EFFECTS OF PINEALECTOMY ON SERUM LEVELS OF THYROIDAL IODOHORMONES AND THEIR CIRCADIAN RHYTHMS IN CHICKENS

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CHAPTER I

INTRODUCTION

The Greek anatomist Galen described the pineal gland in 177 A.D. Since then, the pineal has been the target of much speculation regarding its possible functions. Early investigators attributed the gland with such diverse functions as secreting and regulating the flow of cerebrospinal fluid, secreting lymph, and being the seat of the soul (Rolleston, 1936). In the late nineteenth and very early twentieth century, investigators concluded that the pineal was the vestigial third eye of lower vertebrates. During the first half of the twentieth century, general debate centered around whether the pineal gland was a useless evolutionary holdover or a functioning endocrine gland. Renewed investigative effort in the past twenty years has suggested that the pineal is involved in at least three physiologic areas: 1) regulation of reproduction in birds and mammals (Reiter, 1969; Wurtman, et al., 1963; Orts, 1973); 2) extraretinal photoreception in birds, suggested by the photoreceptivity of the pineal of lower vertebrates (Dodt, et al., 1971); and 3) control of circadian rhymicity of various types (Menaker and Asche, 1974). In addition, the possibility exists that the pineal gland may be directly involved with the control of serum concentrations of thyroidal iodohormones. It is with this latter aspect of pineal function that this thesis is concerned, i.e., the possible relationship between the pineal gland and rhythms of thyroid function.

CHAPTER II

LITERATURE REVIEW

Only during the last decade has the study of rhythms of thyroid function shown any promise. J. Dainat, et al. (1969) published work which indirectly described an ultradian rhythm of ^{131}I uptake by duck thyroid glands. These investigators measured radiation from duck thyroids in vivo at various intervals up to 72 hours after radioactive labeling. When these data were subjected to spectral analysis, a rhythm with an approximate 12 hour period was noted. The maximum deviations from the level were determined to be at 0400 hours and 1600 hours. Attempts to fit these data to a 24 hour sinusoidal curve failed.

Sadovsky and Bensadoun (1971) demonstrated a diurnal rhythm of plasma iodohormones in four ten month old roosters. Plasma iodohormones were separated by cation exchange chromatography and estimated by iodine analysis. Following recovery of iodine concentrations from thin layer plates, appropriate conversion factors were used to determine the amounts of iodine in the form of T_4 and T_3. These conversion factors were 1.69 for T_3 and 1.53 for T_4. The values of T_4 and T_3 determined by this method of analysis were higher by factors of 2 and 10, respectively, than hormone levels reported in the other literature (May, et al., 1973). In demonstrating a diurnal rhythm of T_4 and T_3, these investigators sampled their birds every four hours for twenty hours and interpreted their results as showing a T_3 rhythm with maxima at 0800 and 1600 hours. The T_3

levels at these times were reported to be significantly greater ($P < .05$) than the levels at other test times. T_4 levels were also reported to follow a rhythmic pattern with maximum values at 0800 and 1600 hours but with much less amplitude. In addition, Sadovsky and Bensadoun reported that total iodohormone ($T_4 + T_3$) concentration followed a similar rhythmic pattern, with maxima at the same times as those exhibited by T_3 and to a lesser extent T_4. The methods of analysis used by these writers were analysis of variance and the Duncan Multiple Range Test. These methods of analysis are of little value in attempting to determine the true form of the plasma T_4 and T_3 curves. The fact that two peaks are reported by these writers indicate that their diurnal rhythm is characterized by a wave form that is more complex than a 24 hour sinusoidal curve.

In 1974, Newcomer published work showing a diurnal rhythm in several parameters of thyroid function in chickens. At three hour intervals for 30 hours, eight birds were bled for determination of serum T_4 and T_3 by RIA and 20 birds were sacrificed for ^{131}I uptake, binding rate constant (K_b), and effective clearance constant (Ce/m) determinations. Data for each parameter were then analyzed with respect to statistical fit to a theoretical 24 hour sinusoidal curve by the method of least squares (Halberg, et al., 1972). Of these five parameters, all exhibited a sinusoidal wave form with statistical significance except Ce/m. The acrophases of serum levels of T_4 and T_3 were 0704 hours and 1616 hours, respectively; the acrophases of ^{131}I and K_b were 0657 hours and 1414 hours, respectively. The magnitudes of serum concentrations of T_4 and T_3 were in line with those previously reported (Singh, et al., 1967; Refetoff, et al., 1970; May, et al., 1973). As was indicated by

Newcomer, the fact that the data for these parameters statistically fit sinusoidal curves with a period of 24 hours does not preclude the possibility that the data may fit a sinusoidal curve with some other period. The possibility also exists that the data may fit some entirely different curve. The basic differences between the Newcomer study and that of Sadovsky and Bensadoun are techniques of measuring T_4 and T_3, magnitudes of serum T_4 and T_3 concentrations, number of birds studied, and statistical analysis. In addition, the sinusoidal wave form with a 24 hour period in the parameters of thyroid function mentioned above is quite different from the biphasic wave form that Sadovsky and Bensadoun interpreted from their data. An interesting similarity between the data of these two different investigators is that the acrophase of T_4 reported by Newcomer (0704 hours) is very close to the first peak reported by Sadovsky and Bensadoun (0800 hours) for T_4. Likewise, the acrophase of T_3 from the Newcomer data (1614 hours) is very close to the second peak reported by Sadovsky and Bensadoun (1600 hours) for T_3.

The search for a functional relationship between the pineal gland and the thyroid glands of birds and mammals has been complicated by the failure of investigators to obtain consistent data. In 1963, Scepovic described an exaggerated hypertrophy of thyroid cells coupled with an increase in ^{131}I upstake in pinealectomized rats.

Scepovic (1963) using another group of rats, detected hypertrophy and hyperplasia of the thyroidal cells and colloidal resorption two months after pinealectomy. After longer periods of time, the thyroid showed a polymorphous structure. The administration of pineal extracts to these rats diminished ^{131}I fixation by the thyroid.

Csaba (1969) reporting similar results, stated that the thyroids of

pinealectomized rats had a 50% greater incorporation of ^{131}I than controls. In a 1966 study, Ishibashi, et al. demonstrated that pinealectomy tends to cause an 11.8% increase in thyroid secretion rate (TSR) in female rats.

The employment of pineal extract injection has done much to help formulate the theory that the pineal and thyroids are at least partially related in mammals. In 1961, De Luca, et al. demonstrated that bovine pineal extracts given in large doses decreased thyroid weight and ^{131}I uptake in rats. In 1960, Lerner, et al. suggested that the pineal gland secretes a hormone, melatonin, and that this hormone is responsible for any action that the pineal may have. While this statement may not be entirely true, the administration of melatonin has been shown to reverse many of the above noted effects. In the aforementioned report by Ishibashi, et al. (1966), daily injections of 20 µg/day of melatonin in young female rats decreased TSR by 16%. The opposite effects were noted by Thieblot (1966), namely that administration of melatonin in high doses induced obvious hypertrophy of the thyroid glands of prepuberal rats.

In a 1963 study, Baschieri, et al. reported that daily injections of 150 µg of melatonin (1 µg/gram body weight) modified the morphology of the rat thyroid and its uptake of radioactive iodine. These injections reduced thyroidal cell height and uptake of ^{131}I in control as well as methylthiouracil-treated animals. Wurtman and Axelrod (1965) demonstrated that melatonin depressed the uptake of ^{131}I in rats maintained on a low iodine diet. Another group of investigators (Houssay, et al., 1966) reported that pinealectomy increased thyroid gland weight and that melatonin blocks this effect.

In 1967, Narang, et al. reported results in agreement with those of Ishibashi (1966). These writers reported that high doses of melatonin given subcutaneously reduced TSR in male rats. In a 1972 study, Singh and Turner tested the effects of administration of melatonin on TSR of male rats. Two groups of rats were given daily injections of melatonin subcutaneously at doses of 50 and 100 µg/100 grams body weight and their thyroid secretion rates were determined and compared to controls. The higher dosage of melatonin significantly reduced the TSR from 1.20 µg L-T_4/100 grams body weight to 0.95 µg L-T_4/100 grams body weight while the lower dosage had no significant effect. A recent report has shown that there is present in ovine, bovine, and porcine pineal glands 4-10 times as much gonadotropic releasing factor and at least as much thyroid releasing factor as is present in the hypothalami of the respective species (White, et al., 1973). This work has not been duplicated but the inference of these authors' interpretation of their data is that the pineal may serve as a constant low level source of releasing factor for a tonic or maintenance effect on the pituitary or on some unknown endocrine target. These findings, while inconclusive, and contrary to those reported by De Luca (1961), do seem to indicate the existence of a pineal-thyroid axis in mammals.

The search for a functional relationship between the pineal and thyroid glands of birds has yielded even less conclusive evidence than it has in mammals. In 1953, Shellabarger reported that the removal of the pineal caused hypertrophy of the testes of 40-65 day old male chicks but had no effect on the adrenals or thyroid glands of these chicks. Singh and Turner (1967) demonstrated that melatonin given at two doses had no effect on TSR in chickens. This observation is opposite to that seen in

rats.

In attempting to clarify the physiological role of the pineal, one is inevitably drawn to the theory that the pineal is involved in the control of circadian rhythmicity. This theory is supported in large part by the effect of pineal ablation on two well established circadian rhythms. In 1968, Gaston and Menaker demonstrated that pineal removal abolished the circadian rhythm of locomotor activity in house sparrows. Birds were constantly monitored from day one until day 19. During this time, normal locomotor activity cycles were recorded by telemetry. On day 19, the birds were pinealectomized and within two days became arhythmic. Binkley, et al. (1971) reported similar effects on the circadian rhythm of body temperature in house sparrows. Deep body temperature was continuously monitored by radiotelemetry and following pinealectomy, the body temperature cycle in constant darkness was abolished. While in constant darkness, the pinealectomized birds maintained a continuously high body temperature, i.e., elevated about 4°C, with no fluctuation, but when exposed to alternating light and dark periods, pinealectomized birds entrained within four or five cycles. However the minima of the rhythm exhibited by these birds never fell as low as those of controls. In at least these two circadian rhythms the pineal is involved in the ability of the rhythms to persist in constant conditions.

Since the existence of thyroidal circadian rhythms has been established and since the pineal has been shown to be involved in at least two other circadian rhythms in birds, one is tempted to speculate that the pineal may be effecting the thyroidal circadian rhythmicity and consequently the serum levels of iodohormone as well.

CHAPTER III

METHODS AND MATERIALS

Animal Management

White Leghorn cockerels were obtained from a local hatchery on the day they were hatched (designated day 1). They were immediately divided at random into two groups and housed in commercial battery-type brooder cages under constant light and an ambient temperature of 37°C. The birds received standard broiler ration and tap water ad lib. One group was pinealectomized (see below) while the other group served as either normal controls or sham-operated controls, depending on the experiment. Following surgery, the birds were allowed to recover for two weeks, during which time both groups were exposed to constant light. At the end of this recovery period, both groups were moved to separate floor pens and exposed to an alternating 16 hours of light-8 hours of dark photoperiod (16L:8D). The dark phase of the photoperiod began at 2200 hours. The floor area of the room housing the control group measured 135.38 square feet and the area of the room housing the pinealectomized birds measured 209.58 square feet. A Weston Illumination meter, model 756, was used to determine the average illumination four inches above the floor in each room. The floor area for the control birds was found to receive 0.0753 foot-candles of light per square foot while that for the pinealectomized birds received 0.0622 foot-candles of light per square foot. The ambient temperature of each room was maintained at 24.0°C.

Prior to sampling, birds were removed at random from their respective floor pens and placed in wooden coops, eight birds from each group in separate coops. At the conclusion of each experiment, the birds were sacrificed and grossly inspected for completeness of pinealectomy. Sham-operated controls were also inspected. Blood samples were taken from the heart using a 2cc syringe and a 22 gauge needle. Blood taken in this manner was placed in small test tubes and allowed to clot at room temperature. Shortly after placing blood in the collection tubes, the inside of each tube was "ringed" with a Puritan applicator stick, thus facilitating the retraction of the clot from the wall of the tube and allowing a larger volume of serum to be collected without centrifugation. Serum collected in this manner was stored frozen until the time of hormonal analysis.

Experimental Design

In the course of this thesis problem, three experiments were carried out to test the effect of ablation of the pineal gland on the circadian rhythms of thyroidal iodohormones in the serum of White Leghorn cockerels in a 16L:8D photoperiod. In each experiment, animals were sampled in essentially the same manner. Eight birds from each group, i.e., controls and pinealectomized, were caught and sampled every three hours for thirty hours or until all available birds had been utilized. The time required to take blood samples from the sixteen birds was approximately fifteen minutes. This amount of time was centered around the designated hour and birds were alternated such that first a control was bled and then a pinealectomized bird and so forth.

