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I am submitting herewith a dissertation written by Stephen A. Osei entitled "Photoperiod, Adrenal Corticosterone and the Development of Avian Glaucoma." I have examined the final copy of this dissertation for form and content and 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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PHOTOPERIOD, ADRENAL CORTICOSTERONE AND THE DEVELOPMENT OF AVIAN GLAUCOMA

A Dissertation

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Doctor of Philosophy

Degree

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ABSTRACT

Three hundred and fifty Rhode Island Red cockerels were maintained under three lighting conditions from day one after hatching to 10 weeks of age to investigate the possible relationships between photoperiod, avian glaucoma and adrenal function. Other parameters studied included body, comb, testicular and thyroid weights. The lighting treatments included (1) diurnal light/dark or 12L:12D, (2) continuous light or 24L:0D, and (3) continuous dark or 0L:24D.

Both 24L:0D and 0L:24D conditions significantly increased avian eyeball weight, adrenal weight and plasma content of corticosterone compared with the 12L:12D regimen ($P > 0.05$). There was a highly significant reduction in eye depth ($P < 0.01$) when birds were raised under continuous light or continuous darkness. A non-significant reduction in thyroid weight was produced by keeping chicks under total illumination and also under 24 hours of darkness. Constant light greatly enhanced testicular growth while the continuous absence of light drastically reduced testes weight in comparison with testicular growth under alternating light and dark.

Body weight, comb weight, and intraocular pressure were not significantly affected by any of the treatments.

At least two peaks of plasma corticosterone were seen under each treatment, but the corticosterone diurnal rhythm was not disturbed even though the levels of the hormone were increased significantly ($P > 0.05$) by the absence of an alternating light and dark treatment.

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CHAPTER I

INTRODUCTION

Many physiological functions of animals vary with the time of day or even the time of year. Perhaps the best known example is the daily rhythm of locomotor activity, many animals restricting their periods of activity to particular times of day or night. Thus, there are essentially three types of animals with respect to activity patterns; diurnal animals that are day-active, nocturnal or night-active animals and crepuscular ones which are active at dawn and dusk. Reproductive activity may also be restricted to specific times of day or year depending on the species and on daylength.

In avian species, Rowan's (1926) discovery of a vital relationship between daylength and annual cycles of reproductive activity in the slate-colored junco (Junco hyemalis) was like a floodgate to research in this area. It was not until 1957 that Jensen and Matson pointed out the apparent deleterious effects of what was believed to be excessive lighting. They showed a peculiar eye enlargement coupled with a reduction in corneal curvature in chicks subjected to conditions of total illumination for six weeks. Since these eye lesions also exhibited increased intraocular pressure and retinal damage characteristic of human glaucoma, Lauber and associates (1966, 1970) referred to this pathology as avian glaucoma. It was so strongly thought to be due to excessive lighting that Kinnear et al. (1974) among others called it light-induced avian glaucoma. Work by Voitle (1969) and Chiu et al. (1975) later indicated the inducibility of the condition in

continuous darkness. The common cause then seems to be an alteration of light and dark and not a lack of darkness or the presence of too much light. This alteration may be equivalent to a stress condition according to Buckland et al. (1971). Under conditions of stress (Buckland et al., 1976) the plasma corticoid levels of chickens increase two to four times. This increase in corticoids can then lead to eye abnormalities in as yet unclear ways. Some of these possibilities are presented in the section dealing with the discussion.

CHAPTER II

OBJECTIVES

A group of 350 one-day-old Rhode Island Red cockerels were reared in three lighting conditions to study the possible involvement of the adrenal glands and corticosterone in the genesis and development of photoperiodically induced avian eye enlargement and glaucoma, and the influence of these lighting regimens on other body measurements.

CHAPTER III

REVIEW OF LITERATURE

The effects of light are rather pervasive; from the conversion of solar energy to plant food in the photosynthetic process to the complex processes involved in the reproductive phenomena of higher vertebrates, light is an indispensable commodity. Growth in man and animals relies heavily on the presence of light. It is, therefore, not surprising that so much research has centered on the effects of illumination. In avian species much of this work relates to the influence of light on growth, attainment of sexual maturity, productive efficiency and animal welfare. In this review some of these factors are considered.

Body Weight

Much of the research in this area has been done with broiler-type chickens, and in most cases, no constant darkness treatment was studied. Siegel et al. (1961) reported significantly heavier body weights and improved feed conversion efficiency in White Leghorn pullets at 8 weeks of age when light was restricted to only 6 hours per day compared with 14 hours light per day. Centa et al. (1969) made cumulative sound level recordings of activity patterns and feeding behavior. They showed that activity was drastically reduced during the period of absence of light, but reached maximum in the presence of light. Commercial broilers were raised to 8 weeks by Cain in 1973 under intermittent light schedules of 23-hours light:1-hour dark,

1-hour light:1-hour dark and 3-hour light:1-hour dark. At eight weeks of age the body weights of pullets raised under the long photoperiod were significantly reduced compared with those under the other treatments. Cockerels, however, did not show any significant weight differences. Buckland et al. (1976) suggested that intermittent lighting may be less stressful than continuous light as evidenced by higher plasma corticoid levels in birds on continuous light. In a review of the subject, Buckland and Hill (1970) found great inconsistencies, with about half the studies showing improved growth on continuous light while the rest indicated that selected intermittent light/dark schedules enhanced feed conversion and maximized growth. Work at The University of Tennessee, Knoxville has also proved inconclusive. Osei (1978) observed that birds exposed to continuous darkness throughout an experimental period of 10 weeks were heavier than those reared in either continuous light or a diurnal light/dark regimen. This was in contrast with Voitle's 1969 report which indicated that the diurnal treatment favored maximization of weight gains.

Avian Eye Integrity

The avian eye as in other animals, acts as a photoreceptive organ, directly exposed to light. The effects of excessive lighting or the lack of it were not given great attention until 1957 when Jensen and Matson observed a peculiar eye enlargement accompanied by a reduced depth of the anterior chamber in chicks kept in continuous light for six weeks post-hatching. Besides these eye abnormalities there were also increased corneal curvature and elevated intraocular

pressure or IOP (Lauber et al., 1966). Since the oculopathy displayed features evident in human glaucoma, Lauber and colleagues (1966) called this syndrome avian glaucoma. To this time it was generally believed that the condition was due to excessive light incident on the chick's eyes; Kinnear et al. (1974) referred to it as light-induced glaucoma. In 1967 it was reported that the disease could also be induced in continuous dim light (Harrison and McGinnis). Later work by Voitle (1969) showed that avian glaucoma could result from keeping chicks in 24-hour darkness, a report that was corroborated by Chiu et al. in 1975.

Jenkins et al. (1979) reared 200 broiler-type chicks under either normal lighting or continuous darkness for 16 weeks. Chickens reared under conditions of total darkness showed an abnormal eye enlargement accompanied by elevated IOP. The eyes continued to increase in size from the sixth to sixteenth weeks. Work pre-dating this (Smith, Becker, and Podos, 1969) showed that the mean eye weight from birds raised under continuous light was 4.17 g as against 2.48 g for eyes from chicks under diurnal light/dark. In this connection the work of Jensen and Matson (1957) is very interesting. They drew fluid from the posterior or vitreous chamber of the eye using a hypodermic syringe. Twice as much fluid was collected from the eyes reared under continuous light (0.80 ml) as from chicks receiving the normal light/dark schedule (0.45 ml). After determining the dry weights of the eyeballs and expressing them as a percentage of body weight, they discovered only slight differences. They concluded that the eye hypertrophy was due primarily to an enhanced uptake of fluid. Whether this is associated with an impaired outflow facility is not clearly known (Kinnear et al., 1974).

One of the earliest lesions manifested by photoperiodically induced avian glaucoma is an impaired development of the cornea, particularly an inability of the corneal curvature to increase normally as eye growth occurs. Kinnear et al. (1974) observed that this retarded corneal development began in about nine days and is rapid and conspicuous in its incipient stages. The aqueous space volume is only 75% the normal volume by four weeks of age and declines to only 1/3 normal by 20 weeks of age. In addition, Jenkins et al. (1979) noticed that the corneal thickness of OL:24D eyes increased between four and five weeks, but were not different from the corneas of 12L:12D eyes. From then on the OL:24D corneas decreased in thickness and remained consistently thinner than control eyes.

Reference has already been made to the presence of high IOPs in avian glaucoma (Lauber et al., 1966, 1970; Jenkins et al., 1979). Smith, Becker and Podos (1969) recorded a mean IOP of 33 mm Hg at five weeks for chicks reared in continuous light compared with 23 mm Hg for the control group. While this increased pressure is a feature of glaucoma in birds, there is a question as to when the elevation in IOP actually occurs. Jenkins et al. (1979) determined IOP at weekly and bi-weekly intervals by use of applanation tonometry between 1300 and 1400 hours. There were significantly higher IOPs in chicks reared under OL:24D than in 12L:12D chicks up to the sixth week. Thereafter, OL:24D IOPs progressively declined, reaching significantly lower levels than controls after 12 weeks of age. Smith et al. (1969) showed that IOP was maintained in 24L:0D birds until 15 weeks of age. This was corroborated by the work of Axmith and Morin (1975) which showed that chickens subjected to continuous light did not develop high IOPs at the

end of a 16-week test period, even though eye weight increased. It seems that the strain of chickens used and the methods for determining IOP are important factors in the pressure observed.

