The Protective Effect of Ascorbate in Retinal Light Damage of Rats

Daniel T. Organisciak,* Hih-min Wong,* Zong-Yi Li,† and Mark O. M. Tso†

Cyclic light and dark-reared rats were exposed to intense visible light for various periods and then rhodopsin-measured following recovery in darkness for up to 14 days. Animals were injected with ascorbic acid or ascorbate derivatives at various doses prior to light exposure in green Plexiglas chambers. The results show that ascorbic acid administration elevates retinal ascorbate and reduces the loss of rhodopsin and photoreceptor cell nuclei resulting from intense light. When given in comparable doses, L-ascorbic acid, sodium ascorbate, and dehydroascorbate were equally effective in preserving rhodopsin. The ascorbate protective effect in the retina is also dose dependent in both cyclic light and dark-reared rats and exhibits a requirement for the L-stereoisomer of the vitamin. Ascorbic acid is effective when administered before, but not after, light exposure, suggesting that protection from light damage in the retina occurs during the light period. In some experiments, rod outer segments were isolated from rats immediately after light exposure, lipids extracted, and fatty acid composition determined. As judged by the preservation of rod outer segment docosahexaenoic acid in rats given ascorbate, the vitamin may act in an antioxidative fashion by inhibiting oxidation of membrane lipids during intense light. Invest Ophthalmol Vis Sci 26:1580-1588, 1985

The process of retinal photoreceptor cell degeneration resulting from exposure to intense visible light is currently studied in many laboratories with many different animal models. From these studies it is clear that retinal light damage varies with animal species,1-5 with age and light history,6-10 and with genetic and dietary background.11-15 In the rat, rod cell degeneration by light is rhodopsin-mediated, and the magnitude of damage is a function of the extent of rhodopsin bleaching during exposure and the pre-exposure rhodopsin content.1,13,16-18 Thus, dark-reared rats, which have a higher rhodopsin content than cyclic light reared rats13 and a higher opsin packing density in their rod outer segments (ROS),18 are more susceptible to light damage.

Recent studies indicate that peroxidation may be a contributing factor to the process of light-induced rod cell degeneration.19-21 Other evidence indicates that the retina contains a balance of antioxidative enzymes22-23 and high levels of natural antioxidants24-26 which can diminish peroxidative effects in the tissue. In cyclic light and dark-reared rats, intense light exposure does not decrease the levels of α-tocopherol27-29 or glutathione in the retina, but does lead to a loss of ascorbic acid.26 Retinal ascorbate also decreases in the guinea pig retina30 and monkey retina31 following intense light exposure. Recently, it has been shown that ascorbic acid supplementation of rats prior to intense light treatment diminishes photoreceptor cell death.26 Similarly, more extensive rod cell degeneration from light occurs in scorbatic guinea pigs than in ascorbate adequate animals.32 Thus, despite the diversity of animal models employed, ascorbic acid appears to be involved in the process of retinal light damage. We now present biochemical evidence to substantiate the ascorbate protective effect in the rat retina. In this report we also describe some aspects of the temporal sequence of light-induced retinal degeneration in rats.

Materials and Methods

Animals, Light Exposure

Weanling albino rats were obtained from Harlan Inc.; Indianapolis, IN and maintained in either a cyclic light environment, 5 ft cd, 12 hr per day (lights on 8:00 a.m. off 8:00 p.m.) or total darkness for a period of 30-40 days before use. The dark environment was interrupted by less than 30 min per day dim red light for routine animal maintenance. Twenty-four hours
before and just before light exposure, animals were injected IP with L-ascorbic acid (Sigma Inc.; St. Louis, MO) or ascorbic acid derivatives, at doses ranging from 0.062-1.0 gm/Kg body weight. Control animals were injected with the water vehicle. All animals were fed ad libitum with free access to water and were dark adapted for 16-18 hours before exposure to intense visible light. In these studies, male and female rats were used with no apparent differences.

