 13, 14]. Lipofuscin and fluorescent lipopigments like it are end products of free-radical lipid peroxidation (LPO) in biological membranes as a result of interaction of free amino groups of phospholipids and proteins with aldehydes formed during LPO in the membranes [6, 8]. An important role is ascribed to LPO in the pathogenesis of diseases frequently associated with age, such as atherosclerosis [4]. Accordingly it is interesting to study age changes in the intensity of LPO in myocardial membranes and the character of its cytoplasmic regulation in mammals during aging. Investigations previously carried out in this direction [7, 10-14] do not provide a complete age picture of LPO in myocardial membranes.

In the investigation described below the intensity of accumulation of lipofuscin and of lipofuscin-like pigments in the myocardium, the intensity of membrane LPO in homogenates and mitochondria, and also the activity of several enzymes of the antioxidant system of the myocardial cytosol and mitochondria were studied in rats of different ages.

Department of Biochemistry of Ontogeny, Research Institute of Biology, A. M. Gor'kii Khar'kov University.

(Presented by Academician of the Academy of Medical Sciences of the USSR L. T. Malaya.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 11, pp. 12-14, November, 1982. Original article submitted April 6, 1982.

0007-4888/82/9411-1477$07.50

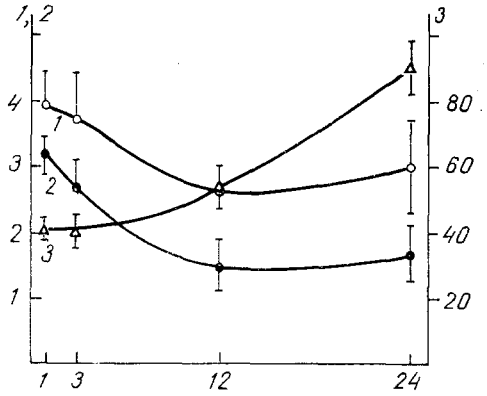

Fig. 1. Effectiveness of MDA accumulation in myocardial homogenates by enzymic LPO (1, 2) and specific lipofuscin content in myocardium (3) of rats of different ages. 1, 2) In nmoles MDA/mg protein/60 min; 1, 2) 0.6 and 3.5 mg protein/ml respectively; 3) in relative units/unit wet weight. Here and in Figs. 2 and 3, abscissa — age (in months).

EXPERIMENTAL METHOD

Male Wistar rats aged 1, 3, 12, and 24 months were used. Mitochondria were isolated from the heart as described previously [15], except that the washing and suspending medium did not contain albumin and EDTA. Cytosol was obtained by centrifugation of the postmitochondrial fraction for 60 min at 105,000g.

The lipofuscin concentration was determined, as in [13], together with other fluorescent myocardial lipopigments.

To investigate membrane LPO a 20% homogenate was prepared in 100 mM Tris-HCl, pH 7.4, which was filtered through three layers of nylon. The intensity of enzymic membrane LPO was determined by incubating the homogenate (0.6 and 3.5 mg protein) with 1 mM NADPH for 60 min at 37°C with constant aeration. Activity of enzymic LPO in mitochondrial membranes was determined by incubating mitochondria (1 mg protein) in the same buffer containing 0.4 mM NADPH, 4 mM ADP, and 50 μM Fe^{3+} at 37°C. The intensity of ascorbate-dependent LPO was determined in 100 mM Tris-HCl, pH 7.4, containing 0.5 mM ascorbate, 12 μM Mohr's salt, concentration of mitochondria 1 mg protein/ml, incubation time 40 min. In all cases the quantity of malonic dialdehyde (MDA) formed during incubation was measured [2].

The glutathione transferase activity of the cytosol and mitochondria was measured spectrophotometrically [15] at 37°C in 100 mM K^+-phosphate buffer, pH 6.5, containing 5 mM reduced glutathione, 1 mM 1-chloro-2,4-dinitrobenzene, and 0.4 mg cytosol protein in 1 ml, and also in buffer containing 10 mM reduced glutathione, 0.1% solution of Triton X-100, 1 mM 1-chloro-2,4-dinitrobenzene, and 0.8 mg mitochondrial protein in 1 ml.

