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To the Graduate Council:

I am submitting herewith a dissertation written by Edd Coolidge Hogg entitled "Ophthalmacrosis of the Chick." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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OPHTHALMACROSIS OF THE CHICK

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Edd Coolidge Hogg

August 1967

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INTRODUCTION

A peculiar enlargement of the eyes of certain groups of chickens has been observed by a number of poultry researchers at The University of Tennessee Agricultural Experiment Station and elsewhere. This condition of eye enlargement, referred to as ophthalmacrosis in this study, is characterized in the live bird by an elongation of the eye. The space between the inner canthus and the edge of the iris is increased; thus, resulting in a much greater exposure of the sclera or white of the eye. This gives the eye the appearance of being slanted. The eyeball itself is greatly enlarged, and the apparent increase in vitreous humor results in an outer displacement of the lens against the cornea with the obliteration of the anterior chamber.

The few reports found in the literature pertaining to the enlargement of the eyes of chickens attribute the development of the condition to excessive exposure to light (Jensen, 1957, Lauber et al., 1961, McCluskey and Arscott, 1967). However, the studies made by Shirley and Voitle, The University of Tennessee Agricultural Experiment Station, dating back to 1955 (unpublished), show that this eye enlargement not only occurs when the birds are exposed to constant light, but to an even greater extent in constant darkness. This indicates that light per se is not the causative factor, but that the absence of a diurnal light-dark cycle may account for the eye abnormality.

This poses the question of the physiological processes involved in the maintenance of the osmotic integrity of the ocular fluids and

their regulation by the photoperiod. Can the dynamics of the ocular fluids be added to the list of several physiological functions of animals now known to be under the influence of photoperiodism?

The experiments reported herein were designed to determine certain physiological relationships involved in ophthalmacrosis of chickens.

OBJECTIVES

The purpose of this study was to determine the physiological effects of photoperiods on the avian eye.

Experiments were conducted to obtain information regarding the effects of various photoperiods on (1) body, eyeball, adrenal, and thyroid weights; (2) corticosterone levels in blood plasma; (3) the osmotic pressure, and the sodium and potassium content of ocular fluid from the posterior chamber; (4) eosinophil count; and (5) the histology of the eyeball, thyroid, adrenal, pituitary, and kidney.

REVIEW OF LITERATURE

The eye of the bird differs primarily from the mammalian eye in that it has a pecten and relatively fewer rods. The eyeball consists of the cornea, lens, anterior chamber containing aqueous humor, vitreous humor, iris and pupil, ciliary body, pecten, sclera, choroid, and retina (Sturkie, 1954).

The anterior chamber lies between the cornea and lens. This part of the eye contains aqueous humor, a proteinaceous watery substance. Suerdlick (1960) suggested that three hypothesis have been developed to explain the formation of the aqueous humor: (1) the ciliary body is a gland (secretary theory), (2) the filtration theory, and (3) the dialyzation theory (dialysate of the plasma).

Bloom and Fawcett (1962) stated that the aqueous humor in the human eye is believed to originate through secretion or transudation from the ciliary processes. It is a clear, water fluid of slightly alkaline reaction, with an index of refraction of 1.33. The drainage of the aqueous humor from the anterior chamber is effected through the canal of Schlemm. The normal intraocular pressure (28 mm. of mercury), which causes the spherical form of the eyeball, is the resultant of the rates of transudation and aqueous humor drainage.

Coulombre (1956) has shown that the role of intraocular pressure in the development of the chick eye is a cardinal factor in the increase of eye size in embryos.

Barany (1951) in his study, Rate of Flow of the Aqueous Humor in the Chicken, stated that "practically nothing is known concerning the physiology of the ocular fluid in birds." He found that the water content in three samples of chick plasma was 92 percent and freely diffusable. The rate of flow of aqueous humor in the chick eye was approximately 1.7 percent per minute of volume of the anterior chamber.

Allimuddin (1956) stated that normal intraocular pressure in the human varies from right eye to left eye, from time to time. Pressure is usually highest in the early morning; lowest in the evening between 6 and 8 p.m. All cases showed a rise in ocular pressure after water-drinking tests. After 1000 ml. of water was drunk, the intraocular pressure increased and diuresis started within thirty minutes. The intraocular pressure reached its peak within two hours and then declined.

Friedenwald (1949) experimented with the formation of intra-ocular fluid. He stated that persistent changes in blood pressure or in osmotic pressure of plasma colloids caused very little effect on intraocular pressure. Furthermore, if the intraocular fluid was filtered through a barrier of small pore size to retard the transport of the larger solute molecules, an osmotic pressure difference rose on two sides of the filtration boundry, retarding the movement of water relative to the solutes.

Becker (1954) reported that Diamox (Acetazolamide, Lederle), caused intraocular pressure to fall within 60-90 minutes and reached

a minimum in 3-5 hours; thereafter, it returned to the initial level within 8-12 hours. The fall of intraocular pressure was accomplished without measurable change in facility of outflow. This would suggest that the decreased pressure induced by Diamox was a result of inhibitation of rate of inflow of aqueous humor.

Seaman and Storm (1963) discussed the effects of decreased intraocular pressure induced by intravenous injection of acetazalamide (Diamox). He used one-year old male and female chickens, anesthetizing the eyes with pontocaine, and intravenously injecting Diamox into the brachial vein. His results revealed that the intraocular pressure, as measured by a Schiotz tonometer, dropped 60 percent within 60-90 minutes. Some birds were refractory to Diamox.

According to Friedenwald (1955), Diamox is an inhibitor of the enzyme, carbonic anhydrase. It prevents renal tubular reabsorption of sodium and potassium, resulting in diuresis. Becker and Constant (1955) reported that Diamox appeared to lower intraocular pressure by directly inhibiting the secretion of aqueous humor by the ciliary body.

According to the Physicians Desk Reference (1964), Diamox, a diuretic and acid-base regulator, is different from myotic agents which contract the pupil of the eye thereby increasing outflow.

A substance similar to aqueous humor, vitreous humor, is found in the vitreous body of the eye. It is more refractive than the aqueous humor. These fluids serve to keep the eyeball distended, and they change the refractive index of light passing through the eye.

Changes in the pressure of these fluids may cause disturbances in vision (Sturkie, 1954).

All the fluid contained in the anterior and posterior chambers of the eye, that which percolates the vitreous body, as well as the tissue fluids of the eyeball coats, should be considered as a physiological unit. The intraocular fluid resembles cerebrospinal fluid in composition and physical characteristics. The vitreous humor is held by a clear gel composed of muco-protein and a residual protein. This jelly is in an unstable state of dynamic equilibrium. Thus, the fluid easily separates, leaving a shrunken gel with opaque granules and strands (Duke-Elder, 1934).

Glaucoma, a disease of the human eye, is characterized by an increase in intraocular pressure. Duke-Elder (1941) stated that attacks of glaucoma are infrequently induced by conditions which upset the vaso-motor balance and are particularly associated with worry, sleeplessness, fatigue, or constipation. Schoenbery (1940) stressed the role of anxiety in the pathogenesis of primary glaucoma. He stated that mental and emotional conflicts, whether conscious or not, may produce a disturbance of the physiological processes.

Crolle and Crolle (1960) stated that the role of emotional glaucoma as a causative factor in acute, closed-angle glaucoma is not fully understood. Unfortunately, this type of emotion cannot be measured by a tonometer. Acute glaucoma can be precipitated by dilating the pupil of the eye. For example, if a patient with shallow chambers is placed in a dark room, the intraocular pressure may

increase. When the patient returns to light, the tension is lowered, due to miosis. The pupil will also become dilated in times of stress, excitement, and anxiety. The degree of dilation will depend on the nature, degree, and duration of the stimulus.

Armaly (1963), Becker and Mills (1963), and Nicholas (1964) stated that adrenocorticosteroids, when used topically, caused an increase in intraocular pressure.

Research has shown that circadian rhythms affect virtually all physiological functions from cellular to glandular. Bernhard (1966) pointed out that we experience an alternation of light and darkness with an almost constant cycle length of twenty-four hours. Since many habits are affected by circadian periodicity, it is not surprising that so many phsyiological functions display the same rhythmic behavior. Diurnal variations are commonplace from the cellular level up through the level of organs coupled by mutually interacting hormones.

Bunning (1964) reported that the hormones, produced by the pituitary gland, fluctuate diurnally in the blood. Since these fluctuations are controlled by the changes of light and darkness, they may well be the basis of some physiological phenomena which are dependent on the light-dark cycle. He assumed that the increase in body temperature, pulse rate, blood pressure, and oxygen-consumption are paralleled by an increase of adrenaline in the blood. The opposite phase, during the night, is marked by an increase in the pituitary hormone.

Bunning (1964) investigated the influence of periodical activity changes in the adrenal gland. The adrenal cycle controls various diurnal functions in the body of vertebrates including humans. The number of eosinophils circulating in the blood reflects the course of this adrenal cycle. The hormones of the adrenal gland tend to diminish the number of eosinophils. Stamler (1950) reported that ACTH injected into the chick did not produce eosinophenia (an abnormally small number of eosinophils in the peripheral blood). Extirpation or pathological loss of the adrenal gland abolished the eosinophil rhythm. There was antagonism between the eosinophils and ketosteroids. Eosinophil count was highest around 2 to 3 a.m. and lowest around 11 a.m.

According to Bunning (1964), the science of aviation and space medicine shows that human test subjects, as well as test animals, are unable to adjust themselves when the light and dark periods deviate too much from the usual 24-hour cycle. In most animals, this period is between 23 and 26 hours. It is not so much the quantity and intensity of light as it is the duration of light. The accuracy with which animals maintain this photoperiod is remarkable. The deviations are usually less than 1 hour; even less than 15 to 20 minutes.

Bernhard (1966) reported that certain epidermal tissues exhibit rates of cell division peaking in the late evening, coinciding with the nocturnal sleep habit. He explained that there is circadian periodicity in hemoglobin concentration, red cell count, and plasma protein with high values by day and low values by night. Body

temperature characteristically shows a circadian periodicity. This temperature depends on the balance between heat production and heat loss. Heat production varies within a twenty-four hour cycle, since the major source of the variation is muscular rest and activity. Even in subjects at complete rest and fasting, the circadian periodicity remains at the lowest values in the early morning which closely follows the normal twenty-four hour rhythm in body temperature.

There is circadian rhythm in the activity of the adrenal cortex. This organ releases a fascinating variety of substances (more than one hundred different hormones of the sterol class) of which cortisone is the best known. The cortical hormones control kidney function (and thus regulate the body's water balance), promote retention of sodium and chloride ions and the excretion of potassium ions, regulate fat, protein and carbohydrate metabolism, and influence the sex organs. They have an additional effect on membranes in the joints, the eyes, and the skin; this is a consequence of cortical control over water, ion, and carbohydrate metabolism (Bernhard, 1966).

