

RELATIONSHIP BETWEEN DIETARY RETINOL AND LIPOFUSCIN IN THE RETINAL PIGMENT EPITHELIUM

MARTIN L. KATZ*, CHRISTINE M. DREA and W. GERALD ROBISON, Jr.

National Eye Institute, National Institutes of Health, Bldg. 9, Room 1E104, Bethesda, MD 20892 (U.S.A.)

(Received April 8th, 1986)

SUMMARY

A variety of evidence suggests that autoxidation of cellular components probably plays a significant role in the age-related accumulation of lipofuscin, or age-pigment, in the mammalian retinal pigment epithelium (RPE). Among the likely candidates for conversion into RPE lipofuscin fluorophores via autoxidative mechanisms are vitamin A compounds, which are present in the retina and RPE in high concentrations. Vitamin E, an important lipid antioxidant, is likely to inhibit vitamin A autoxidation. Experiments were conducted to evaluate the significance of vitamin A autoxidation in the deposition of lipofuscin in the RPE. Albino rats were fed diets either supplemented with or lacking vitamin E. Each of these two groups of animals was further subdivided into three groups which were fed different levels of vitamin A palmitate: none, 14.0 $\mu\text{mol/kg}$ diet, and 80.5 $\mu\text{mol/kg}$ diet. After 26 weeks, the animals were killed and the RPE lipofuscin contents were determined by both fluorescence measurements and quantitative ultrastructural morphometry. Vitamin A palmitate deficiency led to significant reductions in RPE lipofuscin deposition, relative to the amounts of this pigment present in the groups receiving vitamin A palmitate in their diets. The relative magnitude of the vitamin A effect was greater in the vitamin E-supplemented groups than in the groups fed the diets deficient in vitamin E. This finding suggests that vitamin E interacts with vitamin A ester metabolites *in vivo* in a more complex manner than simply acting as an antioxidant protectant. Rats fed the diets containing the higher level of vitamin A palmitate failed to display elevated RPE lipofuscin contents relative to those in the rats fed 14.0 μmol of vitamin A palmitate/kg diet. Failure of high vitamin A intake to enhance RPE lipofuscin deposition may have been due to the fact that intake of vitamin A above normal levels did not lead to an elevation in vitamin A content of the retinal tissue. Establishing an effect of vitamin A deficiency on RPE lipofuscin deposition and charac-

*To whom correspondence should be addressed. *Current address:* University of Missouri School of Medicine, Mason Institute of Ophthalmology, Columbia, MO 65212, U.S.A.

terization of the interactions between vitamins E and A are important steps toward defining precisely the molecular and cellular mechanisms underlying age-pigment accumulation in the RPE.

Key words: Vitamin A; Vitamin E; Lipofuscin; Retinal pigment epithelium; Aging; Autoxidation

INTRODUCTION

Although intracellular deposition of autofluorescent lipofuscin pigments has long been known to accompany the aging process, the precise mechanisms leading to formation of these pigments have yet to be elucidated. The most widely accepted theory at present is that age-pigments form as a result of autoxidation of the molecular components of cells [1]. A variety of experimental evidence supports this theory. Autoxidation of cellular components *in vitro* leads to the formation of products with fluorescence properties that appear to be similar to those of fluorophores extracted from lipofuscin [2]. Additional support for the theory that lipofuscin forms as the result of autoxidation comes from the finding that rates of lipofuscin deposition *in vivo* can be altered by manipulating dietary factors that influence *in vivo* antioxidant status [1].

Substantial evidence has been obtained suggesting that polyunsaturated fatty acids (PUFAs) play major roles in lipofuscin formation [1]. PUFAs are highly susceptible to autoxidation because they are capable of propagating free radical-mediated chain reactions [3]. In the eye, vitamin A compounds might also be expected to contribute to lipofuscin formation via autoxidative mechanisms. Vitamin A plays a central role in visual transduction, and is present in the photoreceptor cells and retinal pigment epithelium (RPE) in relatively high concentrations. Vitamin A is itself quite easily oxidized, and under certain conditions is capable of photosensitizing singlet oxygen formation [4], which can initiate autoxidation of other molecular species.

If vitamin A acts to promote lipofuscin formation in the eye via an autoxidative mechanism, one would expect the effect of vitamin A to be enhanced under conditions where antioxidant protection was impaired. The major natural lipid antioxidant that has been characterized in biological systems is vitamin E. Since vitamin A is a lipid, it is likely that vitamin E plays a significant role in preventing its autoxidation *in vivo*. In order to determine whether vitamin A can influence lipofuscin deposition rates, experiments were conducted in which the influence of dietary vitamin A on RPE lipofuscin content was examined. The influence of dietary vitamin E on the relationship between vitamin A and RPE lipofuscin content was also examined in order to determine whether a potential vitamin A effect was mediated via an autoxidative mechanism.

MATERIALS AND METHODS

Animals and diets

Male Fisher 344 albino rats were obtained from Harlan Sprague-Dawley Inc. (Indiana-

polis, IN) at 21 days of age. The animals were divided immediately into six dietary groups. The composition of the basal diet, described elsewhere [5], was designed to meet all of the nutritional requirements of the rat [6], other than the requirements for vitamins E and A. The six experimental diets were designated: -E-A; -E+A; -E highA; +E-A; +E+A; +E highA. The -E diets contained no added tocopherol, while the +E diets contained 250 mg of dl- α -tocopheryl acetate/kg diet. The - less A diets contained 4 mg retinoic acid/kg diet, but no retinyl palmitate. The +A diets contained vitamin A only in the form of retinyl palmitate (14.0 μ mol/kg diet), as did the high A diets (80.5 μ mol retinyl palmitate/kg diet). Diets were prepared in pellet form by Teklad Inc. (Madison, WI), and were stored refrigerated. Diets and tap water were provided *ad libitum*.

Rats were housed in suspended wire cages and were maintained under 12 h cyclic illumination. General Electric F15T12-CW fluorescent tubes produced an illumination of 1–10 foot-candles at the cage bottoms during the light phase of the lighting cycle.

Tissue levels of vitamins E and A

After the rats had been fed the experimental diets for 20 and 26 weeks, blood samples were obtained from the tail veins of 5 animals from each dietary group, and plasma vitamin E and A concentrations were determined at both time points as described elsewhere [5]. Plasma pyruvate kinase activities were also determined on the same blood samples [5], since plasma pyruvate kinase is an indicator of overall tissue vitamin E status [7].

