# Retinal Light Damage in Rats With Altered Levels of Rod Outer Segment Docosahexaenoate

*Daniel T. Organisciak,* Ruth M. Darrow,* Yih-Ling Jiang,* and Janet C. Blanks†*

***Purpose.*** To compare retinal light damage in rats with either normal or reduced levels of rod outer segment (ROS) docosahexaenoic acid.

***Methods.*** Weanling male albino rats were maintained in a weak cyclic light environment and fed either a nonpurified control diet or a purified diet deficient in the linolenic acid precursor of docosahexaenoic acid (DHA). Half the rats on the deficient diet were given linseed oil, containing more than 50 mol% linolenic acid, once a week to maintain ROS DHA at near normal levels. Diets and linseed oil supplementation were continued for 7 to 12 weeks. To replenish DHA in their ROS, some 10-week-old rats on the deficient diet were given linseed oil three times a week for up to 3 additional weeks. Groups of animals were killed at various times for ROS fatty acid determinations or were exposed to intense green light using intermittent or hyperthermic light treatments. The extent of retinal light damage was determined biochemically by rhodopsin or photoreceptor cell DNA measurements 2 weeks after exposure and morphologically by light and electron microscopy at various times after light treatment.

***Results.*** Rats maintained for 7 to 12 weeks on the linolenic acid-deficient diet had significantly lower levels of DHA and significantly higher levels of n-6 docosapentaenoic acid (22:5n-6) in their ROS than deficient-diet animals supplemented once a week with linseed oil or those fed the nonpurified control diet. As determined by rhodopsin levels and photoreceptor cell DNA measurements, deficient-diet rats exhibited protection against retinal damage from either intermittent or hyperthermic light exposure. However, the unsaturated fatty acid content of ROS from all three dietary groups was the same and greater than 60 mol%. In 10 week-old deficient-diet rats given linseed oil three times a week, ROS DHA was unchanged for the first 10 days, whereas 22:5n-6 levels declined by 50%. After 3 weeks of treatment with linseed oil, ROS DHA and 22:5n-6 were nearly the same as in rats supplemented with linseed oil from weaning. The time course of susceptibility to retinal light damage, however, was different. Hyperthermic light damage in rats given linseed oil for only 2 days was the same as for rats always fed the deficient diet. Six days after the start of linseed oil treatment, retinal light damage was the same as in rats given the linseed oil supplement from weaning. Morphologic alterations in ROS of linseed oil-supplemented rats immediately after intermittent light exposure were more extensive than in either the deficient-diet animals or those fed the control diet. The deficient-diet rats also exhibited better preservation of photoreceptor cell nuclei and structure 2 weeks after exposure.

***Conclusions.*** Rats fed a diet deficient in the linolenic acid precursor of DHA are protected against experimental retinal light damage. The relationship between retinal light damage and ROS lipids does not depend on the total unsaturated fatty acid content of ROS; the damage appears to be related to the relative levels of DHA and 22:5n-6. Invest Ophthalmol Vis Sci. 1996;37:2243–2257.

Vertebrate photoreceptor cell rod outer segment (ROS) membranes have an unusually high content of polyunsaturated fatty acids, with docosahexaenoic acid (DHA) comprising nearly 50 mol% of the total fatty acids.[1–3] Docosahexaenoic acid is formed from the essential fatty acid linolenic acid (18:3n-3) and accumulates in ROS in an age-dependent manner.[4] Unlike the shorter chain-saturated fatty acids, which are renewed by molecular replacement as well as by de novo synthesis,[5] DHA incorporated into newly synthesized disks appears to remain in the ROS membrane and to be displaced apically at a rate similar to that of rhodopsin.[6,7] Although the functions of DHA in photoreceptor cell membranes are not well understood, in several species, dietary restrictions that decrease retinal DHA have been shown to affect visual

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acuity and electroretinographic function.[8-12] It is also known that DHA is conserved in ROS lipids when rats are fed essential fatty acid-deficient diets,[13-15] possibly by recycling from the retinal pigment epithelium.[16] However, when animals are maintained on an 18:3n-3-deficient diet containing a sufficient level of linoleic acid (18:2n-6), DHA is gradually replaced by n-6 docosapentaenoic acid (22:5n-6).[8,15,17-19] The findings that DHA levels in ROS also are reduced by prolonged light exposure[20] and by keeping rats from birth in high-light-rearing environments[21] suggests a relationship between light exposure and DHA content.

A number of laboratories have studied experimental retinal light damage in rats maintained on n-3 fatty acid-deficient diets or on diets supplemented with oils containing high levels of 18:3n-3 or 18:2n-6.[22-26] Despite the use of different techniques to measure photoreceptor cell damage, these studies all found that a reduction in retinal DHA imparted protection against intense light-induced degeneration. This protection may be related to a decrease in the oxidative potential of ROS lipids. However, light damage susceptibility was not increased in rats fed fish oil rich in highly polyunsaturated eicosapentaenoic acid (20:5n-3) and DHA,[26] despite the fact that their retinas were found to contain higher than normal levels of 20:5n-3 and rhodopsin, one determinant of light damage.[27] In vitro oxidation was also greater than in retinal homogenates from rats fed an 18:3n-3 rich soybean oil diet, but retinal light damage was greater in the soybean oil-fed animals.

In the current study, we used a variety of intense light treatments to investigate retinal damage in rats fed a nonpurified control diet or a purified diet deficient in 18:3n-3. Biochemical and morphologic measures of photoreceptor cell loss were performed on rats subjected to intermittent light exposures or hyperthermic light, both of which accelerate the rate of retinal damage.[28,29] We also measured retinal light damage in deficient-diet rats supplemented with 18:3n-3, in the form of linseed oil, to determine the time course of damage susceptibility in relation to the replacement of DHA in ROS lipids.

*From the *Petticrew Research Laboratory, Departments of Biochemistry and Molecular Biology, and Ophthalmology, Wright State University, Dayton, Ohio and †Doheny Eye Institute and the Departments of Ophthalmology and Cell and Neurobiology, University of Southern California School of Medicine, Los Angeles, California.*

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*Reprint requests: Daniel T. Organisciak, Department of Biochemistry and Molecular Biology, Wright State University School of Medicine, 3640 Colonel Glen Highway, Room 234, Biological Sciences Building, Dayton, OH 45435.*

**TABLE 1.** Major Fatty Acids in Rat Diets

| *Fatty Acid* | *Nonpurified Control Diet* | *Purified Diet* | *Linseed Oil†* |
|---|---|---|---|
| 14:0 | 1.8 ± 1.0 | 1.6 ± 0.8 | 0.5 |
| 16:0 | 19.7 ± 1.3 | 24.7 ± 5.6 | 6.5 |
| 16:1 | 2.0 ± 0.5 | 0.4 ± 0.2* | — |
| 18:0 | 2.7 ± 0.3 | 2.0 ± 1.1 | 3.3 |
| 18:1 | 18.9 ± 0.4 | 22.0 ± 3.1 | 18.2 |
| 18:2n-6 | 48.8 ± 2.2 | 48.7 ± 2.1 | 16.8 |
| 18:3n-3 | 5.7 ± 0.8 | 0.7 ± 0.3* | 53.2 |
| 20:4 | 0.2 ± 0.2 | 0.1 ± 0.1 | — |
| 22:4 | — | — | — |
| 22:5n-6 | — | 0.1 ± 0.1 | — |
| 22:6n-3 | 0.3 ± 0.3 | 0.1 ± 0.1 | — |

Values are the mean ± SD (mol/dl) for ($n$ = 4) separate determinations for each diet.
— = < 0.1 mol/dl.
* Value significantly less than for nonpurified diet; Student's $t$-test, $P < 0.001$.
† Average fatty acid composition of raw linseed oil.

## MATERIALS AND METHODS

### Animals, Diets, Light Environment

Weanling male albino Sprague–Dawley rats (Harlan, Indianapolis, IN) were fed a nonpurified control Rat Chow diet (Teklad, Madison, WI) or a purified diet deficient in the 18:3n-3 precursor of DHA. The purified diet (TK79384; Teklad) contained (per kilogram) 180 g vitamin-free casein, 150 g corn starch, 547 g sucrose, 30 g nonnutritive fiber, 35 g mineral mix AIN-76, 3 g DL-methionine, and 5% cottonseed oil.[19] The fatty acid contents of the nonpurified control diet and the purified diet are shown in Table 1. Although both diets contain high levels of 18:2n-6, the precursor of 22:5n-6, 18:3n-3 was less than 1 mol% in the purified diet. The nonpurified control diet contained more than 5 mol% 18:3n-3 but was otherwise similar in fatty acid composition to the purified diet.