There is much evidence that circadian rhythms have an internal origin. It has been suggested that all living organisms are sensitive to circadian cycles. The importance of light in controlling rhythms has been mentioned in connection with factors affecting urine flow. Still, there is no substantial evidence to support any observed cycles and the circadian variation in environmental factors (Bernhard, 1966).

Profound difficulties arise from the possibility that a rhythm might not have its own clock but might be controlled by some other

function which has its own circadian timekeeper. Such a situation is seen in fluctuating level of eosinophils in the circulation. Although the eosinophil level is under control of cortisol, a hormone whose level in the blood plasma has a circadian periodicity, cortisol secretion in turn is mediated by a twenty-four hour variation in adrenocorticotropic hormone (ACTH) which is controlled by similar variation in corticothropin releasing factor (Bernhard, 1966).

Limited research has been reported on the enlargement of the avian eye by exposing chickens to continuous incandescent illumination.

Jensen and Matson (1957) found that the average weight of eyeballs removed from chickens that had received continuous light was about 38 percent greater than the weight of eyeballs of chickens that had received only 12 hours of artificial light each day. The increased size of the eyeballs was brought about largely by an increase in tissue water. It was found that the average diameter of eyeballs from chickens that received continuous light was 2.4 mm. larger than those from chickens not receiving continuous light.

Lauber, Shutze, and McGinnis (1961) reported that a profound enlargement of the eyeball was associated with an accumulation of fluid in the vitreous body. Histological changes were evident in several layers of the eye; the greatest effect was a thinning of the retina.

The influence of incandescent and infrared light upon chickens was studied by McCluskey and Arscott (1967). They found that chicks exposed to infrared lamps were lower in body weight than chicks

given other lamp treatments. There was an apparent trend of increased body weight as the intensity of the light decreased with groups exposed to 20 hours of light. All light treatments, except the intermittent (12 hour) incandescent lamp exposure, caused the chicks to develop abnormal eyes. The condition was more common in birds under the infrared lamps than those under the incandescent lamps.

MATERIALS AND METHODS

Experimental Animals

Four experimental groups of chicks were studied to investigate the effects of light-dark treatments on the physiology of ophthalmacrosis in the chick.

Day-old, Dekalb No. 101, White Leghorn-type cockerels were used in each experimental group. They were housed in windowless pens with thermostatically-controlled ventilation and heating, from which all natural light was excluded. All chicks were brooded in electrically-heated pens approximately five feet square with wood shavings as litter. The formula for the diet that was fed to the chicks is given in Table XLI, Appendix A. It was considered to be adequate in all known nutrients. Feed and water were available to the chicks ad libitum. The chicks were randomized at one day of age, wing banded, and vaccinated by a combination of infectious bronchitis-newcastle vaccine by the intraocular method.

Experimental Treatments

Three basic light-dark treatments were used in the experiments. All chicks in Experiments I, II, III, and part of IV, were grown in one of the following basic light-dark treatments: Treatment one--continuous darkness, Treatment two--continuous light (with a 60-watt light bulb approximately 50 inches above the litter), or Treatment

three-12 hours of light (with a 60-watt light bulb approximately 50 inches above the litter) alternating with 12 hours of darkness. In Experiment IV, the following light-dark cycles were used in addition to the foregoing ones: 20 hours of darkness alternating with 4 hours of light, 4 hours of darkness alternating with 20 hours of light, 72 hours of darkness alternating with 72 hours of light, and 24 hours of darkness alternating with 24 hours of light.

The following procedures were used for each experiment: gross observations of eyeballs were made, body weights were determined, chicks were sacrificed, eyes were enucleated and weighed.

Gross observations. Gross observations of the chicks' eyes were made while they were intact. These observations included size, shape, and the relationship of the iris to the cornea.

Fluorometric determinations. In preparation for the fluorometric readings to determine corticosterone concentration in blood plasma, 5 cc. of blood was taken from each chick by heart puncture, using a syringe and 18 gauge needle. The chicks were handled with great care to avoid overexcitement. The blood was placed in heparinized tubes, and centrifuged at 3000 r.p.m. for 45 minutes. Thereafter, the blood plasma was frozen. The sample was prepared according to the procedure described by Guillemin et al (1959).

After the samples were prepared, the fluorometric determinations were made in a Model III Turner Fluorometer.

Ocular fluids studies. Ocular fluids were removed by syringe and frozen for the osmotic pressure, sodium and potassium determinations.

One sample from each chick was used to make all three determinations.

The osmotic pressure was determined by placing a 2 ml.-sample of ocular fluid in the Model 31K Advanced Osmometer. The freezing point depression method was used.

The stock solution of 1000 p.p.m. sodium was made from sodium chloride and glass-distilled water. Sodium standards of 25 ml. solutions containing 25, 50, 75, and 100 p.p.m. sodium were made from the stock solution. The sodium sample was prepared by diluting the ocular fluid 50 times with glass-distilled water. Thereafter, the solutions were run in the Perkin-Elmer Atomic Absorption Spectrophotometer in the following sequence: standard, sample, standard, sample, standard, and the readings recorded. The readings for each solution were averaged to determine the concentration of the sodium in each sample.

The stock solution of 1000 p.p.m. potassium was made from potassium chloride and glass-distilled water. Potassium standards of 25 ml. solutions containing 1, 2, 3, 4, and 5 p.p.m. of potassium were made from the stock solution. The potassium sample was prepared by diluting the ocular fluid 50 times with glass-distilled water. Thereafter, the procedure used to determine sodium concentration was repeated for the potassium solution.

Histological studies. The adrenal, thyroid, pituitary, kidney, and eyeball of each chick were removed, weighed, and fixed in 10 percent formalin. Standard histological techniques were used in preparing the slides. A detailed microscopic study was made of each slide.

Evaluation of Data

The data were statistically analyzed by the analysis of variance procedure according to Snedecor (1956) and Cornell (1956), and significant treatment mean differences were determined by Duncan's Multiple Range Test (1955).

TREATMENTS AND RESULTS

Experiment I

The first experimental group of 150 day-old chicks was housed in three pens, with 50 chicks per pen. The three basic light-dark treatments were used. At 30 days of age, 42 chicks, 14 from each pen, were selected at random and sacrificed for the following determinations: gross observations, body and eyeball weights, and fluorometric reading of corticosterone concentration.

Gross observations of eyeballs revealed that chicks grown in continuous darkness or in continuous light had greatly enlarged eyeballs; whereas, the chicks grown in the 12 hours of darkness alternating with 12 hours of light had "normal" eyeballs.

Body weights, eyeball weights as a percentage of body weights, and fluorometric readings of corticosterone concentration for Experiment I are shown in Table X, Appendix A.

Analysis of variance and F value were made to determine if there were significant differences in body weights, eyeball weights, and fluorometric readings of corticosterone concentration among chicks in the three basic light-dark treatments.

The resulting F value for body weights, shown in Table XI, Appendix A, was not significant at the 5 percent level of probability. However, the F values for eyeball weights, shown in Table XII, Appendix A, and fluorometric readings of corticosterone concentration,

shown in Table XIII, Appendix A, were highly significant at the 1 percent level of probability.

Average body weights, eyeball weights, and fluorometric readings of corticosterone concentration for Experiment I are given in Table I. Duncan's Multiple Range Test showed: (1) that the differences in eyeball weights of chicks grown in continuous darkness or in continuous light were significantly ($P = .01$) larger than those grown in 12 hours of darkness alternating with 12 hours of light, (2) that the differences in fluorometric readings of corticosterone concentration of chicks grown in all treatments were highly significant ($P = .01$), with chicks grown in continuous darkness having the largest amount of concentration, followed by the chicks grown in continuous light, with chicks grown in 12 hours of darkness alternating with 12 hours of light having the smallest amount of concentration.

Experiment II

The second experimental group of 75 day-old chicks was housed in three pens, with 25 chicks per pen. The three basic light-dark treatments were used. At 30 days of age, 21 chicks, 7 from each pen, were selected at random and sacrificed for the following determinations: gross observations, body and eyeball weights, fluorometric readings, osmotic pressure, sodium and potassium concentrations of ocular fluid.

Body weights, eyeball weights as a percentage of body weights, and fluorometric readings of corticosterone concentration of blood plasma for chicks in Experiment II are given in Table XIV, Appendix A.

TABLE I

AVERAGE[1] BODY WEIGHTS, EYEBALL WEIGHTS, AND FLUOROMETRIC READINGS,[2] EXPERIMENT I

Treatments	Number of chicks[3]	Body weight	Eyeball weight		Fluorometric determinations of corticosterone	
			Absolute	Percent body wt.		
		gm.	mg.	%	Readings	µg.
Continuous dark	14	$323.57^{a} \pm 9.22^{4}$	1450.21	$.446^{A} \pm .010^{4}$	$88.42^{A} \pm 3.46^{4}$	2.85
Continuous light	14	$305.64^{a} \pm 12.52$	1272.93	$.424^{A} \pm .017$	$82.51^{B} \pm 3.74$	2.25
12-hour dark 12-hour light	14	$301.50^{a} \pm 13.32$	1102.06	$.354^{B} \pm .010$	$77.00^{C} \pm 2.84$	1.75

[1]Averages with different superscripts differ significantly: A,B,C (P = 0.01); a,b,c (P = 0.05) as determined by Duncan's Multiple Range Test (1955).

[2]Direct readings taken from Turner's Fluorometer.

[3]Chicks 30 days of age.

[4]Mean ± standard error of the mean.

The analysis of variance and F value of significance, Tables XV, XVI, and XVII, Appendix A, indicated that body weights were significantly different at the 5 percent level of probability, eyeball weights were significantly different at the 1 percent level of probability, and fluorometric readings of corticosterone concentration were significantly different at the 1 percent level of probability.

The average body and eyeball weights and fluorometric readings of corticosterone concentration are given in Table II. Duncan's Multiple Range Test showed that (1) body weights of chicks grown in continuous darkness or in continuous light were significantly ($P = 0.05$) larger than those grown in 12 hours of darkness alternating with 12 hours of light, (2) eyeball weights of chicks grown in continuous darkness were significantly larger ($P = 0.01$) than those grown in the other treatments, (3) fluorometric readings of corticosterone concentration of chicks grown in continuous darkness were significantly higher ($P = 0.01$) than those grown in the other treatments (with chicks grown in 12 hours of darkness alternating with 12 hours of light having the smallest amount of concentration).

