Intrinsic, light-independent, regional differences in photoreceptor cell degeneration in vitamin A--deficient rat retinas. LOUVENIA CARTER-DAWSON, TOICHIRO KUWABARA, AND JOHN G. BIERI.*

The retinas of retinol-deficient rats reared in darkness or cyclic light were examined to determine whether regional degeneration of photoreceptor cells was induced by environmental lighting or resulted from intrinsic differences between ocular hemispheres. Weanling male Sprague-Dawley rats were fed a retinol-adequate or retinol-deficient diet and were reared in either cyclic light or darkness through 29 weeks. The nasal retinal quadrants were examined by light microscopy and the number of photoreceptor nuclei was counted in a 630 μm segment beginning 200 μm from the optic nerve. Retinol-deficient rats reared in darkness for 29 weeks lost 24% of their photoreceptors in the inferior nasal quadrant but only 11% in the superior nasal quadrant. Deficient rats reared in cyclic light for 29 weeks lost 39% of their photoreceptor cells in the inferior nasal quadrant and only 16% in the superior nasal quadrant. Photoreceptor cells degenerated faster in the inferior nasal quadrant than in the superior nasal quadrant in darkness or cyclic light. These results indicate that regional differences in rate of photoreceptor cell loss in retinol-deficient rats are not induced by the lighting conditions but occur as a result of intrinsic differences between the ocular hemispheres. (Invest Ophthal Mol Vis Sci 22:249-252, 1982.)

Light has been shown to influence the pattern of photoreceptor cell degeneration in retinas of albino or pink-eyed rats with inherited retinal dystrophy.1 In cyclic light, photoreceptor cells are lost at about the same rate in the inferior and superior retinal hemispheres. However, dark-rearing slowed the rate of cell loss in the superior hemisphere of the eye but not in the inferior hemisphere.

It has been shown recently that photoreceptor cells in albino rats with experimentally induced vitamin A deficiency degenerated sooner in the inferior hemisphere of the eye than in the superior hemisphere.2 The rats used in the study were reared in dim illumination (1.5 to 2 ft-c). Whether or not this differential rate of degeneration between the two hemispheres was induced by dim illumination, as seen in rats with inherited retinal dystrophy reared in darkness, or resulted from intrinsic differences between the two hemispheres was not determined. To examine the influence of light on the pattern of photoreceptor cell loss, vitamin A--deficient and vitamin A--adequate rats were reared in cyclic light or darkness and the number of photoreceptor cells surviving in the superior and inferior nasal quadrants compared.

Materials and methods

Animals. Male Sprague-Dawley rats, 21 days old, were fed a basal diet6 supplemented with either retinyl palmitate (retinol-adequate; 4 mg/kg of diet) or retinoic acid (retinol-deficient; 4 mg/kg of diet). Rats receiving the retinol-adequate or retinol-deficient diets were maintained in 10 ft-c cyclic light (12 hr light, 12 hr dark) or in complete darkness except for the use of red light (60 W ruby bulb; General Electric; approximately 1 to 2 feet from the animal) for about 0.5 hr (three to four times a week) during the maintenance period. The rats were reared through 29 weeks on the dietary regimens.

Histology. Both eyes were enucleated from two rats in each group receiving retinyl palmitate and from three rats in each group receiving retinoic acid after 9, 13, and 29 weeks. The corneas were punctured and the eyes were immersed in 4% paraformaldehyde and glutaraldehyde in phosphate buffer. Sections were cut at 2 to 3 μm and stained with toluidine blue.

It was included in these analyses because photo-

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Fig. 1. Light micrographs of retina from a retinol-deficient rat reared in cyclic light for 29 weeks. A, Segment of retina near the optic nerve head with a portion of the superior (S) and inferior (I) retina. (×75.) B, Higher magnification of inferior retina within the box shows a reduction in photoreceptor nuclei (PN) to three to four rows. Only remnants of outer segments (OSR) remain. The inner nuclear layer (INL) and inner plexiform layer (IPL) of the retina are apparently thinner. (×470.) C, Higher magnification of the enclosed area of superior retina shows six to eight rows of photoreceptor nuclei. The inner nuclear and inner plexiform layers of the retina are apparently thicker in this region than in the inferior region (compare with B). OS, Outer segment. (×470.)