Light exposures were performed in a series of chambers composed of 6" OD circular green Plexiglas (Dayton Plastics; Dayton, OH) (#2092) cylinders surrounded by six 12" circular fluorescent tubes. Light intensity was uniform among the chambers but varied within each from 200-250 ft cd illumination. Rats moved freely within the cylinders during exposure periods and had access to food and water. Generally, three animals were exposed in each chamber simultaneously. Environmental temperature was maintained at 24-26°C by airflow in and around the cylinders. Under these conditions, rectal temperatures were found to be normal for experimental animals. In most experiments, light exposures were for 24 hr with cyclic light-reared rats and for 12 hr with dark-reared rats. However, some light exposures were for longer or shorter time periods. Depending on the measurements to be performed, following light exposure animals were either killed immediately or placed into the dark environment.

Biochemical and Histological Analysis

To optimize the concentration rhodopsin in the eyes of light-damaged rats, animals were maintained in darkness for up to 14 days after light exposure. During the dark recovery period following intense light, necrotic photoreceptor cell material is removed from the eye and rhodopsin maximized in the surviving rod cells. To estimate the percent photoreceptor death in these experimental rats, control (non-light exposed) littersmates were also placed into the dark environment at the same time, and rhodopsin determinations performed simultaneously.

Rhodopsin was measured by detergent extraction of whole eyes enucleated upon death by methods previously described. Briefly, in dim red light, the lens was excised from each eye via a corneal cut and the remaining eye (retina, RPE, choroid, sclera) placed into 1.5 ml of Krebs-Ringer phosphate buffer (KRP) pH 7.4. The tissue was disrupted by vortexing with a siliconized glass rod and treated sequentially with 4% alum followed by two KRP washes. A 1% Emulphogene BC-720 detergent extract of each tissue was then prepared, and the change in optical density (Δ A 500) of each extract determined from the A 500 readings taken before and after bleaching with light. A second detergent extraction of each sample was performed overnight, and the Δ A 500 determined. The concentration of rhodopsin per eye (nmol) was then calculated from the total Δ A 500 of the two extracts, their volumes and an extinction coefficient of 42,000.

In some experiments, the fellow eye of animals used for rhodopsin measurement was enucleated, the lens excised, and tissues placed into fixative. These eyes were fixed overnight in 4% formaldehyde, 1% glutaraldehyde in phosphate buffer, placed into rinsing buffer and sent to Dr. Tso and associates for histological analysis. The number of photoreceptor nuclei were counted in each of the four quadrants at loci 0.178 mm apart. Measurements included retina extending from the posterior pole to the ora serrata. For complete details of the histological procedures see Li, et al.

ROS isolation, lipid extraction, and analytical procedures for fatty acid determinations have been described. These measurements were performed on purified ROS isolated from ascorbate treated and control rats immediately after light exposure periods. In other experiments, retinas were excised from animals and reduced ascorbic acid was determined by high pressure liquid chromatography. For these measurements, the upper phase of retinal lipid extracts washed with 20% v/v water was used.

The use of animals in this investigation conformed to the ARVO Resolution on the Use of Animals in Research.

Results

Light Damage in the Rat as a Function of Pre-exposure Rhodopsin Levels

As measured by the rhodopsin content in the retinas of rats 14 days after exposure to light of constant intensity, photoreceptor cell loss depends on the previous light history of the animals and the duration of exposure. In these studies 50-60 day old rats reared in the cyclic light environment had 1.75 ± 0.15 nmol rhodopsin/eye compared to 2.05 ± 0.2 nmol/eye in dark reared rats (±SD, n = 6). Cyclic light-reared rats exposed to intense light for 12 hr had rhodopsin levels of 2.0 ± 0.3 nmol/eye after 14 days in darkness, while dark reared rats recovered only 0.7 ± 0.3 nmol/eye after a comparable exposure and dark recovery period. In other words, cyclic light reared rats were unaffected by a 12-hr light exposure, but dark reared rats lost 50-65% of their visual cells. Extending light exposure to 24 hr (at a constant 90-95% rhodopsin bleach) resulted in a rhodopsin recovery of 0.9 ± 0.2 nmol/eye in cyclic light reared rats, or about 55% photoreceptor cell loss compared to those exposed for 12 hr. Twenty-four hour-light exposed-dark reared rats recovered only 0.35 ± 0.2 nmol rhodopsin or, less than 20% of the original
rhodopsin level in their eyes. Thus, rats reared in continuous darkness are more susceptible to intense light than cyclic light reared rats.

