PEROXIDATION OF LIPIDS AND DEGENERATION OF PHOTORECEPTORS IN THE RETINA OF RATS WITH AVITAMINOSIS E

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The formation of peroxidation products of lipids (hydroperoxides, dialkyl peroxides, "intermolecular cross linkages") is sharply intensified in the retina of rats with alimentary avitaminosis E *in vivo* and degeneration of the photoreceptors (mainly the layer of outer segments of the rods) develops. The experimental results are discussed on the basis of views regarding the antioxidant mechanism of action of α-tocopherol *in vivo*.

KEY WORDS: *rat retina; avitaminosis E; peroxidation of lipids; degeneration of photoreception layer of the retina.*

The effectiveness of the reactions of the visual cycle depends on the "fluidity" of the photoreceptor rhodopsin-containing membranes, which is achieved by the incorporation of highly unsaturated aliphatic acyl groups into phospholipids (in the vertebrate retina, chiefly docosahexaenoate $C_{22:6}$ [8]). The abundance of polyene phospholipids easily oxidized by molecular oxygen [7] creates the threat of degradation of the membrane through the development of a process of free-radical oxidation of lipids. Experiments *in vitro* have in fact shown that peroxidation of lipids in the outer segments of the rods of the frog retina can be induced by light [18] or by Fe^{2+} ions (+ ascorbate) [3]. The structural and functional integrity of the outer segments can thus be maintained only in the presence of a reliable system of protection against the harmful action of O_2, and in the photoreceptors this is provided by the very high content of the natural antioxidant α-tocopherol [11], high activity of superoxide dismutase [13], and the inaccessibility of the hydrophobic regions of the disk membranes of the outer segments for active forms of oxygen on account of the high orderliness of the molecular movements of the phospholipids [5]. A previous investigation showed that a deficiency of α-tocopherol (vitamin E) is a cause of degeneration of the photoreceptor layer of the monkey retina [15].

In this investigation, besides the study of the morphological picture of the retina in rats during the development of alimentary avitaminosis E, a quantitative analysis was made of the content of endogenous primary (hydroperoxides), secondary (dialkyl peroxides), and final (fluorescent Schiff bases) molecular products of peroxidation of lipids *in vivo*.

EXPERIMENTAL METHOD

Noninbread male albino rats (weighing 70-80 g at the beginning of the experiments) were used and were kept on a diet deficient in vitamin E with the addition of retinyl palmitate (Table 1) [17]. The content of α-tocopherol in the blood serum, determined by the method in [24], was lowered from 0.76 ± 0.06 mg % in the control to 0.06 ± 0.01 mg % after induction of avitaminosis E for 2 months. During the same period the rate of spontaneous hemolysis of the erythrocytes [16] of the experimental animals increased by 70%.

Lipids were extracted from the retina by a mixture of chloroform and methanol (2:1) [12] with the addition of ionol (1 mg to 100 ml). The content of peroxidation products

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TABLE 1. Composition of the Diet

Component	Quantity of components, g/kg of mixture
Starch	600
Casein	220
Linetol	100
Salt mixture [17]	40
Cellulose	30
Choline	2
Water-soluble vitamins	1
Retinyl palmitate	0,001
α-Tocopherol aectate*	0,5

*Added only to the diet of the control animals.

TABLE 2. Accumulation of Peroxidation Products in Lipids from Retina of Rats Kept on Diet Deficient in Vitamin E

Peroxidation products of lipids	Group of animals	Stage of development of avitaminosis E, days			
		32nd	48th	61st	83rd
Hydroperoxides of lipids*	Control			0,99±0,08	
	Experimental	1,33	1,54	1,16	0,96
Peroxides of lipids†	Control			1,7	
Fluorescent products‡	Experimental	12,0	15,0	17,3	4,1
		191	—	200	128

*From spectrophotometric data in optical density units/mg lipids/ml solution.
†From polarographic data in 10^{-8} mole peroxides/mg lipids ($E_{1/2}$ = −1.3 V).
‡Intensity of fluorescence (in %, control 100%).

