A STUDY OF THE RELATIONSHIP BETWEEN THE PINEAL GLAND AND PHOTOPERIODISM

THESIS

Presented to the Graduate Council of

Southwest Texas State College

in Partial Fulfillment of

the Requirements

For the Degree of

MASTER OF ARTS

By

James G. Benskin, B.S.
(San Marcos, Texas)

San Marcos, Texas

August, 1968

ACKNOWLEDGMENT

The author wishes to extend his appreciation to Dr. Don Green for his technical guidance and inspiration during the duration of this research project and in the preparation of this thesis. To Dr. David Whitenberg and Dr. Charles Willms a special thanks is extended for their professional review in the preparation of this paper.

Acknowledgements are also extended to fellow graduate student and friend George Barnes for his assistance in the operations performed on the quail.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Previous research has demonstrated that there is a relationship between daily photoperiods and annual reproductive cycles in animals, and, in recent years, a carry-over period has been described in bird photoperiodism.[1] Recently, physiological and biochemical studies have been conducted on the functions of the pineal gland, and there have been some attempts made to relate the pineal to photoperiodic phenomena.[2] The objective of this study is to determine whether the pineal gland is the mediator of the carry-over period noted in birds and, if so, the optimum interruption time.

Basis of this Study

This study is based in part on D. S. Farner's[3] work in which he found that a given total duration of light becomes more effective in the stimulation of testicular development if it is broken into shorter periods. A correlation with Axelrod and Wurtman's[4] work on the pineal gland was attempted. Axelrod and Wurtman's studies contend that the pineal produces a substance (melatonin) which under diurnal photoperiods stimulates gonadal development in Aves.

---

[1]Farner, D. S., "Photoperiodic Control of Annual Gonadal Cycles in Birds," pp. 717-750.

[2]Wurtman, R. J., J. Axelrod, L. Phillips, "Melatonin Synthesis in the Pineal Gland: Control by Light," pp. 1071-72.

[3]_____, "Photoperiodism or Effect of Interrupted Light in Photoperiodically Induced Testicular Development," p. 725.

[4]_____, "Melatonin Synthesis in the Pineal Gland: Control by Light," pp. 1071-72.

This problem was planned in an attempt to isolate the effect of various light and dark sequences on the pineal to determine if the pineal could be a mediator in bird photoperiodism. Pen-raised bobwhite quail (Colinus virginianus texanus) were used in the experiment. The birds were divided into three groups: Pinealectomized birds (birds from which the pineal had been removed), sham-operated birds (birds on which a simulated operation had been performed without removal of the pineal), and normal birds. The sham-operated and normal birds were used as controls to compare with the pinealectomized birds.

Three groups of birds were placed in five light boxes set up to sustain photoperiods consisting of light and dark cycles in ratios of 10L2D1L11D, 10L3D1L10D, 10L4D1L9D, 10L5D1L8D, and 10L6D1L7D respectively as illustrated in Figure 1. After 33 days of the light treatment the birds were sacrificed and a histological study was made to determine testicular development. Statistical analysis of data was carried out and was based on four criteria: testes weight, seminiferous tubule numbers, seminiferous tubule diameters, and cell count of cells undergoing spermatogenesis.

Limitations of the Problem

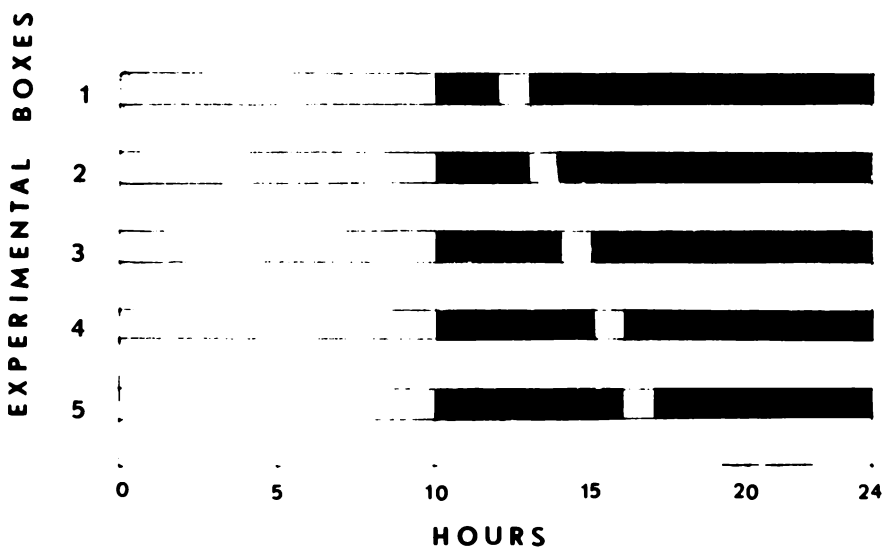
The following are considered to be limitations of the study.

1. The all-male population was purchased from a commercial game bird dealer from east Texas. All the birds, therefore, had had the same environment since hatching.
2. All the birds purchased were born in August of 1967, and thus were seven months old at the time they were placed in the experimental boxes. (At seven months of age, quail have

Figure 1. Diagrammatic schedule of light treatments. White areas represent time lights were on in each 24 hour period.

FIGURE 1

ARRANGEMENT OF LIGHT SEQUENCE



not reached full sexual development.[6])

3. The animals were subjected to the same light intensities, in identical boxes, with a constant supply of food and water.

---

[6]Marshall, A. J., Biology and Comparative Physiology of Birds, p. 419.

CHAPTER II

LITERATURE REVIEW

As early as the seventeenth century it was well known to bird watchers that the song ordinarily associated with vernal reproductive activity could be obtained in autumn by holding males on reduced light through spring and early summer, and then exposing them to the long days of late summer.[1]

The first scientific investigation of photoperiodism in Aves was begun about 45 years ago by William Rowan[2] who worked with the slate colored Junco (Junco hyemalis) and other species. These investigations were followed by those of Bissonnette[3] and colleagues on starlings (Strunus vulgaris). Among the more recent reviews of the photoperiodic phenomena are those of J. W. Burger.[4]

In 25 years of research Burger was able to assimilate the following data pertaining to the male starling:

1. Spermatogenesis can be induced by daily illumination of from 9.5 to 24 hours. Daily illumination of 8.5 hours acting over 20 to 22 weeks did not induce sperm but did induce the production of sperm up to the spermatocyte state of spermatogenesis.

