1974

Circadian Mechanisms in the Hormonal Control of Annual Cycles of Fat Storage and Reproduction in the Lizard, Anolis Carolinensis.

Terry Ned Trobec

Louisiana State University and Agricultural & Mechanical College

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IN THE LIZARD, ANOLIS CAROLINENSIS.

The Louisiana State University and Agricultural
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Physiology

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Circadian Mechanisms in the Hormonal Control of Annual
Cycles of Fat Storage and Reproduction in the Lizard,
Anolis carolinensis

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Zoology
and Physiology

by

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I would like to express my appreciation to Dr. Albert H. Meier whose persistent encouragement, advice, and warm friendship made my graduate education the rich experience that it has been. I also extend thanks to Dr. Prentiss Schilling for his help with the statistical analyses. I wish all the best for the friends and colleagues who made my graduate education enjoyable. Love to my wife and daughter, Maureen and Kerry, for making it all worthwhile.
Abstract

Section I: Fat body weights in groups of lizards maintained on either a 12L:12D photoperiod or a 12(20°C):12(30°C) thermoperiod were minimal in lizards receiving injections of prolactin 4 to 8 hours after the onset of the light period or 8 hours after the onset of the cold period. Maximal fat body weights occurred in groups of lizards receiving injections of prolactin 16 hours after either the onset of the light period or the cold period. A bimodal rhythm of testes weight responses was entrained by the thermoperiod. Maximal testes weights occurred in lizards receiving injections of prolactin at 4 or 20 hours after the onset of the cold, and minimal testes weights occurred in lizards receiving injections of prolactin at 0, 8 or 12 hours after the onset of the cold.

Fat storage and reproduction were also significantly affected by repetitive daily disturbances (saline injections) at specific times of day over a 16L:8D photoperiod. Lizards disturbed 20 hours after the onset of light had suppressed fat body and testicular weights, whereas lizards disturbed 4 hours after the onset of light had fat body weights 10-times greater and testicular weights 3-times greater than the 20-hour group.

Section II: Corticosterone synchronized marked circadian rhythms of ovary weight responses to FSH or LH. Peak responses occurred in groups of lizards receiving either FSH or LH 24 hours after daily corticosterone injections, while no stimulatory effect occurred in groups
receiving either FSH or LH 8 hours after corticosterone injections. Oviducal weight responses also peaked in lizards receiving either gonadotropin 24 hours after daily corticosterone treatment but differed in the time of minimal response, 16 hours after corticosterone/FSH treatment and 8 hours after corticosterone/LH treatment. Circadian rhythms of testicular weight responses to daily prolactin injections were phased by daily injections of corticosterone or thyroxin. Corticosterone treatment phased a bimodal rhythm of testes weight responses, whereas thyroxin phased a unimodal response rhythm. Circadian rhythms of fattening responses to daily prolactin injections in the male anole and granite night lizard were phased by corticosterone treatment. The fattening response curves were unimodal with inhibitory effects occurring in groups of lizards receiving prolactin from 4 to 8 hours after corticosterone in both species and stimulatory effects 16 hours after corticosterone in the granite night lizard and 24 hours after corticosterone in the green anole.

**Section III:** A study was undertaken to determine the daily fluctuations of plasma corticosterone levels in male and female anoles at 5 times of year: February, April, July, September, and November. Mean daily plasma corticosterone levels varied on a seasonal basis according to sex. In females, corticosterone levels were high in April and July and low throughout the rest of the year, while corticosterone levels in males were high in February and July and low in April, September, and November. Distinct circadian variations in plasma corticosterone levels were determined for males in November and April and females in November.
A comparison of the circadian patterns of adrenal steroid levels for males in November and April illustrates a profound shift in the circadian rhythms. Male and female patterns of circadian fluctuations of corticosterone in November are also distinct from one another.
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SECTION I.

The Effect of Thermoperiod and Photoperiod on
Patterns of Fat Body Weight and Gonad Weight Responses
Introduction

Often the word reptile is associated with the phrase "cold blooded." What is meant by this term is that the body temperature of the reptile is directly related to the temperature of the surrounding environment. Bogert (1949) was one of the first investigators to clearly observe and explain behavioral thermoregulation in reptiles and more specifically in lizards. He observed that many lizards were able to maintain relatively stable body temperatures by selectively inhabiting microenvironments of varying temperatures throughout the day. The diurnal lizard emerging during the hours shortly following dawn exposed its head to the sun first and then the rest of its body. This behavior allowed the lizard to rapidly raise its body temperature to that level which it would attempt to maintain throughout most of the day by seeking out cooler or warmer microclimates.

Cloudsley-Thompson (1965) experimentally manipulated the thermoperiod while maintaining the photoperiod constant in order to test whether or not the daily activity rhythm of the nocturnal gecko *Tarentola annularis* and the diurnal skink *Mabuya quinquetaeniatus* could be entrained to a thermoperiod. The circadian rhythms of activity of both lizards were entrained by the thermoperiod although the activity rhythm of the diurnal lizard was more readily entrained to the thermoperiod than that of the nocturnal lizard. By advancing a 12L:12D light cycle and delaying a temperature cycle in daily increments, Evans (1966) was able to demonstrate that the circadian activity pattern of the diurnal lizard *Uta stansburiana* was entrained by the thermoperiod and not by the
photoperiod. On the other hand, in some lizards the time of daily emergence (Taylor and Tschirgi, 1960; Heath, 1962) or the time of emergence from hibernation (Mayhew, 1963) was closely coupled to the photoperiod and seemed to be independent of temperature.

Previous studies have shown that the annual cycles of fat storage and reproduction in some ectothermic and endothermic vertebrates may be regulated in part by circadian rhythms of fattening and reproductive responses to prolactin (see review, Meier, 1972). The daily rhythms of fattening responses to prolactin are entrained in several species by the daily cycle of light and dark. Circadian systems that are entrained by the daily photoperiod are also involved in stimulatory and inhibitory effects of prolactin on the avian reproductive system (see review, Meier and MacGregor, 1972).

The care and treatment of experimental animals in the laboratory may entail repeated disturbances at the same time each day. These disturbances may mimic the effects of the naturally occurring environmental stimuli of thermoperiod and photoperiod by synchronizing circadian rhythms of fattening and reproductive responses, or they may have their own profound effects on fat storage and reproduction.

The following studies were performed to test whether or not daily cycles of either light or temperature can synchronize daily rhythms of fattening and reproductive responses to prolactin and whether or not repetitive disturbances at the same time of day might also influence fat storage and reproduction in the lizard *Anolis carolinensis*. 
Materials and Methods

Adult male green anole lizards, *Anolis carolinensis*, were collected in the vicinity of La Place, Louisiana. Groups of lizards were placed in screen-covered plastic cages and kept in incubators that afforded adjustable light and temperature regimens. The animals were provided daily with an abundance of crickets and water sprinkled onto foliage in the cages.

In one experiment, July 8 to July 23, 1971, lizards were maintained on continuous light with a daily thermoperiod of 12 hours at $20^\circ \pm 2^\circ C$ and 12 hours at $30^\circ \pm 2^\circ C$ for one week prior to experimentation and throughout the experimental period. Three groups of animals were housed in each of two separate incubators in which the temperature cycles were set 180 degrees apart, i.e., while the temperature of one incubator was $20^\circ C$, the temperature of the other incubator was $30^\circ C$. Changes in temperature were made daily at 0700 and 1900 hours. Temperature shifts to $30^\circ C$ and $20^\circ C$ were both accomplished in 60 minutes. Daily injections of prolactin (1.0 $\mu g/g$ body weight) were administered at 0700, 1100, or 1500 to three groups of lizards in each of the two incubators. This regimen provided 6 separate injection times during a 24-hour temperature cycle, i.e., 0, 4, 8, 12, 16, or 20 hours after the onset of the "cold" period ($20^\circ C$). This relationship made it feasible to discern possible manifestations of a free-running rhythm of daily responses to prolactin injections. Prolactin injections were continued for 8 days, and the experiment was terminated on the 9th day for the examination of testes and fat body weights.
In another experiment, July 30 to August 13, 1971, lizards were maintained at a constant temperature of $30^\circ \pm 2^\circ$C and on a 12L:12D photoperiod for one week prior to the initiation of prolactin injections and for the duration of the experimental period. The experimental procedure was the same as that of the first experiment except that the daily injections of prolactin were given at 0, 4, 8, 12, 16, or 20 hours after the onset of light. Daily injections of prolactin were also administered for 8 days in this experiment.

At the termination of the experiments, the lizards were killed and the fat bodies and testes were removed and weighed. A number of lizards maintained under the experimental conditions of the respective studies during the one week acclimation period were killed at the beginning of the experiments, July 15th for the thermoperiod study and August 5th for the photoperiod study. These groups are referred to as the initial controls for the respective studies. The final controls for these experiments were maintained under experimental conditions during the acclimation periods and throughout the experimental periods, but did not receive prolactin injections. They were killed at the end of the experiments, July 23rd for the thermoperiod study and August 13th for the photoperiod study.

Because it seemed possible the numerous daily disturbances (handling and injections) of the lizards in the aforementioned experimental designs might have important effects, an experiment was performed to test whether there were daily variations in the effects of handling on fat storage and testicular weights in the green anole. The adult male lizards were maintained on a 16-hour daily photoperiod at $30^\circ \pm 2^\circ$C for at least 10 days prior to and throughout the experimental period. The lizards were housed
in two separate incubators where the photoperiods were staggered. The 16 hour photoperiod began in one incubator at 0800 and in the other at 2000 and the disturbances were made at 0800, 1200, or 1600. Thus, disturbances were made at 3 times of day and at 6 times relative to a 16 hour daily photoperiod. The daily disturbances were carried out for 10 days and consisted of subcutaneous injections of small amounts of 0.67% saline (0.03 ml). Food and water were provided at 3 times each day corresponding with the times of the treatments. The lizards were killed on the 11th day and the fat bodies and testes were removed and weighed.

The data from the experiments in this section were analyzed statistically by the least-squares analysis of variance and by orthogonal comparisons.
Results

Patterns of daily variations in fattening responses to prolactin were found under both the temperature cycle regimen and the light cycle regimen (Figure 1, and Tables I and II). Weights of fat bodies in a group of lizards that received daily injections of prolactin administered 16 hours after the onset of light were almost 2 1/2 times greater than fat body weights of the untreated controls, whereas those groups that received daily hormone injections administered at 4 or 8 hours after onset of light had fat stores comparable to fat stores of the untreated controls. In the experiment with a thermoperiod, those lizards that received daily prolactin injections 8 hours after the onset of the cold had fat bodies that weighed 1/3 of those of the untreated controls.

