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Light Induced Exophthalmos in the Domestic Fowl.*† (32432)

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Eye enlargement in chickens exposed to continuous light during the growing period has been described(2,3,4). This condition has been characterized by elongation of the eyelids, increased size and weight of the eye (mainly the vitrous body), decreased lens size, and decreased corneal curvature. Pathologically, this condition has shown an increase in the number of eye lesions and a subsequent blindness at arount 2 years after commencement of treatment. Histological examination of the enlarged eyes revealed a thinning of the nerve fiber and rod and cone layers of the retina. Retinal damage has also been found in rats exposed to short periods of excessive light(1,5,6).

In this laboratory, an eye enlargement was also observed in birds subjected to low intensity light (0.01 foot-candles) by Lauber (unpublished). This eye abnormality has the feature of exophthalmia as opposed to the buphthalmia produced in continuous "bright" light (1-10 foot-candles). This paper is concerned with changes occurring in the eyes of birds exposed to low intensity specific spectra light.

Materials and methods. Day-old White Leghorn chicks were randomly assigned to 4 light-control pens. Feed and water were supplied *ad libitum*. Feed used was chick starter for 8 weeks, then chick developer for 10 weeks, and finally, a breeder hen diet for the remainder of the experiment.

Pens were assigned light treatments of clear (controls), blue, green, and red light. Clear light was supplied by plexiglass $CuSO_4$ filtered incandescent light. The colored light was produced by filtering incandescent light through a $CuSO_4$ bath and filters obtained from the Carolina Biological Supply House. These filters transmit narrow, non-overlapping spectra of equivalent energy (microwatts/cm^2/sec.). The blue, green, and red filtered light had a maximum intensity of .006-.009 microwatts/cm^2/millimicron, while the intensity of the clear filtered light was around 0.96 microwatts/cm^2/millimicron, which corresponds to an intensity of 5.5 foot-candles.

At 20 weeks of age, half the birds from each light treatment were transferred to normal

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(4-12 foot-candle) incandescent light, and the remaining half were kept in their respective specific spectra treatments until 40 weeks of age. The light period was the same in all treatments. The lighting schedule was: 16 hours of light per day from day-old to 14 weeks of age, 9 hours of light per day from 14 to 20 weeks of age, and 16 hours of light per day throughout the remainder of the experiment.

Eye measurements were sampled throughout exposure to the various spectra of light. At periods following transfer to incandescent light, eye measurements were taken to determine if the change caused by the low intensity light could be reversed by normal incandescent light. After 60 weeks of exposure to normal light, the birds were sacrificed and comprehensive eye measurements were taken. The fundus was examined for abnormalities and the refractive error was assessed by observing the lens power in the opthalmoscope required to focus on the center of the fundus. Eye protrusion was determined on the live birds by subtracting the distance between the two lacrymals from the distance between the apex of the cornea to the opposite lacrymal.

After sacrificing the birds, the eyes were removed from the orbits, cleaned of extra-orbital fat and muscle, and fixed in 10% formalin. After fixation, eyes were weighed and measured for width and depth along transverse and sagittal planes. The orbits were halved and lens diameter and thickness, pecten width and heght, and corneal radius of curvature were recorded.

After staining with hematoxylin and eosin, slides of the rear of the eye were examined. Thickness of the various layers of the eye wall was measured at between 1 and 2 mm from the optic papilla. Further staining of the gross eye with oil red stain and sectioned eye with periodic acid schiff stain were carried out for histocemical purposes.

Results and discussion. Pullets reared to 20 weeks of age on the low intensity light developed exophthalmos (Fig. 1). The enlargement of the eye was great enough to cause a change in the normal configuration of the head. The extent of protrusion at 80 weeks of age was between 1.7 and 2.0 mm greater in the low intensity colored light as compared to the control light treatments (Table I). Among the treatment groups, blue light showed the greatest amount of protrusion, followed by red and green, respectively. All these differences were significant at the 1% level.

FIG. 1. Photograph of mature Leghorn hens. Control on right, bird on the left reared to 20 weeks of age in the low intensity specific spectra light and maintained from 20 to 80 weeks of age in normal incandescent light.