Receptor cells degenerate sooner in the central region and only a small number of rods are affected in the periphery even after 29 weeks on the deficient diet.2

Results. Retinol-adequate rats reared in darkness or cyclic light showed no significant loss of photoreceptor cells in the nasal inferior and superior quadrants at any time period. In dark-reared retinol-deficient rats, changes in the inferior nasal quadrant had not occurred by 13 weeks, but by 29 weeks there was a 24% reduction in the inferior quadrant and a 11% reduction in the superior nasal quadrant (Table I). Similarly, retinol-deficient rats reared in cyclic light showed no significant change in photoreceptor cell number at 13 weeks, but by 29 weeks, 39% of the cells had degenerated in the inferior quadrant and only 16% in the superior quadrant (Table I and Fig. 1, A to C). These data
show that in retinol-deficient rats, twice as many photoreceptor cells had degenerated in the inferior nasal quadrant than in the superior nasal quadrant of rats reared in darkness or cyclic light.

Other histological differences were seen between the inferior and superior retinal hemispheres of retinol-deficient rats. The inner plexiform and inner nuclear layers were thinner in the inferior retina than in the superior retina by about 10 and 4.0 μm, respectively (Fig. 1, B and C). Photoreceptor outer segments were also markedly shortened. Total retinal thickness was about 22 μm thinner in the inferior quadrant than in the superior quadrant.

Discussion. In our previous study with vitamin A-deficient rats, a more rapid degeneration of photoreceptor cells in the inferior retinal hemisphere than in the superior retinal hemisphere was obtained with a light intensity of only 1.5 to 2 ft-c. The present study clearly shows that this differential pattern of cell loss occurs whether the animals are exposed to cyclic lighting of 10 ft-c or kept in darkness. Thus regional differences in the degeneration of photoreceptor cells in vitamin A deficiency do not appear to depend on the conditions of environmental lighting but rather on regional differences intrinsic to the eye.

Some intrinsic differences are known to exist between the inferior and superior hemispheres of the eye. Several studies have shown that more pigment is present in the inferior half of the eye than the superior half. The two hemispheres react to a different extent to metabolic poisoning, retinal dystrophy, and light damage. It has also been shown that outer segments in the superior hemisphere are longer than those of the inferior hemisphere in pigmented and dark-reared albino rats. Perhaps in dark-reared rats, the outer segments are longer in the superior retina before retinol is depleted from the eye. Thus it might take a longer period of time for the cells to degenerate. Although longer outer segments in the superior retina may provide an explanation for regional differences in rate of cell loss in dark-reared rats, the length probably is not a factor in rate of cell loss in rats reared in cyclic light, since regional differences in outer segment length are not consistently seen.

Other factors must be considered that may explain the regional differences in photoreceptor cell loss in vitamin A deficiency. The two hemispheres may differ in the ability of the pigment epithelium to store vitamin A, in retinal metabolism, or in interactions between the pigment epithelium and photoreceptor cells. Additional studies are needed to examine the spectrum of possible differences between the two retinal hemispheres.

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Key words: vitamin A deficiency, photoreceptor degeneration, retinal hemispheres, rat

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Table I. Number of photoreceptor cells

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<thead>
<tr>
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<th>Inferior hemisphere</th>
<th>Superior hemisphere</th>
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<tbody>
<tr>
<td></td>
<td>9 wks</td>
<td>13 wks</td>
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<tr>
<td>Darkness</td>
<td></td>
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<tr>
<td>Retinol-adequate</td>
<td>97.2 ± 1.1</td>
<td>98.6 ± 1.6</td>
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<tr>
<td>Retinol-deficient</td>
<td>98.1 ± 0.8</td>
<td>95.2 ± 1.5</td>
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<tr>
<td>Cyclic light</td>
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<tr>
<td>Retinol-adequate</td>
<td>96.4 ± 1.1</td>
<td>97.1 ± 1.5</td>
</tr>
<tr>
<td>Retinol-deficient</td>
<td>97.4 ± 3.0</td>
<td>95.3 ± 2.1</td>
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Values are mean ± S.E. for 2-3 animals. *Significantly different from control at less than the 0.05 level of significance (Student t test).