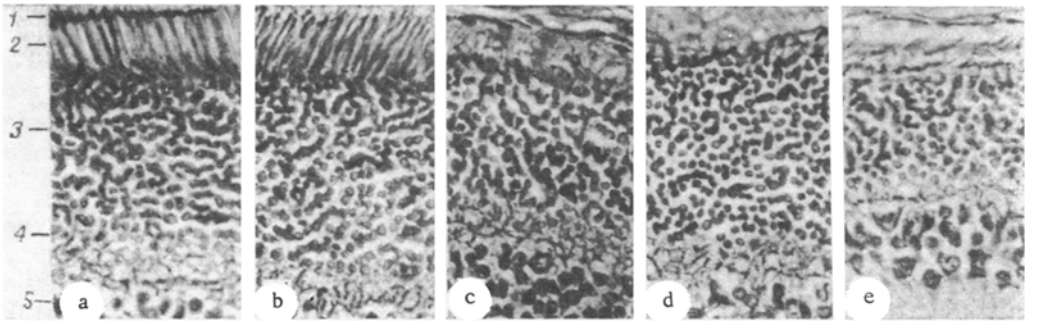

Fig. 1. Morphological picture of the retina of rats with avitaminosis E: a) control; b) 32 days, c) 48 days, d) 61 days, and e) 83 days of feeding on diet deficient in vitamin E. 1) Pigmented epithelium; 2) layer of photoreceptor cells; 3) outer nuclear layer; 4) outer plexiform layer; 5) inner nuclear layer. 400×.

of lipids was determined spectrophotometrically (hydroperoxides of polyene lipids; λ_{max} = 232 nm) [10], polarographically (hydroperoxides and dialkyl peroxides of lipids; $E_{1/2}$ = +0,9 to −1.3 V) [2], and also from their characteristic fluorescence ("intermolecular cross linkages;" λ_{excit} = 360 nm) [9].

The rat's eyes were fixed with a mixture of formalin, ethanol, and acetic acid (3:1: 0.3). The lens was removed immediately after fixation. Paraffin sections were stained with Carrazzi's hematoxylin.

EXPERIMENTAL RESULTS

It will be clear from the results in Table 2 that keeping the rats on a diet deficient in vitamin E led to substantial accumulation *in vivo* of various peroxidation products of lipids in the retina (hydroperoxides of polyene lipids, dialkyl peroxides of lipids, fluorescent products), which was extremal in character. Incidentaly, the level of peroxidation

products of lipids remained virtually unchanged throughout the experiment in the retina of rats kept on the same diet, but with the addition of vitamin E. Comparison of these results with the morphological picture of the retina (Fig. 1) shows that the accumulation of products of free radical oxidation of lipids is accompanied by the appearance of distinct signs of degeneration of the photoreceptor layer in the retina, with damage chiefly to the outer segments of the rods, whereas the outer and inner nuclear layers remained virtually intact. The degeneration continued to progress during the experiment and led eventually to a decrease in the thickness of the layer of outer segments, and in the later stages, to disappearance of the outer segments of the photoreceptors. Whereas in the retina of the control rats the outer segments accounted for about 70% of the total thickness of the photoreceptor layer, on the 83rd day of the experiment this proportion in the experimental animals did not exceed 20%. This may have been the result of elimination of the peroxidation products of lipids from the retina at these times, especially if it is remembered that hydroperoxydiacylglycerophosphatides can serve as substrates for endogenous phospholipases [6] and peroxidases [23].

A deficiency of the natural antioxidant vitamin E thus causes the accumulation of peroxidation products of lipids *in vivo* and degeneration of the photoreceptor layer of the retina. What is the possible connection between the accumulation of autooxidation products of lipids and degeneration of the retina in alimentary avitaminosis E? Previous experiments *in vitro* showed that the appearance of polar peroxidation products of lipids in the membranes of the outer segments leads to weakening of lipid–lipid and protein–lipid interactions in the photoreceptor membrane, with the consequent increase in the extractability of rhodopsin [4] and fragmentation of the membranes of the outer segments [1]. This disturbance of the structural organization of the photoreceptor membranes as a result of the development of peroxidation of lipids can be presumed to make their lipid and protein components accessible for phospholipases and proteolytic enzymes (for example, cathepsin D present in the pigmented epithelium [14]).

The loss of one component of the system protecting the membranes of the photoreceptors from activated forms of O_2, namely α-tocopherol, the natural inhibitor of free-radical reactions, is thus a cause of degeneration of the photoreceptor layer of the retina and the accumulation of peroxidation products of lipids *in vivo*, confirming the antioxidant mechanism of the action of vitamin E in the cell. In the writers' view, it is very important to solve the problem of the extent to which the disturbance of the molecular organization of photoreceptor membranes and lowering of superoxide dismutase activity may be the causes of the oxygen-dependent injury to the retina associated with chronic exposure to light [19, 22] or γ rays [21] and in hereditary degeneration [20].

