The Effects of Photoperiod and Lid Suture on Eye Growth in Chickens

George Osol, Bernard Schwartz, and Donald C. Foss

Three models of postnatal eye enlargement in leghorn chicks (surgical fusion of the eyelids, MES; exposure to continuous light, 24L; prolonged darkness, OL) were characterized on a morphologic and temporal basis, and a relationship between photoperiod and eye growth was described. While pronounced enlargement of the eye was evident in the OL and MES groups after 6 wk, a 15-wk experimental period was necessary to produce significant \( P < 0.05 \) eye enlargement under 24L. This enlargement was unaffected by pinealectomy and was characterized by increased equatorial diameter and decreased axial length. The macrophthalmos resulting from both MES and OL was characterized by increases in absolute and relative eye weights, axial length, and equatorial diameter. The MES eyes, however, showed a pronounced bulging of the cornea and increased anterior chamber depth and equatorial diameter, while those from the OL group had a flattened cornea and decreased anterior chamber depth. Finally, a relationship between photoperiod and eye growth was established and was best described by an inverse, continuous semilogarithmic function. Invest Ophthalmol Vis Sci 27:255–260, 1986

Many factors influence the growth rate of the vertebrate eye during embryologic development, and detailed studies with chick embryos have demonstrated the complexity of interactions between genetic, nutritional, and physical forces in regulating ocular morphogenesis. Conversely, little is known about postnatal regulation of eye growth. Understanding the regulatory mechanisms could be of clinical value for ameliorating conditions such as myopia, which frequently is characterized by macrophthalmos and for which pathogenesis and etiology are unknown. Animal models in which abnormal eye growth can be experimentally induced provide an opportunity for investigation of postnatal regulatory mechanisms.

Three distinct macrophthalmic syndromes, each produced by experimental manipulation of normal light perception, have been described in various vertebrate species: avian glaucoma, which is induced by prolonged exposure to continuous bright light; avian macrophthalmos from prolonged exposure to darkness or dim light; and macrophthalmos from eyelid suture in monkeys, tree shrews, and chickens. The objectives of this study were (1) to establish all three syndromes in one species and characterize differences in rate and morphologic patterns of enlargement, (2) to examine the relationship between photoperiod and ocular growth, and (3) to determine if the pineal gland plays a role in the pathogenesis of avian glaucoma. This latter hypothesis was strengthened by an earlier observation that pinealectomy had a protective effect on the retinopathy induced by continuous light in rats.

Materials and Methods

Single-comb White Leghorn cockerels (ISA Babcock Breeders; Ithaca, N.Y.) were used for these studies. Feed (a nutritionally complete 20% protein corn/soy diet) and water were provided ad libitum throughout the experimental period, and all surgery was performed on 3- to 6-day-old chicks. During the first wk, the birds were housed in electrically heated starting batteries illuminated with 15-W cool-white fluorescent bulbs under a photoperiod of 14 hr of light followed by 10 hr of darkness (14L:10D). When 7 days old, chicks were allotted to specially designed environmental chambers.
that were identical except for lighting. Each chamber could comfortably accommodate up to 30 birds and was thermostatically controlled with continuous flow-through ventilation. The two overhead 40-W, cool-white fluorescent bulbs (F40HUCW, General Electric; Cleveland, OH) delivered an intensity of 760 /μW/cm² at bird height, as measured with an Ealing Model 920 radiometer (Ealing Corp.; Natick, MA).

Experiments 1 and 2: Models of Eye Growth and Effects of Pinealectomy

In these studies, we sought to measure and compare the ocular enlargement that resulted from exposure to continuous light (24L), darkness (0L), or monocular eyelid suture (MES). For the MES group, in which the experimental eye was randomly chosen in each bird, we used a variation of the surgical technique described by Yinon, Rose, and Shapiro. Three- to six-day-old chicks were randomly divided between the 14L:10D; 15 under 24L) was conducted with an environmental chamber that was kept on the 14L:10D schedule. The remaining 30 unoperated chicks were randomly allotted to 2 additional chambers: 1 was continuously illuminated (24L), and the other was kept in complete darkness (0L). A 24-hr period with covers raised and room lights on was allowed for acclimation to the new environment. After this time, the chambers were closed and the experimental period began. After 6 wk, birds were weighed and killed by cervical dislocation. The eyes were immediately enucleated, cleaned of extraocular tissue, and weighed to the nearest tenth of a gram. Using a micrometer, axial length, equatorial diameter, horizontal corneal diameter, and anterior chamber depth were measured. Because the avian eye is not perfectly spherical, equatorial diameter measurements were always taken across the horizontal axis, using the position of the optic nerve for reference. Anterior chamber depth was determined by measuring, with an offset micrometer, the axial distance between the apex of the cornea and the corneoscleral junction. Three randomly chosen pairs of eyes from each treatment group were placed into 10% buffered formalin for subsequent dehydration, clearing, and paraffin embedding. Each eye was sectioned along the axial plane at a thickness of 5-μm and, after staining with hematoxylin and eosin (H and E) or periodic acid-Schiff (PAS), corneal and central retinal and scleral thicknesses were measured with an eyepiece micrometer at a magnification of 160X. We obtained five measurements from each eye at randomly chosen points within several adjacent fields.