Experiment I consisted of sampling normal control birds and

pinealectomized birds after six weeks of exposure to 16L:8D. Experiment II contained three trials, one after 3 weeks, one after 4 weeks, and one after 6 weeks of exposure to 16L:8D and contrasted sham-operated controls with pinealectomized birds. Experiment III was a duplication of experiment II except normal controls were substituted for sham-operated controls. Following completion of all analyses of all samples taken during the seven trials, means and standard errors of the mean were calculated for each eight-bird group of each of the seven trials. In addition, means and standard errors of the mean for each trial were calculated. Using these calculations, serum levels of thyroidal iodohormones could be studied.

Individual data points were punched onto standard computer cards and then fit to a theoretical 24 hour sinusoidal curve by the method of least squares (Halberg, 1972). This fit was statistically tested by the use of the null hypothesis. The equation $Y(t) = C_0 + C \text{ cosine } (\omega t + \phi)$ describes the form of the theoretical sinusoidal curves generated by the data from the seven trials. In this equation, C_0 is the level of the trial, C is the amplitude of the curve from the level (C_0) to the maximum, ω is the angular frequency, and ϕ is the acrophase of the cosine function. Normal time plots were prepared from the information supplied by the computer analysis.

Surgical Technique

Between one and three days of age, White Leghorn cockerel chicks were anesthetized with sodium thiamyl (Surital) at a dosage of 3 mg/100 grams body weight administered intraperitoneally. A surgical plane of anesthesia was achieved within 5 minutes. The dorsal portion of the

chick's head was clipped of down and the bird was placed in a rat stereotaxic instrument specifically modified to immobilize chicks less than two weeks of age. A mid-dorsal incision was made through the skin from the caudal end of the comb to the base of the skull (approximately 3 cm in length). The skin was retracted laterally to expose an area of about 7 mm square over the area of the skull where the transverse and mid-saggital sutures cross. A rectangular bone flap approximately 7 mm by 5 mm was cut in the skull with a cautery needle and raised with two pairs of jewelers forceps. Next, the meninges were incised and lifted to expose the pineal gland. Hemorrhage became a problem at this point since incising the meninges necessarily resulted in the opening of the transverse and mid-saggital sinuses. This bleeding was controlled by the application of gentle suction to the meningeal flap. A second suction cannula was used to remove the gland. Once the gland had been removed, a small piece of Gelfoam (Upjohn) was placed in the cavity left by the removal of the gland and the meningeal and bone flaps were placed over it. The skin was closed with three 11 mm wound clips. The bird was removed from the stereotaxic instrument and placed in an area warmed by a high intensity lamp. After recovery from anesthesia, all birds were returned to their brooder cages. Sham-operations were performed in the same manner with the exception that the glands were not removed and the Gelfoam sponge was not inserted. In the course of the three experiments, the mortality rate from surgery was between 20 and 25 percent.

Antibody Preparation

Antisera to T_4 and T_3 were prepared by a previous investigator according to the methods of Gharib, et al. (1971). L-T_4 and L-T_3 in the

free acid form were conjugated to human serum albumin (HSA). Following dialysis for 72 hours and lyophilization, the conjugate was taken up in Freund's complete adjuvant (Difco Laboratories) and aliquots of the suspension were injected weekly for eight weeks intradermally into two female rabbits. The total of each weekly injection was three ml and was injected at multiple sites. One week after the eighth injection, blood samples were taken and the serum was removed and stored in the frozen state. At this time, a booster injection was given and one week later, final blood samples were taken and the serum was stored at -10°C. Both the pre-booster and post-booster sera were tested for binding capacity. Prior to use in the RIA, the antisera to T_4 and T_3 were diluted 1:70 and 1:3000, respectively.

Reagents for Hormone Analysis

Barbital Buffer

Barbital buffer (0.08 M) was prepared by dissolving 32.992 grams of 5-5'-diethyl barbituric acid (Barbitone, Verdnal; Sigma Chemical Company) and 2 grams of bovine serum albumin (BSA) in 2 liters of glass distilled water. The pH of the resulting solution was adjusted to 8.4 with 4 N NaOH and the solution was filtered through a Whatman #1 filter. The buffer was then stored at 4°C until used.

Dextran Charcoal

Dextran charcoal, stock solution, was prepared by adding 2.5 grams of Norit A decolorized charcoal (Pfanstiehl Laboratories) and 0.25 grams of dextran (M.W. 86,000; K and K Laboratories, Inc.) to 300 ml of cold barbital buffer. This suspension was prepared on the day it was to be

used and stored at 4°C on a magnetic stirrer to prevent settling.

8-Amiline-1-Naphtalene Sulfonic Acid (ANS) Solution

ANS (Eastman Kodak Company) reagent was prepared by dissolving 0.0438 grams of ANS in 50 ml of cold barbital buffer. The solution was stored at 4°C until used.

(^{125}I) Thyroxine (T_4-^{125}I) and Triiodothyronine (T_3-^{125}I)

Radioactively labeled (^{125}I) Thyroxine (T_4-^{125}I) and Triiodothyronine (T_3-^{125}I) were obtained from Abbott Laboratories in the form of Tetramet-125(Thyroxine) and Triomet-125(Liothyronine).

Standard Hormone Solutions

Thyroxine (T_4) Stock I. One mg of L-Thyroxine, free acid (Sigma Chemical Co.) was accurately weighed on a Cahn electrobalance and dissolved in 3 drops of 0.4 N NaOH and 3 drops of 70% ethanol. The resulting solution was then brought up to 100 ml with cold barbital buffer.

Thyroxine (T_4) Working Solution. One ml of T_4 stock solution was diluted to 100 ml with cold barbital buffer. From this working solution, standards of 0, 0.1, 0.5, 1, 2, 3, and 4 nanograms per assay tube were prepared.

Triiodothyronine (T_3) Stock I. One hundred mg of 3,3,5' T_3 were weighed out and dissolved in small amounts of 70% ethanol:1 N HCL (2:1). This solution was brought up to 10 ml with cold barbital buffer.

Triiodothyronine (T_3) Stock II. One ml of T_3 stock I was diluted to 100 ml with cold barbital buffer.

Triiodothyronine (T_3) Stock III. One ml of T_3 stock II was added to 99 ml of cold barbital buffer.

Triiodothyronine (T_3) Working Solution. One ml of T_3 stock solution III was diluted to 100 ml with cold barbital buffer. From this solution, standards of 0, 10, 25, 50, 100, 200, and 400 picograms per assay tube were prepared.

Radioimmunoassay for Serum Thyroxine and Triiodothyronine

The RIA for T_4 was performed in duplicate in 10 x 75 mm disposable test tubes which were initially placed in an ice bath. To each tube was added 75 µl of cold barbital buffer, 25 µl of unknown serum, 100 µl of T_4-^{125}I, 200 µl of ANS, and 100 µl of antiserum to T_4. This mixture was vortexed and incubated at 37°C for 90 minutes. Following incubation, the assay tubes were placed in a 4°C cold room for ten minutes. One ml of dextran charcoal was then added, the mixture vortexed, and allowed to equilibrate in the cold for 20 minutes. Following equilibration, the mixture was centrifuged at 2000 rpm for 15 minutes. The supernatant, which contained the bound fraction, was decanted off of the charcoal pellet which contained the free hormone. Both fractions were counted in an automatic gamma counter.

The assay for T_3 was performed in the same basic manner as that of T_4 except that antiserum to T_3 and iodinated T_3 was used. In either case, following counting, bound to free ratios were calculated and

compared to those taken from the standard curves for the respective hormones. Standard curves were prepared by assaying solutions containing known concentrations of either T_4 or T_3. A "recovery solution" was prepared and assayed with unknown samples in each assay. Data were corrected for recovery prior to statistical analysis.

Miscellaneous Materials

All pipetting for the radioimmunoassay (RIA) for thyroid hormones was done with Eppindorf pipettes (Brinkman). Assay tubes (10 mm x 75 mm; Corning) were counted in a 300 sample gamma counter (Nuclear Chicago). Pinealectomy was performed on birds anesthetized with thiamyl sodium (Surital; Parke, Davis, and Company). All centrifugation was performed in International centrifuges, either the model HN or the model UV.

CHAPTER IV

RESULTS

The results of experiments designed to test the possible role of the pineal gland in the control of circulating thyroid hormone levels will be presented in the first of two sections of this chapter; the second section will deal with the possible effects of pineal ablation on the circadian rhythms of these circulating thyroid hormones. Groups will be designated by an abbreviation for the length of exposure to the photoperiod and the treatment, i.e., 3C would be a control group exposed for 3 weeks. When it becomes necessary to state the experiment from which a group of data has come, this information will follow the group abbreviation.

Effect of Pinealectomy on Circulating Levels of Thyroidal Hormones

Effects of Pinealectomy on Circulating Thyroxine

The results of experiments performed to test the effect of pinealectomy on the thyroxine (T_4) concentration in the serum of White Leghorn cockerels are summarized in Table I and depicted graphically on Figure 1. After three weeks exposure to 16L:8D, the mean serum T_4 level of the pinealectomized (PX) group (1.13 µg%) was significantly greater ($P < .05$) than that (1.07 µg%) of the control (C) group. After four weeks exposure to the photoperiod, the situation was reversed. The serum T_4 in the PX

TABLE I

EFFECTS OF PINEALECTOMY ON SERUM THYROXINE (µg%)

Experiment	Weeks of Exposure to 16L:8D	n	Controls Means ± S.E.	Pinealectomized Means ± S.E.	t[1]	P of Larger Value
II A	3	80	1.03 ± 0.03	1.41 ± 0.01	12.240	> .001
III A	3	88	1.11 ± 0.02	0.88 ± 0.02	7.184	> .001
		168[2]	1.07 ± 0.02	1.13 ± 0.02	2.100	> .05
II B	4	88	1.27 ± 0.02	1.05 ± 0.15	1.482	> .200
III B	4	88	1.25 ± 0.02	1.09 ± 0.02	5.367	> .001
		176[2]	1.26 ± 0.01	1.07 ± 0.08	2.465	> .025
I A	6	96	1.39 ± 0.02	1.39 ± 0.02	0.462	ns
II C	6	96	1.63 ± 0.04	1.68 ± 0.03	1.253	< .200
III C	6	88	1.03 ± 0.02	1.50 ± 0.02	18.146	> .001
		180[2]	1.36 ± 0.02	1.53 ± 0.02	6.702	> .001

[1]t test between T_4 levels of corresponding control and pinealectomized groups.

[2]Mean T_4 levels of all birds exposed to 16L:8D for 3, 4, or 6 weeks.

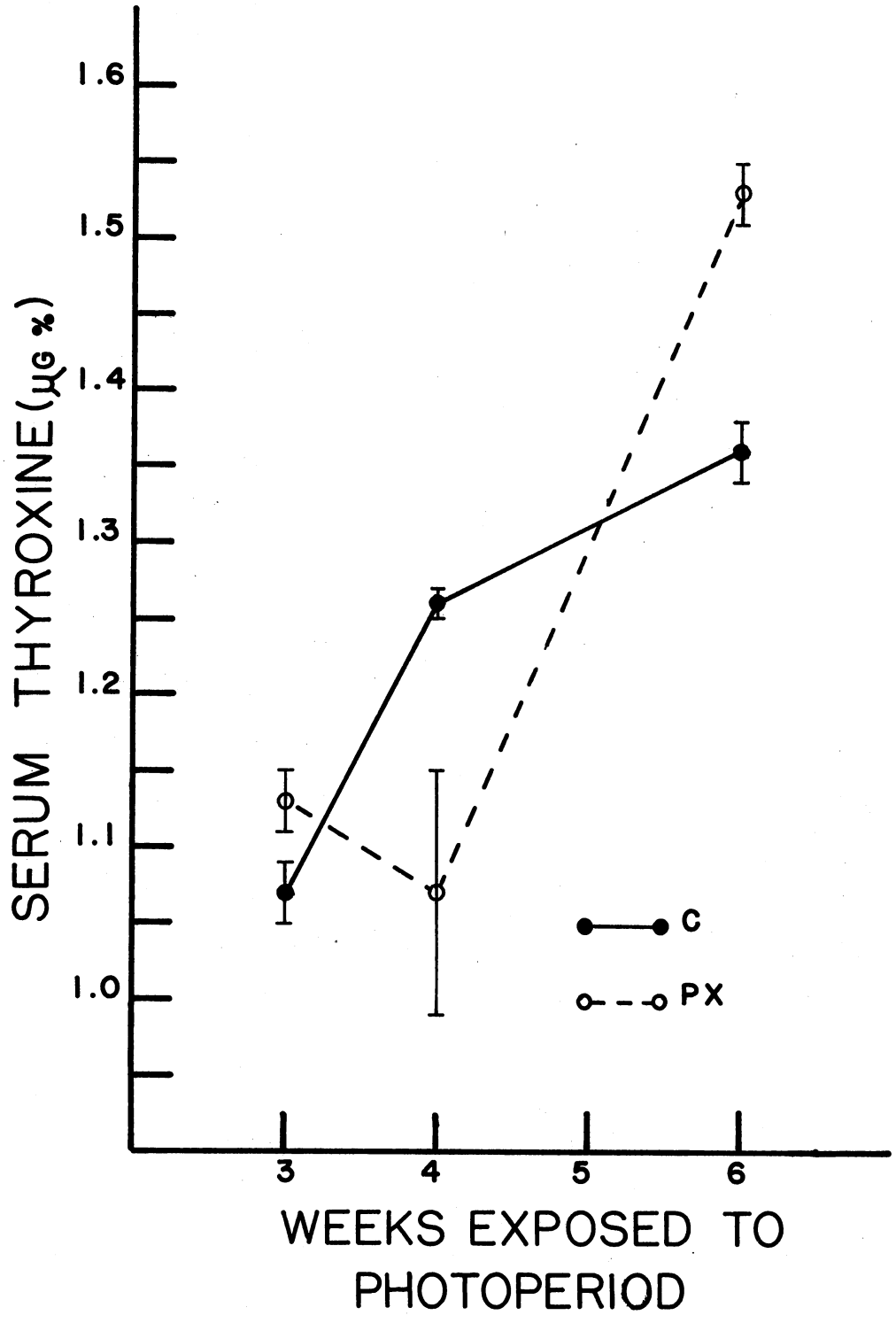

Figure 1. Serum T_4 Concentrations Versus Time (Means $\pm$ S.E.)