There is little doubt that the photoperiodically induced glaucomatous state in chickens is systemically induced. Lauber et al. (1965) discovered that if they kept the eyes of birds covered with an opaque vision occluder and reared them under 24L:0D treatment, these experimental birds showed as much ocular hypertrophy as those not similarly covered. They reached the conclusion that the condition was induced systemically rather than locally. In later studies, Chiu et al. (1975) covered one eye of birds and left the other intact and exposed the birds to 14 hours of light alternating with 10 hours dark or continuous light. When comparing the 24-hour light eyes they observed that the covered eyes showed morphological changes similar to the exposed eyes, although the changes in the former were more severe. Among the 14L:10D eyes, they found the covered eyes to have increased weights and reduced anterior chamber depth. Consequently, they also concluded that a systemic rather than a local etiology was responsible for the eye condition.

Corticosterone Levels in Plasma

The existence of a circadian rhythm of corticoid concentrations in plasma is now an indisputable fact. Plasma levels of corticosteroids reach a zenith in the period preceding awakening or activity and then show a progressive and gradual decrease over the rest of the day. The initial work in this area involved the determination of corticoids every four or six hours over a 24-hour period. The impression from these

earlier works was that peaks and lows in corticoid levels followed a rather smooth curve. In 1971, Krieger et al. determined corticoid levels in human plasma over 20 minute intervals and produced a more substantive and informative description of the corticoid rhythm. Their studies showed unequivocally that the corticoid circadian rhythm occurred as episodic, relatively synchronous peaks throughout the 24-hour day, although the majority of the peaks still occurred in the time period before awakening or activity.

Daily rhythms of corticosterone content in avian plasma have been described by Meier and Fivizzani (1975). The periodicity is entrained by the daily photoperiod, the offset of light being the primary time giver or zeitgeber. In fact, in pigeons, which may represent an extreme case, the rhythm disappears after two weeks in continuous light (Joseph and Meier, 1973). In addition to the circadian periodicity of plasma corticoids, several authors have reported a daily pattern of urinary excretion of these hormones. Characteristic of this diurnal rhythm is a peak of corticoid excretion between 2:00 and 4:00 a.m. and a minimal excretion at noontime (Giedke and Fatranska, 1974). This periodicity is maintained even under conditions of continuous light or darkness; however, darkness increased total excretion and the peak excretion rate tended to occur a little later (Fatranska, 1971). According to Hollwich (1979), 17-hydroxycorticosteroids are excreted in the same fashion as the corresponding corticoid levels in the plasma, but about two hours later.

As is true of many other hormones, the regulatory mechanism for the plasma corticoid rhythm is commonly accepted to reside in the hypothalamus and adenohypophysis. Rhythms of hypothalamic corticotropin

releasing hormone (CRH) and pituitary adrenocorticotrophic hormone (ACTH) drive corticoid rhythms (Sato and George, 1973). The same authors observed in the common pigeon, Columba livia, that the plasma corticosterone rhythm is preceded by about four hours by a hypothalamic rhythm of CRH.

Corticosterone levels have been measured in diverse ways. The earliest methods involved fluorometry, but were not sufficiently accurate and highly variable results were obtained. Prior purification, while lowering the values did not remove the inadequacy of fluorometric methods, so that Boissin and Assenmacher (1968) obtained values as high as 50 ng/ml in mature quail after thin layer chromatographic purification of the samples. Later work by Culbert and Wells (1975) involving the use of applied column chromatography gave a range of values between seven and 20 ng/ml in laying hens. The use of competitive protein binding technique and radioimmunoassay methods have further improved the determinations, reducing values to between 2.3 ng/ml in 30-week-old White Leghorns and 4.0 ng/ml in 6-week-old chickens (Buckland et al., 1973). Buckland et al. (1974) found the basal level of corticoids in 39-day-old White Leghorns to be 6.77 ng/ml.

Photoperiod, Adrenals and Avian Glaucoma

The work of Voitle in 1969 and later Chiu et al. (1975) showed conclusively that photoperiodically induced avian eye enlargement was due to an absence of alternating light and dark. Voitle, in fact, speculated that there might be an underlying stress condition resulting from the unnatural nature of either continuous light or dark. Jenkins et al. (1979) reached the same conclusion. They observed a lethargic

behavior in birds kept under OL:24D and suggested that the lack of social interaction was a result of a generalized metabolic stress. Under conditions of stress the plasma corticoid levels of chickens can increase up to fourfold (Buckland et al., 1976). Increased corticosterone levels due to abnormal lighting conditions have been reported by Meier and Fivizzani (1975) and by Fatranska (1971). Other evidence strongly correlates the onset of glaucoma with adrenal function. Boyd and MacLeod (1964) showed that the peak of the plasma corticoid rhythm in humans preceded the peak of IOP in glaucomatous eyes by nearly four hours, and that an interference with the corticoid rhythm caused a concomitant disruption of the IOP rhythm. Ballintine (1966) correlated the phasic variation in IOP with a phasic variation in sodium concentration and suggested that this relationship resulted from the underlying adrenal cortical rhythm. Khasanova and Posmochnikova (1971), quoted by Rosenberg and Levene (1974), have observed increased plasma and urine levels of free 17-hydroxycorticosteroids in glaucoma patients, while the latter authors have found corticoid levels to increase with age in glaucoma patients, but not in normal individuals. In addition, topical administration of corticoids have induced symptoms of glaucoma (Podos et al., 1971). They demonstrated a dose-response relationship between IOP and dexamethasone concentrations varying from 0.1 to 0.001%.

Thyroid

Harrison et al. (1968) described a hypothyroid condition coupled with lowered oxygen utilization in birds that had been kept under diurnal dim light. The thyroid glands of Sprague-Dawley rats kept

under 24-hour light weighed more while they were lower in weight under continuous darkness than the controls exposed to normal lighting, according to DeProspo et al. (1969). Moreover, constant light yielded a significantly increased, and constant darkness a reduced uptake of radioiodine.

Daily rhythms of pituitary and plasma contents of thyroid stimulating hormone (TSH) have been recorded by Retienne et al. (1968) as cited by Hollwich (1979), and in humans, by Vernikos-Danellis et al. (1972). Hypothyroidism in humans has also been associated with an altered pattern and diminished amplitude in the plasma corticoid rhythm (Meier, 1975), although this is not true in other animals; this may reflect a species difference.

Reproductive Performance

The importance of daylength in the regulation of annual cycles of reproductive functions was discovered by Rowan in 1926. Since then there has been a great accumulation of research data and knowledge relevant to reproductive physiology, not only in birds, but also in other animal species. While the specific minute details are still to be worked out, it is known for certain that the photoperiodic regulation of reproduction involves both the production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary glands (Follett et al., 1975). Actually before this, Bunning (1960) had theorized that light acts in two ways to stimulate reproductive responses in animals. This involves a daily rhythm of reproductive photosensitivity which in turn is entrained by the daily photoperiod. This daily interval of reproductive photosensitivity or the

photo-inducible phase occurs during the light phase when there is a long enough duration of light.

In the male animal the testes serve as the reproductive gonads and may thus be used to ascertain the effects of light on reproductive function. The effects of ocular light perception on testicular (and ovarian) function have been vividly demonstrated in both Peking ducks and chickens by Benoit et al. (1959). While light enhanced sexual maturation in young male ducks, constant darkness had an inhibitory effect. Radnot (1961) utilized periodic nocturnal illumination of the eyes of ducks during the gonadal dormancy period, and was able to stimulate testicular growth and spermatogenesis in the drake (and egg production in the duck). The favorable effect of light in stimulating testicular growth has also been shown by Oishi and Lauber (1973). Continuous light produced gonads of maximal size, which then regressed when the animals were transferred to a short photoperiod or to darkness.

Comb Growth

The comb is an accessory sex characteristic which owes its development to the male hormone testosterone. Any treatment that influences sexual function will consequently be expected to affect comb growth. Thus in 1961, Radnot induced a noticeable development in the cock's comb by the use of intermittent light manipulation in chickens. Osei (1978) produced significantly higher comb weight in Hyline W-36 cockerels by keeping them under either 24-hour light or diurnal light/dark conditions compared with similar birds kept under continuous darkness.

This study was undertaken to investigate the involvement of photoperiod and adrenal corticosterone in the development of avian glaucoma.

CHAPTER IV

MATERIALS AND METHODS

Three hundred and fifty-day-old Rhode Island Red cockerals were randomly, but equally allotted to three lighting treatments and individually wing-banded for easy identification. They were placed in windowless, electrically heated and ventilated pens. There were five replicate pens per treatment (15 pens total). Each pen measured 150 x 150 cm and the floor litter consisted of about 12 cm of wood shavings. Artificial lighting was provided in those pens requiring it. Feed and water were supplied ad libitum.

The trial was carried out from July to September, 1980.

Treatments

Three lighting treatments were utilized and consisted of (1) diurnal light/dark, that is 12 hours light followed by 12 hours darkness (12L:12D), (2) continuous or total darkness (0L:24D), and (3) continuous or total light (24L:0D). Light was provided by means of incandescent light bulbs rated at 60 watts and were regulated by means of time clocks.

Parameters Measured

Parameters measured in this work included:

(a) Live body weights: ten birds from each treatment were individually weighed at the end of the ten-week experimental period prior to slaughter.

(b) Eye weights: the right eye was removed after slaughter; after fat and other extraneous matter had been removed the eyes were weighed.

(c) Eye depth or the distance between the outer surface of the cornea and the iris laterally was measured by use of a micrometer gauge under a microscope.

(d) Intraocular pressure or IOP was measured with a Physiograph 4 connected to a pressure transducer (Narco Model RP 1500) calibrated against a column of water. A 24 gauge needle was inserted lateral to the scleral ring of the eye into the vitreous body. Pressures were allowed to equilibrate for a minute before being recorded.

