

tration of 85 mEq. per kilogram of lens water in 165 mOsm. medium.²

The concentration of sodium should be 16 mEq. per kilogram of lens water or less after osmotic equilibration in a 185 or 165 mOsm. medium. In rats and in rabbits² the concentration of sodium does not change and is the same or slightly higher in a hypotonic solution than it is in an isotonic medium.

Isosmotic volume equilibration. The activities of cation "pumps" and "leaks" are determined by ionic concentrations as distinct from ionic content. The decrease in volume of a cell or lens in hypotonic solution occurs while the concentration of ions is constant. Therefore, an additional factor is influencing volume.

The decrease in volume is associated with a loss in potassium content. In duck red cells this is due to the fact that potassium permeability is related to volume and therefore its efflux is accelerated until the volume returns to its initial value.³ A similar increase in permeability with hypotonic swelling has been reported for lens.^{2, 4}

The change in potassium content and water volume requires 30 minutes for the red blood cell,³ 13+ hours for the rat lens, and 24+ hours for the rabbit lens.⁵

Lens volume. The observations of Cotlier, Kwan, and Beaty² were limited to a 4 hour period, which permitted a completion of the osmotic equilibration but not the isosmotic volume equilibration of rabbit lenses in hypotonic medium. That the second process was in effect, however, is indicated by the fact that osmotic lens swelling at 4 hours was only 63 and 78 per cent of theory for lenses in 270 and 238 mOsm. media. The rabbit lenses incubated by Kinoshita, Merola, and Hayman¹ in hypotonic media for 24 hours had 40 per cent of the volume expected for osmotic swelling. Rabbit lenses incubated for 48 hours in a 220 mOsm. medium had a volume increase equal to 24 per cent of theory.⁵

Conclusion. This work was undertaken to reproduce the magnitude of swelling found in sugar cataracts so that the effects of swelling could be studied in the absence of polyols. Osmotic swelling, however, was counteracted by another process associated with a loss of lens potassium and lens water. The loss of potassium in 24 hours in 185 mOsm. medium equaled 33 per cent of the initial content and could be due to a loss of cell substance or to a cellular adaptation to hypotonic medium. The dry weight of the lens, which is almost entirely lens protein, remains constant during the experimental period. The potassium concentration in hypotonic medium agrees with what would be expected for an intact lens without an increase in extracellular space. The sodium content at the most would allow an extracellular space of 11 per cent. Therefore, it is concluded that the observed loss in water and potassium content is not due to a loss in lens substance but is a cellular adaptation to hypotonic medium similar to that reported for duck red cells.³

The authors are grateful for the technical assistance provided by Miss Janis Langston.

From the Department of Physiology, The University of Connecticut Health Center, Farmington, Conn. This research was supported in part by research grant EY-00902 from the National Eye Institute, National Institutes of Health, Bethesda, Md. Submitted for publication March 16, 1976. Reprint requests: John W. Patterson, M.D., Department of Physiology, The University of Connecticut Health Center, Farmington, Conn. 06032.

Key Words: volume regulation, lens, hypotonic medium, cations, cataract.

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Hormonal influences on photoreceptor damage: the pituitary gland and ovaries.

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To determine whether the absence of pituitary or ovarian hormones would influence retinal degeneration, female albino rats were either hypophysectomized (HYPEX) or ovariectomized (OVEX) before puberty. Later, they were exposed to continuous light for periods up to 45 days. Retinas evaluated by light microscopic measurements showed damage to the outer nuclear layer (ONL) and photoreceptor layer in both the operated and intact, control rats. However, the degree of damage observed in retinas of HYPEX and OVEX rats was significantly less than that observed in retinas of intact rats exposed to the same lighting conditions. Therefore, hypophysectomy and ovariectomy, which influence the normal development of sexual maturation when performed on immature rats, significantly reduce photoreceptor damage in adult rats exposed to continuous light.