Photoreceptor Cell Preservation by Various Forms of Ascorbic Acid

In comparison with non-exposed control rats, four different ascorbic acid compounds and the water vehicle were tested for their ability to reduce the effects of intense light exposure. Cyclic light reared rats were given various forms of the vitamin, exposed for 24 hr to intense light, and rhodopsin determined after 14 days of dark recovery. As shown in Table 1, L-ascorbic acid, Na-ascorbate and dehydroascorbic acid reduced the extent of rod cell loss from intense light. Two weeks after exposure, the eyes of these animals had significantly higher levels of rhodopsin than the vehicle treated rats (57–62% vs 38% of control), $P < 0.001$. Table 1 also shows that D-ascorbic acid is ineffective in reducing the extent of retinal light damage. Furthermore, there was no difference between animals treated with the free acid and the sodium salt of ascorbate, suggesting that the ascorbate effect does not arise from an alteration of intestinal pH or osmotic differences between the two forms of the vitamin. In these experiments, dehydroascorbic acid was given at $\frac{1}{2}$ the dose of L-ascorbic acid because of difficulties in dissolving the dehydroascorbate. Yet administration of both the reduced and oxidized form of the vitamin resulted in the same rhodopsin per eye.

Dose Response of Ascorbic Acid

Figure 1 indicates that ascorbic acid given over a broad concentration range is effective in preserving rhodopsin in both cyclic light and dark-reared rats exposed to intense light. In cyclic light-reared rats, an average of 1.5 nmol rhodopsin/eye was recovered from animals given 0.125–1.0 mg ascorbic acid/gm body weight, compared to 0.9 nmol/eye in vehicle-treated rats. At each concentration of ascorbate, the difference between rhodopsin levels in vehicle-treated rats and those given ascorbate was significant at the $P < 0.001$ level. Figure 1 also shows that up to a concentration of 0.25 mg/gm, dehydroascorbic acid is no more effective than an equivalent dose of L-ascorbic acid in preserving rhodopsin.

In dark-reared rats exposed to intense light for 12 hr, ascorbate administration (0.25–1.0 mg/gm) resulted in the recovery of twice as much rhodopsin as in those given the water vehicle (1.2 vs 0.6 nmol/eye $P < 0.001$). A comparison of the two dose response curves in Figure 1 reveals that the minimum dose of ascorbate required to reduce rhodopsin loss in dark-reared rats is two times higher than in cyclic light animals. The shorter exposure period for dark-reared rats was chosen because of the extensive loss of photoreceptor cells in these animals after 24-hr exposures and to approximate 50% rod cell death from light.

Photoreceptor Nuclei Counts in Light-exposed Rats

To correlate rhodopsin recovery with the loss of photoreceptor cell nuclei in dark-reared rats exposed to intense light, the eyes of ascorbate treated animals were processed for histological analysis and rhodopsin extracted from the fellow eyes. As shown in Table 2, fourteen days after a 12 hr-light exposure, the retina of an untreated rat had an average of two photoreceptor cell nuclei per vertical column, while the fellow eye contained 0.5 nmol rhodopsin. Control rat eyes, unexposed to intense light, contain an average of nine photoreceptor nuclei per vertical column and 2.0–2.1 nmol rhodopsin.