group was significantly less ($P < .025$) than that of the control group with means of 1.07 µg% and 1.26 µg%, respectively. Following six weeks of exposure to the photoperiod, the serum T_4 concentration of the pinealectomized group was again significantly larger than that of the control group ($P < .001$). The mean serum T_4 levels for the control and pinealectomized groups were 1.36 µg% and 1.53 µg%, respectively. Analysis of variance, Table II, indicated highly significant differences due to length of exposure to the photoperiod alone but no significant difference due to pinealectomy alone. However a high degree of significance was also shown to be due to interaction between length of exposure time and pinealectomy. All significant differences were at the .001 level. Further, comparison of differences between serum T_4 levels by a protected least significant difference test (LSD), Table III, strongly supported the statistical evidence provided by the analysis of variance (Table II). The serum T_4 level of the 6PX group was shown to be significantly greater than that of all the other groups ($P < .001$) while the thyroxine concentration of the 6C group was significantly greater than that of the 3C, 3PX, 4C, and 4PX groups ($P < .001$). Although the T_4 level of the 4PX group was not significantly different from the T_4 level of the 3C group, it was significantly less than that of the 3PX group ($P < .01$) and the 4C group ($P < .001$). In addition, the T_4 level of the 4C group was significantly greater than that of the 3C group ($P < .001$) as well as that of the 3PX group ($P < .001$). Finally the serum T_4 level of the 3PX group was significantly greater than that of the 3C group ($P < .01$).

The significant interaction indicated by the analysis of variance is readily apparent from Figure 1 which shows that the two curves intersect at two points.

TABLE II

ANALYSIS OF VARIANCE OF SERUM THYROXINE (T_4) DATA

Source	d.f.	SS	MS	F
Treatment	5	37.7009	7.54019	35.9499[1]
Times of Exposure	2	30.0337	15.0168	71.5970[1]
Treatment	1	0.4780	0.4780	2.2791
Interaction	2	7.18925	3.5946	17.1384[1]
Error	1242	260.4983	0.2097	
Total	1247	298.1992		

[1]P < .001.

TABLE III

COMPARISON OF DIFFERENCES IN MEAN SERUM T_4 LEVELS BY LSD

	3C 1.0699	3PX 1.1336	4C 1.2626	4PX 1.0698	6C 1.3578	6PX 1.5280
6Px 1.5280	0.4581 P < .001	0.3944 P < .001	0.2654 P < .001	0.4582 P < .001	0.1702 P < .001	0
6C 1.3578	0.2879 P < .001	0.2242 P < .001	0.0952 P < .001	0.2880 P < .001	0	
4PX 1.0698	0.001 ns	0.0638 P < .01	0.1928 P < .001	0		
4C 1.2626	0.1927 P < .001	0.1290 P < .001	0			
3PX 1.1336	0.0687 P < .01					
3C 1.0699	0					

Effects of Pinealectomy on Circulating Triiodothyronine

The results from experiments carried out in an attempt to determine if pinealectomy has any effect on circulating levels of triiodothyronine (T_3) in the serum of White Leghorn cockerels are shown in Table IV and depicted graphically on Figure 2. After three weeks exposure to 16L:8D, the mean T_3 level of the control and the mean T_3 level from pinealectomized birds are not significantly different (means of 203.28 ng% and 206.17 ng%, respectively). However, after four weeks of exposure to 16L:8D, the mean T_3 concentration of the controls was significantly greater than that of the corresponding pinealectomized group ($P < .001$). The mean T_3 level of the control group was 329.24 ng% and that of the PX group was 257.25 ng%. Following six weeks exposure, the mean T_3 level of the control group (191.61 ng%) remained significantly greater ($P < .025$) than that of the pinealectomized group (180.11 ng%).

Analysis of variance of these data indicated highly significant differences ($P < .001$) in serum T_3 concentrations due to weeks of exposure to the photoperiod as well as due to pinealectomy (Table V). In addition, a significant difference was indicated due to interaction between length of exposure time and pinealectomy ($P < .001$). Comparisons of differences between mean serum T_3 levels, as shown by LSD, are listed on Table VI. Although not significantly different from that of the 6C group, the serum T_3 level of the 6PX group was significantly less than that of the 3C and 3PX groups ($P < .05$) as well as that of the 4C and 4PX groups ($P < .001$). The T_3 level of the 6C group, while not significantly different from that of the 3C and 3PX groups, was significantly less than that of the 4C and 4PX groups ($P < .001$). The T_3 level of the 4PX group

TABLE IV

EFFECTS OF PINEALECTOMY ON SERUM TRIIODOTHYRONINE (ng%)

Experiment	Weeks of Exposure to 16L:8D	n	Controls Means ± S.E.	Pinealectomized Means ± S.E.	t[1]	P of Larger Value
II A	3	80	264.26 ± 6.37	264.24 ± 6.53	.003	ns
III A	3	88	147.83 ± 3.57	1.5337 ± 1.83	1.379	> .20
		168[2]	203.28 ± 5.73	206.17 ± 5.37	.367	ns
II B	4	96	252.24 ± 6.02	217.23 ± 7.51	3.635	> .001
III B	4	88	413.25 ± 8.30	300.91 ± 6.65	10.563	> .001
		184[2]	329.24 ± 7.80	257.25 ± 5.91	7.358	> .001
I A	6	96	154.59 ± 4.26	166.30 ± 3.92	2.022	< .05
II C	6	96	202.69 ± 7.74	191.66 ± 8.17	.980	ns
III C	6	88	219.90 ± 3.29	182.58 ± 3.92	7.284	> .001
		280[2]	191.61 ± 3.59	180.11 ± 3.39	2.327	> .025

[1]t test between T_3 levels of corresponding control and pinealectomized groups.

[2]Mean T_3 levels of all birds exposed to 16L:8D for 3, 4, or 6 weeks.

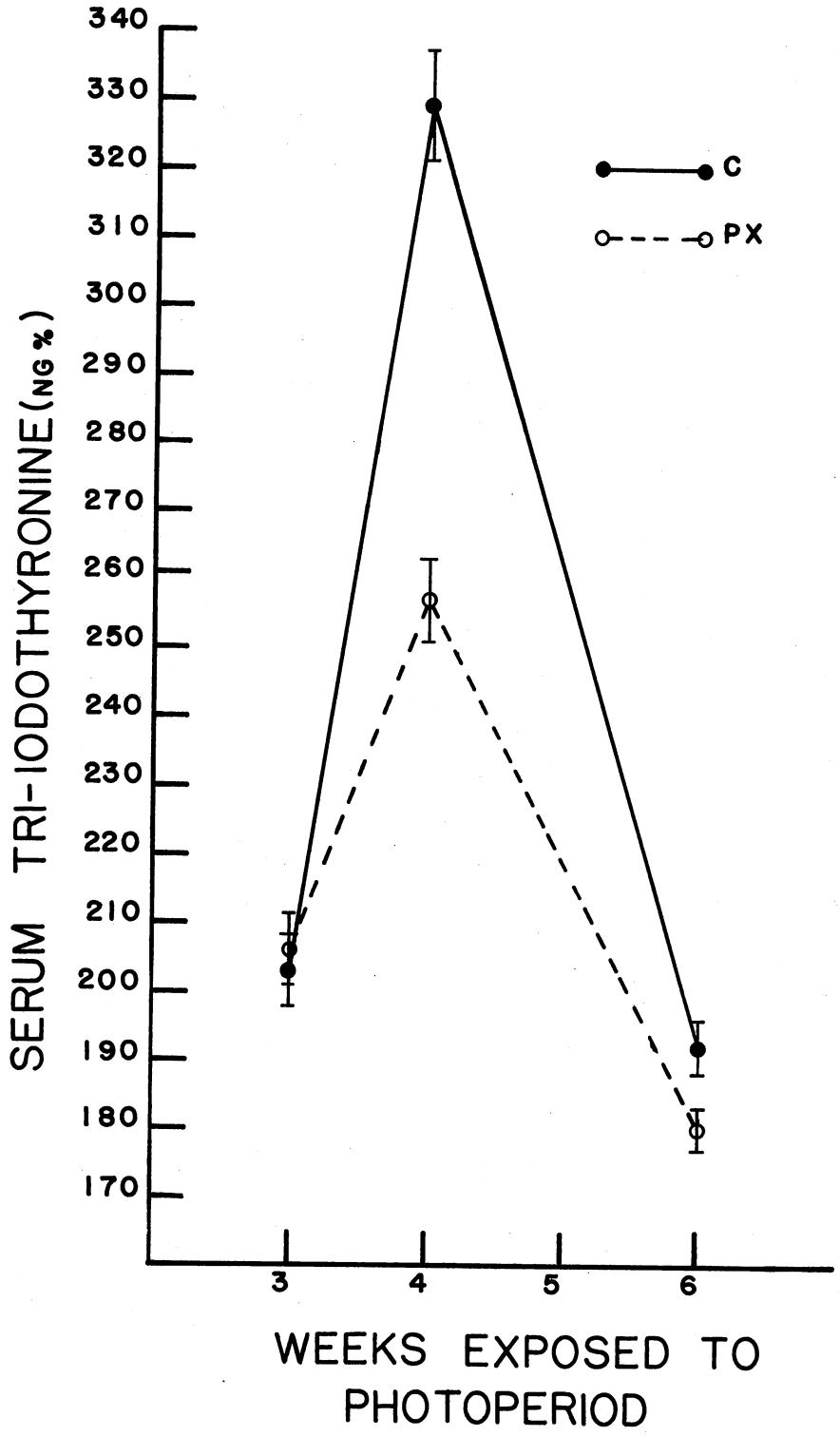

Figure 2. Serum T_3 Concentrations Versus Time (Means $\pm$ S.E.)

TABLE V

ANALYSIS OF VARIANCE OF SERUM TRIIODOTHYRONINE (T_3) DATA

Source	d.f.	SS	MS	F
Treatment	5	2,530,001.67	506,000.33	66.2389[1]
Times of Exposure	2	2,074,855.80	1,037,427.90	135.8063[1]
Pinealectomy	1	187,792.47	187,792.47	24.5853[1]
Interaction	2	267,353.41	133,676.71	17.4992[1]
Error	1258	9,609,891.79		
Total	1263	12,139,893.46		

[1]P < .001.

TABLE VI

COMPARISON OF DIFFERENCES IN MEAN SERUM T_3 LEVELS BY LSD

	3C 203.2783	3PX 206.1607	4C 329.2446	4PX 257.25	6C 191.6107	6PX 180.1107
6PX 180.1107	23.17 P < .05	26.05 P < .05	149.13 P < .001	77.14 P < .001	11.50 ns	0
6C 191.6107	11.67 ns	14.55 ns	137.63 P < .001	65.64 P < .001	0	
4PX 257.25	53.97 P < .001	51.09 P < .001	71.99 P < .001	0		
4C 329.2446	125.97 P < .001	123.08 P < .001	0			
3PX 206.1607	2.88 ns	0				
3C 203.2783	0					

was significantly greater than that of the 4C and 4PX groups ($P < .001$), but was significantly less than the T_3 level of the 4C group ($P < .001$). In addition, the serum T_3 concentration of the 4C group was significantly greater than that of the 3C and 3PX groups ($P < .001$). Finally, the T_3 levels of the 3C and 3PX groups were not significantly different. When serum T_3 levels from controls and pinealectomized birds are plotted against weeks of exposure to 16L:8D, the graph shown on Figure 2 is obtained. The significant interaction indicated by analysis of variance is not readily apparent but is strongly suggested by the lack of a parallelism between the control and pinealectomized curves. Since serum T_4 levels are greater than serum T_3 levels by a factor of at least 3, nothing is gained by combining the two hormone concentrations.

Ignoring the effect of age on serum hormone levels and calculating a grand mean hormone level, the results shown on Table VII were obtained. T_4 concentrations were significantly different ($P < .05$) with the control hormone level being less (1.25 µg%) than the pinealectomized level (1.29 µg%). This was an unexpected result since analysis of variance across all groups failed to indicate a significant difference due to pinealectomy. This significant difference was representative of a 3.17% increase over controls. In addition, the serum T_3 levels were different with the control level of 243 ng% being significantly greater than that of the pinealectomized birds (209 ng%). This difference was on the order of a 14.07% decrease from controls and is in concurrence with the analysis of variance of the T_3 data in indicating an effect due to pinealectomy alone.