(e) Testicular weight: the right testes were weighed.

(f) Comb weight.

(g) Adrenal gland weight: as in the case of eyes, thyroids and testes, only the right adrenal gland was weighed.

(h) Plasma corticosterone levels.

All data were analyzed and tested at the one and five percent levels of probability using the randomized block design. Significant differences were separated by means of Duncan's Multiple Range Test.

CHAPTER V

DETERMINATION OF PLASMA CORTICOSTERONE

Plasma corticosterone levels were determined by the method developed by Abraham, Manlimos and Garza (1977).

Reagents

The following reagents were prepared:

(a) Assay Buffer: The assay buffer consisted of 0.1 M sodium phosphate, 0.9% sodium chloride and 0.1% sodium azide. One liter of deionized water was warmed to 50°C. Two g of gelatin and 2.0 g of sodium azide were added, stirred in and allowed to dissolve. Thereafter 17.4 g of sodium phosphate dibasic (MW 142), 10.8 g of sodium phosphate monobasic (MW 138) and 18.0 g of sodium chloride (MW 58) were added, the whole mixture swirled until complete dissolution. The solution was made to a total volume of two liters with deionized water and stored at 4°C until used.

(b) Charcoal Suspension: Fifty ml of assay buffer was placed in a 100 ml flask and 625 mg of Norit A and 62.5 mg of Dextran T-70 (Sigma) added. After stirring to dissolve, another 50 ml of assay buffer was added. The mixture was vigorously stirred for about 30 seconds after stoppering the flask. It was stored at 4°C until used. A volume of 0.2 ml was used to separate the free from the bound corticosterone later on.

(c) Corticosterone Working Standards: 40 ul of corticosterone stock solution in ethanol (Radioassay Systems Lab.) equivalent to 1 ul/ml

was used to prepare serially diluted standard solutions as follows, and used to construct a standard curve:

Solution A: 40 ul of stock in 10 ml assay buffer
(1 ml = 4.0 ng)

Solution B: 5 ml solution A + 5 ml assay buffer
(1 ml = 2.0 ng)

Solution C: 5 ml solution B + 5 ml assay buffer
(1 ml = 1.0 ng)

Solution D: 5 ml solution C + 5 ml assay buffer
(1 ml = 0.50 ng)

Solution E: 4 ml solution D + 6 ml assay buffer
(1 ml = .20 ng)

Solution F: 5 ml solution E + 5 ml assay buffer
(1 ml = .10 ng)

Solution G: 5 ml solution F + 5 ml assay buffer
(1 ml = .05 ng)

Solution H: 4 ml solution G + 6 ml assay buffer
(1 ml + .02 ng)

(d) Tritiated Corticosterone: tritiated corticosterone in a dose of 10 uCi/ml (Radioassay Systems Lab. purified) was used. One-tenth ml was withdrawn into a glass vial and evaporated. Ten ml of assay buffer was added and incubated for one hour at $37^{o}C$ (0.1 ml = approximately 10,000 counts/minute). A further 4 ml of this was diluted 1:10 and used for extraction and chromatographic purification of corticosterone from plasma.

(e) Antisteroid: 1.0 ml of anti-corticosterone (Radioassay Systems Lab, RSL) obtained at an initial dilution of 1:15 was further

diluted 1:100 upon receipt. One-tenth ml of this dilution was added to each assay tube for the radioimmunoassay step.

Blood Sample Collection and Preparation

(a) Blood Collection and Storage: blood samples were collected from 10-week-old male Rhode Island Reds at two hourly intervals over a 24-hour period, collection occurring only at odd numbered hours. Four ml of blood were drawn using a heparinized needle and syringe by cardiac venipuncture into NH_4 - heparinized sample tubes (Sarstedt). Red blood cells were removed by centrifugation at 200 x g for five minutes and the plasma samples were cooled in an ice bath before storage. Samples were stored at -20°C until used. Blood was taken from 96 chicks per treatment.

(b) Extraction and Purification of Plasma Samples: 1000 cpm of tritiated corticosterone (0.1 ml) was added to each 0.2 plasma sample as an internal standard. After letting it set for 30 minutes, 5.0 ml of dichloromethane (Mallinckrodt) was added and shaken for 30 seconds. Centrifugation was done at 500 x g for five minutes and the yellow supernatant aspirated. The dichloromethane was evaporated to dryness. One-tenth ml of mobile phase (20% ethyl acetate in iso-octane) was added to the dry residue and transferred to a celite column for chromatographic purification. It was eluted to the top under nitrogen gas. Three and one-half milliliters of mobile phase was pipetted onto the top of the column and again eluted under nitrogen. A third elution of 3.5 ml mobile phase was followed by collection into 20 ml glass scintillation vials and evaporated to dryness.

(c) Celite Column Chromatography: celite analytical filter aid from Johns-Manville was prepared prior to use in the columns by heating at 550°C for 16-18 hours in batches of 100 g. After letting it cool, each 20 g of celite was mixed thoroughly with 10 ml ethylene glycol which served as the stationary phase. A small, 3 mm diameter bead was placed in each 5 ml disposable Kimble glass pipette. About 0.2 g of celite was added to each pipette via a funnel, first loosely by free packing, then tight packing using a glass rod. This was repeated until the celite column reached the 3.5 ml mark on the pipette, giving a celite column of approximately 0.5 x 5.0 cm (diameter by height). The columns were eluted twice with 3.5 ml of 20% ethyl acetate in iso-osctane under nitrogen gas before use. Speed of elution or flow rate was maintained within a range of five to ten drops per minute. Each column was used only once.

Radioimmunoassay of Corticosterone

The assay step consisted of incubation, separation of free from bound hormone, counting of the bound portion and mathematical calculation of hormone concentrations.

(a) Incubation: the dry residue from the chromatographic step was dissolved in 2.5 ml assay buffer. A 0.5 ml fraction from this reconstituted volume was used for the extraction and chromatographic efficiency. For the actual assay of corticosterone 0.025 ml of reconstituted volume was drawn into 0.475 ml of assay buffer to make a total volume of 0.5 ml. To each tube, including standards and samples, was added 0.1 ml of anti-corticosterone and 0.1 ml tritiated corticosterone containing 10,000 cpm except for total tubes which received no antisteroid. The tubes were incubated at 4°C overnight.

(b) Separation of Free from Bound Hormone: a charcoal separation technique was employed for this step. After incubation the assay racks containing the tubes were placed in an ice bath on an electric stirrer and mixed for 30 minutes. Two-tenths ml of the charcoal suspension was added to each tube. The racks were shaken for 20 seconds to mix the assay mixture well. After letting them set for another 20 minutes in the ice bath, the tubes were centrifuged for 10 minutes at 1100 x g in a PR-J refrigerated centrifuge. The entire supernatant was then decanted into another set of glass scintillation vials containing 10 ml Scintisol (Biolab) counting fluid.

(c) Counting of Bound Portion: Counting was done in a Searle Analytic liquid scintillation counter for two minutes; each sample was counted in duplicate.

(d) Calculations: Duplicate counts were averaged and the blank value subtracted from them to give actual counts. The counts were then divided by the counts registered by total tubes and converted to percent bound. The percent bound values were plotted against corticosterone standards on semilog paper and the actual amounts of corticosterone in the various samples read off:

$$\text{Formula: } \left[\frac{\frac{(a+b)}{2} - \text{mean blank values}}{\text{mean total values} - \text{mean blank values}} \right] \times 100 = \text{Percent Bound}$$

where a + b = counts for each pair of duplicate tubes.

Hormone concentration in ng/ml = counts/duplicate x recovery factor.

Characterization of the Radioimmunoassay Technique

The following characteristics of the assay method were studied:

(a) Titer, defined as the dilution (or reciprocal thereof) of the antiserum at which 50% of the tritiated steroid hormone is bound. The antiserum was serially diluted from 1:100 to 1:100,000 with assay buffer. One-tenth ml of this diluted antiserum was added to 0.5 ml assay buffer in a 10 x 75 mm glass test tube in duplicate. One-tenth ml of the tritiated hormone was then added and mixed. This mixture was incubated overnight at $4^{o}C$ after which 0.2 ml charcoal suspension was added, mixed and incubated for a further 20 minutes. This was followed by centrifugation at 850 x g for 10 minutes. The supernatant containing the bound fraction was decanted into a glass counting vial, mixed with 10 ml counting fluid and allowed to equilibrate for 30 minutes. Counting was done for two minutes.

(b) Recovery Efficiency, defined as the amount of hormone measured by a combination of dichloromethane extraction and chromatographic purification expressed as a fraction or percent of the amount measured with dichloromethane extraction alone. Ten samples of plasma were randomly selected from each lighting treatment; 0.5 ml fractions in duplicate were either extracted with dichloromethane alone or followed by celite column chromatographic purification. The levels of corticosterone were determined as previously described.

(c) Sensitivity of the Standard Curve, defined as the dose of steroid corresponding to the mean cpm bound at zero dose minus two standard deviations of the mean cpm bound at zero dose. The antiserum concentration defined as the titer (above) was used to run a standard curve with doses of tritiated corticosterone ranging from 0.010 to

2 ng in duplicate. Incubation, separation and assay procedures were performed as already described. The counts per minute bound were then plotted against the dose of steroid on semilog paper, and the sensitivity calculated as defined.

CHAPTER VI

RESULTS

Figure 1 is the standard curve of corticosterone while Figure 2 is a presentation of the antiserum titer curve; the radioimmunoassay sensitivity curve is shown in Figure 3. Summaries of results are presented in Tables I to III and the analyses of variance (ANOVA) for the respective parameters in Appendix Tables IV, V, and VI. Figure 4 is a graphic representation of the relationship between photoperiod, time of day and plasma concentrations of corticosterone.