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**Table 1. Rhodopsin in ascorbate treated intense light exposed rats**

<table>
<thead>
<tr>
<th>Form</th>
<th>Rhodopsin (nmol/eye)</th>
<th>% of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-light exposed†</td>
<td>2.1 ± 0.1 (10)</td>
<td>100</td>
</tr>
<tr>
<td>Water Vehicle</td>
<td>0.8 ± 0.2 (15)</td>
<td>38</td>
</tr>
<tr>
<td>L-Ascorbic Acid</td>
<td>1.3 ± 0.2(13)</td>
<td>62</td>
</tr>
<tr>
<td>Na+ Ascorbate‡</td>
<td>1.2 ± 0.2(7)</td>
<td>57</td>
</tr>
<tr>
<td>Dehydroascorbic§</td>
<td>1.3 ± 0.2(10)</td>
<td>62</td>
</tr>
<tr>
<td>D-Ascorbic Acid‡</td>
<td>0.8 ± 0.2(12)</td>
<td>38</td>
</tr>
</tbody>
</table>

* Results are the mean ± SD for the number of experiments in parenthesis.
† Non-light exposed control: placed into darkness at the same time as experimental rats and maintained for two wk before rhodopsin determinations.
‡ Dose 0.5 mg/gm body weight injected 2X before light exposure of cyclic light reared rats for 24 hr.
§ Dose 0.25 mg/gm body weight.
|| Value significantly higher than water vehicle at the $P < 0.001$ level.

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**Fig. 1.** Whole eye rhodopsin recovered after two wk in darkness for cyclic light reared rats (---) exposed for 24 hrs to intense light. Results are the mean ± SD of 6–16 separate measurements for rats given L-ascorbic acid (○) or dehydroascorbic acid (□) at the dose indicated. Dark reared rats (-----) given L-ascorbic acid (●) and exposed for 12 hr to intense light ($n = 4–12$ determinations).
rhodopsin in the fellow eyes. On the average, therefore, intense light resulted in a loss of approximately 75% of the photoreceptor nuclei and rhodopsin in the untreated rat. In one ascorbate treated rat (+C), six nuclei per vertical column were found in one eye and 1.0 nmol rhodopsin in the other eye. A second +C rat had 1.7 nmol rhodopsin/eye and an average of 7.4 rod cell nuclei. By linear regression analysis, these results correlate at a value of 0.98. Thus, the administration of ascorbic acid prior to intense light exposure is effective in reducing the damaging effects of intense light in dark-reared rats. Histological measurements of photoreceptor cell nuclei in cyclic light-reared rats exposed to intense light indicate that ascorbate treated rat retinas retain 35% more nuclei than unsupplemented animals.34

Ascorbate Effect on the Rate of Rhodopsin Loss from Light

To measure photoreceptor cell (rhodopsin) loss as a function of the duration of light exposure, rats were exposed to intense light for various periods and rhodopsin determined after 14 days of darkness. As shown in Figure 2, ascorbic acid supplementation reduced the rate of rhodopsin loss from intense light in both cyclic light and dark reared rats. In cyclic light-animals, no difference in the extent of rhodopsin loss was detectable for intense light exposures of up to 12 hr. Compared to 2-wk, dark-adapted, non-exposed control rats (2.1 nmol rhodopsin/eye), rhodopsin recovery in both +C and untreated rats was 100%. Measurable, but nonsignificant, differences in rhodopsin content between cyclic light reared +C and untreated rats were found after 16 hr of intense light. Following 24 hr of light, however, ascorbate treated rats recovered an average of 67% rhodopsin, while untreated animals recovered only 43% rhodopsin (1.4 ± 0.1 vs 0.9 ± 0.3 nmol/eye n = 16, P < 0.001). Although the extent of visual cell death was much greater after 48 hr of exposure, significantly higher levels of rhodopsin were also found in +C rats, compared to untreated animals (0.6 ± 0.1 vs 0.2 ± 0.1 nmol/eye n = 6 P < 0.001). Figure 2 also shows that ascorbic acid supplementation had no effect on the pre-exposure level of rhodopsin in these rats.