Data regarding osmotic pressure, sodium and potassium concentrations of ocular fluids for Experiment II are given in Table XVIII, Appendix A. The analysis of variance and F value indicated that all three measurements were statistically significant at the 5 percent or 1 per cent levels of probability. The resulting F value for osmotic pressure of ocular fluid, shown in Table XIX, Appendix A, was significant at the 5 percent level of probability. Whereas, the F values

TABLE II

AVERAGE[1] BODY WEIGHTS, EYEBALL WEIGHTS, AND FLUOROMETRIC READINGS,[2] EXPERIMENT II

Treatments	Number of chicks[3]	Body weight	Eyeball weight		Fluorometric determinations of corticosterone	
			Absolute	Percent body wt.		
		gm.	mg.	%	Readings	µg.
Continuous dark	7	$350.71^{a} \pm 11.83$[4]	1491.14	$.42^{A} \pm .0167$[4]	$87.08^{A} \pm .3361$[4]	2.70
Continuous light	7	$338.14^{a} \pm 17.79$	1201.42	$.36^{B} \pm .0182$	$82.78^{B} \pm .5224$	2.30
12-hour dark 12-hour light	7	$297.42^{b} \pm 10.31$	1058.71	$.35^{B} \pm .0075$	$74.28^{C} \pm .6348$	1.45

[1]Averages with different superscripts differ significantly: A,B,C (P = 0.01); a,b,c (P = 0.05) as determined by Duncan's Multiple Range Test (1955).

[2]Direct readings taken from Turner's Fluorometer.

[3]Chicks 30 days of age.

[4]Mean ± standard error of the mean.

for the sodium and potassium concentrations of ocular fluids, given in Tables XX and XXI, Appendix A, were significant at the 1 percent level of probability.

The average osmotic pressure, sodium and potassium concentrations of ocular fluid are shown in Table III. Duncan's Multiple Range Test showed that (1) the osmotic pressure of the fluids secured from the eyeballs of chicks grown in continuous darkness or in continuous light were significantly ($P = 0.05$) higher than those grown in 12 hours of darkness alternating with 12 hours of light, (2) sodium and potassium concentrations of the ocular fluid from chicks grown in continuous darkness were significantly ($P = 0.01$) higher than those grown under other treatments, with chicks grown in 12 hours of darkness alternating with 12 hours of light producing the smallest concentration.

Experiment III

The third experimental group of 54 day-old chicks was housed in three pens, with 18 chicks per pen. The three basic light-dark treatments were used. Eighteen chicks, six from each pen, were selected at random and sacrificed at 90 days of age for the following determinations: gross observations, body and eyeball weights, osmotic pressure, sodium and potassium concentrations of ocular fluid, adrenal and thyroid weights.

Gross observations of eyeballs in Experiment III revealed that chicks grown in continuous darkness had the largest eyeballs, followed by those grown in continuous light; whereas, the chicks

TABLE III

AVERAGE[1] OSMOTIC PRESSURE, SODIUM AND POTASSIUM CONCENTRATION OF OCULAR FLUID, EXPERIMENT II

Treatments	Number of chicks[2]	Osmotic pressure	Sodium		Potassium	
		Readings[3]	Readings[4]	p.p.m.	Readings[4]	p.p.m.
Continuous dark	7	$319.9^{a} \pm 3.72$[5]	$57.0^{A} \pm .689$[5]	350	$16.0^{A} \pm .533$[5]	175
Continuous light	7	$310.4^{a} \pm 4.43$	$53.7^{B} \pm 1.05$	325	$13.0^{B} \pm .084$	125
12-hour light 12-hour dark	7	$303.7^{b} \pm 5.08$	$48.2^{C} \pm 2.97$	220	$9.9^{C} \pm .939$	78

[1]Averages with different superscripts differ significantly: A,B,C (P = 0.01); a,b,c (P = 0.05) as determined by Duncan's Multiple Range Test (1955).

[2]Chicks 30 days of age.

[3]Direct readings taken from the Advanced Osmometer Model 31K.

[4]Direct readings taken from the Perkin-Elmer Atomic Absorption Spectrophotometer.

[5]Mean ± standard error of the mean.

grown in 12 hours of darkness alternating with 12 hours of light had normal eyeballs.

Body weights, eyeball weights expressed as a percentage of body weights, and osmotic pressure of the ocular fluid for chicks in Experiment III are shown in Table XXII, Appendix A.

The analysis of variance and F value revealed that body weights, Table XXIII, Appendix A, were not significantly different among the three treatments at the 0.05 level of probability, but that eyeball weights were significantly different among the three treatments at the 0.01 level of probability, Table XXIV, Appendix A. Osmotic pressure of ocular fluids, shown in Table XXV, Appendix A, was not significantly different among the three treatments at the 0.05 level of probability.

The average body weights, eyeball weights, and osmotic pressure of ocular fluid for Experiment III are given in Table IV. Duncan's Multiple Range Test showed that eyeball weights of chicks grown in continuous darkness were significantly ($P = 0.01$) larger than those grown under the other conditions. Chicks grown in 12 hours of darkness alternating with 12 hours of light had the smallest eyeballs.

The results of the measurements pertaining to sodium and potassium concentrations of ocular fluid, adrenal and thyroid weights are given in Table XXVI, Appendix A.

The resulting F values of the sodium (Table XXVII, Appendix A) and potassium (Table XXVIII, Appendix A) concentration of ocular fluid were highly significant ($P = 0.01$) for chicks in the basic light-dark treatments.

TABLE IV

AVERAGE[1] BODY WEIGHTS, EYEBALL WEIGHTS, AND OSMOTIC PRESSURE READINGS[2]
EXPERIMENT III

Treatments	Number of chicks[3]	Body weight	Eyeball weight		Osmotic pressure
			Absolute wt.	Percent body wt.	
		gm.	mg.	%	Readings
Continuous dark	6	$1579.5^{a} \pm 90.32$[4]	3.8	$.24^{Aa} \pm .0118$[4]	$327.8^{a} \pm 2.213$[4]
Continuous light	6	$1541.1^{a} \pm 49.54$	2.9	$.18^{Bb} \pm .0070$	$320.2^{a} \pm 1.702$
12-hour dark 12-hour light	6	$1388.3^{a} \pm 50.78$	2.2	$.15^{Bc} \pm .0077$	$305.5^{a} \pm 1.072$

[1]Averages with different superscripts differ significantly: A,B,C (P = 0.01); a,b,c (P = 0.05) as determined by Duncan's Multiple Range Test (1955).

[2]Direct readings taken from the Advanced Osmometer, Model 31K.

[3]Chicks 90 days of age.

[4] Mean ± standard error of the mean.

The adrenal weights (Table XXIX, Appendix A) and thyroid weights (Table XXX, Appendix A) were significantly different (P = 0.05) for chicks in the basic light-dark treatments.

The average sodium and potassium concentrations of ocular fluid, adrenal and thyroid weights for chicks in Experiment III are given in Table V. Duncan's Multiple Range Test showed that sodium concentrations of the ocular fluid from chicks grown in continuous darkness were significantly greater (P = 0.01) than those grown in the other two treatments. Chicks grown in the continuous light treatment had a higher sodium concentration of ocular fluid than did those grown in 12 hours of darkness alternating with 12 hours of light (P = 0.05). The potassium concentrations were significantly greater (P = 0.01) in the eye fluids for chicks grown in continuous darkness followed by those grown in continuous light, than for those grown in 12 hours of darkness alternating with 12 hours of light.

Adrenal weights of chicks grown in continuous light or 12 hours of darkness alternating with 12 hours of light were significantly (P = 0.05) larger than those grown in continuous darkness. Adrenal weights of chicks grown in 12 hours of darkness alternating with 12 hours of light were significantly (P = 0.05) larger than those grown in continuous light. Thyroid weights of chicks grown in continuous light were significantly larger (P = 0.05) than those grown in continuous light. Thyroid weights of chicks grown in continuous light were significantly larger (P = 0.05) than those grown in either continuous darkness or 12 hours of darkness alternating with 12 hours

TABLE V

AVERAGE[1] SODIUM AND POTASSIUM CONCENTRATIONS, ADRENAL AND THYROID WEIGHTS, EXPERIMENT III

Treatments	Number of chicks[2]	Sodium		Potassium		Adrenal weight		Thyroid weight	
						Absolute wt.	Percent body wt.	Absolute wt.	Percent body wt.
		Readings[3]	p.p.m.	Readings[3]	p.p.m.	mg.	%	mg.	%
Continuous dark	6	$80.8^{Aa} \pm .407^{4}$	575	$15.8^{A} \pm .482^{4}$	155	52.1	$.0033^{a} \pm .00018^{4}$	74.8	$.0047^{a} \pm .00022^{4}$
Continuous light	6	$77.1^{Bb} \pm .482$	550	$13.3^{B} \pm .316$	140	56.9	$.0036^{b} \pm .00018$	122.3	$.0079^{b} \pm .00130$
12-hour dark 12-hour light	6	$74.8^{Bc} \pm .316$	537	$8.8^{C} \pm .336$	70	60.8	$.0044^{b} \pm .00041$	59.8	$.0043^{a} \pm .00022$

[1] Averages with different superscripts differ significantly: A,B,C (P = 0.01); a,b,c (P = 0.05) as determined by Duncan's Multiple Range Test (1955).

[2] Chicks 90 days of age.

[3] Direct readings taken from the Perkin-Elmer Atomic Absorption Spectrophotometer.

[4] Mean ± standard error of the mean.

of light. No significant differences were found at the 5 percent level of probability, in thyroid weights of chicks grown in continuous darkness and those grown in 12 hours of darkness alternating with 12 hours of light.

Experiment IV

The fourth experimental group of 432 day-old chicks was housed in 16 pens, with 27 chicks per pen. The specific treatments for Experiment IV are given in Table VI. The first part of Experiment IV was designed to determine body weights and eyeball weights of chicks grown in the three basic light-dark treatments combined with corticosterone and Diamox treatments.

Forty-two chicks were used in the corticosterone study. Seven chicks from each of the 6 pens were injected with 0.2 cc. of corticosterone-corn oil mixture daily for 42 days. The concentration of the solution was calculated to provide 0.5 mg. per 100 gm. of body weight per day. The injections were made subcutaneously in the pectoral area using a 20-gauge needle. Fresh solutions were prepared each week.

Forty-two chicks were used in the Diamox treatment. Seven chicks were selected from each of the 6 pens for the Diamox treatment. They were given 2.5 mg. of Diamox mixed with water per 100 gm. of body weight daily for 42 days. The mixture was administered by a tube inserted into the crop.

Body weights and eyeball weights of chicks grown in the three basic light-dark treatments, combined with corticosterone and Diamox,

TABLE VI

DESIGN OF EXPERIMENT IV

Number of pens	Chicks per pen	Chicks per observation	Treatments	Measurement or observation
			Continuous darkness	
2	27	13	Diamox	Body and eyeball weights
2	27	13	Corticosterone	Body and eyeball weights
			Continuous light	
2	27	13	Diamox	Body and eyeball weights
2	27	13	Corticosterone	Body and eyeball weights
			12-hours dark, 12-hours light	
2	27	13	Diamox	Body and eyeball weights
2	27	13	Corticosterone	Body and eyeball weights
2	27	18	Continuous darkness	Eosinophil count
2	27	18	Continuous light	Eosinophil count
2	27	18	12-hours dark, 12-hours light	Eosinophil count
1	27	6	20-hours dark, 12-hours light	Body and eyeball weights
1	27	6	4-hours dark, 20-hours light	Body and eyeball weights
1	27	6	72-hours dark, 72-hours light	Body and eyeball weights
1	27	6	24-hours dark, 24-hours light	Body and eyeball weights

are given in Table XXXI, Appendix A. Body weights and eyeball weights of chicks receiving corticosterone injections were significantly different at the 1 percent level of probability (Tables XXXII and XXXIII, Appendix A).