---

[1]Farner, D. S., "The Photoperiodic Control of Reproductive Cycles in Birds," p. 138.

[2]Rowan, W., "Relation of Light to Bird Migration and Developmental Changes," p. 494.

[3]Bissonnette, T. H., "Studies on the Sexual Cycle in Birds. I. Sexual Maturity: Its Modifications and Possible Control in European Starling," p. 289.

[4]Burger, J. W., "The Effects of Photic and Psychic Stimuli on the Reproductive Cycle of the Male Starling, Strunus vulgaris," p. 227.

2. Longer daily lighting increases rapidity of induction of testicular activation.
3. The duration of active spermatogenesis and the onset of subsequent testicular involution is related to the length of daily illumination. With long daily periods of light -- 15 hours, 17 hours, or 24 hours -- activation is rapid, but of short duration, and is followed by prompt involution.
4. Maximum testicular activation as judged by testicular volume, duration of the spermatogenic period and rate of activation occurs at about 12.5 hours of daily illumination.
5. Natural involution is followed by a period when photoperiodic stimuli no longer are effective in maintaining spermatogenesis. This refractory is reinforced by longer days and weakened by shorter days. (Wolfson[5] found similar results with other species.)
6. The amplitude of spermatogenesis in laboratory birds is less than in wild birds as reported (Bissonnette and Chapnick[6] and Bullough[7]) in Burger's review.
7. Presence of females plays a marked role in the male cycle. The female increases the magnitude of testicular response by about 45%.

---

[5]Wolfson, A., "Day Length, Migration, and Breeding Cycles in Birds," pp. 191-200.

[6]Bissonnette, T. H. and M. H. Chapnick, "Studies on the Sexual Cycle in Birds. II. The Normal Progress Changes in the Testes from November to May in the European Starling (Strunus vulgaris), An Introduction Now - Migratory Bird," pp. 307-343.

[7]Bullough, W. S., "The Reproductive Cycle of the British and Continental Races of the Starling," pp. 165-246.

Experimental data on the male starling indicate that several major factors are involved in the annual reproductive cycle. There is an inherent tendency to gonadal activation leading to testicular involution and to the development of a refractory state. These "built in" tendencies do not seem capable of duplicating the natural spermatogenic cycle by themselves. Form seems to be given to these tendencies by the daily illumination. All phases of the cycle - progressive, regressive, and refractive - have been shown to be responsive experimentally to photoperiodic manipulations.

In 1935 Benoit[8], with the use of domestic ducks, demonstrated encephalic photoreception other than that involved in vision. In a review of literature Farner[9] states that a given total duration of light becomes more effective in the stimulation of testicular development if it is broken into shorter periods. He cites Benoit (1936), Burger, Bissonnette, and Doolittle (1942), Kirkpatrick and Leopold (1952), Kirkpatrick (1955), Jenner and Engels (1952), Farner, Mewaldt, and Irving (1959) as evidence for this effect. In general the effectiveness of light stimulation appears to increase as the duration of the intervening dark period is reduced.

Farner[10] hypothesizes that somewhere in the response mechanism there

---

[8]Benoit, J. and L. Ott, "External and Internal Factors in Sexual Activity. Effect of Irradation with Different Wave-Lengths on the Mechanisms of Photostimulation of the Hypophysis and on Testicular Growth in the Immature Duck," pp. 27-46.

[9]Farner, D. S., "Photoperiodic Control of Annual Gonadal Cycles in Birds," pp. 717-750.

[10]_____, "Photoperiodic Control of Annual Gonadal Cycles in Birds," pp. 717-750.

is a rate-limiting reaction during the light period. This produces very rapidly a substance which is essential for the photoperiodic response. During the dark period this essential substance decays at a rate much slower than its rate of formation so that the stimulation of testicular development persists temporarily after the end of the photoperiod. Farner refers to this period of continued effect during the dark as the "carry-over" period.

Even a casual review of reproduction periodicity in birds reveals that day length is only one of several environmental factors that may be used in the control of reproductive cycles.[11] Much is yet to be learned about these factors and how they produce their effect on reproductive cycles. Current investigations suggest that the pineal may have an important role in the effect of such environmental factors on the organism.

Until recently most investigators thought that the pineal was simply a vestige of a primitive light-sensing organ: the "parietal eye" found in certain cold blood vertebrates such as the frog. Other workers, noting the precocious sexual development of some young boys with pineal tumors, proposed that in mammals the pineal might be a gland.[12] In 1917 C. P. McCord and F. P. Allen[13] observed that extracts of cattle pineals added to a media which contained tadpoles resulted in bleaching of the skin of the tadpoles. Aaron B. Lerner[14] and his co-workers in 1958 identified

---

[11]Farner, D. S., "Photoperiodic Control of Reproductive Cycles in Birds," p. 137.

[12]Wurtman, Richard J. and Julius Axelrod, "The Pineal Gland," pp. 50-60.

[13]McCord, C. P. and F. P. Allen, "Evidences Associating Pineal Gland Function with Alterations in Pigmentation," pp. 207-244.

[14]Lerner, A. B., "Hormones and Skin Color," pp. 99-108.

this bleaching agent and named it melatonin for its effect on cells which contained the pigment melanin.

