hydrogenase resembles aldose reductase in its ability to reduce glyceraldehyde yet shows no hexitol-producing activity.10

One might speculate that hexitol production plays a role in the pathogenesis of diabetic retinopathy, perhaps via a mechanism analogous to that responsible for the formation of sugar cataract. In the present study, hexitol-producing activity by canine retinal microvessels is found to be several-fold less than that in lens epithelium. It is not known whether this activity is concentrated in a particular cell type in the microvessels or is distributed throughout all the cell types. Endothelial cells cultured from puppy retinal capillaries have been reported to possess hexitol-producing activity.5

Cerebral microvessels, like those from the retina, have hexitol-producing activity, and this activity is inhibited with Sorbinil. This similarity to the retinal microvessels was not to be expected necessarily, since cerebral vessels seemingly are spared many of the lesions characteristic of retinal vessels in diabetes.11 It would be inappropriate to conclude, based on the limited data presented herein, that retinal and cerebral hexitol-producing activities are different.

The presence of hexitol-producing activity within retinal microvessels is consistent with a possible role of hexitol production and accumulation in the etiology of retinal vessel disease in diabetes and experimental galactosemia. Although the hexitol-producing enzyme remains to be identified, its activity, like that of lens aldose reductase, can be inhibited with a known aldose reductase inhibitor. Effects of an aldose reductase inhibitor on the development of retinopathy in diabetic dogs and in galactosemic dogs are under investigation.

Key words: microvessels, polyl, hexose metabolism, diabetic retinopathy, retina

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References


DNA Repair Synthesis in the Rat Retina Following In Vivo Exposure to 300-nm Radiation

Laurence M. Rapp,* Jule Griebrok Jose,† and Donald G. Pirt†

Quantitative autoradiography was used to study the incorporation of 3H-thymidine into the retina of albino rats following in vivo exposure to 300-nm radiation. Relative to background labeling in unexposed eyes, there was 8–20 times as much label per unit area in the outer nuclear layer, inner nuclear layer, and ganglion cells of 300-nm exposed retinas. The photoreceptor inner segments also showed thymidine labeling in both control and exposed retinas. Invest Ophthalmol Vis Sci 26:384–388, 1985

Previous studies have shown that DNA repair synthesis occurs in ocular tissues in response to damaging levels of radiant energy. DNA repair in
tissues of the anterior segment of the eye has been well documented. Jose and Yielding \(^1\) reported repair of DNA in frog and rat lens epithelium after in vitro exposure to 254-nm radiation. Repair also was demonstrated in human lenses following exposure of either enucleated eyes or isolated lenses to 295-nm radiation.\(^3\)

The retina is also subject to DNA damage and repair. In vivo exposure of rabbits to ionizing radiation (\(^{60}\)Co-gamma rays) produced lesions in retinal cells followed by DNA repair.\(^4\) In vitro exposure of isolated rat or mouse retinas to 254-nm radiation stimulated DNA repair synthesis.\(^5\)\(^6\) In these autoradiographic studies, repair was determined by following the incorporation of \(^3\)H-thymidine into DNA.

Whether or not damaging levels of UV-B radiation (290–320 nm) can be transmitted through the anterior segment of the eye to the retina in living animals is an unresolved question. Zigman and Vaughn\(^7\) showed that exposure of albino mice to “black” light (290–425 nm) for 12 hr per day over a period of 90 weeks caused degenerative changes in the retina including severe loss of most of the photoreceptor cells. However, because a broad band radiation source was used, the precise wavelengths responsible for inducing retinal damage were not known. Pitts et al\(^8\) reported a vast increase in the number of electron-dense bodies in the photoreceptor inner segments of the pigmented rabbits exposed to 300-nm (5-nm waveband) radiation.

The present study examines the effects on retinal cells by acute, in vivo exposure of albino rat eyes to narrow-band 300-nm radiation. Evidence is presented that DNA repair synthesis is induced by radiation transmitted through the intact eye.