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LITERATURE CITED

1. L. V. Belousova, L. B. Bratkovskaya, et al., in: Structure, Biosynthesis, and Conversion of Lipids *in vivo* [in Russian], Leningrad (1975), p. 28.
2. V. S. Danilov, V. E. Kagan, et al., Izv. Akad. Nauk SSSR, Ser. Biol., No. 4, 584 (1972).
3. V. E. Kagan, A. A. Shvedova, et al., Biofizika, No. 6, 1043 (1975).
4. K. N. Novikov, V. E. Kagan, et al., Biofizika, No. 6, 1039 (1975).
5. L. A. Sibel'dina, V. E. Kagan, et al., Dokl. Akad. Nauk SSSR, 224, 228 (1975).
6. A. A. Shvedova, "Modification of polyene lipids of the photoreceptors by molecular oxygen and its role in retinal function," Candidate's Dissertation, Moscow (1975).
7. N. M. Émanuel', E. G. Denisov, and Z. K. Maizus, Chain Reactions of Carbohydrate Oxidation in the Liquid Phase [in Russian], Moscow (1965).
8. R. E. Anderson et al., Exp. Eye Res., 18, 205 (1974).
9. W. R. Bildlack and A. L. Tappel, Lipids, 8, 203 (1973).
10. J. L. Bolland and H. P. Koch, J. Chem. Soc., 7-12, 445 (1945).
11. R. A. Dilley and D. G. McConnell, J. Membr. Biol., 3, 317 (1970).
12. J. Folch, M. Lees, et al., J. Biol. Chem., 191, 883 (1951).
13. M. O. Hall and D. O. Hall, Biochem. Biophys. Res. Commun., 67, 1199 (1975).
14. S. Hayasaka, S. Hara, and S. Mizuno, J. Biochem. (Tokyo), 78, 1365 (1975).

15. K. C. Hayes, Invest. Ophthal., 13, 499 (1974).
16. F. G. Jager, Bibl. Nutr. Dieta (Basel), 10, 215 (1968).
17. J. Jones and C. Foster, J. Nutr., 24, 245 (1942).
18. V. E. Kagan et al., Biochim. Biophys. Acta, 330, 76 (1973).
19. T. Kuwabara and R. A. Gorn, Arch. Ophthal., 77, 115 (1967).
20. R. J. Mullen and M. M. Zavail, Nature, 258, 528 (1975).
21. W. K. Noell, in: Biochemistry of the Retina, Academic Press, New York (1965), pp. 51-72.
22. W. K. Noell, V. S. Walker, et al., Invest. Ophthal., 5, 450 (1966).
23. P. J. O'Brien and R. C. Green, Biochem. J., 103, 32 (1967).
24. M. T. Quaife, N. S. Scrimshaw, and O. H. Lowry, J. Biol. Chem., 180, 1229 (1949).

RESPONSE TO A COMBINATION OF SEVERE HEAD INJURY AND ACUTE MASSIVE BLOOD LOSS

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Acute experiments on dogs showed that the character of the response to a combination of craniocerebral trauma and acute blood loss depends on the order of infliction of these extremal conditions. Craniocerebral trauma inflicted in the early period of hemorrhagic shock does not significantly affect the dynamics and outcome of the underlying pathological process. Hemorrhagic shock arising after craniocerebral trauma does so as a result of a much smaller blood loss and it follows a more severe course.

KEY WORDS: *craniocerebral trauma; acute blood loss; hemorrhagic shock.*

The writers showed previously that shock does not develop in severe craniocerebral trauma [1]. Only if craniocerebral trauma is combined with mechanical trauma to the femur did shock develop, and it was marked by certain special features [2].

In this investigation the character of development of shock arising in response to a combination of craniocerebral trauma and acute massive blood loss was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 54 adult dogs. The animals were bled as a one-stage procedure from the femoral artery until the blood pressure was 40-45 mm Hg.

Craniocerebral trauma consisted of infliction of 300-350 blows with an iron hammer (weight 450 g) on the vault of the skull through a rubber pad 8 mm thick. The changes in blood pressure in the femoral artery, pulse rate, and respiration, circulating blood volume (by the T-1824 dye dilution method), bioelectrical activity of the brain (unipolar recording with needle electrodes from the parietal region), ECG (standard lead II), and EMG (bipolar recording from the posterior cervical muscles) were recorded on the 4EEG-1 electroencephalograph throughout the experiment. Changes in spontaneous activity and the effect of photic (10 flashes/sec) and acoustic stimulation (2000 Hz) were investigated. To obtain more reliable data on the dynamics of the process, the arterial blood pressure was recorded simultaneously on two kymographs: on one continuously throughout the experiment with a paper-winding speed of 14 cm/h, and on the other at the most important stages of the experiment, with a paper-winding speed of 1 mm/sec. The conjunctival blood vessels were

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