



# Constant Light Produces Severe Corneal Flattening and Hyperopia in Chickens

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**In this study we report on the effects of constant light (CL) on the refractive development and ocular morphology of White Leghorn chicks (Cornell K-strain). Refractive state and corneal curvature were measured by IR photoretinoscopy and IR keratometry respectively. The axial lengths of the ocular components were measured by A-scan ultrasonography. We find that constant light produces significant hyperopia compared to controls in as few as 10 days (7.4 vs 4.0 D). This is apparently the result of flatter than normal corneal curvature (radius of curvature: 3.22 vs 3.08 mm) as vitreous chamber depth is significantly deeper in CL eyes than controls at that age (5.6 vs 5.1 mm). In contrast to other reports, if CL rearing is continued for longer periods the hyperopia progresses, even though vitreous chamber depth continues to increase. After 11 weeks of CL severe hyperopia was observed (18.2 vs 2.8 D). Long term CL is also found to produce shallow anterior chambers, corneal thickening, lenticular thinning and cataracts, and damage to the retina, pigment epithelium, and choroid.**

Chicks Eye growth Constant light Ocular morphology Refractive state

## INTRODUCTION

A variety of animal models have been used for research on the regulation of eye growth and the development of ametropia. Among these, chicks are the most frequently used due largely to the remarkable responsiveness of their eyes to experimental manipulations of visual experience. Refractive errors (myopia and hyperopia) have been induced in chick eyes using a variety of environmental conditions such as visual field occlusion (Wallman & Turkel, 1978), constant darkness ("CD", Gottlieb, Wentzek & Wallman, 1987), defocussing spectacle lenses (Schaeffel, Glasser & Howland, 1988; Irving, Callender & Sivak, 1991), and constant light ("CL", Lauber, Boyd & Boyd, 1970). Lauber *et al.* reported that raising chicks under conditions of CL results initially in hyperopia (after 3 weeks) that subsequently becomes myopia (after 6 weeks) as abnormal ocular enlargement continues (Lauber *et al.*, 1970; Lauber, 1987, 1991a). Based on these results it has been suggested that chicks reared under CL can be used as a model of myopia (Lauber, 1991a, b).

We raised White Leghorn chicks (Cornell-K strain) under CL for up to 120 days and compared the results with chicks reared under normal light/dark cycles. We observed abnormal corneal flattening and hyperopia after as few as 2 weeks of CL. Unlike the results reported by

Lauber (1991a), corneal flattening and hyperopia increase with continued CL in Cornell-K strain White Leghorn chicks. No myopia, nor even a reduction of hyperopia, has been observed under these conditions. This report also gives a complete description of the effects of CL on gross ocular morphology using slit-lamp and fundus photography, and corneal and retinal histology. A preliminary report of this work was given at the annual meeting of the Association for Research in Vision and Ophthalmology (Li, Troilo, Glasser & Howland, 1992).

## MATERIALS AND METHODS

Thirty-eight White Leghorn chicks (Cornell-K strain) were used in this study. At 1 day of age they were randomly divided into control ( $n=20$ ) or CL ( $n=18$ ) groups. The control group was maintained under a 14 hr light/10 hr dark cycle. The CL group was maintained in continuous light under otherwise identical conditions. The ambient illumination level in the aviary was an average of 700 lx. Illumination was supplied by fluorescent lamps (Sylvania 40 W, Cool White). Both groups were raised for the first 4-6 weeks in temperature controlled brooders (30°C). They were later maintained in large cages at room temperature (21°C). Food (Agway), crop gravel, and water were provided *ad libitum*.

