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THE EFFECT OF DEFICIENCY OF VITAMINS E AND A ON THE RETINA

Vitamin E deficiency causes an extensive accumulation of lipofuscin in the pigment epithelium of the retina. Rats deficient in vitamins E and A, had less retinal lipofuscin than rats deficient in vitamin E only.

Key Words: vitamin E, vitamin A, retina, rod outer segment, retinal pigment epithelium, photoreceptor cells, antioxidant, peroxidation, lipofuscin

The membranes of the rod outer segments (ROS) of the retina have served as models for the elucidation of the structure of membranes since Brown[1] showed that rhodopsin rotates freely in the lipid environment of the ROS membrane. Recently, the antioxidant theory of the function of vitamin E in membranes received direct and unexpected support from the work of Robison, Kuwabara and Bieri.[2,3] Again, the membranes of the rods of the retina served as a model. The outer segment of the vertebrate rod is made up of a stack of disk membranes containing an exceptionally high concentration of polyunsaturated fatty acids (nearly one-half of the ROS fatty acids contain six double bonds).[4] Since the peroxidizability of unsaturated fatty acids is directly proportional to the number of double bonds they contain,[5] the ROS is particularly susceptible to lipid peroxidation. To aggravate this situation, the retina also is served with a plentiful supply of oxygen since it has an unusually high rate of oxidative metabolism. To make matters worse, light is known to enhance lipid peroxidation in the retina.[6]

Vitamin E, a free-radical scavenger, is thought to protect membrane lipids from peroxidation. The concentration of vitamin E is exceptionally high in ROS, 1 mole of α-tocopherol for each 36 moles of rhodopsin.[7] A seasonal dietary decline in vitamin E intake in cattle was found reflected in increased lipid oxidation products in retina in vitro, together with some destruction of ROS membrane structures.[7] In vivo, Hayes[8] observed degeneration of the macular region of retina when monkeys were fed a vitamin E-deficient diet for two years.

The formation of a yellow autofluorescent pigment termed lipofuscin is correlated with polyunsaturated fatty acid oxidation and accumulates as the end product of lipid peroxidation. This pigment is found in many tissues with advancing age and especially in the pigment epithelium of the retina. Physiological mechanisms that prevent lipid auto-oxidation also decrease lipofuscin accumulation. Thus,

vitamin E protects membrane lipids from autooxidation and at the same time retards accumulation of lipofuscin.

Katz et al.[9] showed that rats maintained on a diet deficient in vitamin E accumulated large amounts of lipofuscin in the pigment epithelium of the retina (RPE), especially in diets also high in polyunsaturated fatty acids. The RPE seemed to be especially sensitive to vitamin E deficiency, since the lipofuscin accumulation in other tissues was less severe. It is known that the RPE absorbs the material released during breakdown and turnover of ROS disk membranes. The oldest disks are shed from the ROS tips, absorbed and catabolized by the RPE.[10] In fact, the only other part of the retina in which Katz et al.[9] detected a small amount of lipofuscin was at the ROS tips.

Robison, Kuwabara and Bieri[2] investigated the effect of vitamin E deficiency together with normal or marginal vitamin A intake in the retina of the rat. The reason for considering marginal vitamin A in conjunction with vitamin E deficiency is that vitamin A depletion is known[11] to result in deterioration of the ROS disk structure, due to loss of rhodopsin. Female rats were given a vitamin E-deficient diet. One-half received adequate vitamin A (8.0 mg retinol per kilogram diet), -E+A, and one-half received a vitamin A-marginal diet (0.8 mg per kilogram), -E-A. Control rats received adequate vitamin E (250 mg dl-α-tocopheryl acetate per kilogram diet), with normal or marginal vitamin A (+E+A and +E-A). Tissue vitamin A levels were affected by vitamin E deficiency. After five months, the -E-A group had one-tenth the liver concentration of retinol compared to the -E+A group. The RPE cells of all the -E rats had five times the number of lipofuscin granules (identified by their characteristic fluorescence and PAS staining). Electron microscopy revealed more secondary lysosomes in the RPE in the -E rats. Clearly, the great accumulation of lipofuscin in RPE and disruption of the rods in all -E rats is the end result of the oxidation of polyunsaturated fatty acids in the absence of antioxidant protection. The simultaneous breakdown of the rods and accumulation of lipofuscin suggests that the breakdown products of the rods contribute to the substance of the lipofuscin granules. The RPE merely continues its normal function of phagocytizing and degrading pieces of ROS membrane disks and thus accumulates the end products of lipid peroxidation.

