Analysis of these experimental data thus shows that a change in the intensity of LPO, characterizing the level of oxidative free-radical processes in the graft, in the recipient's tissues adjacent to the graft, and in the recipient's blood depends on the type of transplantation and correlates with the functional state of the graft at all stages of its survival.

LITERATURE CITED

1. A. V. Alekseenko, M. V. Bilenko, and L. N. Shelenkov, in: Free-Radical Oxidation of Lipids under Normal and Pathological Conditions [in Russian], Moscow (1978).
2. A. I. Dzhafarov, Byull. Eksp. Biol. Med., 88, No. 11, 513 (1979).
3. A. I. Dzhafarov, V. M. Kasumov, and B. N. Tarusov, Biofizika, 12, No. 4, 744 (1967).
4. L. B. Dudnik, M. V. Bilenko, and A. V. Alekseenko, Byull. Eksp. Biol. Med., 89, No. 5, 556 (1980).
5. Kh. A. Kibedmagomedova, Yu. L. Mel'nikov, and Yu. A. Vladimirov, Biofizika, 15, 1022 (1970).
6. É. M. Kulieva, V. V. Perelygin, and A. I. Dzhafarov, Dokl. Akad. Nauk Azerb. SSR, 34, No. 2, 85 (1978).
7. L. F. Panchenko, A. M. Gerasimov, and G. A. Nozdracheva, Vopr. Med. Khim., No. 3, 321 (1974).
8. B. N. Tarusov, A. I. Polivoda, and A. I. Zhuravlev, Biofizika, 6, No. 4, 490 (1961).
9. A. G. Chogoshvili, A. I. Dzhafarov, and O. R. Kol's, Tr. Mosk. Ova. Estestvoisp. Prir., 50, 47 (1974).
10. W. R. Bidlack and A. L. Tappel, Lipids, 8, 177 (1973).
11. L. T. Brosnan, H. A. Krebs, and D. H. Williamson, Biochem. J., 117, 91 (1970).
12. L. Stroznajder and Z. Dabrowiecki, Bull. Acad. Pol. Sci., Cl. 2, 23, 9 (1975).
13. A. L. Tappel and H. Zalkin, Arch. Biochem., 80, 326 (1959).

EFFECT OF ANOXIA UNDER DIFFERENT CONDITIONS ON KINETICS OF LIPID PEROXIDATION IN CELL ORGANELLES

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Facts indicating that anoxia is the main cause leading to intensification of lipid peroxidation (LPO) in cells of isolated tissue have recently accumulated. During survival after death, products of LPO accumulate in isolated organs and tissues and antioxidative activity is reduced [2-4, 6]. Inhibition of the functional activity of isolated tissues also have been demonstrated during induced lipid peroxidation, i.e., in the presence of an increased concentration of LPO products [5].

LPO products are known to be capable of causing an increase in the membrane permeability of organelles, the release of enzymes from them, inactivation or transformation of enzymes, oxidation of thiols, and polymerization of proteins; for that reason the intensification of LOP during postmortem survival may aggravate damage to the cell membranes of isolated tissues [1, 9]. Meanwhile, free-radical injury to the whole membrane system of the cells of isolated surviving tissues has not been adequately studied. There have been few investigations primarily aimed at studying changes in the intensity of LPO in organelles exposed to ischemia in tissues [4].

During ischemia in the tissues, because of different velocities of LPO in different organelles, the possibility of diffusion of LPO products from some areas of the cell to others

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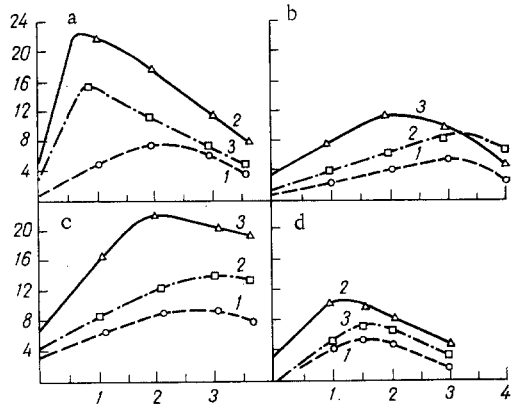

