

STUDIES OF THE PINEAL FUNCTION IN JAPANESE QUAIL
(COTURNIX COTURNIX JAPONICA)

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Louis C. Arrington
1966

THESIS

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LOUIS C. ARRINGTON

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Poultry Science

Robert H. Ringer
Major professor

Date July 22, 1966



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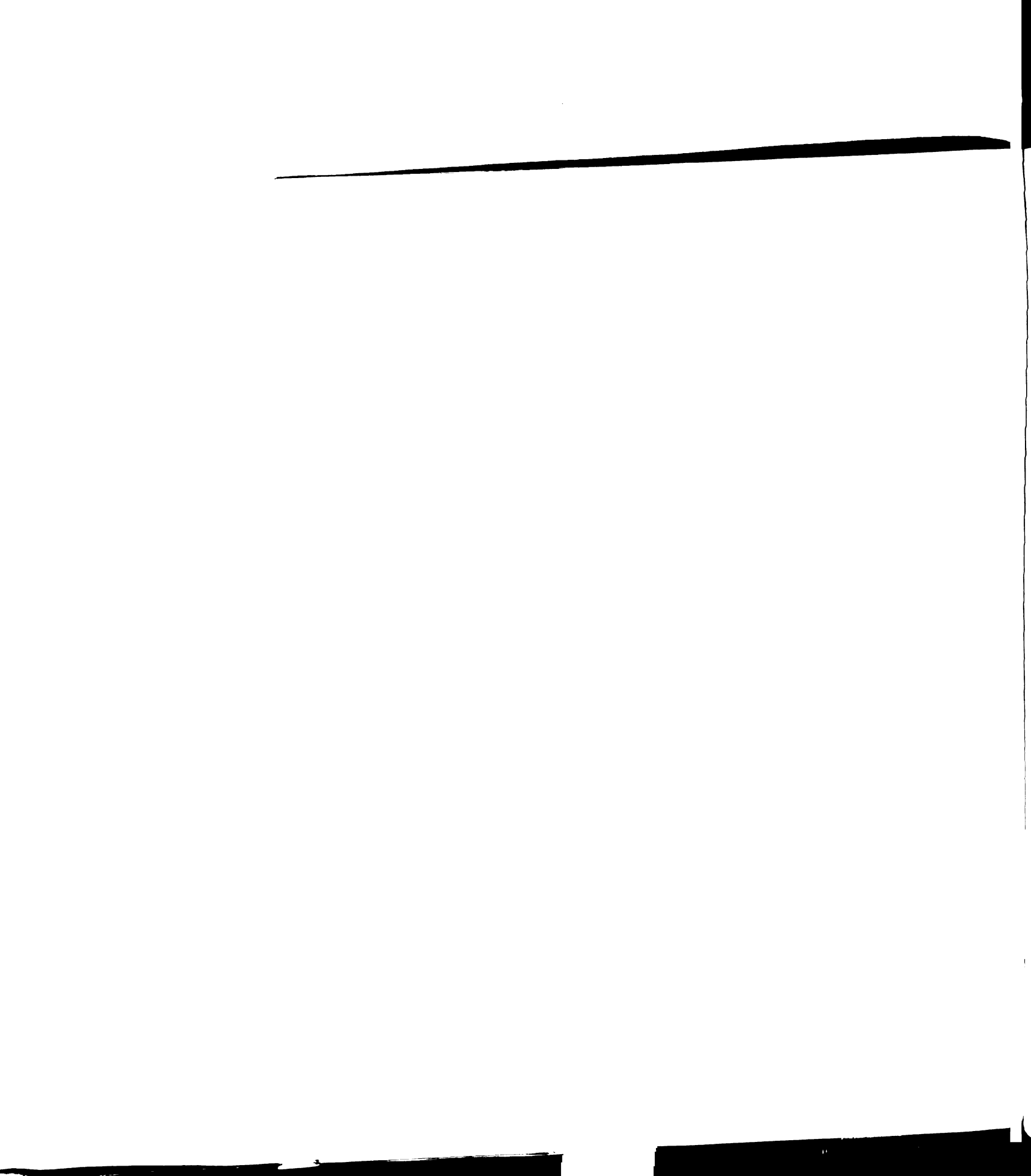
ABSTRACT

**STUDIES OF THE PINEAL FUNCTION IN JAPANESE QUAIL
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by Louis C. Arrington

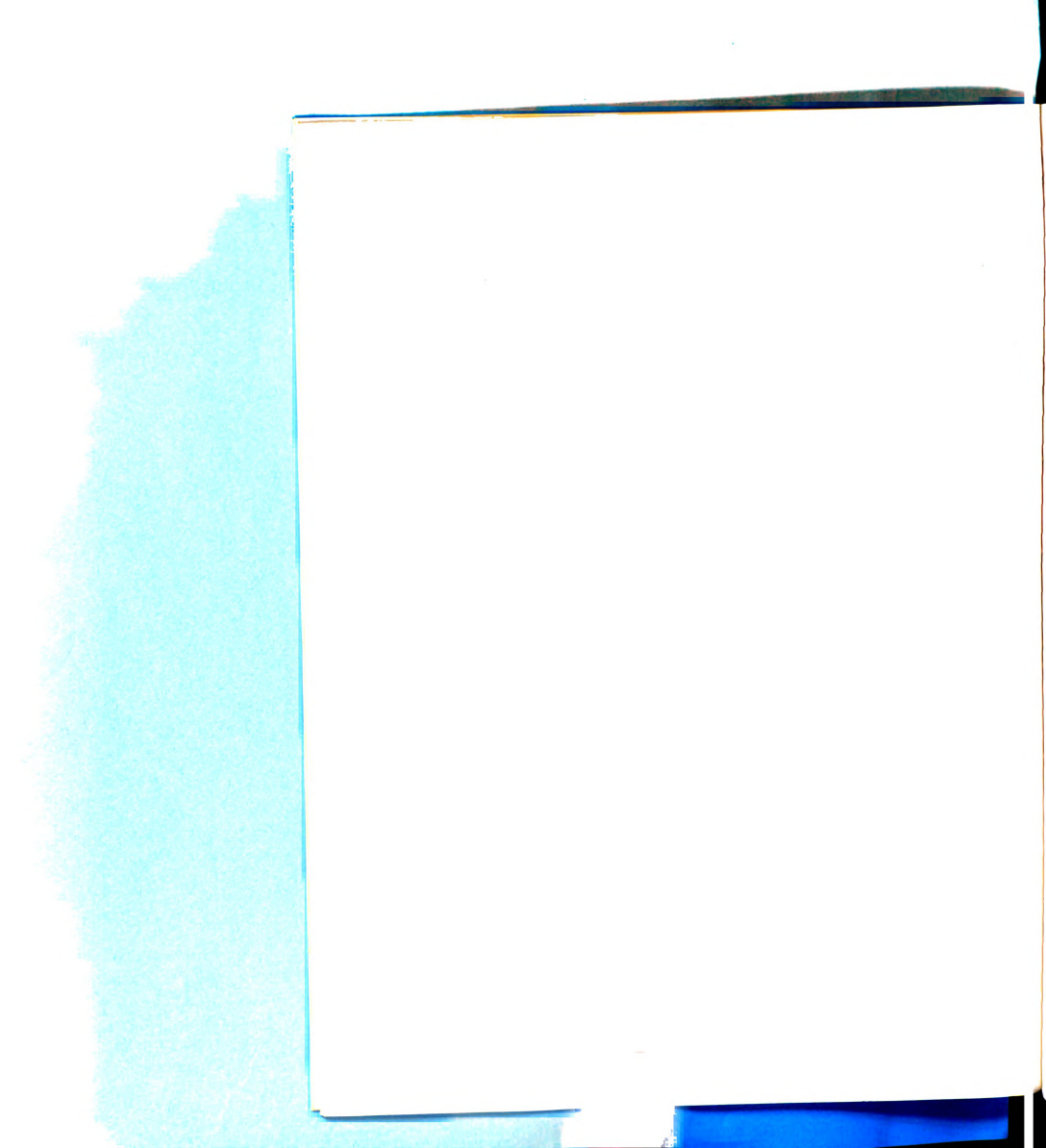
The pineal body is one of the few remaining organs of the body which has not been assigned a definite function. The sparse coverage of this gland in textbooks on avian physiology emphasizes the lack of knowledge about the pineal function in birds. The literature has suggested an involvement of the pineal in the relationship between photoperiods and reproductive performance. It was considered that since avian reproduction is strongly influenced by light, a determination of pineal function would be of importance to avian physiologists.

Newly hatched Japanese quail (Coturnix coturnix japonica) were pinealectomized and grown under inhibitory or stimulatory photoperiods. At various ages they were sacrificed and their body weight, organ weights and age at sexual maturity were compared with unoperated controls. The organs weighed were the pituitary, thyroids, adrenals, spleen, bursa, testes, ovary and oviduct. The success of the pinealectomy operation was determined by macro- and microscopic examination. Daily injections of melatonin, presumed to be a pineal hormone, were given to immature Coturnix quail to determine its effect on body and organ weights. In addition, adult quail hens were injected daily to observe the effects of melatonin on maintenance of egg production.



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The results of pinealectomy experiments indicate that the pineal has little, if any, effect on body weight or on the weight of the pituitary, thyroids, adrenals, spleen or bursa. The differences obtained in these parameters were inconsistent and usually nonsignificant. Complete retardation of growth, and probably atrophy, of the testes was observed in control quail when grown under photoperiods consisting of two hours of light and 22 hours of darkness per day from 3 to 4, 5, 6 and 7 weeks of age. Removal of the pineal glands did not prevent or reduce the inhibitory effect of this photoperiod. Under stimulatory photoperiods (16 hours of light and 8 hours of darkness per day), 5-week old male pinealectomized quail had slightly larger testes. This effect was not present in the 6- and 7-week old males. The possible production of a gonadal inhibitor by the pineal is suggested. However, the results indicate that somewhere near the time of sexual maturity this inhibition ceases due to decreased inhibitor production or decreased response to the inhibitor by the gonads. The responses of females in these tests were not consistent. The only significant response was an increased mean ovary weight in the 7-week pinealectomized group. The 5-week data showed a nonsignificant difference with the control gonads being slightly larger. The mean ages at which egg laying started were not significantly different.





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Adult pinealectomized and control quail hens showed no difference in the number of days required to respond (by commencing egg production) to a change from inhibitory to stimulatory light cycles. Reversing the change in photoperiods produced no difference in the time of cessation of egg production. Sexually mature pinealectomized males showed less atrophy of the testes when switched to an inhibitory light schedule. Due to a large variation in individual results, significance was not obtained, but it is suggested that the presence of the pineal gland may hasten testicular atrophy. The results of the pinealectomy experiments suggest that the pineal modifies, rather than controls, the gonadal response to photoperiods.

Melatonin injections of 50 to 500 micrograms daily produced a slight retardation of gonadal development in prepuberal quail of both sexes. It appeared that the gonads of male quail at or near the puberal phase of growth were not affected by melatonin injections. Daily doses of 50 to 2000 micrograms of melatonin given for 12 to 24 days failed to alter the egg-laying capacity of adult Coturnix hens. These results support the suggestions that the pineal produces an inhibition of gonadal growth in prepuberal quail and that the mature quail gonad is less responsive to pineal inhibition.

STUDIES OF THE PINEAL FUNCTION IN JAPANESE QUAIL
(COTURNIX COTURNIX JAPONICA)

by
Louis C. Arrington

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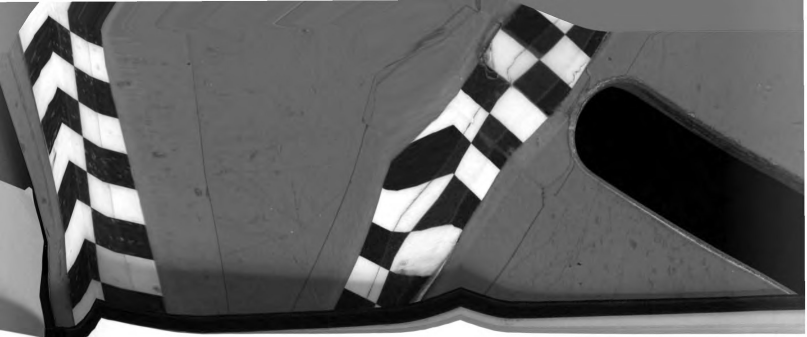
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INTRODUCTION

The pineal body or gland is one of the few remaining organs of the body which has not been assigned a definite function. The most recent textbooks on avian physiology devote only a few sentences to the pineal, stressing the lack of recent work in this area. Even textbooks on general mammalian endocrinology provide little information on the possible role of the pineal. Previous research with both avian and mammalian species has yielded little definitive information due to the inconsistent, and often non-conclusive, results obtained.

Rats have been used in several studies relating the pineal to the effects of light on reproductive parameters. It was considered that since the relationship between light and avian reproductive functions is of economic importance, studies elucidating any pineal involvement would be of importance in interpreting the mechanism by which light mediates its effects on reproduction.

This study is an attempt to determine some of the relationships between the pineal gland and physiological parameters in avian species. The importance of the pineal gland in reproductive function was of particular concern.



LITERATURE REVIEW

A. Description of the Pineal Gland

1. Embryology

Patten (1951), Romanoff (1960, Spiroff (1958) and Tilney and Warren (1919) have described the embryological development of the pineal gland in the chicken. Krabbe (1955) discussed the variations between avian species. Other animals show a similar pattern of development with variations primarily in the appearance time of each stage. At about 52 hours of incubation the epiphysis appears as an evagination in the mid-dorsal wall of the diencephalon. It is destined to differentiate into the pineal gland. By the 36 somite stage (about 72 hours) it is a small hemispherical protuberance. It continues to proliferate dorso-caudally. By the 8th day, the epiphyseal attachment has shifted caudally and the epiphysis has grown out into a long, narrow tube, dilated distally. Proliferation of the ependymal cells in the distal enlargement begins the formation of hollow buds of follicles. Leptomeningeal mesenchyme, containing blood vessels, grows in between the follicles, providing the epiphysis with a connective tissue framework and an extensive vascular network. As the follicles and framework develop, the epiphysis continues to grow dorso-caudally, forming a long, very thin stalk.

2. Morphology

Quay and Levine (1957) showed that the postnatal growth of the rat pineal has a proliferative phase extending to about two weeks after birth. Studies by Dill and Walker (1966) confirmed this report. A cellular hypertrophy phase occurs during the 10 weeks following birth.

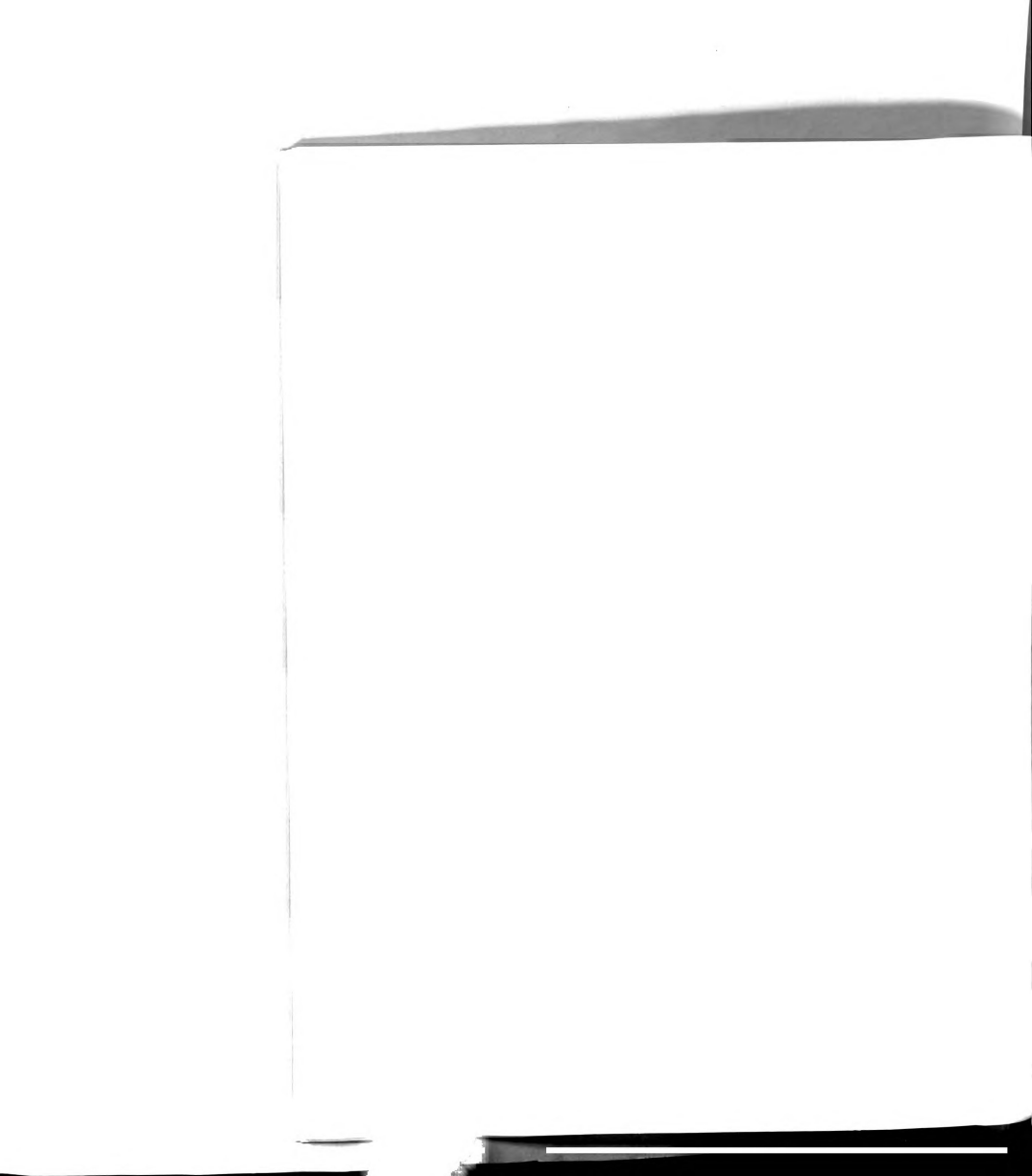
Benoit (1950), Cobb and Edinger (1962), Kappers (1965), Spiroff (1958) and Tilney and Warren (1919) describe the pineal in birds. The pineal is fixed to the arch of the diencephalon by a long, thin stalk. The stalk leaves the brain at a point just rostral to the junction of the forebrain and midbrain, maintaining an attachment in the commissural region, over the 3rd ventricle of the brain. The body of the pineal is superficially located, usually adhering to the dura and sometimes lying in a depression of the cranial roof between the anterior and posterior fossae. It is somewhat teardrop-shaped, although the sides may be partially flattened. The body is situated between the posterior parts of the two cerebral hemispheres and the cerebellar vermis. Its yellowish color distinguishes the pineal from the surrounding whitish brain tissue.

3. Histology

The histology of the avian pineal gland has been described by Basrur and Winget (1963), Benoit (1950),

Kappers (1965), Renzoni and Quay (1963), Spiroff (1958), Stammer (1961) and Tilney and Warren (1919). They demonstrate a specialized, primarily secretory structure, with no clear evidence of photoreceptors. Numerous vesicles are composed of ciliated, cylindrical cells (ependymocytes), small ovoid cells situated between the cylindrical cells (hypendymocytes) and a few peripherally located pinealocytes. These three cell types are of ependymal origin. The thin connective tissue capsule is lined with blood vessels and extends between the parenchymal vesicles. This interstitial connective tissue contains lymphocytes, undifferentiated glial cells and a few large epithelioid cells. Spiroff (1958) describes a lymphocytic invasion of the pineal, seen primarily at 1 week to 6 months of age in domestic fowl.

Electron microscopic studies of the pineal body of rats by De Martino et al. (1964) revealed that the pinealocytes in impuberal rats are small and clearly outlined, with few cytoplasmic processes. These processes contain a few granules of irregular shape. In adult rats, the size and shape of the pinealocytes are less uniform, with numerous cytoplasmic processes extending towards the perivascular spaces. Granules, presumed to be secretory, are more numerous. Anderson (1962) made similar studies of the fine structure of pinealocytes of sheep. The nucleus was described as being large, with infoldings and



a prominent nucleolus. The basophilic cytoplasm contains large amounts of mitochondria in addition to clumps of dense particles, numerous cisternae of the endoplasmic reticulum and a system of fine caualiculi. The golgi complex, centrioles, vesicles, and lipid inclusions were observed in some photo-micrographs.

Kappers (1960, 1964, 1965) described the innervation of the pineal gland in fishes, amphibians, reptiles, birds and mammals. Stammer (1961) gave additional observations from birds. The stalk contains nerves derived from the commissure, which were termed the commissuro-epiphyseal tract. These fibers do not penetrate the bulbous portion of the pineal and are thought to be of no functional significance for the innervation of pinealocytes. The pineal body shows extensive autonomic innervation principally supplied by the two nervi conarii. These are postganglionic fibers, originating in the superior cervical ganglia. The terminal autonomic innervation occurs via perifollicular strands of fibers from which thin single fibers or small groups of fibers branch and penetrate the follicles. Structures thought to be autonomic motor terminals have been observed in relation to the pinealocytes only--not the vascular walls. This autonomic innervation coming from the superior cervical

ganglion appears to be the major, if not the only, functional nervous supply to the pineal. Sensory innervation of the pineal has not been shown.

