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PHYLOGENETIC AND ONTOGENETIC ASPECTS OF LIPID PEROXIDATION IN THE VERTEBRATE RETINA

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Investigations of free-radical oxidation of lipids in biological membranes have shown that lipid peroxidation (LPO) products participate in various physiological processes (pinocytosis, phagocytosis, incorporation of iodine into thyroxine [13-15], regulation of membrane permeability, oxidative phosphorylation, and so on [2, 12]). It has also been shown that uncompensated activation of LPO plays an important, and sometimes decisive, role in the pathogenesis of certain diseases [2].

Having regard to the urgency of this problem, the role of LPO in visual function has been the subject of intensive study in recent years. It has been shown, in particular, that the action of visible light increases the rate of LPO in the photoreceptor membranes of the frog and pollock [4, 6, 8]. However, there have been very few studies of phylogenetic and ontogenetic aspects of LPO in the retina.

In the investigation described below LPO activity was studied in the vertebrate retina during phylogeny and ontogeny.

EXPERIMENTAL METHOD

Retinas were obtained from representatives of various classes of vertebrates: fishes (carp), amphibians (frog), reptiles (turtle), birds (pigeon), and mammals (guinea pig, rabbit). In experiments with dark-adapted animals (2 h) all operations were conducted in weak red light, and with light adapted animals, in daylight. The retina was illuminated by means of an incandescent lamp (1200 lx, 30 min).

The ontogenetic studies were conducted on retinas of chick embryos and newborn rabbits. The chick embryos were obtained from poultry factories in light- and heat-proof boxes. At the time of decapitation the embryos were alive.

The intensity of LPO was judged from changes in concentrations of hydroperoxides [3] and malonic dialdehyde (MDA) [2]. Glutathione peroxidase (GP) activity in retinal homogenates was determined by known methods [5, 11] and the protein concentration by the biuret reaction [1].

EXPERIMENTAL RESULTS

Retinas of dark-adapted representatives of different classes of vertebrates differed in their MDA and hydroperoxide concentrations (Fig. 1). It will be clear from Fig. 1 that the intensity of LPO in the dark-adapted retina decreased appreciably in order from fish to mammals (the pigeons were an exception). For example, whereas the MDA concentration in the carp retina was 0.880 ± 0.085 nmole/mg protein, in the rabbit retina it was 0.450 ± 0.040 nmole/mg protein.

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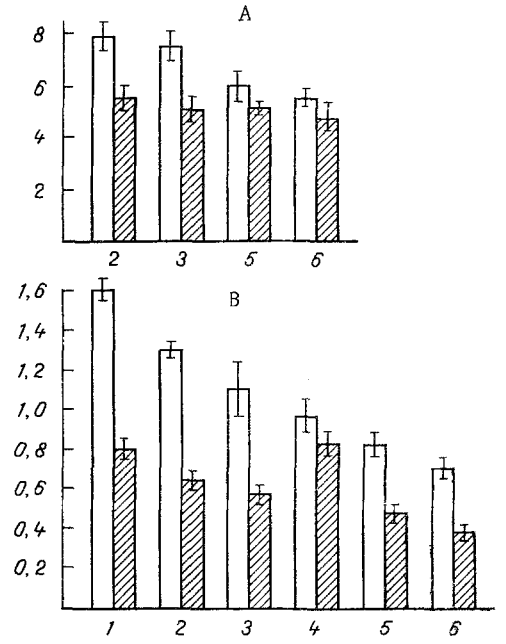

Fig. 1. Concentrations of hydroperoxides (A) and MDA (B) in representatives of different classes of vertebrates. Ordinate: A) concentration of hydroperoxides (in relative units), B) MDA concentration (in nmoles/mg protein). 1) Carp, 2) frog, 3) turtle, 4) pigeon, 5) guinea pig, 6) rabbit. Here and in Figs. 2 and 3: unshaded columns — light-adapted retinas; shaded columns — dark-adapted retinas.

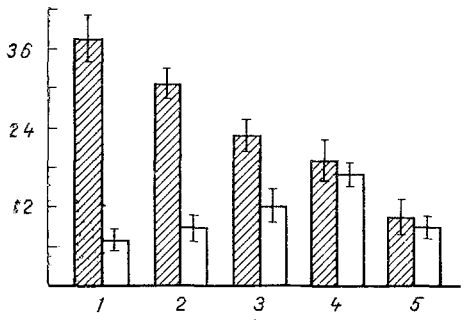

Fig. 2. GP activity (in relative units/g tissue) in the retina of representatives of different classes of vertebrates, depending on the conditions of adaptation. 1) Carp, 2) frog, 3) turtle, 4) pigeon, 5) rabbit.

The action of visible light increased the intensity of LPO in the retina of all the vertebrates studied; maximal MDA accumulation, moreover, was observed in the fish (carp) retina: 1.58 ± 0.12 nmole/mg protein ($n = 8$, $P < 0.001$), minimal in the mammalian (guinea pig and rabbit) retina: 0.87 ± 0.08 nmole/mg protein ($n = 8$, $P < 0.01$), and 0.78 ± 0.04 nmole/mg protein respectively ($n = 8$, $P < 0.001$) (Fig. 1).

This pattern of change in the intensity of LPO in the reintas of representatives of different classes of vertebrates also was preserved when LPO was induced by an iron-ascorbate system: The greatest increase in the intensity of LPO was observed in the retinas of lower vertebrates, compared with birds and mammals.