The body weights of chicks receiving the Diamox treatment were significantly different ($P = 0.01$) among the three basic light-dark treatments (Table XXXIV, Appendix A). However, eyeball weights of chicks receiving the same treatments were not significantly different ($P = 0.05$) among the three basic light-dark treatments (Table XXXV, Appendix A).

The average body weights and eyeball weights of chicks given corticosterone and Diamox treatments in addition to the three basic light-dark treatments are shown in Table VII. All chicks treated with Diamox had considerably larger body weights than those treated with corticosterone. The body weights of chicks treated with Diamox or corticosterone combined with continuous darkness were significantly larger ($P = 0.01$) than those grown in continuous light or 12 hours of darkness alternating with 12 hours of light. The 12 hours of darkness alternating with 12 hours of light, combined with either Diamox or corticosterone produced the smallest chicks.

When eyeball weights were expressed as a percentage of body weights, the chicks treated with corticosterone had considerably larger eyeball weights than those treated with Diamox. Duncan's Multiple Range Test revealed that eyeball weights of chicks grown in continuous darkness combined with corticosterone, were significantly

TABLE VII

AVERAGE[1] BODY AND EYEBALL WEIGHTS FOR CHICKS IN THE CORTICOSTERONE AND DIAMOX INTERACTION STUDIES, EXPERIMENT IV

Treatments	Number of chicks[2]	Corticosterone treatment			Diamox treatment		
		Body weight	Eyeball weight		Body weight	Eyeball weight	
			Absolute wt.	Percent body wt.		Absolute wt.	Percent body wt.
		gm.	mg.	%	gm.	mg.	%
Continuous dark	13	$391.3^{A} \pm 10.77$[3]	1570.7	$.40^{A} \pm .0105$[3]	$598.7^{Aa} \pm 15.97$[3]	2012.8	$.33^{a} \pm .01232$[3]
Continuous light	13	$329.7^{B} \pm 13.35$	1173.6	$.35^{B} \pm .0092$	$492.1^{Bb} \pm 26.12$	1455.1	$.30^{a} \pm .01483$
12-hour dark 12-hour light	13	$305.2^{B} \pm 13.19$	1075.6	$.35^{B} \pm .0100$	$481.1^{Bc} \pm 23.32$	1428.9	$.29^{a} \pm .01050$

[1]Averages with different superscripts differ significantly: A,B,C (P = 0.01); a,b,c (P = 0.05) as determined by Duncan's Multiple Range Test (1955).

[2]Chicks 42 days of age.

[3]Mean ± standard error of the mean.

larger (P = 0.01) than the eyeball weights of chicks grown in continuous light or 12 hours of darkness alternating with 12 hours of light.

The second part of Experiment IV was designed to determine body weights and eyeball weights under a variety of additional photoperiod treatments. The number of pens, chicks per pen, and specific treatments are given in Table VI, page 29. The body weights and eyeball weights for chicks from the four additional photoperiod treatments are given in Table XXXVI, Appendix A. Analysis of variance and F value for body weights (Table XXXVI, Appendix A) and eyeball weights (Table XXXVII, Appendix A) revealed no significant differences among body weights or eyeball weights within the four photoperiod treatments, at the 5 percent level of probability.

The average body and eyeball weights for chicks in the four additional photoperiod treatments are given in Table VIII. Within the four photoperiod treatments, chicks grown in 20 hours of darkness alternating with 4 hours of light and those grown in 4 hours of darkness alternating with 20 hours of light had considerably larger body weights than did those grown in 72 hours of darkness alternating with 72 hours of light or 24 hours of darkness alternating with 24 hours of light.

The third part of Experiment IV was designed to study the influence of photoperiodism on the eosinophil diurnal rhythm. This group of 108 chicks was housed in 6 pens, with 18 chicks per pen. Two pens were used for each of the three basic photoperiod treatments.

TABLE VIII

AVERAGE[1] BODY AND EYEBALL WEIGHTS FOR PHOTOPERIOD TREATMENTS DIFFERING FROM THE THREE BASIC CYCLES, EXPERIMENT IV

Treatments	Number of chicks[2]	Body weight	Eyeball weight	
			Absolute wt.	Percent body wt.
		gm.	mg.	%
20-hour dark 4-hour light	6	538.28[a] ± 31.94[3]	1.795	.299[a] ± 1.736[3]
4-hour dark 20-hour light	6	537.33[a] ± 33.81	1.594	.266[a] ± 1.187
72-hour dark 72-hour light	6	471.71[a] ± 36.37	1.788	.298[a] ± 1.299
24-hour dark 24-hour light	6	418.42[a] ± 20.27	1.784	.297[a] ± .7674

[1]Averages with different superscripts differ significantly: a,b,c (P = 0.05) as determined by Duncan's Multiple Range Test (1955).

[2]Chicks 42 days of age.

[3]Mean ± standard error of the mean.

Blood was taken from a cubital vein of each chick at three and six weeks of age. The blood smears were made at the following times: 6 a.m., 10 a.m., 2 p.m., 6 p.m., 10 p.m., and 2 a.m. The chicks, in the dark rooms, were maintained in total darkness until the sample was taken; at which time, a 15-watt, blue bulb was used. The blood smears were stained with Wright's stain. The eosinophil count (number of eosinophils in 200 white blood cells) was made when chicks were 3 and 6 weeks of age (Tables XXXIX and XL, Appendix A). The average eosinophil count for chicks at 3 and 6 weeks of age are given in Table IX. The averages revealed no substantial differences in regard to eosinophil count among chicks receiving the three light-dark treatments.

TABLE IX

AVERAGE EOSINOPHIL COUNT[1] FOR CHICKS AT THREE WEEKS AND SIX WEEKS OF AGE, EXPERIMENT IV

Time taken	Treatments						Average
	Continuous dark		Continuous light		12-hour dark 12-hour light		
	3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks	
6:00 a.m.	3.0	3.1	3.1	2.5	3.0	2.8	2.9
10:00 a.m.	3.1	3.1	3.6	2.8	3.0	2.8	3.1
2:00 p.m.	3.1	3.3	3.5	2.8	3.0	2.8	3.1
6:00 p.m.	2.6	3.3	3.8	2.5	3.0	2.8	3.0
10:00 p.m.	3.5	3.6	3.8	3.3	3.6	3.5	3.5
2:00 a.m.	3.0	3.3	3.0	3.3	3.5	3.3	3.2
Average	3.5	3.3	3.7	2.9	3.2	3.0	

[1]Per 200 white cells.

DISCUSSION

Body weights. The body weights of birds grown in the three basic light-dark treatments and those combined with corticosterone or Diamox treatments were largest in the continuous darkness, followed by those grown in the continuous light treatment. The body weights of birds grown in the 12 hours of darkness alternating with 12 hours of light resulted in the smallest body weights. The corticosterone treatment resulted in reduced body size compared to the Diamox treatment. Even though the absolute body size differed in the order given for all five groups of birds, the differences in body weights were not significant.

There were no significant differences found in body weights of chicks subjected to the four additional photoperiods. These data, however, may suggest that birds receiving the longer light cycles failed to grow as rapidly as those receiving shorter light cycles. It might be that birds receiving cycles of alternate light and dark would not acclimate to darkness and thus feed consumption could be reduced if intervals between light periods are too great.

Eyeball weights. The eyeball weights of chicks grown in the three basic light-dark treatments were largest for those grown in continuous darkness. They were definitely enlarged and abnormal. Photographs illustrating these conditions are shown in Figure 1, Appendix B. The space between the inner canthus and the edge of the iris was increased, giving the eyeball an appearance of being slanted.

The lens were pushed against the cornea obliterating the anterior chamber. Continuous light generally resulted in some enlargement of the eyeball, but not to the extent of that found in chicks grown in continuous darkness. Photographs illustrating these conditions are shown in Figure 2, Appendix B.

Jensen and Matson (1957), Lauber, et al. (1961), and McCluskey and Arscott (1967) reported that birds under continuous light had larger eyeballs than did those subjected to 12 hours of darkness alternating with 12 hours of light. These workers concluded that excessive light is the factor responsible for eye enlargement in chickens.

The results of this study support those of Shirley and Voitle (1965) in the conclusion that excessive light per se cannot be considered the only causative factor in eye enlargement of chickens. Continuous darkness resulted in eye enlargement to a greater extent than did continuous light.

The corticosterone treatment did not prevent eye enlargement under those light treatments normally resulting in eye enlargement; nor did it induce eye enlargement in the groups receiving the light treatment (12 hours of darkness alternating with 12 hours of light) that invariably resulted in "normal" eyes.

The Diamox treatment prevented the development of ophthalmacrosis. This finding supports the proposition that the development of ophthalmacrosis is related to the rate of secretion of aqueous humor (Friedenwald, 1955). Since Diamox is effective in relieving

or preventing both eye enlargement in chickens and glaucoma in humans, a relationship between these two conditions is considered to be established.

Fluorometric readings. As indicated by the fluorometric readings, the chicks receiving the continuous dark treatment had the highest blood plasma corticosterone level. Continuous light resulted in the next highest level, while 12 hours of darkness alternating with 12 hours of light resulted in the lowest blood plasma corticosterone level.

Osmotic pressure. The osmotic pressure of the ocular fluid secured from chicks at 30 days of age was lowest for the group receiving the 12 hours of darkness alternating with 12 hours of light treatment as compared to those receiving continuous darkness or continuous light. However, there were no significant differences found among the three groups when osmotic pressure of ocular fluid was determined from chicks at 90 days of age. The differences that did exist in chicks at 90 days of age were of the same order relative to light treatment as those that existed at 30 days of age. The fact that the osmotic pressure of the ocular fluid of chicks receiving 12 hours of darkness alternating with 12 hours of light was significantly less than that of the other two treatments at 30 days of age, but not significantly different at 90 days of age may be explained by a consideration of the physical changes noted in the structure of the eyeball. By 90 days of age, the structural integrity of the eyeball

had been partially destroyed; this was evidenced by the thinning and tearing of the retina that was observed in many of the enlarged eyes.

Sodium and potassium concentrations of ocular fluid. Sodium and potassium concentrations of the ocular fluid secured from the chicks were highest from those grown in continuous darkness, followed by the continuous light treatment. The sodium and potassium concentrations of this fluid secured from chicks grown in the 12 hours of darkness alternating with 12 hours of light resulted in the lowest concentrations.

In view of the well-established role of the adrenal cortical hormones in the regulation of electrolytes in the body fluids, these relationships between sodium and potassium concentrations in ocular fluids and of certain photoperiod treatments point to the probable involvement of the adrenal glands in the development of ophthalmacrosis. It may be that continuous darkness or continuous light act as a stress on the chicks resulting in a greater secretion by the adrenal glands.