Optometric measures were made in 6 chicks from each group every 3-5 days for the first 3 weeks of life, then weekly until they were 80 days old. Refractive error was measured, without cycloplegia, using an infrared photorefractor (Schaeffel & Howland, 1987). A

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TABLE 1. Ocular dimensions [mean  $\pm$  SE (mm)] measured from frozen-sectioned eyes of 120-day-old chicks

Measurement	Constant light eye	Control eye	P-value*
Corneal diameter	9.29 $\pm$ 0.24	10.30 $\pm$ 0.12	0.0008
Lens diameter	7.53 $\pm$ 0.10	7.45 $\pm$ 0.10	0.6087
Eye diameter	19.32 $\pm$ 0.41	17.54 $\pm$ 0.24	0.0018
Radius of:			
corneal curvature	8.25 $\pm$ 0.31	5.18 $\pm$ 0.09	0.0001
ant. lens curvature	7.86 $\pm$ 0.72	6.22 $\pm$ 0.34	0.0436
post. lens curvature	-6.65 $\pm$ 0.75	-4.45 $\pm$ 0.21	0.0079
Anterior chamber depth	0.84 $\pm$ 0.04	2.37 $\pm$ 0.04	0.0001
Lens thickness	326 $\pm$ 0.14	3.81 $\pm$ 0.05	0.0010
Vitreous chamber depth	10.45 $\pm$ 0.47	8.46 $\pm$ 0.16	0.0008
Axial length	14.62 $\pm$ 0.36	14.57 $\pm$ 0.16	0.9053
Number of chicks	6	8	

\*Two-sample unpaired *t*-test.

neutralizing lens technique was employed (just as in conventional retinoscopy). The most hyperopic value at which the retinal reflex was reversed was taken to be the resting refraction. Corneal radii of curvature were measured with an infrared keratometer (Schaeffel & Howland, 1987). Axial length, anterior chamber depth, lens thickness, and vitreous chamber depth were measured with A-Scan ultrasound (3M-Biosound). The ultrasound probe (10 MHz) was extended using a 10 mm length of soft rubber tubing filled with ultrasound transmission gel (Aquasonic). The open end of the tube was placed on the corneal surface near the optic axis. Proparacaine HCl (0.5%) was used as a corneal anesthetic.

Fundus and slit lamp examinations of the eyes were made in randomly selected birds at various ages. Fundus photographs were taken with a hand-held camera (Kowa) following cycloplegia with vecuronium bromide to dilate the pupils. Slit lamp examination of the anterior segment was made using an ophthalmic slit lamp (Bausch & Lomb) with camera attachment.

#### Histology and frozen sections

Anterior segment histology was performed on 6 control and 6 CL eyes at 120 days of age. The eyes were enucleated and the anterior segments were removed. The tissue was stored in 4% buffered paraformaldehyde under refrigeration. After dehydration in graded alcohols and xylene the anterior segments were embedded in paraffin and cut using a rotary microtome into 10  $\mu$ m horizontal sections parallel with the optic axis. Sections were stained with Harris' hematoxylin and eosin, and photographed on an Olympus BH-2 microscope. To view the retina, choroid and sclera, the posterior segments of 2 normal and 2 CL eyes were embedded in paraffin, sectioned horizontally, and stained with Harris' hematoxylin and eosin.

Gross ocular morphology was further examined from photographs of frozen-sectioned eyes. At 120 days of age, 6 CL and 8 normal eyes were enucleated and immediately frozen in isopentane cooled by liquid nitrogen to about  $-30^{\circ}$ C. The eyes were positioned dorsal side up in a cryostat and sectioned horizontally. To assure that the eye

was hemisected several photographs were taken as the sections approached the mid-point of the eye. The photograph showing the largest axial lens thickness was used to measure the parameters given in Table 1. The radii of curvature were measured from 3 equally spaced points subtending  $90^{\circ}$  on the cornea, or  $120^{\circ}$  on the respective lens surfaces, as measured from the point where the equatorial axis of the lens intersects the pupillary axis.

Statistical comparisons of CL to control eyes for any of the measures presented in this study were made using two-sample unpaired *t*-tests.

## RESULTS

Constant-light-rearing results in a generally enlarged posterior segment, flattening of the corneal and lenticular surfaces, and high levels of hyperopia which persisted throughout the duration of our study. Despite significantly larger vitreous chambers, we never observed a myopic refraction as measured through 80 days of age.