Although the patterns of fattening responses that were set by the temperature and light cycles were similar when the light period is aligned with the cold period, the patterns of gonadal responses to injections of prolactin may vary. There was a distinct bimodal pattern of gonadal responses in those lizards maintained on a thermoperiod (Figure 2 and Table II). The lowest weights of paired testes were found in the two groups of lizards that received prolactin at 0 and 12 hours after the onset of the cold period. In those lizards maintained on a photoperiod, there was an apparent inhibition of testes weights in lizards that received prolactin injections only at one time of the day, at 16 hours after the onset of light (Figure 2 and Table I). However, because of large intragroup differences in gonadal weights, the pattern of gonadal responses was not statistically verified, and therefore it cannot be concluded that the gonadal response rhythms obtained under a photoperiod or
a thermoperiod differ.

Daily handling disturbances (saline injections) markedly affected both fat body weights and testes weights of male anoles (Figure 3 and Table III). The weights of the testes and of the abdominal fat bodies were greatly suppressed by the disturbances in the 20-hour group. The peak for both response rhythms was in the 4-hour group. The magnitude of the differences resulting from the times of disturbances is illustrated by the fact that the fat bodies were 10 times heavier in the 4-hour group than in the 20-hour group. Similarly, the mean testes weights were more than twice as heavy in the 4-hour group as in the 20-hour group. The shapes of the two response rhythms have quadratic relationships (p<0.01) and the differences resulting from handling at various times are both highly significant (p<0.01).
Discussion

The annual cycles of fat storage and reproduction in the green anole *Anolis carolinensis* are regulated apparently by seasonal changes of both temperature and daylength (for data and reviews: Dessauer, 1955a, b; Licht, 1971). Licht believes that temperature is the more significant environmental determinant from October to June, and that the length of the photoperiod is important from June to October. The results of this study indicate that daily cycles of both light and temperature can synchronize rhythms of fattening and reproductive responses to prolactin. Either a photoperiod or a thermoperiod may entrain daily rhythms of fettening and reproductive responses. These results reflect the circadian nature of the organizational structure of lipid metabolism and reproduction.

Injections of corticosterone can entrain rhythms of fattening responses to prolactin injections in *Anolis carolinensis* (Section II of this paper; Meier, Trobec, Joseph and John, 1971; Meier, 1972; Trobec, 1974) and in the granite night lizard *Xantusia henshawi* (Section II of this paper; Meier, 1972; Trobec, 1974) maintained in continuous light, it is possible that the rhythms of fattening responses synchronized by the photo- and thermoperiod were mediated by the interrenal system. The adrenal steroids also synchronize daily rhythms of fattening and of stimulatory and inhibitory responses of testes weights in some birds (Meier and Martin, 1971; Meier, Martin and MacGregor, 1971; Meier, 1972) and fish (Meier, Trobec, Joseph and John, 1971; Meier, 1972).
A possible mechanism of action in the case of disturbance is that the release of the corticosterone would occur shortly after the disturbance as noted in many species, including mice (Haus and Halberg, 1962), or it might also occur at a later time if the disturbance acts as an entraining agent which indirectly adjusts the daily rhythms of corticosterone. Because daily rhythms of prolactin (Clark and Baker, 1964; Kent, Turnbull, and Kirby, 1964; Meier, Burns and Dusseau, 1969; Butcher, Fugo and Collins, 1972) peak at specific times with respect to the photoperiod, the disturbances may set up temporal patterns between corticosterone released by disturbance and the daily rhythm of prolactin. When temporal relations between these hormones are established by daily injections of prolactin or adrenal corticoids, the fat stores and reproductive system are stimulated or inhibited depending on the temporal pattern (for review see, Meier, 1972).

The release of corticosterone as a result of handling might also affect the reproductive system by entraining the photoinducible phases (Meier and MacGregor, 1972). According to this concept, corticosterone sets the times when light can stimulate the release of the gonadotropic hormones. Depending on the time of handling, then, the photoinducible phases may be set so that they occur during the light (resulting in gonadal stimulation) or during the dark (resulting in gonadal inhibition).

In the quail Coturnix c. japonica, daily disturbances (injections of saline) produce responses similar to those caused by injections of corticosterone (Meier, Trobec, Haymaker, MacGregor and Russo, 1973). However, disturbances may also cause the release of prolactin in some animals (Nicoll, Talwalker, and Meites, 1960). Consequently, the dis-
turbance effect may be a complicated one and further study is needed to establish the mechanism by which disturbance affects the anole. The results of the disturbance experiment serve as a warning to minimize handling effects in experimental animals. The experimental animals in all studies reported in this paper were handled with great care.
Table I. Daily Variations in Fattening and Gonadal Responses to Prolactin Injections in Lizards Maintained on a Photoperiod, 12L:12D.

<table>
<thead>
<tr>
<th>TIME (hrs after onset of light)</th>
<th>(N)</th>
<th>BODY WEIGHT (gm)</th>
<th>FAT BODIES (% body wt.)</th>
<th>PAIRED TESTES WEIGHTS (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Controls(^b)</td>
<td>(5)</td>
<td>4.11</td>
<td>1.17 ± 0.36(^a)</td>
<td>19.2 ± 2.2(^a)</td>
</tr>
<tr>
<td>Final Controls(^c)</td>
<td>(5)</td>
<td>4.05</td>
<td>0.71 ± 0.33</td>
<td>26.7 ± 3.5</td>
</tr>
<tr>
<td>0</td>
<td>(6)</td>
<td>4.53</td>
<td>0.90 ± 0.21(^d)</td>
<td>23.2 ± 6.0</td>
</tr>
<tr>
<td>4</td>
<td>(6)</td>
<td>4.17</td>
<td>0.64 ± 0.28</td>
<td>23.9 ± 2.6</td>
</tr>
<tr>
<td>8</td>
<td>(4)</td>
<td>3.89</td>
<td>0.68 ± 0.27</td>
<td>21.9 ± 1.1</td>
</tr>
<tr>
<td>12</td>
<td>(7)</td>
<td>4.53</td>
<td>1.05 ± 0.23</td>
<td>18.6 ± 2.9</td>
</tr>
<tr>
<td>16</td>
<td>(7)</td>
<td>4.80</td>
<td>1.84 ± 0.26</td>
<td>15.6 ± 2.5</td>
</tr>
<tr>
<td>20</td>
<td>(6)</td>
<td>4.79</td>
<td>1.04 ± 0.42</td>
<td>23.5 ± 4.0</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± Standard Error.

\(^b\) Lizards maintained under experimental conditions during the one week acclimation period were killed at the beginning of the experiment, August 5, 1971.

\(^c\) The final controls were maintained under experimental conditions during the acclimation period and throughout the experimental period, but did not receive prolactin injections. They were killed at the end of the experiment, August 13, 1971.

\(^d\) The pattern of fat body weight responses to prolactin is significant at p<0.05.
Table II. Daily Variations in Fattening and Gonadal Responses to Prolactin Injections in Lizards Maintained on a Thermoperiod, 12 Hours at 20°C and 12 Hours at 30°C.

<table>
<thead>
<tr>
<th>TIME (hrs after onset of cold)</th>
<th>(N)</th>
<th>BODY WEIGHT (gm)</th>
<th>FAT BODIES (% body wt.)</th>
<th>PAIRED TESTES WEIGHTS (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Controls b</td>
<td>(6)</td>
<td>4.71</td>
<td>0.43 ± 0.13**</td>
<td>45.5 ± 4.7**</td>
</tr>
<tr>
<td>Final Controls c</td>
<td>(7)</td>
<td>4.58</td>
<td>0.69 ± 0.15</td>
<td>43.7 ± 3.8</td>
</tr>
<tr>
<td>0</td>
<td>(14)</td>
<td>4.24</td>
<td>0.67 ± 0.14</td>
<td>39.2 ± 3.4</td>
</tr>
<tr>
<td>4</td>
<td>(8)</td>
<td>4.72</td>
<td>0.61 ± 0.12</td>
<td>48.4 ± 1.8</td>
</tr>
<tr>
<td>8</td>
<td>(9)</td>
<td>4.66</td>
<td>0.26 ± 0.09</td>
<td>38.7 ± 2.2</td>
</tr>
<tr>
<td>12</td>
<td>(15)</td>
<td>4.29</td>
<td>0.69 ± 0.11</td>
<td>36.2 ± 3.4</td>
</tr>
<tr>
<td>16</td>
<td>(6)</td>
<td>4.72</td>
<td>0.91 ± 0.34</td>
<td>41.3 ± 4.1</td>
</tr>
<tr>
<td>20</td>
<td>(7)</td>
<td>4.67</td>
<td>0.69 ± 0.16</td>
<td>50.7 ± 5.0</td>
</tr>
</tbody>
</table>

* Mean ± Standard Error.

** A number of lizards maintained under experimental conditions during the one week acclimation period were killed at the beginning of the experiment, July 15, 1971.

* The final controls were maintained under experimental conditions during the acclimation period and throughout the experimental period, but did not receive prolactin injections. They were killed at the end of the experiment, July 23, 1971.

* The pattern of fat body weight responses to prolactin is significant at p<0.05, and the pattern of testes weight responses to prolactin is significant at p<0.01.
Table III. Daily Variations in the Effects of Handling on Fat Storage and Testicular Weights in the Lizard, *Anolis carolinensis*.

<table>
<thead>
<tr>
<th>TIME (hrs after onset of light)</th>
<th>(N)</th>
<th>BODY WEIGHT (gm)</th>
<th>FAT BODIES (% body wt.)</th>
<th>PAIRED TESTES WEIGHTS (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Controls (^b)</td>
<td>(11)</td>
<td>4.91</td>
<td>0.65 ± 0.08(^a)</td>
<td>77.7 ± 3.7(^a)</td>
</tr>
<tr>
<td>Final Controls (^c)</td>
<td>(9)</td>
<td>4.72</td>
<td>1.02 ± 0.18</td>
<td>59.8 ± 3.8</td>
</tr>
<tr>
<td>0</td>
<td>(11)</td>
<td>4.67</td>
<td>0.80 ± 0.17</td>
<td>64.2 ± 4.8</td>
</tr>
<tr>
<td>4</td>
<td>(8)</td>
<td>5.40</td>
<td>1.18 ± 0.13</td>
<td>69.0 ± 3.0</td>
</tr>
<tr>
<td>8</td>
<td>(10)</td>
<td>4.87</td>
<td>0.84 ± 0.17</td>
<td>64.8 ± 5.9</td>
</tr>
<tr>
<td>12</td>
<td>(10)</td>
<td>4.54</td>
<td>0.45 ± 0.08</td>
<td>57.0 ± 4.1</td>
</tr>
<tr>
<td>16</td>
<td>(8)</td>
<td>4.15</td>
<td>0.35 ± 0.13</td>
<td>50.6 ± 4.6</td>
</tr>
<tr>
<td>20</td>
<td>(7)</td>
<td>3.70</td>
<td>0.12 ± 0.02</td>
<td>27.7 ± 2.8</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± Standard Error.

