Thyroid Hormones and Seasonality in the White-Throated Sparrow and Green Anole.

Blaine Richard Ferrell
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THYROID HORMONES AND SEASONALITY IN THE
WHITE-THROATED SPARROW AND GREEN ANOLE

A Dissertation

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Doctor of Philosophy

in

The Department of Zoology and Physiology

by

Blaine R. Ferrell
B.S., University of Pennsylvania, 1973
M.S., Western Kentucky University, 1975
August, 1979
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This treatise is dedicated to my most patient companion, Priscilla J. Ferrell, whose encouragement was instrumental in completing this work.
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ABSTRACT

Part IA: Photo-thermoperiodic Effects on Fattening and Testicular Growth in the Green Anole, Anolis carolinensis. Two circadian oscillations are involved in regulating fattening in the Green Anole, Anolis carolinensis. One oscillation is entrained by the photoperiod and the other by the thermoperiod. Changing interactions between these oscillations may represent a mechanism whereby seasonal conditions of fattening are regulated. Fat stores increased or decreased in response to a 6-hour period of 30±2°C. The response depended on the timing of the 30±2°C treatment relative to the photoperiod of a LD 12:60 cycle. Anoles were kept at 20±2°C the remaining hours of each light-dark cycle. The fattening response to a single 6-hour period of 30±2°C (thermoperiod) during each LD 12:60 cycle was similar in groups receiving treatment at 24-hour intervals. Testicular growth may be regulated in a similar manner. However, this possibility needs to be explored further.

Part IB: Thyroxine and the Fattening Response to Warm Temperature in the Green Anole, Anolis carolinensis. Thyroxine has a permissive influence on warm temperature sensitive mechanisms, such as those regulating lipogenesis and/or lipid transport, which affect lipid deposition into fat bodies in A. carolinensis. The rate of 14C-acetate incorporation into fat body lipid was similarly increased in re-
sponse to warm temperature in untreated and thiourea-thyroxine-treated anoles whereas there was no response to warmth in thiourea-treated anoles. Because a phase shift in the rhythm of $^{14}\text{C}$-acetate incorporation into fat body lipid occurred in response to warm temperature, it is conceivable that thyroxine may be involved in phasing circadian rhythms important in regulating fat stores. The permissive influence of thyroxine in mechanisms regulating fattening may be dependent on the occurrence of warm temperature. Fat stores were increased in thiourea-thyroxine-treated anoles kept at $30\pm 2^\circ\text{C}$, whereas fat stores were not increased in thyroxine-treated anoles kept at $20\pm 2^\circ\text{C}$. The influence of constant warm temperature on testicular growth is not consistent and may depend on photoperiodic entrainment. Testicular growth occurred during December in anoles kept under LD 6:18 but not in anoles kept in continuous light. Thyroxine may influence testicular growth resulting from constant warm temperature treatment. Further experimentation is necessary before this explanation can be accepted.

Part IIA: Thyroxine and Seasonality in the White-throated Sparrow, *Zonotrichia albicollis*. Measurements of endogenous levels of plasma thyroxine were made throughout the day in White-throated Sparrows, *Zonotrichia albicollis* in different seasonal conditions. The results support the concept that circadian hormone rhythms have important consequences in
physiological mechanisms regulating seasonal conditions of fattening, migration and reproduction, possibly through temporal interactions with other circadian hormone rhythms. The photoperiod has an important effect on the daily pattern of plasma thyroxine concentrations. However, the interpretation of daylength, reflected in the daily pattern of plasma thyroxine, changes seasonally and may be influenced by physiological (endogenous circannual mechanism) or other environmental (temperature) changes. A low amplitude daily rhythm of plasma thyroxine concentration occurred in photosensitive sparrows held on LD 10:14. In contrast, a high amplitude daily rhythm was associated with vernal conditions of fattening, nocturnal locomotor activity and gonadal development in the same sparrows transferred to long daylength (photostimulated: LD 16:8) conditions. The daily rhythm disappeared after a period of time under long daylength conditions in sparrows which had become photorefractory.

Part IIB: Resetting the Endogenous Circannual Mechanism in White-throated Sparrows with Corticosterone and Prolactin. Changing temporal relations of two circadian neuroendocrine oscillations comprise the circannual mechanism that controls the annual cycle of molt, migration and reproduction in the White-throated Sparrow. Daily injections of corticosterone and prolactin in specific temporal relations for 11 days reset the circannual clock of late summer birds so that their annual cycle is reinitiated from spring condition.
GENERAL INTRODUCTION

The iguanid lizard *Anolis carolinensis* and the migratory White-throated Sparrow (*Zonotrichia albicollis*), like many temperate zone species, have marked seasonality of metabolic and behavioral conditions such as fattening and gonadal development. The annual cycle of these events appears to involve an endogenous circannual mechanism in that events continue to be expressed on a circannual basis in animals kept under constant daylengths. Because the annual cycle has a period approximating one year, exogenous cues are involved in synchronizing events to coincide with appropriate environmental conditions.

Photoperiod is an important synchronizer of seasonal metabolic and behavioral events. According to an hypothesis of photoperiodism first proposed by Bünning (1936), the photoperiod entrains a rhythm of sensitivity to light. If daylength is sufficient such that light occurs during the light sensitive phase of the photosensitivity rhythm, a physiological event is induced (e.g., fattening). Once entrained by the photoperiod, the rhythm of sensitivity to light occurs on a circadian basis without additional photoperiodic entrainment. Because the photoperiod entrains a daily rhythm of fattening and testicular growth sensitivity to warmth in anoles, I hypothesized that photoperiod-thermoperiod interactions affecting these events may represent a mechanism similar to that proposed to account for photoperiodism. If
so, the photoperiod should entrain a rhythm of fattening and testicular growth responsiveness to warmth which occurs on a circadian basis without additional photoperiodic entrainment.

Because warm temperature apparently is a prerequisite for thyroxine to have an influence on various facets of metabolism in a variety of ectothermic vertebrates, I hypothesized that thyroxine may be involved in mechanisms whereby warmth effects fattening and testicular growth in anoles. In addition, I wanted to determine if the influence of thyroxine on such mechanisms was temperature dependent.

Although exogenous cues (i.e., photoperiod and thermoperiod) may synchronize events of the annual cycle with appropriate environmental conditions, the principal timer is the endogenous circannual mechanism. Changing temporal relations among circadian hormone rhythms may be involved in this mechanism. Measurements of endogenous hormone titers throughout the day during several different seasons in White-throated Sparrows support this notion. Circadian rhythms of corticosterone and prolactin occur in the White-throated Sparrow. The pattern of these rhythms and the phase relation to the photoperiod and to each other changes seasonally. The relevance of changing temporal relations between circadian hormone rhythms to mechanisms regulating reproduction, fattening, and migration in the White-throated Sparrow has been demonstrated through injection studies. Thyroxine has been implicated in mechanisms involved in regulating seasonal
events. However, its role in these events is still unclear despite intensive investigation. Therefore, measurements of plasma thyroxine were made throughout the day during several different seasons to determine if the influence of thyroxine on mechanisms regulating seasonal events depended on the temporal relation of its rhythm with other circadian hormone rhythms. The influence of photoperiod on thyroxine titers was tested also.

Because daily injections of corticosterone and prolactin in a specific temporal relation produce the full complement of metabolic and behavioral conditions appropriate for a specific season, the hormone injections influence central neural mechanisms which are responsible for the organization of these events. If hormone injections do reset central neural mechanisms involved in the circannual mechanism, then these same hormone injections should reset the annual cycle of photorefractory birds in late-summer such that events of the annual cycle will proceed in the natural sequence from the point at which the annual cycle has been reset.
INTRODUCTION

The iguanid lizard *Anolis carolinensis* of the coastal southeastern United States has an annual cycle of gonadal development, fattening, and hibernation. Gonadal regeneration is initiated during the cooler months in winter but spermatogenesis is not completed until a few weeks after arousal of anoles from hibernation in spring (Fox, 1958; Licht, 1967a, b) when conditions of food resources and climate are supportive for reproductive success. As the gonads regress in mid to late summer, body fat stores begin increasing (Dessauer, 1953, 1955a; Fox, 1958) in preparation for periods of reduced feeding experienced during hibernation in winter (Dessauer, 1953, 1955a, b; Licht and Jones, 1967). Two discrete abdominal fat bodies represent the main lipid storage site although some fat is also stored in the liver (Dessauer, 1953, 1955a, b). Anticipatory physiological changes are necessary in order to achieve both reproductive readiness and increased fat stores coincident with the onset of appropriate environmental conditions. How are these preparatory changes induced?

Many experiments have tested the effects of differing photoperiods and/or temperatures on the reproductive cycle of *A. carolinensis* (Clausen and Poris, 1937; Fox and Dessauer, 1958; Licht, 1966, 1967a, b, 1969a, 1971; Noeske, 1974; Noeske and Meier, 1977), and other lizard species (Mayhew, 1964; Licht et al., 1969; Botte et al., 1978). The rate of testicular development in anoles maintained at 28°C can be accelerated
at all seasons by long daily photoperiods (Fox and Dessauer, 1958). Low temperatures, independent of daylength, enhance testicular growth during the regenerative period in late fall and winter (Licht, 1969a), whereas warm temperature (30°C) is necessary during part of the day for long photoperiods to be stimulatory (Licht, 1966, 1967a, b, 1969a). The photoperiod becomes the dominating factor during regression of the testes. Testicular involution is promoted when the daylength decreases below 13.5 hours in late summer (Licht, 1969a, 1971). The effects of photoperiod-temperature interactions on body fat stores have been considered secondarily during investigations of the influence on the reproductive cycle of anoles (Licht, 1967a, b, 1971; Noeske and Meier, 1977) and other lizard species (Licht et al., 1969).

Green anoles are rarely exposed to constant temperature conditions in nature (Licht, 1967 a, b, 1969a). Therefore, a simple interaction between photoperiod and constant temperature is not likely. Results from investigations using different photoperiod-thermocycle combinations clearly indicate that body fat stores and gonadal growth in green anoles (Licht, 1966, 1969a, 1971; Noeske and Meier, 1977), and in goldfishes (Carassius auratus) (Spieler et al., 1977) are either stimulated, depressed, or unchanged depending on the time relative to the photoperiod these animals are exposed to a particular thermoperiod (i.e., duration of warm temperature).

These findings bear striking similarities to those
supporting the hypothesis explaining photoperiodism proposed by Bünning (1936, 1960). According to this hypothesis, a circadian rhythm of photosensitivity is entrained by a daily photoperiod. If light coincides with a photosensitive phase (photoinducible phase) of the photosensitivity rhythm, it induces a physiological process (e.g., hormone release). Once entrained by a period of light, the photoinducible phase will continue to be expressed on a circadian basis for several days. This effect has been demonstrated in many avian species (Hamner, 1963, 1964; Farner, 1964, 1965; Follett and Sharp, 1969; Turek, 1974; see also Meier, 1976; Meier and Ferrell, 1978), and in photoperiodic teleosts (Baggerman, 1973) and mammals (Elliott et al., 1972).

The influence of photoperiod-thermoperiod interactions on body fat stores and gonadal growth in anoles may involve a similar mechanism. Conceivably a daily photoperiod may entrain a circadian rhythm of sensitivity to warm temperature. When warm temperature (instead of light) coincides with this temperature sensitive phase (thermoinducible phase), a physiological event is induced (e.g., hormone release). The following two experiments were designed to test this hypothesis with regard to the influence of photoperiod-thermoperiod interactions on body fat stores (fat body weights) and testicular growth (paired testes weights) in the Green Anole, *Anolis carolinensis*. 
MATERIALS AND METHODS

Large male *A. carolinensis* were captured near Baton Rouge, Louisiana (Experiment I) or purchased from a local biological supply house (Experiment II). Only males which had been through one reproductive season (average snout-vent length of over 58 mm: Dessauer, 1955a) were used in these investigations. Experimental groups of 7 or 8 anoles each were housed in screen-covered plastic tubs. A thin layer of sand covered the floor of the cages. Food (crickets) and water (in gravel filled petri dishes) were available at all times. The cages were placed inside incubators equipped with 20 watt fluorescent lights mounted above the cages. Light from this source is sufficient for photoperiodic effects in *A. carolinensis* (Licht, 1969b).

Temperatures inside the incubators were maintained at either 20±2°C (minimum temperature at which *A. carolinensis* are active in the field and feed: Gordon, 1956; Licht, 1966) or 30±2°C (near the mean preferred body temperature of *A. carolinensis*: Licht, 1968; Licht et al., 1966). Thermocycles were established by transferring cages between incubators.

Each anole was toe clipped for subsequent identification and weighed before initiating each experiment. One group (initial group) of anoles was killed before experimental conditions were initiated and paired testes and fat body weights were determined.
Measurements of testes and fat bodies were also taken from anoles after experimental treatment. Anoles were denied food two days prior to taking weight measurements to allow voiding of the digestive tract.

Experiment I was initiated 29 April, 1978 and terminated three weeks later on 21 May. Anoles were captured near Baton Rouge on 26 and 27 April and kept in CC-LD 12:12 until experimental conditions were initiated. The experimental design is represented in figure 1 and closely resembles that used in a photoperiodism experiment (Hamner, 1960). Anoles were exposed to a 6-hour thermoperiod (30±2°C) at various times during a 72-hour day (LD 12:60). During the remaining 66 hours of each light-dark cycle anoles were maintained at 20±2°C. These thermoperiod treatments will subsequently be represented by WC; hr.-hr., where hr.-hr. is the interval of warmth after light onset.

Experiment II was initiated on 6 June, 1978 after receiving a shipment of anoles from a biological supply house on 4 June. The experimental design is depicted in figure 2 and is similar to that of Experiment I.

Data of both experiments were analyzed statistically with a completely randomized design Analysis of Variance for comparing WC treatment groups by using an SPSS computer program for unequal sample sizes. Treatment groups were ranked according to Student-Newman-Keuls procedure in order to identify significant differences among treatment means. This range test helps protect against determining significant
Figure 1: Experimental Design - Experiment I.

The numbers above the light (L: unshaded) - dark (D: shaded) cycle representations (bars) indicate hours after the onset of light. CC represents a treatment of constant 20+2°C. WC represents treatment of 20+2°C interrupted by a 6-hour period of 30+2°C; hr.-hr. represents the interval of warmth after the onset of light indicated by the brackets ([30°C]) underneath the light-dark cycle bars.
Figure 2: Experimental Design - Experiment II.

All anoles were maintained under LD 12:60. See the legend of Figure 1 for an explanation of the symbols.
differences by chance when making comparisons among treatment groups. Differences among treatment means were considered significant at the 95% confidence level ($p<.05$).
RESULTS

Experiment I was performed to test for circadian rhythms of fattening and gonadal growth responses to warm temperatures. The results are presented in Table I and Figures 3 (fat body weights) and 4 (testicular weights). Consistent with other reports (Dessauer, 1953, 1955a, b; Licht, 1971), anoles appear to have low body fat stores (initial group) in April (Figure 3). Fat bodies from anoles maintained in continuous 20±2°C (CC) and LD 12:12 were similar in weight to those of the anoles in the initial group whereas the fat bodies of anoles maintained in CC and ahemeral photoperiodic conditions were heavier (p<.05 Student "t", Figure 3). Fat storage was affected by photoperiodic conditions (LD 12:60). The influence (stimulatory or inhibitory) of experimental thermo­periods on fat stores was assessed by comparing fat bodies' from anoles in WC treatment groups with fat bodies from anoles maintained under CC-LD 12:60 conditions. In this way, effects attributed to the photoperiodic regimen were minimized when analyzing the influence different thermoperiods had on fat stores. Body weight changes within treatment groups were not significant although they appeared to reflect changes in fat body weights in most cases (Table I). Statistical differences among treatment means of final body weights were detected (ANOVA; Table I). However, these weight differences did not correspond to fat body or testicular weight differences.

WC; 24-30, WC; 36-42 and WC; 48-54 did not stimulate
TABLE I

Thermoperiod effects on fat body and paired testes weights in A. carolinensis maintained under LD 12:60. Anoles were kept at 20+2°C during the remaining 66 hours of each light-dark cycle. Experimental conditions were maintained for 21 days beginning 29 April, 1978.

<table>
<thead>
<tr>
<th>GRP (N)</th>
<th>Treatment</th>
<th>Initial Body Wts. (g)</th>
<th>Final Body Wts. (g)</th>
<th>Paired Testes Wts. (mg)</th>
<th>Fat Bodies (% Body Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>5.04 + .20</td>
<td>85 + .05</td>
<td>.05 + .02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LD 12:12</td>
<td>5.49 + .16</td>
<td>5.35 + .16</td>
<td>95 + 5x, y</td>
<td>.08 + .06x, y</td>
</tr>
<tr>
<td>3</td>
<td>LD 12:60</td>
<td>5.26 + .23</td>
<td>5.65 + .25</td>
<td>88 + 6x, y</td>
<td>.23 + .04x, y</td>
</tr>
<tr>
<td>4</td>
<td>LD 12:60</td>
<td>5.45 + .23</td>
<td>4.82 + .21</td>
<td>83 + 4x</td>
<td>.21 + .07x, y</td>
</tr>
<tr>
<td>5</td>
<td>LD 12:60</td>
<td>5.27 + .15</td>
<td>5.39 + .12</td>
<td>102 + 4y</td>
<td>.69 + .09z</td>
</tr>
<tr>
<td>6</td>
<td>LD 12:60</td>
<td>4.57 + .20</td>
<td>4.97 + .19</td>
<td>86 + 4x</td>
<td>.32 + .07y</td>
</tr>
<tr>
<td>7</td>
<td>LD 12:60</td>
<td>5.04 + .19</td>
<td>4.89 + .24</td>
<td>82 + 2x</td>
<td>.00 + .00x</td>
</tr>
<tr>
<td>8</td>
<td>LD 12:60</td>
<td>5.73 + .61</td>
<td>5.80 + .52</td>
<td>91 + 5x, y</td>
<td>.24 + .07x, y</td>
</tr>
</tbody>
</table>

ANOVA d P < .05 P < .05 P < .05 P < .05

a. The number (N) of anoles in each group (GRP).

b. Treatment symbols: I = initial group; CC = constant 20+2°C; WC; hr.-hr. = treatment of 20+2°C interrupted by a 6-hour period of 30+2°C; interval after the onset of light.

c. Mean weight + one standard error about the mean.

d. Analysis of variance among WC treatment groups.

x, y, z Ranking of treatment means using the Student-Newman-Keuls procedure. Means without a letter in common are statistically different at the 95% confidence level.
Figure 3

Thermoperiod effects on fat body weights in A. carolinensis maintained under LD 12:60. An initial group (I) of anoles was killed before photoperiod-thermoperiod treatments were initiated. Another group of anoles was kept at 20±2°C (CC) under LD 12:12. Thermoperiod treatments (6 hours at 30±2°C) were initiated 29 April, 1978 and continued for 21 days. Anoles were kept at 20±2°C the remaining 66 hours of each LD 12:60 cycle. Treatment means were ranked according to the Student-Newman-Keuls procedure. Bars (treatment means) without hatching in the same direction are statistically different at the 95% confidence level.
Figure 4

Thermoperiod effects on paired testes weights in A. carolinensis maintained under LD 12:60. An initial group (I) of anoles was killed before photoperiod-thermoperiod conditions were established. Treatment groups and symbols are the same as those of figure 3. Thermoperiod treatments were initiated 29 April, 1978 and continued for 21 days.
increases in fat body weights. However, compared with all other groups, fat stores were markedly greater in anoles which received WC; 30-36 (Figure 3). WC; 42-48 depressed body fat stores (Figure 3). These results indicate there is a daily variation of fattening responsiveness to warm temperature entrained by the photoperiod (Figure 3) supporting previous findings (Noeske and Meier, 1977). In addition, the fact that fat bodies from anoles exposed to WC; 24-30 were similar in weight to fat bodies from anoles receiving a similar thermoperiod treatment 24 hours later (WC; 48-54) indicates the daily pattern of fattening responsiveness to warmth is expressed on a circadian basis (Figure 3).