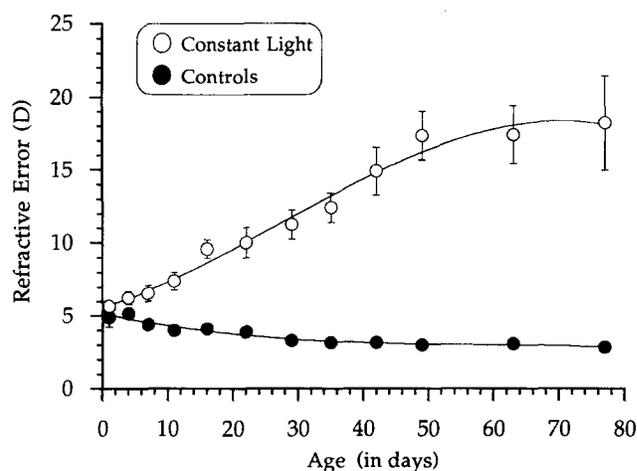


FIGURE 1. Change in refractive error with age for control and CL chicks. The constant light produced significant hyperopia compared to controls in as few as 10 days. Error bars represent standard errors of the mean. Note that here, and in Figs 2 and 3, in some cases the standard errors are smaller than the symbols on the graph. Third order polynomials were used to fit lines to the data points here and in Figs 2 and 3.

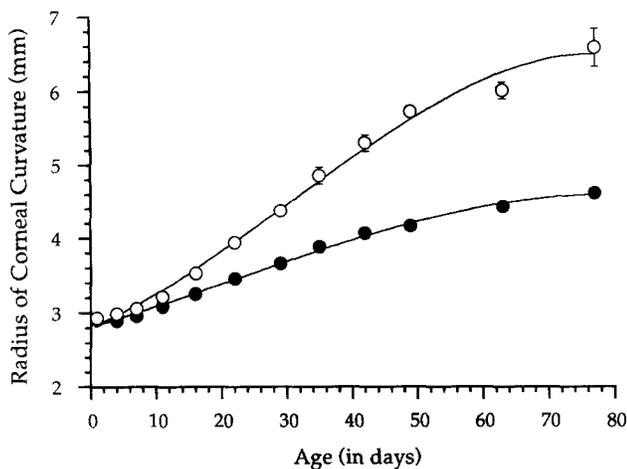


FIGURE 2. Change in radius of corneal curvature with age for control and CL chicks. With the chicks growing under constant light, the radius of corneal curvature increased significantly relative to controls.

### Refractive state

Constant light produced significant hyperopia compared to controls in as few as 10 days (7.4 vs 4.0 D, two sample unpaired *t*-test,  $P < 0.01$ , Fig. 1). As CL rearing is continued the hyperopia progresses; after 80 days of CL more severe hyperopia was observed (18.2 vs 2.8 D,  $P < 0.01$ ).

### Radius of corneal curvature

The corneas of chicks raised under constant light treatment became progressively flatter than normal control chicks as indicated by the increasing mean radius of corneal curvature (Fig. 2). After 10 days CL, the radius of curvature of experimental eyes was significantly greater than controls (3.22 vs 3.08 mm,  $P < 0.05$ ). The corneas continued to flatten and remained significantly flatter than controls during the entire experimental period (80 days: 6.58 vs 4.62 mm,  $P < 0.01$ ).

### Ultrasound measurements

Figure 3 shows all ultrasound data over time for both CL and normal chicks. The anterior chamber depth of chicks growing in constant light decreased with time. After 7 weeks of constant light treatment the depth of the anterior chamber was less than half that of normally reared chicks (0.8 vs 1.66 mm,  $P < 0.01$ ). Lens thickness in CL chicks gradually decreased relative to controls (49 days: 2.76 vs 2.95 mm; 80 days: 2.75 vs 3.42 mm,  $P < 0.01$ ).