The glutathione reductase activity of the cytosol was determined fluorometrically from the decrease in NADPH [3] in 50 mM K^+-phosphate buffer, pH 7.4, containing 0.16 mM NADPH, 1 mM oxidized glutathione, 1 mM EDTA, and 0.6 mg cytosol protein in 1 ml at 30°C.

The velocity of reduction of $NADP^+$, catalyzed by cytosol, was determined fluorometrically in medium containing 25 mM Na^+-phosphate buffer, pH 7.4, 3 mM $MgCl_2$, 0.4 mM $NADP^+$, 1 mM glucose-6-phosphate, 2 mM malate, and 0.2 mg cytosol protein in 1 ml at 30°C. Protein was determined by Lowry's method in Miller's modification [9].

EXPERIMENTAL RESULTS

Changes with age in the specific lipofuscin content in the rat myocardium, calculated per unit wet weight, are shown in Fig. 1. The rise in the lipofuscin level in animals between the ages of 1 and 12 months clearly was less than between 12 and 24 months. This, however, does not mean that the rate of its formation is higher in old age, for during the first half of life the weight of the organ increases by 3.7 times compared with the weight of the heart in rats aged 1 month, but thereafter it remains almost unchanged. Allowing for dilution of

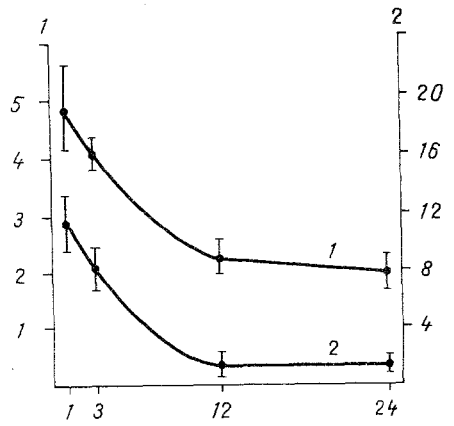

Fig. 2. Efficiency of MDA accumulation (in nmoles/mg protein/40 min) by NADPH-dependent (1) and ascorbate-dependent (2) LPO of myocardial mitochondrial membranes of rats of different ages.

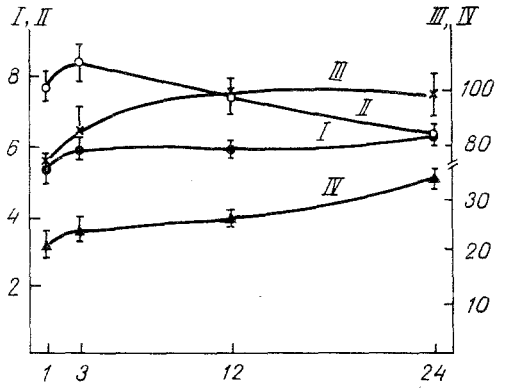

Fig. 3. Rate of reduction of $NADP^+$ by glucose-6-phosphate with malate in cytosol (I), glutathione reductase activity of cytosol (II), and glutathione transferase activity of cytosol (III) and mitochondria (IV) of myocardium from rats of different ages. I, II) In nmoles NADP/mg protein/min; III, IV) in nmoles 1-chloro-2, 4-dinitrobenzene/mg protein/min.

lipofuscin by the newly formed mass of the heart, it can be concluded that the rate of formation of lipofuscin, together with other fluorescent lipopigments, in the myocardium is not less in youth than in old age. This is confirmed by data on MDA accumulation in myocardial homogenates during enzymic membrane LPO (Fig. 1). By the age of 12 months the intensity of membrane LPO declines, as can be seen more clearly in a relatively concentrated homogenate (Fig. 1). The velocity of LPO, both enzymic and nonenzymic, in mitochondrial membranes was higher in rats aged 1 and 3 months than in rats aged 12 and 24 months (Fig. 2).