Dark-reared rats exposed to intense light exhibit marked rhodopsin loss after only eight hr. At this time, ascorbate supplemented rats recovered 1.4 ± 0.25 nmol rhodopsin/eye compared to 1.05 ± 0.15 nmol in the untreated animals (P < 0.005). After 12–16 hr of light, dark reared +C rats recovered 45–50% rhodopsin, but untreated rats recovered only 25–30% of the level in non-exposed controls. The differences in these values for +C and untreated rats were significant at the P < 0.001 level. The extent of rhodopsin loss in the untreated rats after 24 and 36 hr light was 90% and 100% respectively. At these longer time periods, +C animals recovered at least two times more rhodopsin/eye than untreated rats.

Retinal Ascorbate in Light-exposed Rats

As shown in Table 3, ascorbic acid supplementation of cyclic light and dark-reared rats resulted in retinal ascorbate levels which were greater than in the retinas from non-supplemented animals. Following four hr of light exposure, ascorbate treated rats had 38–41% more ascorbic acid/retina than untreated animals (cyclic light and dark reared respectively). In both types of rats, ascorbic acid levels declined as a function of the duration of light exposure. After a 24-hr exposure, retinal ascorbate in +C cyclic light rats was 34% higher than in untreated rats, but 14% lower than in the +C, four-hr exposed rats. In dark-reared rats exposed for 12 hrs, the levels of retinal ascorbate were 33% and 18% lower than after four hrs of light exposure (+C and untreated).

![Graph showing rhodopsin levels over time](image)

**Fig. 2.** Rhodopsin measured 14 days after light exposure for the times indicated. Cyclic light reared rats (○), plus L-ascorbic acid (O) at 0.5 mg/gm body weight injected the day before and day of light exposure; untreated (●). Dark reared rats (●—●) plus ascorbate (●), untreated (▲). Results are the mean ± SD of from 6–16 separate measurements. All animals were between the ages of 50–60 days.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>No light exposure</th>
<th>12-hr light exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodopsin</td>
<td>Untreated</td>
<td>Untreated</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/eye</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>number of Nuclei</td>
<td>9.4 ± 0.1</td>
<td>9.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>(92)</td>
<td>(90)</td>
</tr>
</tbody>
</table>

Table 2. Mean number of photoreceptor nuclei in dark reared rats exposed to intense light*
In addition, following 24-hr light exposures, the levels of retinal ascorbate in dark-reared rats were significantly lower than in the four-hr light exposed animals. At this time ascorbate was also 46% and 30% lower in dark-reared rat retina than in retinas from cyclic light animals (+C and untreated respectively). Thus, a comparable intense light exposure results in a greater loss of retinal ascorbate in the dark-reared rats than in their cyclic light reared counterparts.

**Photoreceptor Cell Loss during Dark Recovery**

To further define the temporal sequence of rod cell loss from intense light, rhodopsin measurements were made at various times during the dark recovery period following light exposure (Fig. 3). Six to 12 hr after light exposure, which bleached over 90% rhodopsin, cyclic light and dark reared +C animals regenerated 70 and 80% of their respective pre-exposure rhodopsin levels. Unsupplemented rats had slightly lower levels of rhodopsin at these time points. In both types of rats, rhodopsin levels declined thereafter and reached a minimum six–seven days after exposure. These levels were 50% of control for +C cyclic light reared rats and 37% for the untreated animals. In dark reared rats six–seven days after exposure, the retinas of both C+ and untreated animals contained only 25–27% of the pre-exposure rhodopsin levels. Thereafter, rhodopsin increased in cyclic light reared rats to a level which after 13–14 days was 75% and 55% (+C and untreated) of the pre-exposure level. Ascorbate treated, dark-reared rats also recovered rhodopsin to an average of 42% of control 14 days after exposure, but the untreated animals failed to increase their rhodopsin levels. In these rats the rhodopsin level was only 5–15% of the level in the non-exposed control. Rhodopsin levels in dark-reared rats were not further increased by a longer dark recovery period. The data in Figure 3 indicate, therefore, that the light induced degeneration of photoreceptor cells in rats proceeds during the dark period following intense light; and that rhodopsin in the surviving photoreceptor cells is maximized after 13–14 days in darkness. Furthermore, these measurements indicate that at all time points, ascorbate treated rats had rhodopsin levels which were equal to or greater than those in the untreated animals. It should also be noted that for the cyclic light-reared rats in these experiments, the fellow eye was processed for histological verification.

