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Location and Severity of UVB Irradiation Damage in the Rat Lens

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Abstract: We investigated the location and severity of lens opacities and epithelial alterations following ultraviolet-B (UVB) irradiation in vivo, using Brown Norway rats. A group of 9 rats received 65 mJ/cm^2 UVB irradiation from overhead lamps every 6 days. Lens changes were documented and evaluated by an anterior eye segment analysis system. Lens epithelial cells were examined postmortem in flat preparations. After 8 weeks of the irradiation schedule (total dose: 0.6 J/cm^2), an anterior polar opacity was apparent; at 16 weeks, the opacities had progressed more deeply into the cortex. At postmortem examination, cells in the central region displayed disorganization, clumping, some pyknotic nuclei and mitosis. There were deeper opacities and cell damage was more severe above the central horizontal plane than below it. This present study demonstrated that UVB damage differed in the superior and inferior parts divided by a horizontal plane through the lens anterior pole, when the UVB source was above and there was no reflection from below or laterally. The lens epithelial cells, and associated lens fibers, are the first target of UVB irradiation. **Jpn J Ophthalmol 1997;41:381–387** © 1997 Japanese Ophthalmological Society

Key Words: Cataract, lens epithelium, rat, Scheimpflug imaging, ultraviolet-B irradiation.

Introduction

Epidemiological studies have indicated that ultraviolet-B (UVB) irradiation is a risk factor in human age-related cataract formation. Most studies reported that cortical opacities were related to solar UVB irradiation[1,2]; cortical opacities also have been reported as occurring more frequently in the lower lens regions than the upper,[3–5] in human age-related cataracts.

In animal model studies, UVB irradiation induced anterior polar cataract in rats,[6–8] rainbow trout,[9] mice,[10] and rabbits[11]; posterior cataract in albino mice,[12] and a pre-nuclear haze in rainbow trout.[9] However, in most of the animal cataracts, opacification types differed from human cataracts, in which UVB-related changes occur as a wedge-shaped opacity in the periphery. Few, if any, in vivo studies have addressed the topographical aspects within the lens involved in the UVB-irradiated cataract model.

In order to have a direct effect on the lens, the UVB must be transmitted through the cornea and aqueous humor and absorbed by the lens; the lens will absorb all the UVB that reaches it in vivo,[13] and the lens epithelium is the primary target of UVB irradiation.[14,15] It is still unclear whether cortical opacity is a consequence of epthielial damage or a direct effect of UVB on the lens fibers. Part of the UVB transmitted through the cornea is absorbed by the aqueous humor.[13,16] A possible photochemical reaction in the aqueous humor and iris, and the breakdown of the blood-aqueous barrier could produce a toxic effect on the lens and induce lens opacity.[17,18]

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To investigate the local morphological changes within the lens, it would be helpful to describe the mechanism of a UVB-induced cataract. In a previous study, Hatano and Kojima observed lens changes with time after exposure to 0.067 J/cm^2 and 0.2 J/cm^2 UVB.[7] In this report, we will show the location and severity of anterior polar opacities and the epithelial alteration in rat lenses after UVB irradiation, using identical conditions.[7]

Materials and Methods

Animals

Male Brown Norway rats, 6 months old, were used in this study. The animals were cared for and handled in accordance with the ARVO resolution on the use of animals in vision research.

UVB Irradiation

The rats were divided into the UVB-irradiated group (UVB; 9 animals) and the nonUVB-irradiated group (CTL; 8 animals). UVB irradiation was produced as described earlier, but with a higher intensity[7]; UVB was provided by ten 20 W lamps fixed in a 60 × 55 cm plane above the rats. Two to three rats were placed in an uncovered cage with non-reflecting walls; UVB intensity was 0.15 mW/cm^2. After dilation of pupils, rats in the UVB group received 65 mJ/cm^2 UVB every sixth day. During the UVB irradiation term, the cornea was carefully inspected to avoid photokeratitis which would make lens observation impossible. When we observed corneal edema, the interval between UVB irradiation was prolonged until healing of corneal damage. Total UVB dose was 0.6 J/cm^2 at 8 weeks and 0.9 J/cm^2 at 16 weeks.