In contrast to the reduction in anterior chamber depth and lens thickness, vitreous chamber depth became significantly deeper in CL eyes than in control eyes. Significant differences were observed as early as 10 days of age (5.6 vs 5.1 mm,  $P < 0.01$ ). The vitreous chamber continued to enlarge in CL eyes. By 80 days the vitreous chambers of CL eyes were, on average, more than 2 mm longer than controls (10.0 vs 7.6 mm,  $P < 0.01$ ).

Axial length never differed significantly between CL

and normal eyes during the duration of the experiment (at 80 days: 13.45 vs 13.22 mm,  $P = 0.541$ ). The shortening of the anterior chamber and thinning of the lens was compensated by the elongation of the vitreous chamber giving axial lengths in the CL and normal eyes that were approximately the same.

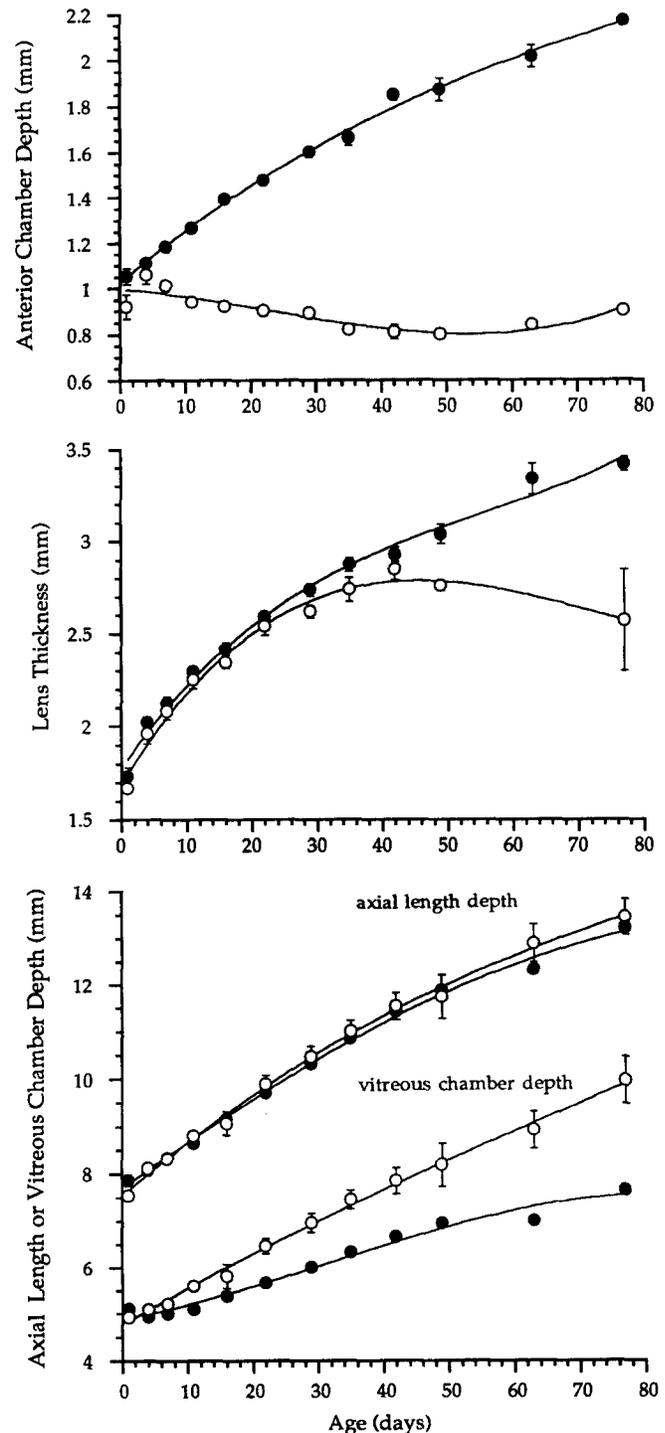


FIGURE 3. Change in ultrasound measurements of anterior chamber depth, lens thickness, vitreous chamber depth, and total axial length with age for control and CL chicks. Anterior chamber depth decreased significantly with the chicks growing in constant light. Lens thickness became thinner in CL eyes. Vitreous chamber depth was significantly deeper in CL eyes than in control eyes as early as 10 days of age. Axial length was not significantly different between CL and normal eye at any age.