TABLE VII

TOTAL T_4 AND T_3 LEVELS IGNORING THE EFFECT OF AGE

	n	Control	Pinealectomized	t	P of Larger Value
Serum T_4 (ng%) Mean ± S.E.	624	1.25 ± 0.01	1.29 ± 0.01	2.217	> .05
Serum T_3 (ng%) Mean ± S.E.	632	243.78 ± 3.98	209.49 ± 2.98	6.086	> .001

Effect of Pinealectomy on the Circadian Rhythms of Thyroxine and Triiodothyronine

Data obtained to ascertain the possible effect(s) of pineal ablation on the circadian rhythms of T_4 and T_3 in the serum of White Leghorn cockerels were subjected to an electronic computer program and tested in terms of their statistical fit to a theoretical sinusoidal curve by the method of least squares (Halberg, et al., 1972). For each set of data, this program gives certain characteristics which are essential in identifying and comparing a certain type of circadian rhythm. These characteristics are level, amplitude, and acrophase. The level, also termed the mesor, is defined as the mean of all experimental observations being analyzed. The amplitude is defined as the magnitude of the greatest difference between the level and the maximum of the curve and the acrophase is defined as the time at which the amplitude occurs. These characteristics and their values for each experiment are shown in Tables VIII and IX. In addition, this computer program performs two "F" tests. For the first, the program forces the data into a 24 hour period and then tests the null hypothesis: β and/or $\gamma = 0$. β and γ are estimators of the horizontal and vertical components of the theoretical sinusoidal curves calculated from the respective sets of data. The results of this "F" test are in a column headed F^a. The second "F" test is performed in order to determine the statistical "goodness of fit", i.e., how well the experimental data fit the theoretical curve once they have been forced into a sinusoidal curve with a period of 24 hours. The results of this test are listed in a column headed F^b. The column headed "change in acrophase" was constructed to indicate the magnitude of the difference between the acrophase of the control group and that of the corresponding

TABLE VIII

CHARACTERISTICS OF THE THEORETICAL SINUSOIDAL CURVES CALCULATED FROM THE T_4 DATA

Experiment	Weeks of Exposure to 16L:8D	Treatment	n	Level µg%	Amplitude µg%	Acrophase CST	P>F[a][1]	P>F[b][2]	Change In Acrophase
II A	3	C	80	1.02	0.14	0515	.0027	.001	2' 43"
	3	PX	80	1.41	0.07	0232	.0043	.001	
III A	3	C	88	1.11	0.15	0545	.0001	.005	2' 10"
	3	PX	88	0.87	1.13	0755	.0002	.001	
II B	4	C	88	1.27	0.14	0453	.0001	.001	1' 24"
	4	PX	88	1.05	0.10	0617	.0006	.001	
III B	4	C	88	1.26	0.07	0317	.1061	.005	1' 07"
	4	PX	88	1.12	0.09	0210	.0130	.001	
I A	6	C	96	1.39	0.10	0619	.0006	.100	2' 50"
	6	PX	96	1.39	0.10	0909	.0170	.001	
II C	6	C	96	1.64	0.23	0645	.0001	.001	2' 10"
	6	PX	96	1.71	0.18	0435	.0001	.250	
III C	6	C	96	1.03	0.03	0437	.8186	.005	2' 55"
	6	PX	96	1.51	0.07	0142	.0319	.005	

[1]Tests null hypothesis: horizontal and/or vertical component(s) = 0.

[2]Tests goodness of fit, i.e., how well the experimental data fit the theoretical sinusoidal curve.

TABLE IX

CHARACTERISTICS OF THE THEORETICAL SINUSOIDAL CURVES CALCULATED FROM THE T_3 DATA

Experiment	Weeks Exposure to 16L:8D	Treatment	n	Level ng%	Amplitude ng%	Acrophase CST	$P>F^{a1}$	$P>F^{b2}$	Change In Acrophase
II A	3	C	80	260.29	46.37	1510	.0001	.250	0' 54"
	3	PX	80	262.05	59.16	1416	.0001	.025	
III A	3	C	88	153.95	31.44	2103	.0001	.005	1' 05"
	3	PX	88	155.28	16.50	1958	.0001	.001	
II B	4	C	88	253.92	47.63	1949	.0001	.050	0' 29"
	4	PX	88	220.21	65.93	2018	.0001	.005	
III B	4	C	88	404.28	47.80	1405	.0004	.001	0' 02"
	4	PX	88	296.64	22.62	1403	.0566	.001	
I A	6	C	96	158.28	27.08	2055	.0001	.100	0' 42"
	6	PX	96	167.71	8.86	2137	.3000	.100	
II C	6	C	96	203.81	75.41	1945	.0001	.001	1' 14"
	6	PX	96	186.94	69.33	1831	.0001	.001	
III C	6	C	96	224.44	25.88	2352	.0001	.005	2' 54"
	6	PX	96	186.37	24.54	2058	.0001	.100	

[1]Tests null hypothesis: horizontal and/or vertical component(s) = 0.

[2]Tests goodness of fit, i.e., how well the experimental data fit the theoretical sinusoidal curve.

pinealectomized group in any given experiment. Since no statistical method for determining significant differences between two values given by one observation each is available, an arbitrary decision must be made in order to interpret the results in this column. It is therefore assumed that unless two acrophases differ by more than six hours, they will not be considered statistically different.

Effect of Pinealectomy on the Circadian Rhythm of Thyroxine

The results of experiments performed to test the effect of pinealectomy on the circadian rhythm of circulating T_4 are shown in Table VIII. The statistical methods of analysis of variance and LSD applied to the serum T_4 mean concentration (Tables II and III) also apply to the level since by definition the level and the mean are identical. Briefly, from the statistical analysis of the T_4 means, there existed a significant difference between the T_4 levels of the controls and pinealectomized groups due to length of time exposed to photoperiod alone ($P < .001$) but not due to pinealectomy alone. A significant difference due to interaction between exposure time and pinealectomy ($P < .001$) was also indicated.

With respect to the amplitudes calculated by the method of least squares analysis, there appeared to be no significant differences between the amplitudes of the control groups and those of the pinealectomized groups. Statistical analysis of these amplitudes is virtually useless due to the lack of significant degrees of freedom. The importance of the analysis of the amplitudes lies in the fact that as the amplitude approaches zero, the statistical significance given by the first "F" test (F^a) decreases. Of the fourteen theoretical curves given by these

data, only two failed to meet the requirements for rejection of the null hypothesis. These two sets of data were from the 4C group of experiment IIIB and from the 6C group of experiment IIIC ($P < .1061$ and $P < .8186$, respectively). In all other cases, the horizontal and vertical components of the respective theoretical curves were significantly different from zero as is indicated by significance levels with greater than 95% confidence. The second "F" test indicated that all groups fit their respective curves with 99% or better confidence with the exception of group 6C of experiment IA and group 6PX of experiment IIC ($P < .1$ and $P < .25$, respectively). Despite the fact that the range of acrophases throughout all groups was 0142 hours to 0909 hours, no two acrophases from corresponding groups, i.e., control and pinealectomized groups from the same experiment, differed by more than six hours and therefore the acrophases of these corresponding groups are assumed not to be significantly different.

Rather than diagram each of the fourteen sinusoidal waves given by the least squares analysis of the serum T_4 data, two corresponding sets of data have been selected as representative of all other sets of data. These two sets of data are from the control and pinealectomized groups of experiment IIB. Figure 3 is a time plot of the data from group 4C of experiment IIB. The result of F^a indicated that the null hypothesis was to be rejected ($P < .001$). The second "F" test indicated that the experimental data fit the theoretical curve with 99.9% confidence. A time plot of the data from group 4PX of experiment IIB is found in Figure 4. The "F" tests indicated that the null hypothesis was to be rejected ($P < .0006$) and that the experimental data fit the theoretical curve with 99.9% confidence ($P < .001$).

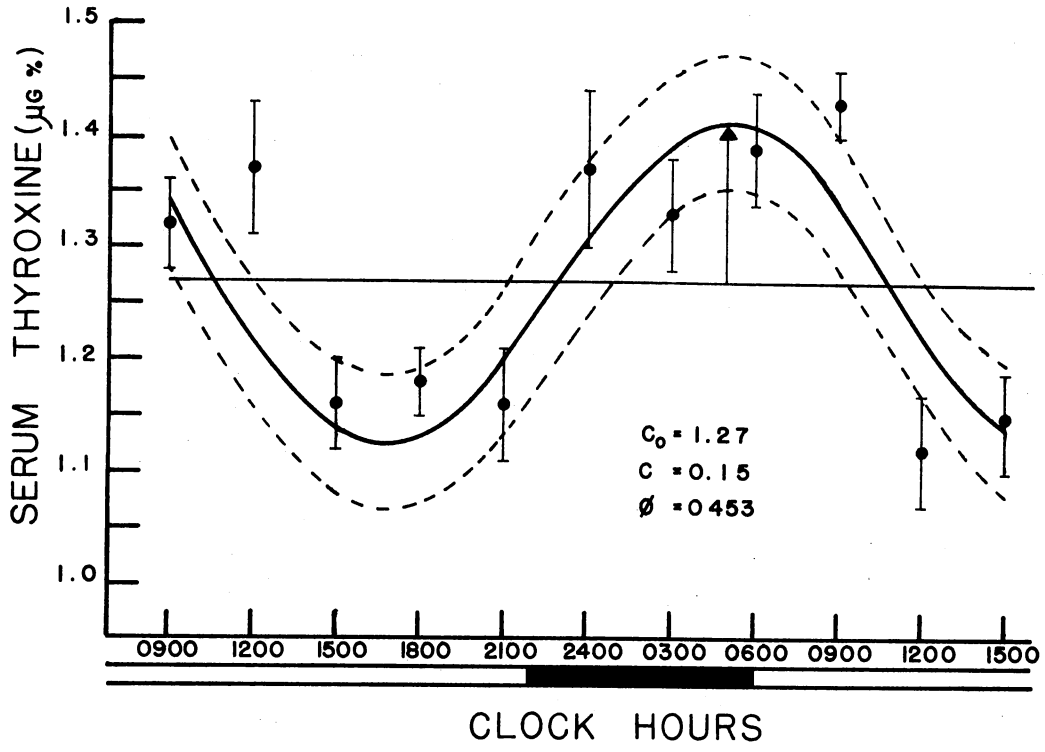

Figure 3. Plot of Serum T_4 of Group 4C of Experiment IIB Against Time

Solid curved line is the best fitting theoretical sinusoidal curve; dashed lines are upper and lower 95% confidence limits; horizontal solid line is the level of all observations and the acrophase is indicated by the arrow. C = level and C_0 = amplitude. Experimental points (means $\pm$ S.E.) are shown as solid dots.

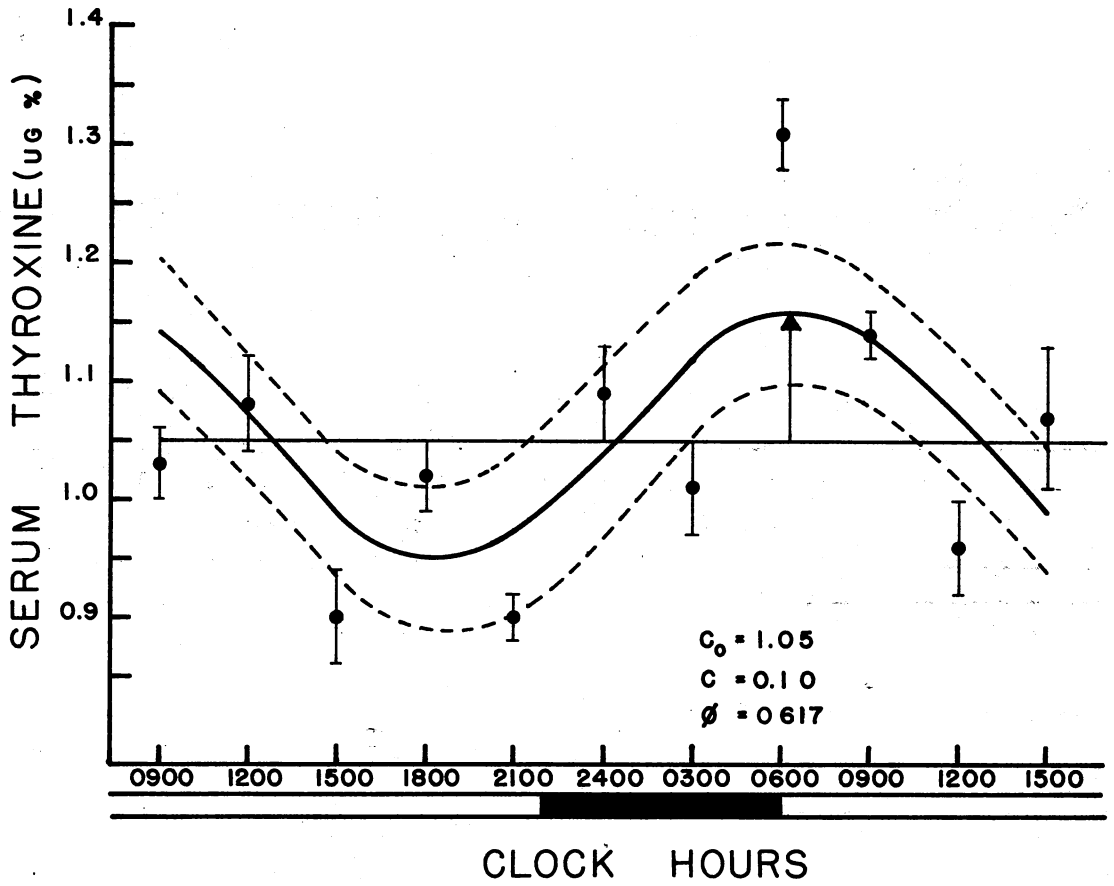

Figure 4. Plot of Serum T_4 of Group 4PX of Experiment IIB Against Time

Representations are as in Figure 3.