In 1959 Giarman found that the pineal contains large stores of serotonin[15]. In 1959 and 1960 Axelrod and Weissbach[16,17] worked with enzymes in an attempt to convert serotonin (the most likely precursor of melatonin) to melatonin. They found that the enzyme, hydroxyindole O-methyltransferase (HIOMT), converts N-acetylserotonin to melatonin. (See Figure 2.) The activity of HIOMT is regulated by light, and in rats synthesis of melatonin is depressed in animals kept in continuous light and increased in animals maintained in darkness.[18]

Melatonin has been found to have an inhibitory effect on gonadal development.[19] Since the action and disposition of melatonin seem to be related to light exposure in the mammal, studies were undertaken to determine whether the capacity of the rat pineal gland to synthesize melatonin from its immediate precursor, as evidenced by testicular development, was also subject to photic regulation.[20] In their study, Wurtman,

---

[15]Giarman, J. J. and S. Schanberg, "The Intracellular Distribution of 5-Hydroxytryptamin (HT: Serotonin) in the Rat's Brain," pp. 301-306.

[16]Axelrod, J. and H. Weissbach, "Enzymatic-o-methylation of n-actyl-serotonin to Melatonin," p. 1312.

[17]_____, "Purification and Properties of Hydroxyindole-o-methyl Transferase," p. 219.

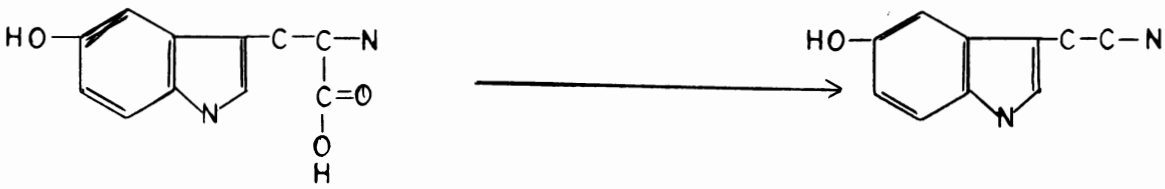
[18]Wurtman, R. J., J. Axelrod, L. Phillips, "Melatonin Synthesis in the Pineal Gland: Control by Light," pp. 1071-72.

[19]Wurtman, R. J., J. Axelrod, E. W. Chu, "Melatonin, A Pineal Substance: Effect on the Rat Ovary," p. 277.

[20]Wurtman, R. J., J. Axelrod, L. Phillips, "Melatonin Synthesis in the Pineal Gland: Control by Light," pp. 1071-72.

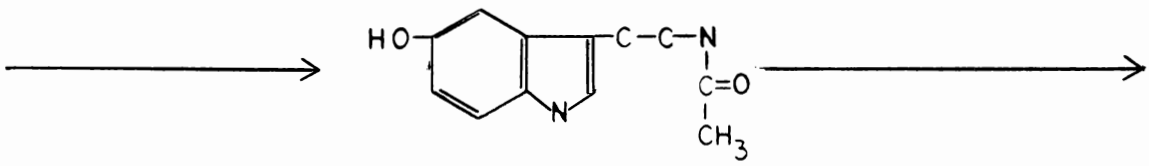
FIGURE 2

SYNTHESIS OF MELATONIN

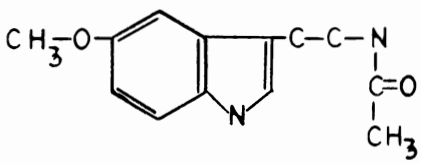


5-Hydroxytryptophan

Serotonin



N-Acetylserotonin



Melatonin

Axelrod, and Phillips[21] suggest that the light-induced inhibition of HIOMT activity may constitute a mechanism of neuroendocrine regulation of gonadal function. In addition, Wurtman, Axelrod, and Fisher[22] seem to believe that the pathway by which information about environmental illumination reaches the pineal gland involves the eyes and the sympathetic nervous system.

Experiments on pineal physiology in Avian species are not entirely in agreement with the results obtained in mammals and seem to be contrary to the results found in experiments in rats. Axelrod et al.[23] report that chickens kept under diurnal photoperiods had higher HIOMT activity during the light period than during the dark period. In addition, the high rate of melatonin production is known to stimulate gonadal development in the chicken.

Shellabarger[24,25] in 1951 and 1953 reported that pinealectomy in chickens caused hypertrophy in old cockerels and gonadal inhibition in young cockerels. Work by McFarland, Homma, and Wilson[26] illustrated sex dependent influences on the pineal. The removal of the superior

---

[21]Wurtman, R. J., J. Axelrod, L. Phillips, "Melatonin Synthesis in the Pineal Gland: Control by Light," p. 1071.

[22]Wurtman, R. J., J. Axelrod, J. E. Fisher, "Melatonin Synthesis in the Pineal Gland: Effect of Light Mediated by the Sympathetic Nervous System," p. 1328.

[23]Axelrod, J., R. J. Wurtman, C. W. Winget, "Melatonin Synthesis in the Hen Pineal Gland and Its Control by Light," p. 1134.

[24]Shellabarger, C. J., "Pinealectomy vs. Pineal Injection in Young Cockerel," pp. 151-154.

[25]_____, "Observations of the Pineal in the White Leghorn Capon and Cockerel," pp. 189-197.

cervical ganglia in mature female Japanese Quail (Coturnix coturnix japanica) resulted in cessation of egg laying for up to 13.2 days, but it failed to produce an effect on the testes in mature males. According to Homma, the pineal is related to certain pathways which transmit environmental stimuli to the pituitary, but the participation of the pineal is not strong enough to modify the entire gonadal response to various environmental stimuli. Sayler and Wolfson[27] confirmed reports by Shellabarger and Homma that the pineal's function ceased after four weeks of age and that its function was also sex dependent.

---

[26]Homma, K., L. Z. McFarland, W. O. Wilson, "Response of the Reproductive Organs of the Japanese Quail to Pinealectomy and Melatonin Injections," pp. 314-318.

[27]Sayler, A. and A. Wolfson, "Avian Pineal Gland: Progonadotropic Response in the Japanese Quail," pp. 1478-1479.