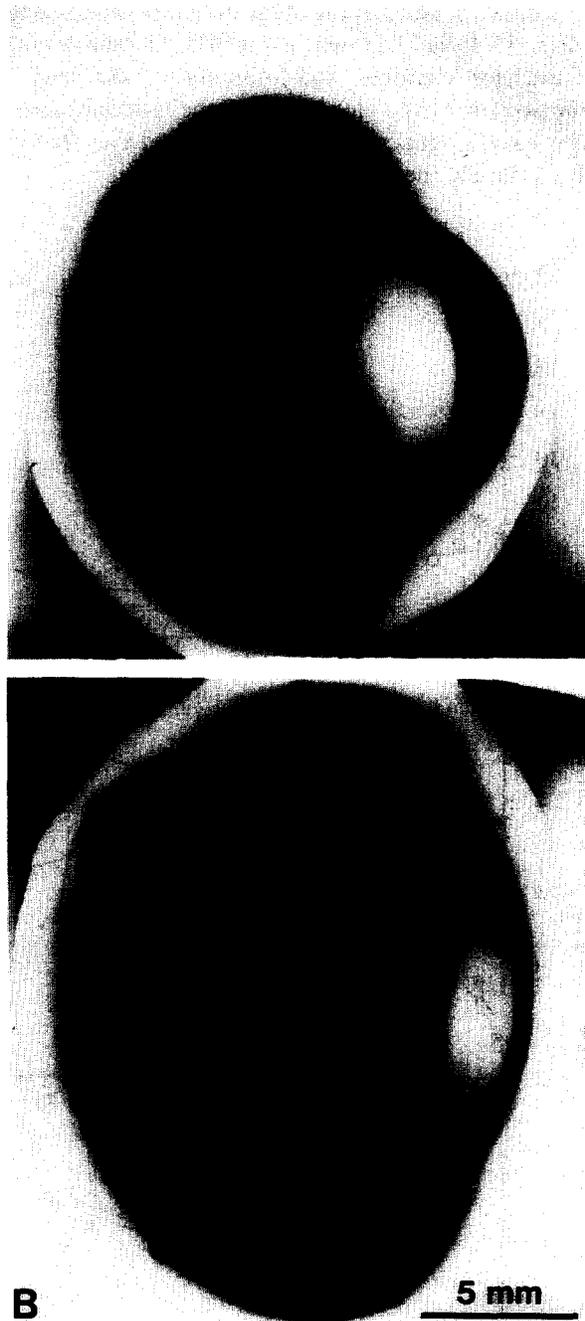


FIGURE 4. Examples of horizontal cross sections through frozen eyes of 120-day-old chicks (A = Control; B = CL). Serial photographs are taken as the sections approach the center of the eye. The photograph showing the largest lens thickness of the series is used for measurement to assure that the section is through the center of the eye. Data collected from such photographs are summarized in Table 1.

FIGURE 5. Examples of slit lamp photographs of the anterior segment of the eye. (A) 2-month-old control chick. The slit lamp camera was focused approximately in the center of the lens so that the reflections from the various ocular surfaces can be observed in a single photograph. Light reflections are, from left to right, posterior lens surface, anterior lens surface, and cornea. Note the clarity of the lens and the depth of the anterior chamber (cornea to anterior lens surface). (B) Slit lamp photograph of the eye of a 3-month-old chick reared in constant light. The camera was focused on the approximate center of the anterior chamber so that the anterior lens surface and corneal surface are clearly visible. Note that the reflections from the cornea and anterior lens surface are extremely close indicating a very shallow anterior chamber. (C) Slit lamp photograph of the same eye shown in (B), but with the camera focused on the posterior lens surface. Note the cataractous opacity in the posterior lens cortex.



