institution. Further investigations and clinical trials are necessary to appreciate the full diagnostic value of this technique.

From the Department of Ophthalmology, University of Illinois Eye and Ear Infirmary, Chicago. Research funded by Grants EY 1107-02 and EY 24-16 from the National Institutes of Health, and in part by grants from the Illinois Lions Club and the National Retinitis Pigmentosa Foundation. Submitted for publication March 18, 1975. Reprint requests: Dr. C. A. Peyman, University of Illinois Eye and Ear Infirmary, 1855 W. Taylor St., Chicago, Ill. 60612.

Key words: scleral-chorioretinal biopsy, retinal dystrophies, malignant melanoma, sympathetic ophthalmia.

REFERENCES

The response of mouse ocular tissues to continuous near-UV light exposure. S. ZIGMAN, J. GROFF, T. YULO, AND T. VAUGHAN.

Continuous exposure of mice to near-ultraviolet (UV) light (black light) over a period of 19 weeks induces adverse alterations in lens protein chemistry, in lens epithelial cell differentiation, and in retinal photoreceptor structure at more than a doubled rate as for 12 hours a day of intermittent exposure. No histologic changes were found in the cornea. The results may indicate the presence of repair mechanisms in these ocular tissues for damage induced by radiant energy.

We have shown that intermittent exposure of mice to black light for many weeks resulted in damage to their lenses and retinas. These included severe thinning of the outer segments and macular degeneration between 10 and 17 weeks, and total destruction of the photoreceptors by 60 to 70 weeks.1 Between 30 and 60 weeks, lens epithelial cells lost their ability to differentiate into fiber cells and pyknotic nuclei permeated the cortex. Lens protein synthesis and growth were also inhibited, and a buildup of insoluble proteins to levels much higher than those of aging controls was found.2 From 60 weeks on, many lens cortical opacities were seen.

In this study, mice of the same strain were exposed to black light 24 hours a day. By comparing the times of appearance of the changes induced by continuous light to those induced by intermittent light, the dose dependency and repair potential of the ocular tissues damaged by near-ultraviolet (UV) light can be assessed.

Materials and methods. Two hundred 8-week old female A/J mice (Jackson Laboratories, Bar Harbor, Me.) were divided into two groups and housed, five per plastic cage. One week after the animals were received, the experimental conditions were imposed. Using heavy black plastic sheeting, the air conditioned mouse room was divided into two separate chambers, each additionally ventilated by a fan. In one chamber, 40 W black light tubes (General Electric, BLB) were fastened to the top of the shelf 3 inches above the cages. The average intensity of the lamps, as measured with an Ultraviolet Products, Inc. Long wave-UV light meter, was 450 µW per square centimeter. All cages received the same amount of UV-irradiation and were periodically rotated. Both chambers had a 12-hour on-off period of incandescent visible light at 50 foot candles. Animals were fed, housed, and cared for as described in Reference 1.

The mice were weighed at time zero, four, eight, thirteen, and nineteen weeks. Four groups of six mice each were then selected at random from the two chambers and were killed with ether. Eyes were immediately removed after death and dissected. Lenses were quickly examined, weighed, and frozen. Eight eyes from each group were removed and fixed in 4 per cent glutaraldehyde for histologic studies. Eye sections of 7 µ were stained with hematoxylin and eosin.

The lenses of each group were pooled and used for biochemical study as indicated in Reference 1.

Results. The only grossly observable differences between the irradiated and control animals were redness and irritation of the skin of the ears and tails, a situation which continued throughout the duration of the experiment. Erosion of the ears and tails after scab formation was common during the second half of the experiment, but no skin tumors were seen.

No differences in body weights were observed between the irradiated and control animals during the 19 weeks of the experiment, but the average control mouse lens weight was greater from 4 weeks on by 0.4 to 0.8 mg (Fig. 1). The mean values are significantly different at a confidence level of one chance in 175.

No significant differences were observed in the total soluble protein increment between control and irradiated mouse lenses during the 19-week
duration of this experiment, nor was there any major change in their distribution as assessed by polyacrylamide gel electrophoretic analysis. Only one component, judged to be a beta-crystallin on the basis of mobility and size, was present at lower levels in the irradiated lenses than in the control lenses starting at 8 weeks. Total insoluble protein levels, however, were appreciably elevated in the near-UV-treated mouse lenses by eight weeks and throughout the duration of the experiment (Fig. 2, A). One of the insoluble protein components was especially elevated as shown by polyacrylamide gel electrophoretic analysis (Fig. 2, B). This component corresponds to the lowest mobility, highest molecular weight water-insoluble protein (HM) of the mouse lens described previously.2

Observable histologic alterations in the lens began after 13 weeks of exposure to near-UV light, at which time an accumulation of undifferentiated epithelial cells with pyknotic nuclei was found (Fig. 3). By 19 weeks the abnormal cells were present in much greater numbers and extended much further into the lens, as shown in Fig. 3. No grossly observable opacities were seen throughout the experimental times.