The results show that the velocity of LPO decreases in the retina in the course of evolution. It is natural to suggest that the phylogenetically dependent change in the intensity of LPO is due to differences in the fatty acid composition, structural organization, and also accessibility of polyenic lipids for catalysts, active forms of oxygen, and radical intermediates of the LPO process, and also to differences in the molecular mobility of membrane components and perfection of the antioxidative defensive system [6, 7, 9, 10].

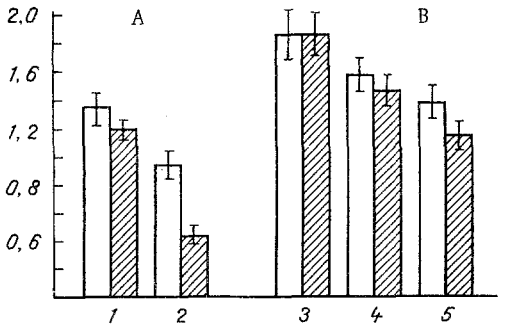

Fig. 3. MDA accumulation (in nmoles/mg lipid) in retinas of young rabbits (A) and chick embryos (B). 1) Blind young rabbits, 2) young rabbits with sight, 3-5) chick embryos at 10th, 15th, and 20th days of incubation respectively.

To study the role of the antioxidative defensive systems, GP activity was investigated in light- or dark-adapted retinas of representatives of the different classes of vertebrates. In the course of evolution GP activity was found to change in the retina of the dark-adapted animals: it decreased appreciably in the series from fishes to mammals (Fig. 2). For example, whereas in the dark-adapted carp retina GP activity was 37 ± 2.9 relative units/g tissue at 24°C in the course of 1 min, in the rabbit retinas it was 11 ± 1.48 relative units/g tissue. It will be clear from these data that the difference between GP activity in dark- or light-adapted retinas decreases in the course of evolution.

It must be pointed out that a change in accumulation of LPO products was observed not only during phylogeny, but also during ontogeny (Fig. 3). As Fig. 3 shows, the initial MDA level in the retinas of blind young rabbits was much higher than in young rabbits which had acquired sight, but the increase in the MDA concentration after illumination was greater in the latter. A similar picture also was observed during ontogeny of the chick embryo. For instance, against the background of a high MDA concentration in 10-day embryos, no significant increase in the concentration of this LPO product was observed in general in response to illumination. Meanwhile light adaptation of isolated retinas of newly hatched chicks led to an appreciable increase in the MDA concentration. Retinas from 15-day embryos occupied an intermediate position as regards both initial level and increase in MDA concentration in response to illumination.

To study the ontogenetic course of formation of defense against the harmful action of LPO, GP activity was determined in the retinas of chick embryos after the 10th, 15th, and 20th days of incubation. The results showed that the enzyme was formed very slowly during the period of incubation. After 10 and 15 days of incubation it was therefore impossible to determine GP activity. Not until after the 20th day of incubation was a very low level of GP activity detectable in the embryonic retina (0.12 relative unit/g tissue at 24°C for 1 min).

The results thus show that both the intensity of LPO and GP activity in retinas of different representatives of vertebrates diminish significantly in the course of evolution. It was also discovered that the higher the class of vertebrates, the smaller the difference in the intensity of LPO and GP activity of the light-adapted or dark-adapted retinas, and this result is evidently associated with perfection of defensive systems against the harmful action of LPO.

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EFFECT OF A PULSED MAGNETIC FIELD ON PERMEABILITY OF THE CORNEA AND SORPTION PROPERTIES OF THE TISSUE STRUCTURES AND REFRACTIVE MEDIA OF THE EYE

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KEY WORDS: pulsed magnetic field; permeability; sorption.

One of the special features distinguishing the action of magnetic fields (MF) on biological objects is their ability to change the permeability of biological barriers and cell membranes [1, 3, 6]. The study of the effect of MF on permeability of the tissue structures of the organ of vision is of great interest.

Motivation for the present investigation was provided by the results of electron-microscopic and radiologic investigations, which yielded indirect evidence in support of increased permeability of the cornea under the influence of a pulsed MF [4, 5]. The writer has attempted to obtain direct proof of increased permeability of the cornea and enhanced sorption properties of the tissue structures and refractive media of the eye under the influence of a pulsed MF, which may be of great importance to clincial ophthalamology.

EXPERIMENTAL METHOD

Experiments were carried out on four groups of rabbits (57 animals, 114 eyes), two of which were experimental and two control. In the course of the investigation the method of radioactive indication of two substances (^{35}S-streptomycin and ^{75}Se-methionine), widely used in ophthalmologic practice, was used. The working solution of the preparations (2 ml), with radioactivity of 300,00 cpm in 0.1 ml, was poured into a lid-retracting bath which, after preliminary local anesthesia, was introduced beneath the animal's eyelids. The system thus formed (the eye with the lid-retracting bath and the radioactive substance poured into it) in the experimental series was exposed for 10 min to the action of a pulsed MF. In the control only the radioactive substances were applied, with the same exposure as in the corresponding experiment. A pulsed MF (pulse duration 0.02 sec) with maximal magnetic flux density in the pulse of 8.5 mT was used, as having the greatest effect on permeability of the cornea in experiments *in vitro* [4].

Changes in permeability of the cornea were assessed on the basis of changes in radioactivity of the aqueous humor. To determine the time of maximal saturation of the aqueous with radioactive substance, samples of aqueous humor were collected immediately after exposure to MF, and 5, 15, and 50 min later.

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