Significant differences in testicular weights were evident (ANOVA; Table I, Figure 4) when comparisons were made among WC treatment groups. Testes weights were heaviest in anoles treated with WC; 30-36. Thus, WC; 30-36 appears to be stimulatory for both fattening and testicular growth (Figures 3 and 4). Testes weights appeared to be somewhat depressed in anoles receiving WC; 24-30 and WC; 42-48. These results confirm previous reports (Noeske and Meier, 1977) that a daily variation of testicular responsiveness to warm temperatures exists in anoles.

The effect on testicular growth produced by the interaction of photoperiod and thermoperiod is not easily interpreted (Table I, Figure 4). Testes weights in anoles maintained under CC-LD 12:12 conditions were heavier when compared with testes
weights from anoles in the initial group or the group maintained under CC-LD 12:60 conditions, but the difference was not significant (Table I). Testes weights from anoles in WC-LD 12:60 treatment groups were compared with testes weights from anoles kept under CC-LD 12:60 conditions. No significant differences were found when making such comparisons using Student-Newman-Keuls ranking procedure (Table I, Figure 4). Testes weights of the initial group are similar to those recorded by others for anoles during April (Dessauer, 1955a,b; Fox, 1958; Licht, 1971) when testicular weight is maximal (Dessauer, 1955a, b; Fox and Dessauer, 1958; Licht, 1969a, 1971). Therefore, the possibility of a daily variation in testes responsiveness to warmth might be explored at a more appropriate season (i.e., lower initial testes weights).

Experiment II was performed to explore further the possibility of circadian responsiveness to temperature. The data are presented in Table II and Figure 5 (fat body weights). As in Experiment I, fat bodies from anoles maintained in CC-LD 12:60 were slightly heavier compared with fat bodies from the initial group (Figure 5). Curiously, fat bodies were not evident in anoles held in WW-LD 12:60. Despite the finding that 30°C is a preferred temperature (Licht, 1968), this photoperiod-temperature combination was unsuitable for healthy maintenance of anoles in that 3 lizards in the WW-LD 12:60 group died (Table II). As anticipated, the previously determined (Experiment I) stimulatory thermoperiod (WC; 30-36) stimulated increases in fat body weights when
Thermoperiod effects on fat body and paired testes weights in A. carolinensis maintained under LD 12:60. Anoles were kept at 20±2°C the remaining hours of each 72-hour light-dark cycle. Experimental conditions were maintained for 21 days beginning 6 June, 1978.

<table>
<thead>
<tr>
<th>GRP (N)</th>
<th>Treatment</th>
<th>Initial Body Wts. (g)</th>
<th>Final Body Wts. (g)</th>
<th>Paired Testes Wts. (mg)</th>
<th>Fat Bodies (% Body Weight)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>I</td>
<td>--</td>
<td>4.63±.15^c</td>
<td>71±3^c</td>
<td>.10±.04^c</td>
</tr>
<tr>
<td>2</td>
<td>CC</td>
<td>4.93±.29^c</td>
<td>5.16±.39</td>
<td>80±6^y</td>
<td>.21±.08^x,y</td>
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<tr>
<td>3</td>
<td>WW</td>
<td>5.69±.37</td>
<td>5.34±.48</td>
<td>37±5^x</td>
<td>.00±.00^x</td>
</tr>
<tr>
<td>4</td>
<td>WC; 30-36</td>
<td>5.20±.23</td>
<td>5.27±.37</td>
<td>77±4^y</td>
<td>.38±.07^y</td>
</tr>
<tr>
<td>5</td>
<td>WC; 54-60</td>
<td>5.01±.16</td>
<td>5.30±.19</td>
<td>85±3^y</td>
<td>.48±.12^y</td>
</tr>
<tr>
<td>6</td>
<td>WC; 6-12;30-36; 54-60</td>
<td>5.10±.26</td>
<td>5.51±.26</td>
<td>76±8^y</td>
<td>.54±.09^y</td>
</tr>
<tr>
<td>7</td>
<td>WC; 42-48</td>
<td>4.59±.24</td>
<td>4.47±.18</td>
<td>73±3^y</td>
<td>.06±.04^x</td>
</tr>
<tr>
<td>8</td>
<td>WC; 66-72</td>
<td>4.68±.17</td>
<td>4.47±.16</td>
<td>81±4^y</td>
<td>.05±.02^x</td>
</tr>
</tbody>
</table>

ANOVA^d  N.S.  P < .05  N.S.  P < .001

\( a \) The number (N) of anoles in each group (GRP).

\( b \) Treatment symbols: I = initial group; CC = constant 20±2°C; WW = constant 30±2°C; WC; hr.-hr. = treatment of 20±2°C interrupted by a 6-hour period of 30±2°C; interval after the onset of light.

\( c \) Mean weight ± one standard error of the mean.

\( d \) Analysis of variance among WC treatment groups. N.S. = no statistical difference at the 95% confidence level.

\( x,y \) Ranking of treatment means using the Student-Newman-Keuls procedure. Means without a letter in common are statistically different at the 95% confidence level.
Figure 5:

Thermoperiod effects on fat body weights in A. carolinensis maintained under LD 12:60. An initial group (I) of anoles was killed before photoperiod-thermoperiod treatments were initiated. Experimental conditions were initiated 6 June, 1978 and continued for 21 days. \[\text{\textbackslash\textbackslash\textbackslash\textbackslash\textbackslash}\] = 30±2°C treatment. The histogram bars are coded according to symbols described in the legend to Figure 3.
compared with fat bodies from anoles maintained at CC (Figure 5). However, the differences were not statistically significant. There was a highly significant difference in fat body weights of anoles held at WC; 30-36 and WC; 42-48 (Figure 5). These thermoperiods were also noted in Experiment I to be stimulatory and inhibitory respectively for fat stores.

The most significant results with regard to the objective of this experiment involve the expression of a circadian rhythm of fattening responsiveness to warm temperature. Fat bodies from anoles exposed to one thermoperiod per experimental light-dark cycle were similar in weight when compared among groups receiving treatments at 24-hour intervals (Figure 5). For example, fat body weights from anoles exposed to WC; 54-60 were similarly increased compared with fat body weights from anoles receiving thermoperiod treatment 24 hours earlier (WC; 30-36) (Figure 5). The depressing effect of the inhibitory thermoperiod treatment on fat stores appeared to be expressed on a 24-hour basis, also. In addition, fat bodies from anoles receiving a 6-hour thermoperiod at 24-hour intervals in relation to the stimulatory thermoperiod (WC; 30-36) on successive days (WC; 6-12, 30-36 and 54-60) were not significantly heavier than fat bodies from anoles exposed to only one stimulatory thermoperiod per LD 12:60 cycle (Table II, Figure 5).

As in Experiment I and in other reports (Dessauer, 1955a, b; Fox, 1958; Licht, 1971), the testes were near maximum
weight during this season (Table I). The only treatment significantly affecting testes weights was WW-LD 12:60 which depressed testes weights compared with all other groups. However, the reliability of this observation is questionable because of the unhealthy conditions of the anoles in this group.
DISCUSSION

Results of this study support the concept that photoperiod-temperature interactions have important effects on the regulation of body fat stores in an ectotherm, A. carolinensis (Noeske and Meier, 1977). The photoperiod entrains a daily rhythm of fattening responsiveness to warm temperatures (30±2°C). Depending on the timing of its occurrence relative to the photoperiod (Noeske and Meier, 1977), a period of warmth can be stimulatory, inhibitory or without consequence to body fat stores in anoles. Similar observations have been reported regarding the influence of photoperiod-temperature interactions on body weight changes in goldfish (Carassius auratus) (Spieler et al., 1977) and changes in gonadal development in anoles (Licht, 1966, 1969a; Noeske and Meier, 1977, and in goldfish (Spieler et al., 1977).

The results also indicate that the photoperiod can set the time of a temperature sensitive phase (thermoinducible phase) which is thereafter expressed on a circadian basis without further entrainment. The occurrence of warm temperature coincident with this thermoinducible phase triggers a physiological event (e.g., fattening). The reduction in body fat stores produced when warm temperature was present during certain time periods relative to the photoperiod (WC; 42-48 and WC; 66-72; Experiments I and II) can be interpreted
similarly. However, this inhibitory effect may have been due in part to the influence of cold temperatures during the thermoinducible phase. For instance, the photoperiod may time the potential for cells involved in lipid synthesis (e.g., liver cells) to respond to warmth with increased lipogenesis, but warm temperature must occur during this period of sensitivity before lipogenesis can take place. Because fat body weights were depressed in anoles receiving the inhibitory thermo-period treatment compared with fat body weights from anoles exposed to constant cold, other factors (i.e., such as lipid mobilization and utilization, appetite) responsive to warmth need to be considered. Warmth present during the inhibitory phase for lipogenesis may actually represent a thermo-stimulatory treatment for lipid mobilization and utilization.

The above explanation for the observed influence of photoperiod-temperature interactions on fat body weights is based on the classical concept of photoperiodism first proposed by Bünning (1936, 1960; see introduction). Noeske and Meier (1977) have suggested two explanations of how photoperiod-thermoperiod interactions may work through the coincidence of circadian systems to regulate physiological mechanisms involved in fattening and gonadal development in anoles. One explanation involves two systems, each having circadian expressions (i.e., hormone rhythms). One system is entrained by the photoperiod (i.e., Corticosterone; Trobec, 1974a), whereas the second system is entrained by the thermoperiod (i.e., prolactin; Spieler et al., 1978). Seasonal
changes in the temporal relations of these two systems produce changes in the temporal relations of endogenous hormone rhythms. The importance of specific temporal relations of hormone rhythms in regulating seasonal metabolic, reproductive and behavioral conditions has been amply demonstrated in members of most vertebrate classes by using daily injections of corticosterone and prolactin in different temporal relations (for review see Meier, 1975). As an alternative explanation, it has been suggested (Noeske and Meier, 1977) that the photoperiod may entrain rhythms of several physiological events, each reaching a maximum potential at a different time of day. The occurrence of temperatures optimal for a particular physiological process (i.e., fat metabolism or gonadal development) coincident with the entrained daily period of potential metabolic activity favoring these processes would produce cumulative results (i.e., increased fat stores and gonadal growth). Results of the present investigation support the first proposition involving interaction of two neuroendocrine circadian oscillations and do not support the alternative explanation for the mechanism by which photoperiod-thermoperiod interactions regulate fat stores in anoles. The cumulative increases in fat body weights from anoles on a LD 12:60 regimen exposed to a stimulatory thermoperiod on successive days were no greater than the increases in body fat stores in anoles exposed to a stimulatory thermoperiod once only during a LD 12:60 cycle (Figure 5). The photoperiod
may set the phase of one neuroendocrine circadian oscillation controlling a daily rhythm of corticosterone in *A. carolinensis* (Trobec, 1974a). Peak levels of plasma corticosterone which occur during a particular time of day appear to entrain a daily rhythm of fattening sensitivity to prolactin, as demonstrated by an injection study in *A. carolinensis* held on LL (Trobec, 1974b). Prolactin administered at the same time as corticosterone stimulated fattening whereas a 4 to 8 hour relation between prolactin and corticosterone injections depressed fat stores. Therefore, a phase shift in a daily rhythm of plasma prolactin with respect to the daily rhythm of plasma corticosterone entrained by the photoperiod might conceivably have important consequences in mechanisms regulating fattening in anoles. Warm temperature reportedly produces an 8-hour phase shift in a daily rhythm of plasma prolactin determined in Gulf Killifish (*Fundulus grandis*) relative to the rhythm found in cold acclimated fish (Spieler et al., 1978).

These data suggest that temperature affects a second neuroendocrine circadian oscillation which controls the daily rhythm of prolactin. Different photoperiod-thermoperiod conditions may produce different temporal relations between the two oscillations. These two oscillations regulate the temporal relation between rhythms of prolactin and corticosterone which in turn affect mechanisms involved in fattening.

A difference is observed in the degree of fattening responsiveness to a particular thermoperiod in comparing
results of both experiments. The increase in fat body weights above initial control values for anoles receiving the same stimulatory thermoperiod treatments was over 2 times greater in Experiment I (.64% of body weight, Table I) than in Experiment II (.28% of body weight, Table II). Approximately one month's time had elapsed between experiments. A shift in the rhythm of fattening responsiveness to warm temperature in anoles maintained under similar photoperiod conditions may have occurred. Such shifts in the daily pattern of testicular growth responsiveness in goldfish (Spieler et al., 1977) and anoles (Noeske and Meier, 1977), body growth responsiveness in goldfish (Spieler et al., 1977) and fattening responsiveness in anoles (Noeske and Meier, 1977) have been reported. For example, testicular growth in anoles maintained under a LD 6:18 regimen was stimulated by an 8-hour period of warmth beginning 8 hours after the onset of light in July but not in October (Noeske and Meier, 1977). These results are significant in that they indicate the presence of an endogenous mechanism modifying the organism's seasonal response to environmental stimuli.

Results of the present investigation indicate that photoperiod-temperature interactions are involved in the regulation of testicular growth in accordance with other reports in A. carolinensis (Licht, 1966, 1967a, b, 1969a; Noeske and Meier, 1977). Depending on the timing of its occurrence relative to the photoperiod (Licht, 1966, 1969a; Noeske and Meier, 1977), a particular thermoperiod may
stimulate, inhibit, or not influence testicular growth. Whether the variation of testicular responsiveness to warm temperature occurs on a circadian basis cannot be determined from the present data (Experiment II). The results, however, do not rule out an explanation based on circadian systems.

The testes of anoles exposed to continuous cold were slightly heavier than initial control values (Table I and II). Cool temperatures have been noted to promote slow gonadal growth in anoles regardless of photoperiod length (Licht, 1967b, Noeske and Meier, 1977). The fact that cold temperatures promoted a somewhat faster rate of testicular growth in anoles maintained on a LD 12:12 regimen than in anoles on a LD 12:60 regimen suggests photoperiod-photoperiod and photoperiod-thermoperiod interactions may be involved in the regulation of gonadal growth. A 12-hour daylength is not considered stimulatory for gonadal recrudescence (Licht, 1969a, 1971) but may be sufficient to allow testicular growth to take place in anoles kept at 20±2°C in April. Testicular growth and spermatogenesis are controlled by separate regulatory mechanisms (Licht, 1967a). The presence of light during a particular time of day may help to accelerate testicular growth promoted by cooler temperatures.

The response of the testes to interactions among environmental cues also depends on the physiological condition of anoles during a particular season (Licht, 1969a). For example, testicular regression occurs in late summer when daylength
falls below 13.5 hours (e.g., 12 hours of light) even though temperatures remain high (Licht, 1969a) whereas the stimulatory effect of warm temperature on testicular recrudescence in spring is not influenced by daylengths of 12 hours (Licht, 1971). Because testicular responsiveness to environmental cues depends on the physiological condition, which changes seasonally in anoles, the difference between testicular growth responses to similar photoperiod-thermoperiod treatments observed in Experiments I and II were expected in that these experiments were carried out approximately a month apart.

In summary, results of the present study confirm reports that fattening and testicular growth in anoles are responsive to photoperiod-thermoperiod interactions. Warm temperature can be stimulatory, inhibitory, or without effect on body fat stores and testicular growth depending on the timing of its occurrence relative to the daily photoperiod. The photoperiod entrains a daily rhythm of fattening responsiveness to warm temperature. Furthermore, the results of the present study indicate that once entrained by the photoperiod the daily pattern of fattening responsiveness to warmth is expressed on a circadian basis without additional photoperiodic entrainment. In addition, the data on fattening supports the hypothesis regarding photoperiod-thermoperiod interactions based on two circadian oscillations. One oscillation is synchronized by the photoperiod and the other influenced by the thermoperiod. Changing interactions between these two
oscillations represent a mechanism whereby seasonal conditions of fattening are regulated. The present investigations emphasize the importance of a chronobiological approach in physiological investigations. Although not considered herein, the implication that the behavior and ecology of a species have for photoperiod-thermoperiod interactions influencing regulatory mechanisms offer a wide array of research opportunities.
Part IB: Thyroxine and the Fattening Response to Warm Temperature in the Green Anole, Anolis carolinensis
Anolis carolinensis undergo a period of inactivity (hibernation) in winter when environmental conditions are unfavorable for foraging and appetites are low (Dessauer, 1955a, b; Fox and Dessauer, 1957; Licht and Jones, 1967). Previously accumulated fat stores serve to meet the energy requirements during this period. Most of this fat is stored in two discrete fat bodies situated caudally within the coelomic cavity (Dessauer, 1955a). Accumulation of fat begins during mid to late summer; the fat bodies reach peak weights in October or November (Dessauer, 1955a, b; Fox and Dessauer, 1957). Anoles are exposed to periods of warm temperatures and shortening daylengths during this time. These conditions are considered favorable for testicular regression (Licht, 1971); their effects on body fat stores have been considered secondarily.