**Time of Ascorbate Administration**

Since rhodopsin levels in the eyes of intense light-exposed rats exhibited time dependent changes during the dark recovery period and ascorbate might be effective in promoting repair processes, it was decided to determine if ascorbate administration during the dark phase would increase the recovery of rhodopsin. The results of these experiments are given in Table 4. In comparison to animals treated with ascorbic acid the day before and the day of light exposure (day 0), ascorbate administered after the light exposure period (day 2–6) was not effective in reducing the loss of ocular rhodopsin. In both cyclic light and dark-reared rats, no differences in rhodopsin levels were measured in rats given ascorbate on days 2–6 after light exposure and in the unsupplemented animals. In cyclic light-reared rats given ascorbate the day of and one day after light exposure (day 1), rhodopsin recovery was less than in day 0 animals but greater than in day 2–6 injected rats.
rats. In this series of experiments, rhodopsin recovery in dark-reared rats receiving ascorbic acid on day 1 was greater than in day 0 rats. It should be noted, however, that in other experiments with 12 hr light-exposed, dark-reared rats given ascorbate on day 0, rhodopsin recovery was 1.2 nmol/eye (Figs. 1, 2). Thus, it appears that the maximum reduction of rhodopsin loss from intense light is achieved in animals given ascorbate during or just after the light exposure period.

Ascorbate Effect on ROS Fatty Acids

To test the antioxidant potential of ascorbic acid in the retina, ROS were isolated from rats following light exposure and total lipid fatty acids measured. Table 5 contains data which indicate that ascorbate reduces the loss of docosahexaenoic acid (22:6) in ROS lipids from light treated rats. In ROS from +C and unsupplemented rats exposed to intense light, the levels of 22:6 were lower than in unexposed animals. In the unsupplemented animals, however, the loss of 22:6 from light exposure was greater than in the ascorbate treated rats and significantly greater than in the unexposed animals (P < 0.005 cyclic light; P < 0.01 dark-reared). The levels of 22:6 in ROS from +C light-exposed rats were not significantly different than the levels in unexposed animals. Table 5 also shows that among the major ROS fatty acids 22:6 is selectively lost, while the concentrations of arachidonic acid and the saturated fatty acids either remained the same or increased as a result of intense light. Increases in the relative concentrations of other fatty acids in light-damaged ROS are not unexpected because of the lower 22:6 content in these rods. In these ROS, light exposure also resulted in a higher cholesterol/phospholipid ratio than in unexposed rats. Thus, the light-damaged ROS may be contaminated with other retinal organelles or retinal pigment epithelium (RPE) plasma membranes. Recent studies with bovine RPE plasma membranes indicate that they have a cholesterol/phospholipid ratio of approximately 1:1 and a 22:6 content of about 2%. 

Table 4. Rhodopsin in rats treated with ascorbic acid at various times after intense light* nmol/eye

<table>
<thead>
<tr>
<th>Day after exposure†</th>
<th>Cyclic light-reared 24-hr exposed</th>
<th>Dark reared 12-hr exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>+C Day 0</td>
<td>1.3 ± 0.2 (12)</td>
<td>0.8 ± 0.2 (10)</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.7 ± 0.3</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.8 ± 0.2</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>Day 6</td>
<td>0.7 ± 0.1</td>
<td>0.1 ± 0.06</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.9 ± 0.3 (12)</td>
<td>0.3 ± 0.2 (12)</td>
</tr>
</tbody>
</table>

* Results represent the mean ± SD (n = 6) unless indicated for rats simultaneously exposed to light.† Day of second IP ascorbate injection (0.5 mg/gm body wt).