The pineal is highly vascular. Goldman and Wurtman (1964) found that the minimum rate of pineal blood flow per gram of tissue exceeds that of most endocrine organs, equals that of the neurohypophysis and is surpassed only by that of the kidney in the rat. The vascularization of the mouse pineal was described by Von Bartheld and Moll (1954). Both the afferent and efferent blood pathways are independent from those of the diencephalic choroid plexus and of the nervous parenchyma. Branches of the posterior cerebral artery supply the pineal after coursing over the lateral brain stem surface. These branches anastomose with arteries arising from the basilar artery. Only very small vessels which have lost most of the arteriolar character actually enter the pineal. No arterioles were found to reach the pineal body via its stalk. The capillaries of the pineal are typical of those of nervous parenchyma. Vanules are found in the pineal parenchyma just beneath the capsule. They empty the pineal blood into large venous trunks which border immediately on the pineal. The pineal stalk has no veins or venules and is, therefore, not thought to be an important vascular link between the pineal and other parts of the brain.

[REDACTED]

[REDACTED]

Quay (1959) first reported finding striated muscle fibers in the rat pineal gland. He found examples in three pineals out of a series of 1200 being examined. Dill (1963) confirmed this observation with a similar report. The occurrence had been reported previously in bovine and human pineals. These pineals appeared histologically normal except for the presence of this muscle. The significance, if any, of these findings is not known.

B. Functions of the Pineal Gland

1. Historical Observations

Men knew of the existence of the pineal gland before 200 A.D. Kitay and Altschule (1954a), Tilney and Warren (1919) and Wurtman and Axelrod (1965) reviewed some of the early historical writings. Early Greek anatomists concluded that the pineal served as a valve over the aqueduct of the cerebrum, regulating the flow of thought. In the 16th century, Galen showed that the pineal was not a valve (he thought that the cerebellar vermi was the thought sphincter), but probably was a gland, perhaps similar to the lymph glands. In the 17th century, Descartes postulated that the pineal contained the seat of the rational soul. In this theory, the eyes perceived the events of the world and transmitted this information to the pineal via "strings" in the brain.

The pineal responded by allowing humors to pass down tubes to muscles which produced the appropriate reaction. These and many other early investigators presented theories which generally fit into one of three categories: 1. The pineal acts as a valve or sphincter, 2. The pineal is the seat of the soul or mind or 3. The pineal is a gland with secretory function.

The name "conarium" was used for the pineal gland, by early dissectionists, according to Galen. This name was derived from the conical shape of the body (Kitay and Altschule, 1954a). Other names reported by Rubin (1952) and Tilney and Warren (1919) include "epiphysis" or "epiphysis cerebri," which are descriptive of the location in the brain. Some writers called it the "glanula superior" in contradistinction to the pituitary gland which was called the "glandula inferior." The terms "corpus pineal," "pineal body" and "pineal gland" resulted from resemblance to a small pine cone.

2. Photoreception vs. Secretory Activity

The pineal gland is primarily a photoreceptive organ in fishes, amphibians, and lacertilian reptiles. These organs are described by Kappers (1965) and Tilney and Warren (1919). Dolt (1963) and Pang (1965) have published recent papers dealing with the direct stimulation of the pineal by light in rainbow trout and



killifish, respectively. Kelly (1962) reviewed the work on pineal photoreceptor function.

Kappers (1965) and Tilney and Warren (1919) reported the lack of discovery of any photoreceptor cells in the pineal bodies of birds or mammals. Being descendants from reptiles, birds might be expected to show some vestige of the parietal eye structure. However, as Cobb and Edinger (1962) state, "...parietal eyes were lost, not within the evolution of birds, but in remote reptilian ancestors some 800 million years before the first, late Jurassic, appearance of feathered animals, and presumably more than 100 million years before the modern type of avian brain was evolved."

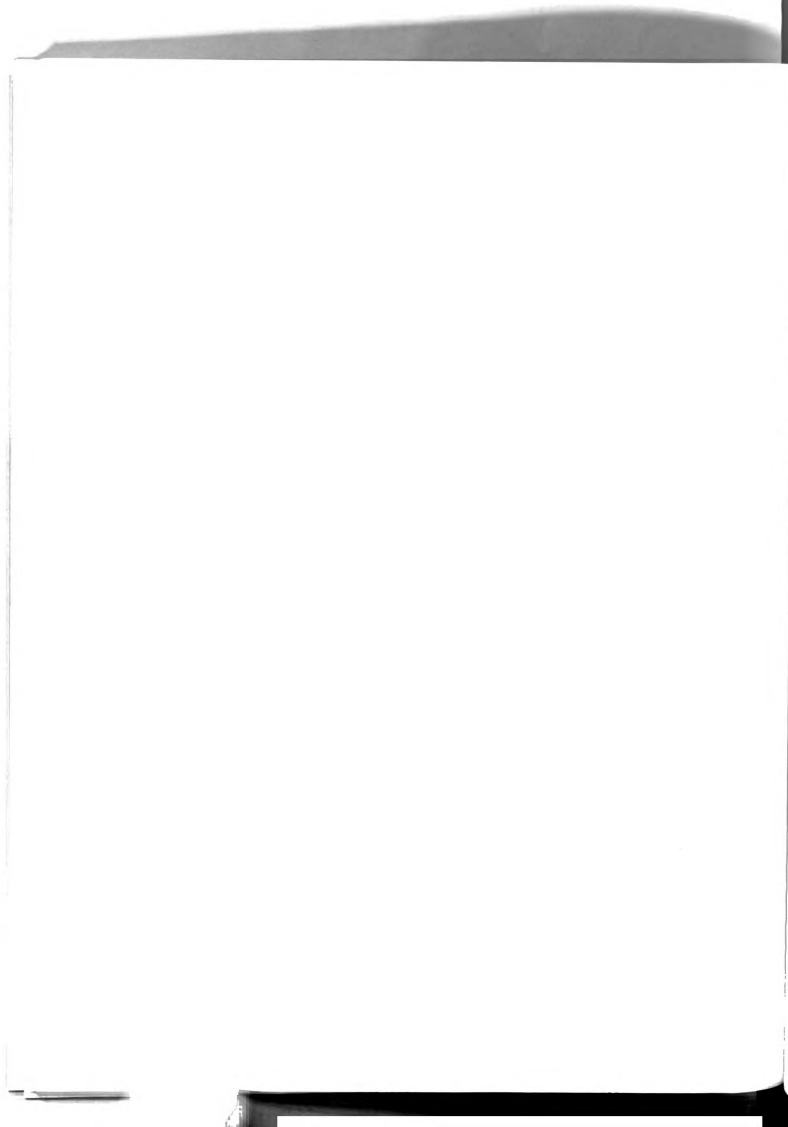
In birds and mammals the specific pineal parenchymal cells, pinealocytes, are secretory. Holmgren (1958) demonstrated the presence of secretory material in the pineal gland of monkeys by aldehyde-fuchsin staining of pineal sections which had been oxidized in performic acid. Quay (1956) used differential staining techniques to demonstrate secretory material in pineal parenchymal cells of rats. Electron microscopic studies by Rodin and Turner (1965) showed the presence of granules in vesicles lying in the perivascular spaces of the rat pineal body.



3. Clinical Observations.

Heubner described the first clinical case in 1898, relating pineal tumors to gonadal function (Kitay, 1954b; Kitay and Altschule, 1954a; Relkins, 1966; Wurtman and Axelrod, 1965). Heubner's report described precocious bodily and sexual development in a young boy who was found to have a pineal tumor. Subsequent reports of clinical cases associated pineal tumors both with precocious and delayed somatic and sexual development. These conflicting reports led to three groups of theories concerning pineal function as follows: that the pineal gland stimulates somatic and sexual development; that it inhibits somatic and sexual development and that it has neither effect.

Kitay (1954b) reviewed 145 reports of verified cases of pineal tumors in boys under 16 years of age. He found that precocious development tended to be associated with tumors of the nonparenchymal tissue, whereas delayed sexual development tended to be associated with tumors of the parenchymal tissue. In the case of nonparenchymal neoplasms, the parenchymal tissue is often destroyed, preventing normal function of the pineal. Parenchymal tumors, on the other hand, increase the pineal activity. The secretion and/or release by the normal pineal of a substance having an inhibitory effect on the gonads is indicated by these findings. One must assume that the pineal function was maintained almost normally in cases



where tumors of either type produced no apparent effect on development. In a few cases precocious puberty was observed with parenchymal tumors and delayed development with nonparenchymal tumors--opposite from the usual results. In the first case, one must assume that parenchymal tumors do not always secrete. In support of this assumption, some pinealomas were found to be composed of cells so undifferentiated and necrotic that secretion was highly unlikely. No evidence was shown to explain the delayed development with nonparenchymal tumors, which would require increased pineal secretion.

4. Results of Pinealectomy

Many investigators have removed the pineal gland in order to determine its function. Stalsberg (1965) removed the pineal from 6-day chick embryos with negative results. No changes were observed in pre- and post-hatching survival, incubation period, body weight, skeletal size, testes, ovary or comb characteristics, when compared to unoperated controls.

Postnatal pinealectomy of birds and mammals has given contradictory results. Foa (1912, 1914) and Izawa (1923) observed stimulated growth, gonadal hypertrophy, increased comb size and early sexual activity in response to pinealectomy of young male chicks. In a similar experiment, Badertscher (1924) failed to obtain any of these results.



Shellabarger (1953) and Shellabarger and Breneman (1949) surgically removed the pineals from male chicks at 4 days of age. Autopsy at various ages showed that the testes of the pinealectomized chicks were significantly smaller at 19 days of age, were not different at 28 to 40 days of age, were significantly larger at 42 to 70 days of age and were again not different at 94 days of age. The reason for the pinealectomized birds having smaller testes at 19 days was unclear. The fact that his sham-operated birds actually had slightly larger testes than those of the controls would indicate that the trauma of the operation was not responsible. He concluded that sometime after 70 days of age, the pineal body has no effect on the testes of chickens. It should be noted that at 94 days, the testes of all birds had gained appreciable weight, indicating that they had started the puberal phase of rapid growth. In another experiment, Shellabarger (1952) again found that pinealectomy reduced the 20-day testes weight of chicks.

Andersen and Wolf (1934) and Davis and Martin (1941) extirpated the pineal of very young rats. They observed no influence on the growth rate, testes weight or age at puberty. In similar pinealectomy experiments, Izawa (1926) obtained accelerated body growth and enlarged testes and epididymides. Dill (1961) pinealectomized

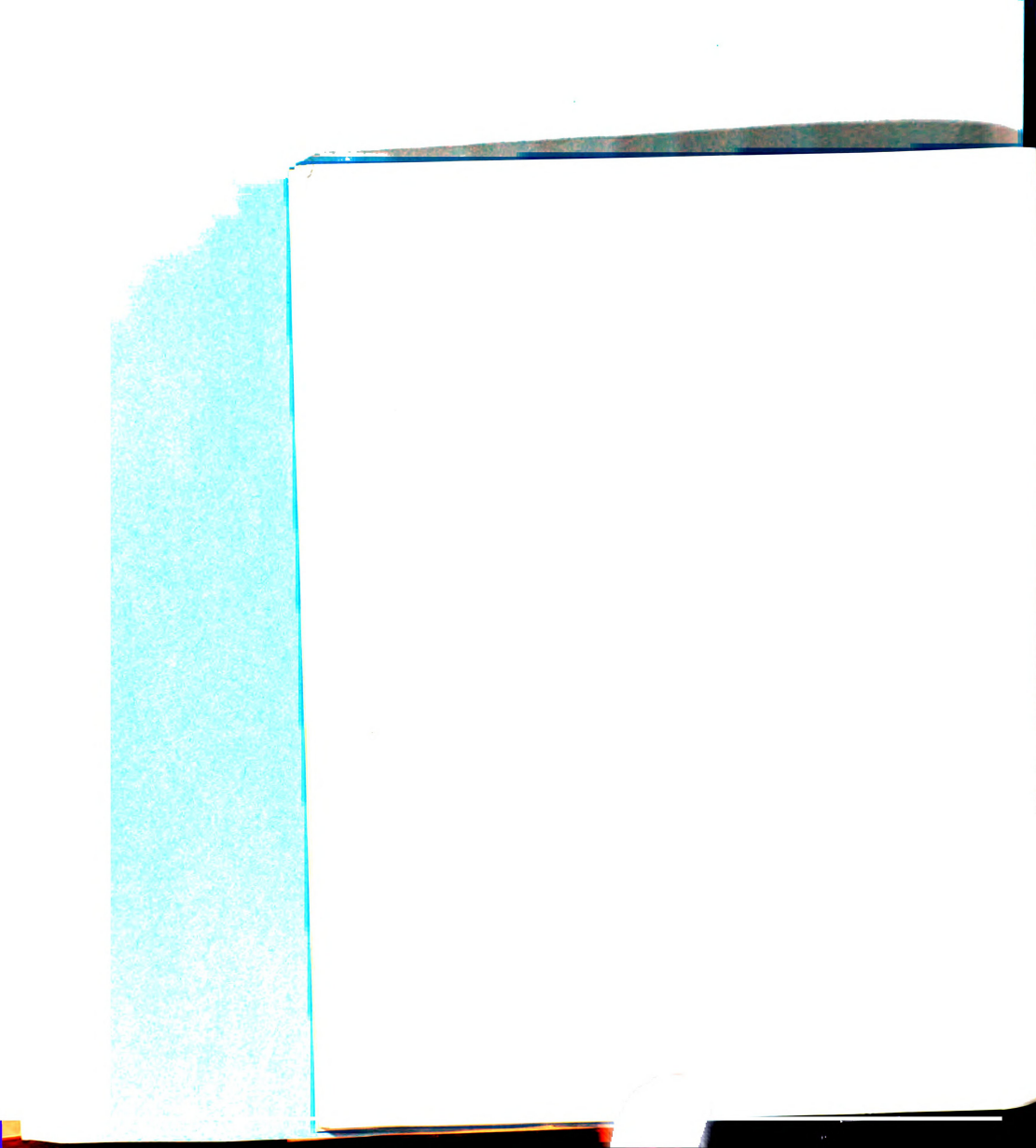
rats at 26 days of age, and observed a significant gonadal hypertrophy at 6 weeks after the operation.

Cutting the mid-sagittal sinus to serve as sham-operated controls also resulted in gonadal hypertrophy (Dill, 1961). The use of these animals as a control could be questioned. Depending on the exact location of the incision, there is a possibility that the nervous supply to the pineal may have been disturbed, which would produce a similar effect to that of pinealectomy.

Renton and Rusbridge (1933) found no consistent or significant change in testicular weights after removal of the pineal of 26-day old rats. Sullens and Overholser (1941) obtained no differences in somatic or sexual development between control rats and those which were pinealectomized at 3 weeks of age for three successive generations. D'Amour and D'Amour (1937) in a similar experiment found no differences in sexual maturity, but observed a distinct weight increase in the third and fourth generations. Although the experimental numbers were small, there was some evidence that this increased growth becomes more marked in successive generations. However, Einhorn and Rowntree (1939) obtained no effect of pinealectomy of successive generations on growth or development of offspring.

Dandy (1915) removed the pineals of young puppies and was unable to show any evidence of somatic, sexual or mental precocity or retardation, nor any macro- or microscopic changes of the testes or ovaries. Davis and Martin (1941) and Martin and Davis (1941) pinealectomized cats by electrocautery at 6 to 7 weeks of age. The operated males were heavier and had larger skeletons than did the controls. The pinealectomized animals also showed sexual interest earlier and had larger external genitalia. The females showed no difference in somatic development and estrus was not observed earlier in the pinealectomized group than in the controls. Pinealectomized mothers had small, weak litters. They often showed little maternal instinct and usually had an inadequate milk supply. Hoffman and Reiter (1965a, 1965b) found that pinealectomy prevented the expected atrophy of the testes of mature hamsters placed in a cyclic photoperiod consisting of 1 hour of light per day. Histological analysis showed the testes to be normal in appearance. Bilateral enucleation of pinealectomized hamsters had no effect on gonadal weight or histology. Bilateral enucleation of control or sham-operated animals resulted in significant atrophy of the gonads.

Both Badertscher (1924) and Foa (1914) were unable to observe any precocious sexual maturity in pinealectomized female chicks. On the other hand, Izawa (1923) obtained



enlarged gonads and combs in 200-day old hens which had been pinealectomized at 24 to 35 days of age.

Pinealectomy of rats caused increased ovarian development in experiments by Gittes and Chu (1965), Izawa (1926), Kitay (1954a) and Wurtman et al. (1959, 1960b, 1961).

Negative results have been reported by Andersen and Wolf (1934), Renton and Rusbridge (1933) and Wragg (1965). Izawa (1926) observed increased uterine growth following

pinealectomy, whereas Wurtman et al. (1960b and 1961) and

Wragg (1965) found no differences. Reiter et al. (1966)

showed that pinealectomy could prevent the expected regression of the uteri of thiouracil-treated hamsters.

Wragg (1965) also reported that pinealectomy had no effect on sexual maturity or the estrous cycle of rats.

Andersen and Wolf (1934) confirmed the lack of effect on both sexual maturity and the estrous cycle. Further sup-

port for the negative results of pinealectomy on the estrous

cycle was given by Ifft (1962). Chu et al. (1964) and

Gittes and Chu (1965), however, observed an increase in the incidence of estrous phases following pinealectomy.

Previous references cited contradictory results on the relationship between pinealectomy and growth rates in males and in mixed groups of animals. Malm et al. (1959) observed significantly greater weight increases in



pinealectomized adult male rats up to the 14th week postoperatively. After the 16th week, the weights did not vary. Gittes and Chu (1965) and Kitay (1954a) removed the pineals of immature rats and found no differences in their adult body weight compared with sham-operated or unoperated controls. Newborn female rats were pinealectomized by Wragg (1965) with negative results on body weight at 60 days of age.

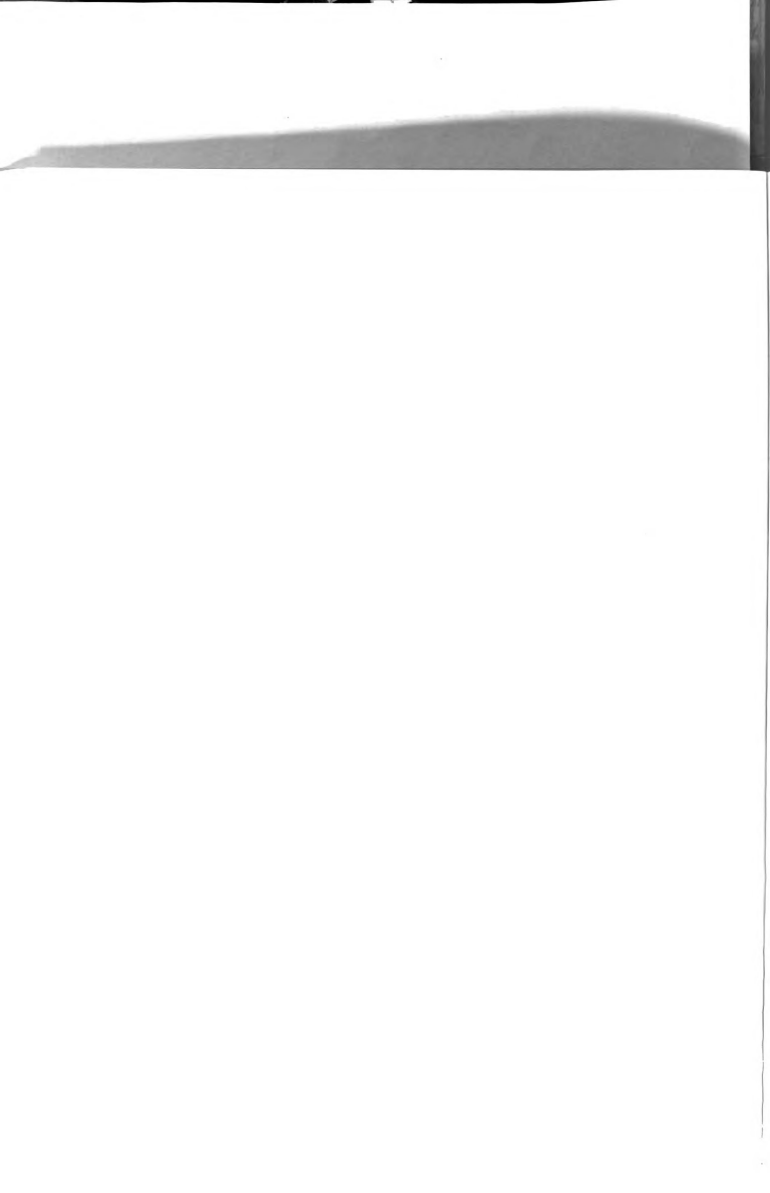
Gonadal changes in response to pinealectomy has been the primary area of study. However, the effect on other parameters has been noted in conjunction with these studies and in separate experiments. Pinealectomy of 6-day chick embryos produced no changes in parathyroid, thymus or spleen weights at 18 or 63 days post-hatching according to Stalsberg (1965). In addition, there were no differences observed in the weight or the histological appearance of the adenohipophysis, thyroids or adrenals. D'Amour and D'Amour (1937) obtained no effect on the weights of endocrine organs of rats pinealectomized at 1 to 3 days of age. Izawa (1923) had similar results with chicks pinealectomized at 24 to 35 days of age and examined at 200 days of age.