FIGURE 5



FIGURE 6. Examples of fundus photographs from a normal control eye (A) and CL eyes (B, C). Compare the smooth fundus of the control eye to the damage dorsal to the pecten (B) in the CL eyes seen in all CL eyes, and tessellation across the retina (C) seen in some of the eyes. These lesions were observed in all continuous-light-reared chick eyes examined (70–80 days of age).

#### Frozen sections

Figure 4 shows representative horizontal frozen sections through control (top) and CL (bottom) eyes. Table 1 gives the means and standard errors of gross morphological measures taken from 6 CL eyes and 8 control eyes at 80 days of age. *P*-values from two-sample *t*-tests are also given and show that the CL eyes had smaller corneal diameters, larger ocular diameters, and larger radii of corneal and lenticular curvatures (i.e.

flatter). As in the ultrasonography data, the CL eyes had shallower anterior chambers, thinner lenses, and deeper vitreous chambers. The axial lengths of CL and control eyes were not significantly different.

#### Slit lamp and fundus photography

Figure 5 shows slit-lamp pictures of the anterior chamber of control and CL eyes. Compared to Fig. 5(A) (control), Fig. 5(B, C) illustrate the shallowness of the anterior chamber of the CL eyes as well as the cataracts of the posterior lens cortex seen in all experimental eyes examined. All the CL animals developed bilateral cataracts after 2 months.

Figure 6 shows examples of fundus photographs. Figure 6(A) shows the smooth appearance of the fundus of the control eyes compared to Fig. 6(B, C) which show the damage and tessellation that appeared in the fundus of every CL eye by 120 days of age. The area just dorsal to the pecten was consistently found to exhibit a vertical tear in the pigment epithelium [Fig. 6(B)] that also affected the outer retina [see Fig. 7(C)]. Besides damage to the area just dorsal to the pecten, some of the CL eyes exhibited widespread damage to the pigment epithelium as indicated by the tessellation across the retina as shown in Fig. 6(C).

#### Corneal histology

We found the corneas of the CL chicks to be significantly thicker than those of the controls (mean  $\pm$  SE:  $165.6 \pm 5.7$  vs  $127.5 \pm 5.3$   $\mu\text{m}$ ,  $P < 0.01$ ). The corneal epithelium of CL chicks was also thicker than controls ( $26.1 \pm 1.0$  vs  $21.1 \pm 0.8$   $\mu\text{m}$ ,  $P < 0.01$ ). These measures were not corrected for tissue shrinkage.

#### Retinal histology

Figure 7 shows representative cross-sections of central retina. Figure 7(A) shows a typical control retina. Figure 7(B) shows the retina from the same region in a CL chick. Note the changes in the outer retina, particularly the loss of oil droplets [arrows in Fig. 7(A)]. We observed these changes across the entire retina of the CL reared chicks. Figure 7(C) shows a section through the region of fundus damage just dorsal to the pecten. This damage was found in all eyes examined and appears to be the result of the enlargement of the vitreous chamber. Note the damage to the outer retina, and the loss of retinal pigment epithelium in the region of the tear.

## DISCUSSION

In this paper we describe the morphological changes and refractive consequences in the eyes of chicks raised under conditions of constant light. We find that severe corneal flattening and reduction in anterior chamber depth more than offset enlargement of the vitreous chamber resulting in significant hyperopia after as little as 1–2 weeks that persisted throughout the duration of the study. The CL-induced changes to the anterior segment include significantly smaller than normal corneal diameter with greater corneal thickness. This suggests