\(^b\) Lizards maintained under experimental conditions during the one week acclimation period were killed at the beginning of the experiment, March 14, 1972.

\(^c\) The final controls were maintained under experimental conditions during the acclimation period and throughout the experimental period, but did not receive saline injections. They were killed at the end of the experiment, March 24, 1972.

\(^d\) Patterns of fat body weights and testes weights responses to handling are significant at p<0.01.
Figure 1.

Diurnal patterns of fattening responses to prolactin entrained by a thermoperiod (A) and/or a photoperiod (B). The thermoperiod consisted of 12 hours at 20°C and 12 hours at 30°C. The photoperiod consisted of 12 hours of light and 12 hours of darkness. Responses are expressed as percentages about the mean of all 6 sampling times.
Fattening Responses to Prolactin (Percentages about the Means)

Time of Prolactin Injections (Hours)

0 3 6 9 12 15 18

SAMPLE

LIGHT

DARK

COLD

WARM

A

B
Figure 2.

Diurnal patterns of testes weight responses to prolactin entrained by a thermoperiod (A) (p<0.05) and/or a photoperiod (B) (p>0.05). The thermoperiod consisted of 12 hours at 20°C and 12 hours at 30°C. The photoperiod consisted of 12 hours of light and 12 hours of darkness. Responses are expressed as percentages about the mean of all 6 sampling times.
Effect of Prolactin on Testes Weights (Percentages about the Means)
Figure 3.

Fat body weight and testes weight responses to disturbance (saline injections) administered daily at 6 times of day on a 16L:8D photoperiod. Fat body weights are reported as % body weight and testes weights as paired testes weights in milligrams. The values are reported as the mean ± the standard error (S.E.). The open circles are fat body weights and solid squares are testes weights.
SECTION II.

The Role of Corticosterone as Phaser of Patterns of Fat Body Weight and Gonad Weight Responses
Introduction

Daily photoperiods and/or thermoperiods can synchronize circadian patterns of fat storage and reproductive responses to injections of prolactin and/or disturbance (Section I of this paper). Daily variations in the responses suggest that another system sets the time of tissue responses. In an earlier study by Meier (1970), this system was affected by thyroxin injections administered to a fresh water killifish, Fundulus chrysotus. On continuous bright light, injections of thyroxin set up a period of fattening sensitivity 18 hours later, a period of sensitivity that recycles every 24 hours with no additional injections of thyroxin. Fattening results when prolactin is given daily during the sensitive period. Injections of saline in place of thyroxin had no entraining activities. It was concluded that injections of thyroxin set the biological time clock of a system capable of circadian oscillations under conditions of continuous light, a system that sets the temporal pattern of fattening responses to prolactin.

Because the adrenocortical hormones have pervasive influences throughout the vertebrate body, and because the plasma levels exhibit circadian oscillations that are synchronized by the photoperiod (Halberg, 1969), it seems possible that a daily rhythm of plasma corticosterone in the green anole may phase rhythms of fattening and reproductive responses to prolactin and possibly other hormones. The experiments reported in this section were designed to investigate the effects of injections of corticosterone on synchronizing circadian patterns of fattening and reproductive responses to injections of prolactin, FSH, or LH.
A. The Temporal Synergism of Corticosterone and Prolactin on Fat Storage

1. Corticosterone/Prolactin in *Anolis* and *Xantusia*

Green anole lizards, *Anolis carolinensis*, were collected in the vicinity of La Place, Louisiana. Granite night lizards, *Xantusia henshawi*, were obtained from Southwestern Herpetological Research and Sales, Calimesa, California. Groups of lizards were kept in screen-covered plastic cages and in incubators at $30^\circ \pm 2^\circ$C with continuous bright light. The animals were provided daily with an abundance of crickets and water sprinkled onto foliage in the cages.

Two experiments were performed to study the effectiveness of corticosterone in entraining daily rhythms of fattening responses to prolactin. In the first experiment, male green anoles were divided into 2 groups receiving daily injections of corticosterone (1 μg/g body weight); one group received the hormone at 0600 and the other received the hormone 12 hours later. Both groups receiving corticosterone were further divided into 4 subgroups receiving daily injections of ovine prolactin (1 μg/g body weight) at 0600, 1200, 1800, or 2400. This method made it possible to compare 2 groups receiving daily prolactin injections at 0, 6, 12, or 18 hours after adrenal steroid treatment and provided a simple means of determining whether the variations in the responses to prolactin were set by the time of injection of corticosterone. The lizards were placed in the incubators on
May 7, 1970. Injections of corticosterone were initiated on May 22 and of prolactin on May 27. Daily injections were continued until June 5 when all the animals were killed and the fat bodies weighed. A group of green anoles (initial controls) maintained under the experimental conditions during the acclimation period were killed at the beginning of the experiment, May 22. The final controls were maintained under experimental conditions during the acclimation period and throughout the experimental period, but did not receive hormone injections. They were killed at the end of the experiment, June 5.

Granite night lizards were the test animals for the second experiment which had a design similar to the first experiment except that the 2 groups that received corticosterone, 0600 or 1800, were each divided into 6 subgroups that received daily injections of prolactin at 0400, 0800, 1200, 1600, 2000, or 2400. The acclimation period was begun on January 7, 1971. Daily injections of corticosterone were initiated on January 29 and of prolactin on February 3. Daily injections were continued until February 9 when all the animals were killed and total ether extractable lipids were determined by means of Soxhlet equipment. Initial and final control values were obtained as in the first experiment.

2. Saline/Prolactin in Anolis

A third experiment was performed to investigate the effect of saline injections on synchronizing daily rhythms of fattening responses to prolactin. Male anole lizards collected as above
were maintained in screen-covered plastic cages with continuous bright light and at 30° ± 3°C. The animals were fed crickets in abundance and water was daily sprinkled onto foliage in the cages. The lizards received daily injections of saline at 2400. These lizards were divided into 4 groups that received daily injections of ovine prolactin (1 μg/g body weight) at 2400, 0600, 1200, or 1800. This resulted in 4 different groups receiving injections of prolactin at 0, 6, 12, or 18 hours after injections of saline. Injections of saline commenced on October 29, 1969, and continued through October 31. Saline injections were again administered on November 4 and 7. The daily injections of prolactin were initiated on November 2 and continued until the end of the experiment on November 8 at which time all the lizards were killed and their fat bodies were dissected out and weighed.

B. Temporal Synergism of Corticosterone and a Second Hormone on the Reproductive System

1. Corticosterone/FSH and Corticosterone/LH in Female Anolis

Two experiments were performed on Adult female green anoles maintained in incubators at 30° ± 2°C and under continuous bright light. An abundant supply of crickets and water were provided daily. In the first experiment, lizards were divided into 2 groups receiving daily corticosterone injections (2 μg/g body weight); one group received the hormone at 0900 and the other received the hormone at 2100. Each group was then divided into 3 subgroups that received daily injections of ovine FSH (2 μg/
g body weight) at 0900, 1300, or 1700. This design created 6 groups that received daily FSH injections at 0, 4, 8, 12, 16, or 20 hours after daily injections or corticosterone. Daily injections of corticosterone were initiated on July 14, 1971, and continued to the end of the experiment on July 28. Daily injections of FSH commenced on July 19 and continued until July 28 at which time all lizards were killed and their ovaries and oviducts were dissected out and weighed.

The second experiment was designed similarly except that the 2 groups receiving corticosterone injections were each subdivided into 3 groups that received daily injections of LH (2μg/g body weight) at 0900, 1300, or 1700. Daily injections of corticosterone were begun on May 19, 1971, and daily injections of LH on May 24. The daily injections continued until the end of the experiment June 1 at which time the lizards were killed and the ovaries and oviducts were dissected out and weighed. In both experiments, lizards were maintained under experimental conditions of light and temperature regimen for at least 2 weeks prior to the beginning of hormone injections.

2. Corticosterone/FSH and Corticosterone/LH in Male Anolis

Adult male anoles were used in the 2 following experiments. One experiment investigated the temporal synergism of corticosterone/FSH and the other investigated the temporal synergism of corticosterone/LH. The experimental designs were like those of the 2 experiments on the female lizards mentioned above. The lizards in these 2 experiments were maintained under experimen-
tional conditions of light and temperature regimen for at least 2 weeks prior to the beginning of hormone treatment. The injection regimen was similar in both of the experiments; 5 days of daily injections of corticosterone prior to the beginning of daily injections of either FSH or LH and then daily injections of both hormones for 8 more days. The animals were killed on the 9th day and paired testes weights were determined. Tables VI and VII may be referred to for further information concerning the experimental regimens.

3. Corticosterone/Prolactin and Thyroxin/Prolactin in Male Anolis

Two experiments were performed to investigate the effectiveness of either corticosterone or thyroxin to synchronize patterns of gonadal responses to injections of prolactin. In the first experiment, lizards were maintained as in the experiments immediately above in Section II. The lizards were divided into 8 groups 4 of which received daily injections of prolactin (2 µg/g body weight) at 0, 6, 12, or 18 hours after daily injections of corticosterone (2 µg/g body weight) and 4 of which received daily injections of prolactin at 0, 6, 12, or 18 hours after daily injections of saline. An acclimation period of 2 weeks preceded the initiation of corticosterone or saline injections. Daily injections of corticosterone or saline were administered for 5 days prior to the start of daily injections of prolactin. Daily injections of both hormones continued for 8 more days. The animals were killed on the 9th day and paired testes weights were determined. Table VIII may be referred to
for further information concerning the experimental regimen.

The second experiment involved male anoles maintained in screen-covered plastic cages with continuous bright light at $30^\circ \pm 2^\circ C$. The lizards received crickets and water daily. The animals were divided into 4 groups that received daily injections of prolactin ($1\mu g/g$ body weight) at 0, 6, 12, or 18 hours after injections of thyroxin ($1\mu g/g$ body weight). Injections of thyroxin were administered on October 29, 1969 through October 31 and again on November 4 and 7. Daily injections of prolactin commenced on November 2 and continued until the end of the experiment on November 8 at which time all the lizards were killed and their testes were dissected out and weighed.

The data presented in this section were analyzed by use of the least-squares analysis of variance and orthogonal comparison.
Results

A. The Temporal Synergism of Corticosterone and Prolactin on Fat Storage

1. Corticosterone/Prolactin in Anolis and Xantusia

Daily injections of corticosterone entrained similar circadian patterns of fattening responses to daily injections of prolactin in both the green anole (p<0.05) and the granite night lizard (p<0.05). In the green anole, injections of prolactin administered 6 hours after corticosterone treatment inhibited fat body growth regardless of whether the adrenal steroid was administered at 0600 or 1800 hours, whereas injections of prolactin administered 18 to 24 hours after corticosterone treatment stimulated fat body growth (Figure 4 and Table IV). However, in the granite night lizard, injections of prolactin administered 4 hours after corticosterone treatment suppressed lipid content, whereas injections of prolactin administered 12 hours later increased lipid content (Figure 4 and Table IV). Thus, the temporal synergism between injections of corticosterone and prolactin has significant effects in the regulation of fat storage in these 2 genera of lizards.