Adrenal weights. The adrenal glands of chicks grown in continuous darkness were significantly smaller than those of chicks grown in continuous light or 12 hours of darkness alternating with 12 hours of light. Based on these results, therefore, a definite relationship is established between the condition of ophthalmacrosis and adrenal cortical function.

The relative gland size is not always indicative of the rate of function. Based upon the results of this study pertaining to plasma corticosterone levels, osmotic pressure, sodium and potassium

concentrations of ocular fluid, it is concluded that an inverse relationship exists between gland size and rate of function. Therefore, it is considered that the smaller gland may be secreting at a greater rate than the larger ones.

Thyroid weights. The thyroid glands of chicks receiving the continuous light treatment were significantly larger than those grown in the other two treatments. The thyroid glands of chicks grown in continuous darkness or 12 hours of darkness alternating with 12 hours of light did not differ significantly from each other. From these results one cannot ascertain with a high degree of reliability the relationship between thyroid size and ophthalmocrosis.

Observations of the birds revealed that those grown in the 12 hours of darkness alternating with 12 hours of light treatment were much more active than those grown in the other treatments. This was exemplified by the amount of litter in the feeders and waterers and activity of the bird. The most docile birds were those grown in the continuous dark treatment.

Eosinophil count. The number of eosinophils in the circulating blood of the chicks grown in the three basic light treatments were determined at 4-hour intervals for a 24 hour period in an attempt to establish the presence or absence of a diurnal eosinophil rhythm. The counts were made when the chicks were 3 and 6 weeks of age.

This study failed to indicate the presence of a diurnal eosinophil rhythm in birds of any of the groups. Distinguishing between certain types of cells in avian blood is extremely difficult.

Olson (1965) reported that the coefficient of variation, or percentage error, in the differential count of eosinophils was approximately 58.8 percent.

No conclusions, therefore, can be made regarding the effects of the photoperiod treatments on the diurnal eosinophil rhythm in chickens. Thus, no relationship was found between the eosinophil rhythm and the physiological mechanism involved in the development of ophthalmacrosis.

Histological studies. Sections of thyroid, pituitary, kidney, adrenal, and eyeball tissue were used to make the histological studies.

Thyroid glands from birds grown in the 12-hour dark, 12-hour light treatment were the smallest of the three treatments. The follicles of these glands were filled with colloid, the epithelial cells were cuboidal-to-columnar, and the colloid was often shrunken from the follicular epithelium. Ham (1965) described this as a normal, active, secreting thyroid. Tissue from birds reared in the continuous darkness and continuous light treatments showed signs of hyperplasia. The cells were squamous, and the lumen was lacking in colloid. Photographs illustrating these conditions are shown in Figure 3, Appendix B.

In the pituitary gland, a high incidence of acidophils was observed in the pars anterior of birds reared in the continuous darkness and continuous light treatments. It appeared that the basophils and acidophils were more evenly distributed in the pars anterior of birds in the 12 hours of darkness alternating with 12 hours of light treatment. Ham (1965) stated that the acidophils secrete somatotrophin,

and the basophils secrete thyrothropin and adrenocorticotrophin.

The researcher was unable to distinguish any morphological or cytological differences in the structure of the kidney or adrenal glands secured from chicks reared in the three basic light-dark treatments.

Histological studies of the eyeballs revealed a difference in the thickness of the wall of the eye among the three treatment groups. The choroid and the retina were thinner in the birds exposed to the continuous dark treatment, considered to be the results of stretching as eyeballs increase in size. Photographs illustrating these conditions are shown in Figure 4, Appendix B.

SUMMARY AND CONCLUSIONS

Four experiments were conducted with Leghorn-type chicks to study the effects of photoperiods, corticosterone, and Diamox administration on body, eyeball, adrenal, and thyroid weights; concentration of corticosterone in blood plasma; osmotic pressure, sodium, and potassium content of vitreous humor; and the eosinophil (number in blood) count. Histological studies were made of the eyeball, thyroid, adrenal, pituitary, and kidney.

The fastest rate of growth was made by chicks subjected to continuous darkness. Chicks subjected to continuous light resulted in the second fastest growth rate, and cycles of 12 hours of darkness alternating with 12 hours of light resulted in the slowest rate of growth. Cycles of light and darkness longer than 24 hours reduced chick growth rate. Corticosterone injections also reduced rate of growth.

Eyeball weights were greatly affected by the photoperiods to which the chicks were exposed. Continuous darkness resulted in greatly enlarged eyeballs; continuous light resulted in less enlargement than did the former treatment, while chicks receiving alternating periods of 12 hours of light and 12 hours of darkness were found to have "normal-sized" eyeballs.

The drug Diamox prevented the development of enlarged eyes in birds receiving light treatments otherwise causing eye enlargement. Corticosterone injections failed to appreciably affect eyeball size.

Adrenal weights were influenced by the basic light-dark treatments. The adrenal size was in reverse order relative to light treatment to that of the eyeball weight. The largest adrenals, as a percentage of body weights, were found in the chicks reared in 12 hours of darkness alternating with 12 hours of light. Continuous darkness resulted in the smallest adrenals while continuous light resulted in adrenals of intermediate size.

Thyroid weights were influenced by the basic light-dark treatments. The largest thyroids, as a percentage of body weights, were found in the chicks reared in continuous light. The thyroids of the birds receiving continuous darkness or 12 hours of darkness alternating with 12 hours of light were the smallest of the three groups. The birds reared in continuous darkness were the most docile of the three groups; whereas, those placed in the 12 hours of darkness alternating with 12 hours of light were the most active.

The fluorometric readings revealed that continuous dark treatment resulted in the highest concentration of blood plasma corticosterone. Birds reared in continuous light had the next highest plasma corticosterone levels, while the 12 hours of darkness alternating with 12 hours of light treatment resulted in the lowest levels.

The osmotic pressure of the ocular fluid secured from chicks at 30 days of age was lowest for the group receiving the 12 hours of darkness alternating with 12 hours of light as compared to those receiving continuous darkness or continuous light. However, there were no significant differences among the three groups at 90 days of age.

The differences that did exist in the chicks at 90 days of age, however, were in the same order, relative to the light treatment, as those that existed at 30 days of age.

Sodium and potassium readings were greatly affected by the basic light-dark treatments to which the birds were exposed. The readings for both sodium and potassium were related to eyeball weights as affected by the light treatments. Sodium and potassium readings were highest from birds receiving the continuous dark treatment, intermediate from those receiving the continuous light treatment, and lowest from those receiving 12 hours of darkness alternating with 12 hours of light treatment. The fact that sodium and potassium was more concentrated in the ocular fluids of the enlarged eyeballs undoubtedly attributed to the enlargement.

The eosinophil count was extremely difficult to measure due to the similarity of the eosinophils and heterophils. It appeared that there was no substantial difference in the eosinophil counts among the basic light-dark treatments. A diurnal rhythm in eosinophil count could not be detected.

The histological studies revealed that the thyroid tissue for birds in the 12 hours of darkness alternating with 12 hours of light treatment was normal; whereas, tissue from glands of birds receiving continuous darkness and continuous light showed signs of hyperplasia, squamous cells, and colloid deficiency.

The pituitary gland revealed a high incidence of acidophils in the pars anterior of birds in the continuous darkness and continuous

light treatments. The basophils and acidophils were more evenly distributed in the pars anterior of birds grown in the 12 hours of darkness alternating with 12 hours of light treatment.

Observation of the kidney and adrenal gland revealed no morphological or cytological differences in the structure of the kidney.

Histological studies of the eyeball revealed a difference in the thickness of the wall. Birds receiving the continuous dark and continuous light treatments had thinner choroid and retina. Frequent tears of the retina were observed in the eyeballs of the birds receiving the continuous dark treatment. The thinning of the eyeball wall and rupturing of the retina very likely was the result of the pressures exerted by the increased volume of fluid in these eyes.

In conclusion, these experiments have established an association between certain photoperiods, physiological factors, and the development of ophthalmacrosis in chickens. In comparing the three basic photoperiod treatments, chicks reared in continuous darkness grew fastest, had greatly enlarged eyeballs, ocular fluid had the highest osmotic pressure, and the highest concentration of sodium and potassium, while the blood plasma corticosterone was highest, and the adrenal weights the lowest. Chicks receiving continuous light were intermediate in respect to these parameters, and chicks receiving the 12 hours of darkness alternating with 12 hours of light had the lowest values for these parameters, with the exception that adrenal weights of chicks from this treatment were the largest of the three treatments.

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APPENDIXES

APPENDIX A

TABLE X

BODY WEIGHTS, EYEBALL WEIGHTS AS PERCENTAGE OF BODY WEIGHTS, AND FLUOROMETRIC READINGS, EXPERIMENT I[1]

Chick number	Treatments								
	Continuous dark			Continuous light			12-hours dark, 12-hours light		
	Body wt.	Eyeball wt.[2]	Fluo. read.[3]	Body wt.	Eyeball wt.[2]	Fluo. read.[3]	Body wt.	Eyeball wt.[2]	Fluo. read.[3]
	gm.	%		gm.	%		gm.	%	
1	348	.42	88.5	366	.42	84.0	336	.33	79.0
2	336	.48	91.0	345	.34	84.0	246	.42	78.5
3	368	.41	88.0	328	.41	83.0	252	.38	75.5
4	384	.38	87.5	190	.52	85.5	260	.39	77.5
5	280	.47	86.5	248	.51	81.0	374	.30	79.5
6	298	.43	90.0	292	.46	82.5	271	.32	79.0
7	318	.50	89.0	318	.37	83.0	334	.32	78.5
8	368	.42	86.5	278	.49	82.5	258	.37	77.0
9	316	.47	88.0	318	.43	82.0	310	.34	76.5
10	292	.41	88.5	344	.35	81.0	342	.34	77.5
11	286	.43	88.0	306	.38	82.5	338	.34	79.0
12	304	.48	88.0	250	.44	80.0	234	.44	77.0
13	288	.45	90.0	308	.46	82.0	272	.35	77.0
14	344	.49	88.5	330	.36	82.0	352	.32	77.5

[1]Chicks 30 days of age.

[2]Expressed as a percentage of body weight.

[3]Fluorometric readings were taken directly from Turner's Fluorometer.