The process of membrane LPO in the cell is controlled by two antioxidant systems: enzymic and nonenzymic [2]. The enzymic system includes a series of enzymes: glutathione transferase, glutathione peroxidase, NADPH-dependent glutathione reductase, superoxide dismutase, and catalase. Figure 3 shows that the rate of formation of NADPH, an electron donor for glutathione, from $NADP^+$ in the cytosol on account of malate and glucose-6-phosphate does not decrease during aging, whereas glutathione reductase activity falls appreciably. The glutathione transferase activity of the cytosol and mitochondria increases with age (Fig. 3). It was shown in [11] that glutathione peroxidase and catalase activity is significantly higher in cardiac mitochondria of rats aged 24 months than in rats aged 3 months, whereas their superoxide dismutase activity is identical. Superoxide dismutase activity in mouse heart homogenates also showed little change with age [12]. The increase in glutathione transferase

(Fig. 3) and glutathione peroxidase [11] activity with age may be one factor which explains why, although the concentration of the myocardial homogenate from rats aged 12 and 24 months was higher, the intensity of LPO in the membranes was reduced more than in young rats aged 1 and 3 months (Fig. 1).

The results of this investigation show that the intensity of free-radical lipid peroxidation in rat myocardial membranes decreases with age. Since free radicals are among the leading factors of aging, the results suggest that the process of aging of the myocardium may follow a more rapid course in youth than in old age.

LITERATURE CITED

1. S. S. Vail' Functional Morphology of Disturbances of Cardiac Activity [in Russian], Leningrad (1960).
2. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
3. A. M. Gerasimov, L. A. Koroleva, O. S. Brusov, et al., Vopr. Med. Khim., No. 1, 89 (1976).
4. V. Z. Lankin, Kardiologiya, No. 8, 42 (1980).
5. V. V. Lemeshko, P. A. Kaliman, L. I. Belostotskaya, et al., Biokhimiya, 47, 552 (1982).
6. D. Mead, in: Free Radicals in Biology [Russian translation], Vol. 1, Moscow (1979), p. 68.
7. A. S. Csallany, K. L. Ayaz, and Su Le-Chu, J. Nutr., 107, 1792 (1977).
8. G. Durand and F. Desnoyers, Ann. Nutr. (Paris), 34, 317 (1980).
9. G. L. Miller, Anal. Chem., 31, 964 (1959).
10. H. Nohl and D. Hegner, Eur. J. Biochem., 82, 563 (1978).
11. H. Nohl, D. Hegner, and K.-H. Summer, Mech. Ageing Dev., 11, 145 (1979).
12. U. Reiss and D. Gershon, Biochem. Biophys. Res. Commun., 73, 255 (1976).
13. A. L. Tappel, in: Pharmacological Intervention in the Aging Process, New York (1978), p. 111.
14. K. T. Tcheng, H. P. Wang, and Y. T. Chu, Sci. Sinica, 10, 445 (1961).
15. M. Younes, R. Schlichting, and C.-P. Siegers, Pharmacol. Res. Commun., 12, 115 (1980).

REDISTRIBUTION OF REGIONAL BLOOD FLOWS AND VOLUMES DUE TO OBTURATION OF THE BILIARY TRACT

O. A. Kovalev, A. I. Gorbashko, G. Ya. Veselov, S. K. Sheremetevskaya, M. A. Parfenova, and O. N. Nepochatov

UDC 616.361-007.272-07:[616.12-008.3+616.151.1

KEY WORDS: bile duct; hemodynamics; blood volume; ^{86}Rb.

Obturation of the biliary tract causes changes in the systemic hemodynamics and the blood volume in individual organs [6]. However, the general structure of the regional distributions of the cardiac ejection — the circulatory minute volume (CMV) and circulating blood volume (CBV) — has not been studied. The aim of the present investigation was an experimental study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighting 180-230 g. The common bile duct was ligated and divided under hexobarbital anesthesia under sterile condi-

Central Research Laboratory and No. 3 Department of Surgery, S. M. Kirov Leningrad Postgraduate Medical Institute.

[Presented by Academician of the Academy of Medical Sciences of the USSR V. K. Kulagin (deceased).] Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 94, No. 11, pp. 14-17, November, 1982. Original article submitted January 13, 1982.

0007-4888/82/9411-1480$07.50