After 26 weeks, vitamin A ester and vitamin E levels in the retina and RPE-choroid were determined. Rats were exposed to bright light from fluorescent tubes which bleached at least 95% of the visual pigment. The animals were then enucleated under CO₂ anesthesia, the retina and RPE-choroid complex were dissected from each eye, and the vitamin A palmitate and stearate contents of each sample were measured [5]. Retinal vitamin E contents were also determined on portions of each retina sample [5].

Ultrastructural analyses

After having been fed the experimental diets for 26 weeks, 5 rats in each group were anesthetized with CO₂ and enucleated. All enucleations were performed between 7 h and 12 h after the onset of the light portion of the daily lighting cycle. The order of enucleation was such that one rat from each group was enucleated prior to enucleating the second rat in any group, and so forth, until all enucleations were completed. Immediately after enucleation, each eye was placed in a fixative consisting of 1.25% glutaraldehyde, 1% paraformaldehyde, 130 mM sodium cacodylate, 130 μ M CaCl₂, and 2% polyvinylpyrrolidone at a pH of 7.4. While maintaining the eyes immersed in fixative, the corneas, irides, and lenses were removed by dissection. The remaining portions of the eyecups were fixed for an additional hour at room temperature with gentle agitation. The tissues were then stored in fixative at 4°C for at least 12 h before further dissection. Portions of the eyecups were removed such that each had one edge extending 2–3 mm superior from the optic nerve head along the superior-inferior meridian. Sections for microscopic analyses

were cut from these edges of the tissues. The eye tissues were post-fixed, embedded, sectioned, and the sections stained and examined as described previously [8].

RPE lipofuscin content was quantified from electron micrographs taken of regions of the RPE extending from approximately 350 to 600 μm superior to the optic nerve head along the superior-inferior meridian. Sequential electron micrographs were prepared representing at least 240 μm of RPE length, excluding areas containing RPE cell nuclei. The electron micrographs were printed at a final magnification of 12 500 times, and RPE lipofuscin content was determined using the Bioquant digitizing morphometry system (R & M Biometrics, Nashville, TN). Lipofuscin granules are part of the RPE phagolysosomal system, which includes primary lysosomes, secondary lysosomes, and residual bodies [9,10]. Since these various components are not clearly distinct from one another, all electron-dense inclusions that appeared to be part of the phagolysosomal system, except for phagosomes (which are easily distinguishable), were designated as lipofuscin for the purposes of this study.

Lipofuscin autofluorescence quantitation

In addition to the ultrastructural measurements, RPE lipofuscin content was also determined microspectrofluorometrically. After being fed the diets for 26 weeks, 3–6 rats in each group were anesthetized with CO_2 and enucleated. The cornea, iris, and lens were dissected from each eye, and the retina was then carefully removed from each eyecup, the inner surface of which was lined with the RPE. A series of radial cuts were made in each eyecup, extending inward about one-third of the distance from the edge of the tissue toward the optic nerve head at the center. Each eyecup was then mounted, RPE-side up, in a drop of buffer on a glass microscope slide. The buffer consisted of 10 mM HEPES, 150 mM NaCl, and 5 mM diethylenetriaminepentaacetic acid (pH 7.4). The eyecups were covered with coverslips and fluorescence measurements were made immediately.

The microspectrofluorometer used for the lipofuscin fluorescence measurements was based on a Leitz Orthoplan fluorescence microscope equipped for epi-illumination, and was described in detail previously [11]. Some modifications in the instrument were made for the purposes of this study. Lipofuscin-specific autofluorescence in the samples was excited with light from a 200 W high pressure mercury vapor lamp used in combination with a 390–490 nm band-pass filter, and a Leitz plan-apo 40 $\times$ objective with a numerical aperture of 0.65. Light emitted from the samples was filtered with a 530 nm barrier filter, and then detected with a Hamamatsu R636 photomultiplier mounted above the camera ocular. The photomultiplier was used in conjunction with an American Instrument Co. (Silver Spring, MD) controller and amplifier. The amplified output signals were recorded relative to background on a strip-chart recorder. All measurements were taken from the central regions of the specimens, and between 10 and 20 intensity determinations were made from different areas of each sample. Areas where the specimens were folded or where the RPE was damaged were not included in the measurements. The fluorescence intensity of a standard was determined just prior to the first

measurement and immediately after the last measurement from each sample. The fluorescence intensity of each sample was expressed relative to the average fluorescence intensity of the standard measured at the same time as the sample. The standard consisted of a mark made by a Blaisdell 365-T red wax pencil on a glass microscope slide. The standard could be positioned precisely in the microscope field, and replicate measurements of the standard fluorescence intensity varied from one another by no more than 5%. The fluorescence intensity of the standard was similar to those of the samples.

The relationship between the photomultiplier response and incident light flux was characterized for the range of light intensities in which the samples emitted. The microscope field was filled uniformly with transmitted light from the tungsten bright-field illuminator which was attenuated with neutral density filters. A rectangular diaphragm was interposed between the camera ocular and the photomultiplier, and the size of the diaphragm aperture was altered in order to vary the amount of light reaching the photomultiplier. The relationship between the area of the aperture and the photomultiplier response was found to be linear in the range of response amplitudes recorded from the tissue samples.

Photoreceptor cell densities

The rate of lipofuscin deposition in the RPE appears to be directly related to photoreceptor cell density [12–14]. In order to determine whether any dietary effects on RPE lipofuscin content were secondary to alterations in photoreceptor cell densities, numbers of photoreceptor nuclei/unit retinal length were determined. Sections were cut at a thickness of 1 μm from the same regions of the retinas used for ultrastructural analysis of RPE lipofuscin content. The sections were stained with toluidine blue and photographed with a Leitz Orthoplan microscope at an initial magnification of 106 times. The micrographs were printed at a final magnification of 800 times, and the number of photoreceptor nuclei in a 125 μm length of retina from each section was determined.

Statistical analyses

Assessments of the significance of dietary effects on RPE retinyl ester and lipofuscin contents and on photoreceptor cell densities were performed using the methods described by Winer [15] for a 2×3 factorial experimental design.