Effect of Pinealectomy on the Circadian Rhythm of Triiodothyronine

Table IX summarizes the results of the experiments performed to test for possible effects of the removal of the pineal on the diurnal rhythm of circulating T_3. As in the case of T_4, the significant differences demonstrated earlier with respect to mean serum T_3 concentrations apply to the levels of the theoretical sinusoidal curves generated by these T_3 data. In short, significant differences were found and ascertained to be due to length of exposure time, pinealectomy, and an interaction between the two ($P < .001$ in all cases). The sinusoidal quality of these data was adequately supported in all cases except two ($P < .0001$ for rejection of the null hypothesis). The null hypothesis could not be rejected with any degree of confidence in the case of the data from group 6PX of experiment IA and with only 95% confidence in the case of the data from group 4PX of experiment IIIB. The T_3 data were found to fit their respective theoretical curves with less statistical confidence than the T_4 data. Only eight of the fourteen groups fit their respective curves with greater than 99.5% confidence. None of the corresponding acrophases differed by more than six hours and therefore were assumed not to be significantly different.

As in the case of the T_4 data, two corresponding sets of T_3 data were selected for illustration of all other sets. These were the data from the 6C and 6PX groups of experiment IIC. Figure 5 is a time plot of the data from group 6C. A highly significant first "F" test (F^a) allowed the rejection of the null hypothesis ($P < .001$) and the second "F" (F^b) test indicated that the experimental data fit the theoretical curve with 99.9% confidence ($P < .001$). A time plot of the data from group 6PX

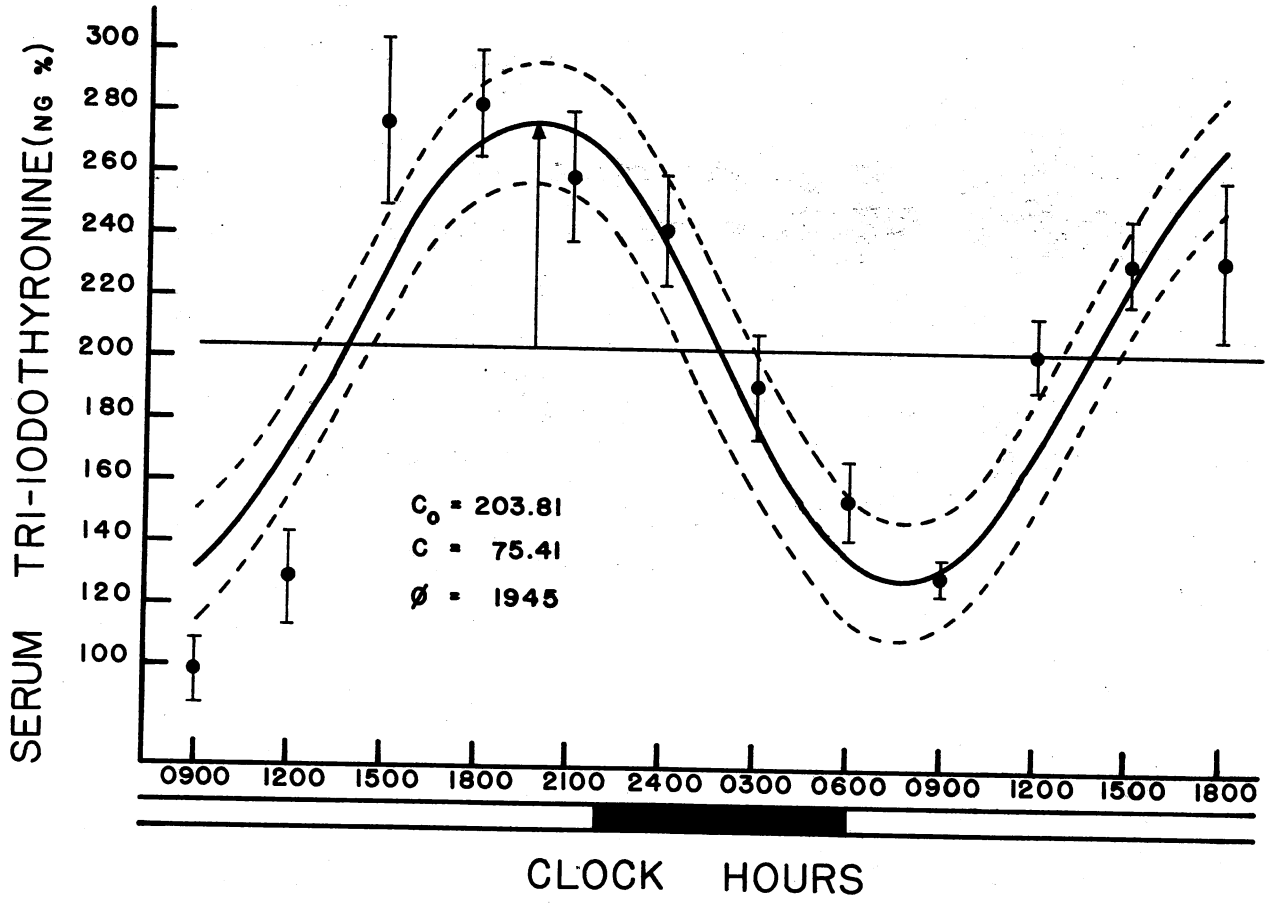

Figure 5. Plot of Serum T_3 of Group 6C of Experiment IIC Against Time Representations are as in Figure 3.

is found on Figure 6. In this case, rejection of the null hypothesis was allowed ($P < .0001$) and the data fit the theoretical sinusoidal curve with 99.9% confidence ($P < .001$).

An interesting observation made on the T_4 and T_3 data was that the acrophases of the circadian rhythms of the two hormones are approximately 180° out of phase, i.e., separated by about 12 hours in all cases. In addition, the acrophase of the T_3 data preceded the onset of the dark phase of the photoperiod (2200 hours) and the acrophase of the T_4 data occurred near or shortly after the onset of the light phase (0600 hours) of the photoperiod.

The raw data for all experiments are presented in the appendix.

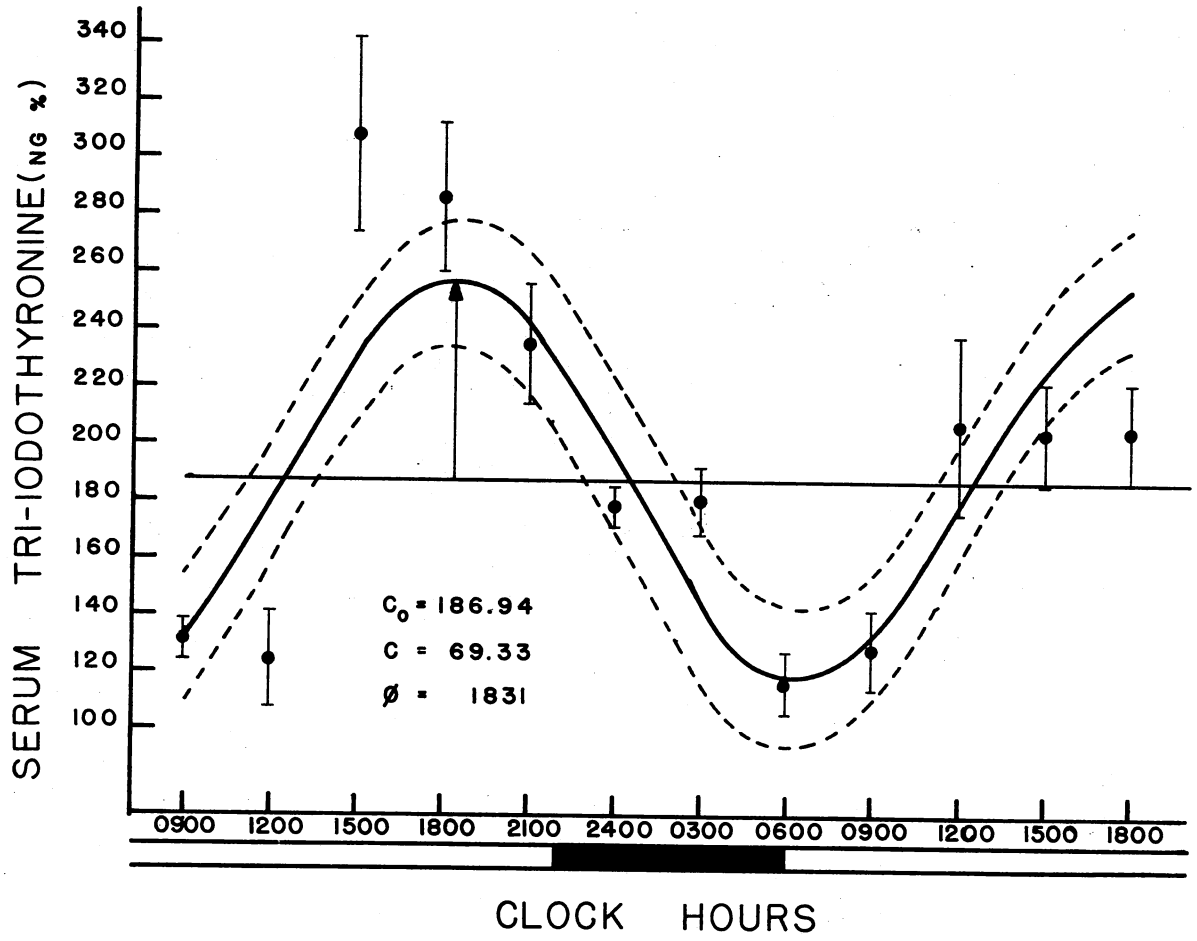

Figure 6. Plot of Serum T_3 of Group 6PX of Experiment IIC Against Time Representations are as in Figure 3.

CHAPTER V

DISCUSSION

Previous attempts to establish a functional relationship between the pineal and thyroid glands have produced little concrete evidence, especially in the area of avain thyroid physiology. This discussion proposes that the pineal is an integral part of a complex control mechanism for thyroid function. The scope of this work does not allow complete formulation of such a theory but perhaps will yield some indication of the direction future research effort should take.

Effects of Pinealectomy on Serum Iodohormones

The serum levels of thyroxine (T_4) determined in this study, both for control and pinealectomized White Leghorn cockerels, are in good agreement with those values previously reported (Newcomer, 1974; May, et al., 1973; Refetoff, et al., 1970). These workers reported serum T_4 values in birds ranging from 1.05 µg% to 3.3 µg% while the T_4 results from this present work cover a range of 0.88 µg% to 1.68 µg%. In the preliminary analysis of the data (Table I), a simple "t" test indicated significant differences between serum T_4 levels of control and pinealectomized birds. However, a "t" test is limited in that it is designed to detect a significant difference between two means without regard for effects that may be present due to levels of treatment. For this reason, a "t" test should not be trusted to demonstrate significant differences

in a factorial experiment such as that used in this work. Therefore, analysis of variance was performed on the T_4 data and indeed, the conclusions drawn from the "t" tests, i.e., significant differences due to pinealectomy alone, were erroneous. The AOV (Table II) indicated significant differences in the data but not due to pinealectomy. Instead, the significant differences suggested by the "t" tests were due to length of exposure time and an interaction between pinealectomy and length of time the birds were exposed to the photoperiod. The length of exposure time is indicative of the birds' age since the birds were two weeks old when initially exposed to 16L:8D. These findings appear to indicate that pinealectomy alone has no significant effect on serum levels of T_4 but when in combination with age, does alter the normal pattern. The control levels of T_4 found in this study significantly increase with age. Results published by May, et al. (1973) show a similar trend but the hormone levels found in the May study do not appear to be significantly different. On the other hand, the serum T_4 values from pinealectomized birds remained essentially the same until after four weeks of exposure when they increased drastically (Figure 1). Between four and six weeks of exposure to 16L:8D, the serum T_4 levels of the pinealectomized birds increased at a much faster rate than those of the control birds during the corresponding time period.