Surprisingly, there was a 46 percent loss of rod nuclei in the -E-A group,whereas the-E+A group lost none, compared to controls. Therefore, the normal level of dietary vitamin A was essential for preserving the number of rod cells. The marginal vitamin A intake had no effect on the rod cells of the +E rats, whereas a marginal vitamin A intake caused a great loss of these cells in the -E group. The animals on the -E-A diet received some dietary vitamin A, and their plasma vitamin A level, though lower than that of the -E+A rats, was still appreciable. Hence, the authors postulate the occurrence of local tissue deficiencies of vitamin A, caused by the lack of antioxidant in the microenvironment of the retina. The decline in vitamin A level in the RPE and the ROS may be such as to mimick the effect of a frank vitamin A deficiency in the whole animal, and thus disrupt the ROS disk structure. The authors are careful to point out, however, that the effect on the ROS of the vitamin E deficiency in conjunction with marginal vitamin A, differs from that of a straight dietary vitamin A deficiency. In the latter, the rods are affected before the cones, whereas in the -E-A rats, rods and cones deteriorated simultaneously and equally.

In their second paper, Robison, Kuwabara and Bieri[3] investigated the effect of vitamin A deficiency on the rod destruction induced by vitamin deficiency. They used vitamin E and frankly (not marginally) vitamin A-deficient rats in the configuration: -E-A, -E+A, +E-A and +E+A. All -A rats received retinoic acid to maintain their general health (retinoic acid cannot reverse the effect of vitamin A deficiency in the retina). After 21 weeks there was, as expected, an accumulation of lipofuscin, the vitamin E-deficient retinas (-E+A and-E-A. The +E-A group had no lipofuscin, but some disk membrane disruption and a 27 percent loss of rod nuclei. The -E-A group showed a 60 percent loss of rod nuclei. After 35 weeks, the vitamin E-deficient retinas showed that the accumulation of lipofuscin was two-fold over control in the -E-A retinas, and more surprisingly, the presence of retinol in the -E+A retinas caused a five-fold *increase* in lipofuscin granules. The

yellow autofluorescence intensity (measuring lipofuscin concentration) also was increased. Thus, the vitamin E-deficient, vitamin A-adequate group not only had the largest number of lipofuscin granules, but also the highest concentration of fluorescent pigment, higher than in the -E-A group. Unexpectedly, though the +E+A group had far fewer lipofuscin granules than the -E-A group, their fluorescence intensity was greater. The fluorescence intensity in the +E-A group was lowest of all, lower than in the +E+A group.

Thus, the massive accumulation of lipofuscin in the RPE of the -E animals is most probably caused by greatly increased peroxidation, resulting in damaged membranes. These membranes would then be phagocytized by the RPE, digested and the "undigestible" remnants packaged as lipofuscin granules. Further, though larger numbers of these granules were formed in vitamin E deficiency, they were less fluorescent in the absence of vitamin A. Possibly, vitamin A is involved in lipofuscin formation in the retina by influencing the composition of the pigment. Alternatively, vitamin A deficiency may decrease the rate of rod phagocytosis by RPE.[12] As a control, the authors compared the uteri of the -E+A and -E-A rats (the vitamin E-deficient uterus is rich in lipofuscin granules). They found no difference in that tissue. Therefore, antioxidant-related pigment formation in tissues outside the retina is not influenced by vitamin A. Possibly, this may be connected to the fact that the uterus responds, but the retina does not respond, to the retinoic acid which was being fed to the -A animals. It should be remembered that retinal, derived from retinol but not from retinoic acid, is a component of the rhodopsin molecule, the principal protein of the rods.

With regard to the structure of the retina, the vitamin E deficiency alone (-E+A), after 35 weeks, caused a disruption of the disk membranes and a 20 percent loss of photoreceptor cells. A vitamin A deficiency superimposed on the vitamin E deficiency (-E-A) led to almost complete destruction of the ROS membranes and loss of more than 90 percent of the photoreceptor cells. As one would expect, vitamin A deficiency alone (+E-A) led to a greatly shortened ROS and an intermediate loss of cells (34 percent). Thus, a -E-A diet produced a greatly accelerated degeneration of the photoreceptor cells compared to a +E-A diet. While vitamin A deficiency leads to some damage of the retina, the disruption caused by a combined vitamin E-vitamin A deficiency is greatly accentuated, apparently more than can be accounted for by an additive effect. The authors are of the opinion that the vitamin E deficiency caused increased oxidation of stored vitamin A in liver and RPE, greatly accelerating the process of rod destruction.

The membranes of the rod disks are unusually sensitive to oxidative damage, yet they are in an environment rich in oxygen. Therefore, the authors suggest, vitamin E appears to be especially necessary for protection of the retina, in particular because of the accentuation of lipid peroxidative damage by light. Moreover, the work reveals that in vitamin E deficiency there may be increased oxidative destruction of the vitamin A stores, leading to more rapid development of damage in the photoreceptor cells. In sum, to quote the authors,[3] "normal dietary levels of both vitamin E and A are essential for the structural maintenance of the neural retina and for a normal pigment epithelium," a discovery of a vitamin interaction of great consequence for the maintenance of normal vision. □

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