peroxidation in the different eye tissues may evidently determine differences in the resistance of the sclera, cornea, and ciliary body to pathological factors. This could be one explanation of the different response of cellular organelles to hormonal and other influences [3]. The effect of hormones on peroxidation of unsaturated fatty acids of lipids in cellular and lysosomal membranes of the eye tissues and also its dependence on the dose of the preparation and the type of tissue must be taken into account when endocrine preparations are used in ophthalmology.

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ROLE OF ANTIOXIDANTS IN THE REGULATION OF LIPID PEROXIDATION OF PRESERVED TISSUES DURING PROLONGED STORAGE

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The effect of natural and artificial antioxidant on accumulation of lipid peroxidation (LPO) products in preserved tissues during prolonged storage was studied. At the end of the characteristic period for each method of preservation the intensity of LPO was shown to switch from a low level to a self-accelerating regime. When antioxidants were used this critical transition appeared much later. Of the antioxidants tested, vitamin E and propyl gallate were the most effective.

KEY WORDS: preservation of tissues; lipid peroxidation; antioxidants.

Bioantioxidants are one of the most important factors in the maintenance of lipid oxidation reactions at a definite, stationary level [1]. The presence of antioxidants is an essential condition for the structural integrity of membranes, and their content in a biosystem characterizes its adaptive possibilities – its ability to function normally under the influence of unfavorable factors also. In the living organism changes in the intensity of lipid peroxidation (LPO) in the tissues are compensated by adequate changes in the content of antioxidants [2]. By contrast, in isolated surviving tissues the content of endogenous antioxidants is limited to the reserves actually present, and for that reason activation of LPO leads to rapid utilization of the antioxidants [7].

It was shown previously that preserved tissues remain capable of survival only as long as the free-radical oxidation reaction is maintained in the steady state [4]. Transition of oxidative processes into an unsteady state leads to undesirable consequences for the cell such as increased permeability of biomembranes, inhibition of activity of many enzymes, depression of synthetic processes, disintegration of cell metabolism, and ultimately necrosis [4, 3].

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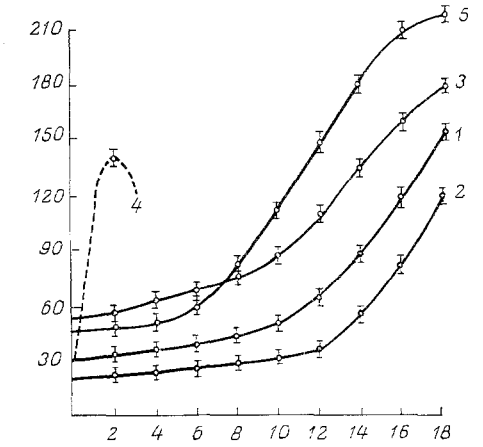

Fig. 1. Kinetics of accumulation of hydroperoxides of preserved tissues during prolonged storage. 1) lyophilized rat liver; 2) frozen rat liver (−30°C); 3) lyophilized human skin; 4) human skin embedded in plastic; 5) human bone embedded in plastic. Here and in Figs. 2 and 3: Abscissa, length of storage (in months); ordinate, accumulation of hydroperoxides (in nmoles/mg lipids).

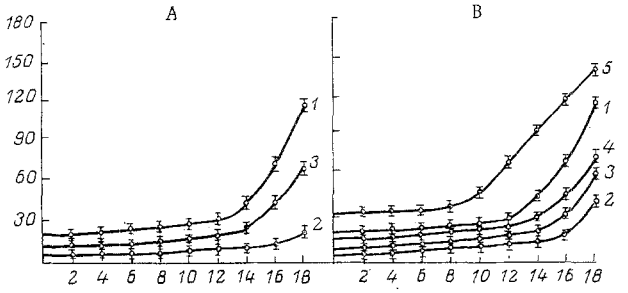

Fig. 2. Kinetics of accumulation of hydroperoxide in liver frozen at −30°C during prolonged storage preceded by antioxidant treatment. A) Preliminary treatment with α-tocopherol: 1) without preliminary treatment, 2) with injection of α-tocopherol into animal, 3) perfusion with α-tocopherol; B) preliminary treatment with propyl gallate (PG) and ionol: 1) without preliminary treatment, 2) injection of PG into animal; 3) perfusion with PG, 4) injection of ionol into animal, 5) perfusion with ionol.

This explains the need for maintenance of the antioxidant level in preserved tissues in order to protect them against oxidative damage during prolonged storage. In order to divide optimal regimes for the use of antioxidants, the kinetic principles governing LPO of preserved tissues, treated with antioxidants before storage, must be studied.

EXPERIMENTAL METHOD

Experiments were carried out on the liver and skin of albino rats and also on tissues (skin, bone) taken from human cadavers. Tissue samples were dissected under sterile conditions and preserved by freezing, lyophilization, and embeddding in plastic [8]. The liver was preserved by freezing and lyophilization, bone simply by embedding in plastic. Skin was preserved by all three methods.