As in the case of the serum T_4 data, simple "t" tests (Table IV) were performed on the serum T_3 data. These tests indicated the possibility of significant differences due to pinealectomy. With the limitations of this preliminary statistical analysis in mind, analysis of variance was performed and significant differences were found to be due not only to pinealectomy but also the length of exposure to 16L:8D and an

interaction between the two. From these statistical inferences, pinealectomy alone appears to effect serum levels of T_3. The range of values obtained in this work are in reasonable agreement with those of Newcomer (1974). The range of most of the serum T_3 values from the present study is from 147.83 ng% to 264.24 ng% with two groups having somewhat higher values. Newcomer reported serum T_3 levels ranging from 171.80 ng% to 259.60 ng%. Control and pinealectomized serum T_3 gave similar graphs when plotted against exposure time (Figure 2). The control serum T_3 level peaked at a higher zenith at four weeks exposure than that of the pinealectomized group. At the time when the serum T_4 level in the control birds was increasing at a moderate rate (between three and four weeks exposure to 16:L8D), the control level of T_3 was increasing rapidly. Conversely, as the pinealectomized serum T_4 level was remaining essentially constant between three and four weeks of exposure, the serum T_3 level also increased but at a slower rate than the corresponding controls (Figure 2). Following four weeks of exposure to 16L:8D, both the control and pinealectomized T_3 levels decreased with the control level decreasing at a faster rate.

In recent years, a considerable amount of work has been done indicating that T_4 is converted extra-thyroidally to T_3 and that T_3 is the active form of thyroid hormone in human beings and other mammals (Braverman, et al., 1970; Pitman, et al., 1971; Shenkman, et al., 1973). The proposal that T_3 is the active form of thyroid hormone is supported by several observations, chief among which are that T_3 has a higher biological potency and a longer half life than does T_4. In addition, T_3 is less tightly bound to its carrier proteins than T_4 and is more widely distributed in tissues. These findings have not been confirmed in birds

but, assuming that T_3 is the major active hormone in birds and that T_4 is peripherally converted to T_3, it is possible to interpret these data as indicative of a pineal role in the control of thyroid hormone release. A possible explanation of this work could be that the pineal is acting as some sort of time modulator, the function of which is to adjust the set point sensitivity of the anterior pituitary to tonic amounts of thyroid releasing hormone (TRH). As developmental needs for thyroid hormone increase, the adjustment of the set point would allow the pituitary to release more thyroid stimulating hormone (TSH), thereby increasing the concentration of thyroid hormone in the blood before the normal negative feedback of thyroid hormone on the pituitary would inhibit TSH. Altering the set point would allow the establishment of a safety factor such that adequate amounts of serum T_4 would always be available to be converted to T_3 in the normal bird. With this proposed pineal-pituitary-thyroid axis in mind, it is readily apparent that the control T_4 level was elevated between three and four weeks of exposure to 16L:8D (five to eight weeks of age) due to developmental needs for T_3. This control serum T_4 level may be approaching a plateau at 6 weeks exposure although this is not known. The pinealectomized serum T_4 level between 3 and 4 weeks of exposure (5 to 6 weeks of age) did not change because the rate of T_4 to T_3 conversion was greater than the rate of synthesis and release of T_4 (no safety factor). The 3 to 4 week control and pinealectomized T_3 levels both increased at the expense of T_4 but the pinealectomized T_3 level could not increase as much as the control T_3 level since much less T_4 was available for conversion in the pinealectomized group. The serum T_4 level of the pinealectomized birds increased between 4 and 6 weeks of exposure because the rate of conversion of T_4 to T_3 in these birds was

less than the production of T_4, resulting in a net gain of T_4. The serum T_3 levels of both the control and pinealectomized birds decreased between 4 and 6 weeks exposure (6 to 8 weeks of age) due to catabolism, peripheral utilization, etc.

A great deal more information is needed to strengthen the argument in favor of the pineal's possible role in the set point regulation of thyroid function. No information concerning changes in secretion rate in birds with age is available and therefore the metabolic status of the thyroid gland under these experimental conditions is uncertain. In addition, it would be helpful if some information were available on changes with age in such indicators of thyroid kinetics as effective clearance constant, ^{131} uptake, and binding rate constant. The serum concentrations of T_4 and T_3 prior to 5 weeks of age (3 weeks exposure to 16L:8D) and after 9 weeks of age (6 weeks exposure to 16L:8D) are unknown and would need to be ascertained in order to complete a thyroid hormone profile.

Consideration of total iodohormone concentration is of little value since serum T_4 makes up the vast majority of the total iodohormone concentration. When the effects of time were ignored and total hormone concentration was calculated (Table VII), highly significant differences were noted between control and pinealectomized serum T_4 and T_3 levels. Since previous analysis (AOV, Table II) of the T_4 data had indicated no significant differences due to pinealectomy, the significant difference ($P < .05$) between control and pinealectomized T_4 levels was quite unexpected. However, this significance is probably due to the interaction of pinealectomy and exposure time rather than simply pinealectomy. In addition, the large number of degrees of freedom (623) may have

contributed to an unreliable estimate of significance. It seems highly improbable that the 3.17% change indicated as statistically significant is meaningful in a practical sense. The results of the above analysis, i.e., "t" tests ignoring time of exposure, on the T_3 data were in agreement with the earlier analysis of the same data (Table V). However, the change noted between the control and pinealectomized total T_3 levels was on the order of 14%, a change likely to be considered physiologically as well as statistically significant. However, in this case also, the large number of degrees of freedom (631) may have contributed to this indication of significance.

Effects of Pinealectomy on Rhythms of Serum Iodohormones

The rhythms exhibited by the serum levels of thyroid hormones from this study are in satisfactory agreement with those previously reported by Newcomer (1974). Newcomer reported that the mean acrophase for the serum T_4 rhythm was near 0700 hours with the majority of the trials exhibiting acrophases early in the diurnal portion of the photoperiod. The acrophases of the serum T_4 levels of this study all fell either in the dark phase of the photoperiod or within three hours of the onset of the light phase. Better agreement with the literature is noted with respect to the acrophases demonstrated by the T_3 data from the present study. Newcomer's data gave a mean acrophase of near 1600 hours while the T_3 data from the present data gave acrophases in that neighborhood (ranging from 1405 hours to 2058 hours). The acrophases of the T_4 and T_3 rhythms from the present study were separated by approximately 12 hours while the corresponding acrophases from the Newcomer study were separated by about

10 hours. This difference in acrophase separation is not considered to be significant.

The only significant differences between the control and pinealectomized rhythms of serum T_4 and T_3 from this study is that the levels or mesors of the control rhythms were significantly different from those of the pinealectomized birds with the same confidence as were the mean serum hormonal levels. This is not surprising since the mesors and the mean serum levels are identical. Other than this difference, the circadian rhythms of serum T_4 of the control and pinealectomized birds appear to be mirror images of each other. The same can be said of the rhythms of serum T_3 from the control and pinealectomized birds. An interesting observation made when comparing the rhythms of T_4 and T_3 of corresponding control and pinealectomized groups is that the rhythms of T_4 and T_3 are approximately 180° out of phase. Since this 180° phase shift is equal to a 12 hour difference, it is impossible to determine which hormone is the leading hormone.

Under the conditions of this work, i.e., exposure to an alternating photoperiod of 16L:8D, the removal of the pineal had no significant effect on the shape or characteristics of the circadian rhythms of these hormones. However, the possibility still exists that the pineal may be involved with the control of these rhythms, especially in view of the previously unreported evidence that the gland is intimately related to changes in serum levels of thyroidal hormones with age. In fact, as has been previously mentioned, the removal of the pineal has been shown to have profound effects on two well established circadian rhythms, i.e., locomotor activity (Gaston and Menaker, 1968) and body temperature (Binkley, 1971). With respect to these rhythms, the pineal was found to

be involved in their ability to persist in constant conditions. Under the experimental conditions of this thesis, this aspect of pineal involvement was not investigated. In order to answer the question of the involvement of the pineal with the rhythms of T_4 and T_3, it would be necessary to establish the effect of pinealectomy on the rhythms of thyroid hormones in birds exposed to constant light and constant darkness.

CHAPTER VI

SUMMARY AND CONCLUSIONS

White Leghorn cockerels were pinealectomized between one and three days of age and, following a two week recovery period, they were exposed, along with a control group of equal size, to an alternating photoperiod of 16 hours of light and 8 hours of dark (16L:8D) for various lengths of time (3, 4, or 6 weeks). At the end of each of these exposure times, blood samples were taken from eight birds from each group every three hours for approximately thirty hours. The serum from these blood samples was analyzed by radioimmunoassay (RIA) for thyroxine (T_4) and triiodothyroxine (T_3). After completion of all experiments, the data were analyzed by two methods. First, serum T_4 and T_3 levels from control and pinealectomized groups were studied in order to determine if the removal of the pineal gland had any effect on the magnitude of these hormone concentrations of these hormones in avian blood. Secondly, the data were subjected to computer program and tested as to their statistical fit to a theoretical curve. By the use of this computer analysis, the character of the circadian rhythms exhibited in these hormones could be studied.

Pinealectomy was found to alter significantly the pattern of serum T_4 levels of the pinealectomized group when compared with corresponding control groups. The significant difference was found to be due to the length of time exposed to 16L:8D. This exposure time is a measure of age

since the birds were allowed a two week recovery period before exposure. A second significant difference was found to be due to the interaction of pinealectomy and age. Serum T_3 levels of pinealectomized birds were also found to be significantly different in most cases from control T_3 levels. This significant difference was found to be due to pinealectomy, length of exposure (age), and an interaction between the two.

The significant differences detected between the mean serum T_4 and T_3 levels of control and pinealectomized birds were reflected in the only significant difference found between the circadian rhythms of the hormones. The control and pinealectomized levels, numerically equal to mean serum hormone levels, were significantly different in the analysis of the rhythms of both hormones. All other characteristics of the hormonal rhythms (both of T_4 and T_3) of the pinealectomized birds were found to not be significantly different from the control animals.

These results lend themselves to the formulation of a hypothesis regarding the pineal's possible role in the control of circulating levels of T_4 and T_3. The pineal gland may serve as some sort of time modulator which adjusts the set point of anterior pituitary sensitivity to thyroid releasing hormone. The utility of this type of mechanism is that sufficient quantities of T_4 would always be present to be converted to T_3. As the developmental needs for T_3 increase with age, significant amounts of the hormone could be obtained by extra-thyroidal conversion of T_4, a manipulation for which ample evidence is provided in mammalian species.

Under the experimental conditions of this thesis, the removal of the pineal gland had no significant effect on the circadian rhythms of T_4 and T_3. Indications from other work lead one to speculate that if the pineal has an effect on the circadian rhythms of T_4 and T_3 it may be to maintain

these rhythms under constant conditions.

In conclusion, the pineal does appear to be an integral part of the control mechanism of serum T_4 and T_3 and possibly could play a role in the control of the circadian rhythms of these hormones.

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APPENDIX

RAW DATA

TABLE X

EXPERIMENT IIA - SERUM T_4 (µg%)

Bird No.	Central Standard Time									
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200
					Controls					
1	1.03	1.10	0.94	1.13	0.52	0.90	1.34	1.36	0.91	1.13
2	1.08	1.01	1.25	1.13	1.10	1.12	1.09	1.30	1.29	1.18
3	1.14	0.64	0.91	1.03	1.18	1.14	1.21	0.92	1.29	1.20
4	0.80	0.93	1.18	0.74	0.71	1.16	1.29	1.44	1.26	1.19
5	0.60	0.99	1.02	0.83	0.77	1.00	1.19	1.47	0.52	1.25
6	0.67	1.01	0.88	0.90	1.01	0.90	1.24	1.31	0.64	1.24
7	0.85	0.72	0.67	0.69	0.80	0.86	1.31	1.78	1.29	1.18
8	0.64	0.94	1.08	0.53	1.06	0.76	1.03	1.31	0.99	1.17
$\bar{X}$	0.85	0.92	0.99	0.97	0.89	0.98	1.21	1.36	1.02	1.19
SEM	0.0745	0.0555	0.0649	0.0765	0.0805	0.0524	0.0381	0.0842	0.1105	0.0136
					Pinealectomized					
1	1.44	1.48	1.19	1.53	1.38	1.31	1.56	1.40	1.38	1.34
2	1.11	1.42	1.47	1.56	1.35	1.45	1.56	1.50	1.39	1.51
3	1.31	1.29	1.23	1.52	1.40	1.32	1.71	1.44	1.44	1.41
4	1.32	1.44	1.29	1.37	1.60	1.41	1.59	1.48	1.32	1.45
5	1.34	1.37	1.41	1.26	1.23	1.40	1.56	1.45	1.34	1.38
6	1.44	1.48	1.36	1.14	1.37	1.50	1.46	1.56	1.38	1.39
7	1.23	1.44	1.23	1.55	1.24	1.40	1.49	1.41	1.25	1.41
8	1.33	1.47	1.38	1.42	1.40	1.52	2.05	1.52	1.33	1.38
$\bar{X}$	1.32	1.42	1.32	1.42	1.37	1.41	1.62	1.47	1.35	1.41
SEM	0.0381	0.0231	0.0353	0.0543	0.0405	0.0267	0.0664	0.0195	0.0202	0.0183

TABLE XI

EXPERIMENT IIIA - SERUM T_4 (µg%)