Fig. 1. Changes in peroxide concentration in suspension of liver cell organelles during incubation at 37°C. a) Mitochondria; b) nuclei; c) microsomes; d) lysosomes. 1) Control organelles; 2) exposed to anoxia in the tissues; 3) exposed to anoxia after isolation. Concentration of organelles in suspension during incubation 11 mg protein/ml. Abscissa, time (in h); ordinate, concentration of LPO (in nmoles peroxide/mg protein).

may arise. It is therefore difficult to determine the true sensitivity of different organelles to ischemia, i.e., the order of intensification of free-radical processes in individual organelles. For that reason, in the present investigation the principles governing accumulation of LPO products in cell organelles (mitochondrial, microsomal, nuclear, and lysosomal fractions) exposed to anoxia under different conditions during incubation were studied.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats weighing 200-250 g and kept under standard animal house conditions. Mitochondria, microsomes, nuclei, and lysosomes were isolated from the liver by differential centrifugation. The experiments were carried out in the following versions: 1) measurement of the intensity of LPO in cell organelles during survival *in vitro* under aerobic conditions at 37°C (the results of these experiments served as the control); 2) measurement of the intensity of LPO in cell organelles isolated from the previously ischemized liver (anoxia) during survival under aerobic conditions; 3) a study of the intensity of LPO of cell organelles previously exposed to anoxia *in vitro* during survival under aerobic conditions.

Lipids were extracted from the cell organelles by the method described in [10]. Ischemia was produced by application of a ligature to the vascular pedicle of a hepatic lobule for 30 min. During creation of anoxia for isolated cell organelles, these were kept in an atmosphere of an inert gas for 30 min. Anoxia and subsequent aerobic incubation of organelles were carried out at 37°C in medium consisting of 0.25 M sucrose, 10 mM Tris-HCl, 110 mM KCl, 5 mM $MgCl_2$, 35 mM $CaCl_2$, pH 7.4. The intensity of LPO was judged from changes in the content of primary peroxidation products and of secondary products, namely malonic dialdehyde (MDA).

The content of lipid peroxides in the organelles was determined by iodometric titration and polarography, using a mercury drop electrode. Accumulation of MDA was judged by the reaction of carbonyl compounds with thiobarbituric acid [11]. The protein concentration in the samples was determined by Lowry's method. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The experiments showed that the kinetics of accumulation of peroxides during incubation was in general similar in all the control organelles: The peroxide content increased to be-

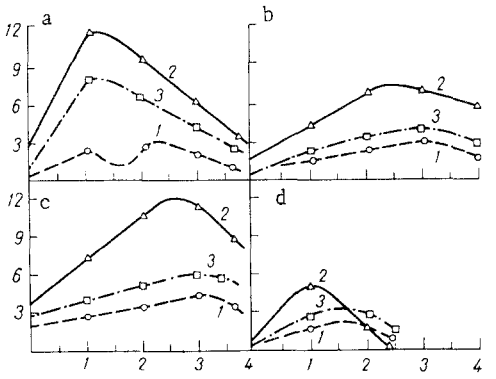

Fig. 2. Changes in MDA concentration in suspensions of liver cell organelles during incubation at 37°C. Ordinate, MDA content (in nmoles/mg protein). Remainder of legend as to Fig. 1.

gin with during incubation, but on reaching a maximum, the rate of their formation decreased. The organelles differed in their levels of this maximum at the time of its achievement. In the control the initial peroxide level was higher in the mitochondria and microsomes than in other organelles, and they were distinguished by the highest rate of accumulation of peroxides. Accumulation of LPO products in lysosomes was inconsiderable but reached a maximum quickly.

During incubation of organelles exposed to anoxia for 30 min within the tissues the character of accumulation of peroxide remained the same on the whole as in the control: Here also peroxides accumulated and their concentration, having risen to a maximum, began to fall. The difference from the control was the higher rate of accumulation of peroxides and the higher level of the maxima. During incubation of cell organelles exposed to anoxia in the tissues for 30 min the concentration of peroxides in the mitochondria reached a maximum after 40 min, in the microsomes and nuclei after 2 h, and in the lysosomes after 1 h; this was quicker than in the control by 3, 1.5, 1.5, and 1.5 times, respectively. The peroxide concentration under these conditions in the mitochondria at its maximum was 3.1 times higher than the control; 2.5 times higher in the microsomal fraction, 2.2 times higher in the nuclei, and 1.8 times higher in the lysosomes (Fig. 1).