RESULTS

Tissue levels of vitamins E and A

The effects of the dietary manipulations used in this study on tissue vitamin E and A levels are presented in detail elsewhere [5], and are therefore given here in summary form only. After having been fed the diets for 20 weeks, rats in the –E groups had no detectable α -tocopherol in their plasmas (<100 pmol/ml), while rats fed the +E diets had average plasma α -tocopherol levels of approximately 50 nmol/ml. Variations in dietary vitamin A levels had no appreciable influence on plasma vitamin E concentrations. Other tissues of

rats fed the $-E$ diets were also apparently depleted of vitamin E by 20 weeks, as indicated by the finding that plasma levels of pyruvate kinase in these rats were elevated an average of almost 30-fold over the levels present in rats fed the $+E$ diets. Plasma α -tocopherol concentrations in all dietary groups were similar at 26 weeks to those observed at 20 weeks. Retinal α -tocopherol levels were determined only at 26 weeks. In animals fed the $-E$ diets, no detectable α -tocopherol was present in the retinal, while α -tocopherol levels averaged 560 pmol/retinal in the $+E$ groups. Vitamin A intake did not have a significant influence on retina α -tocopherol content.

In rats fed the $-A$ diets for 20 weeks, plasma retinol levels were reduced to between 1% and 2% of the levels present in rats fed the corresponding $+A$ diets. Rats receiving the $+E$ high A diet for 20 weeks were found to have plasma retinol levels similar to those of the $+E+A$ rats. In the $-E$ groups, however, animals fed the highA diet had plasma retinol concentrations that were an average of 14% higher than the concentrations in rats fed the $+A$ diet.

Vitamin E deficiency resulted in significant reductions in plasma retinol concentrations in rats fed diets containing all three levels of retinol for 20 weeks. The lower the dietary content of retinol, the more pronounced was the vitamin E effect. In animals fed the highA diets, vitamin E deficiency resulted in an average decrease of 33% in plasma retinol concentrations. The reductions in plasma retinol concentrations due to vitamin E deficiency were an average of 45% in the $+A$ groups and 66% in the $-A$ groups.

After having been fed the diets for 26 weeks, rats in both the $-E+A$ and $-E$ highA groups had plasma retinol concentrations that were only about 50% of the levels present in the corresponding $+E$ groups. Retinol deficiency reduced plasma retinol concentrations to about 1% or less than the levels present in the $+A$ groups at 26 weeks.

Dietary intake of both vitamin E and retinyl palmitate significantly affected RPE retinyl ester content. In the $+E$ groups, animals fed both the $+A$ and highA diets had similar RPE retinyl ester levels, while dietary vitamin A deficiency reduced RPE retinyl ester content by about 90% ($P < 0.005$) (Fig. 1). High dietary vitamin A intake also failed to elevate RPE retinyl ester content in the $-E$ groups, whereas retinol deficiency resulted in a drop of only about 75% ($P < 0.005$) in RPE retinyl ester content in the $-E-A$ group relative to rats fed the $-E+A$ diet (Fig. 1). Unlike the case in plasma, vitamin E deficiency did not result in reductions in RPE vitamin A content. In the highA groups, vitamin E intake had no significant influence on RPE retinyl ester content, but in both the $+A$ and $-A$ groups, vitamin E deficiency actually resulted in significant elevations in RPE retinyl ester contents ($P < 0.05$) (Fig. 1).

RPE lipofuscin content

Vitamin E deficiency resulted in a substantial elevation in RPE lipofuscin content in rats fed all three levels of vitamin A as determined by quantitative ultrastructural morphometric analysis (Figs. 2–5). In the $-A$ groups, vitamin E deficiency resulted in an almost 6-fold increase in RPE lipofuscin content ($P < 0.005$) (Fig. 3). In the $+A$ groups, the $-E$ rats had an average of 3.9 times as much RPE lipofuscin as did the $+E$

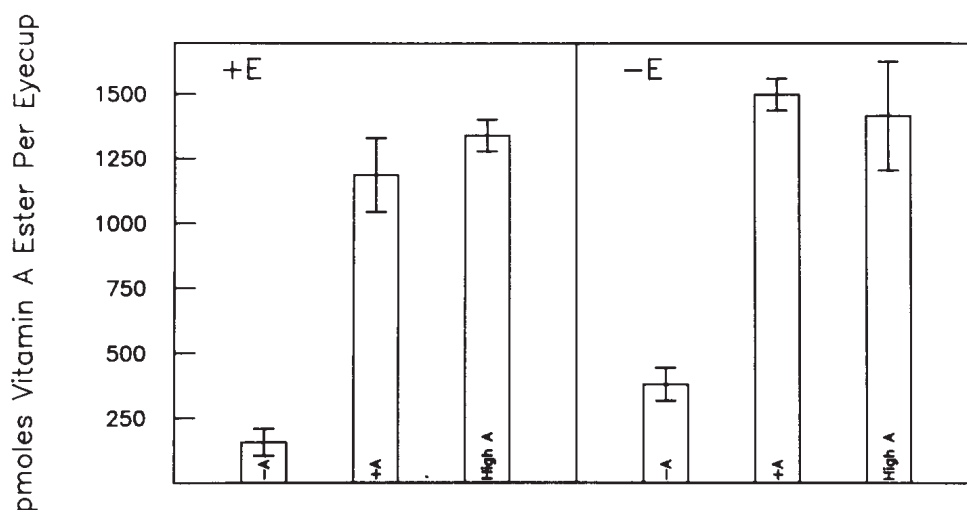


Fig. 1. Influence of dietary vitamins E and A on retinyl ester content of the RPE. Data represent means and S.E.M.s of total retinyl palmitate and stearate per eyecup for 5 to 6 animals per dietary group.

rats ($P < 0.005$), while in the highA groups, the increase in RPE lipofuscin content due to vitamin E deficiency was an average of 4.5-fold ($P < 0.005$) (Fig. 3).

Retinol deficiency was accompanied by a decrease in RPE lipofuscin content in rats fed both the +E and the -E diets, as determined by ultrastructural morphometry (Figs. 2 and 3). In animals fed the +E diets, retinol deficiency resulted in a decrease in RPE lipofuscin content of almost 50% relative to the amount present in the +A group ($P < 0.005$) (Fig. 3). The relative magnitude of the retinol effect was somewhat less in the rats fed the -E diets; in these groups, retinol deficiency resulted in a decrease of about 22% in RPE lipofuscin content ($P < 0.005$) (Fig. 3). Elevated dietary retinol intake had no significant influence on RPE lipofuscin content in animals fed diets either supplemented with or deficient in α -tocopherol (Fig. 3).