During storage for 1.5 years changes in the intensity of LPO of tissues preserved with and without preliminary treatment with antioxidants were studied. The antioxidants used were α-tocopherol, propyl gallate,

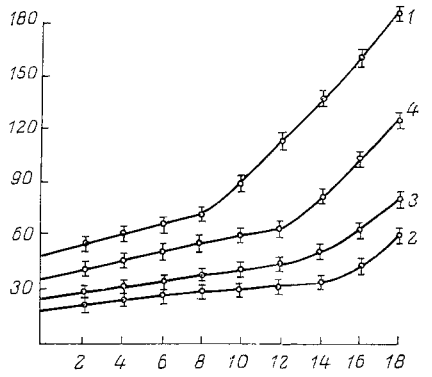

Fig. 3. Kinetics of accumulation of hydroperoxides in lyophilized human skin during prolonged storage after preliminary treatment with antioxidants. 1) Without preliminary treatment with antioxidants; 2) preliminary treatment with α-tocopherol solution; 3) with propyl gallate solution, 4) with ionol solution.

and ionol, which were injected intraperitoneally into the animals or isolated tissues were treated with their solutions. For parenteral use, α-tocopherol was injected in a dose of 200 mg/kg, propyl gallate in a dose of 120 mg/kg, and ionol in a dose of 60 mg/kg. The animals were decapitated 18-20 h after their injection and the liver was examined. With these doses the $K_7 \cdot C$ products of tocopherol and propyl gallate were comparable, but ionol, because of its toxicity, was injected in much smaller doses, equivalent to only 3% of K_7 for tocopherol [5]. Treatment of the isolated tissues with solutions of antioxidant was carried out as follows: The liver was perfused for 40 min with a 0.23 mM solution of tocopherol; 0.45 mM propyl gallate solution and a 1.5 mM solution of ionol in Tween detergent. The skin was kept in these solutions of antioxidant for 4 h and bone tissue for 8 h at 10°C.

During investigation of LPO the preserved tissues were taken out of storage at certain times and incubated at 37°C. Lipids were extracted by Folch's method. The rate of LPO was judged from the accumulation of total hydroperoxides. The content of total hydroperoxides was determined by iodometric titration and by a polorographic method on the ON-104 polarograph.

EXPERIMENTAL RESULTS

The kinetics of LPO during prolonged storage was similar to that of development of chain reactions with degenerate branching – for a long time the accumulation of LPO products proceeds very slowly, then rises sharply (Fig. 1). The length of the induction period depends on the initial velocity of the processes, the method of preservation and storage, the type of tissue and, finally, on whether the tissue was pretreated with antioxidants. Preliminary treatment of the tissues with antioxidants lengthens the induction period and reduces the intensity of accumulation of LPO products.

Curves reflecting the accumulation of LPO products in frozen liver untreated with antioxidants and pretreated with various antioxidants are given in Fig. 2. Clearly the accumulation of LPO products (hydroperoxides) rose sharply after storage for 12 months in liver not treated with antioxidants before freezing. Injection of α-tocopherol considerably delayed the accumulation of hydroperoxides; injection of α-tocopherol into the animal, incidentally, was more effective than perfusion with the solution (Fig. 2A). The same can also be said about propyl gallate – it was more effective by intraperitoneal injection (Fig. 2B). The results obtained by treatment of the liver with ionol differed a little from those described above. First, after treatment with ionol the initial level of the hydroperoxide content was relatively high (Fig. 2B); second, a more rapid increase in the content of LPO product took place in liver taken from animals into which ionol was injected intraperitoneally; this increase was less marked in the perfused liver (Fig. 2B).

The results of the study of the kinetics of accumulation of hydroperoxide during prolonged storage of lyophilized skin showed that with this method of preservation also preliminary treatment with antioxidant solution also lengthened the induction period and lowered the hydroperoxide level. In this case also tocopherol

and propyl gallate were much more effective than ionol (Fig. 3). A similar pattern was observed during the investigation of LPO of bones embedded in plastic, when pretreated with antioxidants.

The high efficacy of vitamin E when injected intraperitoneally can evidently be associated with its more effective embedding into the structure of membranes and the accumulation of other natural antioxidants [1].

The high efficacy of propyl gallate can be explained by the higher value of the $K_7 \cdot C$ product. After its injection into the recipient free radicals from lipids react mainly with it, so that expenditure of total natural antioxidants is limited [5]. By contrast, ionol, because of its low constant K_7, induces increased utilization of endogenous antioxidants [5].

When the efficacy of antioxidants used with individual methods of preservation is compared the following points must be noted: 1) α-tocopherol, when injected intravenously into animals, is most effective with all methods of preservation; 2) when isolated tissues are preserved, propyl gallate as well as α-tocopherol is highly effective, and this is especially so in the case of lyophilization and embedding in plastic.

It can thus be concluded from the results of these experiments that during prolonged storage of preserved tissues their oxidative injury can be prevented to some extent by the use of various antioxidants. Besides natural antioxidants, artificial antioxidants also can be used for this purpose, especially those with high values of their K_7 constant.

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