Half the rats fed the purified diet received raw linseed oil, containing more than 50 mol% 18:3n-3 (Table 1), by gastric intubation on a weekly basis (+L), 0.5 ml the first week and 1 ml per week thereafter.[19] The remaining animals (−L) were sham intubated on the same schedule. All rats were maintained on their respective diets for 7 to 12 weeks in a weak cyclic light environment consisting of 20 to 40 lux white light for 12 hours per day (lights on 8 AM and off at 8 PM). To replenish DHA in their retinas, another group of 10-week-old −L rats was administered 3 doses of linseed oil per week, for up to 3 additional weeks. These rats were given 1 ml of linseed oil by intubation on 3 successive days, followed by 4 days without supplementation.

At various times, some rats were killed for rhodopsin analysis or for the determination of ROS fatty acid profiles. Other animals from the same groups were exposed to intense green light (490 to 580 nm).[29] The

light-exposed animals were either killed immediately in a $CO_2$-saturated chamber or subsequently were placed into a dark environment for a period of 2 weeks. During this period, necrotic photoreceptor cell debris was removed from the light-damaged eye while rhodopsin in the surviving photoreceptors was maximized.[22,23,28] At the end of the 2-week dark-recovery period, experimental animals and unexposed control rats, placed into darkness at the same time, were killed for determinations of the extent of retinal light damage. The use of animals in this investigation conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

## Intense Visible Light Exposures

Two paradigms of intense light exposure were used in these studies. Some rats were treated with intermittent light, beginning at 9 AM and consisting of 1-hour exposures interrupted by 2-hour dark periods.[28] For these exposures, rats from each of the dietary groups were treated simultaneously for 8 to 16 light–dark cycles. Light intensity was 1500 to 1750 lux during exposure; chamber temperature was ambient. Other groups of rats were treated under conditions of hyperthermia.[29] In these experiments, a chamber temperature of 34.5°C was used, resulting in an average rectal temperature of 39°C for the rats. The experimental animals were preheated for 2 hours in darkness, beginning at 8 AM, and then either exposed to light (1100 lux) for 2 hours or kept in the dark for 2 additional hours during continued hyperthermia. These animals were then maintained in darkness at ambient temperature for a 2-week recovery period before rhodopsin or DNA measurements. Histologic determinations were made in another group of rats treated with 9 cycles of intermittent light, or with 2 hours of light during hyperthermia. These animals were killed either immediately after light treatment or after the 2-week dark recovery period. For each of the conditions described, both eyes from two or three −L, +L, control diet, or refed animals were sampled for histopathology. Figure 1 shows the time course of diet administration and the paradigms of light exposure used in these studies.

## Biochemical and Morphologic Estimates of Retinal Light Damage

The extent of visual cell loss resulting from intense light treatment was determined primarily by rhodopsin measurements performed, in dim red light, 2 weeks after exposure. In each case, rhodopsin levels in the eyes of experimental rats were compared with the levels in unexposed control animals maintained in darkness for the same period. For some rats, rhodopsin was measured in one eye, and photoreceptor cell DNA was measured in the fellow eye. Techniques for the measurement of rhodopsin and DNA have been described.[28] After the rats were killed in dim red light, the left eyes were excised and used for whole eye rhodopsin determinations after extraction with 1.5% Emulphogene BC-720 detergent (Sigma, St. Louis, MO).[30] Retinas excised from the fellow eyes were extracted for DNA analysis. Photoreceptor cell DNA measurements were performed with the Hoechst (Calbiochem–Behring, La Jolla, CA) dye-binding assay for DNA, modified for use in the rat retina by Noell et al.[23] Because necrotic photoreceptor material is removed from the eye during the dark recovery period after light exposure, both the rhodopsin and the DNA measurements reflect end point determinations of retinal damage.[22,23,28]

Light and electron microscopy were used to determine the morphologic effects of intense light in the eyes of the three dietary groups of rats. After enucleation of the eye, the cornea and lens were removed, and the eye cups were placed in half-strength Karnovsky's fixative (2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium cacodylate buffer). Twenty-four hours later the eyecups were transferred to 0.1 M sodium cacodylate buffer, pH 7.4. Each eye was then bisected vertically through the optic nerve into nasal and temporal halves. The nasal half was processed for embedding in glycol methacrylate. Toluidine-blue-stained glycol sections 3 $\mu$m in thickness were examined; these included a full length of retina passing through the optic nerve head and ora serrata in both the inferior and superior nasal quadrants. The temporal half was bisected again, horizontally, into superior and inferior quadrants. Each quadrant was then dissected into three pie-shaped wedges (the apex was the most central region). Tissue samples were osmicated, dehydrated in a graded series of alcohols, and embedded in epoxy resin. Thick sections (1 $\mu$m) from both inferior and superior regions were cut on an ultramicrotome and stained with toluidine blue. Thin sections for transmission electron microscopy were obtained and examined under a Zeiss (Thornwood, NY) EM-10 electron microscope.

## Rhodopsin Bleaching and Regeneration

Rats were exposed to green light for as long as 1 hour at ambient temperature in the same chambers used for light damage studies. After various times in light, animals were killed for measurements of rhodopsin bleaching. To determine the rate of rhodopsin regeneration, other groups of rats were exposed to light for 1 hour and then were placed in darkness for as long as 4 hours. At various times, rats were killed for whole eye rhodopsin determinations. In each case, rats from the three dietary groups were treated simultaneously in the same light chamber.

## Fatty Acid Determinations

Rod outer segments were isolated from the pooled retinas of three rats using sucrose density gradient

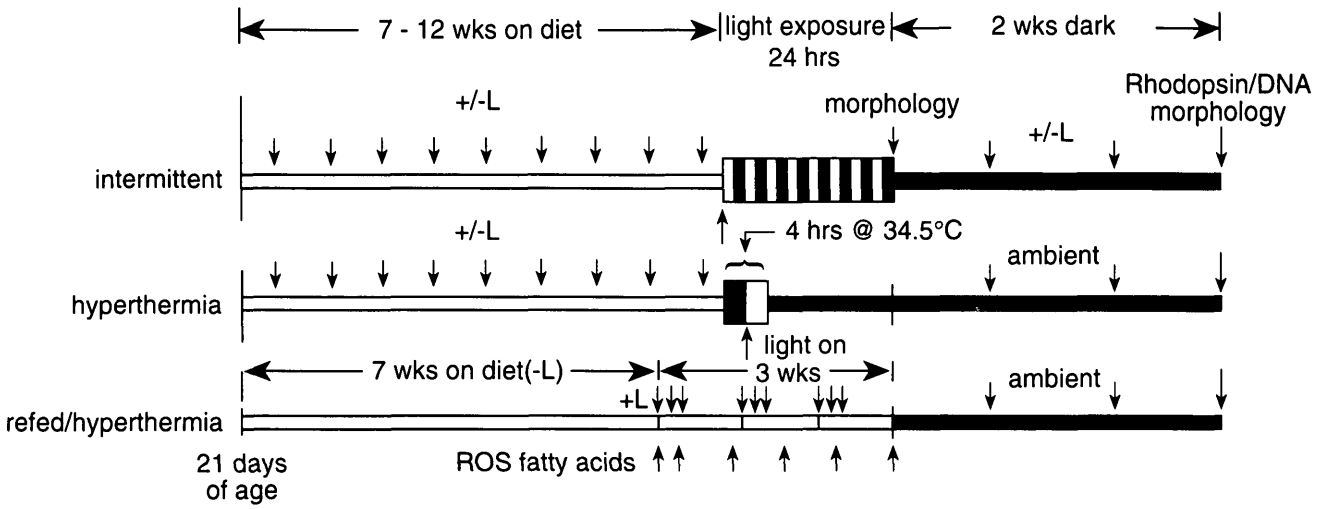


FIGURE 1. Experimental design of studies with rats fed various diets. Weanling rats were maintained on diet for various periods and then were exposed either to intermittent light or to light during hyperthermia. Biochemical measurements usually were taken 2 weeks after exposure. Morphology was performed immediately after exposure or 2 weeks after light treatment. (|) Days of linseed oil treatment for +L and refed rats or sham intubation for −L animals. For refed–hyperthermic-treated rats, rod outer segments fatty acids were at various times (|) during the period of linseed oil treatment. In these animals, hyperthermic light exposures (2 hours) were performed on days 0, 2, 6, 10, and 20.

ultracentrifugation.[31] Band I fractions from control diet, −L, +L, or linseed oil refed rats, unexposed to intense light, were precipitated, extracted with chloroform:methanol 2:1 vol/vol, and total fatty acid composition was determined. The fatty acid compositions of the diets used and of the livers from rats fed the various diets also were determined. Procedures for lipid extraction, fatty acid analysis, and cholesterol determinations have been described.[19]