Bird No.	Central Standard Time										
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500
						Controls					
1	1.17	1.53	0.83	0.91	0.88	1.38	1.31	0.88	1.48	0.93	1.28
2	1.27	1.10	1.10	0.78	0.88	1.00	0.93	1.53	1.05	0.76	1.14
3	1.31	1.22	0.88	0.64	1.19	0.91	1.36	1.66	1.57	0.79	1.14
4	1.03	1.22	0.97	0.88	0.97	1.66	1.22	0.84	1.31	0.74	1.28
5	1.14	1.14	0.79	0.93	1.14	1.10	1.10	1.72	1.22	1.10	0.78
6	1.17	1.36	1.07	1.10	0.97	1.00	1.31	1.22	1.22	0.97	1.07
7	1.03	1.10	1.00	1.05	0.78	1.00	1.17	1.17	1.17	0.86	1.31
8	0.88	1.10	1.03	0.88	1.00	1.14	1.22	1.53	1.28	0.93	1.22
$\bar{X}$	1.13	1.22	0.95	0.90	0.98	1.15	1.20	1.32	1.29	0.89	1.15
SEM	0.0496	0.0543	0.0902	0.0511	0.0482	0.0887	0.0990	0.1210	0.0593	0.0431	0.0609
						Pinealectomized					
1	1.11	1.11	0.52	0.80	0.68	0.66	0.89	1.18	0.96	0.80	0.86
2	1.02	1.11	0.89	0.71	0.77	0.91	0.89	1.29	0.91	0.82	0.82
3	0.95	1.07	0.58	0.64	0.89	0.94	0.85	1.04	1.07	0.82	0.82
4	0.68	1.02	0.82	0.98	0.66	1.11	0.68	1.11	0.95	1.11	0.93
5	0.89	0.98	0.55	0.98	0.61	0.75	0.39	1.21	0.98	1.14	0.80
6	0.85	1.07	0.89	0.80	0.63	1.17	0.85	1.29	0.54	1.07	1.00
7	1.02	0.98	0.63	0.69	0.55	0.86	0.61	0.95	0.98	1.00	0.77
8	0.89	0.80	1.02	0.86	0.57	1.04	0.82	0.96	1.04	0.63	0.95
$\bar{X}$	0.93	1.02	0.73	0.81	0.67	0.93	0.74	1.13	0.93	0.92	0.87
SEM	0.0465	0.0360	0.0671	0.0451	0.0397	0.0616	0.0624	0.0482	0.0583	0.0643	0.0289

TABLE XII

EXPERIMENT IIB - SERUM T_4 (µg%)

Bird No.	Central Standard Time										
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500
						Controls					
1	1.38	1.68	1.31	1.20	1.02	1.45	1.55	1.21	1.53	1.30	1.28
2	1.39	1.31	1.31	1.01	0.95	1.23	1.04	1.40	1.28	1.27	1.07
3	1.27	1.31	1.13	1.07	1.45	1.23	1.44	1.46	1.56	0.86	1.07
4	1.51	1.19	1.14	1.18	1.26	1.79	1.36	1.14	1.46	1.05	1.26
5	1.24	1.20	1.02	1.30	1.20	1.37	1.25	1.54	1.41	1.22	0.97
6	1.28	1.45	1.18	1.29	1.10	1.34	1.30	1.44	1.47	1.10	1.13
7	1.33	1.49	1.20	1.19	1.10	1.25	1.33	1.44	1.35	1.12	1.28
8	1.13	1.34	1.03	1.18	1.18	1.31	1.39	1.49	1.41	1.06	1.16
$\bar{X}$	1.32	1.37	1.16	1.18	1.16	1.37	1.33	1.39	1.43	1.12	1.15
SEM	0.0404	0.0577	0.0399	0.0348	0.0546	0.0656	0.0528	0.0495	0.0325	0.0502	0.0404
						Pinealectomized					
1	1.14	1.14	0.79	1.13	0.90	0.97	1.08	1.36	1.12	0.95	1.04
2	1.01	1.00	0.84	0.94	0.92	1.09	1.10	1.37	1.07	0.86	1.09
3	1.00	1.20	0.89	0.88	0.92	1.18	1.10	1.26	1.23	0.93	1.09
4	0.88	1.01	0.98	1.15	1.00	1.27	0.88	1.20	1.13	1.14	1.15
5	1.10	1.00	0.81	1.09	0.90	0.94	0.87	1.40	1.07	1.06	1.05
6	1.11	1.28	1.04	1.01	0.91	1.08	1.06	1.45	1.21	0.97	1.08
7	1.02	1.02	0.79	0.95	0.78	1.11	0.92	1.18	1.15	0.86	0.99
8	0.95	0.95	1.07	0.97	0.90	1.10	1.09	1.27	1.20	0.87	1.07
$\bar{X}$	1.03	1.08	0.90	1.02	0.90	1.09	1.01	1.31	1.15	0.96	1.07
SEM	0.0309	0.0414	0.0403	0.0346	0.0212	0.0373	0.0365	0.0346	0.0718	0.0357	0.064

TABLE XIII

EXPERIMENT IIIB - SERUM T_4 (µg%)

Bird No.	Central Standard Time										
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500
						Controls					
1	1.40	1.58	1.66	1.34	1.02	1.30	1.58	1.40	1.34	1.52	1.08
2	1.30	1.34	1.34	1.12	0.88	1.30	1.00	1.02	1.34	1.66	0.82
3	1.02	1.20	1.24	1.40	1.52	1.40	1.30	1.00	1.30	0.80	0.82
4	1.82	0.96	1.16	1.34	1.40	1.66	1.30	1.30	1.40	1.24	1.04
5	1.16	1.08	1.12	1.52	1.08	1.46	1.22	1.08	1.40	1.16	1.02
6	1.20	1.58	1.08	1.30	1.08	1.52	1.08	1.46	1.52	1.08	1.04
7	1.46	1.40	1.24	1.16	1.30	1.34	1.30	1.52	1.34	1.24	9.06
8	1.24	1.22	1.04	1.34	1.24	1.30	1.00	1.20	1.34	1.04	0.88
$\bar{X}$	1.33	1.30	1.24	1.32	1.19	1.41	1.22	1.25	1.37	1.22	0.96
SEM	0.0858	0.0789	0.0698	0.0450	0.0752	0.0460	0.0688	0.0718	0.0242	0.0960	0.0369
						Pinealectomized					
1	1.04	1.04	1.00	1.36	1.04	1.20	1.16	1.40	1.16	1.00	1.12
2	0.88	0.76	0.68	1.08	0.98	1.16	1.20	1.30	1.12	0.80	1.26
3	0.94	1.20	1.12	1.04	0.84	1.30	1.04	1.36	1.26	0.94	1.26
4	1.00	0.88	1.04	1.20	1.26	1.30	1.00	1.16	1.20	1.04	1.26
5	1.20	0.80	1.04	1.08	1.12	1.04	1.30	1.46	1.04	0.84	1.30
6	1.26	1.36	1.08	1.12	1.04	0.78	1.16	1.46	1.36	0.74	1.04
7	0.90	0.94	0.88	0.84	0.94	1.26	1.16	1.30	1.20	0.60	1.12
8	0.90	1.00	1.00	0.96	1.16	1.04	1.26	1.46	1.26	1.04	1.08
$\bar{X}$	1.02	0.99	0.98	1.09	1.05	1.14	1.16	1.36	1.20	0.88	1.17
SEM	0.0510	0.0716	0.0496	0.0547	0.0467	0.0625	0.0357	0.0375	0.0344	0.0559	0.0314

TABLE XIV

EXPERIMENT IA - SERUM T_4 (µg%)

Bird No.	Central Standard Time											
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500	1800
						Controls						
1	1.44	1.50	1.32	1.10	1.11	1.30	1.50	1.40	1.50	1.60	1.54	1.32
2	1.40	1.42	1.18	1.34	1.32	1.60	1.54	1.34	1.28	1.22	1.28	1.36
3	1.34	1.34	1.42	1.20	1.44	1.30	1.44	1.40	1.44	1.50	1.76	1.32
4	1.44	1.34	1.34	1.08	1.18	1.18	1.30	1.86	1.36	1.22	1.44	1.40
5	1.80	1.48	1.10	1.14	1.34	1.12	1.48	1.68	1.36	1.28	1.10	1.30
6	1.58	1.36	1.40	1.20	1.40	1.48	1.64	1.50	1.54	1.60	1.42	1.24
7	1.32	1.19	1.44	1.24	1.19	1.34	1.68	1.36	1.18	1.58	1.28	1.44
8	1.36	1.42	1.32	1.44	1.48	1.33	1.54	1.42	1.32	1.76	1.54	1.40
$\bar{X}$	1.46	1.38	1.32	1.22	1.31	1.33	1.52	1.50	1.37	1.47	1.42	1.35
SEM	0.0564	0.0347	0.0420	0.0432	0.0475	0.0541	0.0717	0.0646	0.0417	0.0722	0.0715	0.0230
						Pinealectomized						
1	1.60	1.40	1.64	1.46	1.17	1.46	1.17	1.17	1.36	1.33	1.16	1.26
2	1.70	1.50	2.04	1.20	1.50	1.14	1.24	1.40	1.38	1.38	1.38	1.17
3	1.70	1.80	1.40	1.33	1.16	1.36	1.14	1.44	1.54	1.40	1.26	1.17
4	1.50	2.16	1.14	1.44	1.84	1.60	1.56	1.44	1.26	1.33	1.26	1.16
5	1.94	1.74	1.50	1.36	1.44	1.38	1.56	1.54	1.38	1.26	1.20	1.01
6	1.60	1.44	1.36	1.10	1.40	1.36	1.04	1.80	1.30	1.40	1.33	1.17
7	1.40	1.74	1.44	1.54	1.38	1.70	1.40	1.26	1.33	1.24	1.33	1.21
8	1.84	1.60	1.60	1.54	1.46	1.17	0.68	1.04	1.44	1.33	1.24	1.21
$\bar{X}$	1.66	1.67	1.52	1.37	1.42	1.40	1.22	1.39	1.37	1.33	1.27	1.17
SEM	0.0619	0.0873	0.0928	0.0558	0.0752	0.0678	0.1032	0.0827	0.0306	0.0212	0.0258	0.0257

TABLE XV

EXPERIMENT IIC - SERUM T_4 (µg%)

Bird No.	Central Standard Time											
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500	1800
						Controls						
1	1.84	1.44	1.48	1.44	1.78	2.40	1.52	1.36	1.14	1.52	1.53	1.40
2	2.26	2.00	1.44	1.34	1.48	1.62	1.34	1.62	1.44	1.34	1.20	1.40
3	3.08	2.40	1.48	1.14	1.72	1.72	1.52	2.14	1.62	1.48	1.34	1.52
4	2.40	2.00	1.44	1.34	1.58	1.62	1.44	1.72	1.92	1.40	1.44	1.36
5	1.92	2.14	1.52	1.44	1.58	1.72	2.00	1.92	1.84	1.40	1.36	1.62
6	1.72	1.92	1.58	1.26	1.66	1.52	1.92	1.62	1.84	1.72	1.36	1.18
7	2.14	2.40	1.40	1.40	1.00	1.84	1.58	1.26	1.72	1.34	1.20	1.06
8	2.26	2.14	1.26	1.72	1.66	1.44	1.48	2.26	1.48	1.52	1.20	1.48
$\bar{X}$	2.20	2.06	1.45	1.39	1.56	1.74	1.60	1.74	1.63	1.47	1.33	1.38
SEM	0.1502	0.1082	0.0334	0.0595	0.0861	0.1047	0.0827	0.1244	0.0925	0.0445	0.0432	0.0642
						Pinealectomized						
1	1.80	1.64	1.64	1.32	1.70	1.74	2.00	1.88	1.80	1.40	1.40	1.50
2	1.74	1.64	1.42	1.28	1.54	1.74	1.74	1.60	1.74	1.50	1.50	1.64
3	1.92	1.92	1.16	1.46	1.42	1.88	1.70	1.92	1.54	1.64	1.74	1.42
4	1.80	1.70	1.32	1.80	1.60	1.70	2.08	1.80	2.00	1.10	1.60	1.50
5	1.74	1.50	2.08	1.36	2.00	1.74	1.74	1.50	1.88	1.42	1.24	2.00
6	1.88	1.74	1.28	1.64	1.50	1.74	1.64	1.70	1.36	1.42	2.00	1.64
7	1.92	1.74	2.16	1.40	1.64	1.88	1.64	2.24	2.16	1.60	1.64	1.70
8	2.32	2.00	1.32	1.60	1.74	1.92	2.16	2.08	1.70	1.60	1.54	1.40
$\bar{X}$	1.89	1.74	1.55	1.48	1.64	1.79	1.84	1.84	1.77	1.46	1.58	1.60
SEM	0.0665	0.0565	0.1342	0.0640	0.0631	0.0302	0.0738	0.0866	0.0891	0.0612	0.0804	0.0689

TABLE XVI

EXPERIMENT IIIC - SERUM T_4 (µg%)