The changes in the MDA concentration during incubation of organelles exposed to ischemia in the tissues showed the same rule as the changes in the peroxide concentration. The MDA concentration in the control organelles during incubation, like that of lipid peroxides, increased up to a maximum, and then fell steadily. It should be noted that there were two maxima on the kinetic curve of MDA accumulation in the mitochondria: after 1 and 2.5 h of incubation. After anoxia in the tissues the MDA concentration in all organelles rose appreciably depending on the period of incubation compared with the control and reached the maximum more rapidly: after 1 h in the mitochondria, after 2.5 h in the microsomes, after 1 h in the lysosomes, and after 2 h in the nuclei. The increase in MDA in the mitochondria during incubation was much greater than in the other organelles; whereas the MDA concentration at the first maximum in mitochondria exposed to anoxia was 4.1 times higher than in the control, in the microsomes it was 6.0 times, in the nuclei 2.6 times, and in the lysosomes 2.1 times higher (Fig. 2).

Exposure of the organelles to anoxia outside the tissues also was accompanied by intensification of LPO. In that case, however, during subsequent aerobic incubation the velocity of LPO was appreciably lower than in organelles exposed to anoxia in the tissues. This was particularly noticeable in nuclei or lysosomes exposed to anoxia for 30 min, in which LPO developed at almost the same rate as in the control during incubation. Meanwhile the mitochondria were highly sensitive to anoxia outside the tissues also. In the mitochondrial fraction exposed to anoxia for 30 min the time taken to reach the maximum of peroxide accumulation was reduced by 2.4 times, and the maximum of the peroxide concentration was 2.0 times higher than in the control (Fig. 1). In the remaining organelles (microsomes, nuclei, lyso-

TABLE 1. Changes in MDA Concentration (in nmoles MDA/mg protein) in Suspensions of Mitochondria Exposed to Anoxia for 20 Min before Aerobic Incubation at 37°C (M ± m)

Experimental conditions	Time, in min, after aerobic incubation	
	25	60
Control	1,55±0,05	2,9±0,08
Anoxia	3,7±0,09	12,2±0,12
Anoxia after preliminary treatment with cinchocaine (20 μM)	2,6±0,1	6,4±0,03
Anoxia after preliminary treatment with EGTA (1.5 μM)	2,2±0,08	4,2±0,04

somes) exposed to anoxia outside the tissues, the maximum of the peroxide concentration was only 46, 60, and 30% higher, respectively, than the control level. When organelles were exposed to anoxia outside the tissues the MDA concentration also was appreciably lower during subsequent aerobic incubation than in organelles exposed to anoxia in the tissues. In this case also changes in MDA concentration were most clearly expressed in the mitochondria only (Fig. 2).

The earliest injuries in the mitochondria during anoxia are known [8] to be due to activation of membrane phospholipase A_2 by Ca^{++} ions. Consequently, changes in LPO were studied in a suspension of mitochondria in which phospholipase A_2 activity was inhibited before the creation of anoxia. For this purpose in a special series of experiments either the liver was perfused before anoxia with local anesthetic (20 μM cinchocaine) or the mitochondrial suspension itself was treated with 1.5 μM EGTA. The results of these experiments showed that when phospholipase A_2 activity was inhibited, MDA formation was appreciably depressed during subsequent aerobic incubation in mitochondria exposed previously to anoxia (Table 1). This may be additional evidence that one cause of the intensification of LPO during anoxia may be activation of phospholipase A_2.

Analysis of the kinetics of accumulation of LPO products in cell organelles exposed to anoxia under different conditions enables the role of lysosomes in the production of irreversible changes in the cell to be judged. The results obtained in the present investigation agree with data of those workers [7] who found no activation of acid hydrolases in the prenecrotic period in surviving ischemized tissues, and they are evidence against the view that lysosomes play the leading role in cell death.

We have seen that the intensity of LPO in mitochondria exposed to anoxia both within and outside the tissues is much higher and it begins to rise much sooner than changes in LPO intensity in the lysosomes. Consequently, before labilization of the lysosomal membranes begins, intensification of LPO is taking place in many subcellular membranous formations, functional activity is changing, and ultimately cell death is taking place. It cannot therefore be considered that hydrolases liberated after labilization of the lysosomal membranes were responsible for the earlier ischemic injury to the tissues.