## Statistical Evaluations

Analysis of data presented herein consisted of linear regression analysis and analysis of variance (ANOVA), followed by Tukey's HSD multiple comparison procedure. If more than one ANOVA was performed, a Bonferroni correction was used to maintain an overall significance level of 0.05. Rhodopsin data are presented as the mean ± 1 SD of 3 to 12 separate measurements. Photoreceptor DNA measurements represent 11 separate determinations for each dietary group of rats, with five to six rats in each group exposed to intense light. Rod outer segment fatty acids from control diet −L and +L rats are the mean of 8 to 10 separate determinations ± 1 SD. Dietary fatty acids represent the mean of four separate determinations ± 1 SD, with comparisons by a nonpaired Student's *t*-

TABLE 2. Rhodopsin Levels in Rats Exposed to Intermittent Light

| *Light Exposure* | *Nonpurified Control Diet* | *Purified Diet* | |
|---|---|---|---|
| | | *+Linseed* | *−Linseed* |
| 0 hour unexposed | 1.95 ± 0.11 (8) | 1.96 ± 0.11 (7) | 1.91 ± 0.10 (6) |
| 8 hours + 2 weeks dark | 1.19 ± 0.25* (8) | 1.36 ± 0.28* (12) | 1.73 ± 0.29†* (10) |
| 12 hours + 2 weeks dark | — | 1.36 ± 0.36* (6) | 1.75 ± 0.26* (6) |
| 16 hours + 2 weeks dark | 1.22 ± 0.24* (4) | 1.24 ± 0.31* (6) | 1.54 ± 0.20* (5) |
| Unexposed + 2 weeks dark | 2.24 ± 0.12 (12) | 2.24 ± 0.12 (14) | 2.31 ± 0.10 (13) |

Results are the mean ± SD (nmol/eye) for ($n$) pairs of eyes for rats from the three dietary groups. Rats were fed their respective diets for 9 to 12 weeks before intense light exposure.
* All light-exposed rat rhodopsin values were significantly lower than in the unexposed rats maintained in darkness for 2 weeks ($P < 0.05$).
† Using analysis of variance followed by Tukey's HSD procedure with $\alpha = 0.05$, −L rats have significantly more rhodopsin than +L or normal diet rats; $P < 0.05$.

test. Rod outer segments from rats refed linseed oil and liver fatty acids are the average of two separate determinations. $P < 0.05$ was considered statistically significant.

## RESULTS

### Photoreceptor Cell Loss Occurs in Rats Exposed to Intermittent Light

Whole eye rhodopsin levels were measured to determine the effects of diet and intense light exposure on photoreceptor cell survival. A two-way factorial ANOVA for light exposure and diet revealed a significant interaction effect ($P = 0.02$). As shown in Table 2, rhodopsin levels in rats before light exposure (0 hour) were the same for animals fed the purified diet, with or without linseed oil supplementation, and for those fed the nonpurified control diet. Similarly, there were no significant differences in rhodopsin levels among the three dietary groups of unexposed rats maintained in darkness for 2 weeks before sacrifice. However, in all dietary groups, rhodopsin values for the light-exposed animals were significantly lower than in their respective unexposed controls ($P < 0.05$). Furthermore, rats fed the purified diet for 9 to 12 weeks, without linseed oil supplementation (−L), were protected against retinal light damage compared to the +L rats and to those fed the nonpurified control diet. Two weeks after exposure to eight cycles of intermittent light, −L rats recovered 75% of the rhodopsin level in 2-week dark-maintained control animals. In rats fed the −L diet but supplemented once a week with linseed oil (+L), rhodopsin levels were 61% those of control animals. Rats fed the nonpurified control diet recovered 53% rhodopsin. One-way ANOVA for the eight-cycle light treatment indicated that the recovery of rhodopsin in −L rats was significantly greater than for the +L animals or for those fed the control rodent diet ($P = 0.0008$). Rhodopsin values for +L rats and those fed the control diet were not significantly different from each other. For the −L and +L rats exposed to 12 hours of intermittent light, rhodopsin levels were the same as in rats treated with light for 8 hours. After 16 hours of light exposure, however, rhodopsin recovery was less. Although −L rats retained 67% of the control rhodopsin value, +L rats had only 55% rhodopsin, the same as found in the control diet animals. These values were not significantly different.

### Rhodopsin Levels in Light-Exposed Rats Correlates With Photoreceptor Cell DNA

Another group of rats was maintained on diet for 7 weeks and then was exposed to eight cycles of intermittent light. Rhodopsin and photoreceptor cell DNA were measured 2 weeks after intense light treatment

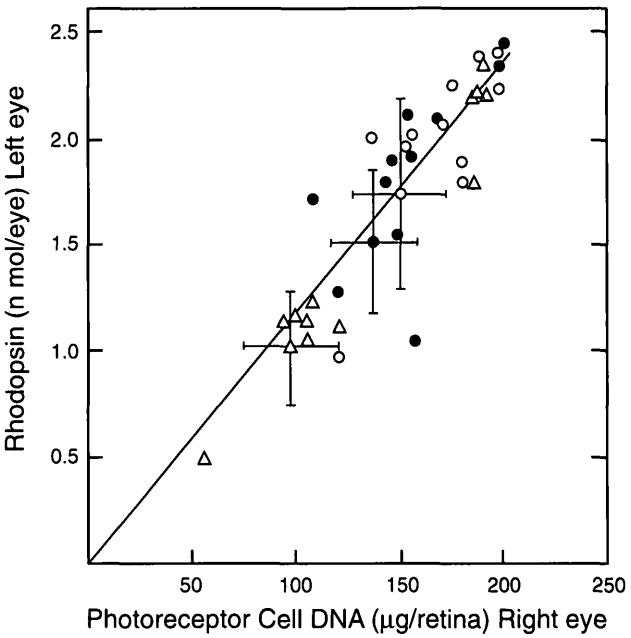


FIGURE 2. Rhodopsin and photoreceptor cell DNA measured in unexposed rats and in light-treated animals 2 weeks after the exposure of rats to eight cycles of intermittent light. Rhodopsin is plotted as a function of photoreceptor cell DNA, measured in the fellow eye ($n = 11$ for each dietary group). The means ± SD ($n = 5$ to 6) for light-exposed rats also are shown. Inner retinal layer DNA (75 μg/retina), measured in 6-month-old Royal College of Surgeons dystrophic rats, was subtracted from total retinal DNA. (△) = control diet rats. For rats fed the purified diet for 7 weeks, (○) = −L and (●) = +L.

(Fig. 2). Data show that photoreceptor cell loss measured by rhodopsin correlates with photoreceptor cell DNA, determined in the fellow eyes of the same rats (Pearson correlation coefficient, $r = 0.8397$; $n = 33$). Also shown in Figure 2 are the mean photoreceptor cell DNA and rhodopsin values for each group of light-exposed rats ($n = 5$ to 6). The respective DNA and rhodopsin values for control diet rats were 52% and 47% of those in the unexposed animals. The +L rats retained 74% DNA and 71% rhodopsin, whereas −L rats had 81% of control for both DNA and rhodopsin. By ANOVA, −L rat DNA and rhodopsin values were significantly higher than those for rats fed the nonpurified control diet ($P < 0.013$ in each case). However, the range of values for +L rats was such that the mean did not differ significantly from −L or control diet animals. In this experiment, rhodopsin was 2.16 ± 0.05 nmol/eye in unexposed control rats. Photoreceptor cell DNA was 185 ± 11 μg/retina; total retinal DNA was 260 ± 15 μg ($n = 16$).

### Rhodopsin Bleaching and Regeneration Are Not Affected by Diet

Because rhodopsin regenerates during the 2-hour dark period between intermittent light exposures and