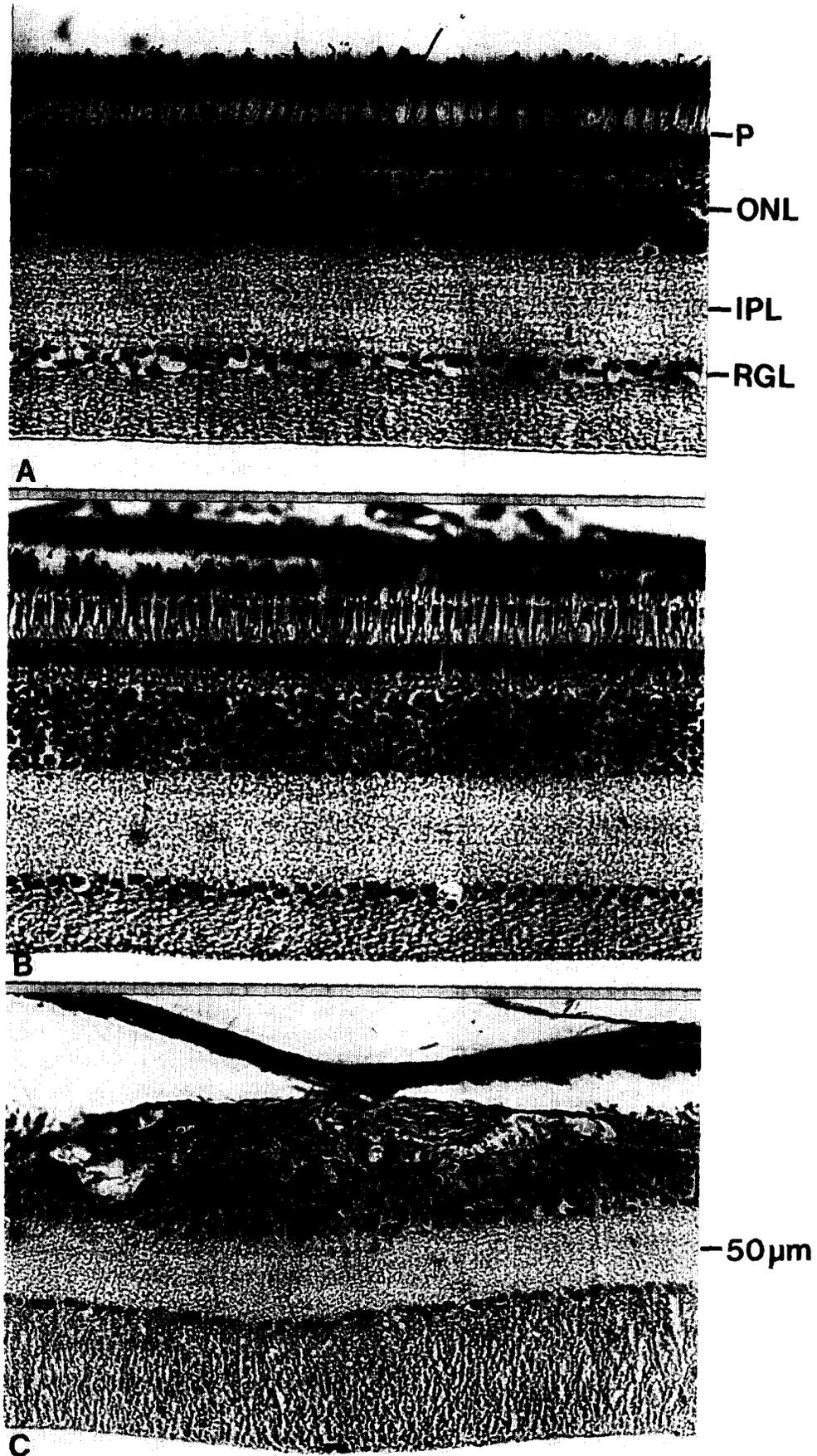


FIGURE 7. Cross sections through the central retinas of control (A) and CL (B, C) eyes. All eyes were treated identically; dehydrated and embedded in paraffin, sectioned at 10  $\mu\text{m}$ , and stained with Harris' hematoxylin and eosin. Labels on (A) show the photoreceptor layer (P), outer nuclear layer (ONL), inner plexiform layer (IPL), and retinal ganglion cells (RGC). The arrow in (A) points to an example of the oil drops normally present in chick retinas. (B) The retina of a CL eye in the area corresponding to that shown in (A) for the control retina. Note the absence of oil droplets. (C) A section through the region of damaged retina just dorsal to the pecten [see Fig. 5(C)]. Note the absence of photoreceptors and pigment epithelium in the damaged region. Scale bar = 100  $\mu\text{m}$ .