Bird No.	Central Standard Time										
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500
						Controls					
1	1.06	0.97	1.22	1.27	0.94	1.08	1.07	1.09	0.90	1.02	0.82
2	1.00	1.11	0.93	1.14	0.79	1.11	0.83	1.14	0.83	1.06	1.09
3	0.91	1.17	1.26	0.98	0.97	1.44	1.04	1.31	0.87	1.06	0.83
4	1.32	0.96	1.23	1.10	0.92	0.94	1.15	1.11	1.17	1.03	1.03
5	0.87	0.92	1.07	1.19	1.02	1.01	0.90	0.84	0.99	0.84	0.83
6	1.22	1.04	1.22	1.08	0.81	1.30	0.98	1.29	1.12	0.99	1.20
7	1.02	0.89	1.01	0.81	0.82	0.89	0.79	1.35	1.03	0.97	0.94
8	0.89	0.97	1.04	1.68	1.07	1.12	1.08	1.15	0.90	1.16	0.99
$\bar{X}$	1.04	1.00	1.12	1.08	0.92	1.11	0.98	1.16	0.97	1.02	0.97
SEM	0.0569	0.0339	0.0441	0.0491	0.0364	0.0645	0.0454	0.0576	0.0434	0.0323	0.0489
						Pinealectomized					
1	1.45	1.43	1.56	1.51	1.18	2.02	1.23	1.62	1.68	1.39	1.64
2	1.13	1.33	1.48	1.59	1.51	1.42	1.58	1.70	1.61	1.49	1.39
3	1.53	1.57	1.58	1.50	1.27	1.67	1.55	1.83	1.67	1.54	1.54
4	1.57	1.21	1.48	1.58	1.57	1.86	1.62	1.63	1.55	1.40	1.69
5	1.53	1.47	1.43	1.65	1.46	1.70	1.38	1.84	1.66	1.45	1.43
6	1.17	1.56	1.54	1.56	1.43	1.69	1.50	1.75	1.52	1.18	0.68
7	1.42	1.43	1.26	1.46	1.53	1.52	1.22	1.84	1.52	1.53	1.13
8	1.48	1.45	1.73	1.66	1.65	1.38	1.28	1.64	1.57	1.20	1.46
$\bar{X}$	1.41	1.43	1.51	1.56	1.45	1.66	1.42	1.73	1.60	1.40	1.37
SEM	0.0593	0.0416	0.0477	0.0250	0.0551	0.0765	0.05770	0.0342	0.0236	0.0492	0.1157

TABLE XVII

EXPERIMENT IIA - SERUM T_3 (ng%)

Bird No.	Central Standard Time									
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200
					Controls					
1	266	279	248	327	244	339	197	270	224	350
2	304	153	315	299	262	227	161	270	321	254
3	248	288	280	292	248	220	197	240	294	288
4	222	336	312	285	232	125	245	256	260	285
5	216	276	352	217	253	261	175	315	214	313
6	355	274	367	294	274	285	154	256	119	217
7	308	365	358	299	223	214	196	286	288	321
8	316	335	334	297	241	255	200	125	245	285
$\overline{X}$	279	288	321	289	247	241	191	252	245	289
SEM	17.402	22.791	14.480	11.134	5.727	21.941	10.016	19.856	22.171	14.493
					Pinealectomized					
1	303	239	310	339	339	212	171	239	229	250
2	303	306	255	297	257	239	179	210	236	180
3	286	341	320	303	236	165	217	186	245	298
4	293	311	488	312	263	219	200	310	207	288
5	288	196	360	310	311	194	213	228	219	234
6	139	284	368	302	277	218	218	234	264	269
7	304	259	304	302	255	177	210	226	297	310
8	316	263	376	345	298	264	190	200	236	300
$\overline{X}$	279	275	348	314	280	211	200	229	242	266
SEM	20.298	16.164	24.546	6.414	12.065	11.392	6.358	13.193	9.921	15.402

TABLE XVIII

EXPERIMENT IIIA - SERUM T_3 (ng%)

Bird No.	Central Standard Time										
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500
					Controls						
1	136	146	193	173	257	154	190	135	91	93	147
2	110	133	128	175	267	168	165	131	100	99	124
3	177	133	141	150	193	169	129	95	102	142	126
4	159	125	147	178	166	127	129	114	90	116	124
5	130	149	164	211	167	179	149	171	131	157	178
6	133	125	163	187	180	179	163	126	123	158	125
7	132	142	157	184	212	142	169	104	126	164	123
8	159	125	160	177	181	164	143	99	87	136	129
$\overline{X}$	142	135	157	179	203	160	154	121	106	133	135
SEM	7.545	3.468	6.795	5.991	13.933	6.452	7.460	8.776	6.279	9.726	6.806
					Pinealectomized						
1	123	137	153	161	181	172	157	132	158	160	158
2	146	154	154	166	188	158	166	137	154	151	167
3	160	161	158	158	173	153	167	140	133	141	152
4	139	151	128	151	166	153	140	153	130	160	161
5	156	151	155	158	172	176	150	134	123	155	158
6	160	161	169	173	196	143	132	125	137	163	169
7	125	143	159	160	224	151	140	149	125	143	147
8	134	150	156	152	198	158	147	128	137	139	154
$\overline{X}$	143	151	154	160	187	158	150	137	137	152	158
SEM	5.307	2.897	4.114	2.531	6.641	3.882	4.486	3.423	4.502	3.349	2.616

TABLE XIX

EXPERIMENT IIB - SERUM T_3 (ng%)

Bird No.	Central Standard Time											
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500	1800
						Controls						
1	272	236	240	377	208	313	212	197	147	204	336	257
2	242	183	298	217	257	276	264	188	374	159	287	255
3	268	202	301	242	308	264	262	225	160	233	283	208
4	238	276	208	304	283	298	169	167	172	219	272	268
5	283	215	217	458	313	262	209	208	215	164	352	308
6	187	240	298	307	415	301	260	204	209	178	344	384
7	225	200	262	238	380	257	196	203	224	194	208	276
8	315	226	242	305	296	301	188	216	242	200	269	292
$\overline{X}$	266	223	259	306	308	284	233	202	218	194	294	281
SEM	10.532	10.269	13.246	28.282	23.156	7.667	15.605	6.274	25.199	9.125	17.062	18.045
						Pinealectomized						
1	199	196	155	266	288	312	204	181	133	102	293	188
2	199	185	293	273	273	252	180	135	120	131	280	408
3	241	241	196	228	273	299	160	224	145	162	138	232
4	214	166	149	264	273	329	199	200	72	89	319	266
5	187	226	199	158	281	320	170	177	278	177	232	307
6	244	118	268	293	133	168	189	143	111	141	297	292
7	133	266	241	259	302	322	183	101	123	123	366	278
8	221	156	218	256	420	278	141	185	99	143	304	302
$\overline{X}$	205	194	215	250	280	285	179	168	135	134	279	284
SEM	12.488	17.168	18.003	14.574	27.355	19.031	7.348	13.952	21.861	10.299	24.055	]].588

TABLE XX

EXPERIMENT IIIB - SERUM T_3 (ng%)

Bird No.	Central Standard Time										
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500
					Controls						
1	419	434	369	373	284	471	330	260	416	394	523
2	498	411	383	461	258	340	264	359	468	384	525
3	514	444	501	429	286	453	370	408	419	375	499
4	448	460	459	421	149	478	365	363	443	446	533
5	316	388	393	463	420	400	464	375	405	460	549
6	386	421	388	389	428	413	264	210	384	370	483
7	451	563	403	318	370	396	354	313	451	438	538
8	486	424	484	470	538	444	420	441	430	415	563
$\overline{X}$	444	443	423	416	342	424	354	341	427	410	526
SEM	23.880	18.739	17.998	18.672	42.988	16.313	24.498	27.045	9.487	12.219	9.138
					Pinealectomized						
1	293	335	308	231	230	248	281	219	243	304	256
2	365	320	156	286	364	294	278	253	352	485	244
3	411	310	297	299	458	268	278	273	301	380	302
4	327	411	264	271	286	298	252	262	241	113	290
5	318	375	302	310	350	275	248	210	296	373	339
6	266	375	315	297	326	404	258	245	298	298	265
7	336	411	340	313	238	259	291	281	169	343	272
8	409	319	310	338	331	275	250	245	281	442	347
$\overline{X}$	340	357	287	293	323	290	267	249	273	343	289
SEM	18.312	14.630	20.078	11.294	26.033	17.287	5.931	8.697	19.357	39.793	13.371

TABLE XXI

EXPERIMENT IA - SERUM T_3 (ng%)

Bird No.	Central Standard Time											
	0900	1200	1500	1800	2100	2400	0300	0800	0900	1200	1500	1800
						Controls						
1	125	203	161	219	164	186	161	152	186	97	117	193
2	89	114	146	171	167	193	167	167	86	108	131	167
3	167	146	164	179	206	203	155	164	125	97	55	179
4	122	268	176	108	193	171	227	143	158	108	161	117
5	108	137	73	242	206	167	167	164	143	167	171	161
6	114	155	179	179	179	155	209	152	89	125	131	161
7	84	103	230	189	216	189	171	183	117	137	167	206
8	86	125	167	183	149	32	183	167	105	124	200	131
$\bar{X}$	111	156	162	184	185	162	185	162	126	121	143	165
SEM	9.731	19.248	15.420	13.764	8.456	19.366	8.928	4.347	12.251	8.337	15.599	10.483
						Pinealectomized						
1	188	185	162	130	144	128	136	203	212	136	200	216
2	118	212	173	161	163	191	219	159	154	128	144	156
3	161	104	151	150	159	143	197	170	144	85	151	161
4	182	188	144	185	194	189	167	179	152	110	128	233
5	185	136	170	147	122	234	197	138	183	124	216	121
6	116	144	212	191	177	243	132	150	62	167	219	180
7	224	210	167	82	184	150	116	159	138	150	188	233
8	159	299	147	141	200	200	222	138	144	163	150	190
$\bar{X}$	166	185	166	148	168	184	173	162	149	133	175	186
SEM	12.915	21.130	7.639	12.042	9.292	14.206	14.641	7.732	15.176	9.722	12.521	14.091

TABLE XXII

EXPERIMENT IIC - SERUM T_3 (ng%)

Bird No.	Central Standard Time											
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500	1800
						Controls						
1	84	108	208	302	302	231	171	238	116	102	320	196
2	101	96	304	218	193	266	236	124	152	196	212	180
3	42	148	306	304	240	200	206	166	117	167	233	252
4	109	92	182	252	364	358	170	170	105	270	248	104
5	167	96	434	237	304	204	232	138	128	228	240	220
6	100	102	256	296	246	220	256	152	156	198	224	312
7	84	206	252	280	198	244	143	123	144	212	204	296
8	96	178	262	368	222	212	122	128	132	188	186	312
$\overline{X}$	97	129	276	282	259	242	192	155	131	203	233	234
SEM	12.268	15.471	27.150	16.637	21.076	18.298	16.946	13.527	6.966	12.298	14.277	25.978
						Pinealectomized						
1	138	130	420	284	234	184	158	104	176	106	166	156
2	109	124	270	292	232	164	178	74	158	328	197	196
3	128	186	408	254	197	204	162	126	104	308	209	146
4	162	90	175	304	202	150	233	68	184	166	156	210
5	110	66	208	436	284	208	184	148	104	218	322	270
6	114	112	416	288	266	172	208	150	98	266	198	272
7	143	200	262	248	140	171	122	112	126	100	194	204
8	143	78	308	177	328	172	197	144	72	162	188	180
$\overline{X}$	131	124	308	286	235	178	180	116	128	207	204	205
SEM	6.710	17.130	34.189	25.779	20.519	6.960	12.007	11.411	14.350	30.993	17.997	16.542

TABLE XXIII

EXPERIMENT IIIC - SERUM T_3 (ng%)

Bird No.	Central Standard Time										
	0900	1200	1500	1800	2100	2400	0300	0600	0900	1200	1500
					Controls						
1	174	207	163	202	279	266	227	257	210	231	260
2	224	192	165	192	284	259	226	222	189	159	226
3	221	213	193	210	193	244	215	227	165	164	232
4	215	206	220	271	255	240	229	228	247	213	204
5	206	190	155	215	273	287	224	227	229	215	210
6	220	183	194	206	300	216	187	229	169	217	218
7	165	209	206	225	219	282	252	226	233	214	213
8	243	223	178	212	253	258	223	223	217	169	219
$\overline{X}$	209	203	184	217	257	256	223	229	207	197	223
SEM	9.306	4.726	8.062	8.481	12.591	8.181	6.357	3.966	10.690	10.122	6.167
					Pinealectomized						
1	163	137	186	193	151	152	145	135	151	187	208
2	154	181	199	187	217	192	204	160	150	127	239
3	160	161	218	155	239	222	117	162	148	145	128
4	159	190	154	215	245	246	195	177	185	156	210
5	162	144	142	198	180	231	187	149	151	101	221
6	179	190	192	246	255	204	220	216	156	116	183
7	199	151	179	214	135	154	215	160	163	149	216
8	247	159	293	211	229	223	223	160	197	186	177
$\overline{X}$	178	164	195	203	206	203	188	164	162	145	198
SEM	11.136	7.286	16.358	9.233	16.037	12.333	13.479	8.436	6.505	10.919	12.206

VITA

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