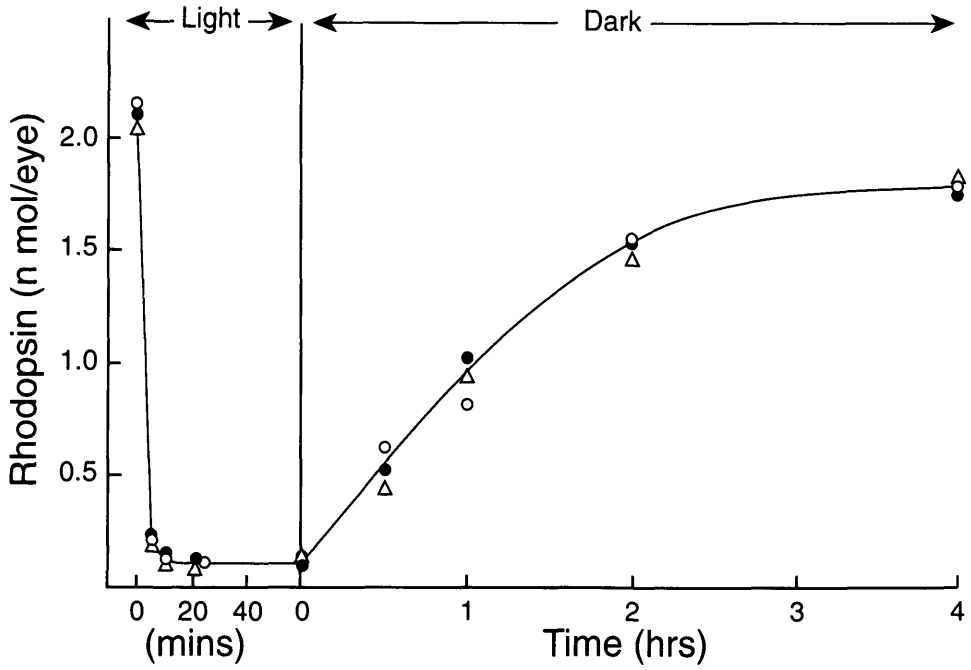


FIGURE 3. Average rhodopsin values measured in control diet rats (△) or in rats fed the purified diet for 12 weeks (○ = −L and (●) = +L) after various times in light or darkness. Rats from each of the dietary groups were exposed simultaneously to light in the same chamber. Values shown are the average of four separate determinations; standard deviations were ±10% of the mean.

because different levels of rhodopsin could affect light damage susceptibility, we measured its rate of bleaching and regeneration. As shown in Figure 3, there were no dietary effects on the bleaching or regeneration characteristics of rhodopsin. In rats maintained for 12 weeks on the −L, +L, or control diet, rhodopsin levels were nearly identical and were greater than 2 nmol/eye. There were no differences in the rate of rhodopsin bleaching, measured after 5, 10, or 20 minutes of light exposure. After 60 minutes of light treatment, rhodopsin levels were 0.12 nmol/eye in all three dietary groups. Small differences in the rate of rhodopsin regeneration were found in light-exposed rats after 30 or 60 minutes in darkness, but these were not significant. After 2 hours in darkness, rhodopsin levels in all three dietary groups were 70% of the unexposed control; rhodopsin levels were greater than 80% of control after 4 hours of regeneration.

## Docosahexaenoic Acid Levels in Retina and Liver Are Affected by Diet

The fatty acid profiles of ROS membranes were determined to characterize the changes associated with feeding rats an 18:3n-3-deficient diet. As expected, ROS from −L rats had lower levels of DHA than found in +L animals or in rats fed the control diet (Table 3). The −L rats had 42 mol% DHA in their ROS, a level significantly lower than for the other dietary groups. The decrease in ROS DHA was also associated with a significant increase in ROS-22:5n-6 in the −L animals. DHA levels were 51.7% and 50.4 mol% for the +L and control diet rats, respectively. However, ROS 22:5n-6 in the +L rats was significantly greater than in control diet animals. By ANOVA, the differences in fatty acid levels were found to represent significant differences ($P < 0.004$). Calculating from the respective levels of 22:5n-6 and DHA, ROS from the +L animals had a three times greater ratio than control diet rats. The ROS 22:5n-6/DHA ratio in −L rats was 11 times higher than in control diet animals and three times higher than in +L rats. Despite these differences, the total unsaturated fatty acid content in all ROS preparations was nearly identical and >60 mol%. As judged by the low cholesterol contents in these preparations and their lipid phosphate levels, ROS purity and yield were also the same for each of the dietary groups of rats.

A comparison of the fatty acid profiles in liver reveals that feeding rats an 18:3n-3-deficient diet results in a systemic effect on the levels of 22:5n-6 and DHA. Although their levels in liver are lower than in retina, rats fed the purified diet had different levels of 22:5n-6 and DHA than control diet animals (Table 4). The −L rats had a 10 times greater level of 22:5n-6 and an eight times lower level of DHA than control diet animals. Intermediate levels of the same fatty acids were present in livers from +L rats. In all rats, liver 18:3n-3 levels were <0.1 mol%. This may be because of its conversion into long-chain n-3 unsaturates, which is known to occur in mammalian liver.[18,32]

## Dietary Influences on Retinal Damage From Hyperthermic Light Exposure

Rats from the three dietary groups were treated with intense light at an elevated body temperature to accelerate the rate of retinal damage.[29] After hyperthermic treatment, the rats were maintained at ambient temperature in darkness for 2 weeks and then were used for rhodopsin determinations. Other animals, unexposed and unheated (0 hour) were killed at the begin-

TABLE 3. Major Fatty Acids in Band I Rod Outer Segments

| Fatty Acid | Nonpurified Control Diet (8) | Purified Diet | |
|---|---|---|---|
| | | −Linseed Oil (10) | +Linseed Oil (10) |
| 16:0 | 13.3 ± 1.9 | 12.3 ± 1.1 | 11.7 ± 1.1 |
| 18:0 | 22.7 ± 3.0 | 23.5 ± 1.9 | 23.0 ± 1.8 |
| 18:1 | 4.9 ± 1.2 | 3.8 ± 1.0 | 3.0 ± 1.0* |
| 20:4n-6 | 4.1 ± 0.7 | 4.8 ± 1.0 | 4.2 ± 0.7 |
| 22:5n-6 | 1.1 ± 0.4 | 10.3 ± 1.5* | 3.9 ± 1.6* |
| 22:6n-3 | 50.4 ± 3.5 | 42.0 ± 2.8* | 51.7 ± 2.5 |
| 22:5/22:6 ratio | 0.022 | 0.245 | 0.075 |
| Total unsaturated | 60.5 | 60.9 | 62.8 |
| Cholesterol (mol/dl) | 9 ± 6 | 10 ± 5 | 10 ± 4 |
| $PO_4$ (nmol) | 242 ± 48 | 234 ± 63 | 242 ± 63 |

Results are the mean ± SD (mol/dl) for ($n$) separate determinations of rod outer segments isolated from the 32/37% interface of discontinuous sucrose gradients. Rats were fed their respective diets for 9 to 12 weeks.

* Using analysis of variance followed by Tukey's procedure 18:1 was significantly lower than in control diet rats, 22:5n-6 significantly higher than control, 22:6n-3 significantly lower than control or for +L rats ($P < 0.05$).

ning of the experiment to establish baseline rhodopsin values. As shown in Table 5, intense light-exposed −L rats had significantly higher rhodopsin levels than either the +L or control diet animals ($P = 0.0154$). Compared with the rhodopsin levels in hyperthermic dark-maintained animals, −L rats recovered 78% rhodopsin, and the +L rats and control diet animals recovered 53% and 51%, respectively. There were no significant differences in rhodopsin levels for the unexposed and unheated 0-hour rats from all three dietary groups. Similarly, rats from all three dietary groups, heated in the dark and then maintained for 2 additional weeks in darkness, had nearly identical rhodopsin levels.

## Linolenic Acid Repletion Alters Retinal Light Damage and Rod Outer Segment Fatty Acids

Based on the differences in retinal light damage between the −L and +L rats, another group of −L animals was given multiple doses of linseed oil to replenish ROS DHA. The time course of DHA repletion was determined, along with light damage susceptibility. Hyperthermic light exposure was chosen because of its short duration compared to intermittent light (2 versus 24 hours or more) and the potential for increased DHA uptake during the longer exposure times. The results of light exposure are shown in Figure 4. As expected, 10-week-old −L rats retained high levels of rhodopsin after hyperthermic light exposure on day 0 or after 20 additional days on the −L diet (13 weeks), whereas +L rats had significantly lower levels of rhodopsin after light treatment at the same times ($P = 0.0001$; two-way ANOVA). When −L rats were given 3 doses of linseed oil per week and then were exposed to light, there were remarkable changes in the extent of retinal light damage. For animals exposed to light 2 days after the start of linseed oil treatment, rhodopsin recovery was nearly the same as found in the −L rats. One-way ANOVA revealed that the 2-day rhodopsin value did not differ from the values found for the 10- and 13-week-old −L rats but that it was significantly higher than the values for +L animals ($P = 0.0049$). However, light exposure 6 days after the start of linseed oil treatment resulted in a rhodopsin level that was the same as in +L animals. This value was significantly lower than for rats on the second day of treatment ($P = 0.0017$). A similar pattern of rhodopsin recovery was found in rats exposed to light 10 or 20 days after the start of linseed oil treatment. These rhodopsin values were also significantly lower than the 2-day value and the value for 13-week-old −L rats. However, the 6-, 10-, and 20-day linseed oil-treated rat rhodopsin values were not significantly different than those for the 10- and 13-week-old +L rats. Figure 4 also shows that multiple doses

TABLE 4. Major Fatty Acids in Rat Livers From Rats Fed Various Diets

| Fatty Acid | Control Diet | Purified Diet | |
|---|---|---|---|
| | | −Linseed | +Linseed |
| 14:0 | 0.3 | 0.5 | 0.6 |
| 16:0 | 23.3 | 23.9 | 25.1 |
| 16:1 | 1.0 | 3.5 | 3.7 |
| 18:0 | 16.7 | 15.5 | 15.5 |
| 18:1 | 9.2 | 13.2 | 13.1 |
| 18:2n-6 | 21.2 | 15.3 | 14.9 |
| 18:3n-3 | — | — | — |
| 20:4n-6 | 21.3 | 23.3 | 21.4 |
| 22:4n-6 | 0.6 | 0.8 | 0.7 |
| 22:5n-6 | 0.3 | 3.1 | 1.1 |
| 22:6n-3 | 6.4 | 0.8 | 4.1 |

Values are the average (mol/dl) of two separate determinations.
— = < 0.1 mol/dl.