Corticosterone Standard Curve

This is illustrated in Figure 1.

Titer of Antiserum

A graphic illustration is shown in Figure 2. The amount of antiserum binding 50% of the tritiated corticosterone is equivalent to 1:15,000.

Sensitivity of Standard Curve

Figure 3 presents the sensitivity data. A sensitivity of 0.020 ng/ml corticosterone was determined in this trial.

Recovery

The recovery data (Table I) show that there was no difference in the efficiency of the extraction of corticosterone from plasma by using either dichloromethane alone or in conjunction with celite column chromatography for each treatment. However, the extraction efficiencies

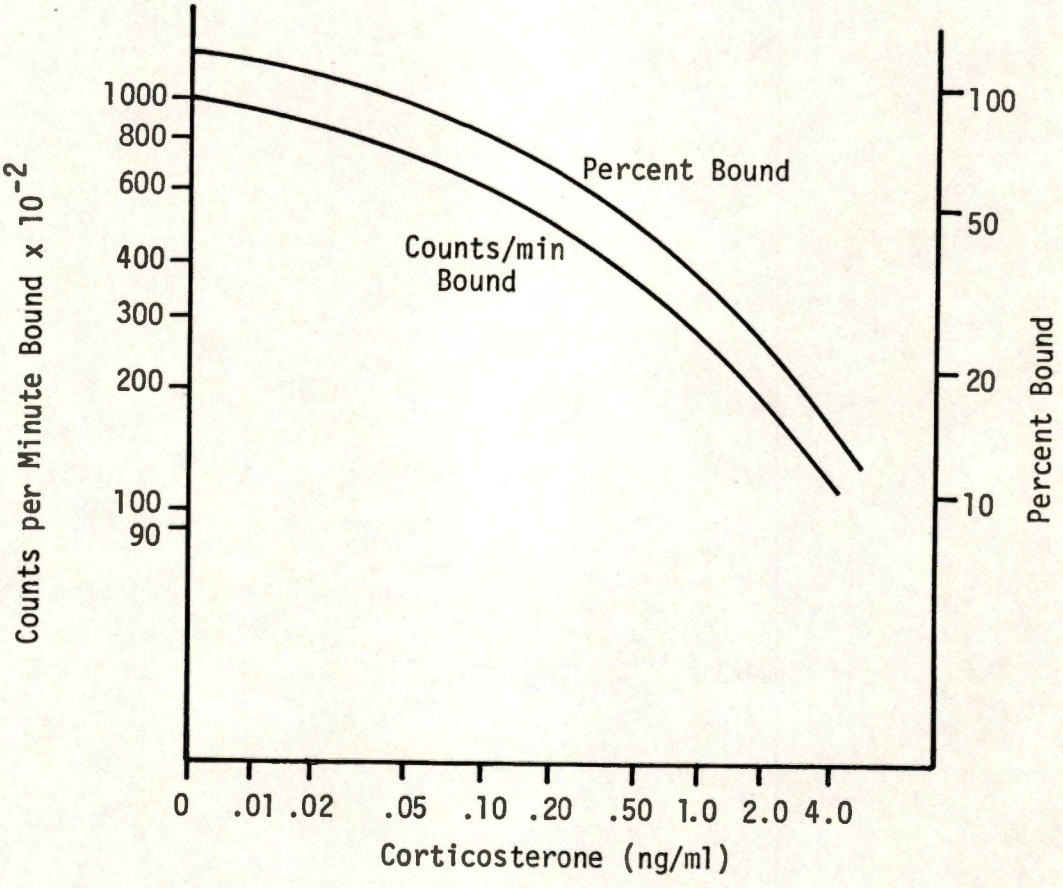


Figure 1. Corticosterone Standard Curve.

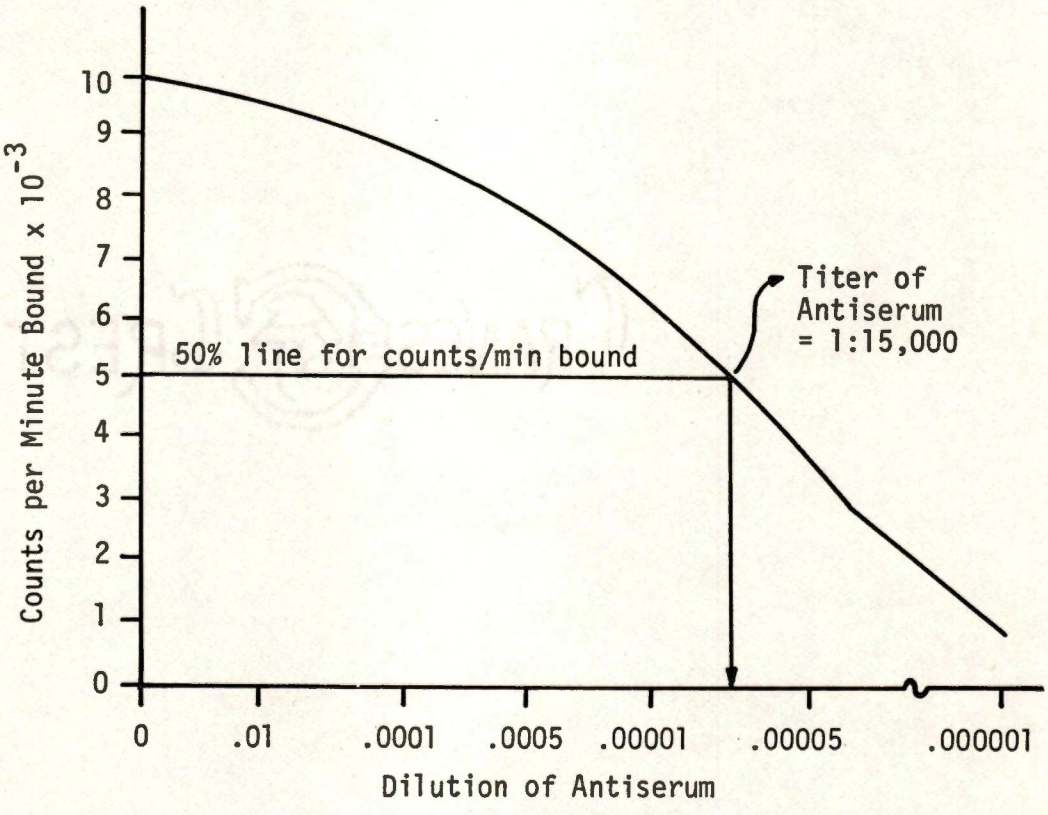


Figure 2. Antiserum Titer Curve.

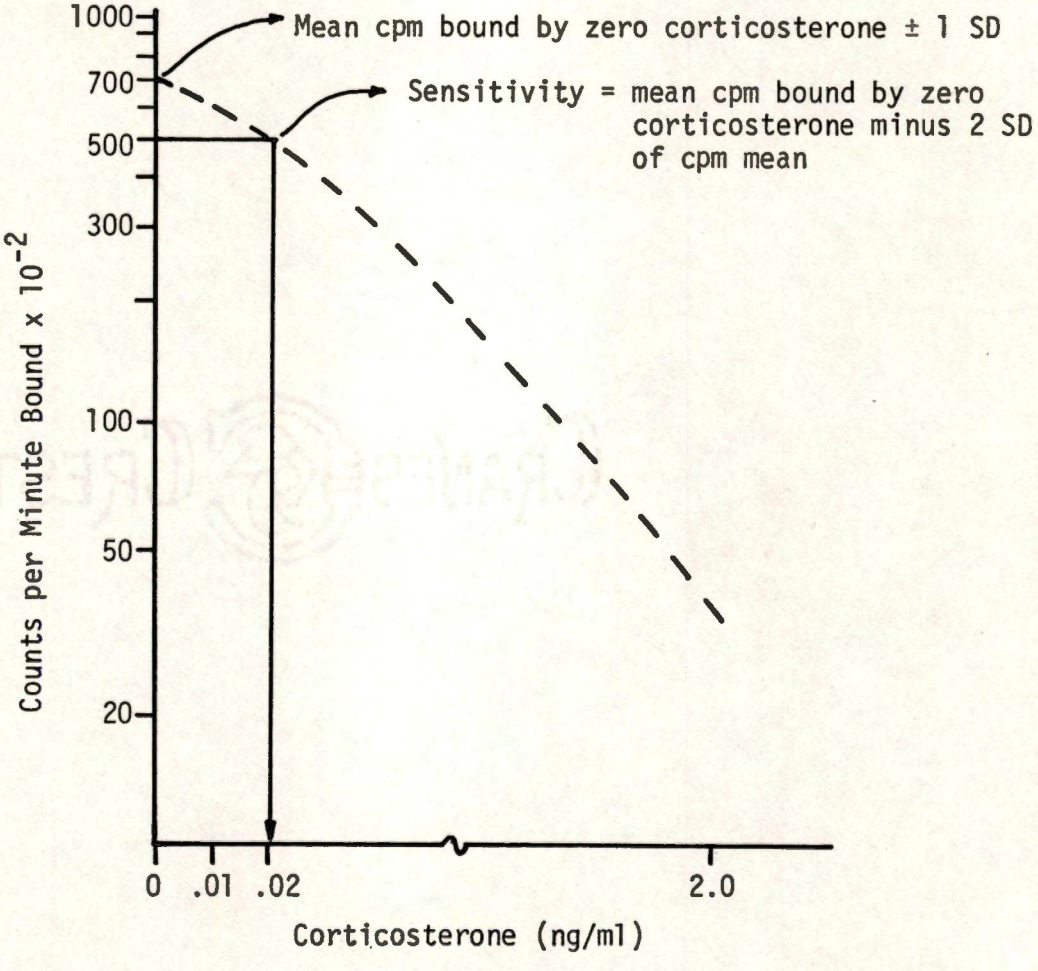


Figure 3. Radioimmunoassay Sensitivity Curve.