In a recent series of experiments (Noeske and Meier, 1977; Noeske, unpublished) differences in fat body weights in response to specific thermoperiods were found in anoles held on various photoperiod regimens (LD 13:11; LD 6:18). Whether fat bodies are heavier or lighter in weight appears to depend on the time of day anoles experience the warmer temperature (30±2°C) (Noeske and Meier, 1977; see part IA). In addition, anoles maintained under continuous warm temperature (30±2°C) had heavier fat bodies than anoles exposed to continuous cold (20±2°C) at all seasons tested
Continuous warm temperature treatment promoted fattening in anoles kept under various photoperiodic conditions when tested during the spring and summer months (Licht, 1971). These results are similar to the findings of Licht and coworkers (1969) in two species of European lizards. They observed that exposure to continuous warmth (33°C) stimulated a faster rate of fat accumulation than exposure to constant cold (20-24°C) in both species of *Lacerta* regardless of whether the photoperiod was long (LD 16:8) or short (LD 8:16).

The magnitude of the differences in fat body weights of anoles held at 20°C and 30°C varied seasonally (Noeske and Meier, 1977), and indicated the presence of a circannual mechanism regulating fat stores. The difference observed during late summer, a time when fat accumulation occurs in nature (Dessauer, 1955a, b; Fox and Dessauer, 1957) was almost 2 times greater than the difference in October, a time when fat stores are maximal. Because values for initial controls were not included, it is impossible to assess whether warm temperature stimulated fat accumulation or cold treatment depressed body fat stores. Interestingly, continuous cold (20°C) rather than continuous warmth (30°C) promoted heavier fat stores in anoles maintained in constant light conditions in October (Noeske, T. 1974. The effects of photoperiod and thermoperiod on the reproductive cycle of the male green anole, *Anolis carolinensis*. M.S. Thesis. LSU.) This response is opposite that noted in anoles held under light-
dark cycles.

The stimulatory influence of constant warmth on body fat stores in *A. carolinensis* (Licht, 1971; Noeske and Meier, 1977) and in other lizard species (Licht et al., 1969) may involve thyroxine in that the activity of this hormone is temperature-dependent in ectothermic vertebrates, including anoles. Perhaps the most studied thyroxine activity in this regard is oxygen consumption which is indicative of an animal's metabolic rate. Magnus-Levy (1895) first demonstrated the now familiar stimulatory influence of thyroxine on oxygen consumption in mammals. Such a simple relationship was not evident in early experiments in ectothermic vertebrates. Thyroid treatment failed to elicit a rise in oxygen consumption in teleosts (Matty, 1957; see Pickford and Atz, 1957) and amphibians (Henschel and Steuber, 1931). On the other hand, many experiments indicate that thyroxine administration does stimulate increased oxygen consumption in fishes (Smith and Matthews, 1948; Müller, 1953; see Pickford and Atz, 1957), amphibians (Taylor, 1939; Warren, 1940; Maher, 1967; Packard and Packard, 1973; Packard et al., 1974; May and Packer, 1976) and reptiles (Maher and Levedahl, 1959; Maher, 1961, 1964, 1965; Wilhoft, 1965, 1966a; Turner and Tipton, 1972; Chandola et al., 1974). The possibility that temperatures to which experimental animals were acclimated might influence thyroxine's effect on oxygen consumption was considered in explaining the observed discrepancy in experimental results (Smith, 1953; Pickford and Atz, 1957; Maher and Levedahl, 1959). Maher and Levedahl (1959) were the first to demonstrate experimentally
in A. carolinensis that body temperature must be elevated above a critical level for thyroxine to stimulate oxygen consumption. Thyroxine treatment stimulated oxygen consumption in anoles maintained at preferred body temperatures (28° or 30°C) but not at 20°C (Maher and Levedahl, 1959; Maher, 1961). Temperature appears to regulate the influence of thyroxine on nitrogen metabolism in fish (Ray and Medda, 1976), as well as nitrogen metabolism (Ashley et al., 1968; McNabb, 1969), carbohydrate metabolism (Smith, 1953; McNabb, 1969) and metamorphosis (Frieden, 1964, 1967) in amphibians. A correlation between warm temperature and thyroid gland activity based on histological evidence from a variety of lizard species (Wilhoft, 1958, 1964; Lynn, 1960; Lynn et al., 1965; Walker, 1973) further suggests that thyroxine is important in physiological events enhanced by warm temperature. Because the stimulatory influence of warm temperature on body fat stores in anoles (Noeske, 1974; Noeske and Meier, 1977) was similar to the thyroxine mediated influence of warm temperature on metabolic rate in anoles (Maher and Levedahl, 1959), a series of experiments was designed to determine if and how thyroxine may be involved in the warm temperature influence on fat metabolism.

Thyroid hormones are important in lipid metabolism (see figure 6). In endothermic vertebrates thyroxine enhances the influence of other hormones on (Goodridge, 1975; Lefebvre, 1975) or directly stimulates (Goodridge, 1978; review; Bernal and Refetoff, 1977) liver lipogenesis, perhaps by inducing
Figure 6

Sites where experimental evidence indicates thyroxine (T4) has stimulatory (+) or inhibitory (-) effects on processes (e.g., the second messenger, cyclic adenosine monophosphate, C-AMP, mode of hormone action) regulating fat metabolism in liver (lipid synthesis) and fat (fat mobilization) cells.
the production of enzymes (i.e. Acetyl-CoA carboxylase and malic enzyme) involved in lipid biosynthesis (Goodridge, 1975; Li et al., 1975; Roncari and Murthy, 1975; review; Bernal and Refetoff, 1977). Thyroid hormones enhance fat mobilization from adipose tissue in a number of endothermic vertebrates (Goodman and Bray, 1966; Fisher and Ball, 1967; reviews; Meier and Burns, 1976; Bernal and Refetoff, 1977). Thyroid hormones may suppress the activity of cyclic-AMP specific phosphodiesterase (Lefebvre, 1975; Bernal and Refetoff, 1977) or augment the activity of adenyl cyclase (Krishna et al., 1968) thereby enhancing the activity of other hormones (e.g. glucagon) which promote lipolysis in adipose tissue via stimulation of cyclic AMP production. The amount of fat stored represents the net result of fat biosynthesis and deposition minus fat mobilization and utilization. Because thyroxine effects the rates of both fat biosynthesis and mobilization, important factors involved in determining net fat storage, two experiments were conducted exploring the possible sites (i.e., liver and/or fat bodies) at which thyroxine influences fattening stimulated by warm temperature in anoles.

Thyroxine treatment can entrain a rhythm of fattening responsiveness to prolactin in at least one ectotherm (Meier, 1970). Prolactin injections made 18 hours after thyroxine injections stimulated fattening in the Golden Topminnow (Fundulus chrysotus kept in LL. A 6-hour relationship
in hormone treatments did not result in fattening. In addition, reports of studies in pigeons held on LL (John et al., 1972) indicate thyroxine may be necessary for the expression of a rhythm of fattening responsiveness to prolactin. A daily rhythm of fattening response to prolactin which had dampened out in control birds persisted in pigeons receiving thyroxine treatment. Prolactin plays an important role in fat metabolism in vertebrates studied (see Meier and Burns, 1976) including A. carolinensis (Trobec, 1974b). Therefore, one experiment was carried out to investigate the possibility that an interaction between thyroxine and prolactin is involved in regulating lipid metabolism.
MATERIALS AND METHODS

Large male anoles were obtained, housed and handled according to procedures detailed previously (Part IA).

Experiment I was initiated 1 December, 1977 when the shipment of anoles arrived from a biological supply house. Anoles in Group 1 (initial group; 1) were killed on 4 December and body, paired testes and fat body weights determined. The remaining anoles were held at 20+2°C (CC) for one week to allow for acclimation to the experimental LD 6:18 regimen. Light was present from 0930 to 1530 hours. After a 1-week acclimation period, Groups 2 (control; 1 week; CC) and 3 (thiouracil-treated (TU): 1 week; CC-TU) were killed and body, gonad and fat body weights determined. After determining body weights the remaining 2 groups (Groups 4; WW and 5; WW-TU) were transferred to an incubator set at 30+2°C (WW) for the remaining 2 weeks. Anoles in Groups 3 an 5 received thiouracil (.08% 2-thiouracil; Sigma Chemical Co.) treatment via their drinking water beginning 4 December. Although the exact dosage received by each animal could not be determined, it was felt that this treatment provided dosages within the range produced by injections shown to have a goitrogenous effect in A. carolinensis (Adams and Craig, 1949, 1951; Ratzersdorfer et al., 1949). Dosages used in this and subsequent experiments were not toxic based on a comparison of mortality rates in this study with
mortality rates in a study investigating the dose response of the thyroid to thiouracil and thiourea treatments in A. carolinensis (Adams and Craig, 1951). Injections were avoided in order to prevent possible entraining influences of handling. Timed daily disturbances (i.e., normal handling) can produce profound physiological changes affecting fat stores in vertebrates (Meier et al., 1972; Horseman et al., 1976) particularly in anoles (Meier et al., 1972). Anoles in Groups 2 and 4 remained untreated and served as controls for Groups 3 and 5, respectively.

Experiment II was initiated 7 April, 1978. Anoles received from a supply house on this date were held for one week in CC while acclimating to a LD 13:11 regimen. Thiouracil treatment (TU) (see Experiment I) was initiated 8 April in order to produce hypothyroidism before experimental treatments were initiated. Goitrogenous effects are not detectable in the thyroid of A. carolinensis until 7 days after initiating thiouracil or thiourea treatments (Ratzersdorfer et al., 1949; Adams and Craig, 1951). Anoles in Group 1 (initial group; I) were killed and weight determinations made 15 April. Thyroxine treatment (0.001% Na\(^{+}\)-L-thyroxine in the drinking water; T4) was initiated on this date. Initial body weights were determined and the following treatments carried out. Groups 2, 3 and 4 were kept in WW and Groups 5, 6 and 7 remained in CC. Groups 2 and 5 were left untreated. Groups 3, 4, 6 and 7 received 2-thiouracil treatment (TU) in their drinking water. In addition, Groups 4 and 7 were treated with thyroxine (T4).
Experiment III, involving 12 groups of anoles captured near Baton Rouge on 2, 3 and 4 June, 1978, was initiated on 5 June and terminated 14 June. All anoles were maintained on a LD 12:12 photoperiodic regimen. Six groups were kept in CC and the rest in WW. Crickets (food) were available at all times until removal at the offset of light 11 June. $^{14}$C-acetate (1μCi/.02 cc of .67% saline/anoles) was injected intraperitoneally 15 minutes before the anoles were frozen in an acetone bath chilled with dry ice. This time interval was sufficient for measurable quantities of $^{14}$C-acetate to be incorporated into liver and fat body lipids which were extracted in a preliminary test of the extraction technique (Folch et al., 1957). A Hamilton syringe volume regulator was used in making injections and proved especially helpful in maintaining equal injection volumes during the dark period. One group of anoles from each temperature treatment was killed at 4-hour intervals throughout the day of 14 June beginning at 0700 hours (onset of light). Frozen anoles were weighed and their livers and fat bodies removed, weighed and packed in aluminum foil over ice as quickly as possible where they remained until lipid extraction was initiated less than an hour later. The extracted lipids from livers and fat bodies were poured into separate pre-weighed scintillation vials. The chloroform/methanol extraction solution was evaporated under a hood and the vial plus dried lipid weight determined. Liver and fat body lipid weights were determined
and the lipids were resuspended in 5 milliliters of scintillation cocktail. The amount of $^{14}$C-acetate incorporation was counted in the $^{14}$C channel of an LS8000 Beckman Scintillation counter.

Experiment IV was initiated simultaneously with Experiment III on 6 June, 1978. Anoles received from a supply house 4 June were kept in WW and LD 12:12. Six groups were established. Two groups (1500 and 1900 hours) remained untreated (controls). The remainder received .1% thiourea (TU) in the drinking water beginning 6 June. Thiourea is an effective goitrogenic agent in _A. carolinensis_ (Ratzersdorfer _et al._, 1949; Adams and Craig, 1951) with the advantage that it dissolves readily in water. Thyroxine (Na$^+$-L-thyroxine; Sigma Chemical Co.) was administered in a novel way, not only to avoid entrainment produced by injections (Meier _et al._, 1972), but to produce more consistent dosage rates as well. Thyroxine was dissolved in ethanol (0.5 µg Na$^+$-L-thyroxine/5 µl of ethanol). Five microliters (0.5 µg Na$^+$-L-thyroxine) were placed between the cerci of crickets (4th week _Acheta domesticus_) using a 5 µl capillary pipette. The ethanol was evaporated and the thyroxine laden crickets were fed to anoles in Group 3. A dosage of .1 µg T4/gram of body weight is effective in stimulating oxygen consumption in _A. carolinensis_ without producing toxic effects (Maher and Levedahl, 1959). Assuming anoles (5 grams approximately) eat approximately one treated cricket per day (the amount provided/day), an appropriate dosage was approximated. No
ill effects were noted in anoles receiving thyroxine. Nervous tremors are obvious in anoles treated with toxic levels of thyroxine (Maher and Levedahl, 1959). Thyroxine was administered to 2 groups (1500 and 1900 hours; TU + T4) of thiourea-treated anoles beginning 12 June.

Crickets were removed two days prior to $^{14}$C-acetate injections on 18 June. Injections and subsequent determinations of $^{14}$C-acetate incorporation into liver and fat body lipids were carried out as described previously (see Experiment III). One group of each treatment type was sacrificed at 1500 hours (8 hours after light onset) and again at 1900 hours; times when acetate incorporation into fat body lipids were low and high, respectively (see results of Experiment III).

Experiment V was carried out using anoles captured locally beginning 2 July, 1978. All anoles were held in CC during acclimation to LD 12:12 regimen. Onset of light occurred at 0700 hours. At the end of one week four groups (Groups 5, 6, 7 and 8) were transferred to incubators set at 30±2°C (WW). Three groups (Groups 2, 3, and 4) remained in incubators set at 20±2°C (CC). Lizards in Group 1 (I) were killed at this time and body, paired testes and fat body weights determined. Groups 2 (FC-CC) and 5 (FC-WW) served as untreated final controls (FC), Groups 3 (PRL-CC), 4 (PRL-T4), 6 (PRL-WW) 7 (PRL-TU), and 8 (PRL-TU-T4) received subcutaneous daily injections of prolactin (5 μg NIH sheep prolactin/.02 cc of .67% saline/anole; PRL) 16 hours after the onset of light for
6 days beginning 13 July, 1978. Prolactin injections given in this relation to a 12-hour photoperiod stimulated body fat stores in *A. carolinensis* when tested at this season (Trobec, 1974a; preliminary test). Thiourea treatment (.1% in the drinking water) was initiated 2 July. Anoles in groups 4 and 8 received thyroxine treatments (see Experiment III) beginning 10 July. The effects of drug and hormone treatments on plasma thyroxine levels were determined in Groups 4, 7, 8 and 9 using a radioimmunoassay technique (Mitsuma et al., 1972; see Part II A).

Experiment VI was initiated 11 December, 1977. Anoles received from a supply house were divided equally into two groups. Anoles in Group 1 (I) were killed 13 December. The remaining group was held in WW under continuous light (LL) until 21 December when anoles were killed.

In Experiment VII, anoles captured near Baton Rouge 2 July 1978 were placed under continuous light (LL) on 4 July. Initial body weights were determined on 4 July and anoles in Group 1 (I) were killed. Anoles in Group 2 (LL:WW) were held in incubators set at 30±2°C. A third group (LL:CC) of anoles were maintained in CC. This experiment was terminated on 19 July.
RESULTS

Experiment I was a preliminary exploration into the possibility that thyroid hormones are involved in the influence of warm temperatures on body fat stores in \( A. \) carolinensis. Data on the effects of antithyroid drug (thiouracil) treatment on fat body and paired testes weights are presented in Table III and Figure 7. Initial testicular weights (I) are similar to those previously reported for anoles during late fall (Dessauer, 1955a, b; Fox, 1958; Licht, 1967a, 1971; Noeske and Meier, 1977). Testes undergo a slow steady recrudescence in anoles studied in late fall when temperatures remain low (Licht, 1967b; 1969a). Fat body weights in December (I; Table III, Figure 7) are near maximal in accordance with other reports (Dessauer, 1955a, b; Licht, 1967a). Thus the condition of anoles received by shipment from a supply house, in this and subsequent experiments, appear to be representative of conditions in nature. It was learned that anoles were captured just prior to shipment. Acclimation to experimental photoperiodic conditions (LD 6:18) for one week in CC did not influence body fat stores or testicular weights (CC and CC-TU; Figure 7).

Fat body weights of anoles which received thiouracil treatment were depressed compared with weights of controls in WW. Under a LD 6:18 regimen (Table III; Figure 7) WW did
TABLE III

Experiment I. The effect of 2-thiouracil treatment on fat body and paired testes weights in A. carolinensis held at 30±2°C and LD 6:18. This experiment was initiated 1 December, 1977. Warm temperature treatment lasted 2 weeks beginning 8 December. Anoles were kept at 20±2°C and LD 6:18 for 1 week prior to warm temperature treatment.

<table>
<thead>
<tr>
<th>GRP (N)</th>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Paired Testes Weights (mg)</th>
<th>Fat Bodies (% Body Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>4.56±.24°C</td>
<td>34±4°C</td>
<td>2.60±.23°C</td>
</tr>
<tr>
<td>2</td>
<td>CC</td>
<td>4.31±.21X</td>
<td>28±3X</td>
<td>2.29±.23Y</td>
</tr>
<tr>
<td>3</td>
<td>CC-TU</td>
<td>4.43±.27X</td>
<td>34±2X</td>
<td>2.41±.23Y</td>
</tr>
<tr>
<td>4</td>
<td>WW</td>
<td>4.77±.23X</td>
<td>48±4Y</td>
<td>2.47±.41Y</td>
</tr>
<tr>
<td>5</td>
<td>WW-TU</td>
<td>4.63±.22X</td>
<td>50±4Y</td>
<td>1.28±.08X</td>
</tr>
</tbody>
</table>

a The number (N) of anoles in each group (GRP).

b Treatment symbols: I = initial group; CC= constant 20±2°C; WW= constant 30±2°C; TU= 2-thiouracil.

c Mean weight ± one standard error about the mean.

x,y Ranking of treatment means using the Student-Newman-Keuls procedure. Treatment means without a letter in common are statistically different at the 95% confidence level.
Paired testes and fat body weight responses to constant 30±2°C (WW) or 20±2°C (CC) in anoles kept under LD 6:18 and the influence of 2-thiouracil treatment on these responses. Experiment I was conducted for 3 weeks beginning 1 December, 1977. One group of anoles (I) was killed before temperature treatments were initiated. Treatment means were ranked using the Student-Newman-Keuls procedure. Bars (treatment means) without hatching running in the same direction are statistically different at the 95% confidence level. S.E.M= one standard error about the mean.
not stimulate fattening however. Testicular weights were heavier in both thiouracil-treated and untreated anoles in response to WW (Table III; Figure 7). The dosage of thiouracil used (0.08%) was not toxic in that a similar low mortality ratio was observed in treated and untreated anoles (Table III). Toxic dosages of thiouracil produce high mortality rates in anoles treated only several days (Adams and Craig, 1951).