2. Saline/Prolactin in Anolis

Daily injections of saline were able to synchronize circadian patterns of fattening responses to daily injections of prolactin in the green anole (p<0.01). Fat Storage was highest in the group receiving injections of prolactin 12 hours after saline
injections and lowest at 18 hours after saline injections (Figure 4 and Table IV). The response curves for lizards that received corticosterone/prolactin treatment and saline/prolactin treatment are both unimodal. However, the time of maximal fat storage in the 2 treatments differs by 12 hours and the time of greatest inhibition of fat storage also differs by 12 hours (Figure 4 and Table IV).

B. Temporal Synergism of Corticosterone and a Second Hormone on the Reproductive System

1. Corticosterone/FSH and Corticosterone/LH in Female Anolis

   The temporal synergism between corticosterone and various hormones also has important effects on the reproductive system of the green anole. Similar circadian patterns of gonadal responses to daily injections of FSH or LH in female anoles were synchronized by daily injections of corticosterone (p < 0.01). Injections of either of the 2 gonadotropic hormones, FSH or LH, 8 hours after corticosterone treatment had no noticeable effect on ovary weights when compared to the untreated final controls (Figure 5 and Table V and Table VI). However, injections of FSH or LH 24 hours after corticosterone treatment caused increases in ovary weights 2 times over those of the untreated controls.

   The circadian response patterns of oviducal weights like those of the ovary weights followed a quadratic curve (p < 0.01). Maximal oviducal weights were obtained in those groups receiving either FSH or LH 24 hours after the administration of cortico-
sterone, while the time of minimal response to gonadotropic hormone injections varied depending on the hormone, 8 hours after corticosterone treatment for lizards that received LH and 16 hours after corticosterone treatment for those lizards that received FSH (Figure 5 and Table V and Table VI).

2. Corticosterone and Thyroxin as Synchronizers in Male *Anolis*

The testes weights of male anoles were also significantly altered by temporal synergisms between 2 hormones. Daily injections of prolactin given at 4 different times of day with respect to daily injections of corticosterone elicited a bimodal pattern of testes weights ($p<0.01$) (Figure 6 and Table IX), whereas daily injections of prolactin administered at 4 different times of day with respect to daily injections of thyroxin or saline elicited a unimodal pattern of testes weights ($p<0.05$ and $p<0.01$, respectively) (Figure 6 and Table IX and Table X).

Prolactin injections administered at 6 or 18 hours after corticosterone treatment increased testes weights, however prolactin administered at 12 or 24 hours after corticosterone treatment had no effect on testes weights when compared with the final controls (Figure 6 and Table IX). A stimulatory effect on testes weights in groups of lizards receiving daily injections of saline/prolactin occurred in those lizards receiving prolactin 18 hours after saline, whereas an inhibitory effect occurred in those receiving prolactin 12 hours after saline (Figure 6 and Table IX).

In the second experiment, testes weights were highest in
those lizards that received injections of prolactin at 12 or 18 hours after treatment with thyroxin and lowest in those lizards that received injections of prolactin 24 hours after thyroxin treatment. Non-hormone injected controls were not included in this experiment. (Figure 6 and Table X).

Because of large intragroup variations, the patterns of testes weight responses to corticosterone and either FSH or LH could not be verified statistically, which precludes any judgment that these response patterns differ from that obtained with corticosterone/prolactin or thyroxin/prolactin (Tables VII and VIII).
Discussion

A. The Effects of a Temporal Synergism of Corticosterone and Prolactin on Fat Storage

The temporal relationship between corticosterone and prolactin is of considerable importance in the regulation of fat storage in both the green anole and the granite night lizard. While injections of corticosterone entrained circadian rhythms of sensitivity in both lizards, the phase angles of the response rhythms differed between the 2 species. Maximum stimulation of fat storage occurred 16 hours after injections of corticosterone in the granite night lizard and 24 hours after injections of corticosterone in the green anole. A possible explanation for these differences may be related to the activity patterns of these animals. The granite night lizard is a nocturnally active lizard, while the green anole is diurnal. The environmental stimulus that synchronizes endogenous circadian rhythms may vary in the 2 genera of lizards, i.e., onset of the photoperiod for one and the termination of the photoperiod for the other or rising temperatures for one and falling temperatures for the other. These environmental stimuli may then synchronize endogenous circadian fluctuations of corticosterone which would set up either inhibitory or stimulatory temporal synergisms with endogenous prolactin.

While injections of saline were able to synchronize a circadian pattern of fattening responses to injections of prolactin in the green anole, the response pattern differed from the response pattern
entrained by corticosterone. As was proposed earlier (Section I), the disturbance of lizards (injection of saline) may produce its effects by triggering a release of endogenous corticosterone, either immediately or at some specific time after disturbance. However, the disturbance response is clearly and distinctly overridden by the injection of exogenous corticosterone in the green anole. Still another explanation for the saline/prolactin results could be that the exogenous prolactin was interacting with endogenous circadian rhythms of corticosterone. Even though continuous light tends to dampen circadian rhythms and for that reason was employed to decrease temporal interference from endogenous systems, there seems to be some evidence that some of these rhythms persisted. In the experiment with corticosterone/prolactin treatment of the anole (Table IV), the fat bodies were heavier in the groups receiving corticosterone at 1800 than at 0600. It appears that daily rhythms of endogenous prolactin were in synchrony among the experimental animals, and that the time of injection of the adrenal steroid with respect to the release of endogenous prolactin was more favorable for fattening at 1800 than it was at 0600.

The role of daily rhythms of prolactin and corticosterone in the control of seasonal levels of fat stores has been studied most extensively in the white-throated sparrow, Zonotrichia albicollis (Meier and Davis, 1967; Meier, Burns, and Dusseau, 1969; Dusseau and Meier, 1971). For each of the 4 seasons when the birds were in 4 distinct physiological conditions, there was a specific rhythm of plasma adrenal steroids having individual phase angles with respect to the photoperiod. During each of the 4 seasons there was also a
specific rhythm of prolactin release from the pituitary. The temporal synergism between these endogenous rhythms varies from season to season. Injections of exogenous corticosterone and prolactin administered in temporal synergisms that mimic the endogenous oscillations during the 4 seasons to sparrows maintained on continuous bright light elicited physiological conditions like those of feral birds at each of the 4 seasons (Meier and Martin, 1971; Meier, 1972).

The green anole also experiences seasonal cycles of fat stores (Dessauer, 1955a,b) and daily rhythms of plasma corticosterone levels that vary from season to season (Section III of this paper). Although there is no present information about the circadian release of prolactin from the pituitary of the anole, it appears that a regulatory mechanism of temporal synergisms between daily rhythms of corticosterone and prolactin might also control seasonal physiological and developmental changes in the green anole.

B. Temporal Synergism of Corticosterone and a Second Hormone on the Reproductive System.

Temporal synergisms involving corticosterone not only cause changes in fat stores in the green anole, but they also affect the reproductive system as well. Corticosterone/FSH and corticosterone/LH treatment elicited similar circadian patterns of ovary weight responses. Maximal stimulation of the ovary, an increase of approximately 100% over controls, occurred when injections of either FSH or LH were administered 24 hours after injections of corticosterone, whereas essentially no increase in ovary weights compared to controls
was observed in groups of lizards receiving injections of either FSH or LH 8 hours after injections of corticosterone.

Chemical and biological studies of the action of mammalian gonadotropins suggest that in reptiles hormones which have FSH-like activities in mammals are able to stimulate gonadal development, ovulation, and steroid biosynthesis under certain conditions (for review see, Callard, Chan, and Potts, 1972). Evans (1935) observed that extracts of mammalian pituitaries elicited marked development of the ovary of the green anole. More recent studies by Licht (1970) have indicated that injections of purified mammalian FSH can stimulate ovary and oviducal development. Studies using mammalian LH have indicated that this hormone can also stimulate ovary and oviducal development but is much less potent than mammalian FSH (Licht and Stockell-Hartree, 1971). On the other hand, another study has indicated that injections of mammalian FSH in dose levels comparable to those administered in the present investigation had no effect on ovary weight in the green anole (Jones, Roth, Gerrard, and Kiely, 1973). Jones and coworkers attributed this response to a temperature effect which prevented the ovaries from responding to the exogenous FSH.

There is still a great deal of confusion about the regulation of seasonal cycles of ovarian development in lizards. Part of this confusion is due to the fact that most of the investigators do not consider the importance of a circadian pattern of sensitivity to exogenous gonadotropins. For instance, the lack of a response to FSH as reported by Jones and coworkers (1973) may have occurred as a result of the time of day when the injections were given. The ovarian response in the present study (0% to 100% increase) depending on the
time of injection again points out the importance of circadian sys-
tems in physiological conditions.

Although corticosterone/FSH and corticosterone/LH treatment re-
sulted in similar response patterns in the male anole, these patterns
could not be verified statistically. Before any judgment is made
about the effectiveness of such temporal synergisms in male anoles,
this study should be repeated using lizards that are not at the peak
of their reproductive cycles.

Corticosterone/prolactin and thyroxin/prolactin treatment both
elicited significant response patterns of testicular development in
the green anole. Temporal synergisms of corticosterone and prolac-
tin resulted in a bimodal rhythm of testes weights, whereas thyroxin/
prolactin or saline/prolactin treatment yielded a unimodal rhythm.

Prolactin has an antagonadal effect in a variety of vertebrates.
Prolactin's antagonadal effect has been demonstrated in an amphibian
(Mazzi, Vellano, and Toscano, 1967) and in female lizards, Dipsosau-
rus dorsalis and Sceloporus cyanogenyes (Callard and Zeigler, 1970;
Callard et al., 1972). However, the antagonadal effect of prolactin
has been most extensively studied in birds (data and review, Meier,
1969). Temporal variations in gonadal responses to daily injections
of prolactin occurred in male green anoles maintained on a photo-
period or the thermoperiod (Section I of this paper) as well as the
present investigations. Temporal variations in gonadal responses
might account for previous lack of prolactin effect on testes in the
green anole (Licht and Jones, 1967).

In an earlier study (Meier, 1970), injections of thyroxin phased
circadian rhythms of fattening responses to prolactin in the fresh
water killifish, *Fundulus chrysotus*. The present study has demonstrated that injections of thyroxin may phase circadian oscillations of tissue sensitivity to prolactin. It has been suggested that thyroxin phases these circadian rhythms by phasing the interrenal system (Meier, Trobec, Joseph, and John, 1971).