CHAPTER III

METHODS AND MATERIALS

The Experimental Animal

The animal used in this experiment was the Bobwhite Quail (Colinus virginianus texanus). Quail were selected because photoperiodic studies on quail were available in the literature. In addition, pen raised quail are easily obtainable from local quail raisers. One hundred seven-month-old males were purchased from a commercial dealer in Naples, Texas.

Experimental Equipment and Procedure

Upon arrival, the birds were divided into three groups and housed in separate holding pens. The first group underwent an operation for the removal of the pineal gland. The second underwent a sham operation (simulated pinealectomy), while the third group was held as a control.

The pinealectomy was performed after a hole was bored through the skull of the quail. The drill consisted of a disc bit made from a #2 brass cork borer mounted in a drillpress. The animal was first anesthetized with Equithesin injected intramuscularly into the breast on either side of the keelbone at the rate of 2.5 ml/kg. Ether was used for brief anesthesia during the operation. The scalp was cut anteroposteriorly along the midline between the eyes to beyond the base of the skull. The skin flaps were retracted and the underlying fascia scraped free in an area wider than the diameter of the bit. Careful removal of these tissues from the field was necessary to prevent entanglement with the drill during the subsequent steps. The drill was centered on the conflu-

ence of the superior sagittal and transverse sinues. A hole was drilled to the desired depth so as not to injure the brain, usually a little less than the thickness of the skull. At this depth, the bone disc which was still adherent to the dura was removed with a probe. The pineal gland, located between the junctions of the two cerebral hemispheres and the cerebellum, was removed by inserting an open pair of microdissecting forceps into the junction, grasping the stalk, and removing gland and stalk in one motion.

Excess hemorrhage was prevented by returning the bone disc to its original position and by applying a cotton pledget with moderate pressure. Sulfanilamide was sprinkled on the wound before the skin flaps were closed and sutured together to prevent further bleeding and possible infection.

The second group of birds as previously mentioned, underwent a sham-operation. The operation was necessary in order to see if the operation itself rather than removal of the pineal glands had an effect on the birds. The sham-operation was performed in the same manner as the pinealectomy except that the pineal gland was left intact.

After surgery, the birds were returned to their holding pens for a week of postoperative recuperation. At the end of the week, the birds were banded and placed in the experimental boxes. These boxes consisted of five 13" x 18" x 24" compartments arranged in a row and lighted by a 15 watt incandescent lamp which provided a range of 40-68 foot candles at different positions in the compartments. The lamp was mounted in the front wall of each box about an equal distance from top to bottom. Movement of the birds in a plane horizontal to the light source provided an intensity range of 40-68 foot candles, with exposure changing with the bird's move-

ment. Five birds from each of the three treatment groups were placed in the compartments. A turkey starter mash and water were available at all times and the temperature ranged from 19-26°C. The birds were subjected to light treatments controlled by electric time switches from March 30 to May 2 (33 days). The light treatments for the five groups are schematically show in Figure 1.

At the end of the 33 day period the animals were removed, weighed and sacrificed. The testes were removed and gently blotted on paper toweling to absorb free blood, then weighed to the nearest 0.1 mg. The testes then were fixed in Formalin-Alcohol-Acetic Acid (FAA) solution, dehydrated, cleaned and embedded in paraffin. The tissue was sectioned at seven microns and stained with hematoxylin and eosin. A microscopic examination was made of seminiferous tubule numbers, seminiferous tubule diameters and the number of cells undergoing spermatogenesis. Statistical tests applied to this data were the analysis of variance, standard deviation and f tests.

CHAPTER IV

RESULTS

A statistical analysis of the data by means of the f test was made in order to determine the effects of the various light and dark sequences on testicular development. In this study, no significant difference was determined by cell count, testes weight, seminiferous tubule number or seminiferous tubule diameter except in the pinealectomized animals. The effects of the removal of the pineal gland on testes development were analyzed. In this manner, the function of the gland could be assayed in birds of six months and older.

All the information obtained from the pinealectomized, sham-operated controls, and normal controls was compiled and treated as one experiment, since compiling the data increased the number of observations and more meaningful conclusions could be reached as to the effect of the removal of the pineal. The results obtained from this composite are cited in Table 1. The means of these numbers were processed by taking the average of the data from the left and right testes and using this average as one number, thus eliminating variance between left and right testicular development on the individual bird.

The results show that the pinealectomized animals had a decrease in number of cells undergoing spermatogenesis, a decrease in testes weight, and a decrease in seminiferous tubule diameters. There was, however, an increase in seminiferous tubule numbers as compared to the sham-operated and normal controls. The over-all results show an atrophy of the testes attributable to removal of the pineal gland.

TABLE I

Mean testicular weight, seminiferous tubule numbers, seminiferous tubule diameters, and spermatogenic cell count of pinealectomized, sham-operated, and normal groups

Groups	No.	Testes Weight mg	Seminiferous Tubule Number	Seminiferous Tubule Diameters	Spermatogenesis Cell Count mg
Pinealectomized	14	713.65±26	397.78±40.03	239.1±30	35.807±4.04
Sham-operated	16	898.12±200	381.12±75.01	261.5±17	44.687±3.36
Normals	24	905.88±200	364.75±81.17	265.5±17	42.229±4.19

The following are graphic representations of the significant data. Figure 3 represents the mean results of the spermatogenetic cell count of the three groups. Figure 4 shows testicular weight, Figure 5 illustrates seminiferous tubule diameters, and Figure 6 demonstrates seminiferous tubule numbers. Each bar represents the mean of the data from 14 pinealectomized, 16 sham-operated, and 24 normal birds, with the line at the end signifying standard deviation. Figures 7, 8, and 9 respectively represent cross sections of testes from normal, sham-operated, and pinealectomized Bobwhite Quail.

Figure 3. Average spermatogenetic cell count of pinealectomized, sham-operated, and normal Bobwhite Quail. Each bar represents the mean and the line at the end signifies standard deviation.