TABLE 5. Rhodopsin Levels in Rats Exposed to Light Under Hyperthermic Conditions

| *Heat + Light Exposure* | *Nonpurified Control Diet* | *Purified Diet* | |
|---|---|---|---|
| | | *+Linseed* | *−Linseed* |
| 0 hour unexposed (ambient) | 1.77 ± 0.06 (4) | 1.82 ± 0.08 (4) | 1.88 ± 0.16 (4) |
| 2 hours at 34.5°C + 2 weeks dark | 1.13 ± 0.37 (6) | 1.20 ± 0.24 (7) | 1.74 ± 0.44* (6) |
| Dark 2 hours at 34.5°C + 2 weeks dark | 2.22 ± 0.07 (5) | 2.25 ± 0.07 (3) | 2.28 ± 0.13 (3) |

Results are the mean ± SD (nmol/eye) for ($n$) pairs of eyes from rats fed the diets indicated for 7 weeks. Rats treated at an elevated temperature were preheated in the dark for 2 hours before either 2 hours of light or 2 additional hours in darkness. These rats were then maintained for 2 weeks in darkness, at ambient temperature, before rhodopsin measurement.
* Values significantly higher than for +L or control diet rats, $P = 0.0154$ (analysis of variance followed by Tukey's HSD procedure).

of linseed oil had no major effect on rhodopsin levels in control rats maintained in darkness at the elevated temperature.

The time course for changes in the major fatty acids of ROS from 18:3n-3 replenished rats is shown in Figure 5. The data in panel A show that intubation of −L rats with linseed oil resulted in an increase in the DHA content of ROS but that the increase first appeared 10 to 14 days after the start of repletion. Although ROS DHA in 20-day replenished rats was similar to the level found in rats supplemented with linseed oil from weaning (+L, 13 weeks), for the first 10 days, DHA levels were nearly the same as in 10-week-old −L animals. During the same 10-day period, ROS 22:5n-6 levels decreased by 50%; after 20 days, the decrease was almost 70%. The ratio of 22-carbon unsaturates in ROS was calculated from the relative amounts of 22:5n-6 and DHA and is shown in Figure 5B. For 10-week-old −L rats, the ROS 22:5n-6/DHA ratio was 0.225, a value similar to that found in other −L rats (Table 3). Rod outer segments from rats treated with linseed oil for 2 days had a 22:5n-6/DHA ratio of 0.210. On the sixth day of repletion, the ROS fatty acid ratio was 0.185; on day 10, it was 0.142, and on day 14 it was 0.097. In animals replenished for 20 days, the ROS 22:5n-6/DHA ratio was 0.058; it was 0.040 in the 13-week-old +L rats. Rod outer segment arachidonic acid (20:4n-6) levels were similar in the −L, +L, and 18:3n-3 replenished animals. During the same 20-day period, the levels of palmitic acid and stearic acid (16:0, 18:0), exhibited changes that appeared to correspond to the periods of linseed oil treatment. This was especially true for 18:0, which increased during the three intubation periods and decreased during the periods between. The average cholesterol content of ROS preparations from the linseed oil-treated rats (11.5 mol%) and the yields of lipid phosphorus (270 nmol) were similar to the values for the −L or +L rats shown in Table 3.

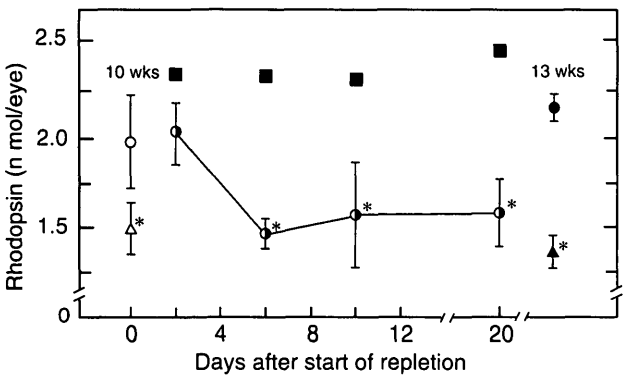


FIGURE 4. Rhodopsin levels in −L and +L rats and in rats given raw linseed oil three times per week, measured 2 weeks after 2 hours of hyperthermic light treatment. Experimental animals were preheated in darkness for 2 hours at 34.5°C and then either treated for 2 hours with light or kept in darkness for 2 additional hours. Values are the mean ± SD for $n = 3$ pairs of eyes from 10-week-old rats fed the purified for 7 weeks. (○) = −L and light exposed; (△) = +L and light exposed; (◑) = 10-week-old −L rats given linseed oil 3 times per week and then exposed to light; (■) −L rats given linseed oil and treated at 34.5°C for 2 hours in darkness. Thirteen-week-old (●) −L rats and (▲) +L rats. *By analysis of variance, rhodopsin values were found to be significantly lower than for −L rats refed linseed oil for 2 days or the 13-week-old −L rats.

## Morphologic Changes Associated With Diet and Intense Light Exposure

There appeared to be no detectable morphologic effect in animals fed either the nonpurified control diet or the purified diet, with or without linseed oil supplementation, as long as the rats were not exposed to intense light (data not shown). However, +L animals exposed to nine cycles of light had slightly more disorganization of the photoreceptor cell nuclear layer (Fig. 6C) than the control diet animals (Fig. 6A) or

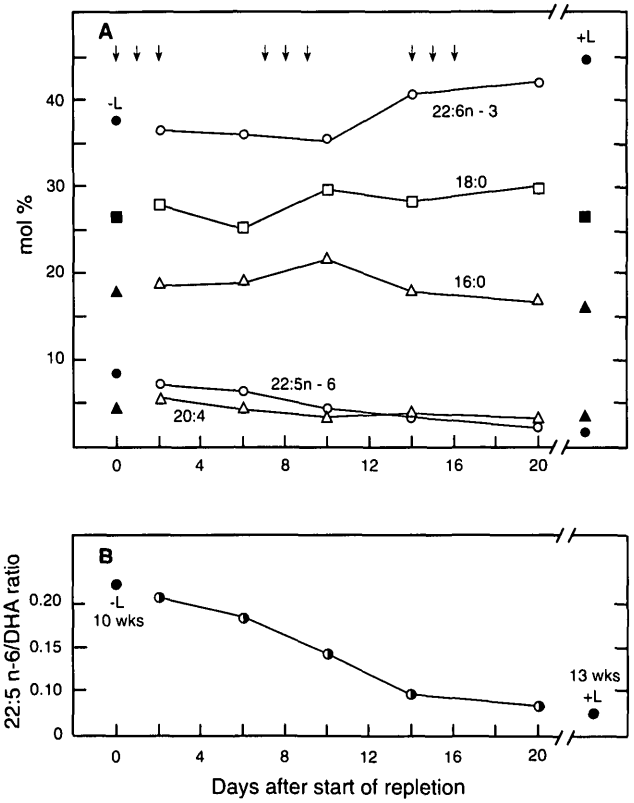


FIGURE 5. (A) Rod outer segment fatty acids in rats fed the purified diet for 7 weeks and then given 1 ml linseed oil (*open symbols*) at the times indicated (↓). Average (mol%) composition is shown, and individual fatty acids are indicated. Day O values for 10-week-old −L rats (*filled symbols*). Values on the far right are for 13-week-old +L rats fed the purified diet, with weekly supplements of linseed oil from weaning. Average of two separate determinations for rod outer segments from three animals, performed in duplicate. (B) Ratio of 22:5n-6/DHA calculated from the data shown in A. (●) = −L and +L at 10 and 13 weeks, respectively. (◑) = refed linseed oil.

the −L rats (Fig. 6B). Both groups of animals fed control diet (Fig. 6A) and the −L rats (Fig. 6B) showed vacuolization of photoreceptor cell terminals within the outer plexiform layer (OPL). The pattern of vacuolization gave a "scalloped" appearance to the interface of OPL with the adjacent outer nuclear layer. Damage to the photoreceptor cells and the retinal pigment epithelium (RPE) occurred under all feeding regimens when animals were exposed to intermittent light followed by a 2-week dark recovery period. However, the extent of the damage varied throughout the retina. Damage was evident in the superior hemisphere but not in the inferior hemisphere. For example, the area approximately 700 μm from the optic nerve in the superior nasal retina from −L rats showed only minimal damage to the photoreceptor outer segments (Fig. 6D), whereas a more peripheral area (3000 μm from the optic nerve) of the same retinal section exhibited extensive damage to the outer nuclear layer. Only a few rows of photoreceptor nuclei were evident with apparent degeneration of all inner segments and outer segments (Fig. 6E).