After analyzing the results of the first experiment, we found that the birds raised under 24L were not macrophthalmic at the end of 6 wk. Consequently, a similar experiment using 30 unoperated chicks (15 under 14L:10D; 15 under 24L) was conducted with an experimental period of 15 wk. Chambers, lighting, and sampling protocols were identical to those in Experiment 1. An additional 30 chicks were pinealectomized at 3 to 5 days of age, using a surgical technique that has been fully described elsewhere. The operated chicks were randomly divided between the 14L:10D and 24L treatments.

Experiment 3: Photoperiod and Eye Growth

Five environmental chambers were used for this study of the effects of photoperiod on eye growth. Each was set to a different photoperiod with individual timers or pecking. The chicks' toenails were clipped as a precautionary measure.
and, dark chamber excepted, the onset of the photophase was synchronized for all. The photoperiods were 0L:24D, 1L:23D, 3L:21D, 9L:15D, and 14L:10D.

Fifteen 1-wk-old chicks were randomly assigned to each chamber and, after an experimental period of 6 wk, all birds were weighed and killed by cervical dislocation. Both eyes were enucleated, cleaned of extraocular tissue, weighed to the nearest gram within 5 min of death, and measured for axial length and equatorial diameter.

All animals were treated according to the ARVO Resolution on the Use of Animals in Research.

Table 2. Effects of unilateral eyelid suture on ocular dimensions in 7-wk-old birds raised under a 14L:10D photoperiod (X ± SE; n = 15)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unsutured eye</th>
<th>Sutured eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye weight (g)</td>
<td>1.5 ± .05**</td>
<td>2.7 ± .13b</td>
</tr>
<tr>
<td>Relative eye weight (g/kg)</td>
<td>2.3 ± .07a</td>
<td>4.1 ± .19a</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>12.6 ± .08a</td>
<td>15.5 ± .16a</td>
</tr>
<tr>
<td>Equatorial diameter (mm)</td>
<td>14.6 ± .14a</td>
<td>18.6 ± .23a</td>
</tr>
<tr>
<td>Anterior chamber depth (mm)</td>
<td>3.2 ± .20a</td>
<td>4.6 ± .21a</td>
</tr>
<tr>
<td>Corneal diameter (mm)</td>
<td>7.9 ± .12a</td>
<td>8.8 ± .16a</td>
</tr>
</tbody>
</table>

* Groups with different superscripts are significantly different (P < 0.05); paired Student’s t-test.13

The ocular dimensions of 7-wk-old chicks grown under continuous bright light for 6 wk did not differ from those of chicks in the 14L:10D control group (Table 1). However, MES resulted in gross unilateral enlargement of the affected eye. Relative and absolute eye weight, axial length, equatorial diameter, anterior chamber depth, and horizontal corneal diameter were all significantly increased in this group by 7 wk of age (Table 2).13

Histologic examination of sectioned stained (H and E or PAS) eyes from each of the treatment groups by two independent observers did not reveal any changes in the cellular appearance of the retina or optic nerve or differences in scleral, retinal, or corneal thickness.

Experiment 2

With a longer experimental period, significant ocular enlargement occurred in chickens raised under continuous light (Table 3). Only absolute eye weight is presented for these data since growth rate is slowed in 16-wk-old birds, and the correlation between body weight and eye weight is small and nonsignificant (r = 0.18 and r = 0.04 for the 14L:10D and 24L groups, respectively).13 The dimensional characteristics of the 24L group show that the increased eye weight was accompanied primarily by an increase in equatorial diameter but not axial length, since the latter actually was somewhat decreased (Table 3). Pinealectomy did not affect eye growth or enlargement, and histologic examination of sectioned eyes did not reveal any significant changes in retinal or scleral thickness between treatment groups.

