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Dimensional and Physiological Lesions in the Chick Eye as Influenced by the Light Environment[1] (38721)

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Domestic chickens reared under continuous light (24L/0D) develop a series of eye lesions culminating in elevated intraocular pressure and blindness (1–3). This light-induced avian glaucoma is characterized by enlargement of the globe (as evidenced by increased eye weight), reduction in anterior chamber depth, and impaired outflow facility (3). Recently we have shown that in preglaucomatous eyes lactic dehydrogenase (LDH) leakage from the cornea is one of the earliest detectable lesions, and that preliminary morphological damage as well can be detected by 4 wk of age (4).

That systemic pathways are involved in the genesis of light-induced avian glaucoma was suggested by a study which showed that bilateral eye enlargement occurs even when light is excluded from one eye of birds reared under 24L/0D (5). Eye enlargement can also be induced in the domestic chicken by greatly reducing the intensity of the environmental light (6, 7). This finding raises the possibility that total exclusion of light from the eye may also have some effect on eye morphology.

The present study involved experiments addressed to the following questions: (a) are all the early ocular morphological changes seen in 24L/0D birds present bilaterally when light is excluded from one eye (Experiment I)? (b) if so, are the effects on the covered eye a result of 24L/0D acting via an extraocular system(s), or are they induced only by lack of light to the eye (Experiment II)? In addition, we have measured aqueous and corneal LDH levels (Experiment III) and here report enzyme changes in 24L/0D birds reared to 8 wk of age, an extension of earlier findings.

Materials and Methods. White Rock chicks (fast growing, broiler type) were reared in light- and temperature-controlled environmental chambers (24 x 24 x 18 in. high). Brooder heat was supplied by warming the inflow air of the positive-pressure ventilation system. Food (chick starter crumbles) and water were available ad lib, the supply of each being placed in the same position in the chamber from day to day, where the chicks had been trained to find them. This is an important aspect of experimental design when chicks are to be reared in total darkness (0L/24D).

Experiments I and II were set up as summarized in Table I. In Experiment I incandescent light was supplied for 14 hr per day (14L/10D, Experiment IA) or continuously (24L/0D, Experiment IB). The light had a peak energy of 2.632 $\mu W/cm^2/nm$ at λmax (Fig. 1), measured at feed-trough height, approximately 22 cm from the light source. The chicks were placed in one of the two lighting treatments from hatching. At 3 days of age, chicks in both Experiments IA and IB were fitted with hoods which excluded light from the left eye while permitting exposure of the rest of the chick (including the right eye) to the light environment. These hoods were fabricated from knitted cotton "infant stockings" sewn to fit the chick's head (Fig. 2). Each hood had a small opening for the beak and a black "iron-on", cone-shaped patch covering the left eye. The eyelids of the left eye were sutured shut. On the right side of the hood a circle of material was removed, thus exposing the right eye to environmental light.

In Experiment II chicks were reared (without hoods) from 1 wk of age in 14L/10D, in "bright" light as in Experiment IA, or in low-intensity light from a "Nite Lite"[2]; maximum intensity at feed-trough height was

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[2] "Panelescent" Nite Lite, Sylvania Lighting Center, Danville, MA.

TABLE I. SUMMARY OF EXPERIMENTAL TREATMENTS.

Experiment	Photoperiod	Maximum intensity (μW/cm^2/nm)
IA[a]	14L/10D	2.632
IB[a]	24L/0D	2.632
IIA	14L/10D	2.632
IIB	14L/10D dim "nite lite"	0.005
IIC	0L/24D (total darkness)	0

[a] Left eye covered, right eye exposed (see text and Fig. 2).

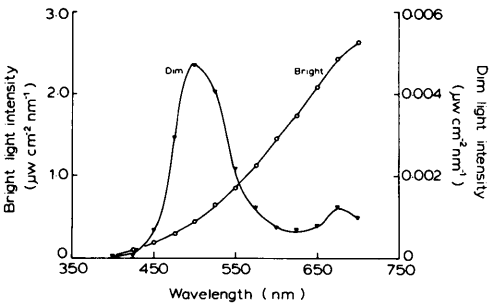

FIG. 1. Spectral distributions and intensities of the lighting treatments used. The left ordinate refers to the bright light curve, and the right ordinate shows the much lower energy range of the dim light source.

