QUANTITATIVE MEASUREMENT BY TELEMETRY OF OVULATION
AND OVIPOSITION IN THE FOWL

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and Oviposition (Fowl)

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ABSTRACT

A radio telemetry system was used to establish a body temperature cycle in the domestic fowl. Certain sex differences were noted. In the female, the maximum body temperature was reached at time of oviposition. The female has a 28-hr cycle and the male a 24-hr cycle. The data were evaluated by autocovariance, power spectral analysis, harmonic regression, and other methods.

INDEX TERMS

circadian rhythm biological cycles sex chicken
gallus domesticus body temperature physiological cycles
biological clocks
The avian reproductive cycle is known to be important in the evaluation of certain mechanisms involved in neuroendocrine cycles (19). Until now, it has been difficult to evaluate ovulation and oviposition in vivo because the exact time of ovulation could not be determined. In birds, the current method for estimating the time of ovulation has been to note the time of oviposition for several weeks and then extrapolate the data (27). A number of situations, however, require a greater degree of accuracy and more speed. For example, Nalbandov (20) discusses the relationship between LH (Leutenizing Hormone) and the circadian rhythm of ovulation, and suggests a need for more quantitative data in this area.

A diurnal change in the body temperature of birds has been reported by many investigators (Woodard and Mather (30); Wilson, (28); Hildén and Stenbäck, (11); Simpson and Galbraith, (23); Simpson, (22); Fronda, (6); Kaupp, (15)), as well as for mammals (Hunter, (13); Jundell, (14); Aschoff, (1); Kleitman and Kleitman, (17); Folk, (6)). However, a change in deep body temperature similar to the one associated with ovulation in mammals (25) has not been observed for class Aves.

The purpose of the present study was to evaluate relatively long-term changes in deep body temperature in the fowl and to equate this temperature with other physiological changes, such as ovulation and oviposition.
METHODS

The telemetry system (5) shown in Fig. 1 is constructed so that the signals from the thermistor are received by an automatic d-c digital voltmeter and recorded in the digital form. The system is capable of continuous operation for over 60 days. The transmitter and the temperature sensor were sutured to the right side of the sternum through an abdominal incision of approximately 3 cm. The laparotomy was performed under sodium pentobarbital (30 mg/kg). This permanent positioning of the sensor insures constant and reproducible results, since it has been shown that abdominal temperatures vary with position (12). The thermistors were standardized and their transmitting characteristics established.

The S. C. white Leghorns in this study were unrestrained and maintained in a specially adapted cage (Fig. 2) on 24 hr of light. Feed and water were provided ad libitum. The environmental temperature remained relatively constant (±1°C) as did the relative humidity (40-50%). Time of oviposition was recorded by time lapse photography (one frame every 14 min).

The reliability of the implanted telemetry system was checked at frequent intervals by a comparison of rectal thermometer readings with the simultaneous telemetry readouts. In no instance was a discrepancy greater than that expected between rectal and abdominal temperatures observed. The differences between the two temperature readings remained constant throughout the experimental period, indicating the stability of the telemetry system.
In order to determine changes in body temperature associated with ovulation and oviposition, deep body temperatures were recorded every 6 min for 14 days starting 7 days after surgery. Data were collected on both male and female chickens approximately 14 months of age so that sex differences could be evaluated.

There were two steps to the analysis: First, the length of the cycle was established by autocovariance (3), power spectral analysis (3), and maximum harmonic intensity tests (9), and correlation coefficients were calculated and the resultant correlogram plotted (16). Second, after the length of the cycle was established, the data were described further by harmonic regression analysis (4).

The data collected from the male were treated in a similar manner.

RESULTS

Changes in deep body temperature of a female bird (14 consecutive days, 3360 observations) are presented in Fig. 3, and for the male (1440 observations), in Fig. 4. The data clearly indicate a 24-hr cycle for the male and a 28-hr cycle for the female. Certain other sex differences are evident. The average diurnal variation in the males is larger (0.85°C) than that in the female (0.58°C). Mellette (18) has reported similar differences in diurnal variations of temperature for men (1.49°C) and for women (1.20°C). The temperatures recorded for the experimental animals generally agree with those reported by a number of earlier investigators, i.e., Simpson and Galbraith (23); Baldwin and Kendeigh (2); Fronda (8);
and Wilson (28). Any differences can be accounted for by sensor location. The maximum temperature in the female chicken is associated with oviposition and ovulation, whereas in the male the peak is around 1200 hr (noon) even after 7 days of continuous light (previous lighting schedule had been 14 hr of light and 10 hr dark). This differs with data of other investigators (i.e., Kaupp (15), Woodard and Mather (30)) in which the maximum temperature responds to changes in lighting.

