some extent the result of the raised level of thyroid hormones which activate the phospholipase molecules *in vivo* in a similar way to what is observed in isolated mitochondria on incubation with thyroxine.

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ACCUMULATION OF LIPID PEROXIDATION PRODUCTS AND DEPRESSION OF RETINAL ELECTRICAL ACTIVITY IN VITAMIN E-DEFICIENT RATS EXPOSED TO HIGH-INTENSITY LIGHT

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Stabilization of membrane structures of photoreceptor cells against induction of lipid peroxidation (LPO) is achieved mainly because of their high content of α-tocopherol (vitamin E) [1], an effective physical and chemical quencher of singlet oxygen [2], an acceptor of superoxide anion-radicals [2], and an antiradical agent [3]. A deficiency of the natural antioxidant, vitamin E, is one cause of degeneration of the photoreceptor layer of the retina [4, 5], accompanied by accumulation of LPO products *in vivo* [5, 6]. Meanwhile induction of LPO in the isolated retina leads to depression of its electrical activity [7]. A comparison of these facts suggests that the retina of vitamin E-deficient animals should be more sensitive to the harmful action of high-intensity light than the retina of animals kept on a diet with the standard concentration of vitamin E. Further evidence in support of the validity of this hypothesis would appear to be given by the fact that photoinduced accumulation of LPO products, preventable by antioxidants, has been demonstrated both *in vitro* [8] and *in vivo* [9].

Accordingly, in the investigation described below, the content of LPO products and the electroretinogram (ERG) were studied in vitamin E-deficient rats exposed to high-intensity light.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male Wistar rats. Alimentary avitaminosis E was induced by keeping the rats on a synthetic diet deficient in α-tocopherol (the composition of the diet is given in Table 1 [10]). The concentration of α-tocopherol fell from 0.74 ± 0.08

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TABLE 1. Composition of the Diet

Component	Quantity of component, g/kg mixture
Starch	600
Casein	220
Linetol	100
Mixed salt	40
Cellulose	30
Choline	2
Water-soluble vitamins	1
Retinyl palmitate	0,001
α-Tocopherol acetate *	0,5

*Added only to diet for control animals.

mg% in the control to 0.06 ± 0.01 mg% on the 60th day of development of avitaminosis E [11]. The ERG of the rats was recorded by means of a wick electrode (active electrode) and a steel needle electrode inserted beneath the skin of the scalp (reference electrode) on an ÉÉGP-4-02 encephalograph. Photic test stimulation was applied by means of the FS-1 photostimulator (flash energy 0.3 J). The pupil was dilated with 1% homatropine and the eye anesthetized with 1% amethocaine. Light-induced injury in the rat retina was induced by exposure for 210 min to daylight lamps (10,000 lx) in a specially constructed chamber. Lipids were extracted from the retina [12] with a mixture of chloroform and methanol (2:1), with the addition of the antioxidant 4-methyl-2,6-di-tert-butylphenol (1 mg/100 ml of mixture). The concentration of LPO products was determined spectrophotometrically (with the Shimadzu MPS-50L spectrophotometer) in relation to the characteristic absorption maximum for diene conjugation products (at 232 nm) in the ultraviolet spectrum of solutions of lipids in methanol—heptane 5:1 [13].

The quantity of accumulated hydroperoxides of polyene lipids was calculated on the basis of differences between the optical densities (E) of solutions of the lipids at 232 nm ($\varepsilon = 2.1 \cdot 10^4$ $cm^{-1}mole^{-1}$).

EXPERIMENTAL RESULTS

Typical traces of the ERG of rats receiving a balanced and vitamin E-deficient diet, before exposure to high-intensity light and 1, 7, and 14 days after exposure are illustrated in Fig. 1. The results of calculation of the amplitudes of the a- and b-waves of the ERG of the two groups of animals are given in Table 2. The following conclusions can be drawn from an examination of these data. The amplitude of the ERG waves of the animals with avitaminosis E was lower than that of the controls. After exposure to high-intensity light a sharp decrease was observed in the amplitudes of the a- and b-waves of the ERG in vitamin E-deficient and control rats 24 h after exposure and this was followed by slow recovery. Depression of the ERG waves in vitamin E-deficient animals as a result of exposure to high-intensity light took place more intensively and recovery was slower than in the group of rats receiving a balanced diet, but 14 days after exposure to light the amplitudes of the ERG waves were practically the same in the control and experimental groups of animals.

Analysis of the content of LPO products in the retina of the rats showed that as a result of the development of avitaminosis E in the rats hydroperoxides of polyene lipids accumulated (15.7 ± 2.4 nmoles/mg lipids). Exposure to high-intensity light also caused a marked increase in the concentration of hydroperoxides in the polyene lipids 24 h after exposure to light in both control and vitamin E-deficient animals. It must be emphasized that light-induced accumulation of hydroperoxides in polyene lipids in the retina of vitamin E-deficient rats (66.8 ± 8.1 nmoles/mg lipids) was more than three times greater than the quantity of hydroperoxides formed as a result of exposure of the control rats to light (21.9 ± 5.8 nmoles/mg lipids). By the time of recovery of retinal function (14 days after exposure to light) the content of LPO products had fallen to the level recorded in both groups of animals before exposure to light.

The high level of correlation between the decrease in amplitude of the ERG waves recorded *in vivo* in the control and vitamin E-deficient animals after exposure to high-intensity light and the light-induced accumulation of endogenous LPO products will be noted. A similar dependence of retinal electrical activity on the quantity of lipid peroxides induced by the Fe^{2+} + ascorbate system was found previously *in vitro* [7].