Adrenal weights were unaffected by pinealectomy in experiments by Shellabarger and Breneman (1949), using chickens; by Andersen and Wolf (1934), Izawa (1926) and Wragg (1965), using rats; and by Hoffman and Reiter (1965b) with hamsters. Experiments by Dill (1961), Farrell (1960b)

and Wurtman et al. (1959, 1960b, 1961) showed a hypertrophic effect of pinealectomy on the adrenal gland of rats, although Dill's results were not significant.

Dandy (1915) and Davis and Martin (1941) were unable to show any histological alterations of the adrenals in pinealectomized dogs and cats, respectively. Renton and Rusbridge (1933) found no differences in weight or in the relative proportion of cortical and medullary substances in adult rats which had been pinealectomized at 26 days of age. Similar results were found by Panagiotis and Hungerford (1961) with rats on both control and low sodium diets. Both pinealectomized animals and their controls responded to the low sodium regimen with equivalent hypertrophy of cells of the zona glomerulosa. These results were confirmed by Wurtman et al. (1960a). Microscopic examination showed no change in the adrenals of rats which had been pinealectomized for three successive generations, according to Sullens and Overholser (1941).

Malm et al. (1959) reported that pinealectomy failed to alter the phosphorus uptake (a measure of metabolic activity) by the adrenal. Keeler (1961) obtained increased sodium excretion in pinealectomized rats. Tanner and Hungerford (1962) observed the opposite effect; that is, decreased sodium excretion following pinealectomy. Farrell (1960b) pinealectomized dogs, resulting in an acute reduction in aldosterone secretion, however, the steroid

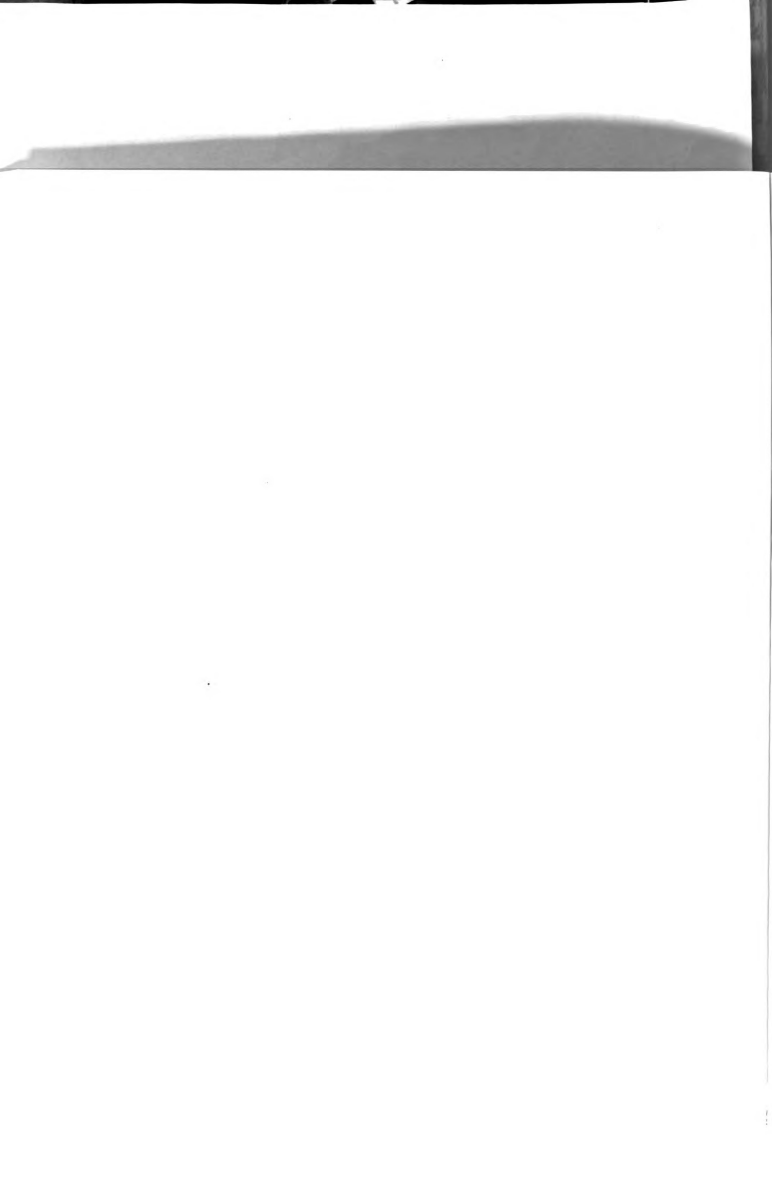


Output returned to or above normal levels within a week.

An attempt by Davis (1960) to confirm these results yielded negative results. Kitay (1963) observed increased levels of ACTH in the pituitary, but no change in plasma corticoids following pinealectomy. These seemingly contradictory observations might be partially explained by the proposed existence of two substances in the pineal and/or surrounding tissue--one being inhibitory and the other stimulatory on adrenal activity. These will be discussed in greater detail in another section.

Andersen and Wolf (1934), Renton and Rusbridge (1933) and Wragg (1965) found no weight change in the pituitary after pinealectomizing rats. Shellabarger and Breneman (1949) obtained confirmatory results with chickens. However, Wurtman et al. (1959) observed significant pituitary hypertrophy in pinealectomized rats, while Izawa (1926) reported retardation in the females only. The absence of histological changes in the pituitary following pinealectomy has been reported in rats (Holmes, 1956; Sullens and Overholser, 1941), and dogs (Dandy, 1915). Malm et al. (1959) found no change in the pituitary metabolic activity of rats compared to sham-operated controls.

Studies of the effect of pinealectomy on thyroid weight (Andersen and Wolf, 1934; Izawa, 1926; Renton and Rusbridge, 1933; Shellabarger and Breneman, 1949), histology (Dandy, 1915; Davis and Martin, 1941; Sullens and Overholser, 1941)



and metabolic activity (Malm et al., 1959) have yielded negative results in birds and laboratory mammals. Likewise, no effect of pinealectomy has been noted for the thymus (Andersen and Wolf, 1934; Dandy, 1915; Malm et al., 1959; Sullens and Overholser, 1941), pancreas (Dandy, 1915; Sullens and Overholser, 1941), parathyroids (Dandy, 1915), liver (Dandy, 1915), spleen (Dandy, 1915), brain (Izawa, 1926; Malm et al., 1959) and other body tissues (Dandy, 1915; Izawa, 1926; Sullens and Overholser, 1941). Two investigators measured the eyeballs of animals. Retardation of eye growth was reported in chicks (Izawa, 1923) and rats (Izawa, 1926) following pinealectomy. Renton and Rusbridge (1933) found no significant effect in rats.

Davis and Martin (1941) noted no behaviour changes in rats, but judged pinealectomized cats to be more aggressive and belligerent. Reiss et al. (1963b) observed that pinealectomized rats were more active than their littermate controls.

5. Results of Pineal Extract Injections and Pineal Transplants

The close anatomical relationship between the pineal gland and the hypothalamus led to the speculation that the effect of pinealectomy actually resulted from the disturbance of nearby hypothalamic centers known to effect endocrine activities. Studies to determine the effect of

Pineal extract injections and pineal transplants in both pinealectomized and unoperated animals have been reported by several researchers.

Takacs (1935) fed dried calf pineal tissue to young fowl at 10 to 35 milligrams per day levels. After 4 months the experimental birds weighed over 200 percent more than the controls. After 5 months, the difference was still marked, but less pronounced. Similar growth promoting effects of feeding pineal tissue were observed by McCord (1914) in chicks and guinea pigs. He also found that pineals from immature donors gave more striking results. Test chicks fed veal pineal for 1 week grew more than their controls. For the next 4 weeks, they were fed pineal tissue from old cattle and failed to gain more than the controls. Switching back to the veal preparation, gains were again increased over those of the controls. McCord (1914) also noted that while feeding pineal tissue growth was stimulated and the material did not cause the animals to grow beyond normal adult size. As the test animals approached maturity, the treatment became less and less effective. There were also less well-established indications of accelerated mental and sexual development in animals fed the pineal tissue.

Lyophilized beef pineal tissue in distilled water was injected into intact and pinealectomized chicks by Shellabarger (1952) at a level of 5 milligrams of pineal



material daily. Non-operated chicks showed increased testis growth, while pinealectomy of chicks prevented the previously obtained inhibition of testis growth. In a subsequent report, Shellabarger (1953) reported the opposite effects; that is, testicular hypertrophy following pinealectomy which was prevented by injections of 5 milligrams of pineal material daily. The author notes that autopsies were made at different ages in the two tests (20 days in the first experiment and 40, 54 and 60 days in the latter one), suggesting, therefore, that the results might not be in conflict.

Four differently prepared pineal extracts were injected into young White Leghorn males by Shellabarger (1953). There were no differences in the testis weights of these experimental groups and their controls. Reiss et al. (1963a) determined the phosphorus metabolism in the gonads of mice, rats, rabbits and chickens as an indicator of the action of pineal extracts. Doses of extracts lower than those required for weight changes produced changes in the phosphorus uptake. Pineal extract inhibited the hypertrophy of the testes and combs of chickens usually produced by pituitary extract injections. Evidence was presented for the existence of two antagonistic fractions in the pineal, one inhibiting and the other stimulating gonadal activity. The effect of simple pineal extracts appeared to depend on the age of the test animals. In immature subjects, whose gonadal function is capable of being

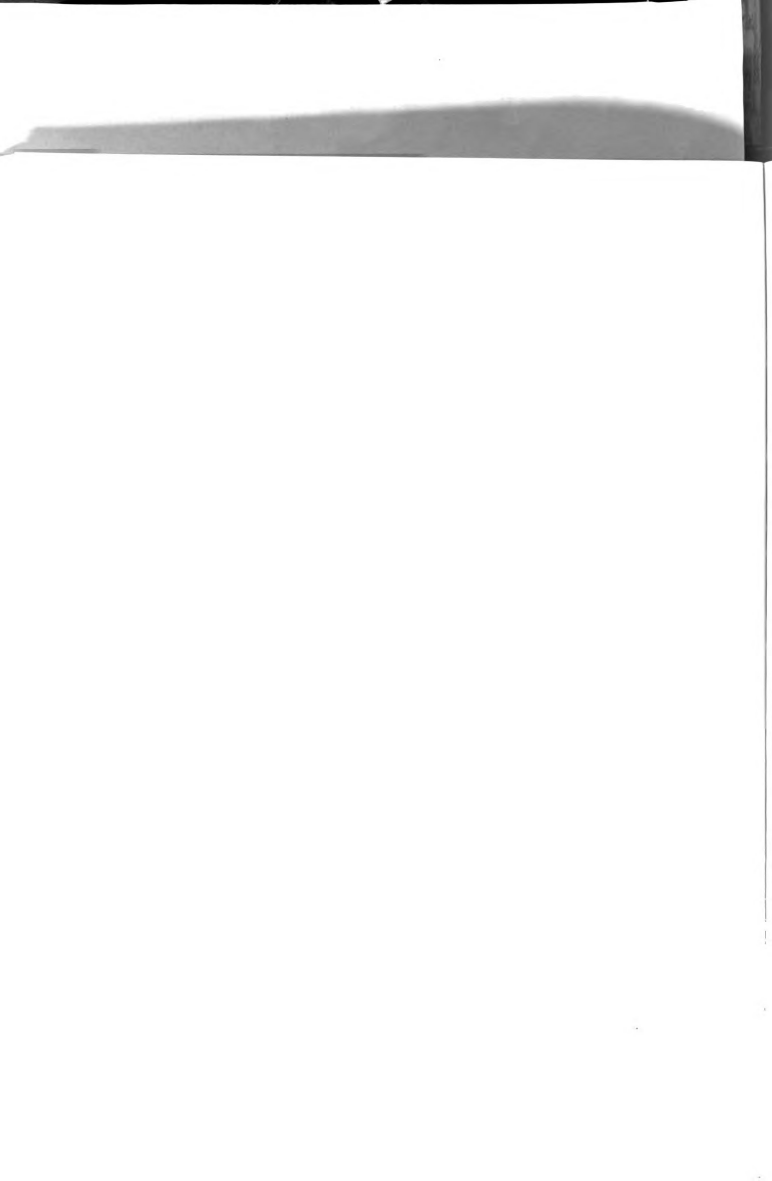
stimulated, both principles could be active, often leading to contradictory results. In adult animals, only the inhibitory effect has been observed.

Rowntree et al. (1936) gave successive generations of parent rats daily intraperitoneal injections of pineal extract while observing both the parents and their offspring. Increased irritability, sexual activity and gonadal weights were observed in the injected parents. Maternal instinct levels became quite variable. In the offspring, growth was retarded, but somatic and sexual development were accelerated. These effects became more marked in succeeding generations. The strength and activity of offspring of injected mothers were decreased, especially during the first 15 to 20 days. In this experiment, the controls were not injected with a carrier or placebo. Although the results were not analyzed, the data appear to be significant. In a later report, Einhorn and Rowntree (1939) gave additional data in support of the previous observations.

Frequent pineal implants in normal rats did not effect the rate of growth or development of their offspring (Einhorn and Rowntree, 1939). These implants did not persist in the test parents. Lahr (1932) implanted his experimental animals with one or two pineals daily, while the controls were similarly implanted with brain tissue. No difference was found in the growth rates. In both males and females, the gonads (testes and ovaries) were lighter

in weight and sexual maturity was delayed by 3 to 5 days after 31 to 41 days of pineal implantation compared to the control implants. Histological studies showed that the seminiferous tubules of the experimental group were consistently smaller and that spermatogenesis was retained. Kozelka (1933) found no differences in body weight or in comb growth when pineals from adults were implanted subcutaneously into young chicks (over 28- and 40-day periods) or when pineals from infant chicks or late stage embryos were implanted into half grown birds (over 47- to 59-day periods). All implants were reported to have been absorbed. It should be pointed out that each experimental group (different treatment procedures) consisted of only one bird.

Kitay and Altschule (1945b) observed decreased ovary weights in response to three experimental plans involving the intraperitoneal injection of extracts from bovine pineals. Rats injected for 2 weeks from 32 days of age had ovarian reductions of borderline significance when the equivalent of 50 milligrams of pineal powder was injected daily. Doubling the dosage (100 milligrams of pineal powder daily) produced highly significant inhibition. Rats given the higher dosage starting at 45 days of age showed only borderline significance, indicating a decreasing effect with animals approaching maturity. Wurtman et al. (1961) showed that the increase in ovarian weight of rats placed in continuous light was prevented by the intraperitoneal



injection of bovine pineal extracts. However, the uterus hypertrophied as usual under these conditions. Wurtman et al. (1959) also found that pineal extract would prevent hypertrophy of the ovary following pinealectomy. The effect of pineal extract on the intact animal varied with the dose. Daily injections of 0.3 milliliters caused significant gonadal atrophy ($P < .05$), while 1.0 milliliters dosage levels caused atrophy, significant at the 0.1 percent level. Soffer et al. (1965) observed the inhibition of the uterine stimulatory effect of both human chorionic gonadotropin and human menopausal gonadotropin by the injection of bovine pineal extracts in laboratory mice. The extract failed, however, to produce any such inhibition against oestrone.

Rats have a post-reproductive period at about 1 to 2 years of age, in which they show persistent estrus. Meyer et al. (1961) altered this persistent state of estrus by injecting 1 or 2 milliliters of a protein-free bovine extract; but they found no influence on the regularity of the estrous cycle in younger adult animals given 0.5 or 1.0 milliliter injections. Exposure to continuous light induces prolonged estrus in rats. Ifft (1962) found that daily 1 milliliter injections of bovine pineal extract did not alter this occurrence, but daily 2 milliliter injections caused a marked increase in the diestrus phase of the estrous cycle. Two milliliters of a brain extract (control injection) was ineffective.

Ifft (1962) also transplanted pineals from newborn rats into the kidney capsules of adult rats in continuous light. Those animals in which the transplanted pineal appeared viable (histological examination) were considered the experimental group while those which did not persist were used as controls. No differences could be shown in the estrous cycles of the two groups. The possibility exists that the transplants which failed to survive the 30 to 50 days of testing, the controls, actually were active for some portion of this time, thus influencing the early results. Moszkowska (1958) caused precocious puberty in female rats by grafting an anterior pituitary from a mature male rat on the ovary. The additional grafting of four pineals clearly decreased this effect. The intramuscular transplantation of 10 or 20 isogeneic pineals into pinealectomized rats consistently reversed the ovarian hypertrophy seen in sham-transplant, pinealectomized control animals (Gittes and Chu, 1965). Histologically, the pineal transplants showed rich vascularization and good overall appearance. However, the authors did show that pineal HIOMT enzyme activity was very low. They suggested that this enzyme level was probably dependent on nervous innervation of the pineal. Holmes (1957) described successful intraocular transplants of pineals in adult rats and rabbits. Autographs attached to the iris, became vascularized and showed persistence of healthy tissue. Homografts of adult tissue became

vascularized and developed, but showed evidence of an antigenic reaction.

Ebels et al. (1965) obtained two active pineal fractions: one capable of stimulating pituitary FSH excretion and the other capable of diminishing pituitary FSH excretion in vitro. Moszkowska (1963) observed an inhibitory effect on FSH excretion when pineals were included in a synthetic incubating medium for pituitary glands. Significant pituitary atrophy resulted when Wurtman et al. (1959) injected 26-day old rats with protein-free bovine pineal extract.

Little work has been done on the effects of pineal extracts on factors other than the gonads and adrenals. Anton-Tay (1965) found that extract of pineal glands can depress thyroid function, presumably through a blockage of the synthesis and/or release of pituitary TSH. Thyroidal response to cold was markedly decreased in rabbits given injections of pineal extracts. Reiss et al. (1963a) suggested that the pineal contains inhibitory and stimulatory factors which act not only on the gonads, but also on the pituitary, thyroid and adrenal glands. An inhibitory factor might also reduce the metabolic activity of the liver.

Kitay (1963) found no effect of pineal gland extracts on the weight of adrenal glands. Daily doses of 0.3

millileters of bovine pineal extract caused insignificant adrenal atrophy in immature rats, according to Wurtman et al. (1959), although increasing the dosage level to 1.0 milliliters daily caused significant atrophy. Dill (1961) obtained adrenal hypertrophy by injecting an aqueous extract of beef pineal glands in immature rats. This was accompanied by a pronounced rise in plasma and adrenal corticosterone. However, he also observed adrenal enlargement when a control extract of calf brain was injected, although this treatment did not affect the corticosteroid levels. An acetone extract of beef pineals showed no effect on the factors measured. Since Wurtman et al. (1960a) found that bovine pineal extract administrations caused no change in the size and lipid content of the adrenal zona glomerulosa, the urinary potassium or the sodium and water intake, they suggested that the pineal hormone probably did not have a specific effect on the secretion or action of aldosterone.

Lommer (1966) reduced the corticosteroid production in incubated slices of bovine adrenal cortex through the addition of a hexane extract of bovine pineal tissue. It was suggested that the pineal contains one or more substances which inhibit the 11-hydroxylation of corticosteroids. Lucis et al. (1961) added homogenized diencephalon tissue (including the pineal gland) to a medium in which quartered rat adrenals were incubated.



The secretion of aldosterone was increased without increasing that of corticosterone. A commercial pineal powder and two extracts of pineal failed to affect the secretion of either aldosterone or corticosterone.

Taylor (1960) suggested that the subcommissural area may be a locus of stimulation, either humoral or neural, whereas the pineal is probably the source of inhibitory influences on aldosterone regulatory mechanisms. In preliminary studies, Everitt and Huang (1962) found evidence of an adrenocortical inhibitory factor in an acetone insoluble extract of sheep pineals.