The effects of injections of corticosterone on the reproductive system of the green anole are dramatic and complex. Circadian oscillations of plasma levels of corticosterone have the potential to organize and regulate the reproductive cycle in the lizard by temporally synergizing with a variety of other hormones.
### Table IV. Temporal Synergisms of Corticosterone/Prolactin or Saline/Prolactin in the Regulation of Fat Storage in Lizards

<table>
<thead>
<tr>
<th>Animal</th>
<th>Corticosterone or Saline</th>
<th>Responses to Prolactin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injection Time</td>
<td>Time</td>
</tr>
<tr>
<td><strong>Anolis carolinensis</strong>a</td>
<td>Corticosterone</td>
<td>0600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1800</td>
</tr>
<tr>
<td><strong>Xantusia henshawi</strong>b</td>
<td>Corticosterone</td>
<td>0600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1800</td>
</tr>
<tr>
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<td></td>
<td>1800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1800</td>
</tr>
<tr>
<td><strong>Anolis carolinensis</strong>c</td>
<td>Saline</td>
<td>2400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2400</td>
</tr>
</tbody>
</table>

a The fattening response to prolactin injections is measured in *A. carolinensis* by the weight of fat bodies as % wet body weight ± the Standard Error. Initial controls = 0.87 ± 0.23% wet body weight. Final controls = 1.70 ± 0.23% wet body weight. The response pattern for this study is statistically significant at p<0.05.

b The fattening response to prolactin injections is measured in *X. henshawi* by the body fat content as % dry body weight ± the Standard Error. Initial controls = 6.30±1.13% dry body weight. Final controls = 9.8±1.22% dry body weight. The response pattern for this study is statistically significant at p<0.05.

c The fattening response to prolactin injections is measured in *A. carolinensis* by the weight of fat bodies as % wet body weight ± the Standard Error. The response pattern in this study is statistically significant at p<0.01.

d The number of lizards sampled to obtain the mean.
Table V.

The Effects of the Temporal Synergism of Corticosterone/FSH on the Ovary and Oviducal Weights of the Lizard, *Anolis carolinensis*.

<table>
<thead>
<tr>
<th>FSH Injections (Hours after Corticosterone)</th>
<th>Paired Ovary Weights (mg)</th>
<th>Paired Oviducal Weights (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.4 ± 22.3&lt;sup&gt;c&lt;/sup&gt; (7)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>34.4 ± 4.1&lt;sup&gt;c&lt;/sup&gt; (7)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final Controls&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.9 ± 22.2 (9)</td>
<td>32.1 ± 3.6 (9)</td>
</tr>
<tr>
<td>0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>195.9 ± 37.6 (6)</td>
<td>44.7 ± 2.6 (6)</td>
</tr>
<tr>
<td>4</td>
<td>148.4 ± 45.4 (8)</td>
<td>42.1 ± 3.5 (8)</td>
</tr>
<tr>
<td>8</td>
<td>95.0 ± 21.9 (9)</td>
<td>40.7 ± 2.6 (9)</td>
</tr>
<tr>
<td>12</td>
<td>123.6 ± 24.9 (6)</td>
<td>41.0 ± 3.6 (6)</td>
</tr>
<tr>
<td>16</td>
<td>115.7 ± 27.5 (9)</td>
<td>30.9 ± 3.6 (9)</td>
</tr>
<tr>
<td>20</td>
<td>130.0 ± 33.4 (8)</td>
<td>38.2 ± 3.8 (8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The lizards in this experiment were acclimated to the experimental regimen of light and temperature for 2 weeks prior to the beginning of corticosterone treatment, which began on July 14, 1971. On that day one group of lizards was killed and the weights of paired ovaries and oviducts were measured. This group was the initial control.

<sup>b</sup> Another group was maintained under acclimation conditions from July 1 to the end of the experiment on July 28, 1971, and received no hormone treatment. This group was killed on the last day of the experiment and the weights of paired ovaries and oviducts were measured. This group was the final control.

<sup>c</sup> Mean ± the Standard Error.

<sup>d</sup> Number of lizards sampled to obtain the Mean.

<sup>e</sup> The patterns of ovary and oviducal weight responses to corticosterone/FSH treatment are statistically significant at $p<0.01$. 
Table VI.

The Effects of the Temporal Synergism of Corticosterone/LH on the Ovary and Oviducal Weights of the Lizard, *Anolis carolinensis*.

<table>
<thead>
<tr>
<th>LH Injections (Hours after Corticosterone)</th>
<th>Paired Ovary Weights (mg)</th>
<th>Paired Oviducal Weights (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.8 ± 18.1&lt;sup&gt;c&lt;/sup&gt; (8)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>27.4 ± 5.0&lt;sup&gt;c&lt;/sup&gt; (8)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final Controls&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104.5 ± 23.9 (9)</td>
<td>33.8 ± 4.7 (8)</td>
</tr>
<tr>
<td>0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>218.6 ± 24.3 (9)</td>
<td>45.6 ± 2.9 (10)</td>
</tr>
<tr>
<td>4</td>
<td>143.3 ± 48.0 (10)</td>
<td>40.1 ± 3.6 (10)</td>
</tr>
<tr>
<td>8</td>
<td>118.8 ± 24.2 (10)</td>
<td>34.4 ± 3.9 (10)</td>
</tr>
<tr>
<td>12</td>
<td>144.7 ± 21.8 (9)</td>
<td>36.7 ± 3.8 (9)</td>
</tr>
<tr>
<td>16</td>
<td>186.0 ± 38.7 (10)</td>
<td>37.5 ± 2.9 (10)</td>
</tr>
<tr>
<td>20</td>
<td>169.4 ± 27.5 (10)</td>
<td>41.7 ± 2.7 (10)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LH Injections (Hours after Saline)</th>
<th>Paired Ovary Weights (mg)</th>
<th>Paired Oviducal Weights (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>103.6 ± 26.2 (10)</td>
<td>33.6 ± 2.8 (10)</td>
</tr>
<tr>
<td>16</td>
<td>109.6 ± 29.9 (10)</td>
<td>36.2 ± 2.9 (9)</td>
</tr>
<tr>
<td>20</td>
<td>107.9 ± 23.9 (9)</td>
<td>38.0 ± 2.5 (9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Saline Injections (Hours after Corticosterone)</th>
<th>Paired Ovary Weights (mg)</th>
<th>Paired Oviducal Weights (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>61.2 ± 17.6 (9)</td>
<td>26.7 ± 4.0 (9)</td>
</tr>
<tr>
<td>16</td>
<td>71.8 ± 18.4 (9)</td>
<td>30.3 ± 3.8 (9)</td>
</tr>
<tr>
<td>20</td>
<td>117.5 ± 27.9 (8)</td>
<td>25.7 ± 3.5 (8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The lizards in this experiment were acclimated to the experimental regimen of light and temperature for 2 weeks prior to the beginning of corticosterone treatment, which began on May 19, 1971. On that day one group of lizards was killed and the weights of paired ovaries and oviducts were measured. This group was the initial control.

<sup>b</sup> Another group was maintained under acclimation conditions from May 5th to the end of the experiment on May 24th and received no hormone treatment. This group was killed on the last day of the experiment and the weights of paired ovaries and oviducts were measured. This group was the final control.

<sup>c</sup> Mean ± the Standard Error.

<sup>d</sup> Number of lizards sampled to obtain the Mean.

<sup>e</sup> The patterns of ovary and oviducal weight responses to corticosterone/LH treatment are statistically significant at p < 0.01.
Table VII.

The Effects of the Temporal Synergism of Corticosterone/FSH on the Testes Weights of the Lizard, Anolis carolinensis.

<table>
<thead>
<tr>
<th>FSH Injections (Hours after Corticosterone)</th>
<th>Paired Testes Weights (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.9 ± 2.8&lt;sup&gt;c&lt;/sup&gt; (8)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final Controls&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.2 ± 3.5 (9)</td>
</tr>
<tr>
<td>0</td>
<td>61.5 ± 6.9 (8)</td>
</tr>
<tr>
<td>4</td>
<td>63.2 ± 3.8 (9)</td>
</tr>
<tr>
<td>8</td>
<td>59.1 ± 4.7 (9)</td>
</tr>
<tr>
<td>12</td>
<td>72.2 ± 5.1 (9)</td>
</tr>
<tr>
<td>16</td>
<td>58.2 ± 3.1 (8)</td>
</tr>
<tr>
<td>20</td>
<td>63.6 ± 2.8 (8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The lizards in this experiment were acclimated to the experimental regimen of light and temperature for 2 weeks prior to the beginning of corticosterone treatment, which began on June 28, 1971. On that day one group of lizards was killed and the weights of paired testes were measured. This group was the initial control.

<sup>b</sup> Another group was maintained under acclimation conditions from June 14th to the end of the experiment on July 12th and received no hormone treatment. This group was killed on the last day of the experiment and the weights of paired testes were measured. This group was the final control.

<sup>c</sup> Mean ± the Standard Error.

<sup>d</sup> Number of lizards sampled to obtain the Mean.
Table VIII.

The Effects of the Temporal Synergism of Corticosterone/LH on the Testes Weights of the Lizard, *Anolis carolinensis*.

<table>
<thead>
<tr>
<th>LH Injections (Hours after Corticosterone)</th>
<th>Paired Testes Weights (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Controls a</td>
<td>46.2 ± 5.6 c (7)</td>
</tr>
<tr>
<td>Final Controls b</td>
<td>54.4 ± 4.7 (7)</td>
</tr>
<tr>
<td>0</td>
<td>59.8 ± 5.5 (7)</td>
</tr>
<tr>
<td>4</td>
<td>55.4 ± 4.3 (7)</td>
</tr>
<tr>
<td>8</td>
<td>66.4 ± 4.8 (7)</td>
</tr>
<tr>
<td>12</td>
<td>56.5 ± 3.4 (7)</td>
</tr>
<tr>
<td>16</td>
<td>54.8 ± 4.1 (7)</td>
</tr>
<tr>
<td>20</td>
<td>54.9 ± 3.1 (6)</td>
</tr>
</tbody>
</table>

a The lizards in this experiment were acclimated to the experimental regimen of light and temperature for 2 weeks prior to the beginning of corticosterone treatment, which began on June 9, 1971. On that day one group of lizards was killed and the weights of paired testes were measured. This group was the initial control.

b Another group was maintained under acclimation conditions from May 27th to the end of this experiment on June 23rd and received no hormone treatment. This group was killed on the last day of the experiment and the weights of paired testes were measured. This group was the final control.

c Mean ± the Standard Error.

d Number of lizards sampled to obtain the Mean.
Table IX.

The Effects of the Temporal Synergism of Corticosterone/Prolactin on the Testes Weights of the Lizard, Anolis carolinensis.