The rise in the female body temperature followed by a perceptible drop is associated with ovulation and oviposition. The authors are not aware of a previous report of this phenomenon. In all but one case (Fig. 3), maximum body temperature is associated with oviposition; however, without exception, oviposition is followed by a decrease in body temperature. The minimum 28-hr period body temperature always follows oviposition.

According to Sykes (24), only a small rise in temperature is caused by the muscular work associated with oviposition. (This observation agrees with those on humans (21) in which daily temperature elevations were not dependent on increases in muscular work.

A more critical evaluation of body temperature and time of oviposition was carried out by scale expansion (see Fig. 5). These data indicate that two increases in body temperature occur during the previous periods of elevated body temperature. The first peak is associated with oviposition as evidenced by time lapse photography. The second peak may possibly be equated with
ovulation; evidence of this (Fig. 6) is a single peak in body temperature accompanying oviposition. The absence of the second peak would indicate no ovulation on this day and therefore no oviposition the following day; this was observed to be the case. Further work is needed to describe the mechanism responsible for these rises in body temperature.

The correlogram constructed from the collected data (serial correlations \( r_k \) for \( k = 1, 2, 3, \ldots, 860 \)) is presented in Fig. 7. The results suggest a cycle of approximately 28-hr duration, with two subcycles, each of about 14-hr duration. Autocovariance of hourly means (336 observations) indicates a major cycle of 28-hr duration (Fig. 8). The power spectral estimates give a basic cycle of 14-hr duration (Fig. 9). The maximum harmonic intensity test, using the hourly temperatures as a harmonic series with a 126-hr fundamental period, shows that period 9 is highly significant \( (p < 0.01, \text{Table 1}) \) and a cycle of 14-hr duration exists. The above analysis shows that each 28-hr cycle consists of two different 14-hr cycles, repeating every 28 hr. For the results of harmonic regression analysis see Fig. 10 and Table 2.

**DISCUSSION**

Birds have an ovarian cycle somewhat different than their mammalian counterpart. Most female birds lay one egg approximately every 24-30 hr for several consecutive days. Ovulation occurs 15-30 min after oviposition (19). The mechanism for controlling ovulation is thought to be LH release. Several other factors
(progesterone, vasotocin) are known to be involved in ovulation (7), and therefore may be the mediators for the rise in body temperature. Gonadal maturation and ovulation are also directly controlled by the hypophyseal gonadotrophic hormones (29). During the period of oviposition the body temperature was always observed to increase. The data do not explain the mechanism responsible for this increase in body temperature. However, the results of this study do provide a quantitative method for establishing the time of oviposition and ovulation, and, consequently, a means for evaluating the mechanism in detail.

The two transient temperature peaks which coincide with time of oviposition and ovulation suggest that the level of LH and probably other hormones are responsible for the elevated temperature. The exact hormones and the extent of their control of temperature cannot be stated; however, the data suggest that those mechanisms associated with ovulation are responsible for the second peak. The fact that the second peak is not observed in birds that miss a day of laying is indirect evidence that LH may be responsible. Further, since LH "peaking" causes ovulation it is reasonable to assume it is responsible for this "peaking" of temperature.

Furthermore, the rise in body temperature associated with oviposition reflects more than the energy calculated (approximately 0.02 cal.) to move the egg from the uterus through the vagina. The data do not indicate the mechanisms responsible for this daily rise
in body temperature associated with oviposition; however, the change in body posture during this period will probably account for a large proportion of heat produced. In the pigeon the amplitude of the heat production cycles can be reduced by a transection of the lumbar and brachial plexi (10), indicating the effect of muscle tone on body heat production.

The length of time between ovipositions remains surprisingly constant within a clutch of eggs. Of course, the actual clock time of laying varies as Warren (26) has stated, indicating that in 24 hr of light, approximately 23 hr are required to produce enough LH to cause an ovulation. Furthermore, the body temperature is starting to increase at the approximate time of onset of LH buildup in the animal. So that the effect of chorionic gonadotropin on the bird's body temperature could be evaluated, a single injection (2400 i.u.) was administered into the pectoral muscle and this was followed by an immediate rise in body temperature.

The data show that the male was less affected by the constant light environment. According to autocovariance and spectral analysis, the periodicity of the male body temperature appears to be more stable than that of the female. The period of the male is also some 4 hr shorter than that of the female.