It is evident from the enlarged orbits and the ophthalmoscopic findings (Table I) that there is a radical change in the development of visual apparatus. The negative power of

TABLE I. Eye Measurements of White Leghorn Hens Reared in Different Low Intensity Light Spectra and Maintained on Clear Incandescent Light from 20-80 Weeks of Age.

	Light treatments			
	Control	Green	Red	Blue
Opthalmoscope lens diopters (left eye)	−.68 ± .17*(22)†	−8.97 ± 2.09 (18)	−8.29 ± 1.29 (19)	−12.47 ± 1.50 (19)
Eye protrusion (mm)	4.48 ± .54 (20)	6.17 ± .19 (19)	6.39 ± .22 (19)	6.52 ± .17 (19)

* Mean and standard error.
† No. of animals measured.

TABLE II. Measurements of Eye from White Leghorn Hens Reared in Different Low Intensity Light Spectra and Maintained on Clear Incandescent Light from 20-80 Weeks of Age.

		Rearing period light treatment		
	Control	Green	Red	Blue
Eye wt (g)	2.34 ± .04* (20)†	2.97 ± .10 (20)	3.62 ± .09 (20)	3.84 ± .04 (20)
Eye width (cm)	1.80 ± .01 (20)	1.94 ± .02 (20)	2.10 ± .02 (20)	2.13 ± .01 (20)
Eye depth (cm)	1.42 ± .01 (20)	1.56 ± .03 (20)	1.56 ± .20 (20)	1.67 ± .03 (20)
Lens diameter (mm)	6.57 ± .16 (12)	6.98 ± .11 (10)	7.26 ± .05 (10)	7.29 ± .09 (10)
Lens thickness (mm)	4.05 ± .14 (11)	4.36 ± .12 (10)	4.34 ± .07 (10)	4.42 ± .09 (10)
Pecten width (mm)	6.31 ± .25 (16)	10.05 ± .41 (10)	7.71 ± .20 (10)	7.95 ± .35 (10)
Pecten height (mm)	4.09 ± .10 (16)	3.34 ± .11 (10)	4.05 ± .19 (10)	3.90 ± .13 (10)
Corneal radius of curvature (mm)	4.85 ± .01 (35)	4.78 ± .05 (34)	5.10 ± .12 (27)	4.89 ± .06 (30)

* Mean and standard error.
† No. of eyes measured.

the opthalmoscope lens required to focus on the center of the retina was significantly less in the control birds.

Gross measurements taken on the enucleated eyes are shown in Table II. Eyes from the low intensity colored light treatments were significantly heavier and had a greater diameter of width and depth than the controls. This enlargement measured after 60 weeks of exposure to normal incandescent light developed during the first 20 weeks of age in the dim colored light. At 12 weeks of age, the eye weights from samples of birds in the blue, green, red, and clear lights were 3.98, 3.38, 3.96, and 2.18 g, respectively.

This eye enlargement differed from the buphthalmos that is developed in continuous light, in that corneal proportions were maintained. In the buphthalmos described by Lauber *et al* and Lauber and McGinnis, there