Documentation and Analysis of Lens Changes

Lens changes were documented and analyzed by an anterior eye segment analysis system (EAS-1000, Nidek, Gamagohri) every 2 weeks.[7,19] Images of each eye were taken by a modified Scheimpflug camera from a vertical slit position. Anterior opacities were analyzed by the same system: a cycle covering the opaque region was set and the opaque area was calculated as in an earlier study.[19] Differences in the distribution of anterior opacities in the superior and inferior parts were measured every 10 pixels from the central line through the anterior pole (Figure 1A). The light scattering intensity was measured to quantitatively evaluate the extent of lens opacity. To measure the depth of the opacity, the width of the light scattering peak was determined at ± 15 pixels from the central line. Data expressed in pixels was converted to millimeters by the EAS-1000 program. Each lens was measured for baseline data at the beginning of the study.

In order to control the quality of the experiment, one experienced camera operator took all the images. Three eyes were used to determine the reproducibility of the documentary technique, taking eight images of each eye on a single day. The coefficient of variation of light-scattering intensities in the opaque areas of the three lenses were 7.4%, 6.5%, and 7.3%. Data are expression as mean values ± standard deviation. Lenses from all animals were analyzed by the Microsoft® Excel program to evaluate the opacity alteration and to compare the difference between the control and experimental rats, using the *t*-test.

Lens Epithelial Alterations on Flat Preparation

At the end of the irradiation period, all animals were sacrificed by an overdose of pentobarbiturate. After enucleation, a mark was made at 12:00 on the limbus; the eyeballs were fixed in ethanol/acetic acid (3:1 V/V) for at least 24 hours. Using an anterior approach to remove the cornea and iris, the lens was removed after making a mark at 3:00 on the periphery. The epithelium was peeled off under a dissecting microscope, stained with hematoxylin, dehydrated and mounted with resin.[20] Superior and a inferior halves of the epithelia could be distinguished under the microscope by the mark on the flat preparations; variations at different locations could be related to the position in vivo. The whole flat preparation (diameter: 6–6.5 mm) was also roughly divided into 2 mm wide peripheral areas and a 2–2.5 mm wide central area to further localize the changes (Figure 1B).

Results

UVB irradiation induced lens anterior polar opacities, as well as corneal edema, with visibly greater intensity in the superior parts of the lenses. There were no apparent abnormalities in the lens nuclear, peripheral, or posterior subcapsular regions of the irradiated lenses, compared with the nonirradiated lenses.

By 6–8 weeks of the irradiation schedule, anterior pole lens opacities in the UVB rats appeared as increases in light scattering along the capsule, gradually developing toward the shallow cortex (Figure 2). To compare the opacities in both groups, the opaque areas were calculated by image analysis (Figure 3). At the start of the experiment, light scattering repre-

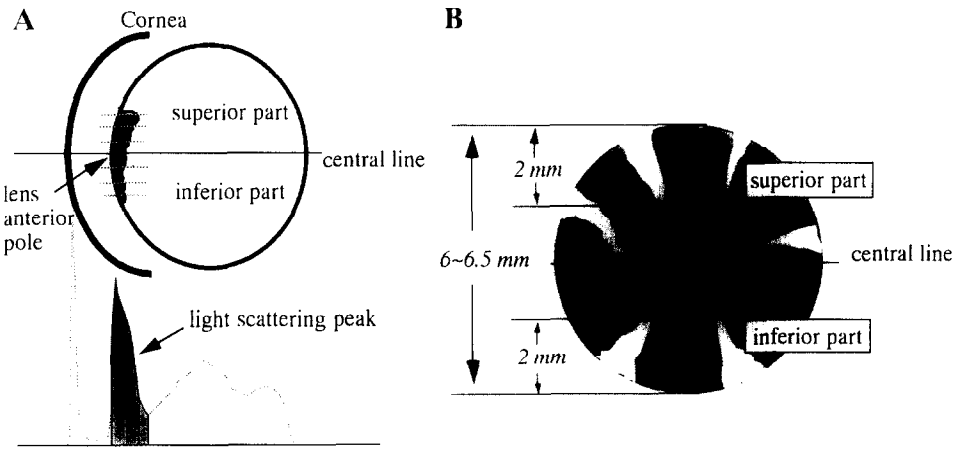

Figure 1. Lens opacities in anterior central region (**A**) and localization of cell changes on flat preparation (**B**). (**A**) Area of light scattering peak was determined every 10 pixels above and below central line. Integrated area of axial densitometry at each measuring point was used for comparing light-scattering intensities. (**B**) In flat preparation, lens was divided by central line into superior and inferior parts with two 2-mm wide peripheral bands and a 2–2.5 mm wide central region, to localize cell changes.

sented only the corneal reflex appearing in the anterior cortex. During 8–16 weeks of irradiation, this increased significantly in the UVB rats, compared with the control rats, in which any increment resulted only from aging of the animals.