Determinations of RPE lipofuscin fluorescence intensity produced data on RPE lipofuscin content that were generally consistent with the data obtained by morphometric analysis. In animals fed all three levels of vitamin A, deficiency in vitamin E resulted in a substantial increase in RPE lipofuscin fluorescence intensity (Figs. 4 and 5). The observed enhancement of RPE lipofuscin fluorescence intensity by vitamin E deficiency (Fig. 5) was not as great as was the enhancement of RPE lipofuscin content as determined by ultrastructural analysis (Fig. 3). This apparent discrepancy probably indicates that the quantum efficiency for lipofuscin fluorescence was lower in the -E than in the +E groups due to the greater concentration of lipofuscin granules in the latter group. Thus, in those tissues with high lipofuscin concentrations, the fluorescence intensity measurements probably underestimated lipofuscin content. Lipofuscin fluorescence intensity in the -E-A group was almost 3-fold that of the +E-A group ($P < 0.005$) (Fig. 5). In the +A groups, vitamin E deficiency resulted in an increase of about

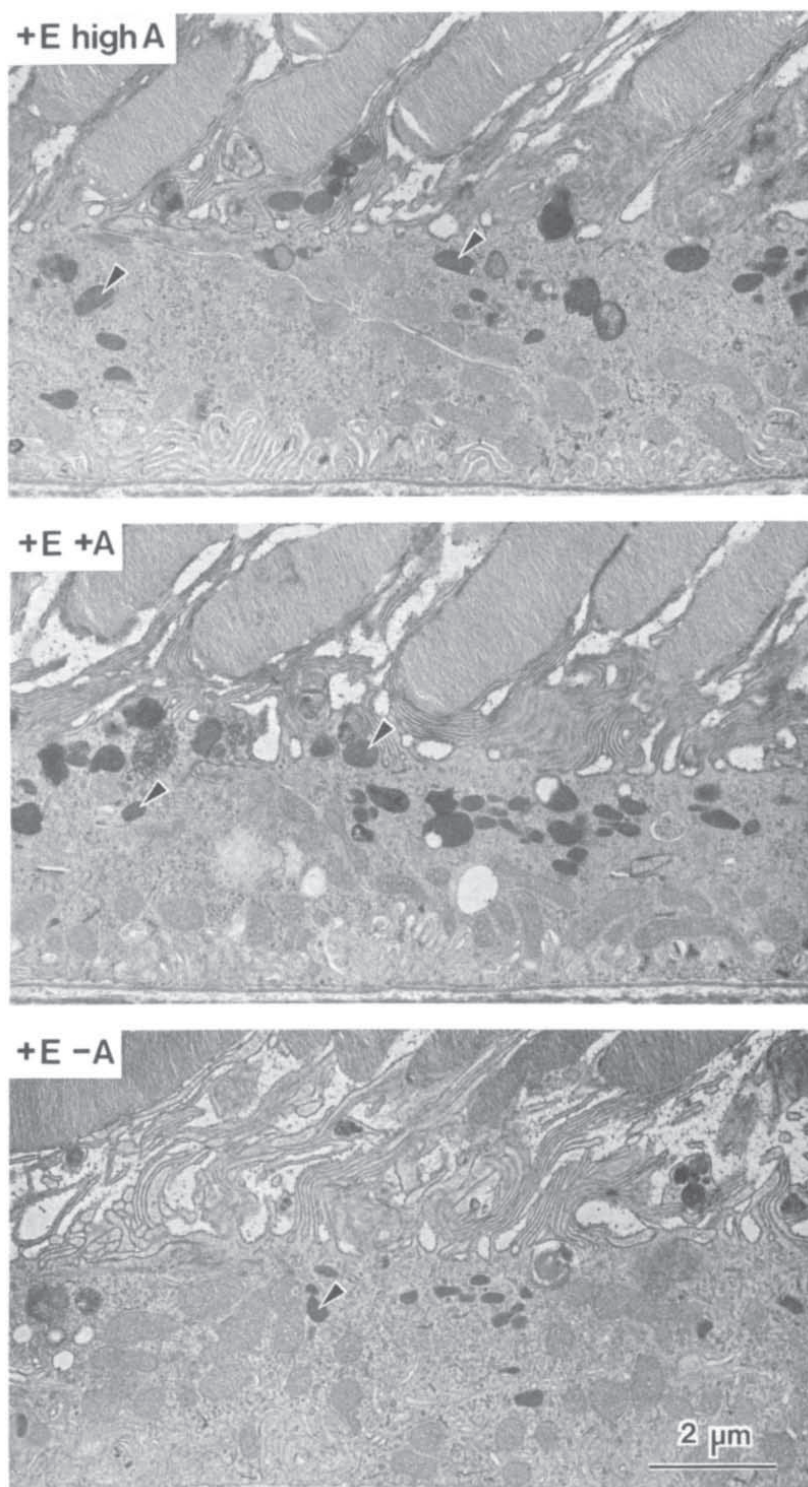
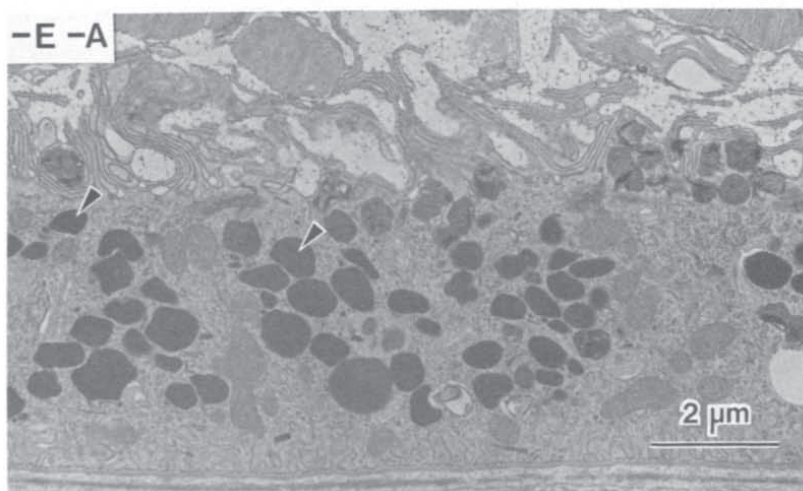
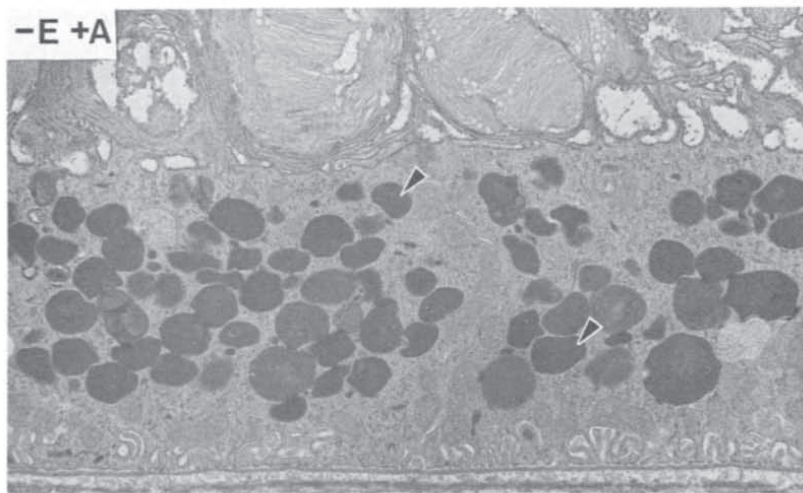
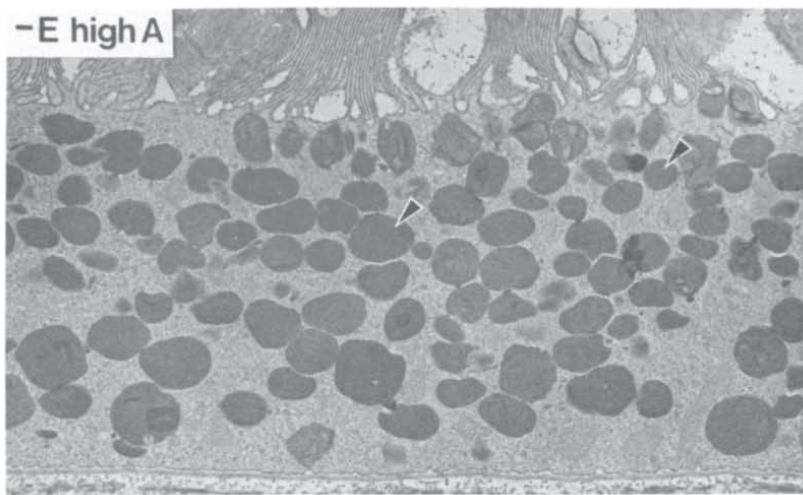