TABLE I

CORTICOSTERONE[1] RECOVERY AS INFLUENCED BY DICHLOROMETHANE EXTRACTION ALONE OR WITH CHROMATOGRAPHIC PURIFICATION

Photoperiod								
12L:12D			24L:0D			0L:24D		
DCMC[2]	DCMO[3]	%DCMC/DCMO	DCMC	DCMO	%DCMC/DCMO	DCMC	DCMO	%DCMC/DCMO
4.10	4.10	100.00	12.00	14.04	85.47	4.84	9.08	53.30
10.70	11.70	91.45	6.82	8.78	77.68	16.03	11.19	143.25
10.70	11.70	91.45	7.80	8.78	88.84	9.68	9.98	96.99
7.95	9.95	79.90	11.30	11.41	99.10	26.03	30.25	86.05
9.95	9.95	100.00	12.40	16.38	75.70	10.50	11.19	93.83
13.60	14.60	93.15	6.00	6.00	100.00	15.75	16.64	94.65
11.00	14.63	95.69	6.70	8.78	76.31	6.05	11.19	54.07
12.33	11.70	105.38	5.80	5.85	99.15	7.87	8.17	96.32
8.75	8.73	99.66	12.80	15.80	81.01	16.05	16.64	96.45
7.00	7.02	99.72	4.10	4.10	100.00	8.75	12.10	72.31
Means:								
9.91	10.42	95.64	8.57	9.99	85.78	12.16	13.64	89.14
Correlation Coefficients, DCMC/DCMO								
	0.82			0.94			0.95	

[1]Corticosterone is in ng/ml.

[2]DCMC stands for Dichloromethane Extraction followed by Chromatographic separation.

[3]DCMO stands for Dichloromethane Extraction only.

TABLE II

PHOTOPERIODIC EFFECTS ON ANATOMICAL AND PHYSIOLOGICAL PARAMETERS OF RHODE ISLAND RED MALES

	Photoperiod		
Structures	12L:12D	24L:0D	0L:24D
Mean body weight, g[1]	933.50±25.20[a2]	968.00±32.40[a]	924.00±23.20[a]
Mean eye weight, g	2.08± 0.04[a]	2.37± 0.90[b]	2.85± 0.10[c]
Mean eye depth, mm	3.00± 0[a]	0.80± 0.28[b]	0.30± 0.14[b]
Mean IOP, mmHg	23.87± 4.38[a]	21.48± 5.07[a]	22.66± 3.92[a]
Mean thyroid weight, mg	37.15± 2.24[a]	34.38± 1.98[a]	34.78± 0.93[a]
Mean testis weight, mg	203.20±44.70[a]	432.20±18.36[b]	124.40±10.30[c]
Mean comb weight, g	1.98± 0.23[a]	2.31± 0.28[a]	1.85± 0.16[a]
Mean adrenal weight, mg	37.42± 2.74[a]	45.66± 3.58[b]	43.49± 5.04[b]
Number of birds in mean	(10)	(10)	(10)

[1]Figures are means ± standard error.

[2]Figures in a row having different superscripts are significantly different ($P > 0.05$).

TABLE III

EFFECTS OF PHOTOPERIOD AND TIME ON CORTICOSTERONE LEVELS IN CHICKEN PLASMA

	Plasma corticosterone in ng/ml		
Time	12L:12D	24L:0D	0L:24D
9 a.m.	$3.86 \pm 1.40^{a1*}$	$7.31 \pm 0.66^{ab1**}$	$7.79 \pm 1.18^{ab1**}$
11 a.m.	$5.66 \pm 1.28^{abcde*}$	$7.82 \pm 0.99^{ab**}$	$8.58 \pm 1.66^{abc**}$
1 p.m.	$10.10 \pm 1.56^{e*}$	$7.35 \pm 0.24^{ab**}$	$12.93 \pm 2.57^{cde**}$
3 p.m.	$9.11 \pm 0.75^{cde*}$	$5.71 \pm 0.79^{ab**}$	$6.58 \pm 1.67^{ab**}$
5 p.m.	$4.80 \pm 0.65^{abc*}$	$7.02 \pm 0.84^{ab**}$	$7.33 \pm 1.30^{ab**}$
7 p.m.	$5.55 \pm 1.88^{abcde*}$	$6.14 \pm 1.29^{ab*}$	$7.14 \pm 0.78^{ab**}$
9 p.m.	$4.90 \pm 0.65^{abc*}$	$7.82 \pm 0.64^{ab**}$	$7.56 \pm 1.15^{ab**}$
11 p.m.	$6.89 \pm 0.80^{abcde*}$	$9.43 \pm 1.34^{abc**}$	$13.68 \pm 1.15^{de***}$
1 a.m.	$7.32 \pm 1.20^{abcde*}$	$8.01 \pm 1.65^{abc*}$	$15.99 \pm 2.30^{e**}$
3 a.m.	$8.78 \pm 0.66^{bcde*}$	$12.17 \pm 1.80^{c***}$	$10.77 \pm 2.43^{abcd**}$
5 a.m.	$9.44 \pm 1.16^{de**}$	$8.04 \pm 0.54^{abc*}$	$13.91 \pm 1.60^{de***}$
7 a.m.	$8.31 \pm 1.50^{abcde*}$	$9.50 \pm 0.63^{abc*}$	$9.79 \pm 0.78^{abcd*}$
No. of birds in mean	8	8	8

Overall means	8.50^{a2}	96	9.70^{b2}	96	12.20^{c2}	96
Daytime means	6.50^{a}	48	6.90^{a}	48	8.40^{b}	48
Night means	7.60^{a}	48	9.20^{b}	48	12.00^{c}	48

[1]Figures in a column having different letter superscripts are significantly different ($P > 0.05$).

[2]Figures (means) in a row having different superscripts are significantly different ($P > 0.05$)--refers only to figures in bottom table.

*Figures in a row having different numbers of asterisks are significantly different ($P > 0.05$)

Note: Number of chicks shown in brackets.

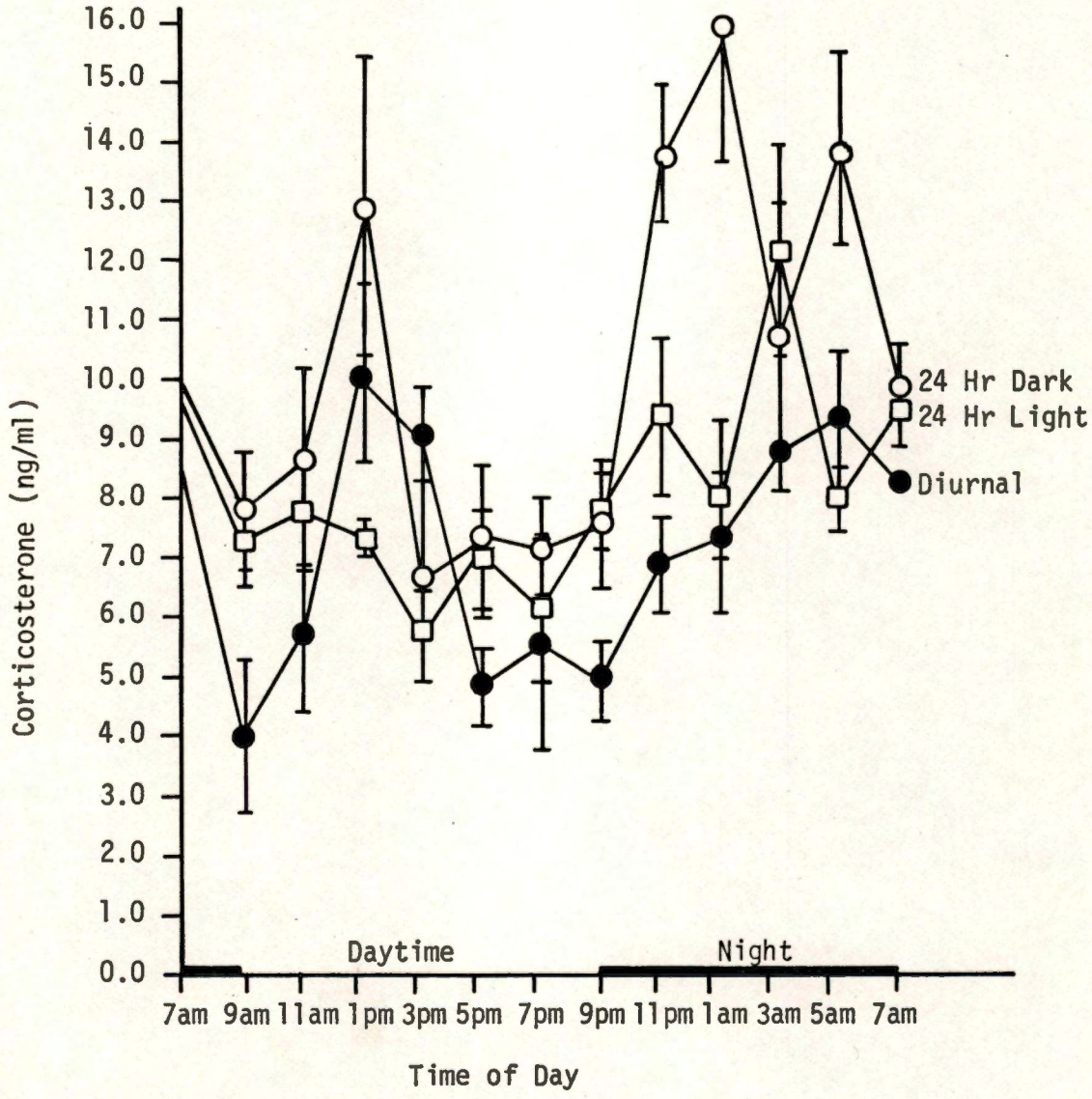


Figure 4. Effects of Photoperiod and Time of Day on Corticosterone Levels in Chicken Plasma.

were different for the various lighting treatments: 95.64% for the 12L:12D treatment, 85.78% for the 24L:0D and 89.14% 0L:24D. Respective correlation coefficients are 0.82, 0.94 and 0.95.