FIGURE 3

SPERMATOGENETIC CELL COUNT

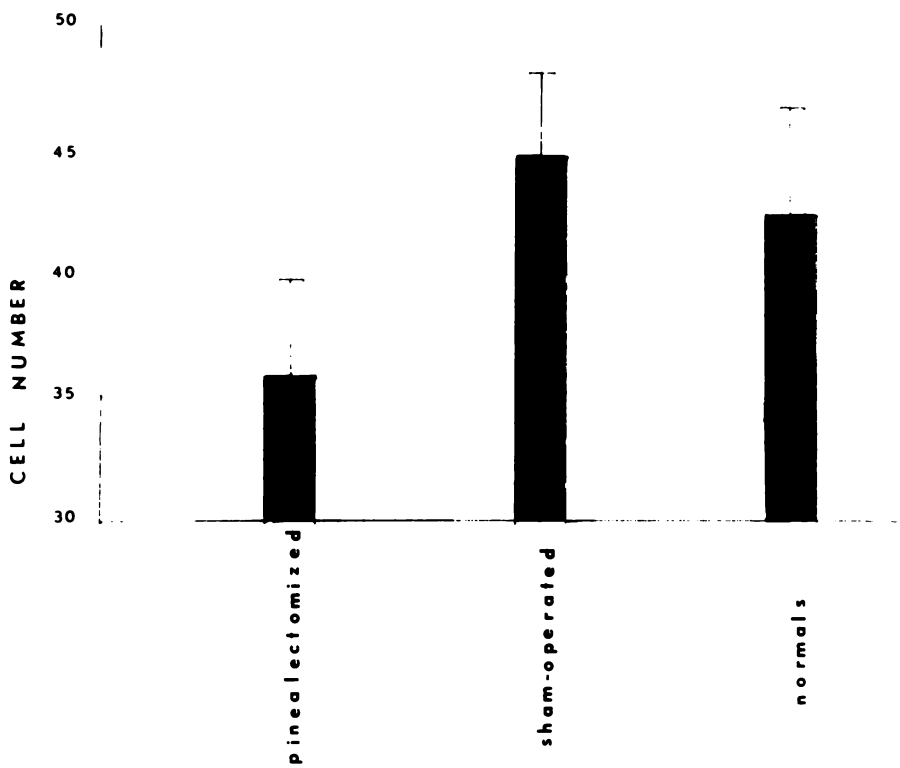


Figure 4. Average testes weight of pinealectomized, sham-operated, and normal Bobwhite Quail. Each bar represents the mean and the line at the end signifies standard deviation.

FIGURE 4

TESTES WEIGHT

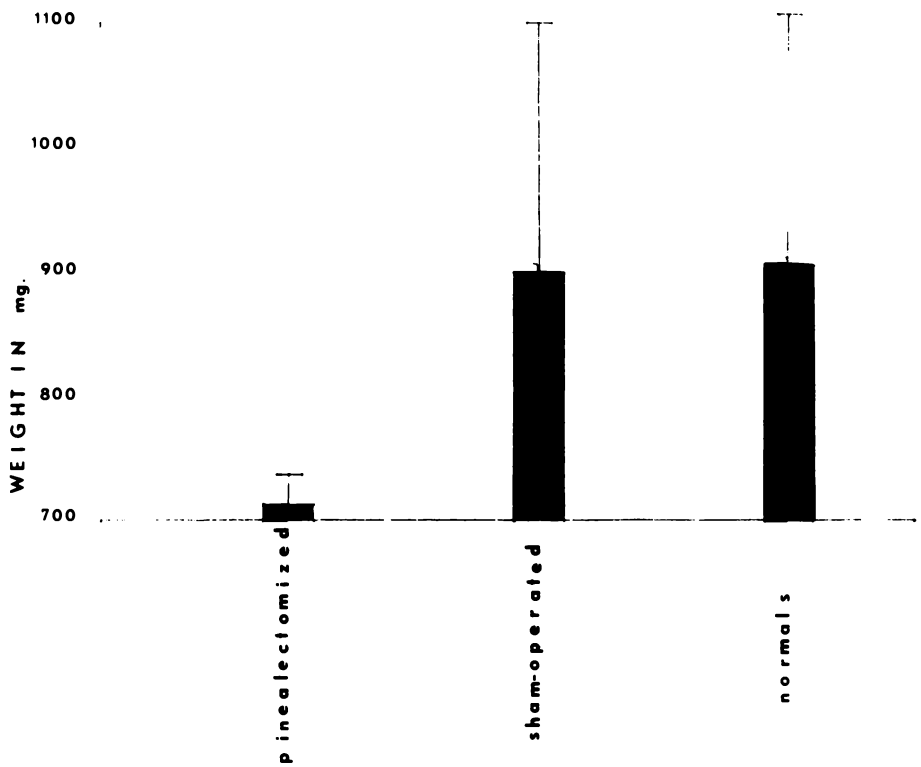


Figure 5. Average seminiferous tubule diameters of pinealectomized, sham-operated, and normal Bobwhite Quail. Each bar represents the mean and the line at the end signifies standard deviation.