Experiment II was carried out to explore further the possibility that thyroxine is involved in warm temperature effects on fat body and testicular weights in anoles. Data are summarized in Table IV and depicted in Figure 8. Body fat stores increased in response to WW compared with fat stores in the initial group (Figure 8). Fat stores were not changed in response to CC. Fat store increases produced in response to WW were not observed in thiouracil-treated animals (Table IV and Figure 8). Thyroxine treatment (0.001% in the drinking water) did not restore fattening blocked by thiouracil treatment (Table IV), perhaps because toxic effects were produced by hormone treatment. Several anoles in the thyroxine-treated group were unable to coordinate body movements when prodded; symptoms reported for anoles treated with toxic levels of thyroxine (Maher and Levedahl, 1959). Thiouracil or thiouracil plus thyroxine treatments did not produce changes in fat body weights compared with fat body weights of untreated anoles exposed to CC (Table IV).
TABLE IV

Experiment II. Effect of 2-thiouracil or 2-thiouracil plus thyroxine treatment on the fattening and testicular growth responses to constant 30+2°C or 20+2°C in anoles held under LD 13:11. Experimental conditions were initiated 7 April, 1978.

<table>
<thead>
<tr>
<th>GRP</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
<th>Paired Testes Weights (mg)</th>
<th>Fat Bodies (% Body Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 7</td>
<td>5.14+.26b</td>
<td>73+3b</td>
<td>.41+.11b</td>
<td></td>
</tr>
<tr>
<td>WW 6</td>
<td>5.76+.14b</td>
<td>87+7</td>
<td>1.46+.16c, x</td>
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</tr>
<tr>
<td>WW-TU 7</td>
<td>5.59+.25</td>
<td>75+3</td>
<td>.37+.16 y</td>
<td></td>
</tr>
<tr>
<td>WW-TU -T4</td>
<td>5.53+.32</td>
<td>75+4</td>
<td>.64+.12 y</td>
<td></td>
</tr>
<tr>
<td>CC 7</td>
<td>5.44+.28</td>
<td>98+5c</td>
<td>.46+.10</td>
<td></td>
</tr>
<tr>
<td>CC-TU 6</td>
<td>5.79+.27</td>
<td>95+10</td>
<td>.28+.08</td>
<td></td>
</tr>
<tr>
<td>CC-TU -T4</td>
<td>5.68+.20</td>
<td>99+9</td>
<td>.46+.16</td>
<td></td>
</tr>
</tbody>
</table>

a The number (N) of anoles in each group (GRP). Treatment symbols: I = initial group; WW = constant 30+2°C; CC = constant 20+2°C; TU = 2-thiouracil; T4 = thyroxine.

b Mean weight ± one standard error about the mean.

c Statistical difference detected using Students "t" test comparing control means (WW, CC) with means of the initial group.

x, y Ranking of treatment means within temperature treatment groups using the Student-Newman-Keuls procedure. Means without a letter in common are statistically different at the 95% confidence level.
Figure 8

The influence of 2-thiouracil treatment (TU) on paired testes and fat body weight responses to constant 30 ± 2°C or 20 ± 2°C in anoles kept under LD 13:11. Experiment II was initiated 7 April, 1978. One group (I) was killed before experimental treatments were initiated. S.E.M. = one standard error about the mean.
Testicular weights were increased in anoles kept in CC compared with initial testes weights (Table IV). Testicular weights in anoles kept in WW were in between values of initial testes weights and testes weights in anoles kept in CC. Drug treatment alone or with thyroxine did not appear to influence testicular weights in anoles held in WW and CC (Table IV).

Experiment III was performed to explore the influence of WW or CC on fat metabolism. Although a similar experiment had been conducted previously (Noeske, unpublished), it was conducted to provide background information necessary to assess if and at what sites (i.e. liver and/or fat bodies) thyroxine is involved in warm temperature effects on lipid metabolism (Experiment IV). Data on $^{14}$C-acetate incorporation into liver (Figure 9) and fat body (Figure 10) lipids are summarized in Table V.

A unimodal daily variation in $^{14}$C-acetate incorporation into liver lipids was found in anoles maintained in CC (Figure 9). $^{14}$C-acetate incorporation into liver lipid was highest at 1500 and lowest at 1100 hours. The daily pattern of $^{14}$C-acetate incorporation into liver lipid appeared to be bimodal in anoles kept in WW (Figure 9). $^{14}$C-acetate incorporation into liver lipid was highest at 0700 hours (light onset) in anoles kept in WW; almost double the amount of incorporation at this time in anoles kept in CC (Figure 9). Following a decrease at 1100, $^{14}$C acetate incorporation into liver lipid appeared to be increased during the afternoon.
TABLE V

Experiment III. $^{14}$C-acetate incorporation into liver and fat body lipid measured at 6 times of day in *A. carolinensis* kept at 30±2°C or 20±2°C under LD 12:12. Experimental conditions were initiated 6 June, 1978.

<table>
<thead>
<tr>
<th>Time (a)</th>
<th>(N)</th>
<th>Body Weight (g)</th>
<th>Liver (% Body Wt.)</th>
<th>DPM/ Liver</th>
<th>Liver Lipids (mg)</th>
<th>Fat Bodies (% Body Wt.)</th>
<th>DPM per Fat Bodies</th>
<th>Fat Bodies Lipid (mg)</th>
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<tr>
<td>0700</td>
<td>6</td>
<td>4.63±.26</td>
<td>2.7±.3</td>
<td>2669±805</td>
<td>7±2</td>
<td>.15±.03</td>
<td>505±139</td>
<td>4±1</td>
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<td>1100</td>
<td>6</td>
<td>5.39±.26</td>
<td>2.8±.2</td>
<td>1309±339</td>
<td>6±1</td>
<td>.14±.05</td>
<td>251±43</td>
<td>2±1</td>
</tr>
<tr>
<td>1500</td>
<td>6</td>
<td>6.48±.40</td>
<td>3.8±.2</td>
<td>5172±632</td>
<td>16±2</td>
<td>.23±.06</td>
<td>2761±778</td>
<td>9±3</td>
</tr>
<tr>
<td>1900</td>
<td>6</td>
<td>5.22±.31</td>
<td>2.8±.2</td>
<td>3248±761</td>
<td>12±1</td>
<td>.16±.10</td>
<td>440±49</td>
<td>4±2</td>
</tr>
<tr>
<td>2300</td>
<td>7</td>
<td>6.18±.24</td>
<td>3.1±.2</td>
<td>3496±406</td>
<td>8±2</td>
<td>.33±.13</td>
<td>962±217</td>
<td>5±2</td>
</tr>
<tr>
<td>0300</td>
<td>7</td>
<td>5.40±.42</td>
<td>3.4±.3</td>
<td>2307±399</td>
<td>12±2</td>
<td>.32±.13</td>
<td>435±110</td>
<td>12±5</td>
</tr>
</tbody>
</table>

ANOVA (d) P<.05 P<.05 P<.05 P<.05 N.S. P<.001 N.S.

(a) Time of day organs were removed and weights determined.
(b) The number (N) of anoles in each group.
(c) Mean weight ± one standard error about the mean.
(d) Analysis of variance among treatment groups: N.S. = no statistical differences at the 95% confidence level.

Symbols: DPM = disintegrations per minute.
TABLE V - continued

30±2°C

<table>
<thead>
<tr>
<th>Time</th>
<th>(N)</th>
<th>Body Weight (g)</th>
<th>Liver (% Body Wt.)</th>
<th>DPM/Liver</th>
<th>Liver Lipid (mg)</th>
<th>Fat Bodies (% Body Wt.)</th>
<th>DPM per Fat Bodies</th>
<th>Fat Body Lipid (mg)</th>
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<tr>
<td>0700</td>
<td>6</td>
<td>5.30±.34</td>
<td>3.9±.2</td>
<td>4997±825</td>
<td>15±2</td>
<td>.93±.15</td>
<td>2714±599</td>
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<tr>
<td>1100</td>
<td>8</td>
<td>5.41±.28</td>
<td>4.0±.2</td>
<td>2889±423</td>
<td>12±4</td>
<td>.81±.20</td>
<td>2176±650</td>
<td>17±7</td>
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<tr>
<td>1500</td>
<td>8</td>
<td>4.92±.34</td>
<td>3.8±.1</td>
<td>4070±579</td>
<td>9±2</td>
<td>.71±.16</td>
<td>1982±425</td>
<td>12±4</td>
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<tr>
<td>1900</td>
<td>7</td>
<td>5.68±.19</td>
<td>3.2±.2</td>
<td>3818±401</td>
<td>17±3</td>
<td>1.21±.15</td>
<td>4161±510</td>
<td>31±6</td>
</tr>
<tr>
<td>2300</td>
<td>6</td>
<td>4.68±.18</td>
<td>4.0±.3</td>
<td>4234±943</td>
<td>13±4</td>
<td>1.10±.26</td>
<td>1117±172</td>
<td>22±10</td>
</tr>
<tr>
<td>0300</td>
<td>7</td>
<td>5.31±.23</td>
<td>4.1±.2</td>
<td>2027±258</td>
<td>18±2</td>
<td>1.25±.21</td>
<td>2090±643</td>
<td>39±6</td>
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</table>

**ANOVA**

<table>
<thead>
<tr>
<th></th>
<th>N.S.</th>
<th>P&lt;.05</th>
<th>N.S.</th>
<th>N.S.</th>
<th>P&lt;.05</th>
<th>N.S.</th>
</tr>
</thead>
</table>

Student "t"^e^ | P<.001 | P<.07 | P<.05 | P<.001 | P<.001 | P<.001 |

^a,b,c,d^  
See legend on previous portion of this table.

^e^  
Statistical difference detected using Student's "t" test comparing the average of means for all sampling times between temperature treatments.
Figure 9:

Effects of constant 30±2°C (WW) or 20±2°C (CC) on the daily pattern of 14C-acetate incorporation into liver lipid. (DPM = disintegrations per minute). Anoles were held under light (L: unshaded portion of the lower bar)- dark (D: shaded portion) 12:12 for 7 days. Livers were removed 15 minutes after 14C-acetate injections at 4-hour intervals throughout a 24-hour period beginning at 0700 hours (onset of light) on 14 June, 1978. S.E.M. = one standard error about the mean.
DPM $\times 10^3$/LIVER

TIME (hours)
0700
1100
1500
1900
2300
0300

JUNE

WWW
CC
S.E.M.
Figure 10

Effects of constant $30+2^\circ C$ (WW) or $20+2^\circ C$ (CC) on the daily pattern of $^{14}C$-acetate incorporation into fat body lipid. (DPM = disintegrations per minute). Anoles were maintained and fat bodies were removed following the procedures mentioned in the legend of Figure 9.
$^{14}$C-acetate incorporation into liver lipid was reduced at 0300 hours in anoles from both temperatures. Total acetate incorporation into liver lipid appeared greater in anoles kept in WW than in CC ($P < .07$).

Similar unimodal daily patterns of $^{14}$C-acetate incorporation into fat body lipids were determined in anoles maintained in CC or WW (Figure 10). However, the daily pattern of incorporation in anoles kept in WW appeared to be delayed 4 hours compared with the pattern in anoles kept in CC (Figure 10). Peak levels of incorporation into fat body lipid were reached at 1500 and 1900 in anoles kept in CC and WW, respectively. In addition, the daily level of $^{14}$C-acetate incorporation into fat body lipid was greater in anoles maintained in WW. This difference in the levels of acetate incorporation is reflected in the difference observed between fat body weights (Table V).

Experiment IV was conducted to further explore the possibility that thyroxine is involved in the fattening response to warm temperature. In addition, the influence of thyroxine on liver lipogenesis and fat storage was investigated. This experiment was carried out in conjunction with Experiment III so that comparisons of results are possible. Liver and fat body weights in anoles from control groups (1500 and 1900 hours) were similar to those determined in anoles from comparable groups in Experiment III (Tables V and VI) indicating a similar acclimation response to experimental conditions (WW; LD 12:12). In addition,
TABLE VI

Experiment IV. The effect of thiourea (TU) or thiourea plus thyroxine (TU-T4) treatments on $^{14}$C-acetate incorporation into liver and fat body lipid in A. carolinensis kept at 30±2°C and LD 12:12. Experiment IV was initiated coincident with the onset of Experiment III on 6 June, 1978.

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Liver Weight (% Body Wt.)</th>
<th>DPM/Liver (mg)</th>
<th>Liver Lipid (mg)</th>
<th>Fat Bodies (% Body Wt.)</th>
<th>DPM per Fat Bodies (mg)</th>
<th>Fat Bodies Lipid (mg)</th>
</tr>
</thead>
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<tr>
<td>1500 hours</td>
<td>Control</td>
<td>5.13±.31</td>
<td>4.0±.2</td>
<td>5726±1056</td>
<td>13±3</td>
<td>.72±.16</td>
<td>1347±338</td>
<td>14±5</td>
</tr>
<tr>
<td></td>
<td>TU</td>
<td>5.36±.30</td>
<td>3.7±.3</td>
<td>3073±897</td>
<td>13±2</td>
<td>.31±.16</td>
<td>436±74</td>
<td>12±7</td>
</tr>
<tr>
<td></td>
<td>TU-T4</td>
<td>5.03±.33</td>
<td>4.4±.2</td>
<td>4353±577</td>
<td>15±3</td>
<td>.72±.19</td>
<td>1409±173</td>
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</table>

1900 hours

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Liver Weight (% Body Wt.)</th>
<th>DPM/Liver (mg)</th>
<th>Liver Lipid (mg)</th>
<th>Fat Bodies (% Body Wt.)</th>
<th>DPM per Fat Bodies (mg)</th>
<th>Fat Bodies Lipid (mg)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>4.82±.31</td>
<td>3.7±.3</td>
<td>4273±650</td>
<td>16±4</td>
<td>.92±.40</td>
<td>2395±697</td>
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<td></td>
<td>TU</td>
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<td></td>
<td>TU-T4</td>
<td>5.74±.20</td>
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<td>3760±591</td>
<td>20±4</td>
<td>1.02±.17</td>
<td>1700±208</td>
<td>25±5</td>
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</table>

a Time of day organs were removed and weights determined.
b The number (N) of anoles in each group.
c Mean weight ± one standard error about the mean.
x,y Ranking of treatment means within a sampling period using the Student-Newman-Keuls procedure. Means without a letter in common are statistically different at the 95% confidence level.
14C-acetate incorporation into fat body lipid was elevated at 1900 hours compared with that at 1500 hours (Table VI, P<.2). These results are similar to the trend observed previously (Experiment III, Table V).

Thiourea treatment depressed 14C-acetate incorporation into fat body lipid when measured at 1500 or 1900 hours (Figure 11). Thyroxine treatment restored 14C-acetate incorporation in thiourea treated anoles (Figure 11). Incorporation of 14C-acetate into liver lipids was not significantly altered by thiourea or thiourea plus thyroxine treatments (Table VI).

Experiment V was designed to investigate the possibility that an interaction between prolactin and thyroxine is important in regulating fat stores in A. carolinensis. Results of this experiment are presented in Table VII and Figure 12. Fat bodies from anoles kept in WW were heavier than fat bodies obtained before experimental treatment (P<.05) (I) or from anoles kept in CC (Figure 12). Prolactin injections (CC-PRL; WW-PRL) did not stimulate fattening above the response elicited by temperature alone (CC; WW) (Figure 12). Fat body weights in thiourea-treated anoles were lower than those in untreated anoles kept in WW whereas, fat bodies in thiourea-treated anoles receiving thyroxine treatment were similar in weight to those in untreated anoles (Figure 12). Fat body weights were not different in thyroxine-treated and untreated anoles kept in CC (Table VII). In anoles kept in WW, plasma
Figure 11

The effect of thiourea (TU) or thiourea plus thyroxine (TU-T4) treatments on $^{14}$C-acetate incorporation (DPM = disintegrations per minute) into fat body lipid in *A. carolinensis* kept at 30±2°C and LD 12:12. Treatment means were ranked using the Student-Newman-Keuls procedure. Bars (treatment means) without hatching running in the same direction are statistically different at the 95% confidence level.
Experiment V. The effect of prolactin, thyroxine and thiourea given alone or in combinations on fat body and paired testes weights in A. carolinensis held at 20±2°C or 30±2°C and LD 12:12. Experiment V was initiated 2 July, 1978.

<table>
<thead>
<tr>
<th>GRP(N)a</th>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Paired Testes Weights (mg)</th>
<th>Fat Bodies (%) Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 7 I</td>
<td>CC</td>
<td>4.79±.36c</td>
<td>68±3c</td>
<td>.19±.09c,e</td>
</tr>
<tr>
<td>2 8 FC</td>
<td></td>
<td>4.44±.22</td>
<td>72±3</td>
<td>.22±.10e</td>
</tr>
<tr>
<td>3 8 PRL</td>
<td></td>
<td>4.90±.17</td>
<td>68±4</td>
<td>.42±.11</td>
</tr>
<tr>
<td>4 8 PRL-T4</td>
<td></td>
<td>5.38±.23</td>
<td>72±6</td>
<td>.51±.12</td>
</tr>
<tr>
<td>5 7 FC</td>
<td>WW</td>
<td>4.75±.26y</td>
<td>63±8y</td>
<td>.80±.18f,y</td>
</tr>
<tr>
<td>6 7 PRL</td>
<td></td>
<td>5.48±.38y</td>
<td>69±4y</td>
<td>1.14±.15y</td>
</tr>
<tr>
<td>7 7 PRL-TU</td>
<td></td>
<td>4.19±.17x</td>
<td>50±7x</td>
<td>.10±.06x</td>
</tr>
<tr>
<td>8 7 PRL-TU-T4</td>
<td></td>
<td>5.48±.23y</td>
<td>67±7y</td>
<td>1.18±.14y</td>
</tr>
</tbody>
</table>

a The number (N) of anoles in each group.
b Treatment symbols: I = initial group; CC = constant 20±2°C; WW = constant 30±2°C; PRL = prolactin injections made 16 hours after the onset of light; T4 = thyroxine; TU = thiourea; FC = controls.
e,f Student "t" test comparing control treatment means with the initial group. Means without a letter in common are statistically different at the 95% confidence level.
x,y Ranking of treatment means within temperature treatment groups using the Student-Newman-Keuls procedure. Means without a letter in common are statistically different at the 95% confidence level.
Figure 12:

The effect of prolactin (PRL; injections given 16 hours after the onset of light), thyroxine (T4) and thiourea (TU) given separately or in combination on fat body weights in A. carolinensis kept at 20±2°C (CC) or 30±2°C (WW) and LD 12:12. Treatment means within temperature treatment groups were ranked using the Student-Newman-Keuls procedure. Bars (treatment means) without hatching in the same direction are statistically different at the 95% confidence level. The concentration of plasma thyroxine determined by radioimmunoassay (Mitsuma et al., 1972) is recorded in µg % above the bars representing appropriate groups.
thyroxine levels determined by radioimmunoassay (PRL; 0.74 ± 0.07 μg T4/100 ml) appeared to be reduced by thiourea treatment (PRL-TU; trace). Thyroxine treatment appeared to restore plasma thyroxine levels in thiourea-treated anoles (PRL-TU-T4; 0.77 ± 0.16 μg T4/100 ml) and these levels were similar to levels in thyroxine-treated anoles (PRL-T4; 0.81 ± 0.03 μg T4/100 ml) kept in CC (Figure 12).