Body Weight

Mean body weights are presented in Table II with the corresponding analysis of variance (ANOVA) in Appendix Table V. No statistically significant differences in body weights were found although birds on continuous light had the highest mean weights (968.0 ± 32.4 g) as against 933.5 ± 25.2 and 924 ± 23.2 g for chicks raised under diurnal light/dark and total darkness, respectively.

Eye Weight

Eye weights are shown in Table II. A highly significant ($P > 0.01$) increase in eye weight resulted from maintaining cockerels under complete darkness for 10 weeks (2.85 ± 0.10 g/eye). This is in comparison with the eye weight of chicks undergoing the diurnal 12L:12D treatment (2.08 ± 0.04 g/eye). Chicks under the 24-hour light treatment had eyes weighing an average of 2.37 ± 0.09 g, and this was significantly higher than those on 12-hour light, but lower than those under continuous darkness. In addition, eyes of birds under either continuous light or darkness tended to protrude out of their socket relative to the eyes of the control (12L:12D) birds.

Eye Depth

As in the case of eye weights (Table II) there was a highly significant effect of photoperiod on eye depths ($P > 0.01$). A very drastic reduction in eye depth was observed in chicks kept in either

total darkness or total light (0.30 mm and 0.80 mm average, respectively). These had a flattened appearance and the lenses appeared to be stretched along the long axis. The eye depths of birds reared under the alternating light/dark schedule averaged 3.0 mm (Table II) and were well rounded and the eyes had a bright appearance.

Intraocular Pressure (IOP)

IOPs are presented in Table II. No statistically significant differences in IOP were found as the pressures were plus or minus only one unit from each other. In fact, all the pressures were within the range of normal IOPs reported for chickens.

Thyroid Weight

Both total light and total dark apparently reduced thyroid weight; however, statistical analysis showed no significant differences (Table II). Birds under continuous light yielded the lowest mean thyroid weights of 34.38 mg each, while birds subjected to 24-hour dark had mean weights of 34.78 mg and those on 12L:12D had thyroids of mean weight of 37.15 mg.

Testis Weight

Cockerels kept under continuous light had testes of maximal weight, 432.20 mg average, and this was significantly different ($P > 0.01$) and twice as large as testes from 12L:12D cockerels, 203.20 mg, and at least thrice as large as testes from cockerels under continuous darkness, as presented in Table I.

Comb Weight

The highest individual comb weight was recorded in chickens reared to 10 weeks under 24 hours of light (4.05 g). Overall, however, there was no significant difference in mean comb weights among treatments, although continuous darkness tended to decrease comb weight (mean weight of 1.85 g) in comparison with 2.31 g for combs from birds on 24-hour light or 1.98 g for combs from the 12L:12D regimen. Combs from the 24-hour light and 12L:12D treatments were more supple and had a bright red appearance.

Adrenal Weight

Birds under the diurnal light/dark treatment had significantly lower adrenal weight (mean of 37.42 mg) than those of the other treatments ($P < 0.05$). The adrenal glands from chicks under 24 hours of light weighed an average of 45.66 mg and this was not different from the 43.49 mg registered by chicks from the continuous darkness treatment (Table II).

Corticosterone Levels

Table III presents data on corticosterone levels of avian plasma. Figure 4 represents the relationship between photoperiod, time of day and plasma corticosterone content. A significant effect due to lighting treatment and time of day is clearly indicated ($P > 0.05$).

Within treatments, chicks reared under a diurnal light/dark regimen had the lowest corticosterone levels at 9:00 a.m. and this was significantly lower than that recorded at 1:00 p.m., the highest level recorded under 12L:12D treatment (3.86 vs 10.10 mg/ml). Two clear peaks of corticosterone levels are observed at 1:00 p.m. and

5:00 a.m. The mean of the daytime corticosterone levels was lower than the night mean, $P < 0.05$ (6.50 vs 7.60 ng/ml). Chicks kept under 24-hour light exhibited three corticosterone peaks. The first peak occurred between 9:00 a.m. and 1:00 p.m., the second between 5:00 p.m. and 9:00 p.m., and the third peak at 3:00 a.m. The night mean peak corticosterone level of 9.16 and 12.17 ng/ml respectively were significantly higher than the daytime mean and first corticosterone peak (6.89 and 7.50 ng/ml, respectively). As in the 24-hour light treatment, birds experiencing total darkness also exhibited three peaks of corticosterone (12.9 ng at 1:00 p.m., 15.99 ng at 1:00 a.m., and 13.91 ng/ml at 5:00 a.m.). These peaks were not different from each other ($P > 0.05$). The night mean (12.00 ng/ml) was also significantly higher than the daytime mean of 8.40 ng/ml ($P > 0.05$).

Between treatment comparisons indicated that keeping birds under totally dark environments significantly ($P > 0.05$) increased plasma corticosterone levels (12.20 ng/ml) over birds kept in a continuous light environment (9.70 ng/ml) or those subjected to a condition of 12L:12D (8.50 ng/ml). The two latter values are different from each other. A similar situation exists with the nighttime means; the values for daytime show a little different picture as the 12L:12D and 24-hour light means are not significantly different from each other, but are significantly lower than the 24-hour dark mean (6.50, 6.90 and 8.40 ng/ml, respectively, $P < 0.05$).

CHAPTER VII

DISCUSSION

Sensitivity of Standard Curve

A high sensitivity of 0.020 ng/ml corticosterone indicates that the assay procedure is accurate enough for the range of values encountered in this trial.

Recovery

Recovery of corticosterone varied from a low of about 86% to a high of approximately 96% which is considered very good (Abraham et al., 1977). Recoveries were lower for plasma samples from the continuous light and continuous dark treatments. It is not clear why, but these samples looked more fatty and the fat may have interfered with the ease of extraction of cortical steroid hormones. While Abraham et al. (1977) recommend a prior chromatographic purification of plasma samples, the results of this trial indicate that there is no significant difference between corticosterone levels determined after dichloromethane extraction alone and those determined after a purification step.

Body Weight

Body weight has probably been the most perplexing aspect of experiments of this nature (Voitle, 1969; Buckland and Hill, 1970; Osei, 1978). In one study, one lighting treatment favors weight gains while in another a different regimen stimulates growth. While no significant differences in body weight were shown in this work, birds

on 24-hour light treatment tended to have the heaviest body weights in agreement with the earlier report of Siegel et al. (1961). Conversely, these results are in contrast with the results obtained by either Voitle in 1969 or Osei in 1978. Jenkins et al. (1979) reported a lethargic behavior in birds kept under extremes of lighting. These results cannot be interpreted in terms of lethargy since the 24-hour dark birds were more lethargic and would, therefore, be expected to weigh the heaviest. It is, however, possible that birds on continuous light spent a lot more time feeding and that might explain the weight gain. It is also possible that birds under 24-hour darkness may have fed less due to vision difficulties in the absence of light. Strain differences may also be a contributory factor to the differences in results reported. In addition, the sample size (10 birds from each treatment group) may have been too small to clearly distinguish small differences in weight.

Eye Weight

Eye weights were maximal under 24-hour darkness (2.85 g each) followed by those under 24-hour light (2.37 g) and the lowest were from chicks under the diurnal light/dark treatment, and these differences were statistically significant. The difference between the eye weights of birds under 12L:12D and those on 24-hour darkness mounted to 37.0 percent. This observation is similar to that reported by Lauber et al. (1965) and Voitle (1969) among others. This increase in eye weight is one of the cardinal features of the avian glaucomatous condition (Kinnear et al., 1974). Eyeball dry matter was not determined in this trial, but Jensen and Matson (1957) demonstrated that this

ocular hypertrophy was due primarily to an enhanced absorption and accumulation of fluid.

Eye Depth

Eye depths of chicks under either continuous light or continuous dark were reduced four and ten times respectively relative to birds that received a normal light/dark treatment (Table II, Page 29). The lenses, on close examination, were shown to be flattened and pushed against the cornea and the anterior chamber was more or less obliterated. This seems to be due to a dimensional change due to a stretching of the longitudinal axis of the lens while the short diameter seemed to be shortened. An accumulation of fluid in the eyeballs could conceivably cause a stretching of the suspensory ligaments which, in turn, will stretch the lens longitudinally.

Intraocular Pressure

The mean intraocular pressures measured in this experiment were not significantly different from each other, and all the readings, in fact, fell within the normal range of avian IOPs (15-25 mmHg). Lauber et al. (1970), Kinnear et al. (1974) and Axmith and Morin (1975) concluded from their separate works that the IOP did not begin to increase until about the 15th week in trials like this. This work was conducted for only 10 weeks. This seems a realistic explanation and an easy one at that until other work is compared. In particular, Smith et al. (1969) and Jenkins et al. (1979) have reported increased IOPs as early as four to six weeks. It is even more complicated in the latter case of Jenkins and colleagues, where the IOP began to fall from the sixth week onward until it became subnormal after 16 weeks. Did the pressures

in this current work rise and fall later (Jenkins et al., 1979) or was 10 weeks too short for the IOPs to be elevated (Axmith and Morin, 1975; and others)? A weekly determination of IOPs in any such work will help to answer this question. It is probable that inherent genetic strain differences may explain part of the problem (Armaly, 1967). Future studies should also include longer time intervals.