FIGURE 5

SEMINIFEROUS TUBULE DIAMETERS

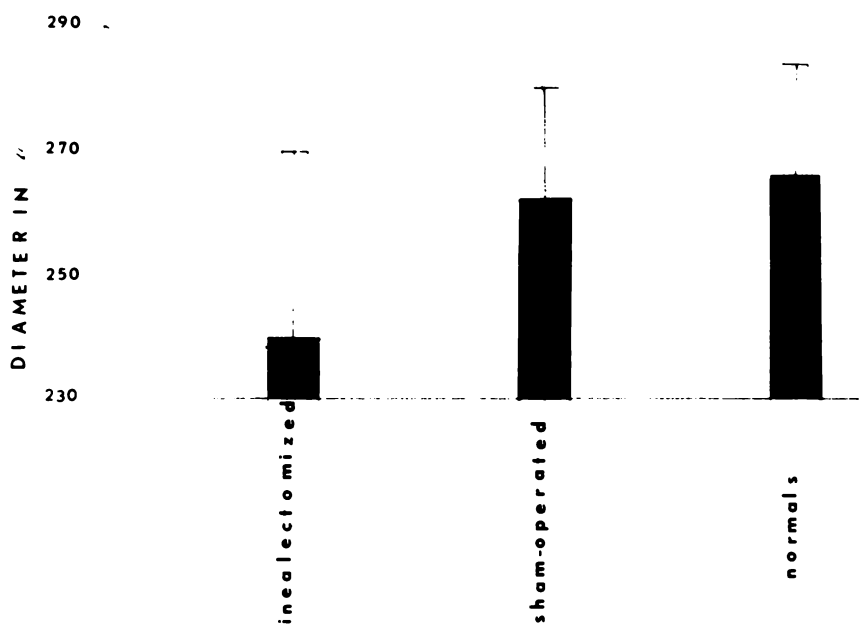


Figure 6. Average seminiferous tubule numbers of pinealectomized, sham-operated, and normal Bobwhite Quail. Each bar represents the mean and the line at the end signifies standard deviation.

FIGURE 6

SEMINIFEROUS TUBULE NUMBERS

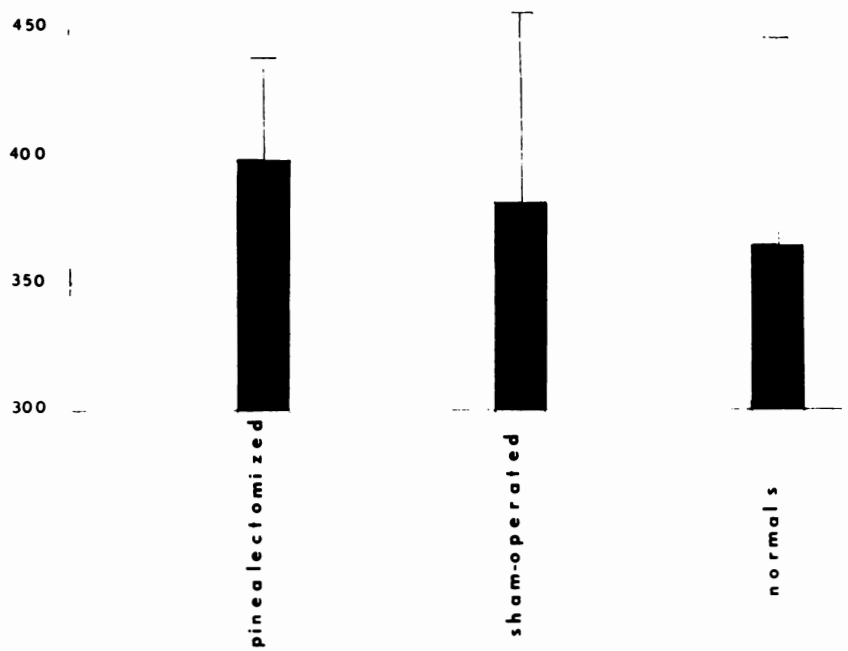
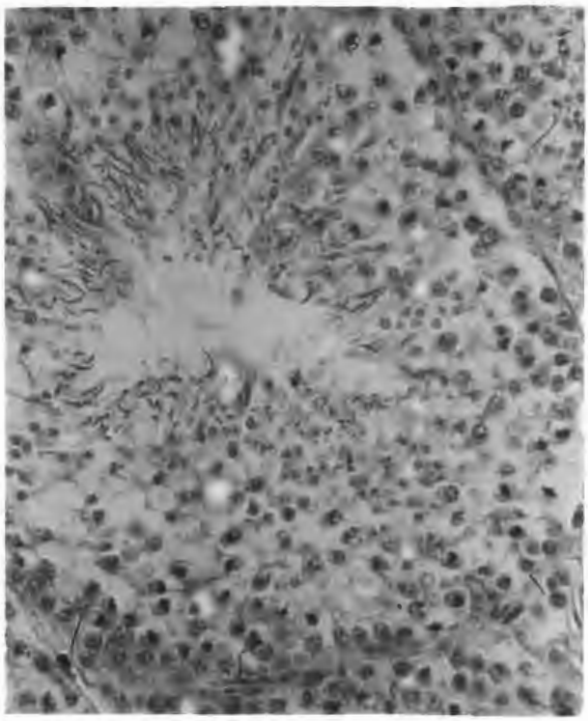


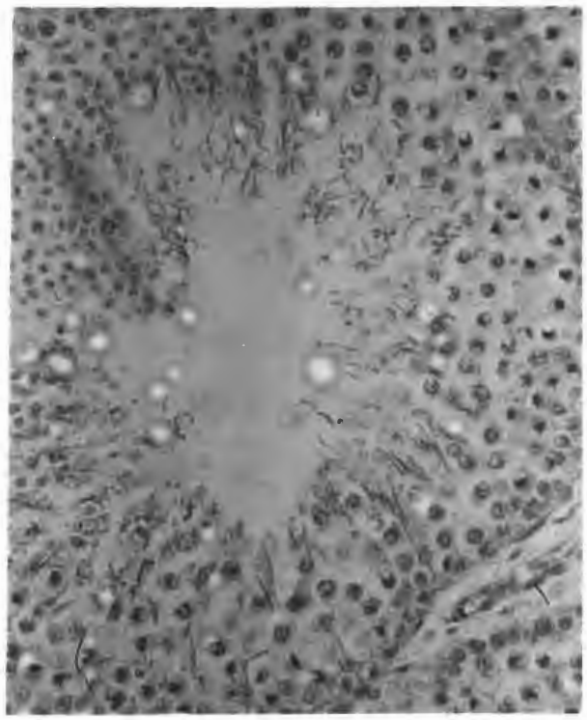
Figure 7. Cross section of the testes from a normal Bobwhite Quail.

Figure 8. Cross section of the testes from a sham-operated Bobwhite Quail.