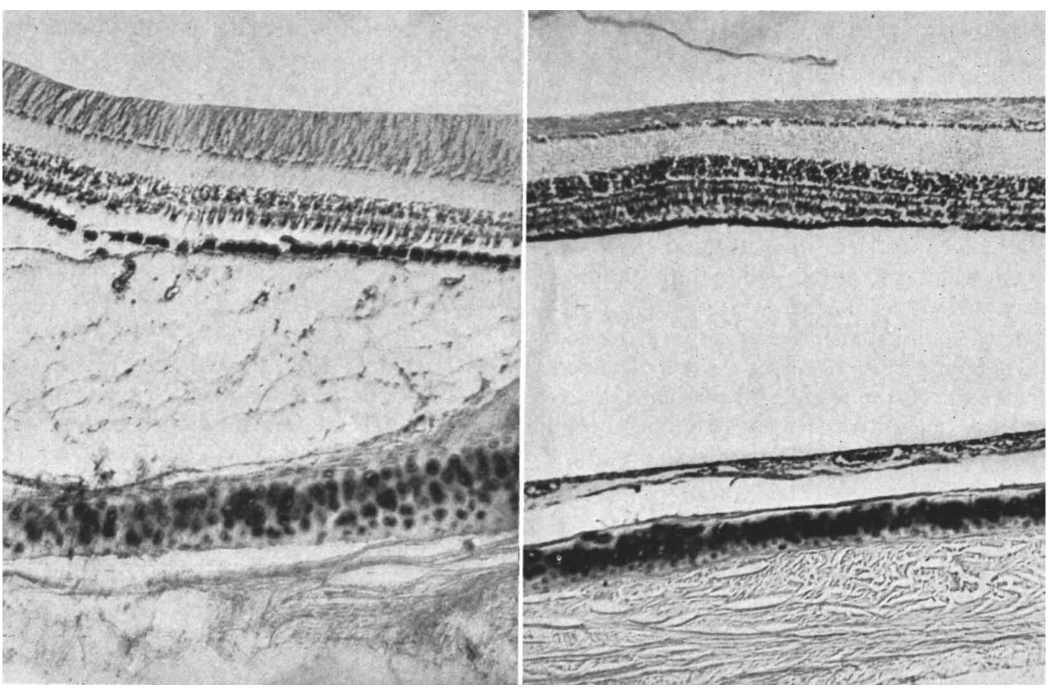

FIG. 2. Cross-section of the eye walls from chickens reared in clear (control) light (right) and in low intensity specific spectra light (left). The area between the detached retina and the choroid of the control group is the area responsible for the thickening of the eye wall, as shown in the treatment group.

was a significant flattening of the cornea. However, in the dim colored light treatments, corneal radius of curvature did not vary from that of control birds. The increase in other parts of the eye (lens and pecten), along with maintenance of normal corneal curvature, indicates that this is a proportional eye enlargement.

Histological examination revealed a thickening of the eye wall in treated birds (Fig. 2). Histological measurements (Table III) show a significant increase in thickness of the eye wall in the colored light, with the greatest increase occurring in the blue light treatment. The tissue layer responsible for the increase in thickness of the eye wall was associated with the choroid. In every treatment group the choroid layer had a 3- to 4-fold increase in thickness over the controls (Fig. 2).

The thickened layer had a gelatinous appearance to gross observation. In attempting to determine the makeup of this substance, two separate stains were employed. Oil red stain for fat was used on the gross cross-sectioned eye and proved negative. Periodic acid schiff stain was used on posterior sections of the eyes and was negative for mucin. To date, the chemical makeup of this substance is unknown. In the treatment groups, an increased amount of hematoxylin staining material was noted in the pigment and in the rod and cone layer.

Measurements randomly taken at 40 weeks of age showed an increase in eye lesions in birds remaining in the low intensity colored light, as compared to the birds transferred to normal light at 20 weeks of age. In the birds remaining in the low intensity light, 51, 43, and 87% of the birds showed retinal detachments in the blue, green, and red light treatments, respectively. Only 4 and 9% of the birds reared on blue and red light, respectively, then transferred to normal incandescent light at 20 weeks of age, showed this abnormality. Lack of pupillary reflex was observed in 2, 1 and 9%, respectively, for those birds remaining in blue, green, and red light, while 1 and 4% of the birds transferred from blue and red light showed no pupillary reflex. Lens opacity was observed in 2, 3, and 4% of the birds maintained on blue, green, and red light, respectively. No lens opacity was observed in the birds transferred to normal incandescent light. The only eye lesions observed at 40 weeks of age in the control (clear plexiglass $CuSO_4$ filtered light) was retinal detachment, which occurred in 5% of the birds. Similar measurements taken on birds reared to 20 weeks of age in the low intensity colored light and subsequently exposed to the normal incandescent light for 60 weeks are shown in Table IV.

TABLE IV. Eye Lesions in White Leghorn Hens Reared in Different Low Intensity Light Spectra and Maintained on Clear Incandescent Light from 20-80 Weeks of Age.