**Materials and Methods.** Animals: Male albino (Sprague-Dawley) rats weighing 100–150 g (5–6 weeks old) were obtained from Timco Breeding Laboratory (Houston, TX). They were acclimated to 12L:12D cycle. The rats were anesthetized with an intraperitoneal injection of Nembutal (50 mg/kg body weight). A local anesthetic (0.5% proparacaine HC1) and a mydriatic (1% Tropicamide) were applied to the cornea of each eye. They then were developed in Kodak Dektol for 2–3 months at 4°C. The tissue then was washed in cacodylate buffer, pH 7.3, overnight at 4°C. After washing in buffer again for 30 min, the tissue was dehydrated by a graded ethanol series, cleared with propylene oxide, and infiltrated with Epon-Araldite. The infiltrated tissue was cured for 36 hr at 65°C.

Plastic sections approximately 1 \(\mu\)m in thickness were adhered to ethanol-cleaned microscope slides by first floating them on drops of distilled water and then drying overnight at 55°C. The slides then were coated with Kodak Nuclear Track Emulsion (NTB2) and exposed in total darkness for 2–3 months at 4°C. They then were developed in Kodak Dektol for 2 min at 15°C, stained with 1% toluidine blue, and cover slipped for light microscopic observation.

The distribution of thymidine labeling in the retina was quantified using a Zeiss Videoplan digitizer tablet and stylus linked to a computer. The microscope slides were visualized over the tablet using a drawing tube attached to a light microscope and, using the stylus, entries were made for the number of silver grains and the area counted. All measurements were taken at \( \times 1,000 \) magnification. For each retinal layer (including the nuclear layers, plexiform layers, and pigment epithelium), silver grains were counted in two to four consecutive regions approximately 100 \( \mu\)m in length in the midperipheral superior retina. With the exception of the ganglion cells, silver grains in a particular retinal layer were counted regardless of whether they were overlying the nucleus, cytoplasm,
or intercellular space. For the ganglion cells, grains were counted only over the area defined by the cell body itself. Grain density in each of the retinal layers was averaged for 3 animals (ie, three 300-nm exposed eyes and three control eyes).

Results. Figure 1 shows an autoradiograph of a nonexposed control rat retina (Figs. 1A, C) and a 300-nm exposed retina (Fig. 1B, D). Incorporation of $^3$H-thymidine into the exposed retina was markedly greater than into the control as evidenced by dense labeling overlying the outer nuclear layer (ONL), the inner nuclear layer (INL), and the ganglion cells (GC). Labeling over the ONL showed a gradient from its proximal to distal borders, with the former being more densely labeled. Exposed retinas also showed a band of labeling over the rod inner segments. In contrast, the nonexposed control retina showed only background labeling throughout, with the exception of what appeared as a greater labeling over the inner segments (Fig. 1A, C).

Quantification of grain density over retinal layers is shown in Figure 2 for control and exposed retinas. The data in this histogram confirm the aforementioned qualitative observation of increased labeling over all three nuclear layers of the exposed retinas. In contrast, all plexiform layers showed comparable

Fig. 1. Light- and dark-field autoradiographs of albino rat retinas exposed to 300-nm radiation. A and C, nonirradiated controls. B and D, 300-nm exposed. RPE: retinal pigment epithelium; ROS: rod outer segments; RIS: rod inner segments; ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GC: ganglion cells (×600).
background labeling in control and exposed eyes. However, relative to the plexiform layers, there was a significantly higher grain density over the photoreceptor inner segments in both control and exposed retinas ($P < 0.001$). The apparently higher labeling observed in the retinal pigment epithelium (Fig. 2) was marginally significant ($P = 0.09$).

Additional experiments were conducted to determine whether DNA repair synthesis also could be induced in the retina by exposing rats to constant light from fluorescent fixtures. Table 1 shows that the grain density did not increase over retinal nuclei following 24 hr of constant exposure to fluorescent light in comparison to dim cyclic light exposure.

**Discussion.** Because the cells of the retina do not ordinarily undergo mitosis, incorporation of $^3$H-thymidine into the nuclear layers was interpreted as evidence for DNA repair synthesis. Relative to background labeling in control eyes, there was 8–20 times as much label per unit area in the ONL, INL, and ganglion cells of 300-nm exposed retinas. An important distinction of the present study as compared with others showing DNA repair in the retina is that exposures were carried out in vivo. This indicates that 300-nm radiation can be transmitted through the anterior segment of the rat eye in quantities sufficient to damage retinal DNA.