<table>
<thead>
<tr>
<th>Prolactin Injections (Hours after Corticosterone)</th>
<th>Paired Testes Weights (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Controls(^a)</td>
<td>35.0 ± 2.2(^c) (11)(^d)</td>
</tr>
<tr>
<td>Final Controls(^b)</td>
<td>64.5 ± 3.7 (11)</td>
</tr>
<tr>
<td>0(^e)</td>
<td>61.5 ± 3.0 (23)</td>
</tr>
<tr>
<td>6</td>
<td>70.2 ± 3.2 (20)</td>
</tr>
<tr>
<td>12</td>
<td>60.4 ± 3.7 (23)</td>
</tr>
<tr>
<td>18</td>
<td>70.5 ± 2.5 (23)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prolactin Injections (Hours after Saline)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0(^e)</td>
<td>59.3 ± 3.1 (10)</td>
</tr>
<tr>
<td>6</td>
<td>59.0 ± 5.1 (9)</td>
</tr>
<tr>
<td>12</td>
<td>57.0 ± 3.3 (9)</td>
</tr>
<tr>
<td>18</td>
<td>71.2 ± 2.8 (11)</td>
</tr>
</tbody>
</table>

\(^a\) The lizards in this experiment were acclimated to the experimental regimen of light and temperature for 18 days prior to the beginning of corticosterone or saline treatment, which began on October 18, 1970. On that day one group of lizards was killed and the weights of paired testes were measured. This group was the initial control.

\(^b\) Another group was maintained under acclimation conditions from September 30th to the end of the experiment on November 1st and received no hormone treatment. This group was killed on the last day of the experiment and the weights of paired testes were measured. This group was the final control.

\(^c\) Mean ± the Standard Error.

\(^d\) Number of lizards sampled to obtain the Mean.

\(^e\) The patterns of testes weight responses to corticosterone/prolactin and saline/prolactin treatment are statistically significant at p<0.01.
Table X.

The Effects of the Temporal Synergism of Thyroxin/Prolactin on the Testes Weights of the Lizard, *Anolis carolinensis*.

<table>
<thead>
<tr>
<th>Prolactin Injections (Hours after Thyroxin)</th>
<th>Paired Testes Weights (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0a</td>
<td>22.9 ± 3.7b (8)c</td>
</tr>
<tr>
<td>6</td>
<td>25.6 ± 4.8 (8)</td>
</tr>
<tr>
<td>12</td>
<td>37.1 ± 4.6 (10)</td>
</tr>
<tr>
<td>18</td>
<td>35.6 ± 2.6 (9)</td>
</tr>
</tbody>
</table>

*a* The pattern of testes weight responses to thyroxin/prolactin treatment is statistically significant at *p* < 0.05.

*b* Mean ± the Standard Error.

*c* Number of lizards sampled to obtain the Mean.
Figure 4.

Fattening responses to prolactin in *Anolis carolinensis* and *Xantusia heneshawi* entrained by corticosterone. Fattening responses for the anole, fat body weights, are reported as % about the mean of all 4 sampling times. Fattening responses for the xantusid, body fat content, are reported as % about the mean of all 6 sampling times. A diurnal pattern of fattening responses to prolactin phased by injections of saline in the anole is also reported.
PROLACTIN INJECTIONS (Hours After Corticosterone) or Saline

BODY FAT CONTENT (% of Mesonephric Fat in Animal)

WEIGHTS OF FAT BODIES

A. carolinensis

X. henshawi

Corticosterone

Saline
Figure 5.

Diurnal patterns of ovary and oviducal weight responses to FSH or LH phased by injections of corticosterone. Weights are expressed as paired ovary or oviduct weights in milligrams.
Diurnal patterns of testes weight responses to prolactin phased by corticosterone or thyroxin. Injections of prolactin were administered daily at 4 times with respect to injections of corticosterone, saline, or thyroxin. Values reported are paired testes weights in milligrams.
Paired Testes Weights (mg)

- Corticosterone/Prolactin
- Saline/Prolactin
- Thyroxin/Prolactin

Time of Prolactin Injections (Hours After Corticosterone, Saline, or Thyroxin)
SECTION III.

Seasonal and Circadian Plasma Corticosterone

Levels in Male and Female Anoles
Introduction

Daily rhythms of adrenal steroids have been reported for a variety of vertebrates (review, Halberg, 1969). However, until the present paper, there has been no report of circadian rhythms of adrenal steroids in reptiles. Seasonal levels of adrenal steroids have been estimated for several vertebrate species. Four-fold increases in the levels of plasma adrenal steroids have been reported in the Pacific salmon and the migratory rainbow trout during the migratory period (Hane and Robertson, 1959; Robertson, Drupp, Favour, Hane, and Thomas, 1961). Seasonal levels of plasma corticosterone in the duck and chicken were found to be higher in the winter than in the spring (Resko, Norton, and Nalbandov, 1964; Assenmacher and Boissin, 1968). However, only one study has investigated circadian rhythms of adrenal steroids at various seasons. Dusseau and Meier (1971) found 4 distinct circadian patterns of plasma corticosterone levels at 4 physiologically distinct times of year in the white-throated sparrow, *Zonotrichia albicollis*. Corticosterone measurements were made in overwintering birds in February, in prenuptial molt in April, in spring migration during May, and in postnuptial molt in August. White-throated sparrows are physiologically different at each of the 4 sampling times. The physiological states of the sparrows differ in fat stores, gonadal development, nocturnal locomotor activity, and condition of molting. Because daily injections of corticosterone and prolactin in various temporal relations have been found to affect fat storage, gonadal development, nocturnal locomotor activity, and a number of other physiological conditions in a variety of vertebrate
species, Meier (1972) suggested that changing temporal relations between the daily rhythms of corticosterone and prolactin may regulate seasonal physiological changes in the white-throated sparrow.

The green anole undergoes seasonal changes in fat storage and reproductive activity (Dessauer, 1955a,b; Licht, 1971), and these physiological parameters are profoundly affected by daily injections of corticosterone, the principal adrenal steroid in reptiles (Phillips, Chester Jones, and Bellamy, 1962; Gist and de Roos, 1966), and prolactin, as well as other hormones (review Meier, 1972; Section II of this paper). Therefore, an investigation of plasma levels of corticosterone was performed to determine if there is a circadian rhythm of plasma corticosterone levels and if the phase of this rhythm is altered from season to season in a manner that might account for seasonal changes in physiological state of the lizard.
Materials and Methods

Green anole lizards, *Anolis carolinensis*, were obtained from a commercial supplier in LaPlace, Louisiana. The lizards were maintained in 2 outdoor cages 3 feet x 3 feet x 5 feet made of hardware cloth. One cage contained only males and the other cage only females. Both cages were positioned so that as the natural photoperiod progressed from dawn to dusk, part of each one of the cages was in the shade and part in direct sunlight. This arrangement allowed the lizards in both cages to position themselves in the shade or sunlight as they preferred. The cages contained a layer of earth and leaves plus a number of logs, sticks, and growing vines in order to simulate the natural surroundings. During the winter months, the lizards utilized the layer of leaves by burying themselves as they might in their natural environment during hibernation (Dessauer, 1953).

A new supply of lizards was used for each of the 5 sampling times throughout the year; September 25 & 26, 1971; November 24 & 25, 1971; February 26 & 27, 1972; April 29 & 30, 1972; and July 26 & 27, 1972. At each sampling time, lizards were maintained in the outdoor cages for one week prior to experimentation. The cages were sprinkled with water daily. Crickets were fed to the lizards approximately every other day.

During each experimental period blood samples were collected from groups of males and females at one of 6 times over 24 hours: 0300, 0700, 1100, 1500, 1900, and 2300 except in February when the sampling times were 0500, 0700, 1100, 1300, 1700, and 2100. Seven to 8 males and 7 to 8 females were sampled at each time spot. Blood was collected from the
lizards by cutting off their tails approximately one centimeter posterior to the vent and allowing the blood to flow into heparinized capillary tubes. Blood collection took no longer than 15 minutes per group. Statistical analysis of each group gave no indication that plasma levels of corticosterone in lizards sampled last were any higher than those sampled first. The capillary tubes were sealed with clay and stored in a refrigerator (10°C) until all of the samples were taken. The blood was then centrifuged and the plasma was frozen (-20°C) and saved for plasma corticosterone analysis. Each lizard was sampled once and was not returned to the holding cages. At the end of each of the 5 experimental periods, one group of males and one group of females were killed and the fat bodies and gonads were dissected out and weighed. This procedure provided data on the fat storage and reproductive condition of the lizards throughout the year.

The concentration of plasma corticosterone was measured by the corticosteroid-binding globulin (CBG) method (Murphy, 1967). The CBG solution contained 2.5 ml of human plasma, 10 µc of 1,2-3H-corticosterone (New England Nuclear Corporation: specific activity = 40 c/mM) and 2.0 ml of absolute ethanol and was brought to a volume of 100 ml by the addition of distilled water. This solution was prepared fresh each week and stored in a refrigerator at 10°C. Corticosterone standards were made by dissolving corticosterone (NIH) into absolute ethanol at a concentration of 10 mg/ml and then diluted with absolute ethanol to a concentration of one µg/ml and held in a freezer at -20°C. A corticosterone standard curve of 0.5, 1.0, 3.0, and 5.0 µg/100 ml, each point prepared in triplicate, was determined for each group of samples analyzed. A blank of absolute ethanol, in triplicate, was also taken through
the procedure for each group. The blank and standards were pipetted into test tubes and all brought to an equal volume of 0.6 ml by the addition of absolute ethanol.

The plasma samples (0.01 ml) were pipetted into test tubes containing 0.3 ml of absolute ethanol to precipitate plasma proteins. The test tubes were centrifuged for 10 minutes and then the ethanol was decanted into a second tube. A second extraction was performed on the sample by adding an additional 0.3 ml aliquot of absolute ethanol to the precipitate in the first test tube. After 10 minutes of centrifugation, the ethanol from the second extraction was pooled with that of the first, total volume 0.6 ml. In most instances duplicate and even triplicate sample preparations could be prepared from a plasma sample collected from a given lizard. A group of samples to be analyzed consisted of plasma of a single lizard from each of the 6 times over the 24 hour period. These groups were from either male or female lizards; sexes were not mixed. By taking a sample from each of the 6 times, any variation in the performance of the CBG assay would not bias the data of one time spot over that of the others. The 0.6 ml preparations of the blank, standards, and samples were then evaporated to dryness in a vacuum oven at 40°C. Upon reaching room temperature, one milliliter of the cold CBG solution was added to each test tube. The tubes were then incubated in a 45°C water bath for 5 minutes followed by 10 minutes in a 10°C water bath in the refrigerator. The tubes were removed from the refrigerator, but maintained in an ice bath while approximately 40 mg of florisil (60/100 mesh) was measured into each tube. The test tubes were shaken for one minute (timing critical) in order to thoroughly suspend the florisil in solution for that amount of time. After shaking,
the florisil settled to the bottom of the tubes, and the tubes were returned to the water bath in the refrigerator for 10 more minutes. An aliquot of 0.5 ml was then pipetted from each test tube into separate liquid scintillation counting vials containing 10 ml of counting solution (a 0.3% solution of 2,5-diphenyloxazole dissolved in toluene was added to Triton-X 100 in the proportion of 2:1 by volume). Each vial was counted on a Beckman liquid scintillation counter until a counting value with only 2% error was attained. The levels of plasma corticosterone were expressed as micrograms corticosterone per 100 ml of plasma.