Table 5. Major fatty acids in ROS lipids of light exposed rats (1) mol%

<table>
<thead>
<tr>
<th>Fatty acid (2)</th>
<th>Cyclic light reared</th>
<th>Dark reared</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hr light exposed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+C or untreated</td>
<td>+C (3)</td>
</tr>
<tr>
<td></td>
<td>untreated</td>
<td>untreated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+C (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>untreated</td>
</tr>
<tr>
<td>16:0</td>
<td>16.0 ± 1.4</td>
<td>16.3 ± 0.8</td>
</tr>
<tr>
<td>18:0</td>
<td>25.4 ± 0.5</td>
<td>24.6 ± 0.6</td>
</tr>
<tr>
<td>18:1</td>
<td>41.1 ± 1.2</td>
<td>5.3 ± 1.2</td>
</tr>
<tr>
<td>20:4</td>
<td>37.0 ± 0.4</td>
<td>41.0 ± 0.9</td>
</tr>
<tr>
<td>22:6 (3)</td>
<td>45.2 ± 3.1*</td>
<td>39.0 ± 2.9</td>
</tr>
<tr>
<td>Chol/PO₄₅</td>
<td>0.09</td>
<td>0.14</td>
</tr>
</tbody>
</table>

(1) Results represent the mean ± SD for the number of determinations in parentheses: 50-60 day old animals; Ohr-light experiments n = 3.
(2) Fatty acid chain length: degree of unsaturation. Other fatty acids collectively represent less than 5 mol% of the ROS fatty acids.
(3) Significance Ohr-light vs. light exposed *p < 0.005 †p < 0.01 ‡not significant.

Discussion

These results demonstrate that ascorbic acid supplementation of rats prior to intense light exposure reduces the extent of photoreceptor cell loss from light. While several forms of ascorbate were effective in preserving rhodopsin in light-exposed rats, the ascorbate effect shows a requirement for the L-stereoisomer of the vitamin. The ascorbate protective effect is also vi-
tamin dose dependent in cyclic light and dark reared rats and appears to exhibit its protective effect during the light exposure period. Ascorbate supplementation decreases the loss of docosahexaenoic acid in the lipids of ROS isolated immediately after exposure, suggesting that ascorbate may function as an antioxidant in the retina. In this study, retinal ascorbate was elevated by supplementation to a level which was about 40% higher than control four hr after a second injection. In both +C and unsupplemented rats, intense light resulted in a decrease in retinal ascorbic acid, which was greater in dark-reared rats than in the cyclic light reared animals (Table 5). In a study with guinea pigs, Woodford and Tso \textsuperscript{32} showed that scorbutic animals incur more extensive photoreceptor cell damage than ascorbate adequate animals. Retinal ascorbate also decreases in the monkey retina during intense light exposure. \textsuperscript{31} In these animal models, as well as the rat, reduced ascorbic acid in the retina following light exposure is greater than 90% of the total of reduced plus oxidized ascorbate. \textsuperscript{26,31,32} Thus, despite the differences in light damage which may exist among the various animal models, ascorbic acid appears to impart a protective effect to the photoreceptor cell.

In the rat, the mechanism of retinal light damage is not well understood, but differences in the extent of photoreceptor cell damage between cyclic light and dark-reared animals are known to occur. \textsuperscript{5,18} These differences can be ascribed to different pre-exposure rhodopsin levels in the two types of rats and to differences in rhodopsin packing their ROS membranes. \textsuperscript{13,15,18} Recently, Noell \textsuperscript{37} classified light damage in the rat as Type I damage, which involves photoreceptor cell loss with extensive RPE involvement and Type II damage in which less extensive or little RPE cell loss accompanies rod cell degeneration. His study also indicates that RPE cell destruction in the rat is probably secondary to the primary damage of rod cells by light. Yet ascorbic acid supplementation is effective in reducing light damage in both dark-reared rats (Type I damage) and cyclic light reared animals (Type II damage). The existing evidence also indicates that the loss of ROS 22:6, which is one manifestation of retinal light damage, \textsuperscript{21} is reduced in the ROS lipids of +C animals exposed to light (Table 5). Furthermore, apparent contamination of the light-damaged ROS membranes by other ocular plasma membranes was reduced by ascorbate administration. How ascorbic acid exhibits its protective effect remains to be elucidated, but histological evidence presented by Li et al \textsuperscript{34} for +C cyclic light-reared rats shows better preservation of rod cell and RPE cell morphology than in unsupplemented light-exposed animals. Similarly, in dark reared +C rats, average photoreceptor cell nuclei loss and rhodopsin loss correlated well and was lower than in the untreated animals.