C. Active Pineal Substances

1. Adrenoglomerulotropin and Anticorticotropin

In 1959, Farrell (1959a) observed that the pineal complex contained a factor capable of stimulating aldosterone secretion. Further studies (Farrell, 1959b, 1960a, 1960b, 1960c) added support to this observation. Farrell first proposed the name "glomerulotropin" for the active factor which he presumed to be a hormone type of substance. The name was derived from the fact that aldosterone is secreted by the zona glomerulosa, therefore, the active substance should be exerting its effect on that portion of the adrenal. The possibility of confusion was recognized in that the name could imply an effect on the glomerulus of the kidney. Thus, the suggested name was changed to "adrenoglomerulotropin." During efforts to purify adrenoglomerulotropin, an inhibitory factor was

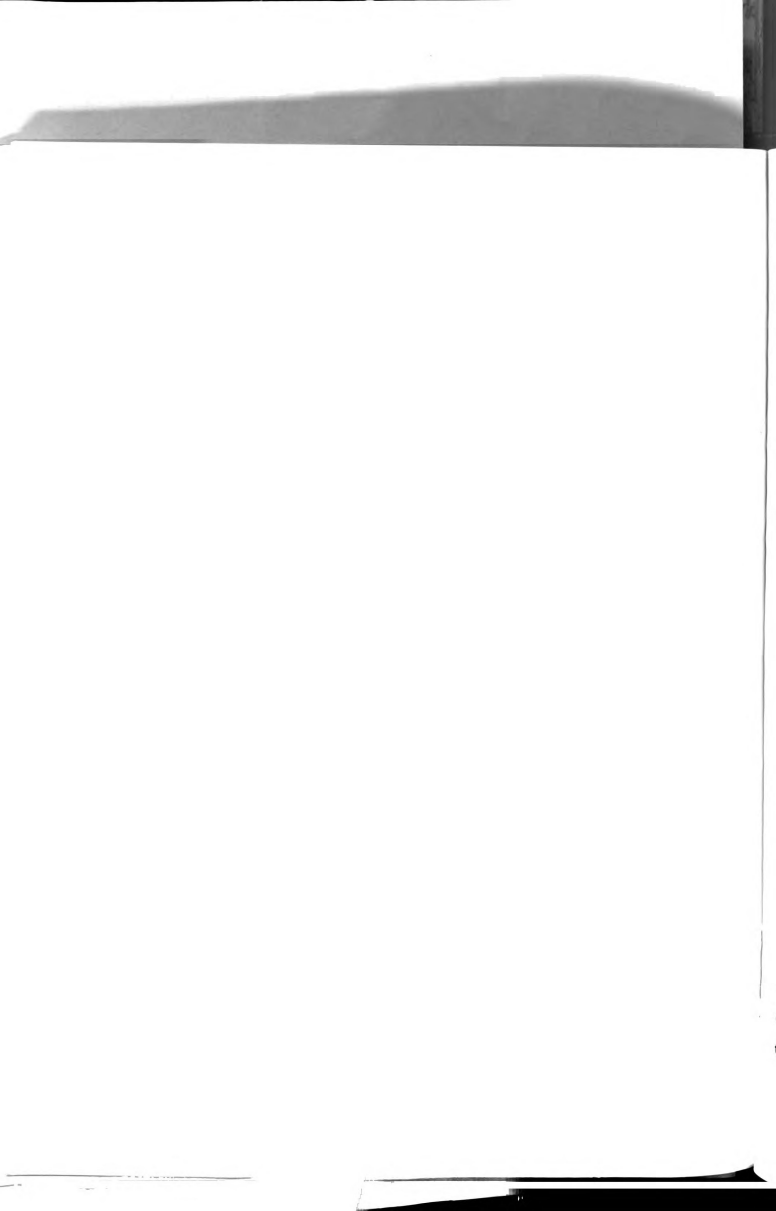
found. This new fraction seemed to inhibit both aldosterone and to a lesser extent, cortisol. This substance was termed "anticorticotropin." This factor appears to compete with pituitary corticotropin and with adrenoglomerulotropin; the rate of adrenal steroid secretion being dependent on the balance between these factors.

Farrell and McIsaac (1961) reported the isolation and identification of adrenoglomerulotropin two years after it was first proposed to exist. It was identified as 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline, which was derived from 5-methoxytryptamine. Taylor and Farrell (1963) pointed out that this may not be the exact structure of the natural substance, but submicrogram doses failed to increase the secretion of cortisol. Panagiotis and Hungerford (1961) observed a loss of stainable fat in histological sections of pineal glands following sodium restriction. This was thought to indicate an augmented activity in the pineal cells, probably in releasing a hormone (adrenoglomerulotropin) involved in salt retention.

Machado and da Silva (1963) showed that rats injected with pineal body extract exhibited strong sodium retention, comparable to that obtained by injecting 10 micrograms of aldosterone. Adrenalectomized animals did not show these results indicating that the extract

acted through the adrenal, rather than directly to cause sodium retention. It was assumed that the pineal extract contained adrenoglomerulotropin, which acted on the adrenal gland. Some doubt remains as to the exact source of adrenoglomerulotropin. Its presence in pineal extracts does not exclude the possibility that it actually comes from adjacent structures which are removed with the pineal. Since pinealectomy and midbrain lesions do not prevent all experimental hyperaldosteronism, Keeler (1961) suggested that the anterior central midbrain area and the pineal cannot be the only sources of the adrenoglomerulotropic hormone. Tanner and Hungerford (1962) noted an increase in sodium retention of pinealectomized rats. If the adrenglomerulotropic effect had been removed by pineal ablation one would have expected the opposite result; increased sodium excretion. Their results suggested the removal of a factor which inhibits aldosterone secretion.

Studies previously cited demonstrated a lipid soluble factor in pineal extracts which inhibits aldosterone activity. Fabre et al. (1965) found ubiquinone in the lipid fraction of pineal extracts and indicated that it reduced aldosterone secretion rates following intravenous infusion of the pineal lipid fraction into intact dogs.



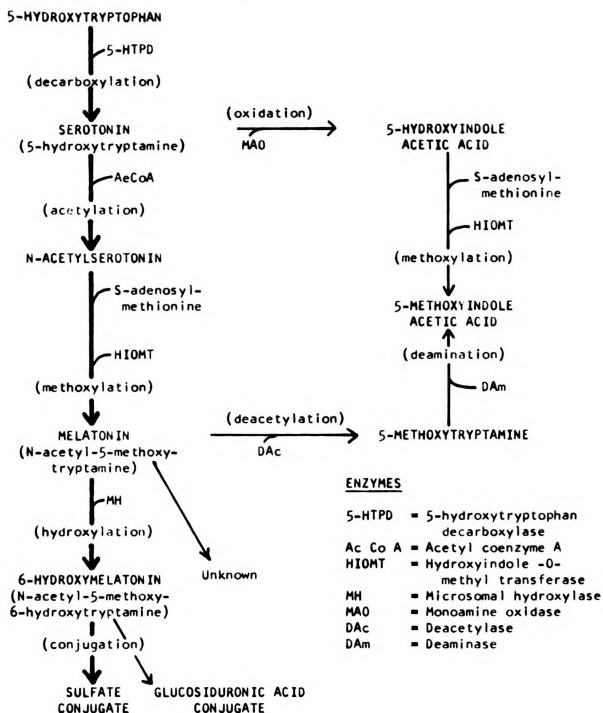
2. Melatonin

Lerner et al. (1958) first isolated the active factor from bovine pineal glands that lightens amphibian skin color and inhibits MSH. They suggested that this substance be called "melatonin." No melatonin activity was found in bovine pituitaries, hypothalamus, thymus, thyroids, adrenals, ovaries, testes or eyes. The following year they identified the structure of melatonin as N-acetyl-5-methoxytryptamine (Lerner et al., 1959a). The isolation of melatonin from other tissues will be described later.

Axelrod and Weissbach (1960) isolated the enzyme hydroxyindole-O-methyl transferase (HIOMT) from the pineals of several mammalian species, and found it to be present only in the pineal. The suggested melatonin metabolic scheme is shown in Figure 1. This was worked out from the findings of many authors (Axelrod and Weissbach, 1960, 1961; Kopin et al., 1960, 1961; Lerner et al., 1960; McIsaac et al., 1965; Weissbach and Axelrod, 1960; Weissbach et al., 1960, 1961).

Tryptophan is the precursor of the melatonin pathway. Under the influence of tryptophan hydroxylase (not shown in Figure 1), it is converted to 5-hydroxytryptophan, which becomes 5-hydroxytryptamine or serotonin in the presence of the 5-hydroxytryptophan decarboxylase (5-HTPD) enzyme. Serotonin is acetylated in the presence of acetyl-coenzyme A to form N-acetylserotonin. The N-acetylserotonin reacts with

Figure 1.--Suggested Melatonin Metabolism Pathway



S-adenosylmethionine in the presence of a methylating enzyme, hydroxyindole-O-methyl transferase (HIOMT), to form melatonin. Three metabolites of melatonin have been described. The primary one is 6-hydroxymelatonin, formed in the presence of microsomal hydroxylase enzyme. The 6-hydroxymelatonin is then conjugated with sulfates or glucosiduronic acid to be excreted. These conjugate forms account for 70 and 6 percent, respectively, of the excreted melatonin derivatives. About 12 percent is excreted in a form which has been only partially characterized. It appears that the indole nucleus of this substance is altered. Trace amounts (less than 1 percent) of the melatonin are deacetylated to form 5-methoxytryptamine and then deaminated to form 5-methoxyindole acetic acid which is excreted. A portion of the serotonin is also excreted as 5-methoxyindole acetic acid, rather than going to form melatonin. This pathway requires monoamine oxidase for conversion to 5-hydroxyindole acetic acid, which is then methylated to form the excretory product. The methylation step requires S-adenosylmethionine and the enzyme HIOMT. Both of these were also used in the conversion of N-acetylserotonin to melatonin.

Cohen et al. (1964) pointed out that melatonin satisfies many of the criteria of a hormone. It is produced by a highly specialized glandular structure; in fact, the HIOMT enzyme has been found only in the pineal gland. It is released into the circulatory system and affects a distant target organ or organs. It can also be taken up

preferentially by the target organ. Additional evidence to support these statements was provided by Lerner et al. (1959b) when they demonstrated the presence of melatonin in the peripheral nerves of man, monkey and cow. Barchas and Lerner (1964) provided additional evidence for the localization of melatonin in the peripheral nerves when they showed that the methylating enzyme, HIOMT, was found in the pineal, but not in other portions of the central or nervous systems. These observations indicated that the pineal must release melatonin into the circulatory system from which it is picked up by peripheral nerves. Axelrod et al. (1963) and Wurtman et al. (1964e) injected labeled melatonin to trace its uptake in body tissues of cats and rats. They found some melatonin in all tissues examined 1 hour after injection. The pineal concentrated the labeled melatonin 40 fold over the plasma level. The ovary and iris-choroid layer of the eye concentrated the hormone 10 fold. Other endocrine glands, peripheral nerve and the sympathetic chain concentrated melatonin 3 to 5 fold. As might be expected, adipose tissue had the lowest concentration of all tissues tested. The high level of melatonin uptake cannot be accounted for by hemodynamic factors, indicating the presence of a selective concentration mechanism.

Following the isolation and identification of melatonin as the factor responsible for the lightening reaction of frog skin to pineal extracts, experiments were carried out

to determine its function in mammals. Body weight was not affected by the daily injection of 20 micrograms of melatonin in rats starting at 27 days of age and continuing for 28 days (McIsaac et al., 1964). Wurtman et al. (1963a) found no difference at the 50 microgram level. Body weight was lowered in both sexes when Tilstra and Prop (1963) gave daily injections of 100 micrograms from birth to 50 days of age. Since both the injection levels and the age of treatment varied, one cannot be sure which factor caused the difference in results.

Gonadal stimulation was observed by Thiebolt et al. (1966a) following the injection of relatively large daily doses of melatonin (100 to 1000 micrograms) for 15 days. In male rats the seminal vesicles were markedly enlarged, while the testis and prostate weights increased somewhat, but remained in the range of individual variation. Similar treatment with serotonin produced no effect. Ebels and Prop (1965) and Dappers (1962) found no changes in testis weights after injecting doses of 30 to 150 micrograms daily. Kappers (1962) did note a possible inhibition of the seminal vesicles with a 500 microgram dosage level, but this involved only one experimental and one control animal. In females, the ovary weight of melatonin-injected rats was 40 to 50 percent larger, but the uterus showed no change in weight compared to the controls.

Serotonin treatment produced a slight, non-significant enlargement of the ovary. Kappers (1962), McIsaac et al. (1964) found no change in ovarian weights after injecting 20 or 100 micrograms of melatonin daily. The 28 day test period was started with 27-day old rats. Similar injections of 5-methoxytryptophol (closely related to melatonin) significantly lowered the ovary weights. Adams et al. (1965), Axelrod et al. (1963), Ebels and Prop (1965) and Wurtman et al. (1963a, 1964b) observed depression of ovary weights with melatonin injections. Dosage levels ranged from 1 to 150 micrograms per day. All rats were immature when treatments were started. Serotonin injections were without effect (Axelrod et al., 1963; Wurtman et al., 1963a) in microgram doses. O'Steen (1965) observed significantly depressed ovarian weights in rats after injecting 10 and 25 milligrams of serotonin per kilogram of body weight. Uterine weight appears to be unaffected by melatonin (Ebels and Prop, 1965; Wurtman and Axelrod, 1964) or serotonin injections (Ebels and Prop, 1965).

Sexual maturity was not affected by daily 100 microgram injections of melatonin given rats from birth to 50 days of age by Tilstra and Prop (1963). Adams et al. (1965), Axelrod et al. (1963) and Wurtman et al. (1963a) delayed sexual maturity by injections of 1 to 100 micrograms of the hormone starting at 2 to 4 weeks of age. Serotonin injections were without effect (Axelrod et al., 1963; Wurtman



et al., 1963a) except when given at levels of 10 and 25 milligrams per kilogram body weight (O'Steen, 1965).

Most reports agree that 1 to 20 microgram injections of melatonin given daily caused a decrease in the incidence of estrus in rats (Axelrod et al., 1963; Chu et al., 1964; McIsaac et al., 1964; Wurtman et al., 1963a, 1964b). Negative results were obtained with serotonin (Axelrod et al., 1963; Chu et al., 1964; Wurtman et al., 1963a), N-acetylserotonin (Chu et al., 1964), 6-hydroxymelatonin (Chu et al., 1964) and 5-methoxytryptophol (McIsaac et al., 1964).

To test for a possible neurotransmission activity of melatonin, segments of intestine and uterine horn were observed in vitro after known neurotransmitters and melatonin were added to the medium. Tilstra and Prop (1963) observed contractions with two neurotransmitters; acetylcholine and serotonin. Melatonin appeared completely inactive alone and did not appear to modify the action of the two active substances. These and similar results from Arutyunyan et al. (1963) support the view of Kappers (1962) that melatonin is not a neurotransmitter substance. However, in a recent paper, Hertz-Eshel and Rahamimoff (1965) reported that melatonin inhibited both spontaneous and serotonin-induced contractions of the rat uterus which had been sensitized during the previous 3 days by diethylstilbesterol treatments.



Two studies of the effect of melatonin injections on thyroid function in rats yielded contradictory results. Baschieri et al. (1963) reported that daily subcutaneous injections of 150 micrograms of melatonin for 10 days resulted in significantly lower thyroid weights and decreased thyroidal uptake of ^{131}I . Histological examination showed the height of the thyroid cells to be significantly reduced. Thiebolt et al. (1966b) administered 100 to 1000 micrograms of melatonin daily to young prepubertal rats and demonstrated marked thyroid cell hypertrophy.

Lerner et al. (1958) had first characterized melatonin as the substance which lightens the skin color of frogs through an aggregation of melanin granules within the cells. No influence was seen on mammalian pigmentation. Additional experiments were carried out by Snell (1965) which supported this lack of effect in mammals. Large doses of melatonin were injected subcutaneously into pure red and pure black male guinea pigs daily for 30 days. He observed no gross changes in skin pigmentation and found no histological evidence that melatonin caused any positional change in melanin-granules.

D. Factors Influencing Pineal Function

1. Alteration of Pineal Anatomy

It is well substantiated that continuous exposure of rats to light causes a decrease in weight of the pineal gland (Fiske et al., 1960; Quay, 1961; Roth et al., 1962;

Wurtman et al., 1960b), compared to that of animals on diurnal lighting schedules. Adrenalectomy (Fiske et al., 1962; Quay, 1961), gonadectomy (Fiske et al., 1962) or hypophysectomy (Fiske et al., 1962) did not prevent the effect of constant light on pineal weight. However, Quay (1961) found that bilateral intraorbital transection of the optic nerves blocked the effect of continuous light. Quay (1963b) also observed cytological and metabolic evidence of pineal inhibition in response to continuous light. Basrur and Winget (1963) compared the histology of the pineal gland of birds kept in constant darkness with that of birds kept under a diurnal light cycle. The only difference observed was the absence of magenta-colored granulations in the large epithelial cells of the pineal septum in birds kept in darkness. They concluded that these cells were light responsive. This report did not indicate the species of bird tested, the length of light treatment period or the age, sex and reproductive state of the birds.

The human pineal undergoes extensive calcification after puberty. Kitay and Altschule (1954a) reviewed some of the early writings in which the presence of calciferous areas, called brain sand or acervulus cerebri, were observed. It was generally thought that this calcification indicated that the pineal ceased to function at or shortly after puberty. Wurtman et al. (1964a) found no correlation



between age of the subject (3- to 70-year old humans) and pineal enzymatic activity. All pineals from subjects over 40 years of age showed obvious calcification. They concluded that the pineal probably remains active throughout life. DeMartino et al. (1963) demonstrated that the number of osmophilic granules in rat pineals increased with age. As these granules are thought to be a product of secretory activity, they suggested a possible increase in pineal activity following puberty.

Everitt and Huang (1962) showed that the pineal gland of the female rat (on a percentage of body weight basis) is larger than that of the male. Pregnancy reduced the pineal weight, an observation also reported by Huang and Everitt (1965). The latter paper indicated that the pineal weight of pregnant rats was inversely proportional to the number of fetuses being carried. Shellabarger (1953) reported hypertrophy of the pineal in White Leghorn capons compared to intact males. Castration of rats was reported to have no effect on pineal weight (Everitt and Huang, 1962).

Everitt and Huang also reported a negative effect of unilateral adrenalectomy on pineal weight (1962). Reiss et al. (1963c) reported that the pineal shows no atrophy following hypophysectomy, as do adrenals, thyroids and gonads. In contrast to the atrophied glands, the pineal phosphorus turnover increases.

2. Alteration of Pineal Metabolic Activity

Experiments have been conducted to determine the factors involved in control of melatonin production and/or release. Quay (1964) showed that the melatonin level in adult rat pineals follows a circadian rhythm and is correlated with light and dark periods. Melatonin content of the pineal rises at the start of the dark and decreases during light. Wurtman et al. (1964d) also observed a reduced synthesis of melatonin in rats when exposed to light.

Hydroxyindole-O-methyl transferase (HIOMT), a melatonin-synthesizing enzyme, also shows a diurnal rhythm when rats are kept under cyclic light treatments. When lighted from 7 a.m. to 7 p.m. enzyme activity was lowest at about 6 p.m. The activity rose about 3 fold to its maximum at midnight, followed by a decrease starting shortly before the next light period started (Axelrod, et al., 1965). Animals kept in constant light show a decrease in HIOMT, whereas animals kept in constant or nearly constant darkness show increased HIOMT activity (Axelrod et al., 1965; Wurtman and Axelrod, 1964; Wurtman et al., 1963b).

Quay (1963a, 1965a), Fiske (1964) and Snyder et al. (1965a, 1964b) showed the diurnal cycle of pineal serotonin content to be approximately opposite that of melatonin; that is, it is at its maximum at about noon, drops quite quickly at the start of the dark period to reach a nocturnal low at midnight and then gradually increases toward the noon level

With most of the increase occurring during the lighted portion of the morning. Quay (1963a) suggested that the sharp decline of pineal serotonin in the early dark period may represent release, destruction or a triggered release of melatonin synthesis from serotonin, with the latter explanation being favored.

The serotonin rhythm persists in constant darkness (Snyder et al., 1965a, 1964b, 1965c) but is abolished in constant light treatments (Quay, 1962; Quay and Halevy, 1962; Snyder et al., 1965a, 1964b, 1965c). The rhythm is abolished by the addition of as little as 4 hours of light, preventing the nocturnal decline in serotonin content of the pineal (Snyder et al., 1965a, 1965c). Snyder and Axelrod (1965) presented evidence to show that serotonin is released from a bound form during darkness, allowing it to be acted on by enzymes for melatonin production or excretion as needed by the body. The binding of serotonin during the light hours accounts for its increasing levels during the day.

The enzyme, 5-hydroxytryptophan decarboxylase (5-HTPD), is involved in the synthesis of serotonin; and monoamine oxidase (MAO) in serotonin metabolism. Effects of light on these enzymes would influence the pineal serotonin levels. Snyder and Axelrod (1965) found no cyclic rhythm in pineal levels of 5-HTPD or MAO in rats in cyclic light. Snyder and Axelrod (1964a) and Snyder et al. (1964a, 1965b)



showed that constant light increased pineal 5-HTPD and constant darkness decreased its level in comparison to an intermediate level in rats under cyclic lighting treatments. No change was effected in the pineal MAO content by continuous darkness (Wurtman and Axelrod, 1965; Wurtman et al., 1963b).

The recognition of light as an important controlling factor of pineal function suggests involvement of the eyes as a receptor. Wurtman et al. (1964d) showed that bilateral enucleation (complete removal of both eyes) resulted in a loss of the capacity of the pineal to respond to altered illumination. Blinding abolishes the HIOMT response to light changes (Axelrod et al., 1965; Wurtman and Axelrod, 1964). The circadian rhythm in pineal serotonin content persisted after enucleation (Snyder et al., 1964b, 1965a). The elevation in pineal 5-HTPD with constant light is eliminated by removal of the eyes.

Removal of the pituitary (Snyder et al., 1965a, 1965c; Wurtman and Axelrod, 1964; Wurtman et al., 1964d), gonads (Snyder et al., 1965c; Wurtman and Axelrod, 1964; Wurtman et al., 1964d, 1965), thyroid (Snyder et al., 1965a, 1965c), or adrenals (Snyder et al., 1965a, 1965c) has not been shown to exert any effect on the pineal metabolic activity. Wurtman et al., (1965) reported that the HIOMT activity increases during diestrus and decreases during proestrus and estrus phases of the estrous cycle in rats.