Experiment VI re-examined the response of body fat stores to warmth in anoles maintained under constant light (LL). Consistent with results from a previous study carried out in October (Noeske, 1974), fat body weights decreased in response to constant warmth in anoles maintained under a LL photoperiodic regimen in December (Table VIII and Figure 13).

Experiment VII was carried out to explore further the influence of constant warmth on fat stores in anoles kept under LL conditions but at a different season. Results are summarized in Table IX and Figure 13. Unlike results determined in December, fat body weights were elevated in response to warmth in July. Interpreting the results is made difficult by the fact that fat stores in cold acclimated anoles (CC) were intermediate in weight between fat stores in warm acclimated anoles (WW) and in the initial group (I).
### TABLE VIII

Experiment VI. The effect of constant 30±2°C (WW) on fat body and paired testes weights in *A. carolinensis* maintained under continuous light (LL). Experiment VI was carried out from 11 December to 21 December, 1977.

<table>
<thead>
<tr>
<th>GRP(N)</th>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Paired Testes Weight (mg)</th>
<th>Fat Bodies ( % Body Wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>5.57±.38&lt;sup&gt;d&lt;/sup&gt;</td>
<td>58±2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.16±.12&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>LL; WW</td>
<td>5.16±.29</td>
<td>58±2</td>
<td>1.56±.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The number (N) of anoles in each group (GRP)

<sup>b</sup> Treatment symbols: I = initial group;

<sup>c</sup> Statistically different compared with the mean of the initial group based on a Student "t" test.

<sup>d</sup> Mean weight ± one standard error about the mean.

### TABLE IX

Experiment VII. The effect of constant 30±2°C (WW) or constant 20±2°C (CC) on fat body and paired testes weights in *A. carolinensis* maintained under continuous light (LL). This experiment was initiated 4 July, 1978.

<table>
<thead>
<tr>
<th>GRP(N)</th>
<th>Treatment</th>
<th>Body Weight (g)</th>
<th>Paired Testes Weight (mg)</th>
<th>Fat Bodies ( % Body Wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>5.23±.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>76±5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.74±.08&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>LL; WW</td>
<td>5.15±.29</td>
<td>71±6</td>
<td>1.43±.22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>LL; CC</td>
<td>5.78±.30</td>
<td>80±5</td>
<td>.99±.13</td>
</tr>
</tbody>
</table>

<sup>a, b, c, d</sup>

See the legend of the table above.
Figure 13:

The effects of constant 30±2°C (WW) and 20±2°C (CC) on fat body weights in *A. carolinensis* maintained under continuous light (LL) in December 1977 and July 1978. See the legend of Figure 12 for the significance of hatching within bars.
DISCUSSION

In accordance with findings from previous studies (Licht and Jones, 1967; Licht et al., 1969; Noeske and Meier, 1977) results of this investigation indicate that exposure to constant warmth favors fat storage provided lizards are maintained under a light-dark cycle. Fat bodies in anoles kept in WW were heavier compared with fat bodies in the initial group or in anoles kept in CC regardless of whether lizards were held under LD 13:11 conditions in April or LD 12:12 conditions in June or July. Fat stores did not increase in response to constant warm temperature in anoles held under short daylengths (LD 6:18) in December (Figure 7). However, seasonally heavy fat stores were maintained in contrast to decreases in fat stores which have been observed in anoles taken from the field during this season (Dessauer, 1955a; Licht, 1967a). Because the photoperiodic regimen provided in December was different from that used at other seasons, it is not clear whether the lack of fattening response to warmth in December was a consequence of photoperiod or whether high seasonal levels precluded additional fattening.

Results of Experiments VI and VII (Figure 13) as well as of previous studies indicate that the fattening response to warm temperature is in part seasonally dependent in anoles. Fat stores in anoles held under continuous light (LL) decreased in response to warmth in December (Noeske, 1974;
Experiment VI) whereas fat stores increased in anoles held under the same conditions in July (Figure 13). The magnitude of fattening in response to warm temperature varied seasonally in anoles kept under long (LD 14:10; Licht and Jones, 1967) or short (LD 6:18; Noeske and Meier, 1977) daylengths, as well. In addition, fat stores did not change in anoles kept in constant warmth regardless of whether the photoperiod was long (LD 18:6) or short (LD 9:15) in experiments carried out in late fall (Fox and Dessauer, 1957). These results are consistent with results of Experiment I carried out in December. Seasonal condition appears to be important in determining the fattening response to constant warmth in other lizard species, as well. Fat bodies in *Lacerta spp.* were heavier in response to constant warm temperature regardless of whether lizards were kept under short (LD 8:16) or long (LD 16:8) daylengths in experiments carried out in the fall (Licht *et al.*, 1969).

Results of this study indicate that thyroxine has an important role in the fattening response to constant warmth. Drugs (2-thiouracil, thiourea) which reduce radioiodine uptake (Nussbaum, 1963) and activity of (Adams and Craig, 1949; 1951; Ratzersdorfer *et al.*, 1949) the thyroid gland in *A. carolinensis* appear to counteract the positive influence of constant warm temperature on body fat stores. Fat stores, near maximal levels in early December (Dessauer, 1955a; Fox and Dessauer, 1957; Licht, 1971) were decreased nearly 50% following thiouracil treatment compared with fat stores in untreated anoles (Experiment I). Drug treatments in April
(Experiment II) and July (Experiment V), times when fat stores are normally low (Dessauer, 1955a, b; Licht, 1971), effectively inhibited fattening in response to constant warmth compared with the response in untreated anoles. Furthermore, thyroxine treatment appeared to permit fattening in response to WW in thiourea-treated animals (Experiment V). Direct measurements of plasma thyroxine levels using a radioimmunoassay technique substantiate the effectiveness of thyroxine administration via crickets in restoring plasma levels of thyroxine (.77 ± .16 μg T4/100 ml) depressed by thiourea treatment (trace amounts).

The permissive influence of thyroxine on fattening appears to depend on the presence of warm temperature. Thyroxine treatment permitted fattening to occur in thiourea-treated anoles kept in WW. In contrast, fattening did not occur in cold acclimated anoles treated with thyroxine. Plasma levels of thyroxine determined by radioimmunoassay in thyroxine-treated anoles kept in WW (.77 ± .16 μg T4/100 ml) and CC (.81 ± .07 μg T4/100 ml) were similar. These results can be interpreted to indicate that WW enhances tissue sensitivity to thyroxine. This interpretation is supported by studies in Rana tadpoles. Thyroxine administration which is normally effective in stimulating metamorphosis in tadpoles kept at warmer temperatures did not do so in tadpoles kept at 5°C (Frieden, 1967). Thyroxine enters the cytosol of tadpole liver cells at 5°C but does not enter the nucleus where it directs RNA production unless temperatures are elevated (Griswold et al., 1972).
Temperature can have dramatic effects on regulatory mechanisms involved in fat metabolism located in liver and adipose tissues which are important sites of lipid synthesis and storage, respectively (see introduction). Experiment III was conducted in cold and warm temperature acclimated anoles kept under LD 12:12 conditions in June. The daily rate of $^{14}$C-acetate incorporation into liver ($P<.07$) and fat body ($P<.001$) lipid in anoles kept in WW was elevated compared with levels determined in anoles kept in CC (Table V). However, these differences can be attributed to greatly increased rates of incorporation at specific times of day in anoles kept in WW. For example, $^{14}$C-acetate incorporation into liver lipids in anoles kept in WW were almost twice the rates in anoles kept in CC when measurements were made at 0700 hours (light onset) whereas at 0300 the levels of $^{14}$C-acetate incorporation were similar in anoles kept at either temperature (Figure 9).

Results of Experiment III indicate that temperature has important effects on the time of day when increased $^{14}$C-acetate incorporation into liver and fat body lipid occur. Peak rates of $^{14}$C-acetate incorporation into liver and fat body lipid occurred simultaneously at approximately 1500 hours (8 hours after light onset) in anoles kept in CC. Peak rates of $^{14}$C-acetate incorporation into liver lipid occurred at 0700 hours (light onset) in anoles held in WW which is 8 hours earlier than peak rates in anoles held in CC. $^{14}$C-acetate incorporation into fat body lipid was also high at this time in
anoles kept in WW, however, peak rates were not reached until approximately 1900 hours which is 4 hours later than peak rates determined in anoles kept in CC (Figures 9 and 10). Rates of incorporation into liver lipid do not reflect absolute rates of hepatic lipogenesis but rather the total lipid synthesized de novo less the amount subsequently transported from the liver. The fact that peak rates of incorporation into liver and fat body lipid did not coincide in anoles kept in WW may indicate that liver lipogenesis and lipid transport are controlled by separate regulatory mechanisms. It is possible, though unlikely, that significant amounts of lipogenesis occur in adipose tissue of green anoles as it does in rodents. The liver is the major site of lipogenesis in most vertebrate species studied (see Meier and Burns, 1976). Further experimentation is necessary to assess the possibility that lipogenesis occurs in reptilian adipose tissue.

Temperature may influence circadian systems involved in regulating fat stores in that WW produced a phase shift in the daily rhythm of acetate incorporation into fat body lipid compared with the rhythm in anoles kept in CC. The possibility that warm temperature can cause a phase shift in circadian rhythms is supported by reports of studies in Gulf Killifish (Fundulus grandis) (Spieler et al., 1978). Warm temperature causes a shift in the daily rhythm of plasma prolactin concentration relative to the onset of light. The peak level of prolactin occurred 8 hours after light onset in fish acclimated to 20°C whereas the prolactin peak in fish acclimated to 28°C
occurred at the onset of light. Results of injection studies in a number of vertebrates (see Meier and Burns, 1976) including *A. carolinensis* (Trobec, 1974) indicate a shift in the phase of a daily prolactin rhythm may have important consequences in the regulation of fattening. Prolactin may be stimulatory or inhibitory to fattening depending on the time of day plasma levels are high. Prolactin injections 16 hours after light onset resulted in increased fat stores whereas injections 4 hours after light onset depressed fat stores in anoles kept in WW and LD 12:12. Therefore, a change in the phase of a circadian rhythm caused by warm temperature may have an important influence on seasonal conditions of fattening.

Thyroxine appears to permit the stimulatory influence of WW on fattening by affecting mechanisms regulating lipid synthesis. Increased deposition of labelled lipid in fat stores is a reflection of increased *de novo* lipid synthesis. Thio-urea treatment inhibited acetate incorporation into fat body lipid induced by warm temperature and thyroxine restored the rate of incorporation in thiourea-treated animals to that observed in untreated controls (Figure 11). This interpretation of the data is supported by studies in endotherms (Roncari and Murthy, 1975; see also Bernal and Refetoff, 1977) where thyroxine appears to have a stimulatory effect on the synthesis of enzymes (e.g. acetyl CoA carboxylase) important in regulating lipogenesis.
As in pigeons (John et al., 1972), thyroxine may have a permissive influence on the fattening response to prolactin in anoles. The results of Experiment VI are suggestive that such an influence occurs. Fattening was not observed in prolactin-thiourea-treated anoles unless thyroxine was provided. Because prolactin injections made 16 hours after the onset of light did not produce fat stores greater than fat stores resulting from temperature conditions alone, it is impossible to assess the possibility that thyroxine and prolactin interact in controlling fat stores. In addition, because fattening normally begins during July and August (Dessauer, 1955a, b; Licht, 1971) when temperatures remain high, endogenous levels of prolactin may have influenced the results of this study. This experiment should be carried out in hypophysectomized anoles and with saline controls in order to establish effects caused by hormone treatments alone.

Testicular growth in response to WW appears to depend on the presence of a light-dark cycle. Testicular growth observed in anoles kept in LD 6:18 was not observed in anoles kept on LL in December. Thyroxine may be involved in mechanisms regulating testicular growth in response to WW but this possibility must be tested further. The fact that testicular growth was promoted by CC may indicate that more than one mechanism may be involved.

Similar to the annual cycles of reproduction, fattening and molt in birds (Gwinner, 1974), the annual cycle of fattening in anoles may be controlled by endogenous circannual
mechanisms. Consistent with results reported in anoles kept on LL:WW in October (Noeske, 1974), fat stores were decreased in anoles kept on LL:WW in December (Table VIII). In contrast, fat stores were increased in anoles kept in LL:WW in July (Table IX). These data are only suggestive and this possibility needs further exploration.

Testicular growth in response to WW appears to depend on the presence of a light-dark cycle. Testicular growth occurred in response to WW in anoles kept under LD 6:18 in December (Figure 7) and LD 13:11 in April (Figure 8) but not in anoles kept under LL in December and July (Tables VIII and IX). A rhythm of responsiveness to warmth entrained by the photoperiod (see Part I A) may be necessary before WW can stimulate testicular growth. The fact that testicular growth was consistently promoted by CC in accordance with previous reports in anoles (Licht, 1969; Noeske and Meier, 1977) may indicate that more than one mechanism is involved in regulating gonadal growth (Licht, 1971).

Thyroxine may influence the testicular growth response to WW in anoles held under light-dark cycles. Testicular growth did not occur in response to WW in thiouracil-treated anoles kept under LD 13:11 in April (Figure 8). However, the influence of thyroxine may depend on daylength or seasonal conditions of anoles. Thiouracil did not inhibit testicular growth in response to WW in anoles kept under LD 6:18 in December. These data are not sufficient and further experimentation conducted in anoles held under the same photoperiodic
conditions through several seasons is necessary before firm conclusions can be reached.

In summary, the fattening response to WW appears to vary seasonally. Fat stores were depleted in December and increased in July in anoles kept under LL. In anoles kept under light-dark cycles, fat stores were maintained in December or increased in April and July in response to WW. Thyroxine may be involved in mechanisms regulating the fattening response to WW during these seasons. This involvement may depend on enhanced tissue sensitivity to thyroxine induced by warm temperatures in that fattening occurred in thiourea-thyroxine-treated anoles kept in WW but not in thyroxine-treated anoles kept in CC. Constant warmth appears to stimulate fattening by influencing regulatory mechanisms involved in fat metabolism located in liver and adipose tissues. Constant warmth appears to influence the rate of lipogenesis at particular times of day. Furthermore, the time of day increased lipid synthesis occurs may be set in response to WW indicating perhaps that WW may shift the phase of circadian (hormone) rhythms important in the regulation of fattening. Thyroxine appears to play an important role in mechanisms responsive to WW which regulate the rate of lipogenesis. Whether thyroxine effects the rate of reactions or influences the phase relation among circadian (hormone) rhythms which in turn regulate fattening remains to be determined. The results of these studies may be correlated with seasonal phenomena observed in anoles. Temperature, appetite and thyroid gland activity are reportedly low in
winter when fat stores are being depleted, whereas temperature, appetite and thyroid gland activity are high in summer when fattening occurs in preparation for winter periods of inactivity.
Part IIA: Thyroxine and Seasonality in the White-throated Sparrow, Zonotrichia albicollis.
Introduction

Thyroxine is a low molecular weight hormone produced in the thyroid gland. Many of its effects are the result of synergisms with other hormones. The lipolytic activities of glucagon, adrenocorticotropin and other hormones are augmented by thyroxine. Such synergisms involve the thyroid hormones in many metabolic and behavioral events. Thyroxine has an important influence on avian reproduction, migration, fattening and molting.

The majority of birds have a restricted breeding season appropriately timed to coincide with optimal habitat conditions for rearing young. Therefore, factors which control the timing of the reproductive effort are important ones to consider. Thyroxine has received considerable attention.

Birds have been categorized into two distinct reproductive types based on the involvement of thyroxine in avian reproduction (see Assenmacher, 1973). Most domesticated species studied (i.e. chickens and ducks) appear to require thyroxine for gonadal development. Thyroidectomy, whether surgical (Aron and Benoit, 1934; Benoit, 1936; 1937; Greenwood and Blyth, 1942) or chemical (Schultze and Turner, 1945) inhibits gonadal growth or activity (i.e. spermatogenesis). Administration of thyroxine appears to accelerate the sexual cycle in House Sparrows (Passer domesticus) (Miller, 1935; Vaugien, 1954). In contrast, thyroidectomy does not interfere
with gonadal development in European Starlings (*Sturnus vulgaris*) (Woitkewitch, 1940; Wieselthier and van Tienhoven, 1971) or tropical weaver finches (*Estrildidae*) (Pandha and Thapliyal, 1964; Thapliyal and Pandha, 1965; 1967 a, b, c; Thapliyal and Garg, 1967, Thapliyal, 1969) and in fact prevents normal gonadal regression. An inverse relation between thyroid activity and gonadal function (i.e. testosterone production) has been reported in Peking ducks as well (Jallageas and Assenmacher, 1972; Assenmacher et al., 1975; Jallageas et al., 1978).

Physiological provisioning must precede migratory flight. This involves substantial increases in the amount of fat stores. Thyroxine stimulates the synthesis of enzymes (e.g. Malic enzyme) involved in liver lipogenesis in vertebrates studied (Lefebvre, 1975; see Bernal and Refetoff, 1977; see also introduction Part IB) including birds (Chandrabose and Bensadoun, 1971; Goodridge, 1975, 1978). In addition, thyroxine administration appears to prevent the dampening out of a rhythm of fattening response to prolactin which occurs in pigeons placed under constant light (Meier et al., 1971; John et al., 1972).

During migratory flight fat stores provide the major source of energy. Corticosterone and thyroxine appear to play supportive roles in fat mobilization from mammalian adipose tissue induced by adrenocorticotropic hormone, growth hormone and glucagon (Goodman and Bray, 1966).