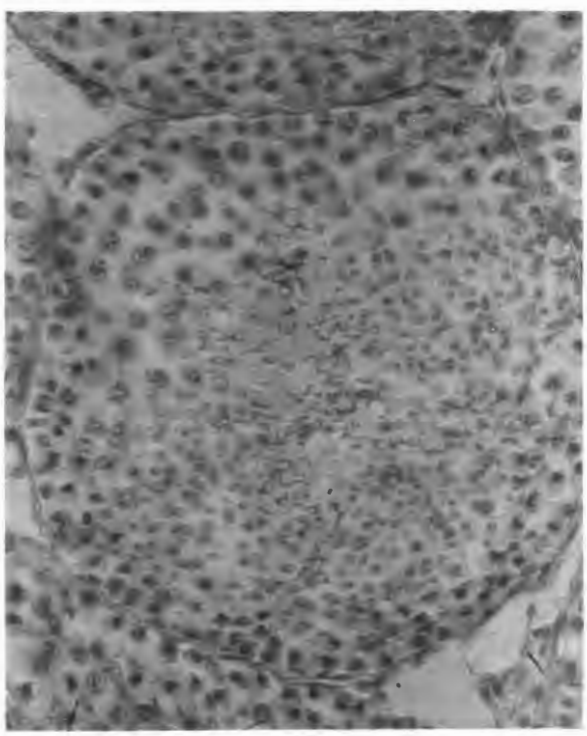
Figure 9. Cross section of the testes from a pinealectomized Bobwhite Quail.



7



8



9

CHAPTER V

DISCUSSION

Previous investigations suggest that the length of the uninterrupted period of darkness is a controlling factor in gonadal development in Aves. There is an indication that darkness affects the reproductive system in a manner directly opposed to the effect of light[1].

An attempt was made to correlate the effects of dark-light cycles on the reproductive system with the function of the pineal gland. Since it has been demonstrated that in quail and in certain other birds a dark period inhibition of gonadal activity is destroyed by short periods of light, a study of the optimum interruption period of darkness is in order. The original purpose of this experiment was to determine whether the pineal gland is the mediator of the carry-over period noted in birds, and, if so, to determine the optimum interruption time.

The optimum interruption time could not be determined from data compiled in this study. Probable reasons for the negative results obtained are:

1. The duration of the experiment was not long enough.
2. The one-hour period of interruption was not sufficient. Axelrod[2] stated that melatonin production is known to stimulate gonadal development in birds and melatonin production is activated during

---

[1]Farner, D. S., "Photoperiodic Control of Annual Gonadal Cycles in Birds," pp. 717-750.

[2]_____, "Melatonin Synthesis in the Hen Pineal Gland and Its Control by Light," p. 1134.

the light period. Farner[3] stated that in the "carry-over" period there is a response mechanism that is rate limiting. Farner also hypothesized that during the dark period there is an essential substance that decays at a rate much slower than its rate of formation. Should a light interrupt the dark period or period of decay, then the essential substance would be activated again. If the essential substance is melatonin and if during the dark period it reverts to N-acetylserotonin, then light would reactivate the production of N-acetylserotonin to melatonin once more. If the light were not of sufficient duration (one hour), however, then there would be no effect on the net production of melatonin which would result in no apparent testicular change.

3. The bird numbers fell to a critical minimum. Initially 15 birds were placed in each experimental box at a ratio of 5:5:5. At the end of the 33 day period, the number of pinealectomized animals had dwindled to two per box except the second box, in which no deaths were recorded. When a study involves only two organisms, individuality plays too large a factor in determining possible light effects.

Since the original objective had to be abandoned as a result of the above factors, an alternate experiment was devised. An analysis was made of the composite data from the three groups to determine if removal of the pineal gland had any effect on birds of this age.

---

[3]Farner, D. S., "Photoperiodic Control of Annual Gonadal Cycles in Birds," pp. 717-750.

Sayler and Wolfson[4], working with Japanese Quail (Coturnix coturnix japanica) in 1967, found the pineal to have no effect on male gonads after 55 days of age. The assumption of Sayler and Wolfson was partly based on a study by Shellabarger and Breneman[5] in 1950, who showed that pinealectomized chickens had smaller testes weight at 19 days. The testes returned to normal at the age of 28 days and remained so until 40 days. Shortly after 40 days the operated chicks were found to have significantly larger testes weight, though this increase was no longer evident at 94 days. Sayler and Wolfson sacrificed their quail at various days up to 55 days old. Finding no significant difference in gonadal weight (the only criteria) and using Shellabarger's work as a reference, they postulated that the pineal does not affect the testes. Instead, according to Sayler and Wolfson, the pineal appears to have a progonadotropic influence on ovarian development during only a narrow phase of growth in birds exposed to a stimulatory photoperiod. Homma, McFarland, and Wilson[6] conducted a similar study in 1967 and made similar statements about the effect of the pineal in relationship to age. Homma, McFarland, and Wilson sacrificed their birds at age four weeks and again used only testes weights as a criterion. It may be concluded that no one has really verified the assumption that the pineal has no effect on older birds. Shellabarger, Homma,

---

[4]Sayler, A. and A. Wolfson, "Avian Pineal Gland: Progonadotropic Response in the Japanese Quail," p. 1478.

[5]Shellabarger, C. J. and W. R. Breneman, "The Effects of Pinealectomy on Young White Leghorn Cockerels," p. 299.

[6]Homma, K., L. Z. McFarland, W. O. Wilson, "Response of the Reproductive Organs of the Japanese Quail to Pinealectomy and Melatonin Injections," p. 314.

and Sayler used only the criterion of testes weight in the formation of their hypothesis, and the duration of Shellabarger, Homma, and Sayler's experiments encompassed respectively 94, 28, and 55 days from birth to the time the birds were sacrificed.