This would appear to indicate a relationship between estrogen output and HIOMT activity. However, injections of estradiol produced no change in HIOMT activity in immature rats and only a slight decrease in mature animals. Thus, the authors concluded that the HIOMT activity changes during the estrus cycle were probably not a consequence of variations in the level of circulating estrogens. There is no definite evidence for a hormonal control of the pineal.

Anatomically it was shown that sympathetic innervation is the primary, if not the only, functional nervous supply to the pineal. This innervation has been interrupted by surgical and pharmacological means in order to determine its function in the control of pineal metabolism. Surgical denervation of the pineal has usually been effected by bilateral superior cervical ganglionectomy. Previous observations showed that this operation effectively destroys the main pineal nervous supply, as evidenced by the degeneration of the two nerve conarii and the loss of intrapineal innervation (Kappers, 1960). Wurtman et al. (1964d) showed that sympathetic denervation of the pineal interrupts the effect of light on melatonin synthesis. The pineal HIOMT cycle in diurnal light is abolished (Axelrod et al., 1965), as is its response to constant light or darkness (Wurtman and Axelrod, 1964). Following bilateral ganglionectomy, the serotonin level of the pineal is reduced (Pellegrino de Traldi et al., 1963) and the cyclic variation in serotonin level is abolished (Fiske, 1964; Snyder and Axelrod, 1965;



Snyder et al., 1965a, 1964b, 1965c). According to Quay and Halevy (1962), transection of the optic tracts also reduced the pineal content of serotonin and related amines. Constant light and superior cervical ganglionectomy both increase the pineal 5-HTPD level (Pellegrino de Iraldi and Rodriguez de Lores Arnaiz, 1964; Snyder and Axelrod, 1964a; Snyder et al., 1964a, 1965b).

Boura et al. (1959) and Boura and Green (1959) described bretylium, a compound which blocks the peripheral sympathetic nervous system without impairing the parasympathetic or central nervous system functions. No effect on the pineal serotonin rhythm was observed by Snyder and Axelrod (1965) following bretylium treatment of rats. This result was unexpected since sympathetic denervation abolishes the serotonin rhythm. Snyder et al. (1965b) found that bretylium-treated rats failed to show an alteration of 5-HTPD enzymatic activity, agreeing with the denervation experiment results.

The negative results with modifications in hypophyseal, gonadal, thyroidal and adrenal endocrines would indicate that the pineal gland is not directly controlled by hormonal factors, although some modifying actions of hormones may be present. The general reduction in melatonin production accompanying bilateral enucleation and interruption of the sympathetic nervous system suggests that the primary control mechanism involves light induced impulses from the retina.



These impulses probably pass through the optic tracts, through unknown central nervous system pathways, through pre- and postsympathetic ganglionic fibers and to the pineal via the nervi conarii. In the pineal gland, neurotransmitters are probably released to influence the activity of various enzymes, especially 5-HTPD and HIOMT, controlling serotonin and melatonin metabolism.

Snyder and Zweig (1966) reported evidence of a non-retinal pathway for light influence on the pineal of very young rats. Extending the light period given blinded, 12-day old rats partially prevented the nocturnal fall in pineal serotonin. Covering the skull of these young rats or the use of 27-day old blinded rats produced the normal serotonin rhythm. These results suggested that light might penetrate the thin skull structure of very young rats and exert an effect directly on some portion of the brain. The subsequent ossification of the skull and growth of the hair covering prevent any appreciable direct influence of light on the brain of older animals.

If the normal pathway of light influence on the gonads involves the retina as a receptor, blinding would be expected to interrupt the results usually obtained. Hoffman and Reiter (1965b) performed bilateral enucleations which resulted in significant atrophy of the testes of hamsters kept in a stimulatory light regime (16 hours light and 8 hours darkness, daily). Wurtman et al. (1964c) prevented the constant light induced hypertrophy of the ovaries and uterus



by removal of both eyes and by bilateral cervical sympathetic ganglionectomy of rats. The associated decreases in pineal weight and melatonin synthesis were also prevented.

Wurtman et al. (1964e) observed a decrease in the melatonin concentration in the ovary when mature rats were placed in constant light. Since no change was observed in the amount of the hormone present in the heart, a light controlled mechanism may influence the concentration of melatonin by the ovary.

There is indirect evidence of a species variation in melatonin synthesizing ability. Snyder and Axelrod (1964b) showed that the rat pineal contained approximately twice the 5-HTPD activity of bovine pineals. The level in quail pineals was slightly less than that of bovine pineals. The enzyme is involved in serotonin production. HIOMT, the melatonin-forming enzyme, is even more variable, as shown by Axelrod and Weissbach (1960) and Axelrod et al. (1964). Monkeys have about 500 times as much pineal HIOMT as rats and birds had 3 to 4 times the activity of monkeys. It is not known whether these enzyme levels reflect a difference in amounts of serotonin or melatonin actually produced.

E. Summary of Literature

1. Bodily growth

Growth is not inhibited by removal of the pineal gland. Papers reporting acceleration of bodily growth or lack of change in growth have appeared in nearly equal numbers for



both fowl and mammals. Most of the researchers who employed statistical analyses reported the lack of a significant difference between pinealectomized, sham-operated and control animals.

The injection of pineal extracts and the implantation of pineal glands has also produced inconsistent results in birds and mammals. Reports of growth stimulation, growth inhibition or lack of effect have occurred with nearly equal frequency. Only three papers have reported the effect of melatonin injections on body weights of rats. Two of the papers reported no significant difference in body weights, whereas the other reported a decrease in both sexes. There is no consistent evidence that the pineal gland has any important influence on bodily growth.

2. Reproductive Function

There is sufficient evidence in the literature to support a proposed inhibitory function of the pineal gland on reproductive function. Extirpation of the pineal gland of immature chicks and mammals has generally resulted in gonadal hypertrophy. In cases where no change or gonadal inhibition were observed, the animals were usually sacrificed after reaching full maturity. While the results were somewhat more variable, there is also good evidence that injections of pineal extract will prevent gonadal hypertrophy in pinealectomized animals and cause some gonadal inhibition in intact animals. Melatonin, a pineal elaboration, appears

to be the factor in pineal extracts which is responsible for the inhibitory action on gonadal development.

The relationship of the pineal gland to sexual maturity of rats is less clearly shown. Pinealectomy caused earlier sexual maturity in some experiments, while producing no change in others. Those finding advanced sexual maturity usually observed a difference of only a few days. However, pineal extract and melatonin injections were reported to delay sexual maturity of rats, with only a few exceptions.

The literature contains strong evidence of a relationship between pineal function and photoperiods. The pineal gland increases in size and produces more melatonin when an animal is housed with photoperiods of short light and long dark periods or total darkness, compared to normal laboratory photoperiods or constant light. Thus, pinealectomy produced more striking results as the length of the dark period was increased. The results were negative with constant light treatment.

Evidence for any pineal influence on other organs of the body is inconsistent and nonconclusive. Adrenal function has been most closely associated with the pineal gland. Further investigation is required to clarify pineal function, if any, on other body organs.

OBJECTIVES

The overall objective of this study was to determine the function of the avian pineal gland as it relates to the control of reproductive function by photoperiods through pinealectomy and melatonin injection experiments.

A. Pinealectomy experiments:

1. To determine whether pinealectomy will permit full or partial gonadal development of immature quail reared under inhibitory photoperiods.
2. To determine whether pinealectomy will alter gonadal development of quail reared under stimulatory photoperiods.
3. To determine whether pinealectomy will prevent or reduce gonadal atrophy in mature quail following a change from stimulatory to inhibitory photoperiods.

B. Melatonin-injection experiments:

1. To determine whether melatonin injections will prevent or reduce the gonadal development of immature quail reared under stimulatory photoperiods.
2. To determine whether melatonin injections will cause atrophy or reduced function in gonads of adult quail housed under stimulatory photoperiods.



EXPERIMENTAL PROCEDURE

A. General Management

Japanese quail (Coturnix coturnix japonica) from miscellaneous genetic lines were used as the test animal in all experiments. All quail chicks were grown in a battery brooder to the age of 5 weeks. Birds remaining on experiment past 5 weeks of age were housed in individual cages, 4 to 5 inches wide, 7 to 8 inches deep (front to back) and $5\frac{1}{2}$ to 7 inches high, with the floors sloping toward the front. Several groups of quail were transferred to a windowless test chamber at 3 weeks or more of age. The chamber is $9\frac{1}{4}$ feet deep by $8\frac{1}{2}$ feet wide. A temperature control unit kept the room temperature between 64 and 70° F. Quail between the ages of 3 and 5 weeks were put in a battery brooder which had non-glowing, encased heating elements and lacked pilot lights. Thus, complete light control was possible. Birds over 5 weeks of age needed no additional heat and were placed in individual cages 4 inches wide, 7 inches deep (front to back) and $5\frac{1}{2}$ to 7 inches high.

A 25 percent protein quail breeder ration and water were provided ad libitum. The composition of the ration was as follows:



Ground yellow corn	412.5 (lbs)
Soybean oil meal, dehulled, 50%	370.0
Alfalfa leaf meal, dehyd., 17%	50.0
Dried whey	25.0
Meat & bone scraps, 50%	25.0
Fishmeal, menhaden, 60%	25.0
Ground limestone	50.0
Dicalcium phosphate	15.0
Salt, iodized	5.0
M-4, Vit. premix (Nopcosol)	2.5
Fat	20.0

All quail were wing banded for identification purposes at 1 to 2 days of age.

B. Pinealectomy Operation

All quail to be pinealectomized were operated on during the day of hatching or on the following day, under light to moderate ether anesthesia. Iris scissors were used to make a longitudinal cut about 10 to 12 millimeters long through the skin on top of the head. The edges of the skin were separated to expose the soft, developing skull bones. The cartilaginous suture line between the frontals was pierced transversely, about 2 millimeters anterior to the frontal fontanelle, using a No. 12 Bard-Parker surgical blade. Care was exercised in this and the following steps in order to avoid cutting or injuring the cerebrum. Fine iris scissors were used to extend the cut transversely to either side, into the frontal bones. The final opening through the skull was about 6 millimeters long. The dura which adheres to the skull was cut at the same time. In a blind operation, fine watchmaker's forceps were inserted through the opening, just under the dura in a caudad direction, and past the frontal

fontanelle to grasp the pineal body and remove it. The stalk remained attached to the pineal body and was removed at the same time. The occurrence of some bleeding could not be prevented, but the amount was not excessive. As soon as the active bleeding had stopped and the blood was removed with a cotton ball dampened with physiological saline, the skin was closed over the skull with three or four sutures.

In preliminary pinealectomy trials, attempts were made to expose the pineal before removal by extending the cut described above caudally on both sides, across the junction of the frontal and parietal bones. The flap of developing bone could then be turned back, exposing the pineal. However, this method markedly increased the amount of bleeding and resulted in excessive mortality. Most of the mortality observed in the test birds during pinealectomy was thought to be due to an overdose of ether. Any birds in which the operation appeared to have resulted in incomplete pineal removal or injury to brain tissue were destroyed immediately. The success of all pinealectomy operations was further checked at the end of each trial. Any suspicious tissue was sectioned and stained for histological observation. Those birds which showed any remaining pineal tissue were eliminated from the experiment.

The successfully operated birds were usually alert and active within 1 or 2 hours following surgery. Mortality in the battery brooder was no greater in the pinealectomized



quail chicks than in their controls. Pinealectomized and control birds of the same age were reared together in the battery brooders. The time required for each operation precluded the use of larger numbers of experimental animals in each test.

C. Melatonin Injections

Melatonin (Nutritional Biochemical Corporation, lot No. 8231) was dissolved in chloroform with subsequent 1:20 dilution in sesame oil (5 percent chloroform solution). An 0.2 milliliter dose contained 500 micrograms of melatonin. There was some tendency for the melatonin to come out of solution when stored at temperatures below 24° C. (75° F.). Subsequent solutions were made in 5 percent ethanol. Levels up to 2000 micrograms melatonin in 0.4 milliliters of solution remained in solution through the experiments. It was necessary to stir and heat this highest concentration to 50° C. on a Temco Stir-Plate (A. S. Aloe Co.) to get all of the melatonin into solution.

All injections were subcutaneous; placed in the area of the lateral body apterium, just anterior to the femoral feather tract. Daily injections were given, alternately on the right and left sides. Melatonin levels and volumes of injections are specified under the respective experiment description. Injections were given with 1 milliliter hypodermic syringes using a 1½ inch, 22 guage needle.

D. Description of Experiments

Experiment 1

Eighteen (18) to 25 pinealectomized and a like number of control quail chicks were started in each of four groups. Mortality and the elimination of unsuccessfully operated birds lowered the numbers of birds completing the experiment. They were grown in brooder batteries with an L:D = 16:8 (light period : dark period = 16 hours : 8 hours, daily) light cycle. At 3 weeks of age prior to puberty, they were moved to a brooding battery located in a windowless chamber. From 3 weeks of age their light schedule was L:D = 2:22. The two groups remaining on experiment past 5 weeks of age were moved to individual cages within the same windowless chamber.

Groups were sacrificed at 4, 5, 6 and 7 weeks of age. The body, adrenal, thyroid, pituitary (6 and 7 weeks only), spleen, bursa, testes, ovary and oviduct weights were recorded. Organs to be weighed were carefully removed and placed on wet paper towels. Excess tissue was dissected away before weighing. Roller-Smith balances were used to weigh all tissues. All birds that had been operated on were macro- and microscopically examined to determine whether the pineal had been completely removed.

Experiment 2

Twenty (20) to 25 pinealectomized and control quail were started in each of 3 groups. They were kept in L:D = 16:8 light cycles throughout the experiment. At the age of 5 weeks, the females were moved to individual cages, while the males remained in the battery brooders until sacrificed.

At the ages of 5, 6 and 7 weeks, the groups were sacrificed, with the exception of one group of females which were kept in individual cages until sexually mature. Thus, only males were sacrificed in the 6 week age group. Body, pituitary, thyroid, adrenal, spleen, bursa, testes, ovary and oviduct weights were recorded as in Experiment 1. Because of size several of the 7 week old quail ovaries and oviducts were weighed on a triple beam balance (Ohaus Scale Corp.). Sexual maturity was assumed when the first egg was laid by each bird. All pinealectomized birds were examined to determine whether the operation was successful.

Experiment 3

Twenty (20) pinealectomized and 25 control Japanese quail were started in the battery brooder under an L:D = 16:8 light schedule. At 26 days of age they were transferred to a battery brooder in a windowless chamber with L:D = 2:22 light cycles. At 38 days of age they were placed in individual cages within the same test chamber. The light period was increased to provide an L:D = 14:10 schedule at the age of 56 days and egg production was recorded to



determine age of sexual maturity. After 28 days of increased light treatment, the photoperiod was abruptly reduced to L:D = 6:18 and gradually lowered (by 15 minutes per day) until a light schedule of L:D = 4:20 was obtained. Egg production records were continued for an 18 day period to determine time of cessation of egg production.

The males were raised under the same lighting conditions, but were sacrificed after 16 days of the final photoperiod. Testes weights and body weights were recorded. Presence or absence of the pineal was determined for all operated birds at the end of the experimental period.

Experiment 4

Sixteen (16) male and 16 female quail were moved from the battery brooder to individual cages at 36 days of age. These were all unoperated birds. Starting at 37 days of age, prior to the puberal phase of gonadal development, half of each sex were injected daily with 0.2 milliliters of carrier solution (chloroform in sesame oil) containing 500 micrograms of melatonin. The control birds were sham-injected with an equal volume of the carrier only.

The quail were sacrificed after 1 week of injections. The body, pituitary, thyroid, adrenal, spleen, bursa, testes, ovary and oviducts were weighed, following the procedure outlined for Experiment 1.

Experiment 5

Twenty-four (24) male quail were randomly divided into three groups at 6 weeks of age. They were individually caged in the test chamber with a light schedule of L:D = 14:10. Daily melatonin injections were given subcutaneously at levels of 0 (control), 100 and 500 micrograms, in 0.2 milliliters of carrier (aqueous ethanol) for 7 days. The birds were sacrificed on the 8th day (age = 50 days, during the puberal phase of gonadal growth), and body, pituitary, thyroid, adrenal, spleen, bursa and testes weights were recorded as in Experiment 1.

Experiment 6

Twelve (12) male and 12 female quail were placed in individual cages at 5 weeks of age. Their light schedule remained at L:D = 16:8. Both sexes were randomly divided into three groups which were given daily injections of 0 (control), 10 and 50 micrograms of melatonin in 0.1 milliliters of carrier solution. The males were injected for 2 weeks, covering both prepuberal and early puberal phases of gonadal growth, and sacrificed the day following the last injection (at 50 days of age). Body, pituitary, thyroid, adrenal, spleen, bursa and testes weights were recorded. The injections were continued in the females until all birds had reached sexual maturity. The age at first egg was recorded.



Experiment 7

Six groups of young mature (in egg production) female Coturnix quail were housed in individual cages. Their photoperiod remained at L:D = 16:8. Daily injections were given to provide dosages of 0 (control), 50, 100, 500, 1000 and 2000 micrograms of melatonin. The levels between 0 and 500 micrograms daily were given in 0.2 milliliters of carrier solution. The two higher doses (1000 and 2000 micrograms daily) were given in a volume of 0.4 milliliters, also in a 5 percent ethanol carrier. Individual egg production records were kept from 4 days before the start of injections to the end of the test period. The injections were given for 12 days, except for the 50 and 1000 microgram levels, which were continued for 24 days.

E. Statistical Treatment

Organ weights were statistically analyzed on a whole organ weight basis and on a milligrams per 100 grams of body weight basis. The F-test was used to detect significant differences between standard deviations. When such differences were found, the approximate t-test was used. All other data were analyzed by the two sample t-test. Standard errors were computed from the pooled standard deviation (s) except in cases where the F-test was significant in which case the error was computed from the individual standard deviations (s_x and s_y) (Gill, 1966; Adler and Roessler, 1958).

All tables of results were constructed on a similar basis. The number of quail (n) is listed for each group. Any exceptions in the number of observations, occurring as a result of the loss or incomplete removal of body organs, are noted in the footnotes. The values listed for each measured parameter give the mean value $\pm$ the standard error of the mean. Body organ weights were calculated on both the whole weight basis and on the adjusted weight (milligrams per 100 grams of body weight) basis. The symbols used to indicate statistical results are:

- † = Significant difference between standard deviations of experimental and control results; $P < 0.10$ (10% level).
- †† = Significant difference between standard deviations of experimental and control results; $P < 0.05$ (5% level).
- ††† = Significant difference between standard deviations of experimental and control results; $P < 0.02$ (2% level).
- * = Significant difference between means of experimental and control results; $P < 0.05$ (5% level).
- ** = Significant difference between means of experimental and control results; $P < 0.01$ (1% level).

Significant differences between standard deviations are indicated, since the statistical treatment of these observations differed as described above. Except in the case

where significant differences between standard deviations were found, the standard errors were calculated from the pooled standard deviations. This statistical procedure gives a more accurate estimate of the expected range, but eliminates its value as an indicator of the variation in the actual data being tested.

RESULTS

Among all quail on which pinealectomy was performed, about 70 percent survived for more than 48 hours. The 30 percent mortality was divided into the following causes: (1) Five (5) percent due to an overdose of ether, (2) Ten (10) percent due to excessive blood loss and (3) fifteen (15) percent sacrificed due to suspected brain damage or incomplete removal of the pineal gland. In the three pinealectomy experiments there were 137 pinealectomized quail which survived to the planned autopsy age. Macroscopic and microscopic examinations showed complete removal of the pineal in 97 of these birds. The remaining 40 quail had tissue varying in appearance from a small, atypical to a definite pineal structure.

Experiment 1

The body and organ weights of the 4-week old quail in Experiment 1 are summarized in Table 1. These birds had been moved abruptly from L:D = 16:8 (light period : dark period = 16 hours : 8 hours, daily) to L:D = 2:22 photoperiods. A general unthrifty appearance of the birds indicated a partial failure to adapt to this new light cycle. Two females (one pinealectomized and one control) appeared obviously weak and emaciated and were eliminated from the experiment. The only statistically significant difference observed was between adrenal weights of the male pinealectomized and control groups at the 5 percent level. This



TABLE 1.-Body weight and organ weights of 4-week old Coturnix quail housed under L:D - 2:22 light cycles at 3 weeks of age as influenced by pinealectomy (mean weight $\pm$ standard error).

