# AGGRAVATING ACTION OF OXYGEN ON LIGHT-INDUCED DAMAGE TO THE ALBINO RAT RETINA

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Investigation of factors determining the degree of injury to the structures of the eye under the influence of increased doses of light is an urgent task in ophthalmology. Light-induced damage of this kind is based on photosensitized free-radical oxygenation [4, 5, 6, 8]. Danger of development of these processes in the retina is determined by several factors: the content of pigmented compounds, which are effective photosensitizers of oxygenation, in the visual cells, the presence of readily oxidized substrates, such as polyunsaturated fatty acids, and the protein rhodopsin, containing thiol groups, and an increased concentration of oxygen, the consumption of which by the visual cells is increased in light.

Retinal, bound with proteins or lipids, behaves as a photosensitizer of oxidation processes in the photoreceptor membrane of the visual cell [5, 7]. Since retinal has a wide absorption band with a maximum in the 380 nm region, as might be expected, the most dangerous region of the radiation spectrum for the retina is the near ultraviolet and the blue-violet [7]. In experiments in vitro on suspensions of photoreceptor membranes, free-radical oxidation of proteins (the SH-groups of rhodopsin) and, in particular, of lipids has been shown to depend significantly on the oxygen concentration [5, 6, 7].

In the investigation described below the effect of increased oxygen concentrations in the inspired mixture was studied on the retina of albino rats, which are exceptionally sensitive to the action of light. In view of the previously determined spectrum of the light damaging action of the molecular components of the photoreceptor membrane [5, 7], rats were irradiated in the 360-380 nm region.

## EXPERIMENTAL METHOD

Experiments were carried out on 43 noninbred albino rats weighing 150-200 g, under pentobarbital anesthesia. The degree of light-induced damage was estimated from the $a$ wave on the electroretinogram (ERG), which reflects activity mainly of the visual cells of the retina. Potentials were recorded from the margin of the retina by means of a wick electrode; the reference electrode, a needle, was inserted subcutaneously into the parietal region. Potentials were recorded on an RM-46 polygraph (Nihon Kohden, Japan), with transmission band of 0.3 to 30 Hz, so that the ERG could be recorded without distortion and to a large extent free from interference.

The saturating flash of white light from an IFK flash tube was used as the photic stimulus. The source of damaging light was a mercury-quartz lamp (PRK-4) with UFS-1 filter, and allowing for the spectral characteristics of the lamp, this emitted light in the 360-380 nm band. The power of the light, estimated actinometrically [2], was 1 mW/cm$^2$.

Another criterion of light-induced damage was the rhodopsin content in the retina of the experimental animal. To determine this parameter the rats were adapted beforehand for 1 h to darkness. All subsequent operations were carried out in dim light. The retinas, taken from the eyes, were washed in 0.9% NaCl solution and then the rhodopsin was extracted with a 2% solution of digitonin for 2 h. The supernatant after centrifugation at 30,000g for 20 min contained rhodopsin. Its concentration was determined from the differential

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TABLE 1. Effect of Oxygen on Light-Induced Damage to the Retina

| Experimental conditions | Dose of irradiation, J/cm² | Amplitude of *a* wave on ERG, % of normal | Rhodopsin content, % of normal |
|---|---|---|---|
| Atmospheric air | 1,8 | 73,0±7,2 | 83,7±6,0 |
| Oxygen, atm | | | |
| 1 | 1,8 | 56,3±4,1 | 42,8±12,1 |
| 2,5 | 1,8 | 42,0±8,0 | 24,3±8,2 |
| 1 | 2,4 | 35,2±1,3 | — |
| 1 (+Phenosan) | 2,4 | 60,1±3,0 | — |

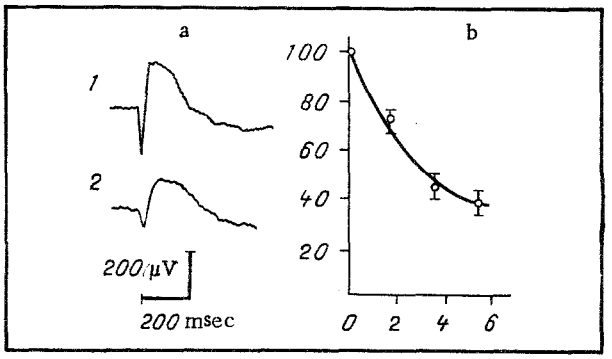


Fig. 1. Effect of light-induced damage on the rat ERG. a) ERG of control rat (1) and after irradiation with a dose of 6 J/cm² in the 360-380 nm region (2); b) change (in %) in amplitude of the *a* wave on the ERG (ordinate) depending on dose of irradiation (abscissa).

absorption spectrum of rhodopsin before and after its decolorization ($\Delta D_{500}$).

Light-induced damage was inflicted on the animals while breathing atmospheric air and also oxygen under a pressure of 1 or 2.5 atm. Animals of the control group were kept under the same conditions, but in darkness.

The rhodopsin content in the retina and the amplitude of the *a* wave on the ERG were determined 24 h after exposure to light.