that the loss of a circadian light cycle affects not only the development of corneal curvature but the development of the limbus as well.

After several months of constant-light-rearing, we also observed obvious changes in the outer retina, particularly damage to the photoreceptor layer. In the area of the fundus directly dorsal to the pecten we consistently observed separation of the pigment epithelium and severe damage to the outer retina. How these changes in the outer retina are related to the altered growth of the CL eyes is unclear. While the changes in the photoreceptor outer segments may be a consequence of constant light, the regions of severe damage may be due to the excessive enlargement of the eye. We have observed the same lesions dorsal to the pecten in eyes raised with visual deprivation under normal light cycle conditions (unpublished observation).

Rearing chicks under constant light has been used as an animal model for glaucoma research (Lauber *et al.*, 1970; Lauber, 1987). Lauber *et al.* report that, in most cases, the eyes of the animals reared in CL enlarge, and their corneas flatten. Refractive errors were variable when reported; hyperopia was reported after 3 weeks and myopia after 6 weeks. Based on this result, Lauber (1991a, b) suggests that rearing chicks in the constant light also can be used as a model of myopia. However, in our chicks we found that CL does not produce myopia, but rather persistent and high hyperopia. We have observed greater hyperopia from constant light rearing in the Cornell-K strain (used here) than in another strain of White Leghorn chicks (Troilo *et al.*, 1992). Such strain differences could explain some of the difference between our results and others (Lauber *et al.*, 1970; Lauber, 1987, 1991a, b).

In normal development, chick eyes are typically about 5 D hyperopic at 10 days of age and gradually become less hyperopic over the next several weeks. The initial ocular effect following exposure to constant light is flattening of the cornea and, consequently, the development of significantly greater hyperopia. Like Lauber, we also observed enlargement of the vitreous chamber, but our CL chicks never became myopic. On the contrary, the hyperopia worsened during 80 days of CL treatment due apparently to continued flattening of the cornea.

We conclude that CL-induced hyperopia is due to severe anterior segment changes induced by the loss of circadian rhythms. During the period of the experiment, the corneal flattening (and later, lenticular flattening) continued and more than compensated for the increasing vitreous chamber depth. What is responsible for the overall enlargement of the posterior segment of the eye is unclear. One possible explanation for the increased vitreous chamber depth is that it results from the active emmetropization of the eye in response to the hyperopia induced by the flattening of cornea and lens. This is suggested by the fact that the eye remains hyperopic throughout the period studied, thus providing a constant

signal for vitreous chamber deepening if emmetropization is taking place. It has also been reported that formoguanamine-induced loss of photoreceptors in chicks prevents axial enlargement in constant-light-rearing but not the flattening of the anterior segment (Lauber & Oishi, 1990). This implies the involvement of vision in the former response but not the latter. In another study (Gottlieb *et al.*, 1987), however, the eyes of dark-reared chicks were also found to develop hyperopia despite ocular enlargement, while at the same time their vitreous chambers became longer. Similar to constant-light-induced hyperopia, the hyperopia induced by constant dark rearing is the result of corneal flattening. In this case, however, the vitreous chamber elongation could not be due to an emmetropization process, as the chicks were in constant darkness. Thus, it is possible that both corneal flattening and vitreous chamber elongation are the result of the lack of a circadian rhythm. Only further experimentation can determine if emmetropization is actually occurring in our constant light experiments.

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