Sex	Males		Females	
	Pinealectomized	Control	Pinealectomized	Control
No. of quail (n)	3	5	4	10
Body weight (gm)	63.30 $\pm$ 4.19	53.10 $\pm$ 3.25	56.22 $\pm$ 5.11	61.49 $\pm$ 3.74
<u>Whole organ weights (mg):</u>				
Thyroids	2.98 $\pm$ 1.17 [†]	3.75 $\pm$ 0.74	3.14 $\pm$ 0.57	3.85 $\pm$ 0.40 ²
Adrenals	3.73 $\pm$ 0.75	4.99 $\pm$ 0.58	3.88 $\pm$ 0.70	4.38 $\pm$ 0.44
Spleen	22.15 $\pm$ 3.62	16.35 $\pm$ 2.81	23.80 $\pm$ 4.45	22.77 $\pm$ 2.81
Bursa	56.78 $\pm$ 8.87	39.08 $\pm$ 6.87	60.48 $\pm$ 12.24	52.64 $\pm$ 7.74
Testes	17.33 $\pm$ 6.46 ^{††}	10.82 $\pm$ 1.18	---	---
Ovary	---	---	14.74 $\pm$ 4.93	23.18 $\pm$ 3.12
Oviduct	---	---	7.95 $\pm$ 1.30	10.07 $\pm$ 0.92
<u>Adjusted organ weights (mg/100 gm body wt.):</u>				
Thyroids	4.34 $\pm$ 2.39 [†]	7.15 $\pm$ 1.51	5.59 $\pm$ 0.79	6.28 $\pm$ 0.56 ²
Adrenals	5.86 $\pm$ 0.28 ^{†*}	9.38 $\pm$ 1.10	7.00 $\pm$ 1.64	7.52 $\pm$ 1.04
Spleen	34.16 $\pm$ 4.47	30.53 $\pm$ 3.46	42.36 $\pm$ 5.69	36.32 $\pm$ 3.60
Bursa	89.09 $\pm$ 12.77	72.95 $\pm$ 9.99	107.03 $\pm$ 17.06	82.91 $\pm$ 10.83
Testes	26.11 $\pm$ 8.84 ^{††}	20.40 $\pm$ 2.09	---	---
Ovary	---	---	26.54 $\pm$ 6.75	37.46 $\pm$ 4.27
Oviduct	---	---	14.17 $\pm$ 1.57	16.32 $\pm$ 0.99

[†] Significant difference between standard deviations, $P < 0.10$.

^{††} Significant difference between standard deviations, $P < 0.05$.

* Significant difference, $P < 0.05$.

1 n = 2

2 n = 8

observation was significant only on the adjusted weight basis, which showed the control quail to have larger adrenals (9.38 ± 1.10 vs. 5.86 ± 0.28 milligrams per 100 grams of body weight).

Tables 2, 3 and 4 summarize the body and organ weights of 5, 6 and 7 week old quail, respectively. The quail seem to be in much better condition than those at 4 weeks of age, as evidenced by appearance and body weights. The only significantly different whole organ weights found were the pituitaries of the 7-week old males (5 percent level). The pinealectomized quail had lower pituitary weights.

The reproductive organs showed little, if any, development in any of the groups tested. In several cases, the F-test showed a significant difference in standard deviations between the weights of pinealectomized groups and those of the controls. However, there was no consistent trend seen in the weights of the testes, ovary or oviduct.

Experiment 2

Coturnix quail in Experiment 2 were raised under L:D = 16:8 light cycles from hatching to the end of the experiment. The results are summarized on Tables 5, 6 and 7 for quail at 5, 6 and 7 weeks of age, respectively. Table 6 contains data for males only, as the females were kept to determine age of sexual maturity.

TABLE 2.-Body weight and organ weights of 5-week old Coturnix quail housed under L:D = 2:22 light cycles at 3 weeks of age as influenced by pinealectomy (mean weight $\pm$ standard error).

Sex Treatment	Males		Females	
	Pinealectomized	Control	Pinealectomized	Control
No. of quail (n)	7	12	4	11
Body weight (gm)	83.69 $\pm$ 3.90	85.62 $\pm$ 2.98	84.40 $\pm$ 3.41	91.26 $\pm$ 2.06
<u>Whole organ weights (mg):</u>				
Thyroids	4.84 $\pm$ 0.87	5.56 $\pm$ 0.67	6.79 $\pm$ 1.09	5.52 $\pm$ 0.66
Adrenals	6.01 $\pm$ 0.93	6.24 $\pm$ 0.71	7.05 $\pm$ 0.72	5.68 $\pm$ 0.44
Spleen	30.21 $\pm$ 7.25	40.40 $\pm$ 5.54	40.46 $\pm$ 19.84	60.93 $\pm$ 11.96
Bursa	76.89 $\pm$ 10.79	83.82 $\pm$ 8.25	72.08 $\pm$ 15.72	104.45 $\pm$ 9.48
Testes	17.10 $\pm$ 1.90 ¹	14.40 $\pm$ 1.40 ²	---	---
Ovary	---	---	29.86 $\pm$ 1.57 [†]	31.40 $\pm$ 3.07
Oviduct	---	---	13.42 $\pm$ 1.40	11.55 $\pm$ 0.84
<u>Adjusted organ weights (mg/100 gm body wt.):</u>				
Thyroids	5.64 $\pm$ 0.79	6.60 $\pm$ 0.60	7.82 $\pm$ 1.11	6.05 $\pm$ 0.67
Adrenals	7.16 $\pm$ 1.29	7.57 $\pm$ 0.99	8.37 $\pm$ 0.92	6.24 $\pm$ 0.56
Spleen	35.36 $\pm$ 8.17	47.36 $\pm$ 6.24	49.39 $\pm$ 19.57	65.05 $\pm$ 11.79
Bursa	93.14 $\pm$ 10.82	96.45 $\pm$ 8.27	84.88 $\pm$ 14.53	113.17 $\pm$ 8.76
Testes	20.42 $\pm$ 1.98 ¹	16.84 $\pm$ 1.46 ²	---	---
Ovary	---	---	35.81 $\pm$ 4.75	34.33 $\pm$ 2.87
Oviduct	---	---	15.93 $\pm$ 1.54	12.67 $\pm$ 0.93

[†] Significant difference between standard deviations, $P < 0.10$.

¹ n = 6

² n = 11

TABLE 3.-Body weight and organ weights of 6-week old Coturnix quail housed under L:D = 2:22 light cycles at 3 weeks of age as influenced by pinealectomy (mean weight $\pm$ standard error).

Sex	Males		Females	
	Pinealectomized	Control	Pinealectomized	Control
No. of quail (n)	8	8	5	14
Body weight (gm)	96.64 $\pm$ 3.16	97.58 $\pm$ 3.16	96.78 $\pm$ 2.15	94.26 $\pm$ 1.27
Whole organ weights (mg):				
Pituitary	0.78 $\pm$ 0.09	0.71 $\pm$ 0.09	0.55 $\pm$ 0.10	0.80 $\pm$ 0.06
Thyroids	6.45 $\pm$ 0.78	6.46 $\pm$ 0.78	6.73 $\pm$ 0.84	6.40 $\pm$ 0.52
Adrenals	9.47 $\pm$ 0.93††	7.92 $\pm$ 0.71	6.48 $\pm$ 0.65	7.25 $\pm$ 0.40
Spleen	57.08 $\pm$ 16.99†††	45.86 $\pm$ 4.03	59.80 $\pm$ 14.87	51.68 $\pm$ 8.88
Bursa	75.60 $\pm$ 6.63††	103.28 $\pm$ 17.45	102.67 $\pm$ 12.74	90.06 $\pm$ 7.62
Testes	13.61 $\pm$ 0.65	13.05 $\pm$ 0.65	---	---
Ovary	---	---	27.72 $\pm$ 3.22	30.19 $\pm$ 1.93
Oviduct	---	---	11.82 $\pm$ 0.51††	12.02 $\pm$ 0.96
Adjusted organ weights (mg/100 gm body wt.):				
Pituitary	0.80 $\pm$ 0.09	0.73 $\pm$ 0.09	0.57 $\pm$ 0.11	0.84 $\pm$ 0.07
Thyroids	6.66 $\pm$ 0.70	6.58 $\pm$ 0.70	6.97 $\pm$ 0.89	6.80 $\pm$ 0.55
Adrenals	9.92 $\pm$ 0.83	8.20 $\pm$ 0.83	6.69 $\pm$ 0.70	7.77 $\pm$ 0.44
Spleen	58.76 $\pm$ 17.64†††	46.68 $\pm$ 3.61	62.01 $\pm$ 15.16	54.36 $\pm$ 9.06
Bursa	78.75 $\pm$ 6.99††	103.80 $\pm$ 15.91	107.19 $\pm$ 13.38	95.17 $\pm$ 8.00
Testes	14.15 $\pm$ 0.67	13.40 $\pm$ 0.67	---	---
Ovary	---	---	28.43 $\pm$ 3.08	31.95 $\pm$ 1.84
Oviduct	---	---	12.21 $\pm$ 0.47††	12.73 $\pm$ 0.96

†† Significant difference between standard deviations, $P < 0.05$.

††† Significant difference between standard deviations, $P < 0.02$.

1 n = 13

TABLE 4.—Body weight and organ weights of 7-week old Coturnix quail housed under L:D = 2:22 light cycles at 3 weeks of age as influenced by pinealectomy (mean weight $\pm$ standard error).

Treatment	Males		Females	
	Pinealectomized	Control	Pinealectomized	Control
No. of quail (n)	6	10	7	10
Body weight (gm)	102.92 $\pm$ 4.31	100.97 $\pm$ 3.34	107.79 $\pm$ 1.75	104.39 $\pm$ 1.46
Whole organ weights (mg):				
Pituitary	0.52 $\pm$ 0.04†*	0.80 $\pm$ 0.08 ¹	0.91 $\pm$ 0.18†††	0.76 $\pm$ 0.05
Thyroid	5.62 $\pm$ 0.42††	6.84 $\pm$ 0.90	5.90 $\pm$ 0.56	6.97 $\pm$ 0.47
Adrenals	10.33 $\pm$ 1.32	8.66 $\pm$ 1.02	7.14 $\pm$ 0.87	8.46 $\pm$ 0.72
Spleen	34.27 $\pm$ 6.80	33.80 $\pm$ 5.26	58.19 $\pm$ 10.05	37.22 $\pm$ 8.40
Bursa	78.48 $\pm$ 14.18	87.72 $\pm$ 11.00	106.00 $\pm$ 10.05	107.60 $\pm$ 8.42
Testes	13.06 $\pm$ 1.14†††	17.26 $\pm$ 3.123	29.60 $\pm$ 3.22	27.42 $\pm$ 2.70
Ovary	---	---	11.41 $\pm$ 0.97†††	13.03 $\pm$ 2.40
Oviduct	---	---	---	---
Adjusted organ weights (mg/100 gm body wt.):				
Pituitary	0.50 $\pm$ 0.03†*	0.78 $\pm$ 0.07 ¹	0.86 $\pm$ 0.18†††	0.70 $\pm$ 0.05
Thyroids	5.26 $\pm$ 0.35††	6.73 $\pm$ 0.85	5.48 $\pm$ 0.51	6.67 $\pm$ 0.42
Adrenals	10.09 $\pm$ 1.31	8.66 $\pm$ 1.01	7.18 $\pm$ 0.78	8.08 $\pm$ 0.66
Spleen	32.74 $\pm$ 6.88	34.22 $\pm$ 5.33	54.78 $\pm$ 10.03	35.71 $\pm$ 8.39
Bursa	76.33 $\pm$ 12.39	85.48 $\pm$ 9.60	97.75 $\pm$ 9.08	103.27 $\pm$ 7.60
Testes	12.76 $\pm$ 1.04†††	17.16 $\pm$ 3.213	27.44 $\pm$ 2.97	26.24 $\pm$ 2.49
Ovary	---	---	10.61 $\pm$ 0.95††	12.45 $\pm$ 2.18
Oviduct	---	---	---	---
¹ n = 8 † Significant difference between standard deviations, P < 0.10. 2 n = 5 †† Significant difference between standard deviations, P < 0.05. 3 n = 9 ††† Significant difference between standard deviations, P < 0.02. * Significant difference, P < 0.05.				

TABLE 5.-Body weight and organ weights of 5-week old Coturnix quail housed under L:D = 16:8 light cycles as influenced by pinealectomy (Mean weight $\pm$ standard error).

Sex Treatment	Males		Females	
	Pinealectomized	Control	Pinealectomized	Control
No. of quail (n)	8	12	5	7
Body weight (gm)	89.15 $\pm$ 1.71 ^{††}	86.38 $\pm$ 3.21	86.38 $\pm$ 2.24*	94.44 $\pm$ 1.89
Whole organ weights (mg):				
Pituitary	0.60 $\pm$ 0.09	0.72 $\pm$ 0.07	1.08 $\pm$ 0.21 [†]	1.06 $\pm$ 0.172
Thyroids	5.97 $\pm$ 0.81	4.95 $\pm$ 0.66	5.44 $\pm$ 0.62	5.24 $\pm$ 0.53
Adrenals	8.45 $\pm$ 0.86	8.95 $\pm$ 0.70	10.26 $\pm$ 1.21	8.40 $\pm$ 1.02
Spleen	56.04 $\pm$ 13.60 ^{†††}	40.04 $\pm$ 3.79	55.38 $\pm$ 13.49	67.24 $\pm$ 11.40
Bursa	84.59 $\pm$ 7.38	75.91 $\pm$ 6.03	97.31 $\pm$ 15.84	93.51 $\pm$ 13.38
Testes	751.70 $\pm$ 73.21 ^{††}	682.40 $\pm$ 133.04	---	---
Ovary	---	---	66.53 $\pm$ 3.92 ^{†††}	70.67 $\pm$ 15.94
Oviduct	---	---	72.77 $\pm$ 16.43 ^{†††}	236.14 $\pm$ 134.91
Adjusted organ weights (mg/100 gm body wt.):				
Pituitary	0.67 $\pm$ 0.12	0.75 $\pm$ 0.10	1.26 $\pm$ 0.34 ^{†††}	1.10 $\pm$ 0.22 ²
Thyroids	6.64 $\pm$ 0.94	5.23 $\pm$ 0.77	6.30 $\pm$ 0.72	5.59 $\pm$ 0.61
Adrenals	9.53 $\pm$ 1.20	9.47 $\pm$ 0.98	12.07 $\pm$ 1.56	8.91 $\pm$ 1.32
Spleen	62.24 $\pm$ 14.66 ^{††}	44.47 $\pm$ 5.73	63.80 $\pm$ 14.73	71.41 $\pm$ 12.45
Bursa	95.18 $\pm$ 10.35	80.70 $\pm$ 8.46	112.09 $\pm$ 18.14	99.74 $\pm$ 15.33
Testes	837.89 $\pm$ 72.73 ^{††}	629.13 $\pm$ 131.53	---	---
Ovary	---	---	77.60 $\pm$ 6.32 [†]	74.02 $\pm$ 15.81
Oviduct	---	---	84.04 $\pm$ 18.68 ^{†††}	238.08 $\pm$ 131.53

1 n = 4

2 n = 6

^{††} Significant difference between standard deviations, $P < 0.05$.

^{†††} Significant difference between standard deviations, $P < 0.02$.

* Significant difference, $P < 0.05$.

TABLE 6.-Body weight and organ weights of 6-week old Coturnix quail housed under L:D = 16:8 light cycles as influenced by pinealectomy (mean weight $\pm$ standard error).

Sex	Males	
	Pinealectomized	Control
No. of quail (n)	8	11
Body weight (gm)	97.48 $\pm$ 2.40	101.27 $\pm$ 2.05
<u>Whole organ weights (mg):</u>		
Pituitary	1.69 $\pm$ 0.36	1.21 $\pm$ 0.31
Thyroids	5.98 $\pm$ 0.63	7.09 $\pm$ 0.54
Adrenals	10.59 $\pm$ 0.94	10.00 $\pm$ 0.80
Spleen	35.96 $\pm$ 3.74 ^{††}	44.02 $\pm$ 7.09
Bursa	72.76 $\pm$ 8.43	85.71 $\pm$ 7.19
Testes	1593.31 $\pm$ 180.00	1584.11 $\pm$ 153.62
<u>Adjusted organ weights (mg/100 gm body wt.):</u>		
Pituitary	1.74 $\pm$ 0.25	1.19 $\pm$ 0.21
Thyroids	6.17 $\pm$ 0.67	7.03 $\pm$ 0.57
Adrenals	11.00 $\pm$ 1.36 [†]	9.92 $\pm$ 0.66
Spleen	36.83 $\pm$ 3.71 ^{††}	42.90 $\pm$ 6.29
Bursa	74.15 $\pm$ 7.31	84.31 $\pm$ 6.24
Testes	1624.51 $\pm$ 172.63	1564.18 $\pm$ 147.31

[†] Significant difference between standard deviations, $P < 0.10$.

^{††} Significant difference between standard deviations, $P < 0.05$.

TABLE 7.-Body weight and organ weights of 7-week old Coturnix quail housed under L:D = 16:8 light cycles as influenced by pinealectomy (mean weight $\pm$ standard error).

Sex	Males		Females	
Treatment	Pinealectomized	Control	Pinealectomized	Control
No. of quail (n)	8	7	4	8
Body weight (gm)	99.50 $\pm$ 2.60	98.26 $\pm$ 2.78	120.32 $\pm$ 4.10	120.91 $\pm$ 2.90
Whole organ weights (mg):				
Pituitary	1.39 $\pm$ 0.15	1.51 $\pm$ 0.16	1.20 $\pm$ 0.08†	1.18 $\pm$ 0.15
Thyroids	6.03 $\pm$ 1.19	8.14 $\pm$ 1.28	7.39 $\pm$ 1.57	8.22 $\pm$ 1.11
Adrenals	13.68 $\pm$ 1.00	10.58 $\pm$ 1.07	9.38 $\pm$ 1.76	12.59 $\pm$ 1.24
Spleen	46.82 $\pm$ 4.75††	62.16 $\pm$ 11.79	87.98 $\pm$ 17.23†††	57.74 $\pm$ 3.91
Bursa	67.56 $\pm$ 10.72	61.09 $\pm$ 11.44	84.75 $\pm$ 11.80	59.81 $\pm$ 8.35
Testes	2347.08 $\pm$ 212.13	2366.80 $\pm$ 226.94	---	---
Ovary	---	---	5025.00 $\pm$ 1118.03*	1651.35 $\pm$ 791.83
Oviduct	---	---	4950.00 $\pm$ 962.29	3121.40 $\pm$ 680.44
Adjusted organ weights (mg/100 gm body wt.):				
Pituitary	1.40 $\pm$ 0.15	1.53 $\pm$ 0.16	1.00 $\pm$ 0.14	0.97 $\pm$ 0.10
Thyroids	6.03 $\pm$ 1.03	8.15 $\pm$ 1.10	6.12 $\pm$ 1.30	6.80 $\pm$ 0.92
Adrenals	13.73 $\pm$ 0.56†	10.75 $\pm$ 1.28	7.83 $\pm$ 1.34	10.36 $\pm$ 0.95
Spleen	47.27 $\pm$ 6.47	63.54 $\pm$ 6.91	73.53 $\pm$ 14.90†††	47.84 $\pm$ 3.30
Bursa	69.06 $\pm$ 11.70	63.18 $\pm$ 12.49	70.44 $\pm$ 9.62	49.36 $\pm$ 6.80
Testes	2357.20 $\pm$ 208.45	2411.49 $\pm$ 222.93	---	---
Ovary	---	---	4129.44 $\pm$ 894.43*	1362.11 $\pm$ 632.46
Oviduct	---	---	4090.90 $\pm$ 772.01	2592.73 $\pm$ 545.89

† Significant difference between standard deviations, $P < 0.10$.

†† Significant difference between standard deviations, $P < 0.05$.

††† Significant difference between standard deviations, $P < 0.02$.

* Significant difference, $P < 0.05$.

At 5 weeks of age, the pinealectomized females weighed significantly less than their control group (5% level). The males were not significantly different at this age, but the pinealectomized males were slightly heavier. The 6-week old control males slightly outweighed their counterparts (nonsignificant). By 7 weeks of age, in both sexes, the operated and control birds showed no difference in body weight.

The pituitary glands and spleen showed no significant or consistent differences in any of the age groups. At 5 weeks of age the thyroids of both sexes were larger in the pinealectomized groups, but the difference was not significant. The 6-week old pinealectomized males had nonsignificantly lower thyroid weights. The adrenals of pinealectomized males at 7 weeks of age were nonsignificantly larger. The other groups had no significant differences and showed no consistent trend. While the bursa weights were not significantly different, there appears to be a trend toward slightly larger bursas in the pinealectomized birds.

The testes weights of male pinealectomized quail were not significantly different from those of their controls at 5, 6 or 7 weeks of age. There was, however, a difference between the standard deviations of the experimental and control groups at 5 weeks of age. The original data show a range in testes weights of 469.0 to 1082.0 milligrams in

the pinealectomized birds versus 89.2 to 1309.8 milligrams in the controls. Six of the individual control observations were less than the minimum experimental observations and three control observations were greater than the maximum experimental observation. The individual testicular weights and the standard deviations of the 6- and 7-week old groups were much less variable.

The mean ovarian weights of pinealectomized and intact control quail were almost equal at 5 weeks of age (66.53 vs. 70.67 milligrams). A significant difference between the standard deviations ($P < 0.02$) resulted from the wide range of individual control observations (32.5 to 133.0 milligrams). At 7 weeks of age the mean ovary weight of the pinealectomized group was significantly higher than that of the control group at the 5 percent level (5025 vs. 1651 milligrams). A part of these quail hens was sexually mature, causing an extremely wide range in individual observations within a treatment group. Individual ovary weights in the pinealectomized group varied from 1800 to 7500 milligrams, while the control group varied from 119.6 to 5100 milligrams. Only two of the 8 control birds had ovarian weights as high as 1800 milligrams, the lowest weight in the experimental group.