Fig. 2. Electron micrographs of representative areas of the RPE from animals fed the six experimental diets. RPE lipofuscin granule (arrowheads) content was enhanced by vitamin E deficiency at all levels of vitamin A intake. Retinol deficiency reduced RPE lipofuscin content relative to that in animals receiving dietary retinol.



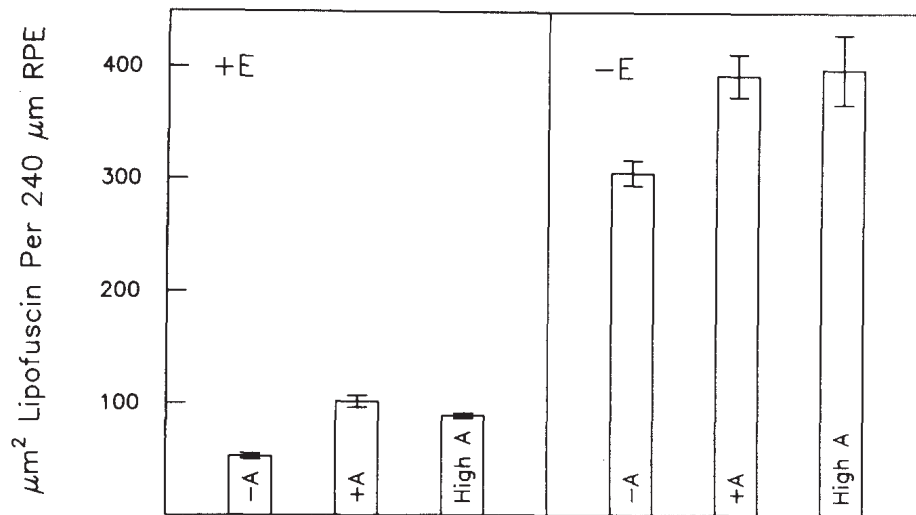


Fig. 3. Influence of dietary vitamins E and A on RPE lipofuscin content, as determined by ultrastructural morphometric analysis. Vitamin E deficiency led to a significant increase in RPE lipofuscin content, regardless of dietary retinol intake. Retinol deficiency led to a significant reduction in RPE lipofuscin accumulation in both the +E and -E groups, while high retinol intake did not elevate RPE lipofuscin content above that present in animals fed normal levels of retinol (+A). Data shown represent means and S.E.M.s for 5 animals per group.

2.3-fold in RPE lipofuscin fluorescence intensity ($P < 0.005$), and in the highA groups, the increase in RPE lipofuscin fluorescence due to vitamin E deficiency was approximately 2.8-fold ($P < 0.005$) (Fig. 5).

Retinol deficiency was accompanied by a decrease in RPE lipofuscin fluorescence intensity in rats fed both the +E and the -E diets (Fig. 5). As indicated by the morphometric data, the relative magnitude of the retinol effect was greater in the +E rats than in the rats fed the -E diet. Retinol deficiency resulted in a decrease of about 44% ($P < 0.005$) in RPE lipofuscin fluorescence intensity in the rats fed the -E diets, while the decrease in fluorescence due to retinol deficiency in the +E groups was approximately 56% ($P < 0.005$) (Fig. 5). Elevated dietary retinol intake had no significant influence on RPE lipofuscin fluorescence in animals fed either the +E or the -E diets (Fig. 5).