TABLE XI

ANALYSIS OF VARIANCE FOR BODY WEIGHTS, EXPERIMENT I

Source of variation	Sum of squares	df	Mean square	F
Between groups	3853	2	1927	.985
Within groups	76295	39	1956	
Total	80148	41		

TABLE XVI

ANALYSIS OF VARIANCE FOR EYEBALL WEIGHTS AS PERCENTAGE OF BODY WEIGHTS, EXPERIMENT I

Source of variation	Sum of squares	df	Mean square	F
Between groups	.0641	2	.032	16.**
Within groups	.0841	39	.002	
Total	.1482	41		

**Significant at 1 percent level of probability.

TABLE XIII

ANALYSIS OF VARIANCE FOR FLUOROMETRIC READINGS, EXPERIMENT I

Source of variation	Sum of squares	df	Mean square	F
Between groups	791.23	2	395.62	244.2**
Within groups	63.23	39	1.62	
Total	854.46	41		

**Significant at the 1 percent level of probability.

TABLE XIV

BODY WEIGHTS, EYEBALL WEIGHTS AS PERCENTAGE OF BODY WEIGHTS, AND FLUOROMETRIC READINGS, EXPERIMENT II

Chick number	Treatments								
	Continuous dark			Continuous light			12-hours dark, 12-hours light		
	Body wt.	Eyeball wt.[1]	Fluo. read.[2]	Body wt.	Eyeball wt.[1]	Fluo. read.[2]	Body wt.	Eyeball wt.[1]	Fluo. read.[2]
	gm.	%		gm.	%		gm.	%	
1	344	.48	86.0	302	.41	82.0	262	.38	76.0
2	380	.36	87.0	315	.39	84.5	348	.33	74.5
3	310	.47	88.5	320	.38	83.0	292	.33	76.5
4	370	.40	87.5	491	.27	81.0	276	.34	73.0
5	309	.45	86.0	344	.32	84.5	296	.37	75.0
6	357	.39	87.5	329	.38	83.0	310	.36	72.0
7	385	.42	87.0	266	.37	81.5	298	.34	73.0

[1]Expressed as a percentage of body weight.

[2]Fluorometric readings taken from Turner's Fluorometer.

TABLE XV

ANALYSIS OF VARIANCE FOR BODY WEIGHTS, EXPERIMENT II

Source of variation	Sum of squares	df	Mean square	F
Between groups	10,005	2	5,002	3.81*
Within groups	23,640	18	1,313	
Total	33,645	20		

*Significant at the 5 percent level of probability.

TABLE XVI

ANALYSIS OF VARIANCE FOR EYEBALL WEIGHTS AS PERCENTAGE OF BODY WEIGHTS, EXPERIMENT II

Source of variation	Sum of squares	df	Mean square	F
Between groups	.0227	2	.0113	7.06**
Within groups	.0282	18	.0016	
Total	.0509	20		

**Significant at the 1 percent level of probability.

TABLE XVII

ANALYSIS OF VARIANCE FOR FLUOROMETRIC READINGS, EXPERIMENT II

Source of variation	Sum of squares	df	Mean square	F
Between groups	592.88	2	296.44	161.1**
Within groups	33.07	18	1.84	
Total	625.95	20		

**Significant at the 1 percent level of probability.

TABLE XVIII

OSMOTIC PRESSURE,[1] SODIUM AND POTASSIUM READINGS[2] OF OCULAR FLUIDS, EXPERIMENT II

Chick number	Treatments								
	Continuous dark			Continuous light			12-hours dark, 12-hours light		
	Osmotic pressure readings	Sodium readings	Potassium readings	Osmotic pressure readings	Sodium readings	Potassium readings	Osmotic pressure readings	Sodium readings	Potassium readings
1	322.0	56	17	326.0	59	13.5	293.0	46	10.5
2	300.0	55	16	299.0	51	13.0	309.0	50	9.0
3	319.0	58	17	318.0	54	12.5	314.0	49	8.0
4	322.0	55	15	319.0	51	12.0	292.0	48	8.5
5	331.0	57	18	314.0	52	14.0	300.0	47	9.5
6	327.0	58	15	302.0	55	12.5	326.0	49	9.0
7	318.0	60	14	295.0	54	13.5	288.0	47	9.0

[1]Direct readings taken from the Advanced Osmometer, Model 31K.

[2]Direct readings taken from the Perkin-Elmer Atomic Absorption Spectrophotometer.

TABLE XIX

ANALYSIS OF VARIANCE FOR OSMOTIC PRESSURE READINGS, EXPERIMENT II

Source of variation	Sum of squares	df	Mean square	F
Between groups	1054	2	521	3.77*
Within groups	2479	18	138	
Total	3533	20		

*Significant at the 5 percent level of probability.

TABLE XX

ANALYSIS OF VARIANCE FOR SODIUM READINGS, EXPERIMENT II

Source of variation	Sum of squares	df	Mean square	F
Between groups	291	2	145.5	33.1**
Within groups	79	18	4.4	
Total	370	20		

**Significant at the 1 percent level of probability.

TABLE XXI

ANALYSIS OF VARIANCE FOR POTASSIUM READINGS, EXPERIMENT II

Source of variation	Sum of squares	df	Mean square	F
Between groups	535.34	2	267.67	923.0**
Within groups	5.21	18		
Total	540.55	20		

**Significant at the 1 percent level of probability.

TABLE XXII

BODY WEIGHTS, EYEBALL WEIGHTS AS PERCENTAGE OF BODY WEIGHTS, AND OSMOTIC PRESSURE READINGS,[1] EXPERIMENT III

Chick number	Treatments: Continuous dark			Continuous light			12-hours dark, 12-hours light		
	Body wt.	Eyeball wt.[2]	Osmotic pressure readings	Body wt.	Eyeball wt.[2]	Osmotic pressure readings	Body wt.	Eyeball wt.[2]	Osmotic pressure readings
	gm.	%		gm.	%		gm.	%	
1	1664	.230	335	1472	.208	316	1201	.175	309
2	1442	.261	330	1376	.184	327	1312	.179	298
3	1786	.244	323	1660	.174	321	1341	.173	316
4	1557	.287	321	1672	.200	319	1477	.132	296
5	1696	.230	332	1462	.213	316	1471	.167	313
6	1332	.203	326	1605	.173	322	1528	.145	301

[1]Direct readings taken from the Advanced Osmometer, Model 31K.

[2]Expressed as a percentage of body weight.

TABLE XXIII

ANALYSIS OF VARIANCE FOR BODY WEIGHTS, EXPERIMENT III

Source of variation	Sum of squares	df	Mean square	F
Between groups	122,038	2	61,019	3.10
Within groups	295,738	15	19,716	
Total	417,776	17		

TABLE XXIV

ANALYSIS OF VARIANCE FOR EYEBALL WEIGHTS AS PERCENTAGE OF BODY WEIGHTS, EXPERIMENT III

Source of variation	Sum of squares	df	Mean square	F
Between groups	.019936	2	.009968	19.94**
Within groups	.007499	15	.000500	
Total	.027435	17		

**Significant at the 1 percent level of probability.

TABLE XXV

ANALYSIS OF VARIANCE FOR OSMOTIC PRESSURE READINGS FOR OCULAR FLUIDS, EXPERIMENT III

Source of variation	Sum of squares	df	Mean square	F
Between groups	1545	2	773	2.00
Within groups	579	15	386	
Total	2124	17		

TABLE XXVI

SODIUM AND POTASSIUM READINGS,[1] ADRENAL AND THYROID WEIGHTS AS PERCENTAGE OF BODY WEIGHTS, EXPERIMENT III

Chick number	Treatments											
	Continuous dark				Continuous light				12-hours dark, 12-hours light			
	Na^2 read.	K^3 read.	Adrenal wt.	Thyroid wt.	Na^2 read.	K^3 read.	Adrenal wt.	Thyroid wt.	Na^2 read.	K^3 read.	Adrenal wt.	Thyroid wt.
			%	%			%	%			%	%
1	81	16	.003	.006	77	14	.004	.006	75	9.5	.005	.005
2	81	15	.004	.004	79	13	.004	.014	75	9.0	.004	.006
3	79	17	.003	.002	76	12	.004	.006	74	8.5	.004	.005
4	82	14	.003	.007	77	13	.004	.006	76	10.0	.006	.001
5	81	17	.003	.005	76	14	.003	.008	74	8.0	.004	.004
6	81	16	.004	.005	78	13	.003	.006	74	8.0	.003	.004

[1]Direct readings taken from Perkin-Elmer Atomic Absorption Spectrophotometer.

[2]Sodium.

[3]Potassium.

TABLE XXVII

ANALYSIS OF VARIANCE FOR SODIUM READINGS, EXPERIMENT III

Source of variation	Sum of squares	df	Mean square	F
Between groups	115	2	57.5	191.7**
Within groups	15	15	3.0	
Total	130	17		

**Significant at the 1 percent level of probability.

TABLE XXVIII

ANALYSIS OF VARIANCE FOR POTASSIUM READINGS, EXPERIMENT III

Source of variation	Sum of squares	df	Mean square	F
Between groups	149.4	2	74.700	83.65**
Within groups	13.4	15	.893	
Total	162.8	17		

**Significant at the 1 percent level of probability.

TABLE XXIX

ANALYSIS OF VARIANCE FOR ADRENAL WEIGHTS, EXPERIMENT III

Source of variation	Sum of squares	df	Mean square	F
Between groups	.000004	2	.000002	4.*
Within groups	.000007	15	.0000005	
Total	.000011	17		

*Significant at the 5 percent level of probability.

TABLE XXX

ANALYSIS OF VARIANCE FOR THYROID WEIGHTS, EXPERIMENT III

Source of variation	Sum of squares	df	Mean square	F
Between groups	.000041	2	.000021	4.2*
Within groups	.000081	15	.000005	
Total	.000122	17		

*Significant at the 5 percent level of probability.

TABLE XXXI

EFFECTS OF PHOTOPERIODS, CORTICOSTERONE, AND DIAMOX ON BODY AND EYEBALL WEIGHTS,[1] EXPERIMENT IV

Chick number	Treatments											
	Continuous dark				Continuous light				12-hours dark, 12-hours light			
	Corticosterone		Diamox		Corticosterone		Diamox		Corticosterone		Diamox	
	Body wt.	Eyeball wt.	Body wt.	Eyeball wt.	Body wt.	Eyeball wt.	Body wt.	Eyeball wt.	Body wt.	Eyeball wt.	Body wt.	Eyeball wt.
	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%
1	344	.409	638	.306	358	.351	651	.233	371	.317	494	.207
2	466	.407	673	.307	362	.316	575	.264	266	.407	432	.317
3	410	.418	636	.293	408	.319	594	.237	259	.415	564	.284
4	375	.365	624	.301	424	.303	466	.281	333	.366	618	.241
5	366	.489	552	.410	293	.366	426	.312	344	.312	468	.313
6	351	.441	551	.406	321	.349	410	.359	385	.302	522	.258
7	408	.352	454	.411	316	.373	356	.388	286	.383	449	.316
8	370	.357	595	.318	330	.342	520	.264	265	.354	552	.278
9	395	.417	630	.298	285	.352	365	.404	300	.364	442	.307
10	360	.415	642	.330	255	.381	610	.282	275	.381	385	.345
11	440	.375	568	.343	295	.369	502	.296	230	.392	548	.295
12	360	.418	635	.317	350	.425	440	.317	345	.345	320	.392
13	440	.374	615	.364	315	.392	490	.287	325	.335	392	.314

[1]Expressed as percentage of body weights.