Recent evidence indicates that $\alpha$-tocopherol in the rat retina and ROS is not diminished during light exposure. \textsuperscript{27-29} However, retinal ascorbic acid decreases during intense light, in an age and previous light environment dependent fashion. \textsuperscript{26} This suggests that ascorbic acid, which is some 30 times higher than $\alpha$-tocopherol in the rat retina, \textsuperscript{26,29} may reduce oxidized $\alpha$-tocopherol formed during light. Since $\alpha$-tocopherol can interrupt the cascade of peroxidation by interacting directly with lipid free radicals, \textsuperscript{38} then the ratio of vitamin C to E may be an important antioxidative force. Tappel \textsuperscript{39} suggested such an interaction, and evidence for a direct and synergistic antioxidative effect between the two vitamins in membranes has been presented. \textsuperscript{40,41} A cooperative effect between the two vitamins may also diminish the extent of RPE cell damage in light-exposed rats, although the water soluble ascorbic acid may also interact directly with peroxides released from the rod cell.

As shown by the lack of an effect when ascorbic acid was given after light exposure, the protective effect of ascorbate does not appear to be due to its ability to enhance repair processes as in corneal wound healing. \textsuperscript{42} The ascorbic acid effect in the retina occurs during light exposure and appears to require the L-stereoisomer Preliminary studies with D-ascorbate treated rats indicate that retinal ascorbate is not elevated three-four hrs after a second injection (data not shown). This may help to explain the inability of the D-stereoisomer to reduce the extent of retinal light damage. In our attempts to maximize the ascorbic effect we also employed dehydroascorbic acid, which enters the eye rapidly. \textsuperscript{53} In the present study we found no greater effect with equal doses of ascorbate or dehydroascorbate injected the day before and day of light damage. It remains to be determined if dehydroascorbate injection at a more appropriate time during light exposure would be more effective than L-ascorbic acid.

If ascorbic acid has an antioxidative role in the retina, then it may be useful for studying the mechanism of light damage. Peroxidation of ocular membranes is but one part of a complex sequence of events which starts with photon absorption by rhodopsin and ends with cell destruction when intense light of long duration is employed. In this regard, ascorbic acid supplementation of rats was effective in reducing the light-mediated loss of 22:6 from ROS lipids. However, ascorbate supplementation does not completely eliminate rod cell loss from light, suggesting that other events may also contribute to the process of retinal light damage. While
ascorbic acid is effective in reducing rod cell loss during the light period and not during the dark recovery phase, we are presently unable to further define the temporal sequence of these light damaging events. Recently, Winkler et al. presented evidence to suggest that light damage in isolated rat retinas after ten-min exposures may be due to elevated photoreceptor cell calcium levels. As originally suggested by Noell et al., metabolic alterations in the retina during intense light exposure may be an important factor in the light damage process. Peroxidation, then, could be a consequence of metabolic alterations in the retina which arise from light exposure for only brief periods. This suggests that intense light exposure is an extreme (pathological) example of the normal rhodopsin-light mediated reactions in the eye. The study of light damage, therefore, may lead to a better understanding of normal light induced processes in the retina. In this regard, ascorbic acid may serve as a useful probe by which we can study these processes.

Key words: rhodopsin, light damage, ascorbic acid, rat

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