Photoreceptor cell densities

Vitamin E deficiency resulted in significant decreases in photoreceptor cell densities in rats fed all three levels of retinol (Fig. 6). The magnitude of the vitamin E effect was similar regardless of dietary retinol content. After having been fed the experimental diets for 26 weeks, the -E rats had an average photoreceptor cell density that was 19% lower than that of rats in the +E groups ($P < 0.001$). No significant influence of dietary retinol on photoreceptor cell density was seen in rats fed either the +E or the -E diets.

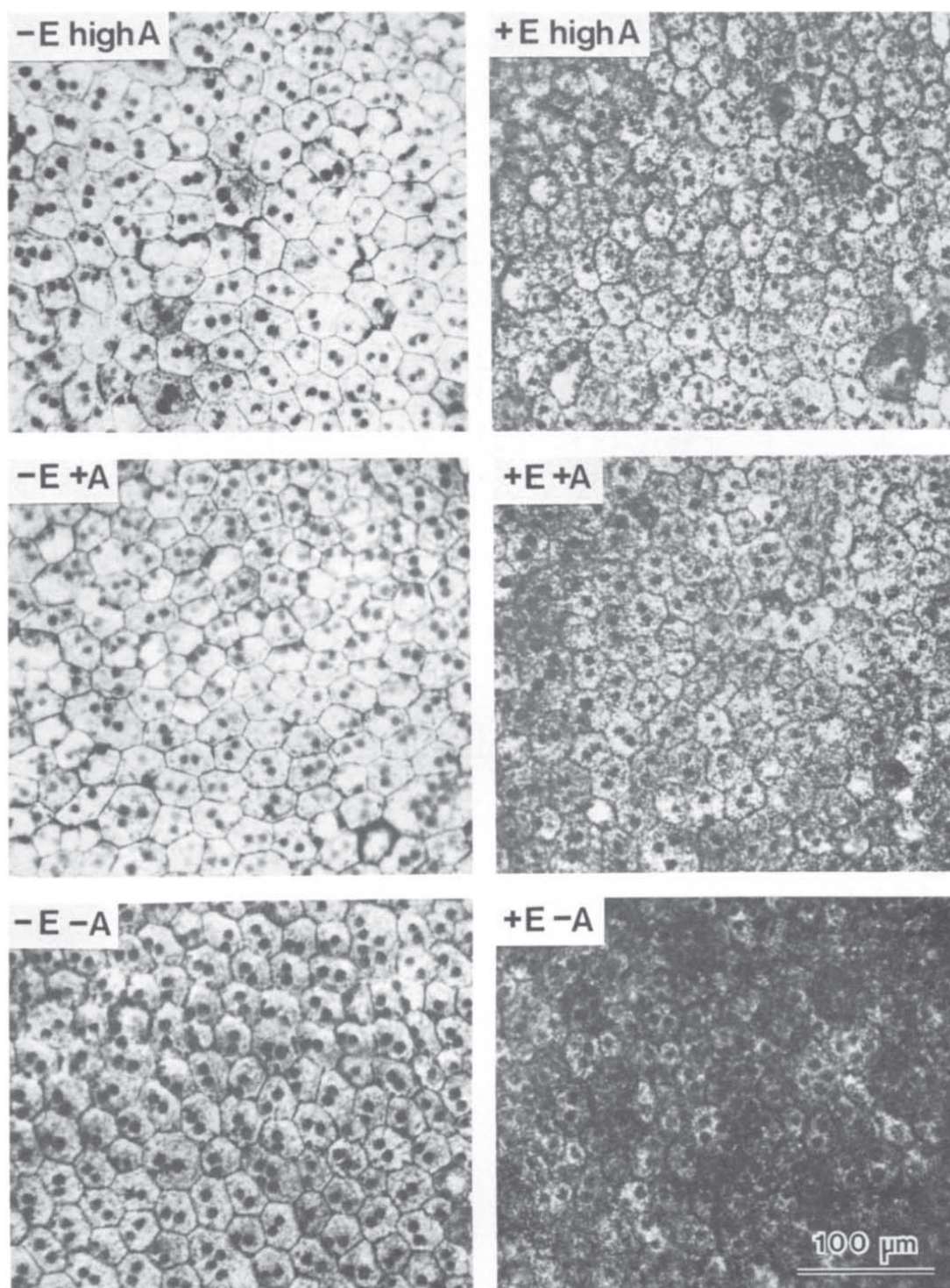


Fig. 4. Fluorescence micrographs of RPE whole-mounts illustrating the influence of dietary vitamin E and vitamin A on RPE lipofuscin fluorescence intensity.

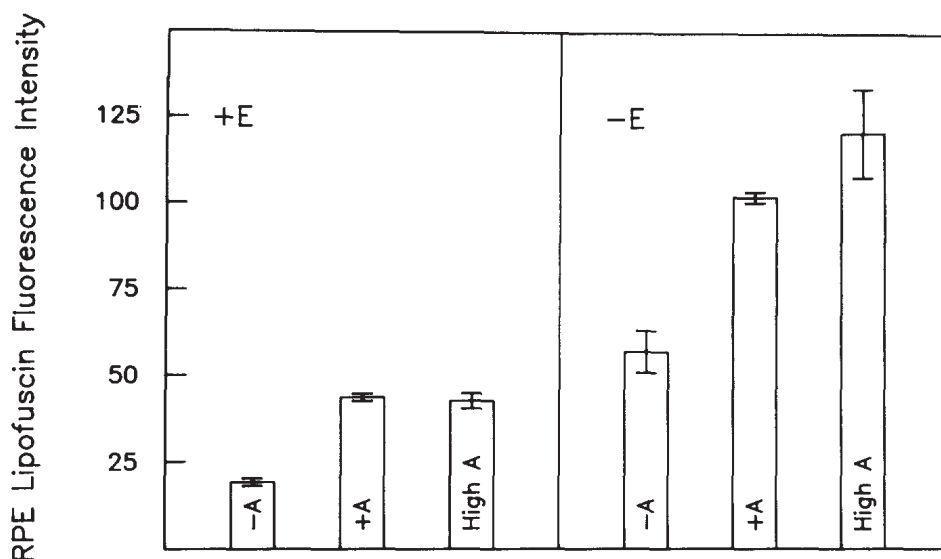


Fig. 5. Influence of dietary vitamins E and A on RPE lipofuscin fluorescence intensity. In general, the fluorescence data were consistent with the morphometric data on RPE lipofuscin content, although the apparent magnitude of the vitamin E effect was lower when determined by fluorescence intensity measurements than it was when measured with ultrastructural morphometry. Data shown represent means and S.E.M.s for 3-6 animals/group.