No significant differences were found in the mean oviduct weights at 5 or 7 weeks, although the latter age had differences approaching borderline significance. At

5 weeks of age the mean oviduct weight of the pinealectomized quail was appreciably lower than that of the control group (72.77 vs. 236.14 milligrams), but extreme variation in the individual data (26.25 to 107.2 and 12.3 to 915.6 milligrams, respectively, prevented statistical differences. The 7-week mean oviduct weights gave contradictory results. The pinealectomized quail had the larger oviducts (4950 vs. 3121 milligrams average). Although their individual variation was reduced (ranges of 3500 to 6700 compared to 300 to 6500 milligrams for the controls) the difference between means was not significant.

Seven pinealectomized and 10 control female quail made up the groups being tested to determine the effect of pinealectomy on age at sexual maturity. The average age of sexual maturity in the pinealectomized quail was 52.1 days, with a range from 48 to 57 days. The control mean age at sexual maturity was 48.9 days and the range was from 43 to 58 days of age. The difference between these means was not significant.

Experiment 3

The test groups in Experiment 3 consisted of 6 pinealectomized males, 12 control males, 6 pinealectomized females and 8 control females. At the end of the stimulatory light treatment (L:D = 14:10) period, all of the quail appeared to be sexually mature as evidenced by the presence of an active cloacal gland in the males and by egg production

in the females.¹ No significant difference was found in the day of first egg for the females. The average time for the beginning of egg production was 18.17 days after being given the stimulatory photoperiod in pinealectomized birds, compared to 18.75 days for the controls. The respective ranges of individual observations were 16 to 20 and 13 to 22 days.

After 28 days of stimulatory light treatment, an inhibitory light cycle was imposed. The males were sacrificed after 16 days of the inhibitory light treatment. There was no significant difference in the mean body weights of pinealectomized and control males (117.73 vs. 115.63 grams, respectively). However, the heavier mean weight of the testes of pinealectomized males approached borderline significance. The mean weight of testes in pinealectomized birds was 868.30 milligrams compared to 616.98 milligrams in the controls. One individual in the control group had relatively large testes (1314.2 milligrams) which probably kept the difference in means from being significant. Seven of the 12 control males had testes weights falling below

¹Externally, the cloacal gland appears as a swelling dorsal and posterior to the cloaca of sexually mature *Coturnix* males. It produces a foamy substance of unknown function, which is excreted with the feces. Wolfson (1952) suggests that activity of this structure is a dependable indicator of sexual function.

that of the smallest weight in the pinealectomized group. The cloacal gland appeared to be inactive in the pinealectomized and control males. The females remained under the inhibitory photoperiod for 21 days and egg production records were kept to determine the time of cessation of egg production. The mean number of days required to inhibit egg production under these conditions was 13.0 days for pinealectomized birds (range of 11 to 17 days) and 12.62 days for the unoperated controls (range of 10 to 16 days). The difference was not significant. The mean number of eggs produced per bird was also not significantly different between the pinealectomized and control quail (20.17 vs. 19.13 eggs, respectively).

Experiment 4

Eight male and 8 female Coturnix quail were given 500 micrograms of melatonin daily from 37 to 43 days of age, in 0.2 milliliters of a chloroform and sesame oil carrier. Similar groups were given the carrier alone as a sham-injected control. Two of the melatonin-injected females died early in the experiment, leaving 6 birds in that group. Table 8 summarizes the results of Experiment 4.

The difference between mean body weights in the melatonin-treated and control groups of males was insignificant. In the females, body weights of the melatonin-injected group were lower than those of the controls at the 1 percent level of significance. Of the endocrine organs,

TABLE 8.-Body weight and organ weights of Coturnix quail as influenced by daily melatonin injections from 37 to 44 days of age (mean weight $\pm$ standard error).

Sex Treatment	Males		Females	
	500 μ g Melatonin	Control (carrier only)	500 μ g Melatonin	Control (carrier only)
No. of quail (n)	8	8	6	8
Body weight (gm)	97.19 $\pm$ 1.72	98.80 $\pm$ 1.72	96.89 $\pm$ 3.54**	113.09 $\pm$ 3.06
Whole organ weights (mg):				
Pituitary	0.99 $\pm$ 0.12 [†]	1.01 $\pm$ 0.11	0.80 $\pm$ 0.13	1.18 $\pm$ 0.12
Thyroids	7.47 $\pm$ 1.12	6.96 $\pm$ 1.12	7.24 $\pm$ 0.82	7.37 $\pm$ 0.71
Adrenals	12.52 $\pm$ 0.93 ^{††}	10.94 $\pm$ 2.30	10.03 $\pm$ 1.17	10.09 $\pm$ 1.02
Spleen	47.67 $\pm$ 6.21	51.89 $\pm$ 6.21	66.69 $\pm$ 8.99	46.82 $\pm$ 7.78
Bursa	63.09 $\pm$ 5.23 [†]	87.82 $\pm$ 10.40	85.97 $\pm$ 10.39	106.19 $\pm$ 9.02
Testes	465.83 $\pm$ 119.61 [†]	838.36 $\pm$ 251.88	56.99 $\pm$ 6.75 ^{†††}	1690.17 $\pm$ 958.12
Ovary	---	---	26.73 $\pm$ 5.66 ^{†††}	1889.64 $\pm$ 933.27
Oviduct	---	---	---	---
Adjusted organ weights (mg/100 gm body wt.):				
Pituitary	1.01 $\pm$ 0.13 [†]	1.03 $\pm$ 0.12	0.83 $\pm$ 0.13	1.04 $\pm$ 0.11
Thyroids	7.83 $\pm$ 2.66 ^{†††}	7.08 $\pm$ 1.08	7.66 $\pm$ 1.04	6.60 $\pm$ 0.90
Adrenals	13.01 $\pm$ 1.24	11.10 $\pm$ 1.24	10.46 $\pm$ 1.14	8.96 $\pm$ 0.98
Spleen	49.42 $\pm$ 6.68	52.83 $\pm$ 6.68	70.12 $\pm$ 9.48*	42.20 $\pm$ 8.21
Bursa	64.91 $\pm$ 5.24 [†]	89.02 $\pm$ 10.50	88.74 $\pm$ 9.62	94.54 $\pm$ 8.33
Testes	472.28 $\pm$ 117.68 [†]	833.93 $\pm$ 245.48	58.65 $\pm$ 5.74 ^{†††}	1395.80 $\pm$ 786.77
Ovary	---	---	27.22 $\pm$ 5.04 ^{†††}	1564.94 $\pm$ 762.89
Oviduct	---	---	---	---

† Significant difference between standard deviations, $P < 0.10$.

†† Significant difference between standard deviations, $P < 0.05$.

††† Significant difference between standard deviations, $P < 0.02$.

* Significant difference, $P < 0.05$.

** Significant difference, $P < 0.01$.

pituitary, thyroids and adrenals, the only difference approaching significance was observed when comparing the mean pituitary weights in females. The experimental group had lower pituitary weights than the controls. When the mean pituitary weights are adjusted to the body weight, the difference was reduced. The mean bursa weights were only slightly smaller in the experimental males and females, compared to the respective sham-injected controls. The average spleen weights of females treated with melatonin were nonsignificantly larger than those of the controls. When adjusted to body weights (milligrams per 100 grams of body weight) the difference was significant at the 5 percent level.

The testes of the melatonin-injected birds were lighter in weight, averaging 465.83 milligrams compared with 838.36 milligrams in the controls; however, the wide range of individual weights in both groups (28.0 to 921.0 milligrams in melatonin-treated birds and 19.5 to 1771.8 milligrams in controls) eliminated any statistical significance. Similar results were found for both ovary and oviduct weights. The mean ovary weights for quail treated with melatonin and the controls were 56.99 vs. 1690.17, respectively, with ranges of 39.5 to 84.5 milligrams and 42.4 to 6700 milligrams. The average oviduct weights were 26.73 (melatonin-injected) and 1889.64 (control). A large range in the individual results (16.0 to 48.9 and 18.25 to 6000 milligrams, respectively), again prevented statistical significance.

Experiment 5

Levels of 0, 100 and 500 micrograms of melatonin were injected in 0.2 milliliters of aqueous ethanol solution daily, from 42 to 49 days of age. The results are summarized in Table 9. Body weights were not significantly affected. The birds receiving 500 micrograms of melatonin had larger adrenals which were significantly different from the controls at the 1 percent level. The birds on the 100 microgram melatonin dosage level had significantly smaller bursae (5 percent level).

Experiment 6

Four male quail in each of three groups were given 0.1 milliliter injections containing 0, 10 and 50 micrograms of melatonin in aqueous ethanol carrier daily from 35 to 49 days of age. The results are summarized in Table 10. Body weights and organ weights except for the spleen showed no significant differences. The spleen of the group receiving the 10 microgram dosage was larger than their controls (significant at the 5 percent level). The testes weights were slightly reduced at the 50 microgram dosage level.

Experiment 7

Twelve daily injections of 0, 100, 500 or 2000 micrograms of melatonin in aqueous ethanol solution did not cause sexually mature Coturnix hens to cease laying eggs. No difference was seen in the number of eggs

TABLE 9.-Body weight and organ weights of male Coturnix quail as influenced by daily melatonin injections from 42 to 49 days of age (mean weight $\pm$ standard error).

Treatment		Control (carrier only)		100 μ g Melatonin		500 μ g Melatonin	
No. of quail (n)		8		7		8	
Body weight (gm)		102.39 $\pm$	1.36	100.30 $\pm$	1.45	100.08 $\pm$	1.10
Whole organ weights (mg):							
Pituitary		0.99 $\pm$	0.11	1.11 $\pm$	0.13 ¹	1.26 $\pm$	0.09
Thyroids		5.15 $\pm$	0.63	7.18 $\pm$	0.73 ¹	5.56 $\pm$	0.50
Adrenals		8.66 $\pm$	0.71	10.37 $\pm$	0.76	11.98 $\pm$	0.64**
Spleen		40.66 $\pm$	4.07	28.17 $\pm$	4.36	41.56 $\pm$	4.54
Bursa		91.77 $\pm$	9.46	56.61 $\pm$	10.10*	68.96 $\pm$	10.91
Testes		2171.08 $\pm$	252.39	2392.74 $\pm$	269.81	2763.68 $\pm$	200.00
Adjusted organ weights (mg/100 gm body wt.):							
Pituitary		0.97 $\pm$	0.13	1.12 $\pm$	0.11 ¹	1.26 $\pm$	0.09*
Thyroids		5.03 $\pm$	0.70	7.09 $\pm$	0.60 ¹ *	5.55 $\pm$	0.49
Adrenals		8.45 $\pm$	0.74	10.35 $\pm$	0.70	11.99 $\pm$	0.64**
Spleen		39.84 $\pm$	4.28	27.99 $\pm$	4.01	41.82 $\pm$	4.70
Bursa		89.62 $\pm$	10.15	56.91 $\pm$	9.50*	69.25 $\pm$	10.86
Testes		2212.32 $\pm$	255.54	2376.83 $\pm$	238.96	2765.36 $\pm$	194.94*

* Significantly different from control, $P < 0.05$.

** Significantly different from control, $P < 0.01$.

¹ n = 6

TABLE 10.--Body weight and organ weights of male Coturnix quail as influenced by daily melatonin injections from 35 to 49 days of age (mean weight $\pm$ standard error).

Treatment	Control (carrier only)	10 μ g Melatonin	50 μ g Melatonin
No. of quail (n)	4	4	4
Body weight (gm)	101.90 $\pm$ 4.68	107.00 $\pm$ 4.42	105.68 $\pm$ 4.93
<u>Whole organ weights (mg):</u>			
Pituitary	1.25 $\pm$ 0.21	1.44 $\pm$ 0.14	1.05 $\pm$ 0.28
Thyroids	7.01 $\pm$ 1.50	8.85 $\pm$ 1.63	7.68 $\pm$ 1.37
Adrenals	13.45 $\pm$ 0.95	11.60 $\pm$ 0.82	13.14 $\pm$ 1.08
Spleen	37.06 $\pm$ 7.48	57.90 $\pm$ 5.60*	42.64 $\pm$ 9.36
Bursa	47.95 $\pm$ 9.14	56.56 $\pm$ 9.98	66.40 $\pm$ 8.29
Testes	2603.10 $\pm$ 445.86	2680.55 $\pm$ 406.20	2217.00 $\pm$ 138.56†
<u>Adjusted organ weights (mg/100 gm body wt.):</u>			
Pituitary	1.22 $\pm$ 0.21	1.37 $\pm$ 0.16	1.01 $\pm$ 0.26
Thyroids	6.91 $\pm$ 1.16	8.49 $\pm$ 1.10	7.22 $\pm$ 1.22
Adrenals	13.21 $\pm$ 1.06	10.93 $\pm$ 0.88	12.64 $\pm$ 1.25
Spleen	36.56 $\pm$ 7.66	54.76 $\pm$ 6.28	40.65 $\pm$ 9.03
Bursa	46.88 $\pm$ 8.88	55.39 $\pm$ 9.47	63.71 $\pm$ 8.28
Testes	2546.92 $\pm$ 412.65	2525.93 $\pm$ 386.01	2105.57 $\pm$ 91.60††

† Significant difference between standard deviations, $P < 0.10$.

†† Significant difference between standard deviations, $P < 0.05$.

* Significantly different from control, $P < 0.05$.

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produced during the treatment period. A dosage level of 50 or 1000 micrograms given for 24 days also gave completely negative results. Only one hen, on the higher dosage level, ceased egg production after 19 days of treatment.





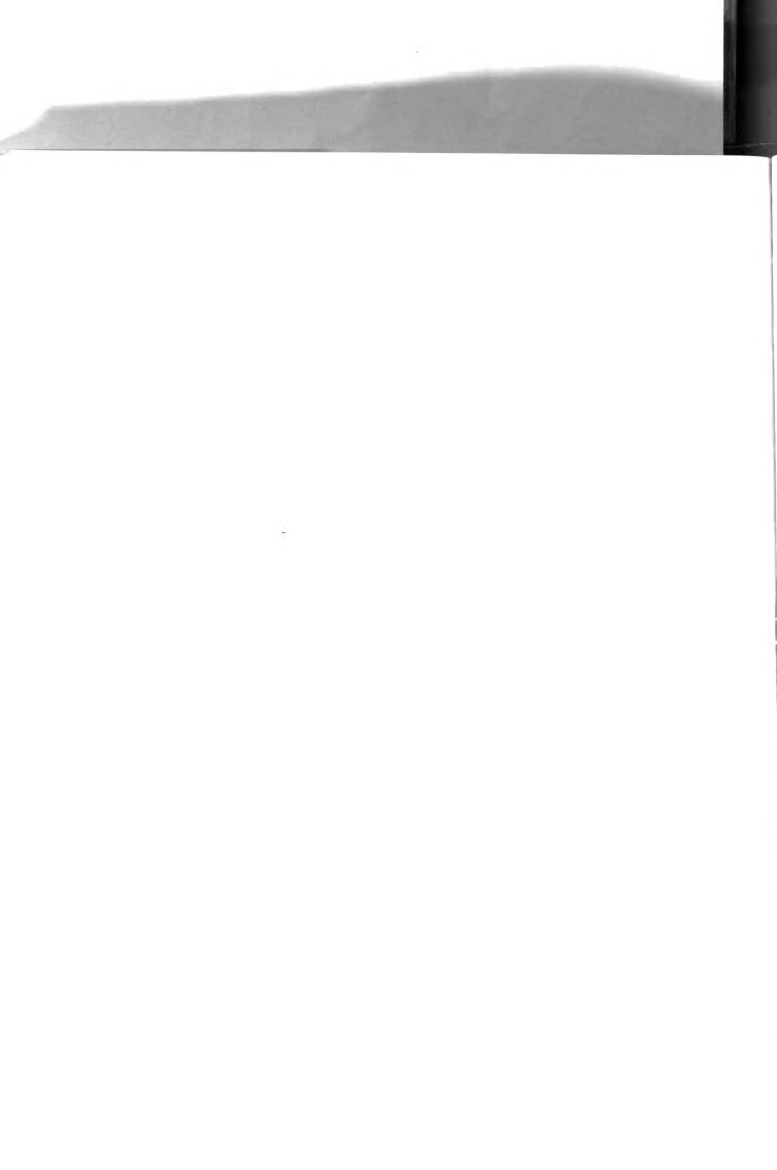
DISCUSSION

The use of Japanese quail (Coturnix coturnix japonica) in avian research has gained tremendous popularity in the last decade. Their use as a pilot animal has been based on the possible saving of time, space and money. Howes and Ivey (1961), Padgett and Ivy (1959), Wilson et al. (1961) and Woodard et al. (1965) describe the coturnix quail and justify their use in avian research. Studies of the function of the pineal gland in coturnix quail have not been previously reported.

The 30 percent mortality rate of pinealectomized quail in this series of experiments compares favorably with that observed in previous avian work reported. Badertscher (1924), Foa (1912, 1914) and Stalsberg (1965) reported mortality rates of nearly 70 percent following the operation. Shellabarger and Breneman (1949) observed less than 25 percent mortality. The only report of pinealectomy success rates among survivors was given by Shellabarger and Breneman (1948). These authors reported the presence of pineal tissue in almost 20 percent of the birds autopsied. Through microscopic examination, almost 30 percent of the quail in the present experiments were judged to have some remaining pineal tissue. Several of the quail judged to be unsuccessfully pinealectomized had very small masses of pineal tissue which lacked the typical follicular appearance of the avian pineal gland. Although these structures appeared to be inactive remnants of the pineal, these observations were

eliminated from the experiment since there is presently no basis for accurately accessing the activity level of pineal tissue.

In Experiment 1 pinealectomized and control quail were kept under L:D = 2.22 photoperiods from three weeks of age until sacrificed. At four weeks of age the quail were not thriving as well as would be expected under stimulatory light schedules. The four-week old quail averaged about 60 grams in body weight. Woodard et al. (1965) show a growth curve indicating a normal weight of about 60 grams at three weeks of age and 75 grams at four weeks of age. Assuming the quail to have been of normal size before being put on short light periods, they failed to gain weight during the first week on L:D = 2.22. It is suggested that they did not eat sufficiently during the first week of reduced light treatment. They probably were unable to find feed and water in the dark in the new surroundings and probably did not have sufficient capacity to eat a 24-hour supply of feed during the 2 hour supply of feed during the 2 hour period of light. Subsequent body weight data at 5, 6, 7 weeks of age showed more or less normal weekly gains, although the body weights remained slightly below the values given by Woodard et al. (1965). After the first week on short light periods, the quail were apparently able to eat adequate amounts of food during the 2-hour light period or were able to find food during the dark period. The latter explanation appears more probable.



The differences in organ weights between pinealectomized and control quail under L:D = 2.22 were not consistent or, for the most part, significant. The adrenal glands of 4-week old pinealectomized males were smaller than those of their controls; barely showing significance at the 5 percent level when calculated on the milligrams per 100 grams body weight basis. Conversely, the 5-week old pinealectomized females had slightly larger adrenals (nonsignificant). The pituitary gland was weighed only in the 6 and 7-week old groups. Pinealectomized females at 6 weeks of age and males at 7 weeks had smaller pituitaries than their respective controls. In the first case the difference was not significant and in the latter case, the 5 percent level was attained. The other two groups (males at 6 weeks and females at 7 weeks of age) showed no significance, but in both, the pituitaries were slightly larger in the pinealectomized birds.

The dependence of gonadal development on the light cycle is well known. Wilson et al. (1961) showed that L:D = 6:18 photoperiods prevented maturation of the testes of coturnix quail when given for the first 49 days post-hatching. Woodard et al. (1965) showed marked retardation of testicular growth in 5-week old coturnix males on L:D = 12:12 light cycles, compared to cycles providing longer light periods. Farner (1964) reviewed the evidence for control of gonadal function in birds by photoperiods.



Hoffman and Reiter (1965a, 1965b) showed that pinealectomy prevented atrophy of the testes of hamsters placed in L:D = 1:23 light schedules for 4 weeks.

Experiment 1 was designed to test the effect of pinealectomy on the rate of gonadal growth of quail raised under an inhibitory light schedule from 3 to 7 weeks of age. There were no significant differences in testes weights at 4, 5, 6 or 7 weeks of age, comparing pinealectomized birds with controls of the same ages. Comparison between the testes weights obtained and the suggested normal weights (quail on L:D = 16:8 light schedule) given by Mather and Wilson (1964) indicate that the testes not only failed to grow in experimental or control birds under L:D = 2:22, but actually atrophied. Assuming that the quail were of normal size when put on the inhibitory light schedule at 3 weeks of age, the mean testes weights should have been about 60 milligrams at that time. The mean weights observed ranged from 10.82 to 17.33 milligrams at 4 to 7 weeks of age. These weights correspond to the 2 weeks-of-age level found by Mather and Wilson (1964).