In the retinas of the mice continuously exposed to near-UV light, a similar progression of photoreceptor damage, as was found in the intermittently exposed mice, occurred, but it took place much earlier (Fig. 4). Outer segments and much of the outer nuclear layer were lost, as shown in Fig. 4.

In neither the continuous nor the intermittent UV-light exposure experiments were the corneas found to be damaged.

Discussion. Continuous exposure of white albino

Fig. 1. Growth characteristics of the lenses of mice exposed to near-UV (Black light) for 24 hours a day, and controls. See materials and methods for other details. Standard errors are shown by long vertical brackets. (Closed circles, controls; open circles, UV.)

Fig. 2, A. Accumulation of the total insoluble proteins in the lenses of mice maintained for 19 weeks under continuous near-UV light plus incandescent light for 12 hours a day compared to controls exposed to the incandescent light only. Each point represents the mean of four separate determinations done on pools of 8 to 10 lenses each, and the standard error is included. (Closed circles, controls; open circles, UV.)

Fig. 2, B. Increase in the level of the highest molecular weight and lowest electrophoretic mobility sulfonated insoluble protein component in the lenses of mice. Comparisons are made between near-UV light exposed and control animals, as in Figs. 1 through 2, A. Each point represents the mean of four separate determinations done on pools of 8 to 10 lenses each, and the standard error is included. (Closed circles, controls; open circles, UV.)
Fig. 3. Sections of the lenses of mice exposed for 24 hours a day to black light (near-UV) and for 12 hours a day to incandescent light and the controls (exposed only to the incandescent light for 12 hours a day) after 13 and 19 weeks. (×450; hematoxylin and eosin.) Note the accumulation of pyknotic nuclei and abnormal fiber cells, especially at 19 weeks.

Fig. 4. Sections of the retinas of mice exposed for 24 hours a day to black light and for 12 hours a day to incandescent light and controls (exposed only to the incandescent light for 12 hours a day) after 13 and 19 weeks. (×450; hematoxylin and eosin.) Note thinning of photoreceptors and presence of wandering cells at 13 weeks and total loss of photoreceptors at 19 weeks.
mice to near-UV light led to similar destructive changes in the lens and retina as found previously for 12 hour a day exposure at the same intensity but, as expected, they occur much sooner. Continuous exposure led to a depression of lens weight increment compared with controls within four weeks. For a similar change to occur in the 12 hour a day exposures, 30 to 35 weeks were required.

No change in soluble protein level from controls was found within the 19 weeks of the continuous exposure experiment reported here, but a 10 per cent lower soluble protein level was observed by 35 to 40 weeks of near-UV light due to 12 hours a day of exposure. An increase of lens insoluble protein content over controls was noted after only 8 weeks of exposure for 24 hours a day, but took 30 to 40 weeks to occur in mice exposed for 12 hours a day. This increase occurred mainly in the component found to represent the major amount of the total insoluble protein. This insoluble protein component does not identify as a specific crystallin, and may be a membrane component. For a lowered lens weight to appear as compared to controls, a fourfold length of time was required in the 12 hour a day exposed animals as in those exposed 24 hours a day. The intermittently exposed animals also required a fourfold longer time period to initiate a similar increase in the level of lens insoluble protein as those exposed continuously.

This study raises the question of whether repair mechanisms exist in certain ocular tissues which aid in their resistance to possible damaging effects of radiant energy. Evidence of this possibility is provided by corneal epithelial cells which were not observed to be adversely influenced throughout the intermittent or continuous near-UV light experiments. Further exploration of this possibility is under way.

From the Departments of Surgery (Ophthalmology), Biochemistry, and Animal Medicine, University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y. 14642. This work was supported in part by Public Health Service Research Grant No. EY-00459 (National Eye Institute) and in part by a grant from the H. M. Pack Foundation. Submitted for publication Dec. 5, 1974.

Key words: near-UV light, continuous exposure, intermittent exposure, mouse, lens proteins, lens epithelial cells, retina photoreceptors.

REFERENCES