0.005 μW/cm^2/nm at λmax (Fig. 1). In a third group chicks were reared in total darkness (0L/24D). In Experiment III chicks were reared from hatching (without hoods) in one of two lighting treatments as described in Experiment I.

All chicks were killed by decapitation at 4 wk (Experiment I), 5 wk (Experiment II), or 8 wk (Experiment III) of age. The eyes were immediately enucleated, trimmed, and weighed. The results have been expressed as relative eye weight (g/kg body weight) in order to circumvent the possible effect (probably irrelevant) of body-weight differences on the results (4). Corneal thickness and anterior chamber depth were measured by the Haig-Streit method (8). Lactic dehydrogenase was measured in aqueous humor and homogenized cornea by use of Boeringer-Mannheim Test Combination reagents[3], as employed by Kim *et al.* (9). The concentration of enzyme is expressed in International Units per unit volume (U/ml) or per unit weight (U/mg) of sample. One International Unit represents the amount of enzyme which catalyzes the conversion of 1 μmole of substrate per minute under standard conditions (25° and pH 7.5).

All data were examined by analysis of variance. Duncan's multiple-range test was used to compare results from the several lighting treatments at the 5% probability level. In Experiment I the exposed and covered eye of each bird were compared by paired *t* test.

Results. Experiment I. Exposure of the eye to 24L/0D (Fig. 3). The exposed right eyes of 24L/0D chicks when compared with the exposed eyes of 14L/10D chicks show: (i) greater eye weight; (ii) reduced anterior chamber depth; (iii) increased corneal thickness (all statistically significant). Thus, as expected, the exposed 24L/0D eyes exhibit the early lesions characteristic of light-induced avian glaucoma (4).

Covering the eye in 24L/0D. When covered and exposed eyes of 24L/0D chicks are compared, the covered eye shows similar morphological changes to the exposed eye, but the changes are more severe. The covered 24L/0D eyes show: (i) greater eye weight ($p < 0.001$); (ii) increased corneal thickness ($p < 0.05$). A slight reduction in anterior chamber depth in the covered eye is not statistically significant.

Covering the eye in 14L/10D. Even in a diurnal photoperiod, covering the eye produces some morphological changes similar to those seen in exposed and covered 24L/0D eyes. Comparison of 14L/10D covered and exposed eyes shows that the covered eyes have: (i) greater eye weight ($p < 0.001$); (ii) reduced anterior chamber depth ($P < 0.05$). Corneal thickness is not significantly affected in the covered 14L/10D eyes.

Comparison of covered 24L/0D and covered 14L/10D eyes shows that the 24L/0D eyes have: (i) greater eye weight; (ii) increased corneal thickness; (iii) reduced anterior chamber depth (all statistically significant). Thus, superimposition of the two experimental conditions, continuous light

[3] Boeringer-Mannheim GBMH, Biochemical Department, Fisher Scientific Co., Montreal, Quebec.