Chronologic analysis of variations in retinal damage in two strains of rats after short-term illumination. W. Keith O’Steen and Jan E. Donnelly.

In order to determine whether an optimal period existed for evaluating photodamage in rats, a chronologic analysis was made of quantifiable changes in the outer nuclear layer (ONL) and retinal thicknesses in Sprague-Dawley (SD) and Wag/Rij rats after a single, 24 hr exposure to fluorescent light. A gradual reduction in ONL and retinal thicknesses occurred between postexposure days 0 and 10 and appeared to stabilize by day 14 in both strains.

The percent difference in both ONL and retinal thicknesses in unexposed and exposed rats was greater in Wag/Rij than in SD rats. This finding emphasizes a significant variability between rat strains and is possibly a manifestation of the slowly progressing genetic retinopathy in the Wag/Rij rats. Results indicated that the optimal period for assessing photodamage under these experimental conditions was not sooner than 10 to 14 days after exposure. Quantifiable analyses prior to that time would not provide an accurate evaluation of the total influence of a short-term exposure period. (Invest Ophthalmol Vis Sci 22:252-255, 1982.)

Exposure of rats to fluorescent illumination results in severe damage to the outer nuclear layers (ONLs) of the retina. The extent of the damage depends on the duration of the exposure and the intensity of the light source and other factors such as age and hormonal status of the animal at exposure time.

The purpose of the present project was to examine and compare quantitatively the damage and subsequent destruction of photoreceptor cells in the ONL after a single short-term light exposure in two strains of rats, Sprague-Dawley (SD) and Wag/Rij, the latter of which has a slowly progressing, spontaneous hereditary retinal degeneration. The rats were exposed to a 24 hr, high-intensity photoperiod, and retinal and ONL thicknesses were measured at subsequent time periods to determine the immediate extent and later progression of the light-induced photoreceptor damage and cell death.

Materials and methods. Adult female rats (10 weeks old, SD and Wag/Rij strains) were kept in a cyclic light environment (14 hr light:10 hr darkness) prior to a continuous 24 hr exposure to 400 ft-c as measured with a Tektronix J16 digital photometer with the illuminance probe directed toward the fluorescent light source. Animals were exposed in pairs in polyethylene cages with wire tops. After exposure, the SD animals survived in cyclic light (as above) for 0, 1, 2, 4, 7, 10, and 14 days, and the Wag/Rij rats survived for 0, 1, 2, 3, 4, 7, 10, 14, 28, 42, and 56 days prior to autopsy. Each group contained at least four animals. Control groups (n = 4) of identical strains were autopsied to ascertain the thickness of the entire retina and ONL in unexposed animals (Tables I and II).

At autopsy the rats were overanesthetized with ether and exsanguinated, and the eyes were enucleated. The superior surface of the eyes was marked with an indelible felt-tip pen for future orientation during sectioning. The eyes were fixed for 4 hr in Bouin's solution, dehydrated in an alcohol and benzene series, and embedded in paraffin. Tissue blocks were sectioned at 7 μm on the anterior-posterior axis, and sections of the central retina, including the optic nerve, were stained with Harris' hematoxylin and eosin. Thickness measurements of the entire retina and ONL were made on each retina as previously described.

Statistical significance was determined by comparing (1) data from two groups of animals in each experiment by Student’s t test or (2) data among groups by Duncan’s multiple range test.

Results. The ONL of photoreceptor nuclei and entire retina of the SD and Wag/Rij strains of rats were reduced significantly in thickness by exposure to the 24 hr photoperiod (Tables I and II). Both measurements differed statistically from those of the cyclic light (LD) control group at each time period (0 to 14 days) in the SD strain and, with the exception of the entire retinal thickness at...