The most extensive damage occurred in +L animals exposed to nine cycles of light, followed by a 2-week dark recovery period (Fig. 7). The damage varied throughout the retina, ranging from unremarkable morphology in the inferior nasal quadrant (Fig. 7A) to degeneration of all but three to four rows of photoreceptor nuclei and a few scattered inner segments in the central superior nasal quadrant (Fig. 7B) to degeneration of all but a few isolated photoreceptor cell nuclei in the mid-peripheral superior nasal region (2000 μm from the optic nerve) (arrows, Fig. 7C). When the morphology in Figure 7 is compared to that for −L animals exposed to nine cycles of light followed by a 2-week dark recovery period, the most striking difference is that all the photoreceptor cells degenerated in the superior nasal quadrant of the retinas from +L animals. In general, the damage was more extensive in the superior peripheral retina, midway between the optic nerve and the ora serrata, than in the central retina. The superior retina was more damaged than the inferior retina in nasal and temporal quadrants. Because the changes observed in the +L and −L rats may represent individual variations, a morphometric study is in progress using wholemounted retinas to quantitate regional damage.

At the ultrastructural level, minimal damage was observed in the outer retina when −L rats (Fig. 8A) were exposed to nine cycles of light. Outer segments appeared disorganized in the region beneath RPE. A few large vacuoles were present between RPE cells (asterisk, Fig. 8A), but the remainder of the RPE cytoplasm appeared relatively normal. Extensive outer segment disruption was apparent in +L animals exposed to nine cycles of light (Fig. 8B). Increased numbers of phagosomes were apparent in the RPE, and vacuolization occurred between adjacent RPE cells. The synaptic terminals of photoreceptor cells were swollen (asterisk, Fig. 8C), and pyknotic nuclei were evident in the outer nuclear layer (white arrows, Fig. 8C).

When −L animals were refed linseed oil for 3 weeks before they were exposed to hyperthermic conditions in darkness, their retinas were almost normal in appearance (Fig. 9A). If, however, they were exposed to even 2 hours of light at this elevated temperature, extensive disorganization occurred in the inner, and especially in the outer, segments (Fig. 9B). In both cases, swelling occurred in the photoreceptor terminals located in the OPL.

## DISCUSSION

This study shows that diet-induced modifications of ROS fatty acids are associated with different degrees of retinal light damage. Whether light-induced photo-

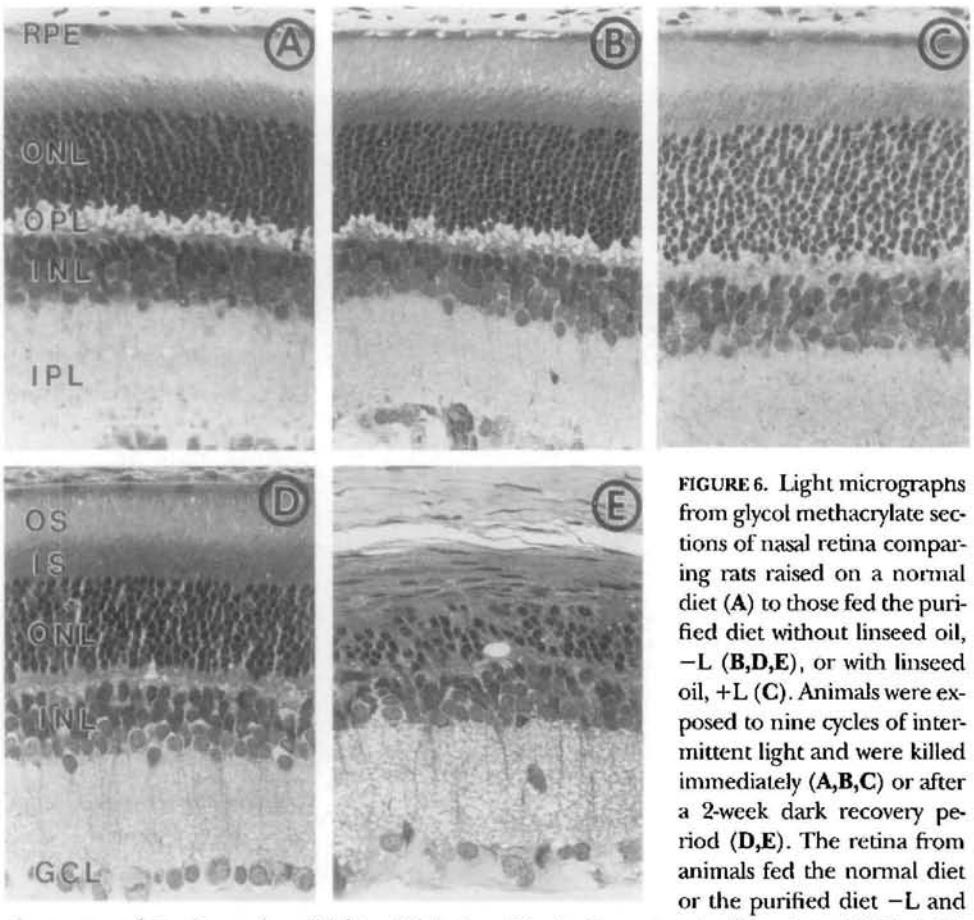


FIGURE 6. Light micrographs from glycol methacrylate sections of nasal retina comparing rats raised on a normal diet (**A**) to those fed the purified diet without linseed oil, −L (**B,D,E**), or with linseed oil, +L (**C**). Animals were exposed to nine cycles of intermittent light and were killed immediately (**A,B,C**) or after a 2-week dark recovery period (**D,E**). The retina from animals fed the normal diet or the purified diet −L and then exposed to nine cycles of light exhibited swelling in the outer plexiform layer (OPL; **A,B**), which gave the layer a scalloped appearance. The swelling in the OPL was less pronounced in animals fed the purified diet, +L (**C**). The outer nuclear layer appears less compact in the +L animals (**C**). All animals exposed to nine cycles of light followed by a 2-week dark recovery period showed normal histology in the inferior retina and in the central superior retina close (700 $\mu$m) to the optic nerve (**D**), as well as areas of photoreceptor degeneration with reduced numbers of photoreceptor nuclei in the outer nuclear layer (ONL) and complete degeneration of both inner (IS) and outer (OS) segments in the mid-peripheral retina (approximately 3000 $\mu$m from the optic nerve (**E**). GCL = ganglion cell layer; INL = inner nuclear layer; IPL = inner plexiform layer; RPE = retinal pigment epithelium. Magnification, ×268).

receptor cell loss was measured by the level of rhodopsin or DNA in the retina or was determined morphologically, rats fed the −L diet exhibited protection against light damage. These findings confirm earlier studies demonstrating a reduction in retinal light damage in rats with low levels of ROS or retinal DHA,[22–25] and they extend that work by describing the same effect in rats treated with paradigms of intense light that accelerate retinal damage. Thus, whether continuous,[22–25] intermittent, or hyperthermic light treatments are used, diets deficient in the 18:3n-3 precursor of DHA have a protective effect.

Retinal light damage resulting from long-term continuous light exposure is known to be associated with an increase in conjugated dienes and a decrease in ROS DHA levels.[20] Furthermore, antioxidant treatment of rats before light exposure reduces retinal damage, regardless of whether the animals are fed 18:3n-3-deficient diets.[22,23] Although a diet-induced decrease in a readily oxidizable polyunsaturated fatty acid such as DHA could impart protection against light damage, the current findings suggest that an overt decrease in fatty acid oxidation is not the reason for the protective effect. In this study, the total unsaturated fatty acid contents of ROS were nearly the same for the −L, +L, or control diet rats (Table 3), whereas the extent of light damage was different among the three dietary groups (Tables 2, 5). In an earlier study with 18:3n-3-deficient diet rats, we found a selective loss of ROS 22:5n-6 and DHA after exposure to continuous light for 24 hours.[22] The light-induced decrease in DHA was greater than the decrease in 22:5n-6 levels, and ascorbate treatment of the rats reduced the losses of both fatty acids. Taken together, this suggests that the two fatty acids may be differentially susceptible to oxidation. However, at this time, it is unclear whether these changes were causative or were the result of prolonged, continuous light exposure.