Statistical analyses of plasma levels of corticosterone and of seasonal changes in fat body and gonad weights were performed using the least-squares analysis of variance and the Student-Newman-Keuls test.
Results

There were distinct seasonal fluctuations in the weights of fat bodies and in the weights of gonads in both male and female anoles (Figure 7 and Table XI). Increased fat storage in preparation for winter hibernation was indicated in both male and female anoles by maximal fat body weights obtained from groups of lizards in September and November. Hibernation markedly reduced fat storage as indicated by weights of fat bodies. In February, the fat stores were 29% of prehibernation levels in males and 24% of prehibernation levels in females. Fat stores continued to decline throughout the breeding season. The lowest fat body weights for both male and female anoles were recorded in July, 6% of prehibernation levels for males and 9% of prehibernation levels for females.

There was an inverse relationship between seasonal variations in gonad weights and seasonal variations of fat body weights. The weights of the ovaries during the breeding season, April and July, were 20- to 25-times heavier than the regressed ovaries weighed in September, November, and February. As noted in earlier studies (Dessauer, 1955b; Licht, 1967a,b, and 1971), gonadal recrudescence in the male anole began shortly after testicular collapse at the end of the breeding season. The time of minimal testes weights (September) coincided with the time of maximal fat body weights for male lizards. Testes weights continued to increase until the beginning of the breeding season in April at which time they were 10-times heavier than they were in September. The weights of fat bodies were minimal at the time of peak testicular weights.

Plasma corticosterone levels for both male and female lizards
varied significantly on a seasonal basis ($p < 0.001$) (Figure 7 and Table XI). The seasonal cycle of plasma corticosterone levels in females closely followed the seasonal cycle of ovary weights. Corticosterone levels were high in April and July and low throughout September, November, and February. However, the seasonal fluctuations of plasma corticosterone levels in male lizards produces a bimodal cycle with high corticosterone levels in February and July and low levels in April, September, and November. The yearly mean of plasma corticosterone levels was significantly different between the sexes ($p < 0.01$). The average of all samples taken from male lizards throughout the year was $14.0 \mu g/100 \text{ ml}$ of plasma and from female lizards was $10.5 \mu g/100 \text{ ml}$ of plasma.

The peak values of the circadian rhythms of plasma corticosterone in male anoles during April ($p < 0.05$) and November ($p < 0.01$) were 2- to 3-times greater than the trough values. Comparable differences in the rhythms of plasma corticosterone for female anoles were found only in November ($p < 0.05$) (Figures 8 and 9, Table XII). Because of marked intragroup variations, circadian patterns at other seasons of the year could not be verified statistically.

The circadian rhythm of plasma corticosterone levels for males in April has peak corticosterone levels at 1900 and trough corticosterone levels 4 hours later at 2300 (Figure 8 and Table XII). Plasma samples from males in November yielded a circadian rhythm of plasma corticosterone levels with a plateau peak from 1900 to 0300 and a trough at 1100. These 2 distinctly different patterns occur at seasons of the year when the male anole is in 2 distinct physiological states. In November, the weights of fat bodies are near maximum and gonadal recrudescence is progressing, whereas in April the weights of fat bodies are minimal and the gonads have reached full reproductive maturity.
The circadian variations of plasma levels of corticosterone in females sampled in November follow a pattern with a peak value at 1100 and a trough value 8 hours later at 1900. This circadian oscillation in plasma corticosterone is distinctly different than that observed for males in November (Figure 9 and Table XII). These distinctly different patterns are coincident with distinctly different seasonal physiological conditions of the 2 sexes. While fat stores for both females and males are at maximal levels in November, the ovaries are in a quiescent period whereas the testes are undergoing recrudescence. The circadian rhythm of plasma corticosterone levels of females in November is also distinctly different than the diurnal rhythm of plasma corticosterone of males in April. Distinct seasonal physiological conditions are noted at these times in each sex, quiescent gonad and maximal fat stores in the female and maximally active gonad and minimal fat stores in the male.
Discussion

Measurements of plasma corticosterone in male and female green anoles indicated that corticosterone levels are highest for both sexes in July with a secondary peak for males in February. The lowest concentrations (mean daily levels) of corticosterone were found in November (males) and February (females) (Figure 7 and Table XI). These seasonal patterns of adrenal steroid levels differ from those reported for several species of birds. Corticosterone levels were highest during the winter and lowest in the spring for the duck and the chicken (Resko, et al., 1964; Assenmacher and Boissin, 1968). Studies of the white-throated sparrow indicated a linear decline in plasma levels of corticosterone from February to August (Dusseau and Meier, 1971). While the seasonal patterns of the lizard differ from those of a number of birds, there is some histological evidence in reptiles that corroborate the present study. Adrenal glands in a number of reptilian species appear to have minimal function during the winter and maximal activity during the spring and summer (Miller, 1955; Martaja, Duguy, and Saint Girons, 1961; Grignon and Grignon, 1965). The reporting of a sexual difference in seasonal levels of adrenal steroids seems to be unique to the present paper.

Of further interest with respect to the regulation of seasonal physiological states of the lizard is the alteration in the circadian pattern of plasma corticosterone levels from one season to another (Figure 8 and Table XII). The pattern of males in April has a sharp peak at 1900 and a distinct trough 4 hours later at 2300, whereas the pattern in November males has a broad peak from 1900 to 0300 with a trough following
8 hours later at 1100. The comparison of these 2 patterns illustrates an 8 to 12 hour shift in phase relation from November to April. Male lizards are physiologically different at these 2 seasons. Males in the fall are undergoing gonadal recrudescence, whereas males in the spring are fully developed for breeding activity. Fat stores in males are maximal in the fall and minimal in the spring (Figure 7 and Table XI). Investigations of temporal synergisms between corticosterone and prolactin or another hormone demonstrated that the time relationship between corticosterone and a second hormone has crucial significance to the physiological state of the animal (Trobec, 1974; Meier, 1972; Section II of this paper).

Either a photoperiod or a thermoperiod can entrain response rhythms to injections of prolactin (Trobec, 1974; Section I of this paper). It is postulated that the environmental cues of light and temperature entrain circadian rhythms of corticosterone which can then work in various temporal synergisms with injections of prolactin. The 8 to 12 hour shift from November to April occurs when the photoperiod is changing from 10.5 to 13 hours of daylight. The increase of 2.5 hours of light would not seem to account for the large phase shift observed. Therefore, the circadian oscillation of plasma corticosterone levels may be only loosely coupled to the photoperiod. This seems also to be the case for the white-throated sparrow (Dusseau and Meier, 1971). An additional consideration is the effect of temperature cycles on circadian oscillations of adrenal steroids. Perhaps thermoperiods and photoperiods work together to phase circadian rhythms of plasma corticosterone. However, some studies have indicated that for certain activities temperature seems more important than photoperiod. Evans (1966), by daily advancing
a 12L:12D light cycle and delaying a temperature cycle at the same time, was able to demonstrate that the circadian activity pattern of the diurnally active lizard *Uta stansburiana* was entrained by the thermoperiod and not the photoperiod. Other studies of locomotor activity in lizards have indicated that photoperiod is the primary entrainer (Taylor and Tschirgi, 1960; Heath, 1962; and Mayhew, 1963). Studies of the male green anole reproductive cycle suggest that light and temperature are both primary regulators depending on the time of year (Licht, 1971).

Licht suggests that temperature has a greater influence on gonadal development than light from early fall until the beginning of the breeding season, while the photoperiod seems to be more important from April until the collapse of the testes in August or September. Photoperiod and/or thermoperiod may operate in yet another way. They may regulate plasma levels of thyroxin. Injections of thyroxin phased rhythms of fattening responses to prolactin in the fresh water killifish, *Fundulus chrysotus*, (Meier, 1970) and rhythms of gonadal responses to prolactin in the green anole (Section II of this paper). Perhaps the environmental cues of light and temperature are phasing 2 systems, one working directly on plasma corticosterone levels, and another working through the thyroid to phase rhythms of sensitivity to various hormones. Thyroxin may also play a permissive role on rhythms of tissue sensitivity (Meier, Burns, Davis, and John, 1971). Meier and coworkers found that administration of thyroxin to pigeons while not phasing rhythms of fat storage, crop sac weight responses, and liver weight responses did permit rhythms of tissue sensitivity to remain under conditions of constant light and temperature, conditions which normally cause endogenous rhythms to dampen out. Thyroxin also changed the period lengths of the free-
running response rhythms. A similar action of thyroxin coupled to either the thermoperiod or photoperiod may account for the phase shift of plasma adrenal steroid levels in the green anole.

Because of the variety of environmental cues that may be affecting hormone rhythms in Anolis, photoperiod and thermoperiod reported in this paper and seasonal cycles of rainfall (Sexton, et al., 1971), and a possible diversity of mechanisms affecting diurnal variations of plasma corticosterone levels as cited above, the individual lizards making up any population at any season may reflect a diversity of circadian fluctuations that are almost synchronized as a population. This could result in wide variability of plasma corticosterone levels in groups of lizards sampled at various times of day, and still allow for the seasonally synchronized development of distinct physiological states. Unfortunately, this source of variability makes it difficult to establish statistical significance of circadian rhythms of plasma adrenal steroid levels. The competitive-protein-binding assay (Murphy, 1967) used in the present study have been reported to yield similar ranges of variability in measurements of plasma corticosterone levels made at a single time of day in the green anole (Licht and Bradshaw, 1969) as well as a variety of other reptilian species (Bradshaw and Fontaine-Bertrand, 1970).

The sex difference in seasonal fluctuations in daily mean levels of plasma corticosterone reported above is also present at the diurnal level. A circadian pattern of corticosterone with a peak level at 1100 and trough level at 1900 has been determined for female lizards in November (Figure 9 and Table XII). This contrasts with the pattern established for male lizards in November, an extended peak at 1900 to 0300 and a trough at 1100. These patterns appear to be 8 to 16 hours out of phase with one another. Differences between males and females during
November are also reflected by the activity of the gonads of each sex. The ovaries are in a quiescent state and will not become demonstrably active until April, whereas the testes have begun the process of recrudescence and are already close to half their maximal size (Figure 7 and Table XI). The states of the respective gonads may be directly related to the different circadian patterns of corticosterone observed in the 2 sexes. The state of fat storage, however, is similar in both sexes during November. Fat body weights are at a peak (Figure 7 and Table XI). This suggests that more than one temporal synergism involving corticosterone may be operating. A temporal synergism between corticosterone and prolactin may be regulating fat storage while a temporal synergism between corticosterone and the gonadotropins may be regulating gonadal development. Another possibility could be that thyroxin is phasing response rhythms involving reproductive development and corticosterone is phasing response rhythms involving fat storage. Any of these possible hormonal systems in turn may be directly or indirectly coupled to the photoperiod and/or the thermoperiod.