Results from studies exploring the possibility that thyroxine plays an important role in migration are inconsistent.
Histological evidence from several studies indicates there is a positive correlation between thyroid activity and migration (Putzig, 1937; 1938; George and Naik, 1964; John and George, 1966; Rolliber, 1977). On the other hand, evidence from histological studies in the White-crowned Sparrow (Zonotrichia leucophrys gambelli) (Wilson and Farner, 1960) as well as actual measurements of plasma thyroid hormone titers in the Canada Goose (Branta canadensis interior) (John and George, 1978) do not support a correlation between thyroid activity and migration. The administration of thyroid hormones produced variable results regarding both locomotor activity and fat stores associated with "Zugdisposition" (i.e. readiness to migrate) (Wagner, 1930; Merkel, 1938, 1958; Schildmacher and Rautenberg, 1952; see also Berthold, 1975). Low doses (.1 mg) of thyroprotein reportedly stimulated "Zugunruhe" (i.e. nocturnal restlessness) in robins (Erithacus rubecula) and white throats (Sylvia communis) in winter. The stimulatory effect of thyroid hormones was short-lived (3 days) and dependent on the presence of winter fat stores. Thyroid hormone administration in lean birds stimulated daytime activity but no nocturnal activity was evident. In contrast, high doses of thyroid hormone preparations (.3 mg) depressed Zugunruhe (Merkel, 1958) and fattening (Schildmacher and Rautenberg, 1952). It is quite evident from inconclusive and contradictory findings that a new approach is needed in this area.

The annual cycle of molt varies greatly among avian species; most often molt occurs outside of the reproductive
and migratory seasons. Thyroxine is believed to play a significant role in the process of molting. Thyroid hormone injections may induce molting prematurely in hens (Tanabe et al., 1957). A direct stimulatory effect of thyroid hormones on feather follicles has been reported in pigeons (Höcker, 1967) and other avian species (Voitkevich, 1966). Besides influencing molt directly, thyroxine may time the occurrence of molt (Voitkevich, 1966). Thyroidectomy in European Starlings inhibited, altered or had little effect on the molt depending on the time between thyroidectomy and the normal expected molt. These data can also be interpreted as indicating that the effect of thyroid hormones on molt depends on the seasonal condition of birds being tested.

The possibility that there is a direct correlation between thyroid activity and molt has been both supported and refuted based on histological evidence in a number of avian species (review; Payne, 1973). These contradictory findings have not been resolved using thyroxine assay techniques. Because high plasma thyroxine levels in early June preceded the postnuptial molt in Peking ducks (Assenmacher et al., 1975; Jallageas et al., 1978), the authors suggested this condition may be prerequisite for the postnuptial molt. On the other hand no correlation between high plasma thyroid hormone titers and the postnuptial molt was evident in Canadian Geese (John and George, 1978).

It is a common assumption that thyroid activity indicated by histological evidence bears a direct relation with plasma concentrations of thyroid hormones and thus the activity of these hormones. A timed release of hormones leading to
elevated plasma hormone levels during a particular period of the day may have important consequences on physiological mechanisms regulating fattening, migration, reproduction and molt. Such a possibility does not seem remote if evidence from studies of prolactin in White-throated Sparrows (*Zonotrichia albicollis*) is considered. Pituitary prolactin levels were high in the morning in May and August. However, the apparent release of prolactin occurred in the afternoon in May but late at night in August (Meier et al., 1969). The difference in the time of prolactin release has important consequences in establishing seasonal conditions in the White-throated Sparrow, as will be discussed shortly. Therefore, even though a hormonal storage organ (e.g. thyroid gland) may appear equally active during different seasons, the timing of hormone release and consequently the time of day plasma hormone levels are elevated may be more important in determining seasonal conditions than overall plasma levels per se.

Organisms must continuously make physiological and behavioral adjustments to meet changing demands placed on them by the environment. In species adapted to seasonally changing climates, physiological adjustments are often anticipatory. The temporal arrangement of seasonal changes associated with reproduction, molting, migration, photo-refractoriness and fattening constitutes the annual cycle. It is evident that the annual cycle is controlled by an endogenous circannual rhythm or sequence (Gwinner,
1973, 1974, 1975). Circannual rhythms are involved in the annual cycles of reproduction, molt and migration in birds (Gwinner, 1971, 1972; Schwab, 1971; Berthold et al., 1972). For example, the annual cycles of molt and nocturnal activity persisted for more than a year in warblers maintained under constant daylengths (Gwinner, 1973).

It was suggested that circannual rhythms are generated by changing temporal relations of two circadian systems (Meier, 1973, 1976; see also Meier and Ferrell, 1978). Changing temporal relations of circadian rhythms of corticosterone and prolactin appear to play an important role in generating annual cycles of migratory disposition (Martin and Meier, 1973), reproduction (Meier and MacGregor, 1972; Meier et al., 1971) and fattening (Meier and Davis, 1967; Meier and Martin, 1971; Meier, 1973, 1975) in White-throated Sparrows. Seasonal changes in the phases of the circadian rhythms of plasma corticosterone (Meier and Fivizzani, 1975) and prolactin (Fivizzani and Meier, 1976) concentrations have been demonstrated and simulation of these hormonal phase relations by hormone injections produce appropriate changes in reproductive and migratory indices in the White-throated Sparrow. Injections of corticosterone followed by injections of prolactin 12 hours later in birds maintained in conditions of constant light induced gonadal development and migratory indices of fattening (Meier et al., 1971), Zugunruhe and a northward orientational preference (Martin and Meier, 1971). Prolactin injections 4 hours after corticosterone induced fall migratory indices.
Although undefined, thyroxine appears to play a role in avian reproduction (Farner, 1964; Thapliyal et al., 1968; Thapliyal, 1969; Jallageas and Assenmacher, 1974), molting (Voitkevich, 1966; Payne, 1973) and migratory disposition (Putzig, 1937, 1938; Merkel, 1958). The timing of the occurrence of daily peaks in plasma thyroid hormone titers may be as important as the basic levels of the hormones as indicated by studies concerning other hormones (e.g. corticosterone and prolactin). Daily variations in plasma thyroxine concentration are known to occur in chickens (Newcomer, 1974; Krebietke, 1975; Klandorf et al., 1978).

The endogenous mechanism controlling the circannual sequence of events has been discussed above. When birds are maintained under constant conditions the endogenous mechanism leads to a circannual sequence which approximates a year's time (Gwinner, 1973). There must be some exogenous cue(s) which entrains the endogenous mechanism so that events of the sequence remain synchronized with the appropriate seasonal conditions. One of the most reliable exogenous cues and one which has been extensively investigated is the daily photoperiod. The circadian rhythm of plasma corticosterone concentrations in the White-throated Sparrow is entrained by the photoperiod (Meier and Fivizzani, 1975). Although the phase of the corticosterone rhythm is probably set by the photoperiodic schedule, the shape and phase relation of the rhythm changes with respect to the daily photoperiod and with respect to other hormone rhythms in time indicating
a difference in the interpretation of photoperiod at different
times of the year (Meier and Fivizzani, 1975). Because
thyroxine is required for the expression of the circadian
rhythm of corticosterone in some other vertebrates (Meier, 1975),
including the pigeon (John et al., 1972), any effect the
photoperiod may have on thyroid hormone levels or daily
pattern may help explain a difference in the interpretation
of the photoperiod with respect to the corticosterone rhythm.

There is evidence that photoperiod length does influence
thyroid gland activity (Follett and Riley, 1976; John and
George, 1978). After ten days the rate of $^{131}\text{I}$ uptake by the
thyroid and $\text{PB}^{131}\text{I}$ levels in the blood were increased in
female Japanese Quail (\textit{Coturnix coturnix japonica}) held under
a long (LD 20:4) photoperiodic schedule compared with values
in quail held under short (LD 6:18) daylengths. A reverse
in this trend observed after 20 days of photostimulation was
attributed to increased estrogen production accompanying gonadal
development (Follett and Riley, 1967). Similar to the effect
of testosterone on plasma thyroid hormone levels reported in
Peking ducks (Assenmacher et al., 1975; Jallageas et al., 1978)
estrogen may suppress thyroid activity (Follett and Riley,
1967). Interestingly, plasma thyroxine levels increased
dramatically as reproductive activity waned in Peking ducks
during early summer when daylengths are long (Assenmacher
et al., 1975; Jallageas et al., 1978). Conclusions based on
correlations between thyroid hormone levels and reproductive
activity reported in the above studies may not be valid because hormone levels were checked at one time of day only. To investigate the influence of photoperiod on daily thyroxine levels and their pattern, measurements of thyroxine taken at 6 times of day from photosensitive birds maintained on a non-stimulatory photoperiod were compared with similar measurements from the same birds after 6 weeks of photostimulation and again with measurements from the same birds in a photorefractory condition.
Materials and Methods

White-throated Sparrows (Zonotrichia albicollis) were captured by mist net in the vicinity of Baton Rouge, Louisiana and held in a large outdoor aviary until initiation of this study. Grower poultry feed mixed with grain was available to the birds.

On 15 February, 1978, 75 White-throated Sparrows were transferred to three indoor cages (25 birds per cage: 76 cm x 100 cm x 70 cm). The birds received 10 hours of light daily (0800-1800 hours) supplied by "daylight" fluorescent bulbs (1000 lux at mid cage perch height) until 10 March, 1978 when the photoperiod was changed to 16 hours of light (0500-2100 hours). The temperature in the room was maintained within a few degrees of room temperature (21°C) until approximately the end of May when temperatures in the room rose above room temperature during the daytime. Blood samples from the wing veins were collected in heparinized capillary tubes on 7 March, 22 April and 1 July, 1978 beginning at the onset of lights and at 4 hour intervals for 24 hours. Six or 7 birds were bled at each sampling time and no birds were bled twice during the day of sampling. Blood samples were centrifuged immediately and the plasma portion was stored at -20°C.

The plasma concentration of thyroxine was determined by radioimmunassay technique (Mitsuma et al., 1972). The procedure was modified slightly in that standards were prepared in buffer solution. The rabbit antibody to thyroxine (Calbiochem) was diluted 1:100 in barbital buffer (pH 8.4).
Na\textsuperscript{+}L-thyroxine (Sigma Chemical Company) was used to prepare the standards. Labelled thyroxine (\textsuperscript{125}L-thyroxine) was obtained from New England Nuclear. Determinations of plasma thyroxine concentrations were made in duplicate for each bird. Counts from the bound fraction determined for standards and samples were expressed as per cents of the standard to which no cold thyroxine was added (0 \( \mu \)g T4/100 ml; maximum counts). Expressing the values as per cents allowed for comparisons of values among runs without considering the rate of decay of \textsuperscript{125}I. The limit of detectability was \( 0.27 \mu \)g thyroxine/100 ml plasma. The inter- and intraassay variabilities were 5.0\% and 4.8\%, respectively. To allow for possible inter-assay variation and prevent bias a few samples from each sampling time were run with each assay. Thyroxine concentrations in the plasma of photosensitive and photostimulated birds were determined simultaneously. Because plasma thyroxine levels in photorefractory birds were determined in a separate assay series, the constancy of the assay was checked by comparing thyroxine levels in plasma samples from several photostimulated sparrows not analyzed previously with levels determined during the previous assay series. The data were analyzed statistically by an analysis of variance and ranked according to the Student-Newman-Keuls ranking procedure at the 95\% confidence level.

One week before each blood sampling a representative group of birds (12 to 14) from the large indoor holding cages were placed in individual activity cages in order to record
the amount of locomotor activity. The perches in these
cages rested on microswitches wired to an Esterline-Angus
20 channel event recorder which recorded each perch hop. Of
particular interest were the recordings of nocturnal activity
indicative of the urge to migrate (Eyster, 1954; Helms, 1963).
Body weight, molt index, and gonadal development were also
determined for these sparrows. The molt index (amount of
head, body, and wing feather loss) and fat index (amount of
visible body fat accumulation) are based on scales used by
Weise (1956). Values of 12 and 4 indicate maximum amounts
of molting and fat stores, respectively. In determining
nocturnal locomotor activity values, each hour of the night
was divided into ten 6 minute intervals. The number of 6
minute intervals with any amount of activity recorded is
expressed as a percent of the total number of 6 minute
intervals per night. Values determined from activity
records for 10 birds over seven nights were averaged. Gonadal
development was determined by laparotomy in at least seven
male sparrows during each season. All data were analyzed
statistically by analysis of variance. The daily pattern
of locomotor activity was determined in a similar manner to
nocturnal activity. However, recordings of 6 minute inter-
vals which were not completely filled with activity were
assigned a value of ½ instead of 1 activity unit. Unlike
nocturnal activity much of the daytime activity does not occur
in intense spurts.
Results

Birds examined in March were in a reproductively photosensitive but not a photostimulated condition. There was no evidence of molt, no evident fat stores, little nocturnal activity and left testes appeared undeveloped (Table X and Figure 14). Plasma thyroxine (T4) levels rose during daylight hours reaching peak levels (5.4 ± 0.7 μg T4/100 ml) 4 hours after light onset (Table XI and Figure 15) and dropped off gradually to a trough (3.5 ± 0.6 μg T4/100 ml) 4 hours before light onset. However, the levels did not vary significantly (P<.23) throughout the day (Figure 15). The mean plasma level of all determinations was 4.4 μg T4/100 ml.

In contrast birds examined approximately 6 weeks after transferral to long (LD 16:8) photoperiodic conditions had heavy fat stores, intense nocturnal activity and left testes were developed (Table X and Figure 14); all attributes generally observed in photostimulated White-throated Sparrows (Meier and Fivizzani, 1975). The presence (P<.001) of a marked unimodal daily rhythm of plasma thyroxine (Figure 16) is in sharp contrast to the shallow daily variation observed in the plasma of non-photostimulated birds in March. A sharp rise in plasma thyroxine to peak levels (10.7 ± 1.6 μg T4/100 ml) occurred coincident with the offset of light (Figure 16). The plasma levels gradually tapered off to a trough (4.3 ± 0.6 μg T4/100 ml) at 1300 hours (middle of the light period; Figure 16). A sight comparison of plasma thyroxine levels with levels of locomotor activity indicates a coincidence of


TABLE X

Indices of seasonal conditions in White-throated Sparrows held under a LD 10:14 regimen in March (Photosensitive: PS) and a LD 16:8 regimen in April (Photostimulated: PST) and July (Photorefractory: PR)

<table>
<thead>
<tr>
<th>INDEX</th>
<th>LD 10:14</th>
<th>LD 16:8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MARCH: PS</td>
<td>APRIL: PST</td>
</tr>
<tr>
<td>MOLT Absent</td>
<td>Absent</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>BODY WEIGHT (grams)</td>
<td>26.7 ± .6</td>
<td>31.4 ± .3</td>
</tr>
<tr>
<td>FAT Absent</td>
<td>Absent</td>
<td>3.8 ± .1</td>
</tr>
<tr>
<td>NOCTURNAL ACTIVITY (% of total)</td>
<td>8.2 ± 1.8</td>
<td>71.0 ± 3.9</td>
</tr>
<tr>
<td>LEFT TESTES VOLUME (mm³)</td>
<td>2.0 ± .3</td>
<td>93.3 ± 20.6</td>
</tr>
</tbody>
</table>

a Analysis of variance among seasonal groups within a given index.

b Indices are based on measurements described in the text.
Figure 14

Indices of seasonal conditions in White-throated Sparrows held indoors under a LD 10:14 regimen in March (Photosensitive: PS) and a LD 16:8 regimen in April (Photostimulated: PST) and July (Photorefractory: PR). Indices are described or referenced in the methods and materials section. Symbols: S.E.M. = one standard error about the mean; P = probability that index values are the same among birds in different seasonal conditions determined by an analysis of variance.
TABLE XI

Plasma thyroxine concentrations and locomotor activity in the White-throated Sparrow in different physiological conditions.

<table>
<thead>
<tr>
<th></th>
<th>March: PS-LD 10-14</th>
<th>April: PST-LD 16:8</th>
<th>July: PR-LD 16:8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hr^a</td>
<td>ug% T4 Units/hr.</td>
<td>ug% T4 Units/hr.</td>
<td>ug% T4 Units/hr.</td>
</tr>
<tr>
<td>0</td>
<td>4.6±1.6 6.3(3.2-8.4)</td>
<td>3.1±1.7 7.4(4.1-5.8)</td>
<td>2.8±1.7 6.9(4.9-8.4)</td>
</tr>
<tr>
<td>1</td>
<td>5.7(3.2-7.4)</td>
<td>6.2(5.4-8.1)</td>
<td>8.0(5.8-9.5)</td>
</tr>
<tr>
<td>2</td>
<td>5.0(3.0-6.2)</td>
<td>7.0(5.6-7.4)</td>
<td>7.2(6.0-8.3)</td>
</tr>
<tr>
<td>3</td>
<td>4.7(2.5-6.4)</td>
<td>7.8(6.5-9.5)</td>
<td>7.1(5.0-8.4)</td>
</tr>
<tr>
<td>4</td>
<td>5.4±1.7 4.1(2.6-6.3)</td>
<td>5.1± 5 4.7(3.7-5.6)</td>
<td>2.3± 4 6.5(4.9-7.9)</td>
</tr>
<tr>
<td>5</td>
<td>3.7(2.6-4.7)</td>
<td>5.0(3.1-6.0)</td>
<td>5.7(4.7-8.1)</td>
</tr>
<tr>
<td>6</td>
<td>4.8(4.0-5.8)</td>
<td>4.3(3.3-6.3)</td>
<td>5.2(4.4-6.0)</td>
</tr>
<tr>
<td>7</td>
<td>4.1(2.0-5.6)</td>
<td>4.9(3.5-6.7)</td>
<td>4.7(3.7-5.5)</td>
</tr>
<tr>
<td>8</td>
<td>5.1± 4 4.0(2.0-5.6)</td>
<td>4.3± 6 3.6(2.9-4.9)</td>
<td>2.6±1.4 5.0(2.1-8.7)</td>
</tr>
<tr>
<td>9</td>
<td>3.7(2.3-7.1)</td>
<td>3.4(2.2-4.3)</td>
<td>5.6(4.2-8.8)</td>
</tr>
<tr>
<td>10</td>
<td>.9(2.2-2.5)</td>
<td>3.3(2.3-4.6)</td>
<td>5.6(4.0-8.6)</td>
</tr>
<tr>
<td>11</td>
<td>.0(0.0-.1)</td>
<td>3.3(2.3-4.6)</td>
<td>5.0(2.3-8.5)</td>
</tr>
<tr>
<td>12</td>
<td>3.7± 8 .1(0.0-.6)</td>
<td>4.6± 6 3.3(2.6-5.1)</td>
<td>3.8±1.5 5.3(3.0-7.2)</td>
</tr>
<tr>
<td>13</td>
<td>.2(0.0-.7)</td>
<td>2.6(2.1-3.2)</td>
<td>4.8(3.5-6.5)</td>
</tr>
<tr>
<td>14</td>
<td>.5(0.0-1.6)</td>
<td>2.6(1.7-3.6)</td>
<td>4.8(4.0-6.6)</td>
</tr>
<tr>
<td>15</td>
<td>.7(0.0-1.6)</td>
<td>3.1(1.9-3.9)</td>
<td>6.2(4.0-7.8)</td>
</tr>
<tr>
<td>16</td>
<td>3.8± 7 .6(0.1-.8)</td>
<td>10.7±1.6 3.8(2.1-6.1)</td>
<td>2.4± 1 2.0(0.0-.3)</td>
</tr>
<tr>
<td>17</td>
<td>.7(.0-2.1)</td>
<td>6.9(3.5-9.3)</td>
<td>.0(0.0-.1)</td>
</tr>
<tr>
<td>18</td>
<td>.2(0.0-.5)</td>
<td>7.6(5.2-9.3)</td>
<td>.0(0.0-.2)</td>
</tr>
<tr>
<td>19</td>
<td>.1(0.0-.7)</td>
<td>7.5(5.7-8.8)</td>
<td>.0(0.0-.2)</td>
</tr>
<tr>
<td>20</td>
<td>3.5± 6 .2(0.0-.6)</td>
<td>8.8± .5 7.6(4.8-8.9)</td>
<td>2.7± .2 1.0(0.0-.2)</td>
</tr>
<tr>
<td>21</td>
<td>.6(.0-1.4)</td>
<td>7.5(4.3-8.5)</td>
<td>1.0(0.0-.2)</td>
</tr>
<tr>
<td>22</td>
<td>1.2(0.0-2.2)</td>
<td>6.2(4.3-8.2)</td>
<td>1.0(0.0-.2)</td>
</tr>
<tr>
<td>23</td>
<td>1.9(0.0-2.6)</td>
<td>4.2(3.1-6.1)</td>
<td>.2(0.0-.4)</td>
</tr>
</tbody>
</table>