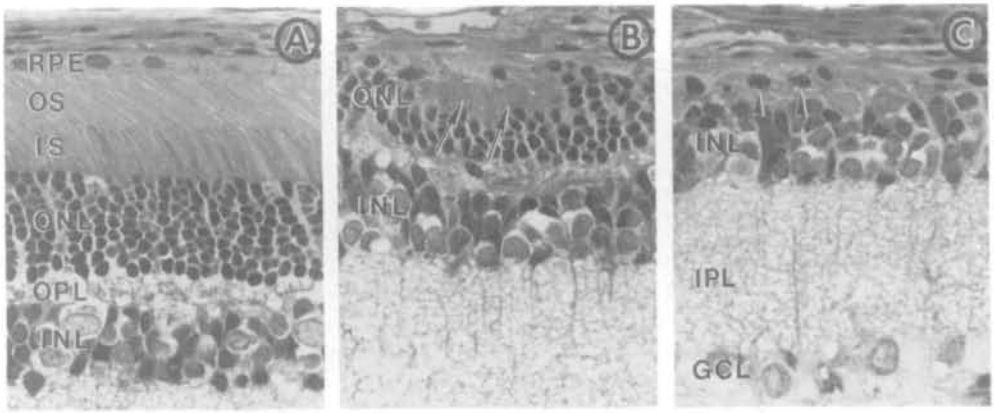


FIGURE 7. Light micrographs from a glycol methacrylate section of the nasal retina from +L animal exposed to nine cycles of light followed by a 2-week dark recovery period. The intensity of damage to the outer retina varied from normal histology in the inferior nasal quadrant (**A**) to the central superior nasal quadrant, approximately one fifth the distance between the optic nerve and the ora serrata (750 $\mu$m), where a few layers of photoreceptor nuclei remain adjacent to few inner segments (*arrows*, **B**). In the mid-peripheral superior nasal region, approximately 2000 $\mu$m from the optic nerve, only a few isolated pyknotic photoreceptor nuclei remain (*arrows*, **C**). Magnification, ×424.

Ten-week-old deficient-diet rats given multiple doses of linseed oil had ROS DHA levels that were the same as in −L rats for the first 10 days, but light damage susceptibility increased 6 days after the start of repletion (Fig. 4). However the linseed oil refed rats exhibited a time-dependent decrease in the 22:5n-6 content of their ROS and a linear decrease in the ratio of 22:5n-6 to DHA. Ten days after the start of repletion, the 22:5n-6/DHA ratio was 37% lower than in −L rats; after 20 days, it was 74% lower and was similar to the ratio in the 13-week-old +L rats (Fig. 5B). Light damage susceptibility, therefore, may depend on the relative levels of 22:5n-6 and DHA in ROS and the kinetics of their changes in the refed animals. Although the 22:5n-6/DHA ratio was clearly lower 10 to 20 days after the start of linseed oil treatment, it was only 18% lower than in −L rats after 6 days of linseed oil treatment. On the other hand, if one assumes a linear increase in ROS DHA during the period of linseed oil refeeding, the 22:5n-6/DHA ratio after 6 days would actually be lower than shown in Figure 5B. Whether this change represents a threshold for retinal light damage will require further study. Changes in ROS 16:0 and 18:0 also were found in the replenished rats and coincided with the days of linseed oil administration. Linseed oil contains >50 mol% 18:3n-3, as well as a variety of saturated and other 18-carbon unsaturated fatty acids (Table 1).[19] Although radioactive 18:3n-3 given to frogs[32] or rats[33] is partially degraded, with label appearing in 16:0 in the retina, it is likely that the increased levels of 18:0 and 16:0 in linseed oil-treated rats resulted from other fatty acids in the oil.

In this study, −L rats, with a high ROS 22:5n-6/DHA ratio, were protected against light damage, whereas +L and control diet animals had much lower ratios and incurred more extensive damage. In rats fed an 18:3n-3-deficient diet, retinal 22:5n-6 levels were higher than in rats fed an 18:3n-3-rich diet,[24] and light-induced ROS disruption was less extensive. Immediately after 24 hours of continuous light exposure, we found similar changes in ROS morphology in the same three dietary groups of rats used in this study.[34] However, using intermittent light exposure, the extent of damage was different when retinas were examined immediately after light exposure (Figs. 6A to 6C) or 2 weeks later (Figs. 6D, 6E, 7). The damage was always more extensive when the animals, regardless of diet, were exposed to intermittent light followed by the 2-week dark period. These findings can be explained by the progression of photoreceptor cell damage and cellular death, which occurs in the retina for a period of days after intense light exposure.[29]

Both the variability in damage severity from animal to animal and the regional differences were underscored by an examination of the nasal and temporal inferior and superior quadrants from the retina of each animal. The +L animals showed total degeneration of photoreceptor cells starting in the superior mid-periphery (3000 $\mu$m from the optic nerve), whereas −L rats were protected against experimental light damage. The inferior hemisphere and central region of the superior hemisphere from these retinas appeared relatively normal (Fig. 6D), whereas the more peripheral region of the same retina showed the outer nuclear layer reduced to four to five rows of photoreceptor nuclei (Fig. 6E). Most damage to photoreceptor cells, regardless of diet, was in the peripheral retina, approximately midway between the optic nerve and the ora serrata. Our data showed that the

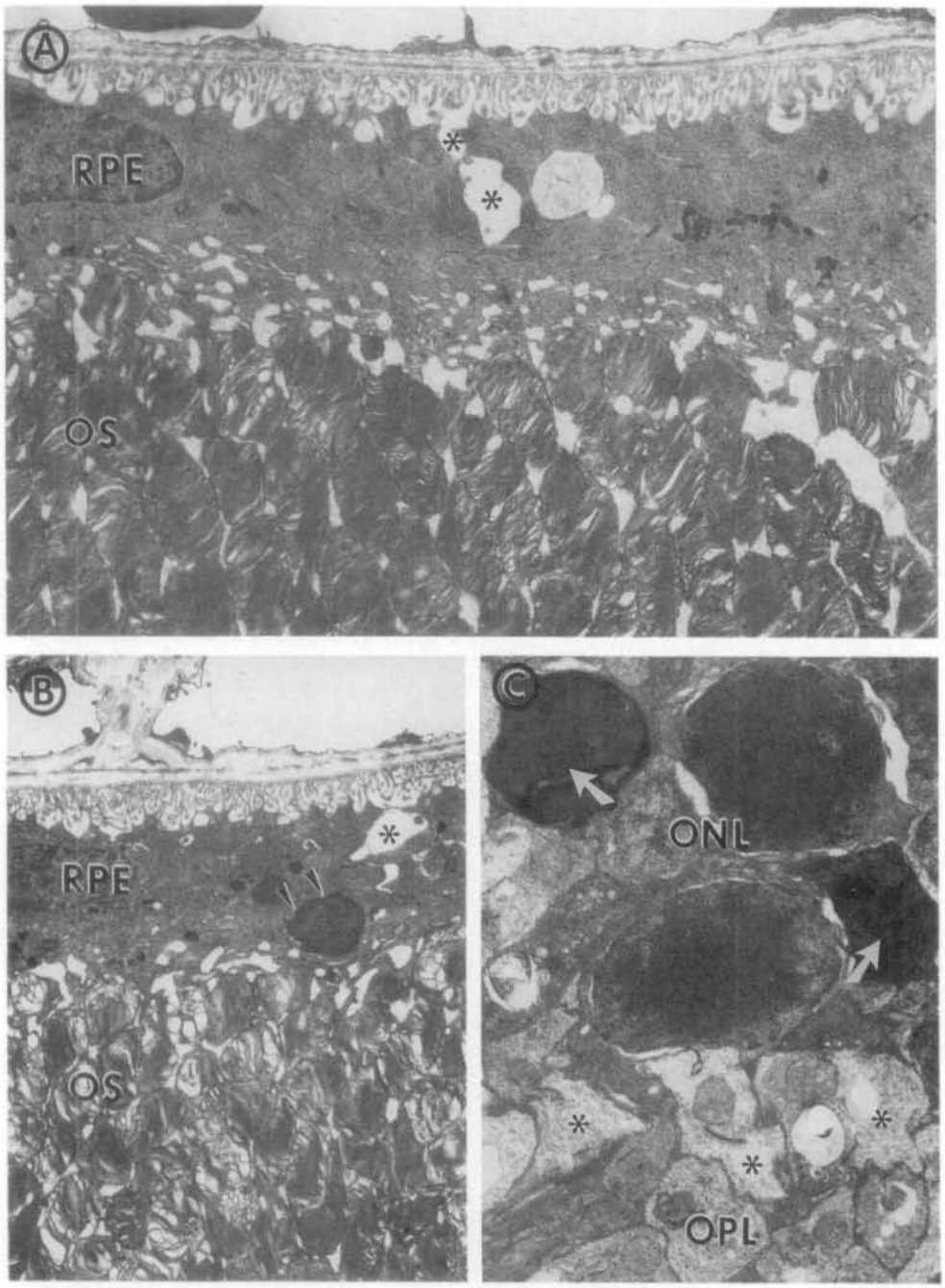


FIGURE 8. Electron micrographs from the outer retina of rats exposed to nine cycles of light and fed the purified diet (−L, **A**; same block as Fig. 6B) compared to rats fed a purified diet (+L, **B,C**; same block as Fig. 6C). Although there was some disorganization in the outer segments of the −L rats, increased swelling in the outer segments existed in +L rats (**B**). There was swelling at the extracellular junction between adjacent RPE cells in both conditions (*) (**A,B**). Phagosomes (*arrowheads*, **B**) were often present in the RPE of +L animals exposed to nine cycles of light. The OPL exhibited increased swelling of the photoreceptor terminals (*) (**C**), as well as numerous darkly stained, pyknotic nuclei in the outer nuclear layer (*white arrow*, **C**). Magnifications, ×5116 (**A**); ×3168 (**B**); ×4864 (**C**).