## EXPERIMENTAL RESULTS

The electrical activity of the retina declined with an increase in the dose of irradiation, and ultimately disappeared (Fig. 1). Dependence of the fall of amplitude of the *a* wave on the ERG on the dose of it to which the animal was exposed was obtained both with a constant intensity of damaging light and an increase in the duration of illumination, and also with a constant period of exposure of the animal to light (2 h) and a change in the intensity of irradiation. A fall of amplitude of the *a* wave on the ERG by 50% 24 h after exposure to light took place with a dose of irradiation of about 3.5 J/cm², measured on the cornea. A fall in amplitude of the *a* wave on the ERG by almost 30% was observed when the dose of irradiation was about 1.5-1.8 J/cm². Under these circumstances an almost 20% decrease in the rhodopsin content was observed (Table 1). A dose of 1.5-1.8 J/cm² corresponds to that which the eye can receive if, for about 1 h, it continuously examines the blue sky on a bright sunny day. In the present case the photic dose was determined for the 360-380 nm spectral region, which corresponds to the maximum in the spectrum of action of photooxidation of the molecular components of the photoreceptor membrane [7]. A similar spectrum of action of light-induced damage to the retina also was obtained in experiments on monkeys: blue light proved to be much more effective than green, and more effective still than red [9]. Naturally during exposure to white light, the threshold dose, estimated in experiments on rats and rabbits relative to the ERG, was significantly higher [3].

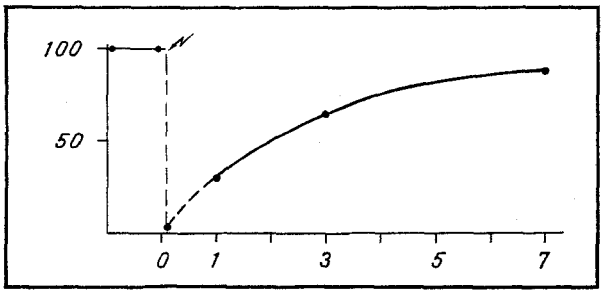


Fig. 2. Kinetics of recovery of *a* wave on ERG of rat after irradiation in a dose of 6 J/cm$^2$. Abscissa, time after irradiation (in days); ordinate, amplitude of *a* wave (in % of normal). Arrow indicates beginning of irradiation.

In experiments on intact animals it was interesting to follow the course of recovery of electrical activity of the retina, when damaged by light.

Light damage was induced in these experiments by irradiating the rats for 2 h in the 360-380 nm band with an intensity of 1 mW/cm$^2$. It will be clear from Fig. 2 that restoration of the amplitude of the *a* wave on the ERG took place only after 7-10 days. About the same length of time is needed, as we know, for complete renewal of the outer segment of the visual cell of the rat retina [10]. It can therefore be tentatively suggested that the membrane structures of the outer segment, when irreversibly damaged by light, are replaced by newly formed structures after this time.

In the next series of experiments the effect of hyperoxia on light-induced damage to the retina was investigated. Besides photosensitizers and oxidation substrates, oxygen itself is a very important factor in the photooxidative process. It must therefore be expected to have an aggravating effect on light-induced damage. The results of electrophysiological and biochemical experiments completely confirmed this hypothesis (Table 1).

Keeping the rats in darkness under conditions of hyperoxia (1 and 2.5 atm) did not affect the electrical activity of the retina or the rhodopsin content of its visual cells. However, in rats irradiated with the same doses (1.8 J/cm$^2$), but with a rising oxygen concentration in the inspired air, there was a marked decrease in amplitude of the *a* wave on the ERG and a decrease in the rhodopsin content. During inhalation of oxygen under a pressure of 2.5 atm, 24 h after exposure to light, only about one-quarter of the initial rhodopsin content still remained in the retina. This is in full agreement with results obtained in our own experiments in vitro. In these experiments photooxidation of rhodopsin in the visual cell led to a disturbance of its most important functional property, namely its ability to regenerate, i.e., to restore the original absorption spectrum ($D_{max}$ = 500 nm) [4].

A combination of exposure to bright light with an increased oxygen concentration in the inspired air thus presents a definite risk for vision: the conditions are created for initiation and development of pathological processes of photosensitized free-radical oxidation in the structures of the eye. As a result of these processes, reversible, or in some particularly severe cases, irreversible damage may take place to the cells of the retina and the pigmented epithelium of the eye. An important condition for the prevention of light-induced damage of this type is the drawing up and observance of health standards for the lighting and the oxygen concentration in the air for persons exposed to the simultaneous action of light and hyperoxia. Reduction or even the complete elimination of ultraviolet and blue-violet radiation in illumination sources must be strictly provided for.

Drugs belonging to the antioxidant class may have a promising role to play in the prevention of light-induced damage to structures of the eye under conditions of hyperoxia. This is shown by the experimental results given in Table 1. Rats were given an intraperitoneal injection of the antioxidant phenosan (the potassium salt of 4-hydroxy-3,5-di-tert-butylphenylpropionic acid), synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR, 24 h before irradiation. In animals of the control group, irradiated with a dose of 2.4 J/cm$^2$, a threefold decrease in amplitude of the *a* wave on the ERG was observed. Meanwhile in animals protected by the antioxidant, electrical activity of the retina,

although depressed, nevertheless remained twice as high as in the control. Consequently, light-induced damage to the retina, developing as a result of a mechanism of free-radical oxidation, can be prevented, although only partially, by means of antioxidants.

Oxygen thus has a distinct aggravating action on the development of light-induced damage to the structures of the eye, and this must be taken into account both in clinical practice and when health standards are drawn up for persons whose occupation is associated with long periods of work under conditions of increased brightness, especially when combined with hyperoxia.

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