- **Hr.** = hours after the onset of light.
- **ug of thyroxine per 100 milliliters of plasma ; mean ± standard error**
- **Means (range of values determined in 12 sparrows monitored 7 days)**
- **Analysis of variance among plasma thyroxine concentrations in samples collected at different times of day; N.S. = not significant at the 95% confidence level.**
- **Symbols: PS = photosensitive ; PST = photostimulated; PR = photorefractory**

*d*  
N.S.  
**P<.001**  
N.S.
Figure 15

The daily patterns of plasma thyroxine concentrations and locomotor activity in photosensitive White-throated Sparrows held under a LD 10:14 regimen in March. Symbols: S.E.M. = one standard error about the mean; μg% = μg of thyroxine per 100 ml of plasma. See text for a description of how activity units per hour were determined.
○ PLASMA THYROXINE (ug %) or LOCOMOTOR ACTIVITY ○
(activity units/hour)
Figure 16

The daily patterns of plasma thyroxine concentrations and locomotor activity in photostimulated White-throated Sparrows held under a LD 16:8 regimen in April. Symbols: see the legend to figure 15.
APRIL: PHOTOSTIMULATED

PLASMA THYROID HORMONE (μg%) or LOCOMOTOR ACTIVITY (Activity units/hour)

TIME (hours)

0000 0500 0900 1300 1700 2100

S.E.M.
daily patterns in these two variables except during the daytime peak of activity. Changes in plasma thyroxine levels
generally preceded corresponding changes in the levels of
locomotor activity by approximately 2 hours. Activity levels
in photostimulated sparrows held under a LD 16:8 regimen were
much lower (2.6 activity units/hour) in the afternoon compared
with peak activity levels (7.8 activity units/hour) in the
morning and nocturnal activity was intense (Figure 16), an
activity pattern characteristic of migratory species in
Zugdisposition (review; Berthold, 1975). This daily pattern
of locomotor activity similar to that reported in White-
throated Sparrows during the vernal migratory period (Eyster,
1954) was in sharp contrast to the daily activity pattern in
photosensitive birds (Figures 15 and 16). Levels of activity
in photosensitive sparrows held under a LD 10:14 regimen were
only slightly lower in the afternoon (4.8 activity units/
hour) compared with levels in the morning (6.3 activity units/
hour) and nocturnal activity was not evident (Figure 15).

Birds examined in July appeared to be in a photorefractory
condition. An almost complete change of plumage characteristic
of the postnuptial molt, nocturnal activity and fat stores
were at pre-photostimulated levels and the gonads were fully
regressed (Table X and Figure 14). A daily rhythm in plasma
thyroxine titers (Table XI and Figure 17) was not evident.
In addition the average plasma level of thyroxine (2.8 μg T4/
100 ml) was lower compared with the mean concentration (6.9 μg
T4/100 ml) in photostimulated sparrows (Figures 16 and 17).
Figure 17

The daily patterns of plasma thyroxine concentrations and locomotor activity in photorefractory White-throated Sparrows held under a LD 16:8 regimen in July. Symbols: see the legend to Figure 15.
JULY: PHOTOREFRACTORY

PLASMA THYROXINE (ug%) or LOCOMOTOR ACTIVITY (activity units/hour)

TIME (hours)

0100 0500 0900 1300 1700 2100

S.E.M.-

The daily activity pattern, similar to the pattern previously reported in photorefractory White-throated Sparrows (Eyster, 1954), resembles the pattern in photosensitive sparrows (Figures 14 and 17).
Discussion

Results of this study indicate that the plasma concentration of thyroxine in White-throated Sparrows may vary on a daily basis (Table XI). A high amplitude, unimodal, daily rhythm in plasma concentrations occurred in photostimulated sparrows (April; LD 16:8; Figure 16). Although the plasma concentration in photosensitive sparrows (March; LD 10:14; Figure 15) did not vary significantly, levels were highest in the daytime in accordance with the daily pattern reported in sexually immature male chickens kept on LD 16:8 (Newcomer, 1974). In contrast, Klandorf and co-workers (1978) reported that plasma thyroxine levels in sexually immature female chickens kept in LD 14:10 were highest at night. The difference in photoperiodic schedules may account for the difference in results of these two studies. Interestingly, the plasma concentration did not vary daily in photorefractory White-throated Sparrows (July; LD 16:8; Figure 17). Daily variations in the plasma concentrations of thyroxine have been reported in mammals (Martin et al., 1978; Meier, unpublished) and fish (Osborn et al., 1978; Spieler and Noeske, in press) also.

Plasma levels of thyroxine determined by competitive protein binding assays reported in large avian species such as chickens (Refetoff et al., 1970; Klandorf et al., 1978), geese (John and George, 1978) and ducks (Assenmacher et al., 1975; Jallageas et al., 1978) are low (range .7 to 1.7 µg T4/100 ml). Refetoff and co-workers (1970) reported higher levels of thyroxine (range 2.4 to 3.3 µg T4/100 ml) in a
smaller avian species, the pigeon. Conceivably, levels of plasma thyroxine, similar to basal metabolic rates, are inversely correlated with body size in endothermic vertebrates. Levels of thyroxine determined by cation exchange techniques (3.5 to 5.6 µg T4/100 ml; Sadovsky and Bensadoun, 1971) and by radioimmunoassay (1.48 to 3.14 µg T4/100 ml; Newcomer, 1974) reported in chickens are higher than levels determined by the competitive protein binding technique. The concentrations of thyroxine in most plasma samples collected from White-throated Sparrows of this study are within the range of levels reported in other avian species. However, the plasma thyroxine concentration in samples collected from photo-stimulated sparrows at 0100, 0500 and 2100 hours are outside this range. The concentration of thyroxine detected in plasma samples collected at 2100 were 2-fold higher than the concentration detected in samples collected at 1300 hours.

The relevance of plasma thyroxine concentrations to physiological mechanisms regulating molt, fattening, migration and gonadal development is being considered in the present study through a new approach. The concentration of thyroxine detected in plasma samples collected from White-throated Sparrows at 2100, 0100 and 0500 hours were higher in comparison with levels detected in plasma samples collected from photosensitive sparrows at these same times (Table XI). Such comparisons might be construed to indicate that there is a direct correlation between plasma thyroxine levels and gonadal development, fattening and migration.
However, plasma levels detected at other times of day are comparable and such an interpretation would not be supported (Table XI). Measurements of plasma hormone levels made at the same time of day during different seasons do not produce relevant results. Instead, the time of day plasma levels are elevated may have more relevance to physiological mechanisms regulating seasonal events than plasma levels per se.

The possibility that seasonal changes may occur in the pattern of the daily rhythm of plasma thyroxine concentration has not been considered previously. The extensive use of non-seasonal laboratory and domesticated animals is at least partially responsible for this phenomenon. The daily pattern in plasma thyroxine concentrations does change seasonally in the White-throated Sparrow. A low amplitude daily rhythm in levels of thyroxine was evident in photosensitive sparrows with peak levels occurring at midday (Figure 15). In contrast, a high amplitude daily rhythm with peak levels at night was found in photostimulated sparrows (Figure 16). A daily rhythm in plasma thyroxine levels did not occur in photorefractory sparrows (Figure 17). Seasonal changes in the pattern of other circadian hormone rhythms have been reported in the White-throated Sparrow (Meier et al., 1969; Dusseau and Meier, 1971; Meier and Fivizzani, 1975; Fivizzani and Meier, 1976) and a lizard (Trobec, 1974a). Circadian rhythms of plasma corticosterone (Meier and Fivizzani, 1975) and prolactin (Fivizzani and Meier, 1976) concentrations have been
determined in White-throated Sparrows during several seasons. Seasonal changes in the temporal relation of corticosteroid and prolactin rhythms are believed to be important in the regulation of seasonal conditions of molt, fattening, migration and gonadal development. Because the daily pattern of plasma corticosterone concentrations differ between photosensitive and photorefractory birds maintained under a constant 16 hour photoperiod, the alterations in hormone rhythms are believed to be the consequences of endogenous rather than environmental changes. Differences in the daily pattern of plasma thyroxine concentrations observed in this study between photostimulated and photorefractory birds kept under a 16 hour photoperiod are consistent with this interpretation.

The importance of temporal relations of hormone rhythms as they relate to physiological mechanisms involved in fattening, migration and gonadal development has been realized through injection studies (reviews; Meier, 1972; Meier, 1973; Meier and Ferrell, 1978). Injections of corticosterone and prolactin in different temporal relations which simulate the relations of peak plasma levels of these two hormones found during various seasons in the White-throated Sparrow have produced relevant physiological and behavioral changes. Results from the present investigation indicate that the relevance of thyroxine to physiological changes may result from a temporal interaction with corticosterone and prolactin.

Thyroxine appears to either drive circadian rhythms such as in Fundulus chrysotus (Meier, 1970) or more likely, in
endoothermic vertebrates, to permit the expression of circadian rhythms (Meier et al., 1971, John et al., 1972; Joseph and Meier, 1973). Adrenocorticotropic hormone treatment restored plasma corticosterone levels in hypophysectomized rats, but thyroxine was necessary before a daily rhythm was reestablished (Meier, 1976b). The daily rhythm of plasma thyroxine concentrations is lost and plasma levels reduced in photorefractory White-throated Sparrows in this study. A close correlation can be drawn between these results and the loss of a daily rhythm of corticosteroid concentrations reported in White-throated Sparrows maintained under conditions similar to those in the present study (Meier and Fivizzani, 1975). Birds were in photorefractory condition as evidenced by the lack of gonadal development, obvious fat stores and nocturnal activity. Furthermore, birds in both studies were undergoing a complete molt characteristic of the postnuptial molt. The simultaneous loss of corticosteroid and thyroxine rhythms may have important implications in understanding the physiological mechanisms of photorefractoriness as well as molt.

Thyroxine also acts permissively in the expression of circadian rhythms of responsiveness to prolactin injections in the pigeon (Meier et al., 1971; John et al., 1972). A rhythm of fattening sensitivity to prolactin was lost in untreated pigeons maintained for two weeks under constant illumination whereas the rhythm of sensitivity was maintained in pigeons which received thyroxine (John et al., 1972).
Furthermore, dampening out of the rhythm of fattening sensitivity to prolactin in birds kept on constant light may have resulted from the loss of a daily rhythm of plasma thyroxine. A daily variation in plasma thyroxine concentration was detected in chickens kept under light-dark cycles (Newcomer, 1974; Klandorf et al., 1978) whereas, a daily rhythm was not present in chickens kept under constant light (May, 1978). The effect of thyroxine in synchronizing circadian rhythms may have important consequences for seasonality in the White-throated Sparrow. The association of a daily rhythm of thyroxine and important vernal events such as fattening, nocturnal activity and gonadal development and the absence of a rhythm at other seasons is intriguing. The rise in thyroxine levels during a particular time of day may influence the phase of the rhythm of prolactin release; shifting the period of prolactin release into the bloodstream such that peak plasma levels occur 12 hours after the rise of plasma corticosterone levels would produce vernal conditions as demonstrated by injection studies (Meier and Martin, 1971; Meier, Martin and MacGregor, 1971; reviews Meier, 1973; Meier, 1976). As mentioned previously, the absence of these rhythms may represent an important aspect of photorefractoriness and molt.

The daily photoperiod is recognized as the most important environmental entraining agent. The daily photoperiod acts both as an entrainer of a photosensitive phase and as a photo-inducer of some event (hormone production or release) when
light coincides with the light sensitive phase (Bünning, 1936). The circadian rhythm of corticosterone is believed to be involved in the mechanism of photoperiodism (Meier and MacGregor, 1972; Meier and Martin, 1971; Meier and Dusseau, 1973). In the White-throated Sparrow, the offset of light is considered the entraining element in the photoperiodic entrainment of the corticosteroid rhythm. Plasma corticosteroid concentrations rose approximately 12 hours after the offset of lights in photosensitive White-throated Sparrows maintained on 10 hour or 16 hour photoperiods (Meier and Pivizzani, 1975). The rise in plasma corticosterone concentrations possibly entrains a photoinducible phase in turn. If light is present during this second phase the release of a hormone (prolactin or thyroxine) may be induced producing physiological changes associated with fattening, gonadal development and nocturnal activity. Interestingly, increases in the amplitude of the daily rhythm and mean plasma levels of thyroxine were directly correlated with daylength, initially. A low amplitude rhythm was determined in photosensitive sparrows held under short (LD 10:14) daylengths, whereas a high amplitude rhythm occurred in the same birds placed under long (LD 16:8) daylengths. Thyroid activity, indicated by mean plasma levels of thyroxine, was higher in sparrows kept under long daylengths (6.9 μg T4/100 ml) than under short daylengths (4.4 μg T4/100 ml) in accordance with reports in other avian species (Follett and Riley, 1967; John and George, 1978).
The influence of daylength on the daily pattern of plasma thyroxine levels may be altered by physiological changes, perhaps associated with the circannual mechanism. The daily pattern of plasma thyroxine concentrations changed seasonally in White-throated Sparrows held under constant LD 16:8 conditions. The rhythm of plasma levels found in photostimulated sparrows disappeared in sparrows believed to be photorefractory. The possibility that other environmental factors such as temperature may have affected the daily pattern of plasma thyroxine concentrations cannot be ruled out.

Warm temperatures are believed to depress thyroid activity in endothermic vertebrates in general. Low plasma concentrations of thyroxine (average; 2.2 µg T4/100 ml) determined in photorefractory sparrows which had been exposed to warm temperatures during June are in agreement with this generalization. Thyroid activity and ambient temperature appear to be inversely correlated in White-crowned Sparrows (Wilson and Farner, 1960), a species closely related to the White-throated Sparrow, and the Canada Goose (John and George, 1978) based on results from histological and assay studies, respectively.

The daily pattern of plasma thyroxine concentration may have important consequences in mechanisms regulating nocturnal locomotor activity associated with migration. There appeared to be a correlation between high plasma thyroxine and nocturnal locomotor activity levels in photostimulated sparrows (Figure 16). Daytime levels of activity did not
seem to reflect changes in the concentration of thyroxine however. The daytime peak of locomotor activity did not appear to be associated with increased levels of thyroxine in photostimulated sparrows or photorefractory sparrows (Figures 16 and 17). These findings are in accordance with results reported in obese robins (Erithacus rubecula) tested in winter (Merkel, 1958). A pulse of thyroid hormones produced by one injection stimulated nocturnal locomotor activity without influencing daytime activity levels. Interestingly, the induced nocturnal activity persisted for several days. Perhaps, peak levels of plasma thyroxine in photostimulated sparrows of this study timed a daily rhythm of nocturnal activity.

The daily pattern of plasma thyroxine concentrations in photostimulated sparrows may have important implications regarding metabolism during the migratory period. Migratory flights require great expenditures of energy. This energy is mainly derived from fat previously stored. Fat deposition, indicated by body and hepatic lipid content measured at various times of day, occurs during and continues for several hours after feeding activity (review Meier, in press). In fact, lipogenesis measured by acetate incorporation in migrant White-throated Sparrows peaks during the afternoon (Meier, 1977). Thyroxine enhances liver lipogenesis in birds (Chandrabose and Bensadoun, 1971; Goodridge, 1975, 1978) and the coincidence in the occurrence of low thyroxine and locomotor activity levels during the afternoon following a
morning activity (feeding) peak in photostimulated sparrows in Zugdisposition is consistent with the concept that low afternoon activity levels help conserve fat stores in migratory species (Dolnik and Blyumental, 1967; review: Berthold, 1975). During the actual migratory flight mobilization of stored fats is necessary to sustain prolonged muscular activity. Lipolytic activity, indicated by plasma levels of free fatty acids, is highest during the period of migratory activity (Yablonkevich, 1975). Thyroxine is a permissive agent effecting fat mobilization from mammalian adipose tissue in response to other hormones (Goodman and Bray, 1966). Because the permissive effect of thyroxine on fat mobilization may involve enzyme synthesis (Krishna et al., 1968) its effect is probably not immediate. Therefore, it is conceivable that the high plasma levels of thyroxine found preceding and coincident with intense nocturnal activity in photostimulated White-throated Sparrows may time the occurrence of adipose tissue sensitivity to other lipolytic hormones, such as glucagon.