Anoxia of cell organelles within the tissues thus causes damage to their structures to different degrees, manifested as a marked intensification of LPO during subsequent aerobic incubation. In the case of anoxia outside the tissues, however, appreciable intensification of LPO was observed only in mitochondria. The freer velocity of LPO, its earlier attainment of its maximum in the mitochondria than in other organelles indicate that their sensitivity is highest to anoxia whether within or outside the tissues, and that they are vulnerable to oxidative destruction before the other organelles. The products of chain oxidation of lipids secreted by the mitochondria under these circumstances may probably be the source of initiation of LPO in other organelles also during anoxia within the tissues.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
2. A. I. Dzhafarov, V. M. Kasumov, and V. N. Tarusov, Biofizika, 12, 744 (1967).

3. A. I. Dzhafarov and S. R. Samedov, Dokl. Akad. Nauk Azerb. SSR, 32, No. 11, 70 (1976).
4. L. D. Dudnik, M. V. Bilenko, and A. V. Aleksenko, Byull. Eksp. Biol. Med., 34, No. 5, 556 (1980).
5. E. M. Kulieva, V. V. Perelygin, and A. I. Dzhafarov, Dokl. Akad. Nauk Azerb. SSR, 34, No. 2, 85 (1978).
6. L. F. Panchenko, A. M. Gerasimov, and G. A. Nozdracheva, Vopr. Med. Khim., No. 3, 321 (1974).
7. A. A. Pokrovskii, A. I. Archakov, and T. V. Alenicheva, Tsitologiya, 10, No. 11, 1467 (1968).
8. V. I. Sorokovoi and Yu. A. Vladimirov, in: Biophysics [in Russian], Vol. 5, Moscow (1975), pp. 12-55.
9. W. R. Bidlack and A. L. Tappel, Lipids, 8, 177 (1973).
10. E. G. Bligh and W. L. Dyer, Can. J. Biochem. Physiol., 37, 911 (1959).
11. A. L. Tappel and H. Zalkin, Arch. Biochem., 80, 326 (1950).

INVESTIGATION OF MITOCHONDRIAL PERMEABILITY FOR LABELED CYCLIC AMP

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The effect of cyclic AMP on various mitochondrial processes has recently been discovered [3] and has made the elucidation of the mechanisms of this effect an urgent task. One fact supporting the view that cyclic AMP can penetrate into mitochondria was activation of the matrix enzyme NAD-dependent isocitrate dehydrogenase by this nucleotide in the mitochondrion [4]. Data indicating that on incubation with mitochondria cyclic AMP binds with the mitochondrial transcription complex [7] have also been obtained. However, no adequate quantitative description of this phenomenon has yet been given and the distribution of labeled cyclic AMP within mitochondria has not been studied. The present investigation was devoted to an examination of these matters.

EXPERIMENTAL METHOD

Experiments were carried out on 16 Wistar rats. Mitochondria were isolated from the rat liver by the usual method of centrifugation of the postnuclear supernatant at 6000g in a sucrose medium of the following composition: 250 mM sucrose, 1 mM EDTA, 10 mM Tris-HCl buffer, pH 7.4 (with one rinsing). The suspension of mitochondria (60-80 mg protein/ml) was incubated for 10 min at 30°C with cyclic AMP in a final concentration of $0.4 \cdot 10^{-6}$ M in the presence of 10 mM theophylline. These conditions are optimal for activation of mitochondrial

TABLE 1. Specific Activity of Submitochondrial Fraction (pmoles cyclic AMP/mg protein) after Incubation of Mitochondria and Mitoplasts with Cyclic AMP (M ± m)

Labeled compound	Incubation with mitochondria						Incubation with mitoplasts	
	whole mitochondria	fraction of mitochondria		Digitonin supernatant	mitoplasts of fraction		fraction	
		solubilized	membranes		solubilized	membranes	solubilized	membranes
^{14}C-cyclic AMP	1,0±0,36	2,7±0,65	0,69±0,08	1,4±0,12	0,40±0,11	0,36±0,12	0,91±0,50	0,12±0,04
^{3}H-cyclic AMP·10^{-1}	0,64±0,22	1,6±0,38	0,39±0,07	0,92±0,08	0,25±0,07	0,26±0,08	0,67±0,31	0.16±0,04

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