The changes in eye weight and dimensions resulting from exposure to darkness, continuous light, or from eyelid suture are compared in Table 4.

Experiment 3

Differences in absolute eye weight, axial length, and equatorial diameter observed in 7-wk-old birds grown under different photoperiods were stratified into 3 statistically discrete levels: 0L:24D > 1L:23D, 3L:21D > 9L:15D, and 14L:10D (Table 5). Generally, the effect was graded, with an inverse relationship between photoperiod and eye size. While this trend persisted when

Table 3. Ocular dimensions in intact (C) and pinealectomized (Px) 16-wk-old chickens raised under a diurnal photoperiod (14L:10D) or in continuous light (24L) (X ± SE; n = 15)

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>Surgical treatment</th>
<th>Eye weight (g)</th>
<th>Axial length (mm)</th>
<th>Equatorial diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14L:10D C</td>
<td></td>
<td>2.47 ± .05**</td>
<td>15.0 ± .26a</td>
<td>18.9 ± .11a</td>
</tr>
<tr>
<td>14L:10D Px</td>
<td></td>
<td>2.55 ± .06a</td>
<td>15.1 ± .14a</td>
<td>19.3 ± .14a</td>
</tr>
<tr>
<td>24L C</td>
<td></td>
<td>2.86 ± .09b</td>
<td>14.5 ± .23a</td>
<td>20.0 ± .20a</td>
</tr>
<tr>
<td>24L Px</td>
<td></td>
<td>2.78 ± .07b</td>
<td>14.0 ± .19b</td>
<td>20.1 ± .16b</td>
</tr>
</tbody>
</table>

* Groups with different superscripts are significantly different (P < 0.05); analysis of variance followed by Duncan's New Multiple Range Test.14
eye weight was expressed relative to body weight, only
the dark-reared group (0L:24D) could be considered
0.42 (r² = 0.176), and the overall correlation coefficient
eye weight and body weight for the 14L:10D group was
(P < 0.05); 7-wk-old birds (n = 15).
† 16-wk-old birds (n = 15).

Table 4. Summary of changes in ocular dimensions resulting from monocular eyelid suture (MES), exposure to darkness (0L), or to continuous light (24L). Results are expressed as a percentage of mean of age-matched controls kept under 14L:10D.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental manipulation</th>
</tr>
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<tbody>
<tr>
<td>Absolute eye weight</td>
<td>MES*</td>
</tr>
<tr>
<td>Relative eye weight</td>
<td>180</td>
</tr>
<tr>
<td>Axial length</td>
<td>178</td>
</tr>
<tr>
<td>Equatorial diameter</td>
<td>123</td>
</tr>
<tr>
<td>Anterior chamber</td>
<td>127</td>
</tr>
<tr>
<td>Corneal diameter</td>
<td>144</td>
</tr>
</tbody>
</table>

* 7-wk-old birds (n = 15).
† 16-wk-old birds (n = 15).

Discussion

The results of these studies show that ocular enlargement can be induced postnatally in both eyes by manipulating the environmental lighting conditions, or in one eye by surgically fusing the lids closed over the selected eye. Furthermore, the temporal and morphologic profiles of the macophthalms differ, depending on the experimental conditions. In a preliminary experiment, significant increases in eye weight were measured in lid-sutured and dark-reared chicks by 3 wk of age (unpublished observations), and differences between control and MES and 0L birds were striking (Tables 1 and 2) and grossly apparent by 7 wk of age. Conversely, a 15-wk experimental period was necessary to show a statistically significant difference in eye weight in birds raised under continuous bright white light (Table 3), and the percent increase in weight was considerably less. Chiu, Lauber, and Kinnear15 measured significant differences in relative eye weight in 4-wk-old broiler chicks raised under continuous light: the differences in results may be related to their use of a faster growing bird or, more intriguingly, to the spectral quality and intensity of the light sources (fluorescent vs incandescent).

All three treatments (24L, 0L, MES) produced an enlargement of the posterior hemisphere evidenced by an increased equatorial diameter; however, compared with age-matched controls, axial length increased in MES and 0L-reared chicks but decreased in the 24L group. It is not known if this difference in eye shape was caused by selective trophic mechanisms or by altered geometric constraints of the orbit in 16- versus 7-wk-old birds.