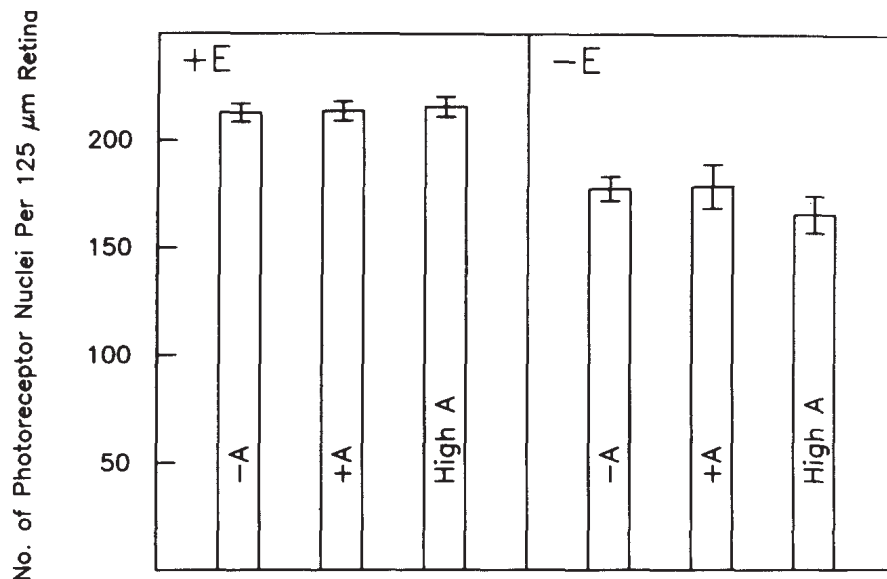


Fig. 6. Influence of dietary vitamins E and A on retinal photoreceptor densities. Variations in dietary retinyl palmitate had no significant effects on photoreceptor cell densities, while vitamin E deficiency resulted in significant reductions in the numbers of photoreceptor cells in the central retinas. Data shown represent means and S.E.M.s for 5 animals/group.

DISCUSSION

Antioxidant nutrient deficiency has been shown to greatly accelerate lipofuscin deposition in the RPE [16–19], as well as in a variety of other tissues [1], suggesting that lipofuscin can be formed via autoxidative mechanisms. Among the likely candidates for conversion into RPE lipofuscin fluorophores via autoxidation are retinol derivatives, which are relatively abundant in the retina and RPE. If vitamin A compounds are indeed precursors of RPE lipofuscin, one would expect that by varying the vitamin A content of the retina and RPE, one could influence the rate of lipofuscin deposition in this tissue. It is possible to deplete the retina of vitamin A while supplying other tissues with adequate levels of this vitamin. The retina requires vitamin A in the form of retinol, which can be derived from dietary retinyl esters, but not from retinoic acid [14,20–22]. The latter compound can apparently satisfy the vitamin A requirements of tissues other than the retina. Thus, by feeding animals diets containing vitamin A only in the form of retinoic acid, the retinas could be rendered retinol-deficient while maintaining the rats in a generally healthy state.

The finding that retinol deficiency resulted in a substantial reduction in RPE lipofuscin accumulation is consistent with the hypothesis that vitamin A compounds can be converted into lipofuscin fluorophores. Excessive dietary intake of retinol, on the other hand, failed to enhance RPE lipofuscin deposition over that seen in animals fed normal amounts of this vitamin. The latter finding is probably related to the failure of high dietary retinol intake to produce elevated vitamin A levels in the retinal tissues.

The question of whether the effect of retinol on RPE lipofuscin content occurred via an autoxidative mechanism was addressed by determining whether vitamin E deficiency enhanced this effect. In relative terms, the magnitude of the retinol effect was actually lower in the vitamin E-deficient animals than in the animals receiving this vitamin in their diets. Ultrastructural analysis indicated that retinol deficiency only lowered RPE lipofuscin content by about 22% in the vitamin E-deficient groups, but by almost 50% in the groups receiving vitamin E. One possible interpretation of these findings is that the effect of retinol on RPE lipofuscin content does not occur via an autoxidative mechanism. This interpretation may not be completely accurate, however. Retinol deficiency resulted in a reduction of about 90% in RPE retinyl ester levels in the vitamin E-supplemented groups, but only 75% in the vitamin E-deficient groups. Thus, there was relatively (and absolutely) more vitamin A available for enhancing lipofuscin formation in the –E–A group than in the +E–A animals. The failure of vitamin E deficiency to enhance the effect of retinol on RPE lipofuscin deposition appears, therefore, to result from the existence of more complex interactions of vitamins A and E *in vivo* than simply a pro-oxidant/antioxidant relationship.

One mechanism by which retinol deficiency could indirectly reduce RPE lipofuscin deposition is by causing photoreceptor cell death [19–22]. Previous investigations have demonstrated that RPE lipofuscin deposition is related to the presence of retinal photoreceptor cells; rats lacking photoreceptor cells due to a genetic defect show substantially

less RPE lipofuscin deposition during aging than do congenic animals with normal retinas [13]. Thus, a reduction in photoreceptor density due to retinol deficiency would be expected to result in reduced lipofuscin deposition in the RPE. In the current experiment, photoreceptor cell density was not affected by dietary retinol intake, indicating that the reduced lipofuscin deposition seen in the retinol-deficient rats was not due to photoreceptor cell death. It is quite possible, however, that retinol deficiency affected various aspects of photoreceptor cell metabolism, such as outer segment renewal, that in turn led to lower rates of lipofuscin formation.

The retinol effect on lipofuscin deposition does not appear to occur in all tissues. Bieri and colleagues [23] reported that vitamin E deficiency resulted in substantial lipofuscin deposition in the uterus, but that dietary retinol had no apparent effect. The effect of dietary retinol on lipofuscin accumulation does not, on the other hand, appear to be restricted to the RPE. Herrmann and colleagues [24,25] reported that retinol deficiency reduced the amount of lipofuscin which accumulated in the choroid and extraocular muscle in response to vitamin E deficiency.