This study provides additional data in support of the concept that circadian hormone rhythms have important consequences in physiological mechanisms regulation seasonal conditions. A high amplitude circadian rhythm of plasma thyroxine concentration appears to be involved in regulating vernal conditions of reproduction, fattening and migration in the White-throated Sparrow, possibly through temporal interactions with other circadian hormone rhythms. The loss of
a circadian rhythm of thyroxine may have important con-
sequences in physiological mechanisms controlling photo-
refractoriness. Daylength apparently has an important effect
on the daily pattern of plasma thyroxine concentrations.
The interpretation of daylength reflected in the daily
pattern changes seasonally and is possible influenced by
physiological (circannual mechanism) or environmental
(temperature) changes. Determining whether or not the daily
rhythm of thyroxine in photostimulated sparrows is causally
related to the activity rhythm associated with migration
could have important implications toward understanding
physiological mechanisms regulating migration and accompany-
ing changes in fat metabolism. These hypotheses need to be
tested further.
Part IIB: Resetting the Endogenous Circannual Mechanism in White-throated Sparrows with Corticosterone and Prolactin
INTRODUCTION

The White-throated Sparrow (Zonotrichia albicollis) like many other avian species of the temperate zone exhibits a well documented annual cycle of gonadal development, fattening, nocturnal activity and molt (Eyster, 1954; Weise, 1956; Shank, 1959; Wolfson, 1959; Helms, 1968; Meier et al., 1969). Although daylength is an important synchronizer of the annual cycle, the principal timer is an endogenous circannual mechanism (Berthold, 1974; Meier and Fivizzani, 1975; Gwinner, 1977). After the establishment of vernal migratory and reproductive conditions in White-throated Sparrows subjected to short daylengths in winter, summer and fall conditions occur sequentially in White-throated Sparrows maintained under constant LD 16:8 in a manner similar to the sequence observed in birds under natural conditions (Meier and Fivizzani, 1975). Although the overt expression of an endogenous circannual mechanism is well documented in a variety of avian species, (Merkel, 1963; Berthold et al., 1972; Gwinner, 1973, 1975; Sansum and King, 1976) few investigations have dealt with the underlying regulatory mechanism. Because the neuroendocrine system has so many regulatory functions in vertebrates it must have an important role in the circannual mechanism.

Previous investigations of White-throated Sparrows in this laboratory demonstrated circadian rhythms in the plasma levels of two hormones (corticosterone and prolactin) which changed seasonally in their phase relations with one another
(Dusseau and Meier, 1971; Meier et al., 1969). Daily injections of corticosterone and prolactin at times which simulate the temporal relations of the daily peaks of the endogenous hormones at several seasons produced appropriate seasonal conditions. The present investigation demonstrates that similar hormone treatment resets the endogenous circannual clock which is responsible for the orderly sequence of physiological and behavioral conditions in White-throated Sparrows.

The first evidence for circadian hormonal components in regulation of the annual cycle came from studies of the White-throated Sparrow (Meier and Davis, 1967; Meier, 1968). Daily injections of prolactin during the afternoon (LD 16:8) induced increased body fat stores (Meier and Davis, 1967) and nocturnal locomotor activity (Meier, 1969) whereas similar injections near the onset of light were ineffective or inhibitory. Furthermore it was found that pituitary prolactin is released in the afternoon during the vernal migratory period when the sparrows have large fat stores and are nocturnally active and near dawn in summer photorefractory birds which have low fat stores and are inactive at night (Meier et al., 1969). This shift in time of day of prolactin release may be an expression of the circannual mechanism in that daylengths are similar at the two seasons tested.

In a subsequent series of experiments corticosterone was found to be an effective entraining agent for a rhythm of fattening responsiveness to prolactin (Meier et al., 1971a).
In White-throated Sparrows maintained under conditions of continuous light in order to remove photoperiodic entrainment daily injections of corticosterone and prolactin for 10 days induced various physiological and behavioral conditions depending on the temporal relationships of the hormone injections. Increases in body fat stores (Meier and Martin, 1971), gonadal recrudescence (Meier et al., 1971a), nocturnal locomotor activity and northward orientation (Martin and Meier, 1974) indicative of vernal migratory conditions were observed in sparrows that received prolactin injections 12 hours after corticosterone. Prolactin administered 8 and 4 hours after corticosterone produced summer and autumnal conditions, respectively (see Figure 1). Assays of plasma corticosterone levels in spring and summer (Dusseau and Meier, 1971) when compared with the prolactin assays (Meier et al., 1971c) further revealed that there is a 12-hour relation between the peak of plasma corticosterone concentration and the release of pituitary prolactin in spring and an 8-hour relation in birds in summer. Furthermore the daily rhythms of both corticosterone (Fivizzani and Meier, 1976) and prolactin shift in phase as birds undergo a circannual sequence of changes while being held under constant photoperiodic conditions (LD 16:8) (Fivizzani and Meier, unpublished). These studies were the basis for an hypothesis which states that changing temporal relations among circadian neuroendocrine rhythms constitute the skeletal framework of the endogenous circannual mechanism (Meier, 1976; Meier and Ferrell, 1978).
Because corticosterone and prolactin administration in a specific temporal relation produce an entire complex of metabolic and behavioral events appropriate for a specific season, the hormone injections appear to influence central neural mechanisms which are responsible for this organization. Perhaps daily hormone injections for several days can reset a circannual clock. If so, we reasoned that vernal conditions induced by hormone injections in refractory birds should persist after the termination of treatments and that treated birds kept on a constant daylength should subsequently undergo an appropriately timed sequence of seasonal conditions corresponding to summer and autumnal conditions, respectively.
MATERIALS AND METHODS

White-throated Sparrows were captured from flocks wintering in the vicinity of Baton Rouge, Louisiana and maintained in a large outdoor aviary. One group (9 birds) was transferred indoors 23 July, 1977. A second group (9 birds) was brought indoors 22 August, 1977. Individual birds of each group were placed in small activity cages (23 cm x 36 cm x 28 cm; see Materials and Methods, section IIA) and maintained under long daily photoperiods (LD 14:10). After five (Group 1: July) or nine days (Group 2: August), light (350-400 lumens/cm²) was made continuous (LL) to remove the interference of photoperiodic entrainment. Injections (subcutaneously in the leg) of corticosterone (25 µg corticosterone/.05 cc of 0.9% saline at 0700 hours) and prolactin (25 µg prolactin/.05 cc of 0.9% saline at 1900 hours) in the 12-hour relation were carried out for 11 days to establish conditions found naturally in spring. After treatment both groups of sparrows were maintained on long daily photoperiods (LD 14:10). Observations of body fat stores and molt indices (see Materials and Methods, section IIA) were made prior to and on a weekly basis following injections. Nocturnal locomotor activity was assessed prior to and at appropriate intervals following injections. Orientational preference was assessed in birds displaying nocturnal activity. Orientational experiments were conducted with Emlen cages (Emlen and Emlen, 1966) on the roof of a building on the campus of Louisiana State University.
Tests were run between 2200-2400 hours on clear nights only. In order to analyze and present orientational data, each funnel was divided into 24 sectors of 15° each. The amount of activity represented in each sector as ink footprints was assigned a subjective value based on comparisons with standards ranging from 1 to 20 (see Emlen and Emlen, 1966). Thus, the preferred direction is represented by vectors with relatively higher assigned values. Testis size prior to injections was assessed in six aviary birds. Following injections, laparotomies were performed on selected birds to determine the volume of the left testis using the formula for an ellipsoid: 

$$\frac{4}{3}\pi \left(\frac{w}{2}\right)^2 l^{(1/2)}$$

where \(w\) = width and \(l\) = length.

Reports of studies in White-throated Sparrows maintained under constant long daylengths (Wolfson, 1960; Martin and Meier, 1973; Meier and Pivizzani, 1976; Meier, unpublished) indicate that events of the annual cycle occur in their natural sequence. However, the gonads once regressed will not redevelop unless the sparrows are kept under short daylengths for a period of time. In addition, exposure to continuous light for 2 weeks does not seem to interfere with the expression of the annual sequence of events (Meier, unpublished). Furthermore, results of previous studies in White-throated Sparrows show that seasonal conditions produced by hormone injections in specific temporal relations result from hormonal effects and not injections per se (Meier, Martin and MacGregor, 1971; Meier and Martin, 1971;
Martin and Meier, 1973; Ferrell, unpublished). Therefore, saline injected controls were not included. Several photo-refractory sparrows maintained on long daylengths were monitored for several months following 14 days of continuous light treatment in November in order to assess the effect of continuous light on the annual cycle of metabolic and behavioral events. Continuous light had no immediate influence on fat stores, molt or gonadal development in White-throated Sparrows during 2 separate experiments carried out in late fall (Ferrell, unpublished).
RESULTS

The postnuptial molt was in progress, there was no observable body fat stores and no nocturnal migratory activity in birds brought indoors in July prior to injections. The left testes were fully regressed (2.0+-.4mm$^3$) in birds taken at random from the aviary in July (Figure 18). Birds brought indoors in late August were in early autumnal migratory condition. There were moderate fat stores, nocturnal migratory activity and left testes were regressed (Figure 19). Although all birds did not respond equally well to hormone treatments, testicular size (left testis) was increased in all but one bird (5 males in each group) tested within four weeks after injection (Figures 18 and 19). One responding male died 5 weeks after injections. Testes of sparrows in Group 2 became larger than those in Group 1. The testis size of one bird was comparable to that found naturally during the peak of reproduction. Because only a few birds were tested at the apparent peak of reproductive response to hormone treatment (within a week post injection)(Figures 18 and 19), it is possible that other birds may have responded fully as well. Enlarged follicles were found in the ovaries of all but one female (4 in each group). Further quantification was unattainable. The gonads regressed to minimal nonstimulated sizes within 4-6 weeks following hormone injections and remained small during the ensuing 5 months (Figures 18 and 19).
Figure 18 - Group 1

Effects of hormone treatment of summer sparrows and subsequent patterns of gonadal development, fat stores, molt and nocturnal locomotor activity.

Photorefractory birds were placed in continuous light in late July and injected daily for 11 days with corticosterone at 0700 followed by prolactin at 1900 (12-hour relation). Following injections the birds were maintained on a long photoperiodic schedule (LD 14:10). Testis size (indicated by $\text{\%}$) prior to injections was assessed in six aviary birds. Laparotomies were performed on selected birds to determine the volume of the left testis using the formula for an ellipsoid: $4/3 \pi (w/2)^2 x (1/2)$. The fat class is based on a subjective index developed by Weise (1956) in which a value of 4 indicates maximal amounts of fat in furcular and abdominal areas, whereas a value of 0 represents no observable fat stores. Molting scores are based on an index in which a value of 12 represents maximal loss of body and wing feathers and 0 represents no observable feather loss. Nocturnal activity is represented as the percent of the total nighttime when activity occurred. Orientational preference as indicated by N (northward) and S (southward) was determined in Emlen cages under the open night sky. The shaded areas represent one standard error about the mean shown by the solid line.
Nocturnal Locomotor Activity (% of total)

Molt Index

Fat Index

Left Testis Volume (mm³ x 10)

TIME (Weeks)
Figure 19 - Group 2

Effects of hormonal treatment of late summer sparrows and subsequent patterns of gonadal development, fat stores, molt, and nocturnal locomotor activity.

Sparrows in this group were brought indoors 22 August 1977 and subjected to conditions and treatments similar to those of Group 1 (see Figure 18). * indicate index values prior to injections.
Nocturnal Locomotor Activity (% of total)

Molt Index

Fat Index

Left Testis Volume (mm³×10)

TIME (Weeks)
Dramatic increases in body fat stores occurred in 7 of 9 birds in Group 1 in response to timed injections of corticosterone and prolactin. Fat stores were gradually depleted over an 8-week period at varying rates for individual sparrows (Figure 18). Another accumulation of body fat stores occurred 16-25 weeks post injection in 5 of the 7 sparrows that responded initially (Figure 18). One of these sparrows escaped during an orientational test run. Another sparrow responding initially died 21 weeks after injections. In Group 2 initial fattening response to injections is difficult to assess because fat stores were present before injections. Fat stores decreased to low levels within 6 weeks. A second peak of fattening occurred in birds beginning 17 weeks following hormone treatment (Figure 19). Two sparrows from Group 1 and 4 birds from Group 2 exhibited nocturnal locomotor activity for 2 weeks following injections (Figure 18 and 19). Orientation was directed to the north (Figure 20). Five birds in Group 1 and 4 birds in Group 2 expressed a second period of nocturnal activity beginning 23 (Group 1) or 19 (Group 2) weeks post injection (Figures 18 and 19). Orientational preferences were directed southward in the birds tested under the open night sky (Figure 20).

Intense molt coincided with periods of low body fat stores, regressed gonads and an absence of nocturnal locomotor activity (Figures 18 and 19). Thus, it is equatable with naturally occurring postnuptial molt. There was major replacement of wing and tail feathers along with substantial replacement of
Figure 20

Orientation of hormone treated sparrows. Tests were conducted between 2200-2400 hours on clear nights only. Each vector of a diagram represents the amount of activity (as footprints) of a given bird recorded in a $15^\circ$ sector. The values of each vector were averaged for birds tested more than one night. The vector with the greatest activity is represented by the radius of each diagram. The number outside the vector diagram is a subjective value of intensity of activity for the vector equalling the radius where 20 is the highest possible intensity discernible. The arrows around the perimeter indicate the mean angular direction taken on a particular night.
body feathers, all characteristics of the postnuptial molt observed in White-throated Sparrows under natural conditions (Helms, 1968). Curiously, 2 birds (1 from each group) which did not respond in other ways to hormone treatment molted during much of the experiment.

Uninjected photorefractory White-throated Sparrows exposed to continuous light apparently remained in photorefractory condition. Left testis volume and fat stores were not increased in response to LL conditions. A minor molt was initiated approximately 3 weeks after LL treatment. This molt does not appear to correspond to the molt observed in hormone-treated sparrows in that the body molt was not intense and wing feathers were not lost. The peak molt index reached was 5.5.
DISCUSSION

Although only several birds in each group exhibited the entire range of conditions examined, it is evident in these birds that the endogenous circannual mechanism was reset by hormone injections in late summer so that the sparrows exhibited late vernal conditions following treatment and subsequently progressed to summer conditions in autumn and to autumnal conditions in winter (Figure 17). Because these hormone treatments have such profound effects on reorganizing entire physiological complexes in the White-throated Sparrow, an explanation for these effects based solely on the peripheral actions of these hormones does not seem sufficient. Rather, we believe such hormone treatments affect, through feedback mechanisms, more central neural organizational centers which in turn organize various physiological complexes necessary to produce seasonal conditions.

Because the temporal relation of corticosterone and prolactin is so important in determining the physiological and behavioral conditions in sparrows, two neural oscillations may represent the central organizational framework with corticosterone affecting one and prolactin the other. It follows that the proper temporal relation of these neural oscillations is required to produce the different seasonal conditions in White-throated Sparrows. If these two oscillations become uncoupled, as may have been the case for two birds which molted continuously, the regulation of the circannual sequence may become disrupted.
Figure 21

Comparison of hormone-induced circannual sequences with the annual cycle of White-throated Sparrows in an outdoor aviary.

Symbols: Pre = prenuptial molt; Post = postnuptial molt; N = northward orientation; S = southward orientation; hatched bars indicate the duration of hormone treatment.
In a few birds it appeared that certain events of the circannual sequence were set by hormone treatments whereas others were not. Apparently all birds initiated a postnuptial molt at approximately the same time, but two birds from Group 1 failed to respond to hormone treatment with increased body fat stores. It appears that there may be several components of the underlying circadian regulatory oscillations of the circannual sequence. These separate components may become uncoupled from the principal neural oscillation. A phenomenon has been observed in other avian species during the circannual cycle which supports this conclusion. The phase relationship among circannual rhythms of testicular size and molt in *Sylvia* warblers kept under constant conditions may change considerably (Merkel, 1963).

We believe that 2 neural circadian oscillations comprise the circannual mechanism controlling the annual cycle of molt, fattening, migration and reproduction. These neural oscillations involve individual circadian components which may become uncoupled. One oscillation controls the prolactin rhythm. Injections of the hormones are thought to set the phases of the two oscillations and thereby determine the temporal relations of the two oscillations and their circadian expressions. The temporal synergism of the two sets of circadian events determine metabolic and behavioral conditions. As the temporal relations change between the two oscillations so also do the conditions of the annual cycle.
GENERAL CONCLUSION

The annual cycle in the Green Anole and the White-throated Sparrow is synchronized by exogenous cues such that metabolic and behavioral events (eg., fattening and testicular growth) occur coincident with appropriate environmental conditions. In anoles, it appears that an interaction between the photoperiod and thermoperiod affect mechanisms which are responsible for this synchronization. This interaction is similar to that proposed to account for photoperiodism. The photoperiod entrains a circadian rhythm of fattening sensitivity to warm temperature. If the thermoperiod is such that warm temperature occurs coincident with the warm temperature sensitive phase of the temperature sensitivity rhythm an event (eg., fattening) is induced. Though the evidence is not conclusive, it appears that warmth present during the warm temperature sensitive phase entrains a rhythm of fattening rather than inducing fattening directly.

Thyroxine appears to have a permissive influence on mechanisms affecting fattening in response to warmth. However, for thyroxine to have this permissive influence, warm temperatures are required. The influence of warm temperature on testicular growth may require the presence of an entraining element such as the photoperiod.

Although synchronized by exogenous cues, events of the annual cycle are principally timed by an endogenous circannual mechanism. Measurements of plasma thyroxine made
throughout the day during several seasons supports the idea that the circannual mechanism is endogenous and involves changing temporal relations of circadian hormone rhythms. Furthermore, daily injections of corticosterone and prolactin in specific temporal relations carried out for 11 days appear to reset the endogenous circannual mechanism of late-summer birds so that their annual cycle is reinitiated from spring condition.

As a model, I propose that seasonality in vertebrates may involve changing interactions between 2 neural circadian oscillations. Each oscillation organizes a number of individual circadian components. One oscillation may control the rhythm of thyroxine. The temporal relation between these neural circadian oscillations may change seasonally thereby altering the temporal relations among the circadian components. The temporal synergism of these 2 sets of circadian systems determines metabolic and behavioral conditions. Furthermore, the circadian components may influence the relationship between these 2 neural oscillations through feedback mechanisms. The data of these studies support such a model.
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VITA

I was born in Upper Darby, Pennsylvania on 3 February 1951 and spent the next 21 years in Springfield, Pennsylvania, a suburb just outside Philadelphia. After graduating from Springfield High School in May 1969, I attended the University of Pennsylvania for the next four years. While at the university, I had the great fortune to work with Dr. Frank Gill and Dr. John Smith on a project involving hummingbird feeding strategies. It was this work which inspired me to continue my education at Western Kentucky University in Bowling Green. Under the guidance of Dr. Herbert E. Shadowen, I received a masters degree in biology in May 1975.

Since receiving my masters degree, I have had the pleasure of working with Dr. Albert H. Meier at Louisiana State University. I have gained a new perspective in viewing biological systems through his guidance. At the present time I am employed as a faculty member at Western Kentucky University.
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