The circadian rhythms of plasma corticosterone levels differ from one season to another and between the sexes. The differences are associated with changes and differences in physiological conditions of fat storage and reproductive activity. Investigations involving temporal synergisms between injections of corticosterone and prolactin or a second hormone offer evidence for the role of corticosterone in organizing various states of fat storage and reproduction. Studies demonstrating the ability of a photoperiod and a thermoperiod to phase rhythms of tissue sensitivity to prolactin imply that the photoperiod and/or the
thermoperiod are involved in synchronizing circadian rhythms of plasma corticosterone levels. The preceding sequence allows for an environmentally sensitive endogenous mechanism for the regulation of seasonal physiological states in the green anole.
Table XI. Seasonal Measurements of Plasma Corticosterone Levels, Fat Body Weights, and Gonad Weights in the Lizard, *Anolis carolinensis*.

<table>
<thead>
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<tbody>
<tr>
<td><strong>Plasma Corticosterone</strong>&lt;sup&gt;a&lt;/sup&gt; (g/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.3 ± 1.4&lt;sup&gt;b&lt;/sup&gt; (47)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.7 ± 1.9 (44)</td>
<td>13.5 ± 1.5 (46)</td>
<td>9.3 ± 1.2 (48)</td>
<td>17.4 ± 1.8 (34)</td>
</tr>
<tr>
<td>Females&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.1 ± 1.2 (38)</td>
<td>16.2 ± 1.9 (28)</td>
<td>8.0 ± 0.5 (33)</td>
<td>8.1 ± 0.8 (46)</td>
<td>7.8 ± 0.9 (49)</td>
</tr>
<tr>
<td><strong>Fat Body Weights</strong> (as % Body Weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.26 ± 0.04 (7)</td>
<td>0.23 ± 0.05 (8)</td>
<td>4.27 ± 0.54 (8)</td>
<td>4.06 ± 0.73 (8)</td>
<td>1.22 ± 0.17 (8)</td>
</tr>
<tr>
<td>Females&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.67 ± 0.11 (6)</td>
<td>0.29 ± 0.13 (4)</td>
<td>3.15 ± 0.53 (6)</td>
<td>3.17 ± 0.25 (8)</td>
<td>0.75 ± 0.28 (8)</td>
</tr>
<tr>
<td><strong>Gonad Weights</strong> (wet weight, mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testes&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79.5 ± 5.1 (7)</td>
<td>30.1 ± 6.5 (8)</td>
<td>7.5 ± 0.62 (8)</td>
<td>30.1 ± 2.6 (8)</td>
<td>61.7 ± 3.0 (8)</td>
</tr>
<tr>
<td>Ovaries&lt;sup&gt;d&lt;/sup&gt;</td>
<td>125.3 ± 29.1 (6)</td>
<td>150.4 ± 19.6 (5)</td>
<td>6.7 ± 0.29 (6)</td>
<td>5.7 ± 0.3 (6)</td>
<td>6.4 ± 0.5 (8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Plasma corticosterone levels reported for the 5 months are values obtained by averaging the corticosterone levels determined for all green anoles sampled at each of the 6 daily sampling times.

<sup>b</sup> All values in this table are expressed as the Mean ± Standard Error.

<sup>c</sup> Numbers in parentheses are the number of lizard samples for the reported Mean.

<sup>d</sup> Pattern of seasonal variations significant at p<0.001.

<sup>e</sup> Pattern of seasonal variations significant at p<0.01.
<table>
<thead>
<tr>
<th></th>
<th>0700 Hours</th>
<th>1100 Hours</th>
<th>1500 Hours</th>
<th>1900 Hours</th>
<th>2300 Hours</th>
<th>0300 Hours</th>
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<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Apr. 29 &amp; 30</td>
<td>10.9±1.6b(8)c</td>
<td>11.7±3.3(7)</td>
<td>13.5±2.5(8)</td>
<td>21.8±5.0(8)</td>
<td>6.9±2.7(8)</td>
<td>9.2±2.3(8)</td>
</tr>
<tr>
<td>Females</td>
<td>13.8±5.5(4)</td>
<td>12.4±1.8(7)</td>
<td>9.0±2.8(7)</td>
<td>14.1±3.9(6)</td>
<td>11.7±2.2(7)</td>
<td>12.5±2.7(7)</td>
</tr>
<tr>
<td>Jul. 26 &amp; 27</td>
<td>25.4±5.4(8)</td>
<td>17.0±3.9(7)</td>
<td>22.7±4.7(8)</td>
<td>18.6±4.2(7)</td>
<td>14.5±4.4(6)</td>
<td>23.7±5.1(8)</td>
</tr>
<tr>
<td>Females</td>
<td>16.8±5.6(4)</td>
<td>14.4±5.1(5)</td>
<td>24.3±7.2(8)</td>
<td>15.3±3.2(4)</td>
<td>12.7±2.8(5)</td>
<td>13.6±2.8(5)</td>
</tr>
<tr>
<td>Sep. 25 &amp; 26</td>
<td>17.0±6.8(7)</td>
<td>13.0±2.9(8)</td>
<td>10.8±1.7(7)</td>
<td>15.9±4.2(8)</td>
<td>12.4±3.1(8)</td>
<td>12.0±3.3(8)</td>
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<td>7.4±1.2(6)</td>
<td>9.5±1.1(7)</td>
<td>6.0±0.9(6)</td>
<td>6.5±1.6(5)</td>
<td>10.1±1.8(4)</td>
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<td>Nov. 24 &amp; 25</td>
<td>5.6±1.0(8)</td>
<td>4.7±0.8(8)</td>
<td>7.6±1.5(8)</td>
<td>13.0±5.3(8)</td>
<td>12.4±3.9(8)</td>
<td>12.4±2.2(8)</td>
</tr>
<tr>
<td>Females</td>
<td>6.5±0.8(8)</td>
<td>12.9±3.3(8)</td>
<td>9.2±1.7(7)</td>
<td>5.2±0.7(8)</td>
<td>7.2±1.1(8)</td>
<td>7.9±1.7(7)</td>
</tr>
<tr>
<td>Feb. 26 &amp; 27</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Males</td>
<td>17.3±6.9(5)</td>
<td>16.4±2.4(9)</td>
<td>11.7±1.7(5)</td>
<td>21.2±1.5(5)</td>
<td>17.3±4.2(5)</td>
<td>21.2±8.4(5)</td>
</tr>
<tr>
<td>Females</td>
<td>7.2±2.2(8)</td>
<td>8.1±1.8(13)</td>
<td>6.5±3.2(6)</td>
<td>9.9±3.6(7)</td>
<td>9.3±1.5(8)</td>
<td>5.4±2.0(7)</td>
</tr>
</tbody>
</table>

All times are Central Standard Time.

b g/100 ml Plasma Corticosterone; Mean± Standard Error.

c Numbers in parentheses are the number of lizards sampled for the reported Mean.

d Diurnal variation of plasma corticosterone levels significant at p<0.05.

e Diurnal variation of plasma corticosterone levels significant at p<0.01.
Seasonal changes in plasma corticosterone levels, fat body weights, and gonad weights in male and female anoles. Lizards were sampled in February, April, July, September, and November. Plasma corticosterone levels are expressed as μg/100 ml plasma and are the average of all individual determinations for the 6 daily sampling times. Fat storage is expressed as fat body weights as % body weight. Gonad weights are expressed as wet weight in milligrams.
Figure 8.

Diurnal plasma corticosterone levels in male anoles in November and April. Values are reported as the mean ± the standard error (S.E.)
Plasma Corticosterone Levels (pg/100ml)

Males

Time of Day

0700 1100 1500 1900 2300 0300

Plasma Corticosterone Levels (pg/100ml)

- April
- November

S.E.
Figure 9.

Diurnal plasma corticosterone levels in male and female anoles in November. Values are reported as the mean ± the standard error (S.E.).
Plasma Corticosterone Levels (µg/100ml)

November

Males

Females

S.E.

Time of Day

0700 1100 1500 1900 2300 0300
Summary

Both a thermoperiod and a photoperiod entrained circadian rhythms of fattening responses to injections of prolactin. Circadian rhythms of testes weight responses to injections of prolactin were entrained by a thermoperiod. Repetitive daily disturbances (saline injections) given at specific times of day on a 16L:8D photoperiod elicited marked increases or decreases in fat body and testes weights.

Injections of corticosterone entrained circadian rhythms of fattening responses to daily injections of prolactin in the green anole and the granite night lizard. Circadian rhythms of ovary and oviducal weight responses to FSH and LH were synchronized by injections of corticosterone. Injections of corticosterone, saline, and thyroxin synchronized circadian rhythms of testes weight responses to daily injections of prolactin. The synchronizing effect of injections of corticosterone or thyroxin on circadian rhythms of gonadal weight responses to FSH, LH, or prolactin are most likely a composite of the endogenous hormone rhythms of the lizards and the temporal synergisms of exogenous hormone treatment. The synchronizing effect may involve a complex relationship between disturbance effects of the injections and the exogenous hormones.

Circadian rhythms of plasma corticosterone levels in the green anole differ seasonally and between the sexes. These rhythms are associated with seasonal physiological states of fat storage and reproduction.

Daily changes in thermoperiods and/or photoperiods throughout the year probably entrain circadian rhythms of plasma corticosterone
levels which result in various temporal synergisms between corticosterone and prolactin or other hormones. These temporal synergisms are responsible for organizing seasonal changes in fat storage and reproduction and are part of a system that synchronizes the physiological condition of the lizard to the environment.


Vita

Terry Ned Trobec was born May 17, 1943, in Lake Forest, Illinois. He attended Franklin & Marshall College in Lancaster, Pennsylvania, where he received the Bachelor of Arts degree in June, 1965. Mr. Trobec entered graduate school in the Department of Biological Sciences at De Paul University, Chicago, Illinois in September, 1967. The following June, he married Maureen L. Gilgore. In June, 1969, he received the Master of Science degree. Mr. Trobec began working toward the doctoral degree in the Department of Zoology and Physiology at Louisiana State University, Baton Rouge, Louisiana, in September, 1969. His doctoral studies were supported by teaching assistantships and by a pre-doctoral research fellowship awarded by the National Science Foundation. The Trobecs were blessed with a baby girl, Kerry Morgan Trobec, on January 6, 1973.

Mr. Trobec is presently employed as an Instructor of Biology at Oakton Community College, Morton Grove, Illinois, where he has taught for the last two years. He is also a candidate for the Doctor of Philosophy degree in the Department of Zoology and Physiology at Louisiana State University.