The light scattering intensities measured at a 10-pixel level above or below the central line indicated the greatest density occurred at +10 pixels (Figure 4). Opacities developed in both intensity and range at both measurement times; there were significant increases of light scattering in the UVB lenses, compared with the controls, at 8 and 16 weeks, at all 7 measurement positions ($P < 0.01$). However, the difference in UVB lens opacities between the superior and inferior paired positions analyzed by the *t*-test was not significant because the mean values had high coefficients of variation, ranging from 9.3–22.7% at 8 weeks and from 14.6–35% at 16 weeks.

Table 1 shows the depth of the UVB lens anterior opacities in the upper and lower parts of the lenses,

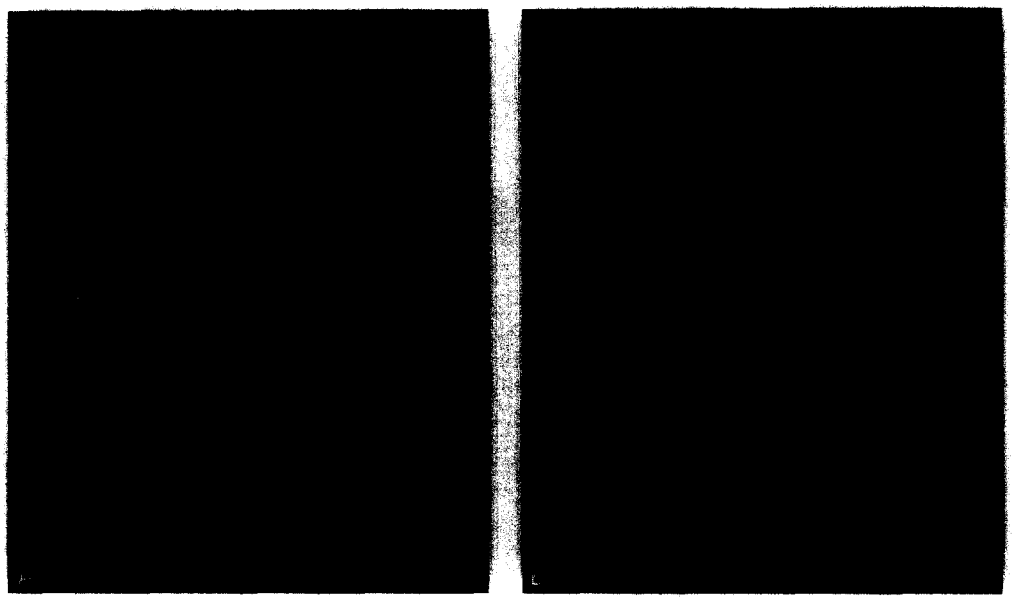

Figure 2. Scheimpflug photos of lenses in two groups at end of study. (**A**) Control lenses did not show obvious opacity. (**B**) Opacity appeared in anterior central region in UVB group.

Jpn J Ophthalmol
Vol 41: 381-387, 1997

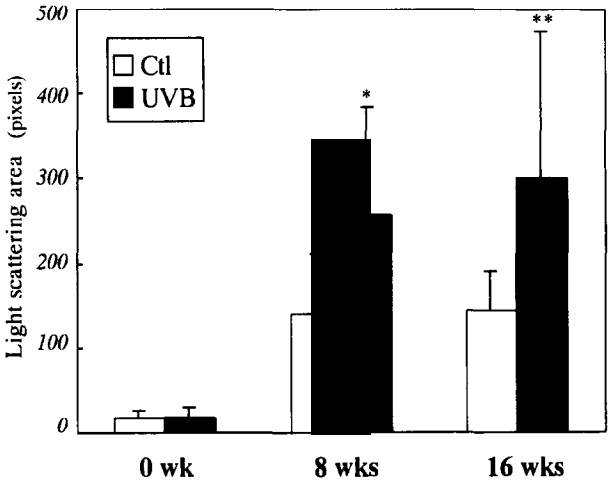

Figure 3. Comparison of light scattering area in anterior central region of UVB-irradiated and nonirradiated eyes. At both 8 and 16 weeks, increases in light scattering were more significant in lenses of UVB group ($*P < 0.05$, $**P < 0.01$, Mean ± *SD*).