Exposure of albino rats to continuous, low-intensity illumination at environmental temperatures commonly used in laboratories and animal

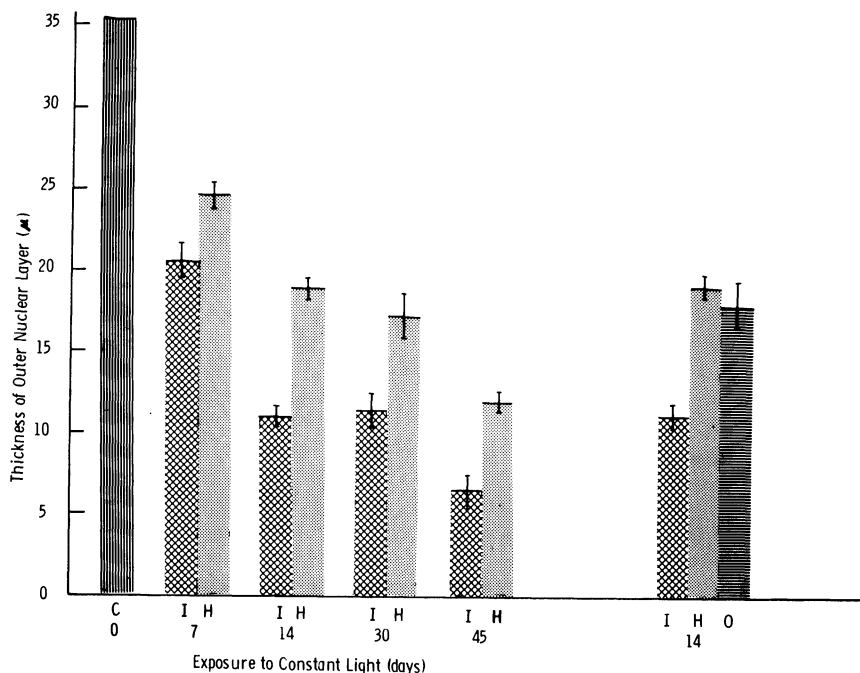


Fig. 1. Thickness of the outer nuclear layer (ONL) of rat retinas exposed to cyclic photoperiod (control, C), or to continuous illuminance (L:L) for 7, 14, 30, and 45 days. Hypophysectomy (H) and ovariectomy (O) were performed at 5 weeks of age. I, Intact L:L group. All rats were 14 weeks old when L:L was begun. Each group represents measurements from at least 6 eyes (mean \pm S.E.M.).

rooms (70 foot-candles, 22° C.) causes destruction of the photoreceptors in the outer nuclear layers (ONL) of the retina.¹⁻³ Both the degree of retinal damage and the rate at which it occurs are dependent on the intensity of the light source, the environmental temperature, and the duration of the exposure period.²⁻⁴ Structural changes are visible in the pigment cell, ONL, and photoreceptor layer at the electron microscopic level after only 6 hours of exposure, but these changes are reversible. However, after 96 hours of exposure the changes are irreversible.⁵ The degree of photoreceptor damage is also dependent on the age of rats when exposed to continuous illuminance. The retinas of sexually immature rats are much more resistant to photoreceptor damage than those of adult rats.⁶ The initial susceptibility to light damage is coincident with sexual maturity, suggesting a possible relationship between the degree of retinal damage and the maturation of the pituitary-gonadal axis. The purpose of this study is to investigate the effects of the removal of the pituitary gland and ovaries on retinal photoreceptor damage caused by exposure of rats to continuous fluorescent illumination.

Materials and methods. Intact and hypophysectomized 35-day-old female CD albino rats were purchased from the Charles River Co. The day

following arrival, groups of intact animals were ovariectomized by the dorsal approach. The animals were housed in clear polyethylene cages with wire tops at 22° \pm 1° C. with a diurnal cycle of 14 hours of light at an intensity of 70 ft.-c. and 10 hours of darkness. They were provided with rat food and water ad libitum. When the animals reached 14 weeks of age, they were placed in continuous fluorescent light with a spectral range of 397 to 732 m μ (maximum at 590 m μ) and an approximate intensity of 70 ft.-c. The total energy flux density of 210 μ w per square centimeter millimicron was measured with a Model SR spectroradiometer (Instrumentation Specialties Co. (ISCO), Lincoln, Nebr.), at animal eye level. Three intact and three hypophysectomized rats were maintained in continuous light for periods of 7, 14, 30, and 45 days. Four ovariectomized (OVEX) and four hypophysectomized (HYPEX) animals were exposed for 14 days. At the end of each exposure period the animals were anesthetized with ether and exsanguinated, and the eyes were enucleated. The eyes were fixed in Bouin's solution, dehydrated with alcohol, and embedded in paraffin. Tissue blocks were sectioned serially at 7 μ thickness on the anterior-posterior axis, and sections of the central retina including the optic nerve were stained with Harris's hema-