	Rearing period light treatment			
	Control	Green	Red	Blue
Retinal abnormality (%)*	.0	33.3	26.3	52.6
No pupillary reflex (%)	4.8	5.5	.0	26.3
Lens abnormality (%)	.0	5.5	15.8	10.5

* Retinal abnormalities consisted of retinal detachments, meaty or avascular appearance, and abnormal pigmentation. Lens abnormalities consisted of lens opacity and a spidered lens appearance. Both eyes were examined on 20 birds from each treatment.

TABLE III. Histological Measurements of Eyes from White Leghorn Hens Reared in Different Low Intensity Light Spectra and Maintained on Clear Incandescent Light from 20-80 Weeks of Age.

		Rearing period light treatment		
	Control	Green	Red	Blue
Eye-wall thickness (mm) (10)*	5.12 ± .018†	.804 ± .045	.802 ± .029	.968 ± .050
Sclera width (mm) (10)	.153 ± .013	.147 ± .008	.170 ± .005	.155 ± .043
Choroid width (mm) (10)	.141 ± .014	.423 ± .032	.395 ± .027	.590 ± .043
Pigment layer width (mm) (10)	.021 ± .001	.022 ± .002	.022 ± .000	.025 ± .002
Retina width (mm) (10)	.200 ± .004	.213 ± .008	.216 ± .003	.202 ± .000

* No. eyes examined.
† Mean and standard error.

From these measurements it appears that the incidence of eye lesions is directly related to length of exposure to the low intensity colored light, with an increase in the incidence of eye lesions occurring in those remaining in the red light.

Summary. Birds reared in low intensity colored light developed greatly enlarged eyes. Associated with the eye enlargement was: (1) a change in dioptrics, (2) increased eye protrusion, (3) increased incidence of eye lesions, especially in the red spectrum, which was directly related to length of exposure to the low intensity light, and (4) a thickening of the eye wall which occurred mainly in the choroid layer. The appearance was characterized by exophthalmos rather than the buphthalmos which occurs in continuous light. Corneal curvature was not changed while other eye parameters increased

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Substitution of Egg Yolk for Serum in Indirect Fluorescence Assay For Rous Sarcoma Virus Antibody. (32433)

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While searching for simpler and more rapid methods of monitoring chicken flocks for evidence of avian leukosis infection, we noted that other investigators had substituted egg yolk for mother hen's serum in neutralization and metabolic inhibition tests with Rous sarcoma virus(1,2). By using egg yolk as antibody reagent in an indirect immunofluorescence system we have developed a simple and rapid test for detection of viral antibody in birds.

Material and methods. Viral antigen. Three strains of Rous sarcoma virus (RSV) were used: Schmidt-Ruppin—lot SR45, Harris—lot HA8, and CT559—lot TV43, all of which were kindly supplied by Dr. R. Holdenried, National Cancer Institute, Bethesda, Md. A strain of avian lymphomatosis virus (RIF), RPL 12-L37, was provided by Dr. W. Okasaki, Regional Poultry Laboratory, East Lansing, Mich.

Chick embryo fibroblasts (CEF), grown in 32 oz bottles as primary culture, were suspended in a medium composed of the following ingredients (parts per 100): Eagle's #2 medium—88; tryptose phosphate broth—5; calf serum—5; stock 3% glutamine solution—1; stock antibiotic solution—1 (to give a final concentration of penicillin 200 u per ml and streptomycin 200 mcg per ml). To infect the cultures with RSV, 0.1 ml of a virus dilution, containing approximately 2-3 logs of virus, as determined by the fluorescent antibody (FA) technique, was added to 10 ml of the cell suspension containing 3×10^6 CEF. The RSV-infected cells were seeded on pre-cleaned coverslips in a Falcon plastic Petri dish (100×20 mm). To infect the cultures with RIF virus, 1 ml of the virus dilution containing approximately 10,000 $TCID_{50}$ as determined by RIF test(3) was added to 50 ml of the cell suspension (15×10^6 CEF) seeded in 32 oz bottles. The RIF virus-infected cells were passed twice after 3 or 4 days' growth in bottles; the third passage cells were seeded in a Falcon plastic Petri dish, as described for the RSV procedure. The Petri dish cell cultures were incubated in a