This experiment, which yielded negative results as far as establishment of the effective light interruption period, shows that the pineal does have a definite effect on the testes of older birds (four weeks of age and older). In quail used in this experiment there was a definite reduction in testes weights, in the number of cells undergoing spermatogenesis, and in seminiferous tubule diameters in older birds. The only criterion that did not support the postulation that absence of the pineal causes atrophy of the testes was that of seminiferous tubule numbers. The data available from this study certainly indicate that the effect of the pineal is not age dependent. There is no evidence that the pineal ceases to function at seven months of age, since removal of the pineal continues to cause atrophy of the testes of older birds.

CHAPTER VI

SUMMARY

Bobwhite Quail (Colinus virginianus texanus) bought commercially were divided into three groups. One group was pinealectomized, a second group of birds was sham-operated, and a third group was used as normal controls. The three groups were placed in experimental light boxes designed to provide light-dark cycles of 10L2D1L11D, 10L3D1L10D, 10L4D1L9D, 10L5D1L8D, and 10L6D1L7D respectively. Thirty-three days later the birds were removed and sacrificed. The testes were weighed and examined histologically for comparison of development. Data were examined statistically in an attempt to determine optimum time of light interruption in conjunction with pineal function. A supplementary analysis was conducted in order to determine if the pineal gland was functional in seven-month-old quail.

Negative results were drawn from experiments involving interrupted light periods. This does not rule out the fact that the pineal could be the coordinating system that regulates the "carry-over" phenomena since future experiments involving larger numbers of birds and a longer duration of treatment could produce meaningful results substantiating this hypothesis.

Data obtained from this experiment demonstrate that the pineal does produce a progonadotropic effect on male Bobwhite Quail. There is a decrease in testes weight, spermatogenetic cell count, and seminiferous tubule diameters of the pinealectomized birds as compared to the controls. These data also demonstrate that the pineal is functional in birds older than four weeks of age.

BIBLIOGRAPHY

Axelrod, J. and H. Weissbach, "Enzymatic-o-methylation of n-actylserotonin to Melatonin," Science, Vol. 131 (1960), 1312.

_____ and H. Weissbach, "Purification and Properties of Hydroxyindole-o-methyl Transferase," J. Biol. Chem. 236 (1961), 211.

_____ , R. J. Wurtman, C. M. Winget, "Melatonin Synthesis in the Pineal and Its Control by Light," Nature, 201 (1964), 1134.

Benoit, J. and L. Otto, "External and Internal Factors in Sexual Activity. Effect of Irradiation with Different Wave-Lengths on the Mechanisms of Photostimulation of the Hypophysis and on Testicular Growth in the Immature Duck," Yale J. Biol. Med. 17 (1944), 27-46.

Bissonnette, T. H., "Studies on the Sexual Cycle in Birds. I. Sexual Maturity Its Modification and Possible Control in European Starling," Am. J. Anat. 45 (1930), 289-305.

_____ , and M. H. Chapnick, "Studies on the Sexual Cycle in Birds. II. The Normal Progress Changes in the Testes from November to May in the European Starling (Strunus vulgaris), An Introduction Now - Migratory Bird," Am. J. Anat. 45 (1930), 307-343.

Bullough, W. S., "The Reproductive Cycle of the British and Continental Races of the Starling," Phil Trans. R. Soc. London. Series B 231 (1942), 165-246.

Burger, J. W., "The Effects of Photic and Psychic Stimuli on the Reproductive Cycle of the Male Starling, Strunus vulgaris," Journal of Experimental Zoology. Vol. 124 (1953), 227-240.

Farner, D. S.,"Photoperiodic Control of Annual Gonadal Cycles in Birds," Photoperiodism and Related Phenomena in Plants and Animals. Ed. R. B. Withrow (1959).

_____ , "The Photoperiodic Control of Reproductive Cycles in Birds," American Scientist. 52 (1964), 137-155.

Giarman, J. J., and S. Schanberg, "The Intracellular Distribution of 5-Hydroxytryptamin (HT:Serotonin) in the Rat's Brain," Biochem. Pharmacology. Vol. 1 (1958), 301-306.

Homma, K., L. Z. McFarland, W. O. Wilson, "Response of the Reproductive Organs of the Japanese Quail to Pinealectomy and Melatonin Injections," Poultry Science. Vol. 46 (1967), 314-319.

Lerner, A. B., "Hormones and Skin Color," Sci. Amer. 205 (1961), 99-108.

Marshall, A. J., Biology and Comparative Physiology of Birds. Academic Press I, (1961).

McCord, C. P. and F. P. Allen, "Evidences Associating Pineal Gland Function with Alteration in Pigmentation," J. Exp. Zool. Vol. 23 (1917), 207-244.

Rowan, W., "Relation of Light to Bird Migration and Developmental Changes," Nature. 115 (1915), 494-495.

Sayler, A. and A. Wolfson, "Avian Pineal Gland: Progonadotropic Response in the Japanese Quail," Science. Vol. 158 (1967), 1478-79.

Shellabarger, C. J., "Observation of the Pineal in the White Leghorn Capon and Cockerel," Poultry Science. Vol. 32, No. 2 (1953), 189-197.

_____ , and W. R. Breneman, "The Effects of Pinealectomy on Young White Leghorn Cockerels," Proceedings of The Indiana Academy of Science. Vol. 59 (1950), 299-302.

_____ , "Pinealectomy vs. Pineal Injection in Young Cockerel," Endro. (1952), 151-154.

Wolfson, A., "Day Length, Migration, and Breeding in Birds," Sci. Mon. 74 (1952), 191-200.

Wurtman, R. J., J. Axelrod, L. Phillips, "Melatonin Synthesis in the Pineal Gland: Control by Light," Science. 142 (1963), 1071-1072.

_____ , J. Axelrod, E. W. Chu, "Melatonin, A Pineal Substance: Effect on the Rat Ovary," Science. Vol. 141 (1963), 277.

_____ , J. Axelrod, J. E. Fisher, "Melatonin Synthesis in the Pineal Gland: Effect of Light Mediated by the Sympathetic Nervous System," Science. Vol. 143 (1964), 1328-1329.

_____ , and J. Axelrod, "The Pineal Gland," Scientific American. Vol. 213, No. 1 (1965), 50-60.