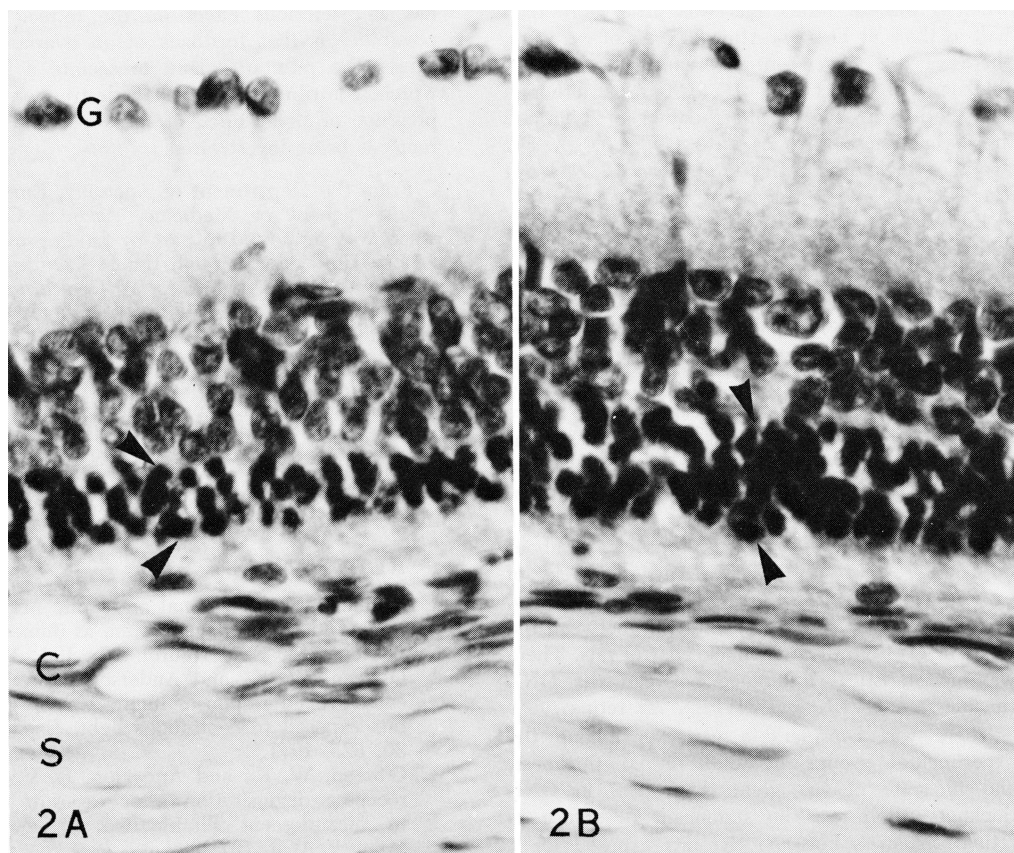


Fig. 2. A, Retina from a continuous light-exposed intact rat after 14 days. Compare the thickness of the outer nuclear layer (between arrows) with that in B. S, Sclera; C, choroid coat; G, ganglion cell layer. (Original magnification, 400 \times .) B, Retina from hypophysectomized rat exposed to continuous light for 14 days. The ONL (between arrows) in this example is approximately 44 per cent thicker than that in A. (Original magnification, 400 \times .)

toxylin and eosin. The following measurements were made on each retina with an ocular micrometer at 400 \times magnification: (1) ONL thickness, measured from the outer limiting membrane inward to include all photoreceptor nuclei, and (2) retinal thickness, the distance from the outer limiting membrane to the inner margin of the ganglion cell layer. The two measurements were taken at six different loci around the circumference of each retinal section, beginning at the periphery. Loci were separated by a distance of approximately 1,078 μ . Statistical comparisons were based on a two-tailed, one-way analysis of variance.⁷

Results. The average thickness of the ONL of retinas from intact and HYPEX rats exposed to continuous low-intensity light for 7, 14, 30, and 45 days and of OVEX rats exposed for 14 days is compared in Fig. 1 with that of control rats maintained in cyclic light. There is a significant reduction in the ONL thickness of all rats exposed to

continuous light when compared with the ONL thickness of rats maintained in cyclic light. Fig. 1 also shows that the longer exposure periods (14 to 45 days) caused greater reduction in the thickness of the ONL than was seen after 7 days.