TABLE XXXII

ANALYSIS OF VARIANCE OF BODY WEIGHTS FOR THE CORTICOSTERONE-PHOTOPERIOD INTERACTION STUDY, EXPERIMENT IV

Source of variation	Sum of squares	df	Mean square	F
Between groups	49,258	2	24,699	12.1**
Within groups	73,132	36	2,031	
Total	122,390	38		

**Significant at the 1 percent level of probability.

TABLE XXXIII

ANALYSIS OF VARIANCE FOR EYEBALL WEIGHTS[1] FOR THE CORTICOSTERONE-PHOTOPERIOD INTERACTION STUDY, EXPERIMENT IV

Source of variation	Sum of squares	df	Mean square	F
Within groups	.017387	2	.008694	6.73**
Between groups	.146444	36	.001290	
Total	.063831	38		

[1]Expressed as percentage of body weights.

**Significant at the 1 percent level of probability.

TABLE XXXIV

ANALYSIS OF VARIANCE FOR BODY WEIGHTS FOR THE DIAMOX-PHOTOPERIOD INTERACTION STUDY, EXPERIMENT IV

Source of variation	Sum of squares	df	Mean square	F
Between groups	119,937	2	59,969	9.33**
Within groups	231,147	36	6,421	
Total	351,084	38		

**Significant at the 1 percent level of probability.

TABLE XXXV

ANALYSIS OF VARIANCE FOR EYEBALL WEIGHTS[1] FOR THE DIAMOX-PHOTOPERIOD INTERACTION STUDY, EXPERIMENT IV

Source of variation	Sum of squares	df	Mean square	F
Between groups	.011058	2	.005529	2.64
Within groups	.075292	36	.002091	
Total	.086350	38		

[1]Expressed as percentage of body weights.

TABLE XXXVI

BODY AND EYEBALL WEIGHTS OF CHICKS GROWN IN VARIOUS PHOTOPERIOD TREATMENTS, EXPERIMENT IV

Chick number	Treatments							
	20-hour dark 4-hour light		4-hour dark 20-hour light		72-hour dark 72-hour light		24-hour dark 24-hour light	
	Body wt.	Eyeball wt.[1]	Body wt.	Eyeball wt.[1]	Body wt.	Eyeball wt.[1]	Body wt.	Eyeball wt.[1]
	gm	%	gm.	%	gm.	%	gm.	%
1	654	.255	598	.245	567	.255	509	.268
2	487	.330	516	.276	432	.311	459	.320
3	618	.243	531	.256	394	.311	400	.325
4	496	.313	387	.320	398	.330	500	.294
5	460	.352	614	.354	596	.261	512	.298
6	566	.302	578	.243	425	.320	539	.289

[1]Expressed as percentage of body weights.

TABLE XXXVII

ANALYSIS OF VARIANCE FOR BODY WEIGHTS OF CHICKS GROWN IN LIGHT-DARK CYCLES DIFFERING FROM THE THREE BASIC CYCLES, EXPERIMENT IV

Source of variation	Sum of squares	df	Mean square	F
Between groups	26,186	3	8,729	1.49
Within groups	116,965	20	5,848	
Total	143,151	23		

TABLE XXXVIII

ANALYSIS OF VARIANCE FOR EYEBALL WEIGHTS IN PHOTOPERIODS DIFFERING FROM THE THREE BASIC CYCLES, EXPERIMENT IV

Source of variation	Sum of squares	df	Mean square	F
Between groups	47.64	3	15.88	1.58
Within groups	201.07	20	10.05	
Total	248.71	23		

[1]Expressed as percentage of body weights.

TABLE XXXIX

EOSINOPHIL COUNT[1] FOR CHICKS AT THREE WEEKS OF AGE, EXPERIMENT IV

Sample number	Continuous dark						Continuous light						12-hours dark, 12-hours light					
	Time taken						Time taken						Time taken					
	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.	2 a.m.	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.	2 a.m.	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.	2 a.m.
1	4	3	4	3	3	3	3	2	3	3	3	3	3	3	3	3	4	3
2	3	5	3	3	4	2	2	3	3	3	3	3	3	3	2	4	3	4
3	3	3	4	5	4	4	4	3	4	2	4	4	4	3	4	2	4	3
4	3	3	4	5	5	3	2	2	3	3	4	2	3	2	3	3	3	3
5	4	3	3	3	3	3	3	3	3	3	3	3	2	4	4	2	5	5
6	4	5	3	4	4	3	4	3	3	2	4	3	3	3	2	4	3	3
Average	3.5	3.7	3.5	3.8	3.8	3.0	3.0	2.7	3.2	2.7	3.5	3.0	3.0	3.0	3.0	3.0	3.7	3.5

[1]Per 200 white cells.

TABLE XL

EOSINOPHIL COUNT[1] FOR CHICKS AT SIX WEEKS OF AGE, EXPERIMENT IV

Sample number	Continuous dark						Continuous light						12-hours dark, 12-hours light					
	Time taken						Time taken						Time taken					
	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.	2 a.m.	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.	2 a.m.	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.	2 a.m.
1	3	3	3	3	3	4	3	3	3	2	3	3	2	4	3	3	3	4
2	4	2	5	4	4	2	2	2	3	3	3	4	3	2	3	2	4	3
3	2	5	3	3	4	3	2	4	4	3	4	3	3	3	2	3	4	3
4	3	3	3	5	5	3	2	2	2	2	3	2	3	3	3	3	3	3
5	3	4	4	2	3	3	3	2	3	3	2	4	4	2	4	3	4	3
6	4	2	2	4	3	5	3	4	2	2	4	3	2	3	2	3	3	4
Average	3.0	3.2	3.3	3.5	3.7	3.3	2.5	2.8	2.8	2.5	3.2	3.2	2.8	2.8	2.8	2.8	3.5	3.3

[1]Per 200 white cells.

TABLE XLI

COMPOSITION OF CHICK DIET

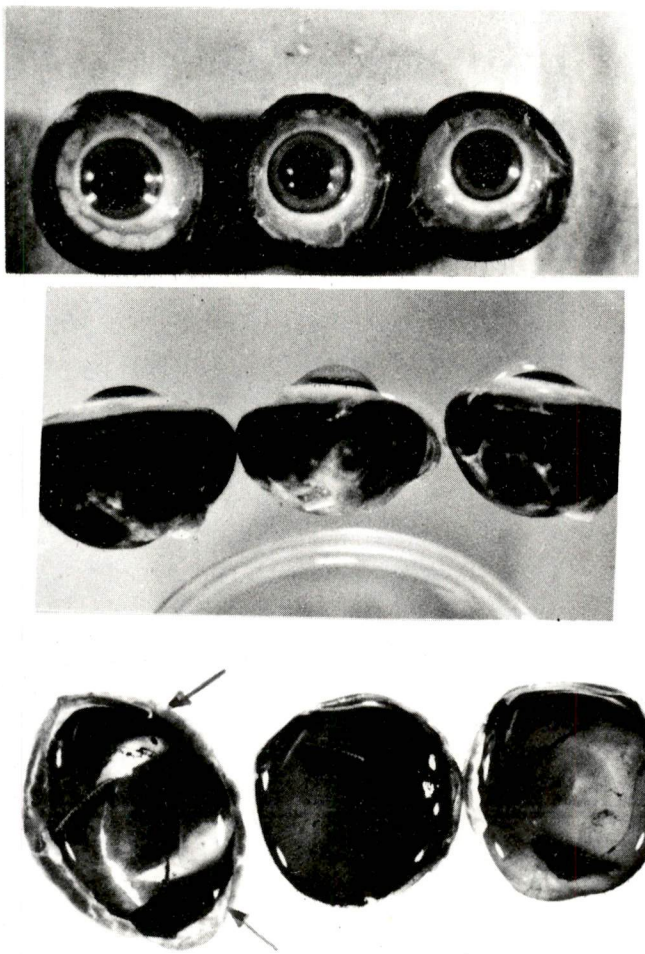
Ingredient	Percent
Yellow corn	63.80
Alfalfa meal (17%)	2.50
Fish meal	2.50
Soybean oil meal (50%)	25.50
Ground limestone	0.60
Defluoronated rock phosphate	1.50
Salt	0.48
Manganese sulfate	0.02
Vitamin mix[2]	0.60
Coccidiostat premix[3]	2.50
Total	100.00

[1]Calculated analysis: crude protein, 21.54 percent, productive energy, 943 calories per pound, C/P ratio, 43.8; Methionine, 0.408 percent; Cystine, 0.313 percent; Calcium, 0.960 percent; Phosphorus, 0.692 percent; Manganese, 31.2 mg./lb.

[2]Calculated analysis: Vitamin A, 5349 I.U./lb.; Vitamin D, 340 I.C.U./lb.; Riboflavin, 3.01 mg./lb.; Niacin, 27.78 mg./lb.; Pantothenic acid, 6.67 mg./lb.; Choline, 718.0 mg./lb.

[3]2.5 pounds of premix supplies 0.013 lbs. of amprolium.

APPENDIX B



(a)

(b)

(c)

Figure 1. Enucleated eyeballs from chickens grown in the three basic light-dark treatments.

(a) Front view.

(b) Lateral view.

(c) Dissected eyes. The arrow indicates torn retina in eyeballs of chicks grown in continuous darkness.

Left to right - continuous darkness, 12 hours of darkness alternating with 12 hours of light, continuous light.

Light spots around periphery are light reflections.