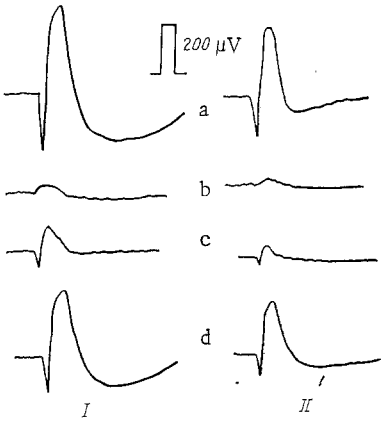

Fig. 1

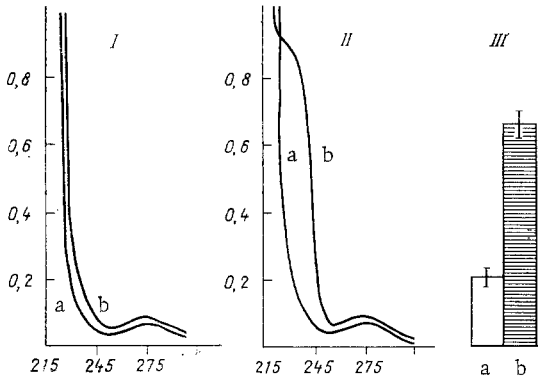

Fig. 2

Fig. 1. ERG of rats before and after exposure to high-intensity light. I) ERG of control animals, II) ERG of animals with avitaminosis E; a) before, b-d) 1, 7, and 14 days respectively after exposure to high-intensity light.

Fig. 2. Absorption spectra of retinal lipids of rats before and after exposure to light. Abscissa, wavelength (in nm); ordinate, optical density (in relative units). I) Lipids isolated from retina of control animals (a) and 24 h after exposure to light (b); II) lipids isolated from retina of vitamin E-deficient animals before (a) and 24 h after exposure to light (b); III) light-induced accumulation of hydroperoxides in retinal lipids of control (a) and vitamin E-deficient rats (b).

TABLE 2. Amplitude of a- and b-Waves of ERG (in μV) of Animals before and after Exposure of High-Intensity Light (M ± m)

Group of animals	ERG wave	Before exposure	After exposure		
			1 day	7 days	14 days
Control	a	267,5±17,0	4,6±0,4	51,8±5,1	168,8±19,1
	b	625,0±47,5	106,3±8,3	224,9±25,0	580,0±37,5
Vitamin E deficient	a	168,3±11,6	Менее 1	10,6±1,2	135,6±10,0
	b	505,0±23,3	31,9±5,1	166,6±15,8	458,3±33,3

Dratz et al. [6] found neither depression of the ERG nor accumulation of LPO products in the retina of vitamin E-deficient rats after exposure to light for 12 h. The reason for the disagreement between these results and those of the present investigation must probably be sought in the different conditions of exposure to light (intensity of photic flux, spectral composition of the emitted light, duration of exposure).

It can thus be concluded from the results of the present investigation that in the presence of a deficiency of the natural antioxidant α-tocopherol, injury to the retina due to the action of high-intensity light (depression of retinal electrical activity, accumulation of LPO products) is much more severe than in animals receiving a diet with a normal vitamin E content. This means that an essential role is played by natural antioxidant systems in stabilizing photoreceptor membranes against light-induced injury accompanied by activation of peroxidation processes in the membranous structures of the visual cells. Without going into a detailed examination of the possible mechanism of photoinduced formation of peroxides in this paper, it should nevertheless be stated that the most likely process responsible is the recently discovered generation of singlet oxygen by retinal, the chromophore of visual pigment [14, 15]; both protein (rhodopsin) and lipid components of photoreceptor membranes may behave as chemical quenchers of singlet oxygen in photoreceptors [14].

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CHANGES IN THYMIDINE-^{3}H CONTENT IN DNA OF LIVER AND SKIN CELLS AFTER ADMINISTRATION OF SYNGENEIC TISSUE EXTRACTS

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The literature on reparative regeneration of organs in fully grown mammals is extensive. One of the most probable mechanisms by which the body recognizes the location of injury and stimulation of cell division in the residual part of an organ is considered to be immunologic reactions [1]. The most demonstrative data in support of this view have been obtained by the use of regenerating rodent liver as the model. Blood, plasma, serum, and spleen cells of animals after removal of about two-thirds of the volume of the liver have been shown to acquire for a certain length of time the ability to stimulate mitotic activity of liver cells in syngeneic recipients [1, 4]. If this effect is assumed to be connected with the development of an immunologic reaction, induced by factors arriving from the injured parenchyma of the liver, their presence in normal liver likewise cannot be ruled out.

The object of the present investigation was to study the effect of parenteral injection of liver and skin extracts on proliferative activity of liver and skin cells in syngeneic mice on the basis of incorporation of thymidine-^{3}H.

EXPERIMENTAL METHOD

Six male CC57 white mice weighing 16 g were given an intravenous injection of 0.2 ml of liver extract from syngeneic donors containing 180 µg protein. The intravenous injection of extract was repeated 40 days later in a dose of 90 µg (as protein) and an intraperitoneal injection of thymidine-^{3}H (5-methyl derivative, specific activity 0.5 mCi/mole) in a dose of 1 µCi/g body weight was given at the same time.

The liver extract was prepared from a native tissue homogenate in distilled water by freezing to —12°C and thawing at 37°C five times, followed by centrifugation at 12,500g.

An extract of skin or physiological saline and thymidine-^{3}H were injected by a similar scheme into two other groups of mice, differing in number.

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