After 1 week of exposure there was a 15.7 per cent difference between the ONL thickness of intact and HYPEX rats. Retinas from intact rats had a reduction in the photoreceptor cell population as compared to those of HYPEX rats, but the difference in the mean measurements was not statistically significant. Focal areas of photoreceptor damage were located in the posterior retinas of intact, control animals approximately 1.2 mm. lateral to the optic disc. At these sites, a statistically significant reduction in thickness of the ONL occurred.

Exposure for 14 days (Fig. 2) produced a highly significant 42.8 per cent difference between intact and HYPEX rats ($p < 0.001$). The ONL thickness of HYPEX rats averaged 19.32 μ ,

whereas that of intact rats was little more than half as thick at 11.06μ . After 30 days of exposure, there was a 37.8 per cent difference between ONL thickness of intact and HYPEX rats, although they both were slightly more damaged in limited areas than those after 14 days ($p < 0.01$). The average thickness of the ONL was 17.32μ for HYPEX rats and 10.71μ for intact rats.

Forty-five days of continuous light exposure caused almost total destruction to the ONL of intact animals. At many areas around the retina of intact rats, the photoreceptor nuclei were totally absent. When ONL nuclei were observed, they were rarely more than two rows deep. The average thickness of the ONL of intact rats was 4.98μ . Retinas from HYPEX rats also showed severe damage, but the average thickness (11.69μ) was more than twice that of intact rats. There was a 56.6 per cent difference in ONL thickness between intact and HYPEX rats ($p < 0.001$).

OVEX slowed the degenerative process to about the same extent as HYPEX. After 2 weeks of exposure, the ONL of OVEX rats was 18.10μ . There was a 38.9 per cent difference between the ONL of OVEX rats and intact, control rats. There was no statistical difference between the ONL thickness in OVEX and HYPEX rat retinas.

There was considerable variation among the experimental groups in total retinal thickness, and in spite of the great differences in ONL thickness, there was no significant difference in retinal thickness between the rats exposed for 1 week and for 45 days. There was, however, a statistically significant decrease in the total retinal thickness of intact rats between the first (87.78μ) and second (73.22μ) week of exposure, followed by an increase in total thickness (87.7μ) at 30 days. In HYPEX rats the pattern was considerably different. Again, there was no difference in total retinal thickness between the animals exposed for 1 week and for 45 days. But, after 2 weeks, instead of a reduction in total retinal thickness, there was a slight increase. Total thickness of the retina was 82.74μ at one week and 89.60μ at 2 weeks. It remained high at 30 days (90.66μ), but was reduced significantly by 45 days of exposure (74.97μ , $p < 0.01$). There was no difference between the intact and OVEX total retinal thickness at the single exposure period, 14 days.

Discussion. Prevention of sexual maturity by removal of the pituitary gland or the ovaries during the fifth week after birth affords a significant amount of protection to the retina of albino rats from the damaging effects of continuous exposure to low-intensity fluorescent light. Possibly, a pituitary hormone acts directly on the retinal receptor cells, or perhaps one of the pituitary hormones, such as follicle-stimulating hormone, stimulates the ovary, and one of the ovarian hormones, in turn,

has a deleterious effect on the retina. A third possibility is that feedback of an ovarian steroid causes the pituitary gland to secrete a hormone which contributes to retinal destruction in the presence of light. Each of these possibilities currently is being investigated.

From the Department of Anatomy, Emory University School of Medicine, Atlanta, Ga. This study was supported in part by funds provided by Emory University through the McCandless Fund, and by National Institutes of Health grant EY 01566. Preliminary results have been previously presented at the annual meeting of the American Association of Anatomists.⁸ Submitted for publication May 18, 1976. Reprint requests: Dr. W. Keith O'Steen, Department of Anatomy, Emory University School of Medicine, Atlanta, Ga. 30322.

Key words: eye, retina, retinal degeneration, ovariectomy, hypophysectomy, aging, sexual maturity, photoperiod, photoreceptor damage.

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Photosensitive pigments formed with rat opsin. ROSALIE CROUCH.

Photosensitive pigments are formed in vitro from rat opsin with the rods in suspension or as digitonin solutions. Rhodopsin, isorhodopsin I, and isorhodopsin II were generated with 11-cis, 9-cis and 9,13-dicis retinal, respectively. An isorhodopsin I analogue was formed on combination with 9-cis 13-desmethyl-14-methylretinal. The absorp-