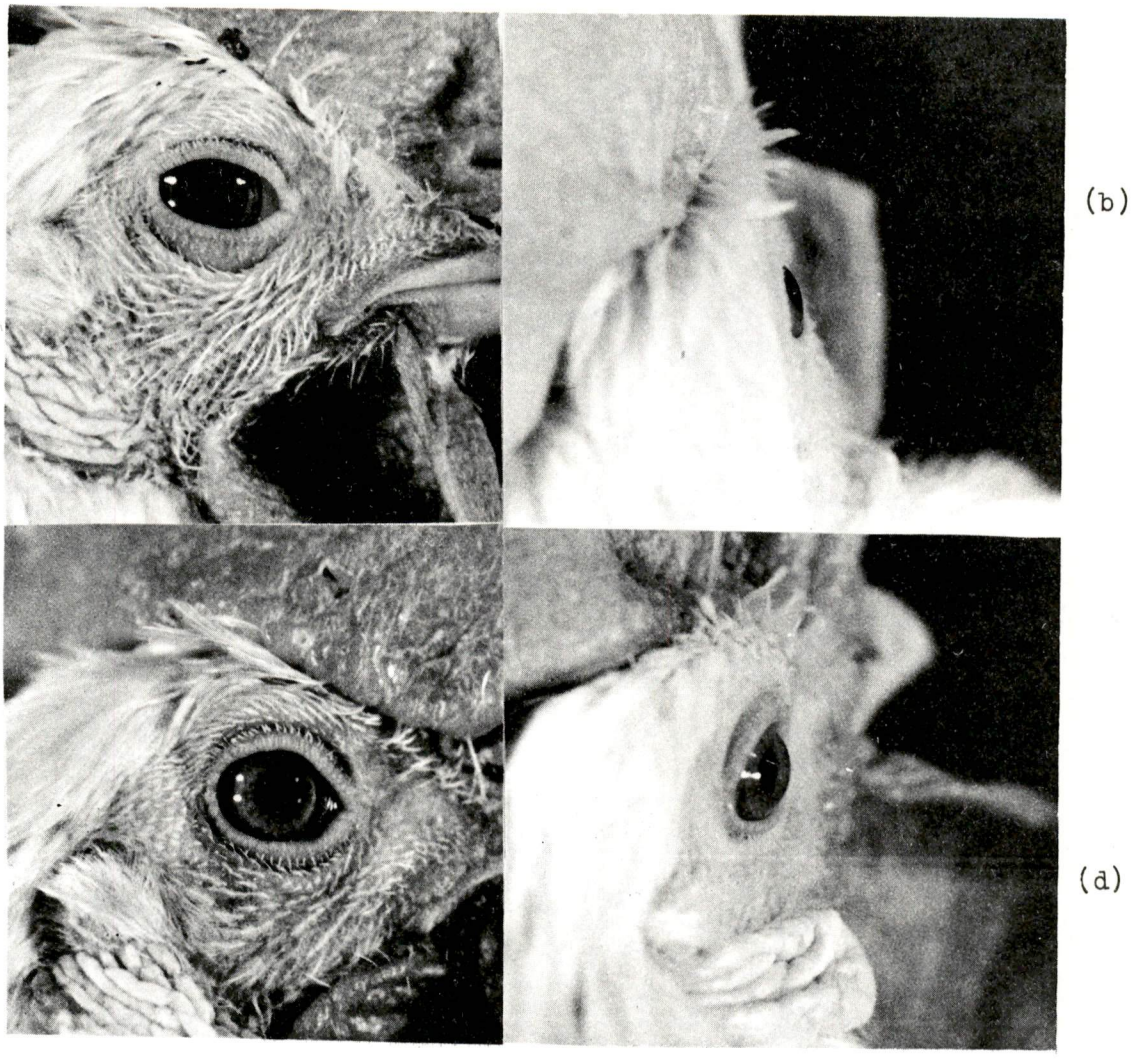


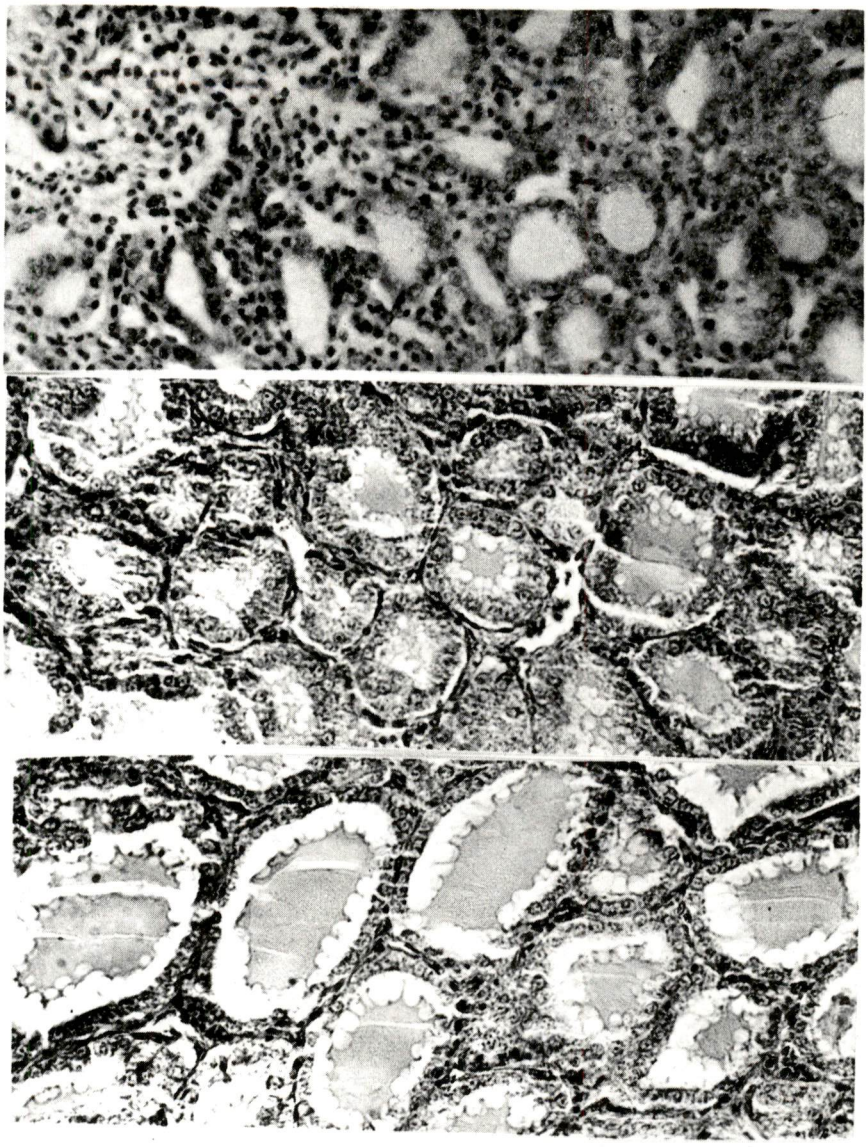
Figure 2. Appearance of chickens with ophthalmacrosis compared to chickens with "normal" eyeballs.

(a) Lateral view of chicken, grown in continuous darkness, with ophthalmacrosis; illustrating the "slanted" eye and the abnormally large space between the lens and anterior canthus.

(b) View from the rear of chicken, grown in continuous darkness, with ophthalmacrosis; illustrating the absence of space between the cornea and the lens.

(c) Lateral view of chicken, grown in 12 hours of darkness alternating with 12 hours of light, with "normal" eyeballs.

(d) View from the rear of chicken, grown in 12 hours of darkness alternating with 12 hours of light, with "normal" eyeballs.



(a)

(b)

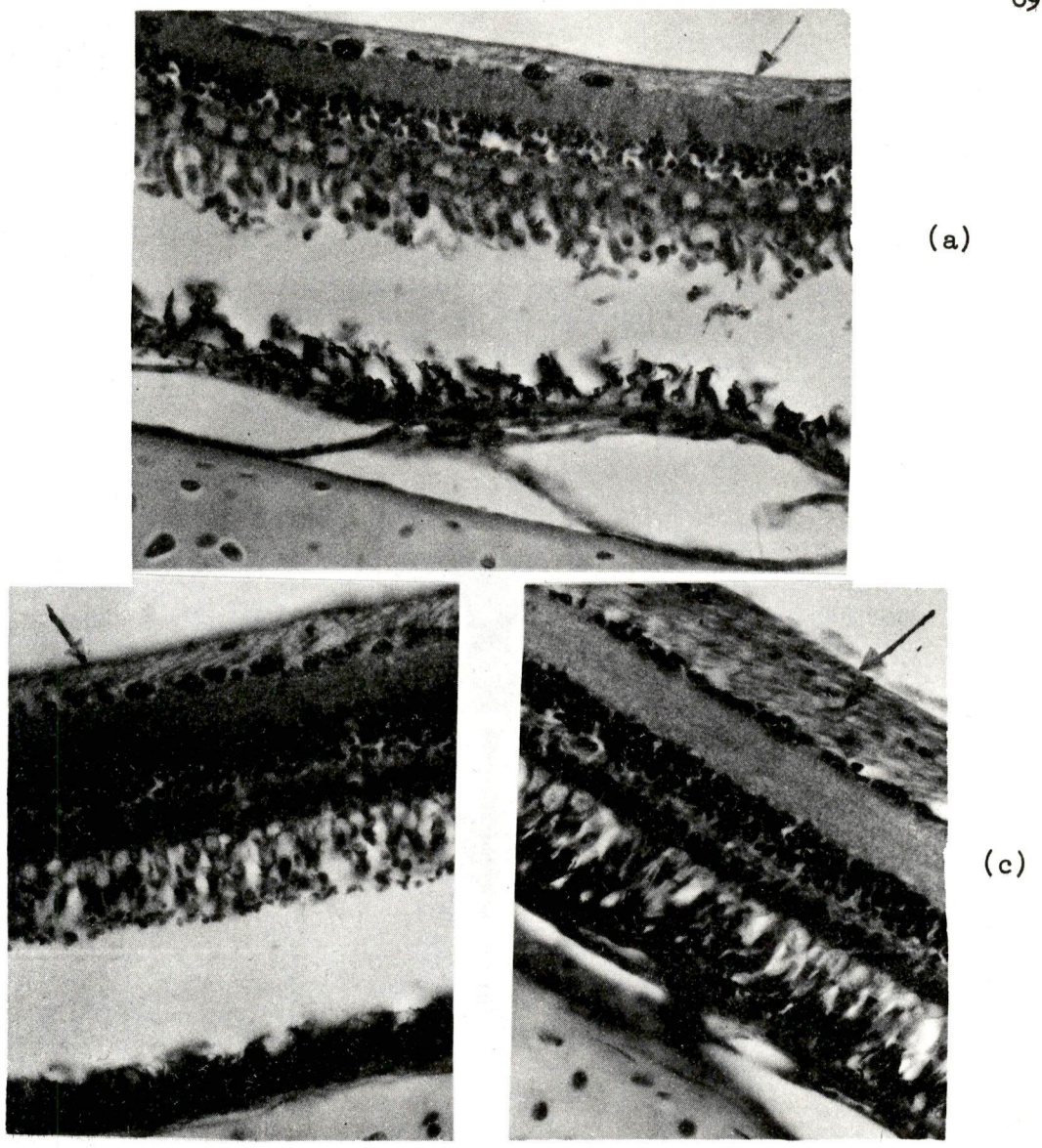
(c)

Figure 3. Photomicrographs of sections of thyroids from chickens receiving the three basic light-dark treatments. x 300.

(a) Thyroid section from chickens grown in continuous light; illustrating hyperplasia and a diminished amount of colloid.

(b) Thyroid section from chickens grown in continuous darkness; illustrating hyperplasia and diminished amount of colloid to a lesser extent than section (a).

(c) Thyroid section from chickens grown in 12 hours of darkness alternating with 12 hours of light; illustrating a "normal" thyroid.



(a)

(b)

(c)

Figure 4. Sections of the wall of the eye from chickens receiving the three basic light-dark treatments. x 300.

(a) Section of eyewall from chickens receiving continuous darkness; illustrating an extremely thin retina. The most striking difference, shown by the arrow, is the inner limiting membrane.

(b) Section of eyewall from chickens receiving continuous light; the arrow illustrating a thin retina, but to a lesser degree than those grown in continuous darkness.

(c) Section of eyewall from chickens receiving 12 hours of darkness alternating with 12 hours of light; the arrow illustrating a "normal" retina.