A possible explanation for the apparently triphasic IOP curve is that initially the pressure tends to rise; however, the rapid growth of the bird, including the eyeballs, will tend to reduce pressure. Later, as growth declines, the increase in eyeball size is not sufficient to counterbalance the elevation in pressure induced by the physiological defects resulting from abnormal photoperiods (Dr. H. V. Shirley, personal communication).

Thyroid Weight

Thyroid weights were not significantly affected by the lighting schedules in this trial, although they were reduced under either total dark or total light. While the former was expected (Harrison et al., 1968; DeProspo et al., 1969), the latter was surprising in light of earlier findings that light enhanced not only thyroid growth, but also the production of thyroid hormones and the uptake of radioactive iodine (DeProspo et al., 1969).

Testis Weight

Continuous 24-hour light greatly increased testicular weight in cockerels. Testes of chicks on this treatment weighed at least twice as much as those on 12L:12D regimen which weighed the next heaviest. The stimulatory effect of long photoperiods on reproductive function of

male birds has been known for a long time (Rowan, 1926; Radnot, 1961; Oishi and Lauber, 1973). This photoperiodic effect on testicular growth and function is mediated through the production and release of sex hormones luteinizing hormone and follicle-stimulating hormone (Follett et al., 1975).

Comb Weight

The lack of a significant effect of lighting treatment on comb weight was unexpected in view of an earlier report by Osei (1978) and the recognized stimulatory influence of light on sex organs. The combs of Rhode Island Red cockerels do not grow as fast as those of White Leghorns, and since only 10 birds per treatment were used, the sample size may have been too small to be statistically valuable in detecting small, otherwise significant differences.

Adrenal Weight

Adrenal weights were significantly increased by total light or total dark over those under 12L:12D. This adrenal hypertrophy is a consequence of a generalized metabolic stress caused by abnormal lighting conditions (Jenkins et al., 1979). According to Selye (1951) the General Adaptation Syndrome or GAS is the sum total of all the non-specific responses to a systemic stressor. In the GAS, the stressor stimulates the anterior pituitary gland to release increased quantitites of ACTH which then induces an adrenal hypertrophy and increased output of corticoids.

Corticosterone Levels

A significant effect of both lighting and time of day on corticosterone levels is clearly evident from the results (Table III, Page 30). These results were not unexpected, and reflect both a circadian rhythm and a stress response (Meier and Fivizanni, 1975; Jenkins et al., 1979). This circadian influence is shown by higher levels of the hormone during the night hours and lower levels during daylight hours. Whether the three peaks seen under either total dark or total light in contrast with the two found under diurnal light/dark has any significant meaning is difficult to tell in view of the fact that the normal circadian periodicity shows an episodic characteristic of peaks and lows throughout the day; however, the majority of the corticosterone peaks occurred in the night period in agreement with earlier reports by Krieger et al. (1971). These daily rhythms of corticosterone are entrained by the daily photoperiod through a delicate relationship between the adenohypophysis, the hypothalamus and the adrenal cortex (Sato and George, 1973).

That an abnormal lighting situation can trigger a hypersecretion of adrenal corticosteroid has been known for some time (Fatranska, 1971). Under Seyle's (1951) General Adaptation Syndrome, stress situations induce a hyperactivity of adrenal glands under a preceding stimulation by hypothalamic CRH and pituitary ACTH. Indeed, under stress the corticoid levels in chicken plasma have been reported to show up to a fourfold increase (Buckland et al., 1976).

CHAPTER VIII

GENERAL DISCUSSION

Figures 5 and 6 represent an attempt to synthesize the relevant literature and thereby put forward a hypothesis to explain the genesis of avian glaucoma. Figure 5 is a modified form of an earlier hypothesis put forward by Osei in 1978, while Figure 6 is adapted from the work of Anderson (1975). Osei in 1978 theorized that abnormal photoperiods elicited a stress situation that stimulated adrenal activity, including adrenal hypertrophy and increased synthesis and release of adrenal hormones, particularly corticosterone, the main glucocorticoid of avian species (Assenmacher, 1973). This photoperiodic effect is exerted by way of the eyes and/or the pineal gland (and in some cases, as in lower vertebrate forms, directly by way of light penetrating the skull) to the hypothalamo-pituitary axis as in Figure 5. A sequential release of hypothalamic corticotropin releasing hormone or CRH and pituitary adrenocorticotrophic hormone, ACTH, will in turn cause an adrenal hypersecretion of hormones. While the corticoid influence on the eyes was suggested in 1978, the exact sequence of events was not fully understood, and indeed, are still not clearly known. However, the separate works of Lieb and Stark (1966), Anderson (1975) and Francois (1975) have clarified some of the events that culminate in the glaucomatous condition.

A cardinal point to remember is that elevated intraocular pressure (IOP) is a primary manifestation of the glaucomatous state, whether in humans or in chickens as reported by Smith, Becker and

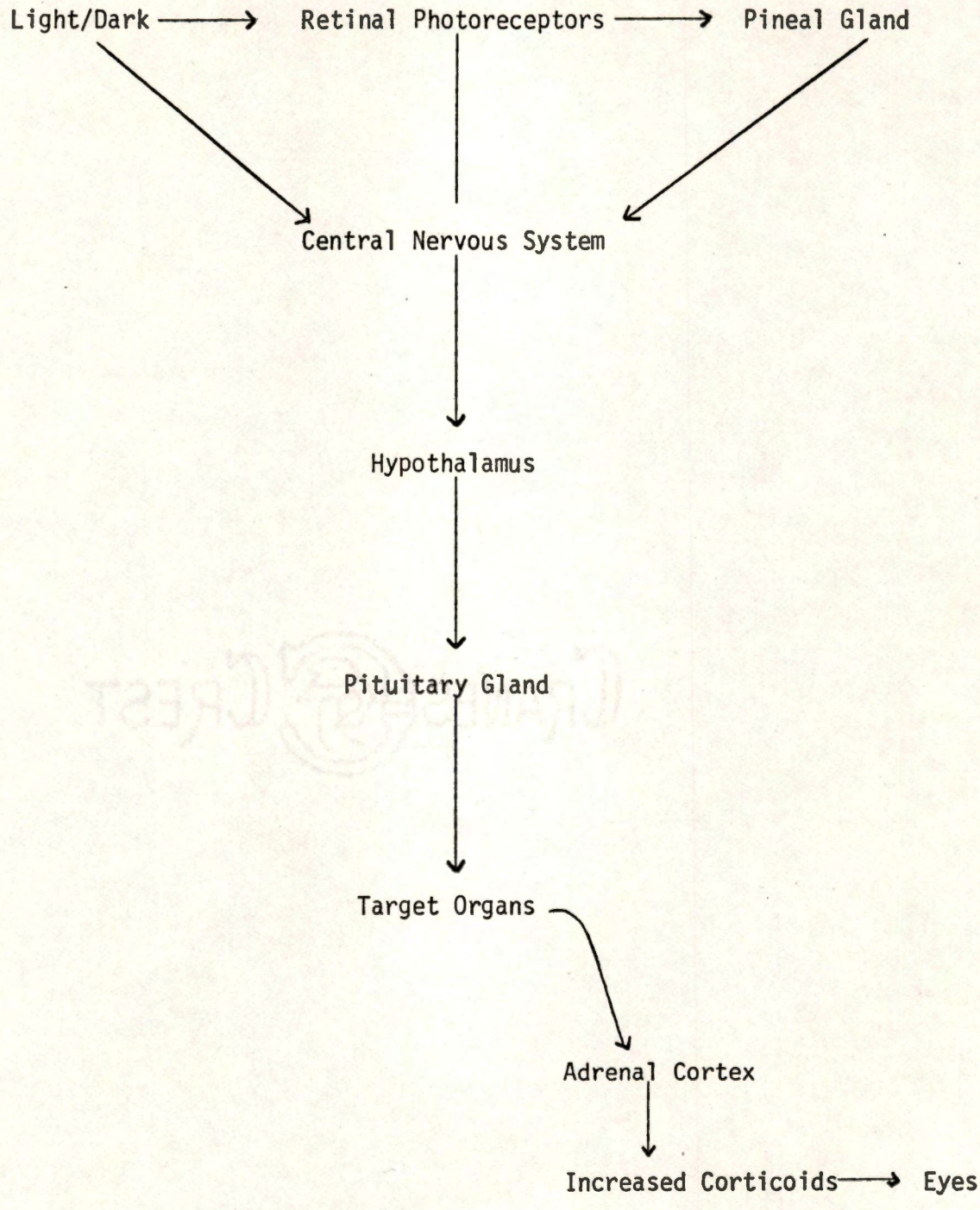


Figure 5. Involvement of photoperiod and adrenal cortex in avian glaucoma. (After Osei, 1978).