The most widely recognized precursors for lipofuscin fluorophores are polyunsaturated fatty acids, the autoxidation products of which are thought to react with other cellular components to generate autofluorescent compounds. Demonstration of an effect of retinol on lipofuscin deposition suggests that vitamin A derivatives may be incorporated into lipofuscin granules as well. Since the effect of retinol on lipofuscin formation may be indirect, however, further research will be necessary to determine whether lipofuscin granules contain compounds derived from vitamin A.

ACKNOWLEDGEMENTS

The expert technical assistance of Anne Groome is gratefully acknowledged.

REFERENCES

- 1 M.L. Katz and W.G. Robison, Jr., Nutritional influences on autoxidation, lipofuscin accumulation, and aging. In J.E. Johnson, R. Walford, D. Harman and J. Miquel (eds.), *Free Radicals, Aging, and Degenerative Diseases*, Alan R. Liss, New York, 1986, pp. 221–259.
- 2 K.S. Chio, U. Reiss, B. Fletcher and A.L. Tappel, Peroxidation of subcellular organelles: formation of lipofuscin-like fluorescent pigments. *Science*, 166 (1969) 1535–1536.
- 3 C.K. Chow, Nutritional influence on cellular antioxidant defense systems. *Am. J. Clin. Nutr.*, 32 (1979) 1066–1081.
- 4 M. Delmelle, An investigation of retinal as a source of singlet oxygen. *Photochem. Photobiol.*, 27 (1978) 731–734.
- 5 M.L. Katz, C.M. Drea and W.G. Robison, Jr., Dietary vitamins A and E influence retinyl ester content and composition in the retinal pigment epithelium. *Biochim. Biophys. Acta* (1986) submitted.
- 6 American Institute of Nutrition, Report of the American Institute of Nutrition ad hoc committee for nutritional studies. *J. Nutr.*, 107 (1977) 1340–1348.
- 7 L.J. Machlin, E. Gabriel, H.E. Spiegel, L.R. Horn, M. Brin and J. Nelson, Plasma pyruvate kinase and glutamic oxaloacetic transaminase as indices of myopathy in the vitamin E deficient rat. *J. Nutr.*, 108 (1978) 1963–1968.

- 8 M.L. Katz and W.G. Robison, Jr., Age-related changes in the retinal pigment epithelium of pigmented rats. *Exp. Eye Res.*, **38** (1984) 137–151.
- 9 L. Feeney, The phagolysosomal system of the pigment epithelium. A key to retinal disease. *Invest. Ophthalmol. Vis. Sci.*, **12** (1973) 635–638.
- 10 L. Feeney-Burns and G.E. Eldred, The fate of the phagosome: conversion to “age pigment” and impact in human retinal pigment epithelium. *Trans. Ophthalmol. Soc. U.K.*, **103** (1983) 416–421.
- 11 M.L. Katz, W.G. Robison, Jr., R.K. Herrmann, A.B. Groome and J.G. Bieri, Lipofuscin accumulation resulting from senescence and vitamin E deficiency: spectral properties and tissue distribution. *Mech. Ageing Dev.*, **25** (1984) 149–159.
- 12 W.G. Robison, Jr., T. Kuwabara and J.G. Bieri, The roles of vitamin E and unsaturated fatty acids in the visual process. *Retina*, **2** (1982) 263–281.
- 13 M.L. Katz, C.M. Drea, G.E. Eldred, H.H. Hess and W.G. Robison, Jr., Influence of early photoreceptor degeneration on lipofuscin in the retinal pigment epithelium. *Exp. Eye Res.* (1986) in press.
- 14 W.G. Robison, Jr. and M.L. Katz, Vitamin A and lipofuscin. In L. Andrews, P. Dayhaw-Barker, R. Hilfer and J. Sheffield (eds.), *Tenth Symposium on Ocular and Visual Development: The Microenvironment and Vision*, Springer-Verlag, New York, 1986, in press.
- 15 B.J. Winer, *Statistical Principles in Experimental Design*, 2nd edn., McGraw-Hill, New York, 1971.
- 16 M.L. Katz, W.L. Stone and E.A. Dratz, Fluorescent pigment accumulation in retinal pigment epithelium of antioxidant-deficient rats. *Invest. Ophthalmol. Vis. Sci.*, **17** (1978) 1049–1058.
- 17 M.L. Katz, K.R. Parker, G.J. Handelman, T.L. Bramel and E.A. Dratz, Effects of antioxidant nutrient deficiency on the retina and retinal pigment epithelium of albino rats: a light and electron microscopic study. *Exp. Eye Res.*, **34** (1982) 339–369.
- 18 W.G. Robison, Jr., T. Kuwabara and J.G. Bieri, Vitamin E deficiency and the retina: photoreceptor and pigment epithelial changes. *Invest. Ophthalmol. Vis. Sci.*, **18** (1979) 683–690.
- 19 W.G. Robison, Jr., T. Kuwabara and J.G. Bieri, Deficiencies of vitamins E and A in the rat. Retinal damage and lipofuscin accumulation. *Invest. Ophthalmol. Vis. Sci.*, **19** (1980) 1030–1037.
- 20 J.E. Dowling and G. Wald, Vitamin A deficiency and night blindness. *Proc. Natl. Acad. Sci. U.S.A.*, **44** (1958) 648–661.
- 21 J.E. Dowling, Nutritional and inherited blindness in the rat. *Exp. Eye Res.*, **3** (1964) 348–356.
- 22 L. Carter-Dawson, T. Kuwabara, P.J. O'Brien and J.G. Bieri, Structural and biochemical changes in vitamin A-deficient rat retinas. *Invest. Ophthalmol. Vis. Sci.*, **18** (1979) 437–446.
- 23 J.G. Bieri, T.J. Tolliver, W.G. Robison, Jr. and T. Kuwabara, Lipofuscin in vitamin E deficiency and the possible role of retinol. *Lipids*, **15** (1980) 10–13.
- 24 R.K. Herrmann, W.G. Robison, Jr. and J.G. Bieri, Deficiencies of vitamins E and A in the rat: lipofuscin accumulation in the choroid. *Invest. Ophthalmol. Vis. Sci.*, **25** (1984) 429–433.
- 25 R.K. Herrmann, W.G. Robison, Jr., J.G. Bieri and M. Spitznas, Lipofuscin accumulation in extraocular muscle of rats deficient in vitamins E and A. *Graefes Arch. Clin. Exp. Ophthalmol.*, **223** (1985) 272–277.