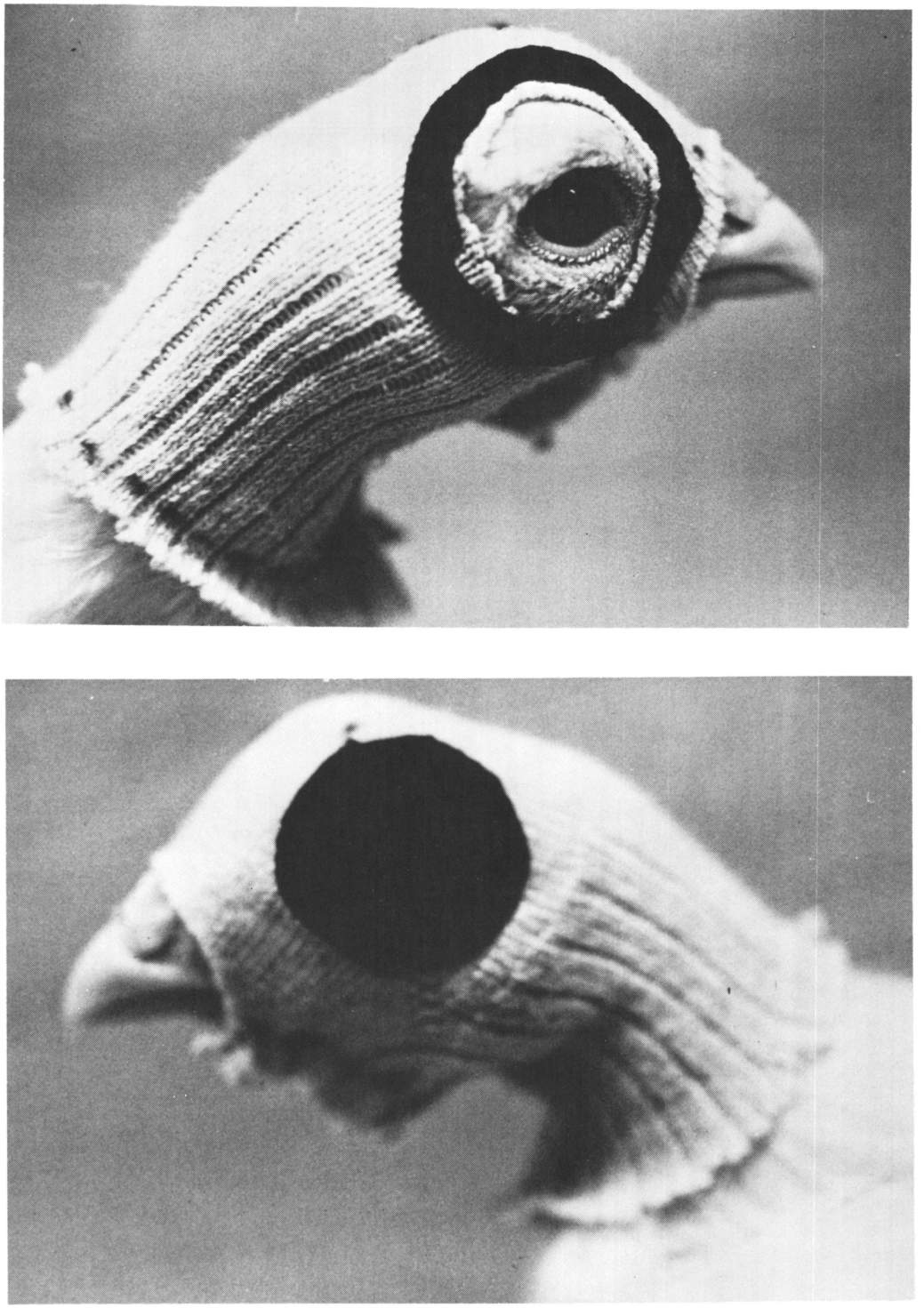

FIG. 2. Chick fitted with a fabricated hood, as for Experiment I, showing the manner in which the left eye was covered while the right eye was exposed to the light environment. For details, see text.

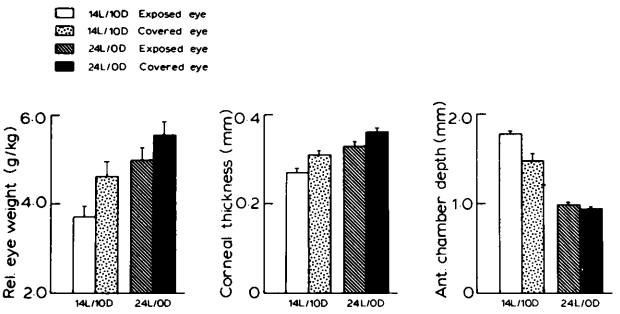

FIG. 3. Physical parameters of the covered and exposed eyes of chicks reared under one of two lighting treatments in Experiment I. Values are means ± SE for n = 11–15 animals at 4 wk of age.

and covering, causes the most severe morphological changes. These data are summarized graphically in Fig. 3.