The ovary and oviduct weights also showed negative results. No differences were found by comparing pinealectomized and control group weights at weekly intervals from 4 to 7 weeks. Data are not available giving the normal weights of coturnix ovaries and oviducts at 2 to 4 weeks of age, however, the mean weights obtained at all four ages

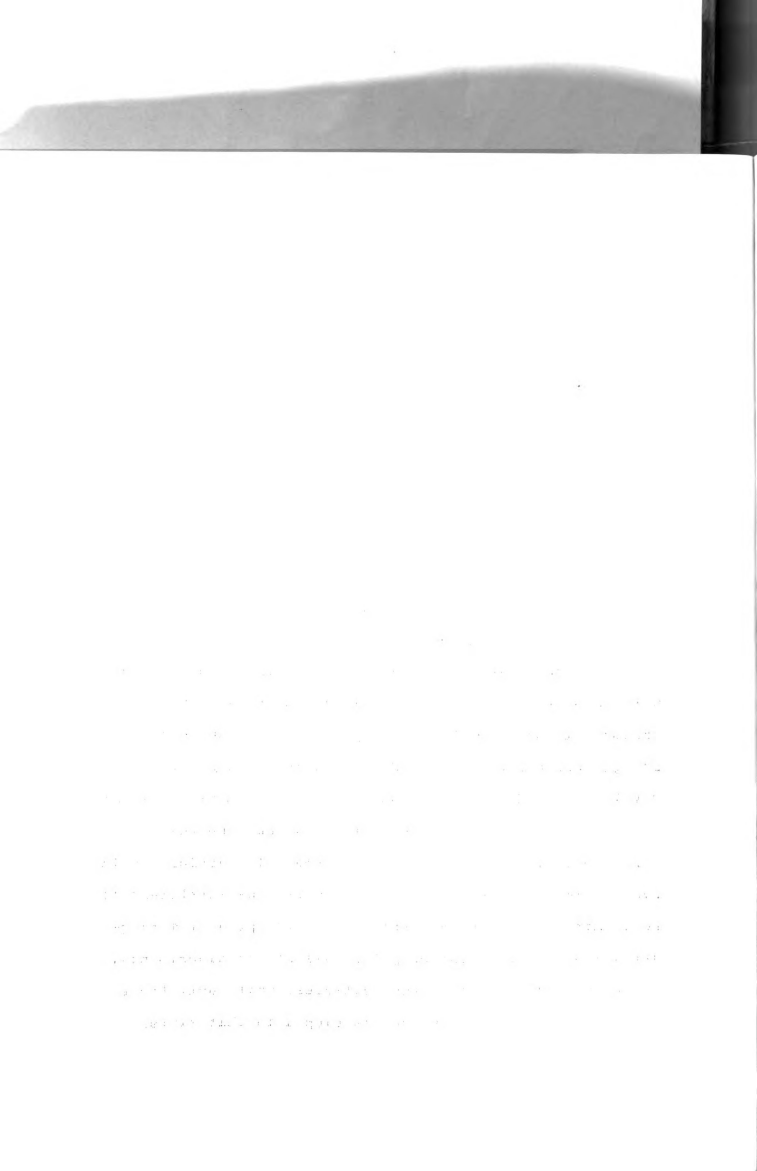
examined fell within the ranges observed by Wilson et al. (1962) in 6 and 7-week old Coturnix raised under inhibitory photoperiods (L:D = 6:18).

Experiment 1 showed that after a short period of adaptation, Coturnix quail continue to gain in body weight at approximately normal rates when given only 2 hours of light daily starting at 3 weeks of age. Removal of the pineal gland immediately after hatching does not affect these results. While a few significant differences were attained, the inconsistency of the data suggests that pinealectomy does not influence the weights of the pituitary, adrenals, thyroids, spleen, bursa, testes, ovary or oviduct under the conditions of this experiment. The short photoperiod appeared to cause an atrophy of the testes. The ovary and oviduct do not gain weight while on the inhibitory light schedule, but it is not known whether any atrophy occurs.

Experiment 2 measured the same parameters as those tested in Experiment 1, but the pinealectomized and control Coturnix quail were kept on L:D = 16:8 light schedules until sacrificed at 5, 6 and 7 weeks of age. Rather than sacrificing the females at 6 weeks of age, this group was kept to 9 weeks of age to determine age of sexual maturity.

The lower mean body weight of the 5-week old pinealectomized females, although significant, is not supported by similar results in the males or in the 7-week old

females. Any effect of surgical trauma would not be expected to be manifested at this age. Much of the difference would be negated by the elimination of one particularly small pinealectomized bird and one particularly large control, suggesting that larger numbers would not show an appreciable difference. As was seen in Experiment 1, there appears to be no consistent effect of pinealectomy on the organ weights except in the ovary and oviduct at 7 weeks of age. At 5 weeks of age, the control group's mean oviduct weight was markedly higher than that of the pinealectomized group, due to an obvious hypertrophy of the oviduct of two control birds preparatory to reaching sexual maturity. Some birds in this group showed no sign of impending sexual maturity, producing an extremely large variation. There were no ovaries with large follicles in the 5-week age groups. In the 7-week age groups several of the hens were in egg production. All birds showed definite gonadal development. The pinealectomized quail had markedly larger oviducts, but a large individual variation made the differences fall short of significance. The difference in ovary weights (pinealectomized controls) was sufficient to be significant at the 5 percent level in spite of a large variation in individual weights. All of the experimental group ovaries had some large follicles, while only three of the eight control hens had developed to that state.



Pinealectomized males had slightly larger testes at 5 weeks of age, but the subsequent age groups showed no difference.

The mean age of sexual maturity was delayed about 3 days in the pinealectomized female quail, compared to their controls. The difference was approaching borderline significance. The apparent inconsistency at 7 weeks of age suggests the use of caution in interpreting the results. While the only significant difference found indicated a hastened gonadal development of birds on L:D = 16:8 photoperiods, the contrary results found when measuring age of sexual maturity were close enough to being significant that one cannot ignore them.

In Experiment 3 pinealectomized and control quail of both sexes were kept on inhibitory light cycles (L:D = 2:22) from 3 to 8 weeks of age. As shown in Experiment 1, this treatment completely retarded sexual development while allowing almost normal somatic development. At 8 weeks of age, a stimulatory photoperiod of L:D = 14:10 was given to induce gonadal maturation. It was thought that this treatment might better show any effects of pinealectomy on age of sexual maturity in the females, since large changes in body weight would not be superimposed. The average number of days required for the onset of egg production, following the start of the stimulatory light treatment, was not significantly different between the pinealectomized and control hens.

The stimulatory light treatment was given for 4 weeks. All males had enlarged and active cloacal glands at the end of this phase of the experiment, indicating that they had reached sexual maturity (Wolfson, 1952). The light schedule was changed abruptly to L:D = 16:18 with gradual additional reduction in the light period to test the effect of pinealectomy in preventing the atrophy of the gonads of mature quail receiving inhibitory photoperiods.

Egg production records showed that pinealectomy caused no significant difference in the time required for cessation of egg production in response to short day-lengths. The males were sacrificed after 16 days of the final light treatment. Mean body weights for the pinealectomized and control males were not different. Differences approaching borderline significance indicated that pinealectomized birds had higher mean testes weights than did the controls. The testes in both groups were lower in weight than those of normal sexually mature *Coturnix* quail (Wilson et al., 1962). The results suggest that pinealectomy failed to prevent atrophy of the testes in response to inhibitory light schedules, but that the atrophy was somewhat delayed or reduced.

The results of these experiments suggest that the pineal gland does not control the influence of photoperiods on body weight, organ weights or reproductive function. It is indicated, however, that the presence

or absence of the pineal may influence gonadal parameters through a modifying effect. Numerous reports (see Literature Review) suggest the production of a gonadal inhibitor by the pineal gland, in response to reduced light. The results of Experiment 1 show that other mechanisms are present in the Coturnix quail which are capable of producing maximum gonadal inhibition in the absence of the pineal gland.

If the insignificant increase in testes weight of pinealectomized males under L:D = 16:8 is a real difference, it would indicate a retarding action of the pineal on sexual development sometime prior to this age. The lack of difference in the 6- and 7-week old birds would indicate that the pineal ceases to act in this manner near the time of sexual maturation. Shellabarger (1953) and Shellabarger and Breneman (1949) suggested that the pineal gland in White Leghorn cockerels might follow this pattern of activity.

All of the 7-week old pinealectomized females in Experiment 2 had enlarged ovaries and oviducts indicating that they were nearing or had already attained sexual maturity. Only 3 of the 8 control quail had gonadal development of the same magnitude. The differences in ovary weights were significant at the 5 percent level, but the oviduct values failed to reach a significant level. These results would justify the suggestion that

the pineal gland inhibits female gonadal development at least until the age of sexual maturity, and possibly retards sexual maturity to some extent. However, the 5-week and the age-of-sexual-maturity data do not support this suggestion. At 5 weeks of age the pinealectomized birds had the lower ovary and oviduct weights (not significant), and two of the control quail were already showing signs of oviduct hypertrophy preparatory to attaining sexual maturity. In the test of age at sexual maturity, the average age at which egg production commenced was delayed by 3 days in the pinealectomized group. These apparently contradictory results point out the problems involved in interpreting results of biological experiments involving small numbers of animals and the measurement of indirect responses. Many of the reports in literature concerning pinealectomy involved less than 5 animals per treatment and measured the results at only one age. Statistical procedures were often ignored in reporting the results. These factors would appear to be partially responsible for the inconsistent results published.

Experiment 3 showed no relationship between presence or absence of the pineal and the control of adult female gonadal function by photoperiods, as judged by the time required for the onset or cessation of egg laying. In adult male quail it was again shown that pinealectomy does not prevent testicular atrophy in response to



inhibitory photoperiods. It was suggested, however, that the gonadal atrophy was somewhat reduced by pinealectomy (approaching borderline significance), supporting the suggestion of a modifying action by the pineal gland. These results are in conflict with those of Hoffman and Reiter (1965a, 1965b), who reported that pinealectomy completely prevented testicular atrophy of adult hamsters placed under L:D = 1:23 photoperiods for a 4-week period.

The Literature Review cites many papers dealing with the action of melatonin. This substance is produced in the pineal area and has been suggested to be a pineal factor involved in gonadal inhibition. Other reports dispute this role of melatonin.

Sexual maturity of Coturnix females has been observed as early as 35 days of age (Howes and Ivey, 1961; Wilson et al., 1962), whereas males appear to mature slightly later (Howes and Ivey, 1961). Daily injections of 500 micrograms of melatonin into males from 37 to 43 days of age resulted in a nonsignificant reduction of testes weights at 44 days of age (Experiment 4). Both groups contained individuals with juvenile testes, but the control groups included four birds in which the combined testes weights ranged from 1144 to 1771 milligrams. It would appear that the injection of high levels of melatonin can either reduce or delay gonadal development in males nearing sexual maturity.

Experiment 5 tested the effect of 100 and 500 microgram doses given daily at 42 to 49 days of age. The high-level-dosage group outweighed the controls (nonsignificant). These quail were in the age range of expected sexual maturity. The apparent conflict of these results with those from the previous experiment could be explained on the basis of a reduction in sensitivity of the testes at or near the puberal age. Such an interaction is not unlikely, but additional investigation is needed before any conclusions can be reached.

In Experiment 6 daily doses of 10 micrograms of melatonin produced no effect on testes weights when given from 35 to 49 days of age. The 50 microgram level resulted in a slightly (nonsignificant) reduced mean testes weight. The smaller degree of difference found in this experiment compared with that of Experiment 4 could be due to the lower dosage or to the increased age at autopsy (the injections were started at 35 and 37 days, respectively; this small difference in procedure is not thought to be a factor). Indirect evidence by Axelrod and Weissbach (1960), Axelrod et al. (1964) and Quay (1965b) suggest that avian species may normally produce more melatonin than mammals do. In this case, the dosage required for maximum response may be in excess of 50 micrograms when given in daily injections. If, on the other hand, 50 microgram doses are sufficient to produce

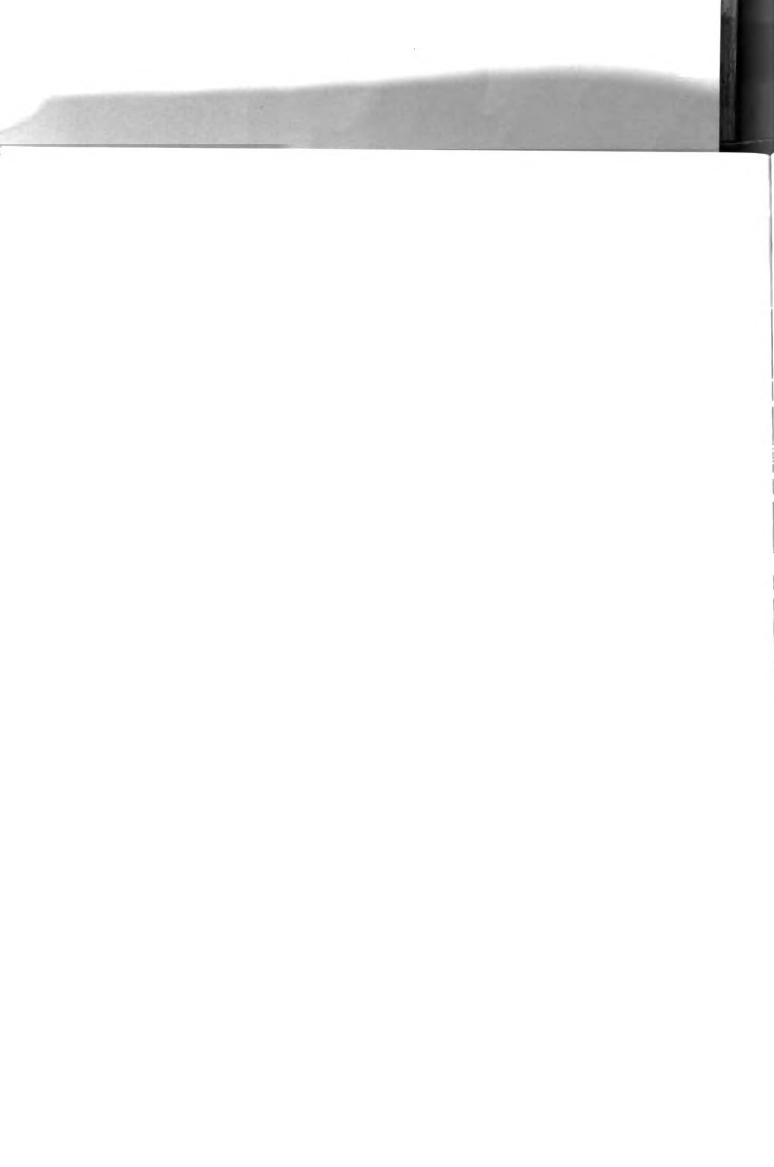
maximal responses, the age-at-autopsy difference might account for the decreased difference in testes weights observed in Experiment 6. This would support the suggestion of a decreased response by the testes as the puberal phase of development is approached. The 50 microgram level of melatonin did not prevent maturation of the testes, but some reduction or delay of gonadal development was indicated.

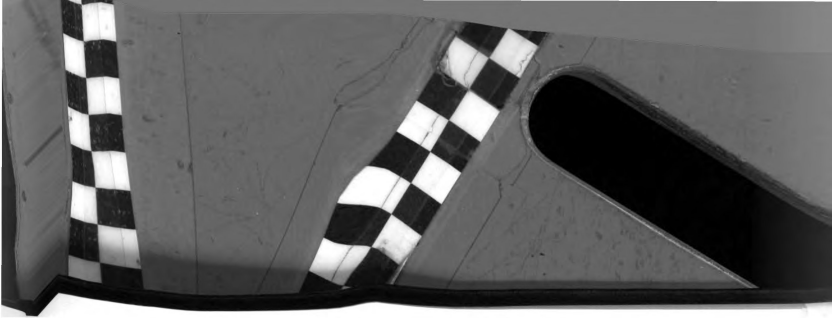
Both the ovary and oviducts were slightly smaller in quail given 500 micrograms of melatonin daily between the ages of 37 and 44 days, compared to their sham-injected controls (Experiment 4). In the latter case the difference approached borderline significance. Two of the control quail had ovaries and oviducts within the weight range of adult quail (4 to 7 grams; Wilson et al., 1961, 1962). Other controls had gonads which varied from juvenile to intermediate in size. None of the melatonin-injected quail showed any appreciable hypertrophy of the ovary or oviduct (maximum individual weights of 48.9 and 84.5 milligrams, respectively). The wide variation in control weights precluded the finding of significance in this experiment. Nevertheless, there is strong evidence of an inhibitory effect of large doses of melatonin on the sexual maturation in prepuberal female Coturnix quail. Several papers cited in the Literature Review report depression of rat ovary weights and delay of sexual maturity by injected melatonin. The lack of development at 44 days of age would suggest

that sexual maturity would have been delayed by melatonin if Experiment 4 were continued until egg production began.

Mature female quail showed no reproductive response to daily injections of melatonin in doses from 50 to 2000 micrograms in Experiment 7. None of the experimental animals ceased to produce eggs during the trial periods of 12 and 24 days. Neither was there any appreciable difference in the total number of eggs laid. It is concluded that the mature *Coturnix* quail ovary is unresponsive to melatonin-injected females of Experiment 4 (1 percent level). A portion of this difference (3.5 grams per bird) can be attributed to the increased gonadal development in the control group. It is not known whether the remaining difference is associated with the onset of puberty or is an increase in somatic development in the controls. The short duration (1 week) of the injection treatment would seem to favor the explanation associating the difference with sexual development. McIsaac et al. (1964) and Wurtman et al. (1963a) obtained no difference in rats injected with 1 to 50 micrograms of melatonin from 27 to 55 days of age. Tilstra and Prop (1963) observed lowered body weights in both sexes when daily 100 microgram doses were injected from birth to 50 days of age. Male rats receiving daily injections of 10 to 500 micrograms of melatonin for up to 2 weeks showed no change in body weights from that of the controls.

Effects of melatonin injections on other organ weights were largely negative. The few differences observed were usually at the borderline level of significance and not consistent in the 3 experiments.





SUMMARY

Newly hatched Japanese quail (Coturnix coturnix japonica) were pinealectomized and grown under inhibitory or stimulatory photoperiods. At various ages they were sacrificed and their body weight, organ weights and age at sexual maturity were compared with unoperated controls. The organs weighed were the pituitary, thyroids, adrenals, spleen, bursa, testes, ovary and oviduct. The success of the pinealectomy operation was determined by macro- and microscopic examination. Daily injections of melatonin, a pineal elaboration, were given to immature Coturnix quail to determine its effect on body and organ weights. In addition, adult quail hens were injected daily to observe the effects of melatonin on maintenance of egg production.

The results of pinealectomy experiments indicate that the pineal has little, if any, effect on body weight or on the weight of the pituitary, thyroids, adrenals, spleen or bursa. The differences obtained in these parameters were inconsistent and usually nonsignificant. Complete retardation of gonadal growth and probably atrophy of the testes was observed in control quail when grown under photoperiods consisting of two hours of light and 22 hours of darkness per day from 3 to 4, 5, 6 and 7 weeks of age. Removal of the pineal glands did not prevent or reduce the inhibitory effect of this photoperiod. Under stimulatory photoperiods (16 hours of light and 8 hours



of darkness per day), 5-week old male pinealectomized quail had slightly larger testes. This effect was not present in the 6- and 7-week old males. The possible production of a gonadal inhibitor by the pineal is suggested. However, the results indicate that somewhere near the time of sexual maturity this inhibition ceases due to decreased inhibitor production or decreased response to the inhibitor by the gonads. The responses of females in these tests were not consistent. The only significant response was an increased mean ovary weight in the 7-week pinealectomized group. The 5-week data showed a non-significant difference with the control gonads being slightly larger. The mean ages at which egg-laying started were not significantly different.

Adult pinealectomized and control quail hens showed no difference in the number of days required to respond (by commencing egg production) to a change from inhibitory to stimulatory light cycles. Reversing the change in photoperiods produced no difference in the time of cessation of egg production. Sexually mature pinealectomized males showed less atrophy of the testes when switched to an inhibitory light schedule. Due to a large variation in individual results, statistical significance was not obtained, but it is suggested that the presence of the pineal gland may hasten testicular atrophy. The results of the pinealectomy experiments suggest that the pineal



modifies, rather than controls, the gonadal response to photoperiods.

Melatonin injections of 50 to 500 micrograms daily produced a slight retardation of gonadal development in prepuberal quail of both sexes. It appeared that the gonads of male quail at or near the puberal phase of growth were not affected by melatonin injections. Daily doses of 50 to 2000 micrograms of melatonin given for 12 to 24 days failed to alter the egg-laying capacity of adult Coturnix hens. These results support the suggestions that the pineal produces an inhibition of gonadal growth in prepuberal quail and that the mature quail gonad is less responsive to pineal inhibition.



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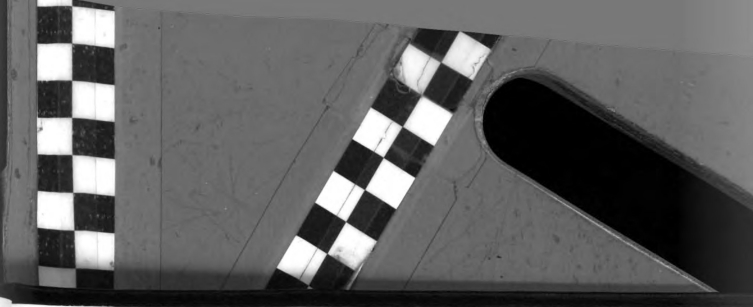
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