Table 1. Depth of Opacities at ±15 Pixels in UVB Lenses (mm)

		8 Weeks ($n = 18$)	16 Weeks ($n = 17$)
Superior	Mean values	0.372	0.456
(+15 pixels)	Standard Deviation	0.041	0.096
Inferior	Mean values	0.316	0.347
(−15 pixels)	Standard Deviation	0.051	0.083
P[a]		0.001	0.0013

Data expressed in millimeters was calculated from EAS-1000 program.
[a] *t*-test: superior vs inferior.

measured at 15 pixels above and below the center line through the anterior pole (Figure 1). At 8 weeks, depths were 0.372 mm (upper) and 0.316 mm (lower); at 16 weeks, they increased to 0.456 mm and 0.347 mm. Depths of the opacities in the superior and inferior parts were significantly different at both measurement times.

The epithelia at various sites (Figure 1, legend) were examined at the end of the experimental period for UVB-induced changes. Figure 5 shows the changes in the central area: the size, density and arrangement of epithelial cells varied significantly. The nuclei were not stained homogeneously (Figure 5B); many damaged cells with pyknotic nuclei had gathered together or spread out (Figure 5C). Clumps of cells appeared as superimposed nuclei, some clumps were elongated (Figure 5D). Acellular areas were also noted (Figure 5E). Cells of less uniform size and

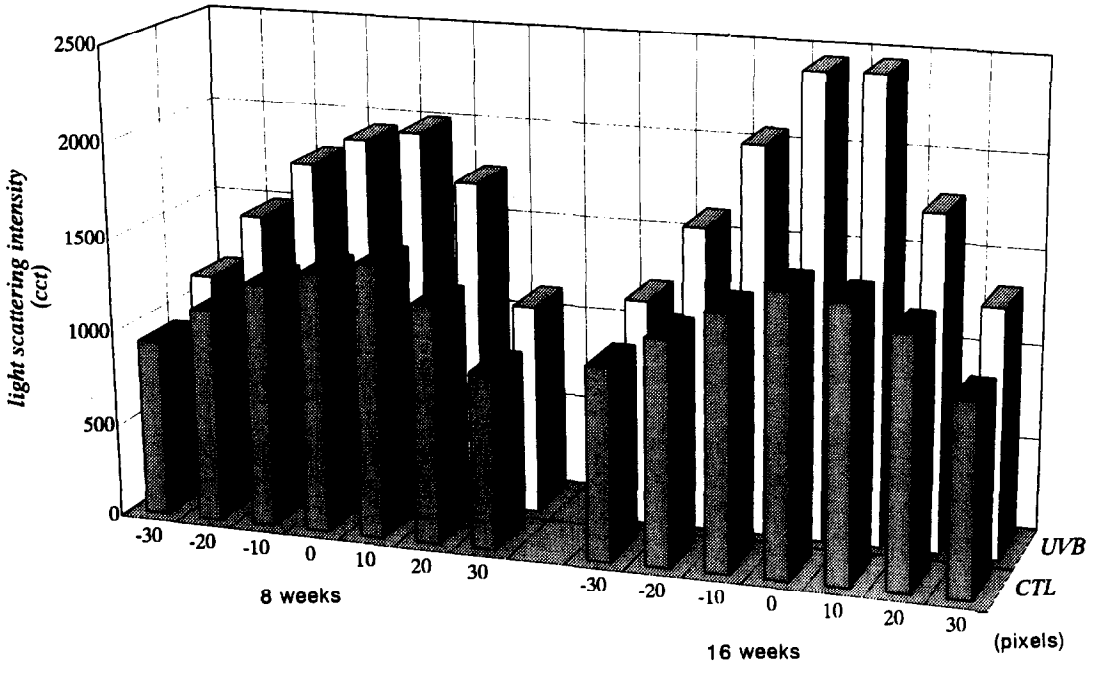

Figure 4. Comparison of light-scattering intensities every 10 pixels at 8 and 16 weeks during experiment in two groups showed significant increases in UVB group at all measurement points; most obvious scattering at +10 pixel points at both measurement times.