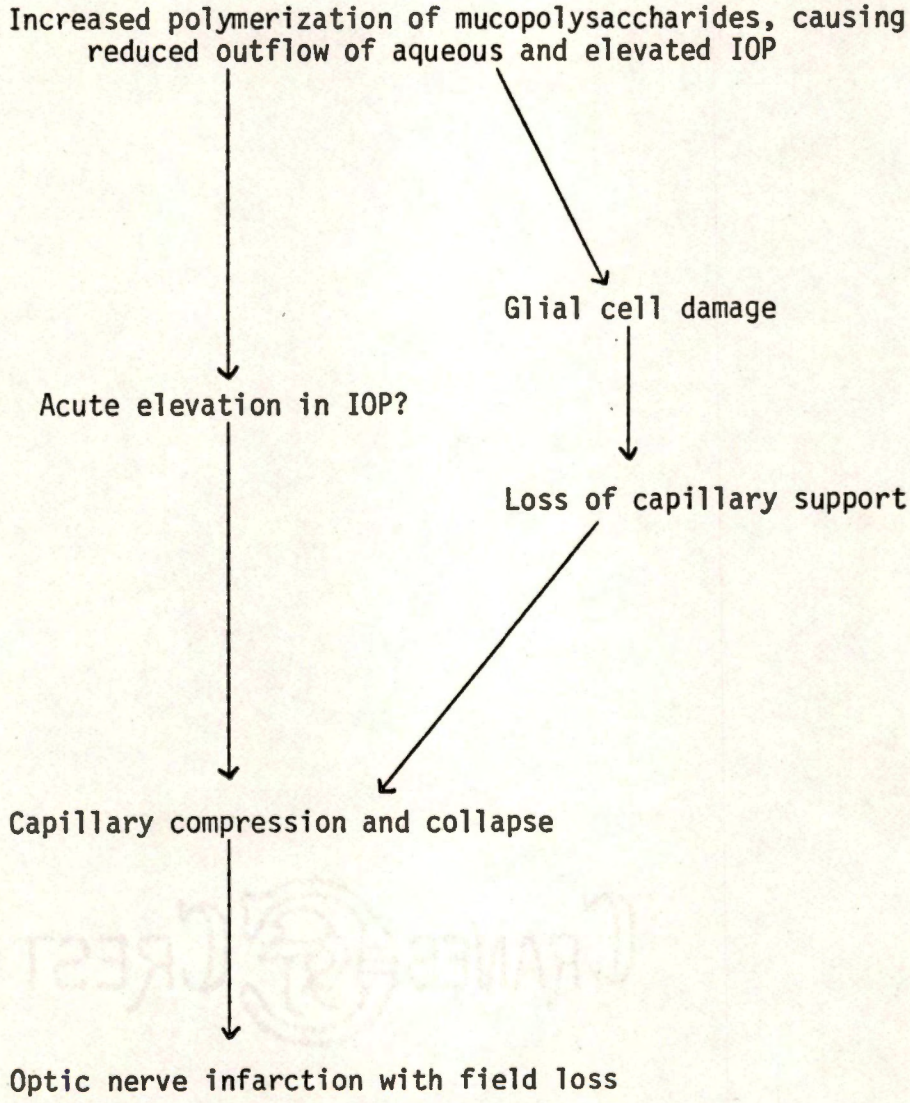


Figure 6. Elevated IOP, glial cell damage and the development of glaucoma (After Anderson, 1975).

Podos (1969) on the one hand and Anderson (1975) on the other. The eye is filled with intraocular fluid of sufficient pressure in the eyeball to maintain ocular distension. This IOP is maintained by a balance between the formation of aqueous humor by the ciliary body of the eye and the outflow of the same through the trabecular meshwork in the eye's anterior chamber (Starka and Obenberger, 1976). The regulation of the aqueous humor outflow is mediated by a steady decomposition or depolymerization of mucopolysaccharides localized in this trabecular meshwork, in the canal of Schlemm and in the walls of the aqueous veins according to Francois (1975). These mucopolysaccharides are highly sensitive to hyaluronidase and are thus probably mainly hyaluronic acid. Polymerization of these mucopolysaccharides has the effect of enhancing their hydration capacity, a situation that induces a narrowing of trabecular meshes and thus increasing the resistance to aqueous outflow. On the other hand, physiological homeostasis is achieved by a constant depolymerization of these macromolecules by the catabolic action of lysosomal enzymes. That corticosteroid hormones can and do induce an elevation of IOP is a well-known fact (Ballintine, 1966; Starka and Obenberger, 1976). However this response of IOP to corticosteroids is an inherited autosomal recessive trait polygenetically determined by the allele pairs P^L, P^H (Armaly, 1967). The mode of action of corticoids may be by their action on lysosomal membranes. Glucocorticoids stabilize these membranes and prevent the release of enzymes and thus keep the mucopolysaccharides in a constant state of polymerization as reported by Lieb and Stark in 1966. Polymerization of the mucopolysaccharides causes a reduction in the outflow of aqueous humor and subsequently a rise in intraocular pressure.

Nevertheless, in vitro studies have shown that very high levels of glucocorticoids are needed to keep the lysosomal membranes stabilized (Starka and Obenberger, 1976) that other possibilities are being explored. In particular, the work of Ballintine (1966) suggested that increased IOP may be caused by an underlying increased uptake of sodium, while Voitle in 1969 showed that the administration of corticoids can increase the levels of sodium in the eye fluid. The possibility of an impairment in glucose metabolism (induced by high levels of corticoid hormones) has also been suggested by Fox et al. (1971) and Varma and Kinoshita (1974). A high level of glucose may stimulate the aldose reductase or polyol pathway of metabolism, and this results in the accumulation of the sugar alcohol sorbitol. Sorbitol is not readily metabolized and, in addition, cannot leak out of cells once it has entered. In the lens sorbitol causes an osmotic swelling of cells (Varma and Kinoshita, 1974). The possibility that all these factors, and in fact other unknown factors, may work together to increase IOP needs further study.

Whatever the cause of elevated IOP, however, a chronically high level of pressure gradually results in a loss of glial cells according to Anderson (1975), perhaps by simple physical distension and rupture of intracellular fibrils and tonofilamints. Glial cell damage causes a subsequent loss of capillary support (Figure 6), capillary compression and closure leading to ischemia. Nutrition of the retina is diminished and this often results in a permanent atrophy of the retina and optic nerve, finally ending in blindness.

CHAPTER IX

SUMMARY AND CONCLUSIONS

An experiment was carried out to investigate possible relationships between the daily photoperiod, adrenal function and the onset of avian glaucoma as well as other physiological functions of domestic chickens.

The body weights of chickens were not significantly affected by the light treatments up to 10 weeks of age, although cockerels under continuous light tended to weigh the heaviest.

Both total darkness and total light significantly improved the size and weight of eyes and adrenal glands at the 5% level of statistical significance.

Eye depth was severely reduced when birds were kept under either continuous darkness or complete light in comparison with a diurnal light/dark cycle.

Twenty-four-hour lighting significantly stimulated testicular growth while depressing thyroid weights; the latter was also true of the 24-hour dark treatment.

Neither intraocular pressure nor comb weight were significantly influenced by the various lighting regimens.

While corticosterone levels in chicken plasma were increased by 24-hour lighting and 24-hour darkness, several peaks of corticosterone were present in all treatments and the circadian rhythm of the hormone was not disrupted.

An attempt has been made to explain photoperiodically induced avian glaucoma in terms of a generalized stress situation; a resulting increase in adrenal hormones may precipitate the lesions associated with the pathology.

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APPENDIX

TABLE IV

ANALYSIS OF VARIANCE FOR CORTICOSTERONE LEVELS IN PLASMA

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatment	35	2046.13	58.46	4.27*
Time (T)	11	892.00	81.09	5.92*
Light (L)	2	509.68	254.84	18.62*
L x T	22	644.45	29.29	2.14
Error	252	3448.50	13.68	
Total	287	5494.63		

*Significant at the 5% level of probability.

TABLE V

ANALYSIS OF VARIANCE FOR ANATOMICAL AND PHYSIOLOGICAL PARAMETERS OF CHICKENS

Function	Source	Degrees of Freedom	Sum of Squares	Mean Squares	F
Body Weight	Treatment	2	10.722.00	5361.00	0.65
	Error	27	222,303.00	8233.00	
	Total	29	233,025.00		
Eye Weight	Treatment	2	3.00	1.50	20.0*
	Error	27	2.03	0.075	
	Total	29	5.03		
Eye Depth	Treatment	2	41.27	20.64	57.48*
	Error	27	9.70	0.34	
	Total	29	50.97		
IOP	Treatment	2	57.36	28.68	0.64
	Error	27	1,205.63	44.65	
	Total	29	1,262.99		
Thyroid Weight	Treatment	2	57.36	28.68	0.26
	Error	27	2,279.86	44.65	
	Total	29	1,262.99		
Comb Weight	Treatment	2	2.44	1.22	16.66*
	Error	27	1.97	0.07	
	Total	29	4.41		
Testis Weight	Treatment	2	1.22	0.61	1.28
	Error	27	12.85	0.48	
	Total	29	14.07		
Adrenal Weight	Treatment	2	4,110.37	2050.69	80.64**
	Error	27	686.61	25.43	
	Total	29	4,796.98		

*Significant at P > 0.05.

**Significant at P > 0.01.

TABLE VI

ANALYSIS OF VARIANCE FOR RECOVERY DATA (TABLE III)

Photoperiod	Source	Degrees of Freedom	Sum of Squares	Mean Squares	F
12L:12D	Treatment	1	1.29	1.29	0.129
	Error	18	179.84	9.99	
	Total	19	181.13		
24L:0D	Treatment	1	10.08	10.08	0.167
	Error	18	1086.20	60.35	
	Total	19	1096.36		
0L:24D	Treatment	1	10.99	10.99	0.266
	Error	18	744.45	41.36	
	Total	19	755.44		

VITA

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He is married to Ama Osei of Accra, Ghana, and they have a son, Kwame Osei.