There were changes also in the anterior portion of the eye; anterior chamber depth and horizontal corneal diameter increased significantly in the MES group but were decreased (anterior chamber depth) or unaltered (corneal diameter) in chicks raised in darkness. In a study with 8- to 25-wk-old chicks, Yinon and colleagues16 observed similar changes in the anterior chamber following lid suture and found the affected eyes to be highly myopic (~21.9 diopters on the average). We did not determine whether these changes in the anterior pole of MES birds resulted from the altered visual input or from localized responses to the thermal and mechanical influences exerted on the eye by the fused lids. By injecting latex into the corneal stroma of monkeys, Wiesel and Raviola17 were able to show that a disturbance in visual input was sufficient to cause an increase in axial length but did not report any differences in anterior chamber depth from lid suture or corneal opacification; hence, this question remains unresolved. The decrease in anterior chamber depth in dark-reared birds supplements earlier observations in which exposure to very dim light for 10 wk increased the corneal radius of curvature.5 Although changes in the anterior chamber of 24L birds were not defined in these studies, others15 have shown that anterior chamber depth is decreased in chickens raised under continuous light.

The mechanisms by which lid fusion or prolonged exposure to darkness, very dim, or continuous bright light augment eye growth are unresolved. It seems likely
that different mechanisms are involved in different conditions. For example, Wallman, Turkel, and Trachtman have provided convincing evidence that altered visual experience can lead to myopia and axial elongation, and that the resulting conditions are qualitatively dependent on the nature of the experimental alteration. In lid suture, the imbalance in light intensity as perceived by the eyes may be a factor in stimulating the accelerated growth of the "deficient" eye, or the lack of visual acuity. Physical factors may also play a role; with respect to darkness, the combination of a shallow anterior chamber with dilatation of the pupil may impair aqueous drainage and increase intraocular pressure.

Based on the initial observations in chickens by Jensen and Matson nearly 30 yr ago, a series of careful experiments by Lauber, Boyd, and Boyd showed that prolonged exposure to continuous light results in a condition reminiscent of human glaucoma in that intraocular pressure is elevated, aqueous outflow facility is impaired, and retinal damage and blindness ensue over time. Nevertheless, eye enlargement precedes changes in intraocular pressure or outflow facility by several wk and, in spite of considerable effort, the primary causal lesion has not been identified. Since macrophthalmos develops in chickens kept under continuous light following optic nerve transection or after daily topical application of atropine, the mechanism does not appear to be cholinergic or to be mediated by retinal afferents passing along the optic nerve. Intrigued by an earlier report in which pinealectomy decreased retinal damage in rats exposed to continuous light, we pinealectomized 3- to 5-day-old chicks prior to exposing them to 14L:10D or 24L environments. After a 15-wk experimental period, there were no differences in eye growth in either group (Table 3), ruling out pineal gland involvement.

Because ocular growth is so profoundly affected by an absence of light, we were interested in determining whether there is a minimum photoperiod required for normal eye growth. The results of experiment 3, shown in Table 5, may be interpreted in two ways, depending on whether absolute or relative eye weight data are used. Analysis of absolute weights shows a graded response, with eye weight, axial length, and equatorial diameter all increasing as the photoperiod is decreased. Given this interpretation, there does not appear to be a minimum threshold photoperiod, and the relationship is best described by an inverse, continuous semilogarithmic function (Fig. 1).

Conversely, if body weight is taken into account and eye weight expressed on a relative basis, conventional statistical analysis suggests that a threshold requirement does exist, and that it lies at or below 1 hr of light per day. We find the former interpretation to be more appropriate for the following reasons: although the correlation coefficients between body and eye weight were positive for all treatment groups (r = 0.44 - 0.10), r² values are on the order of 0.19. Thus, only 19% of the variation in eye weight can be attributed to variation in body weight. Second, the trend toward increasing eye weights with decreasing photoperiod persists when eye weight is expressed relative to body weight, and it is probable that differences would be statistically significant if the sample size were larger. When mean data are plotted semilogarithmically (see Fig. 1), the relationship appears to be graded, regardless of whether absolute or relative eye weight data are used.

To our knowledge, the relationship between photoperiod and eye weight has not been described previously. Future studies could be directed toward an understanding of the ways in which short photoperiods affect the optical properties of the eye; for example, the shallow anterior chamber (Table 1) and increased corneal radius of curvature in dark-reared chicks may indicate an adaptive mechanism designed to forestall the development of myopia, which might otherwise result from the increase in axial length.

Key words: macrophthalmos, eyelid suture, pinealectomy, photoperiod, eye growth, chickens

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References