Ocular media transmission for UV radiation has not been determined yet for the rodent eye. Transmission curves for human and rabbit eyes are similar and indicate that the short wavelength cut-off for UV radiation extends into the UV-B portion of the spectrum. However, the percent transmitted to the retina is relatively low (ca 0.29% at 320 nm for the rabbit eye). Although these data cannot be extrapolated directly to the rat eye, a rough estimate would be that at least 0.01% and perhaps as much as 0.1% of 300-nm radiation is transmitted to the retina. In the present experiment, this would calculate to a retinal dose of between 2.3 and 23 erg/mm². This value is two to three orders of magnitude less than what was used by other in vitro studies, indicating that stimulation of DNA repair does indeed occur at these low irradiation levels.

DNA repair synthesis was found to occur in the present study in all three nuclear layers of the retina. However, the ONL showed a non-uniform distribution of thymidine label. Perhaps the intensity of UV radiation reaching the ONL is attenuated by absorption in the inner retina. This also could account for the comparatively low labeling of the retinal pigment epithelium cell nuclei in the irradiated retinas. In rabbit eyes exposed to $^{60}$Co-gamma rays, thymidine incorporation was also significantly lower in the ONL as compared with the INL and ganglion cells. In that case, however, no gradient of radiant energy would be expected, since gamma rays readily penetrate through retinal tissue. Previous studies demonstrating DNA repair synthesis in retinas exposed in vitro to 254-nm radiation reported uniform thymidine labeling in all three layers of the retina, but no quantitative data were presented.

The finding of thymidine incorporation into the inner segments of the photoreceptor cells is novel. Since the mitochondria of the photoreceptors are localized in the inner segments, this labeling may represent thymidine turnover in the mitochondrial DNA of these actively respiring cells. The labeling is not UV-induced because it is present and equal in both irradiated and control retina (Fig. 2).

In summary, 300-nm radiation is transmitted through the rodent lens and cornea, in vivo, in amounts sufficient to induce DNA repair synthesis in retinal cells. Whether or not damaging levels of UV radiation can be transmitted to the human retina is difficult to predict because of safety factors such as ocular pigmentation, focal length, and angle of incident radiation. Nevertheless, because of the possibility of chronic UV-exposures and accumulated DNA damage, including that from sunlight, further studies

<table>
<thead>
<tr>
<th>Retinal layer</th>
<th>Cyclic light</th>
<th>Constant light (24 hr)</th>
<th>300 nm (15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONL</td>
<td>3.6</td>
<td>3.6</td>
<td>38.6</td>
</tr>
<tr>
<td>INL</td>
<td>7.3</td>
<td>4.4</td>
<td>67.3</td>
</tr>
<tr>
<td>G</td>
<td>3.3</td>
<td>5.5</td>
<td>57.8</td>
</tr>
</tbody>
</table>

* Units are grains/1000 square microns.
are warranted to assess the danger to the human retina.

Key words: DNA, repair synthesis, retina, ultraviolet, 300 nm, thymidine incorporation, nucleus, inner segment

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References

Light Exposure Can Reduce Selectively or Abolish the C-Wave of the Albino Rat Electroretinogram

Adrienne L. Groves, Daniel G. Green, and Leslie J. Fisher

The ERG (electroretinogram) of the albino rat is reported to lack a c-wave. Observations of our own suggested that the conditions of light-rearing are important. Consequently, the authors recorded c-waves in two groups of albino rats. One group was reared from birth in dim illumination (dark-reared) and the other in 12/12 cyclic light (light-reared). Rats were tested after birth from 22 days to about 1 year. All dark-reared animals had a c-wave. Rats reared in cyclic light typically had no detectable c-wave. Physiologic and anatomic evidence suggests this consistent difference, c-waves present in dark-reared animals but absent or diminished in light-reared animals, is probably not due to extensive light induced retinal damage. No consistent differences between the two groups were seen in a- or b-wave thresholds, a- or b-wave intensity-response functions, and in the time-course of b-wave dark adaptation. Invest Ophthalmol Vis Sci 26:388–393, 1985

The c-wave, a slow cornea-positive potential that appears after the a-wave, is a prominent feature of most vertebrate electroretinograms (ERGs). Steinberg, Schmidt and Brown have shown that a component of the c-wave is produced by the retinal pigment epithelial (RPE). Faber provided evidence that the vitreal c-wave is the difference between the epithelial response and slow PII. Both responses are caused by light-evoked decrease in concentration of extracel-