most severe photoreceptor damage consistently was located in the mid-to-far peripheral superior retina. These results are in contrast to earlier research[35,36] suggesting that the most severe damage was localized to photoreceptor cells in the central superior retinas of rats exposed to constant light. The central superior region coincides with that found to have longer outer segments and the highest rhodopsin levels.[37] A more extensive morphometric study using retinal wholemounts is planned to resolve differences between our

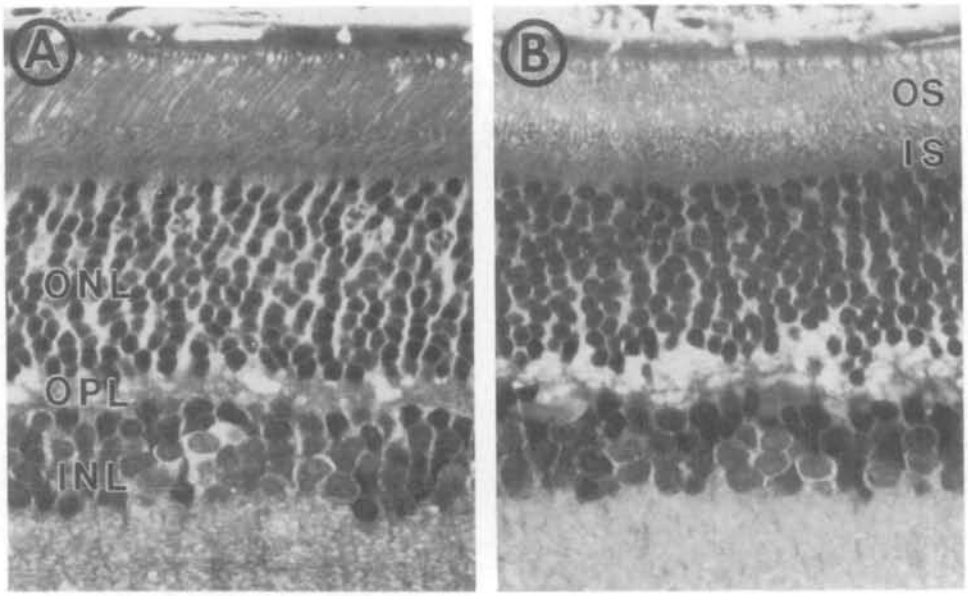


FIGURE 9. Light micrographs from animals fed the deficient diet for 10 weeks, followed by a 3-week period of linseed oil supplementation. (**A**) Retina from a rat treated in hyperthermic conditions for 4 hours in darkness. (**B**) Retina from a rat treated in hyperthermic conditions for 2 hours in darkness, followed by 2 hours of light. Magnification, ×617.

findings and earlier published reports of histopathology in the light-damaged retina.

A consistent morphologic finding was swelling of the outer plexiform layer in the retina of all animals exposed to intermittent light, regardless of dietary regime. Although our findings with +L and −L rats represent individual morphologic variations, similar swelling was evident in other studies, illustrating the effect of high levels of illumination of the retina.[38] The outer synaptic layer may be susceptible to light damage because DHA is relatively abundant in synaptic membranes. It has been shown that injected, tritiated DHA accumulates in the synaptic terminals of photoreceptor cells. It is likely that DHA is incorporated into the membranes of synaptic vesicles as they are formed.[16] Whether the morphologic changes in the OPL of the rats used in this study were simply manifestations of intermittent light exposure[28] or whether the ratio of 22:5n-6/DHA in synaptic membranes is altered by diet are open questions.

At this time, the mechanism by which diet-induced changes in ROS fatty acids protect the retina against light damage is unknown. From a consideration of retinal chromophore levels in the three dietary groups of rats, we found no differences in the respective preexposure levels of rhodopsin or in the 2-week-dark control levels (Tables 2, 5). These findings are in agreement with earlier studies[19,22] and indicate that a difference in endogenous rhodopsin levels is not the reason for the different light damage susceptibilities. In recent studies, rats fed an 18:3n-3-deficient safflower oil diet[24,39] or a diet enriched with fish oil[26] actually had higher levels of rhodopsin than animals on an 18:3n-3-rich soybean oil diet, but light damage was greater in the soybean oil-fed rats. Similarly, Koutz et al[25] found that linseed oil-diet rats reared in a 1 lux cyclic light environment incurred more light damage than rats fed an 18:3n-3-deficient safflower oil diet or a diet rich in saturated fatty acids but deficient in n-3 and n-6 fatty acids. Bush et al[39] also found that rats with lower retinal DHA regenerated rhodopsin more slowly than rats with higher levels. In this study, no significant differences were found between the respective rates of rhodopsin bleaching or regeneration (Fig. 3). Additional work will be needed to resolve the differences between the two studies.

One way by which changes in ROS lipids might alter light-damage susceptibility is the potential to affect subsequent steps in the visual transduction cascade. For example, bovine[40,41] and rat[42] ROS disk membranes contain less cholesterol than is found in the plasma membrane, and a high level of membrane cholesterol reduces the activation of ROS cyclic guanosine monophosphate phosphodiesterase.[41] In model membranes containing rhodopsin, cholesterol and the degree of fatty acid unsaturation in the sn-2 position of glycerophospholipids were found to affect bilayer structure and the meta I to meta II equilibrium.[43] In agreement with Bush et al,[39] we also found no difference in the rate of metarhodopsin II formation in ROS from +L and −L rats (Mitchell D, Litman BJ, personal communication, 1995), but the effects of altered membrane fatty acids on other steps in the visual transduction cascade have not been studied. Numerous ROS proteins are acylated or isoprenylated, including rhodopsin,[44] transducin, and cyclic guanosine monophosphate phosphodiesterase.[45] These lipid residues are thought to serve as membrane anchors and to maximize protein interactions.[44] Recently, Niebylski and Salem[46] found that phosphatidylcholine-containing sn-2 22:5n-6 had significantly lower transition enthalpy and entropy values than phosphatidylcholine with sn-2 DHA. This suggests that the gel-to-liquid crystalline state of membranes may be affected

dramatically by a lipid having only one less carbon–carbon double bond. The potential effects of 22:5n-6 on membrane structure[46] and the membrane-binding properties of ROS proteins containing covalent lipids could help to explain the differences in light damage between +L and −L rats.

Another possible way that 22:5n-6 and DHA may influence retinal light damage is their potential to affect the flux of fatty acid derivatives formed by cyclooxygenase or by lipoxygenase. Both enzymes are present in the retina, and both typically use a variety of polyunsaturated 20-carbon fatty acids.[47] In fact, Remé et al[26] suggest that the n-3:n-6 ratio of eicosanoids may be the reason for less light-induced disruption in basal ROS from fish oil-fed rats. Recently, Bazan et al[48] reported the presence of a retinal lipoxygenase that uses DHA as a substrate. The docosanoid products are hydroxylated DHA derivatives,[47,48] which may affect retinal function in a manner similar to the better known 20-carbon eicosanoids. Although the functions of docosanoids are unknown, it is tempting to speculate that a change in the n-3:n-6 ratio of 22-carbon fatty acids could alter the normal balance of docosanoid synthesis in the retina. This might affect the ionic balance in photoreceptors in the same way that n-3 and n-6 fatty acids alter calcium channels in cardiac tissue.[49] Given the concurrence of results from a number of laboratories showing protection against light damage in rats fed diets that alter ROS 22:5n-6 and DHA, the membrane structural characteristics and metabolic properties of these fatty acids deserve additional study.

### *Key Words*

docosahexaenoate, light damage, rat, retina, rod outer segment

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