Experiment II: Exposure to total darkness (*Fig. 4*). Chicks reared in total darkness (0L/24D) develop eye lesions similar to those induced by covering the eye. When compared with controls (14L/10D), eyes of 0L/24D birds show: (i) increased eye weight; (ii) reduced anterior chamber depth (both statistically significant).

Exposure to low intensity light. Dim light causes a significant increase in eye weight over control (14L/0D "bright" light) values. The increase is not as large as that seen in total darkness. Anterior chamber depth is also intermediate between 14L/10D "bright" and 0L/24D values but not statistically different from either. Neither of these experimental treatments causes an increase in corneal thickness.

Experiment III (*Table 2*). Chicks reared in 24L/0D from hatching to 8 wk of age show a reduction of LDH in the cornea and an increase in the aqueous (both statistically significant) as compared with 14L/10D controls. These changes are in addition to the expected morphological lesions of light-induced avian glaucoma.

Discussion. In light-induced avian glaucoma, enzyme imbalance in the cornea and aqueous has been detected as early as 7 days of age. By 9 days corneal curvature was decreased, resulting in reduced anterior chamber depth. Eye enlargement was apparent at 3 wk (4). Like the morphological lesions (1), the LDH changes persist with age, as our present results with 8-wk-old chicks show. A similar enzyme redistribution has been reported for human eyes during cold storage; it has been suggested that this represents leakage from the cornea due to endothelial damage (9).

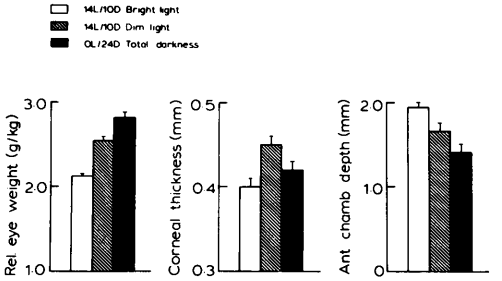

FIG. 4. Physical parameters of the eyes of chicks reared under one of three lighting treatments in Experiment II. Values are means ± SE for n = 11–13 animals, at 5 wk of age (4 wk exposure to lighting treatment).

The results of Experiment I confirm that exposure of the chick to 24L/0D environmental light causes pronounced ocular lesions by 4 wk of age. Experimental hooding itself does not appear to be deleterious. Body weight of hooded birds is slightly below normal, and this is reflected in somewhat higher relative eye weights than have been reported previously (4). However, hooding does not prevent development of the 24L/0D eye lesions outlined above. In experiments with the photogonadal response of Japanese quail, hooding also did not interfere with normal growth and development (10).

The results of Experiment I permit an answer to the first question posed: the early morphological lesions of light-induced avian glaucoma are present bilaterally, even when light is excluded from one eye. The covered eye of 24L/0D chicks exhibits globe en-

TABLE II. LACTIC DEHYDROGENASE (LDH) LEVELS IN CORNEA AND AQUEOUS HUMOR OF CHICKS REARED TO 8 WEEKS OF AGE IN A DIURNAL PHOTOPERIOD (14L/10D) OR CONTINUOUS LIGHT (24L/0D)[a]

	Corneal LDH (U/mg)	Aqueous LDH (U/ml)
14L/10D	0.098 ± 0.007 (10)	2.182 ± 0.452 (10)
24L/0D	0.117 ± 0.004 (10)[b]	0.882 ± 0.217 (10)[c]

[a] Values are means ± SE for numbers of subjects in parentheses.
[b] $P < 0.05$.
[c] $P < 0.02$.

largement, shallow anterior chamber, and corneal thickening by 4 wk of age. Thus, the effect of 24L/0D on the eye appears to be systemic rather than local. However, even in a diurnal photoperiod, covering the eye produces some lesions similar to those induced by continuous light: the globe is enlarged and the anterior chamber is shallow. When the eye is covered and the subject is exposed to 24L/0D, the eye changes are most severe, as if the two effects were superimposed.