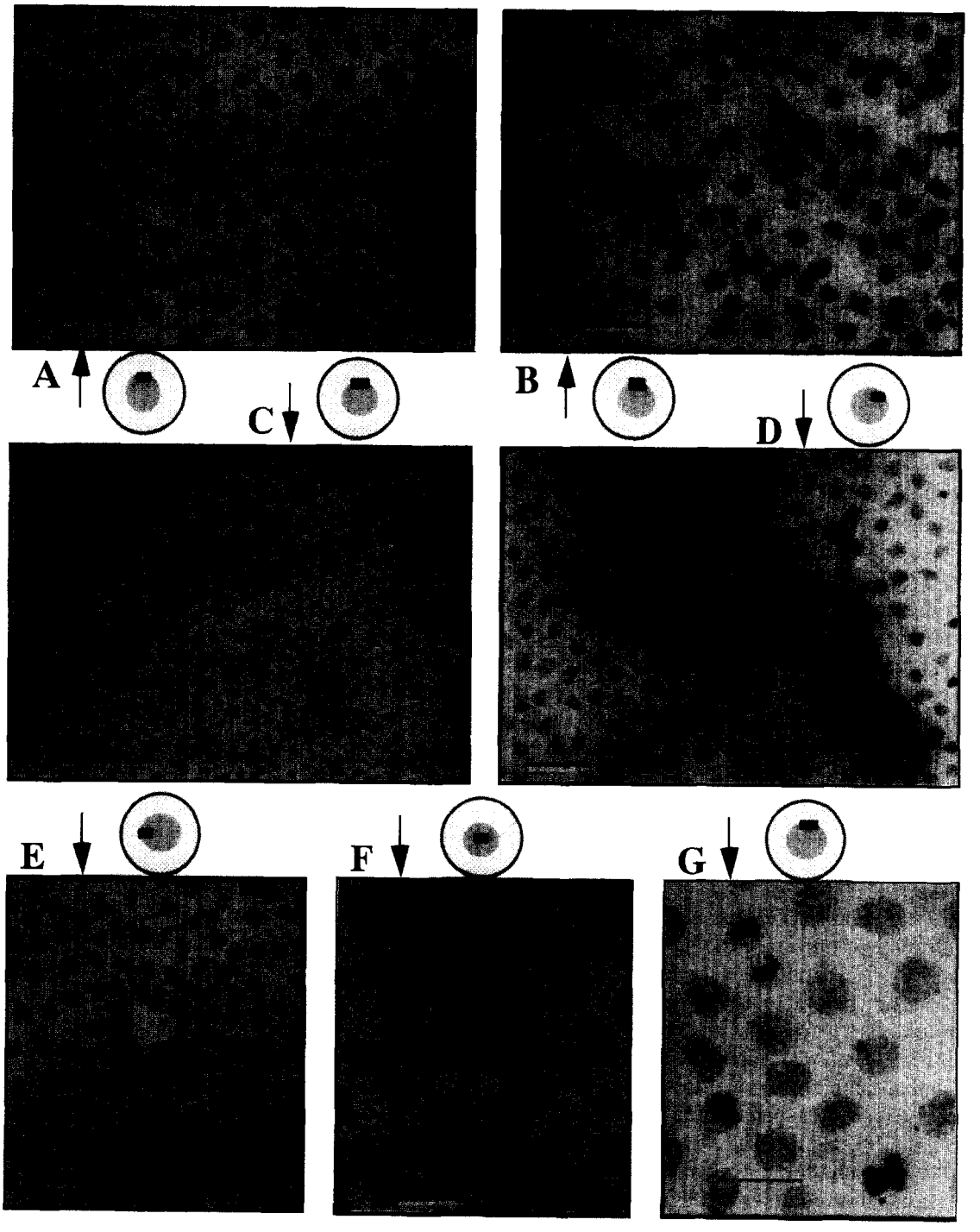

Figure 5. Location of lens epithelial changes. Size and distribution of epithelial cells in superior central region are different in nonirradiated (**A**) and UVB-irradiated (**B**) rat lenses. (**C**) Grouping of damaged cells with pyknotic nuclei and cell loss occurred in some places. (**D**) Clumps of epithelia, some elongated, appeared to be superimposed. (**E**) Acellularity. (**F**) Enlarged cells, small pyknotic cells and normal cells. (**G**) Mitosis occurred in central area. Black rectangles in circles next to photos indicate location on lens photographed. Bar in A, B, D, E: 35 mm; in C: 70 mm; in F,G: 17.5 mm.