The results of Experiment II show that complete absence of light, or drastic reduction of the light intensity under a 14L/10D photoperiod, leads to several ocular lesions similar to those encountered in Experiment I (increased eye weight and shallow anterior chamber). However, as in the covered 14L/10D eyes of Experiment IA, thickening of the cornea is not induced by total darkness or by low intensity 14L/10D light. This suggests an answer to the second question posed: the eye lesions brought on by absence of light appear to constitute a separate phenomenon from that of light-induced avian glaucoma (i.e., induced by 24L/0D).

Very dim 14L/10D light induces changes intermediate in severity between control ("bright" diurnal light) and total darkness values. Previous workers have shown that low-intensity light induced eye enlargement in white leghorn chicks (6, 7). The 15% increase in eye weight seen here (with a light intensity of 0.005 $\mu W/cm^2/nm$ at λmax 500 nm) is of the same magnitude as that reported by Harrison *et al.* (intensity of 0.015 $\mu W/cm^2/nm$ at λmax 450–475 nm) (7) and Bercovitz *et al.* (intensity of 0.017 $\mu W/cm^2/nm$ at λmax 575 nm) (8). In the Harrison and Berkovitz experiments concomitant changes in corneal curvature were not found. Our observations on the effects of very dim light as compared with total darkness suggest that the "dim light effect" may, in fact, represent a response to the absence of light.

The mechanisms by which absence of light affects the eye, and whether this effect is local or systemic, remain unresolved. The greatest increase (28%) in eye weight that we observed is seen in chicks reared in total darkness. The pathological shallowing of the anterior chamber and narrow iridocorneal angle, together with dilatation of the pupil in darkness, could impair aqueous drainage. A resulting accumulation of aqueous may then contribute to severe eye enlargement. However, angle block cannot explain the reduced anterior chamber depth, although a narrow drainage angle might exacerbate impaired outflow. In light-induced glaucoma (i.e., due to 24L/0D light), eye enlargement was evident well before outflow facility was impaired (3, 4). Also, iridectomy did not prevent the eye enlargement and other lesions of light-induced avian glaucoma (11). Thus, angle block does not appear to be responsible for eye enlargement in this syndrome.

Thus, we have shown that the early morphological lesions of light-induced avian glaucoma are systemically rather than locally induced. We further show that absence of light, or very dim light, can induce similar but not identical eye lesions.

1. Lauber, J. K., Schutze, J. V., and McGinnis, J. Proc. Soc. Exp. Biol. Med. **106**, 871 (1961).
2. Lauber, J. K., and McGinnis, J. Vision Res. **6**, 619 (1966).
3. Lauber, J. K., Boyd, J. E., and Boyd, T. A. S. Exp. Eye Res. **9**, 181 (1970).
4. Kinnear, A., Lauber, J. K., and Boyd, T. A. S., Invest. Ophthalmol. **13**, 872 (1974).

5. Lauber, J. K., McGinnis, J., and Boyd, J., Proc. Soc. Exp. Biol. Med. **120,** 572 (1965).
6. Harrison, P. C., Bercovitz, A. B., and Leary, G. A., Int. J. Biometeorol. **12,** 351 (1968).
7. Bercovitz, A. B., Harrison, P. C., and Leary, G. A., Vision Res. **12,** 1253 (1972).
8. Lowe, R. F., Amer. J. Ophthalmol. **62,** 7 (1966).
9. Kim, J. O., Campbell, D. J., and Hassard, D. T. R., Can. J. Ophthalmol. **8,** 132 (1973).
10. Oishi, T., and Lauber, J. K., Amer. J. Physiol. **225,** 155 (1973).
11. Frankelson, E. N., Lauber, J. K., and Boyd, T. A. S., Can. J. Ophthalmol. **4,** 59 (1969).

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