Jpn J Ophthalmol
Vol 41: 381–387, 1997

distribution were accompanied by an accumulation of debris and some pyknotic nuclei (Figure 5F). Mitosis was seen in the central region, 2.5 mm from the mount margin, unlike the control lenses in which mitosis was seen within 1 mm of the margin (Figure 5G). These morphological changes were more prevalent in the superior central area than the inferior central area. Epithelia at the equator had a normal morphological appearance, as did the control lenses.

Discussion

UVB-induced cataract in the rat is one of the most widely used and successful experimental models for ultraviolet cataract study found in the literature.[7,8] In this study, we also used this model to investigate lens opacities developed in the anterior central region by UVB irradiation. These opacities appeared along the anterior capsule and developed toward the deeper cortex as UVB exposure duration increased. They differed from human cortical cataracts, which occur in the periphery as wedge-shaped opacities with higher incidence in the lower lens segment.[3–5]

While cortical cataractous opacities are predominantly localized in the lower nasal quadrants, only a few studies have investigated this.[3–5] Possible explanations of localized lens opacities in the lower part of the lens include effects of blood flow, aqueous composition, or aqueous circulation,[4] and UV irradiation if the UV effect is believed to be the chief cause of cortical cataract formation.[5] The etiology is not yet clear.

To clarify the correlation between UV irradiation and localization of opacities, rats in this study were irradiated by UVB from above, with no reflection from the sides or below. Image analysis showed that the extent of opacity and its development differed in the superior and inferior parts of the opacity when the UV source came only from above. In this experiment, development was faster in the upper segments closer to the UVB source which received longer exposure to UVB radiation. Ultraviolet light is known to scatter much more than visible light: it is also necessary to examine the possible effects of UVB irradiation from other directions such as the nasal, temporal, or lower sides (ground reflection). We suggest that the direction of the UVB source influenced the variations in the severity of opacification in this rat study.

The photochemical effects of UVB on lens cells in vitro have been described.[14,21,22] Lens epithelial cells, including membrane structure, enzyme function, proteins and DNA, would be the initial target of direct UVB irradiation to the lens in vivo since UVB is first transmitted to the capsule and its appended epithelium. Results shown in Figure 5 indicate that the epithelium of the central area is a UVB target in vivo. We saw no significant cell changes in the peripheral region even though the central opacities had developed into the cortex by the end of this experiment. It is possible that the peripheral regions of the rat lens received less UVB exposure because of iris and lid obstruction. It is also possible that the cells in the central region are more susceptible to photodamage in vivo. In cultured rabbit lenses, Reddan et al found that peripheral cells were more resistant to hydrogen peroxide toxicity than cells in the central and preequatorial regions.[20] Our previous study also showed that lens proteins and membrane lipids in cultured lenses or lens proteins from the equator in solution were less insulted by UVB irradiation compared with those of the anterior cortex (unpublished data).

The present study showed, in agreement with many before, that anterior polar opacities developed toward the deeper cortex. The chief factor was UVB directly transmitted to the central lens epithelia and lens tissue, which produced photochemical toxicity. Although we did not measure the UVB dosage reaching the rat lens in vivo, it is clear that the rat lens is exposed to UVB to a much higher degree than the lens of most other animals. Jose and Pitts hypothesized that abnormal differentiation of the epithelia acted as a light-scattering influence.[10] In rats irradiated with 0.15 and 0.2 J/cm^2 of UVB, Schmidt et al showed remarkable histological changes at the anterior pole of the lens, generally spreading toward the deeper cortical regions.[8] In the present study, epithelial mitosis and proliferation were found in the central region, but this was not an adequate explanation of the deep opacity development which was not consistent with the extent of cell proliferation (Figures 2, 5). We therefore assumed that UVB could directly damage lens epithelia and lens fibers in the anterior central area.

What occurs in human eyes remains unknown, since rat and human anatomical characteristics differ, as does the UVB exposure. It has been reported that less than 10% of UVB at 310 nm can be transmitted to the human lens.[13,16] The rat eye is more prominent, with a thinner cornea (1/2) and shallower anterior chamber (1/5) (author's unpublished data) than the human eye. Therefore, under identical irradiation conditions, rat lenses would be exposed to a much greater amount of UVB than human lenses, and the affected sites would also differ.

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