The analysis of the data indicates that the various cycles can be adequately represented by a relatively small number of harmonic terms, but that these terms vary significantly from cycle to cycle; i.e., that the amplitude or range of temperature and the phase or the time of maximum temperature varied significantly from cycle to cycle (Table 2).
Observations made on the male and female bird indicate a daily rhythm in body temperature. The male temperature cycle is shorter and less variable than the female. In female there is a maximum body temperature at time of oviposition followed by the daily minimum. There are two peaks in temperature during the daily temperature maxima in those animals which lay an egg the next day. Ovulation can account for one peak and oviposition for the other. Movement of the egg from the uterus accounts for only a small portion of the rise in body temperature. The female bird has a 28-hr deep body temperature cycle as compared to a 24-hr cycle in the male. Other differences between the male and female cycles are discussed.
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TABLE 1. Results of test for maximum harmonic intensity

<table>
<thead>
<tr>
<th></th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F-ratio*</th>
<th>Probability</th>
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<tr>
<td>Total</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period i = 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; = 2</td>
<td>2</td>
<td>11.26</td>
<td>&lt; 1</td>
<td>N.S.**</td>
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<tr>
<td>&quot; &quot; = 3</td>
<td>2</td>
<td>27.35</td>
<td>&lt; 1</td>
<td>N.S.</td>
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<tr>
<td>&quot; &quot; = 4</td>
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<td>10.12</td>
<td>&lt; 1</td>
<td>N.S.</td>
</tr>
<tr>
<td>&quot; &quot; = 5</td>
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<td>39.98</td>
<td>&lt; 1</td>
<td>N.S.</td>
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<tr>
<td>&quot; &quot; = 6</td>
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<td>59.88</td>
<td>1.159</td>
<td>N.S.</td>
</tr>
<tr>
<td>&quot; &quot; = 7</td>
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<td>26.30</td>
<td>&lt; 1</td>
<td>N.S.</td>
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<tr>
<td>&quot; &quot; = 8</td>
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<td>17.04</td>
<td>&lt; 1</td>
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<tr>
<td>&quot; &quot; = 9</td>
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<td>180.56</td>
<td>3.496</td>
<td>N.S.</td>
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<tr>
<td>Residual</td>
<td>107</td>
<td>51.65</td>
<td>16.067</td>
<td>&lt; 0.01</td>
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*The F-ratio was tested at the $\alpha = 0.01$ level of significance using the $100\alpha/m$ percent point of the F-distribution with 2 and 107 degrees of freedom ($m = 9$). The critical value is approximately 7.40.

**Not significant.
<table>
<thead>
<tr>
<th>Row</th>
<th>Source of Variation</th>
<th>Degrees of freedom</th>
<th>Cycle A</th>
<th>Cycle B</th>
<th>Cycle C</th>
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<tr>
<td>1</td>
<td>Between Replicates</td>
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<td>74.85 &lt; 0.001</td>
<td>8</td>
<td>64.43 &lt; 0.001</td>
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<tr>
<td>2</td>
<td>Effect of (A₁ + B₁)</td>
<td>2</td>
<td>12.84 &lt; 0.001</td>
<td>2</td>
<td>14.49 &lt; 0.001</td>
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<tr>
<td>3</td>
<td>Effect of (A₂ + B₂)</td>
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<td>-</td>
<td>2</td>
<td>4.96 &lt; 0.025</td>
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<tr>
<td>4</td>
<td>Effect of (A₃ + B₃)</td>
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<tr>
<td>5</td>
<td>Effect of (A₄ + B₄)</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>6</td>
<td>Scatter About Curve</td>
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<td>1.16 N.S.</td>
<td>135</td>
<td>0.91 N.S.</td>
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<td>7</td>
<td>Replicate × (A₁ + B₁)</td>
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<td>17.44 &lt; 0.001</td>
<td>16</td>
<td>21.76 &lt; 0.001</td>
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<td>-</td>
<td>16</td>
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</tr>
<tr>
<td>9</td>
<td>Replicate × (A₃ + B₃)</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
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<td>Replicate × (A₄ + B₄)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>11</td>
<td>Replicate × Scatter</td>
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<td>23.89*</td>
<td>1080</td>
<td>28.93*</td>
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<tr>
<td>12</td>
<td>Total</td>
<td>1259</td>
<td></td>
<td>1259</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Replicate × Amplitude₁</td>
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<td>18.42 &lt; 0.001</td>
<td>8</td>
<td>24.90 &lt; 0.001</td>
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<td>38.14 &lt; 0.001</td>
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<tr>
<td>16</td>
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<td>-</td>
<td>8</td>
<td>21.38 &lt; 0.001</td>
</tr>
<tr>
<td>17</td>
<td>Replicate × Phase₂</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>22.13 &lt; 0.001</td>
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<tr>
<td>18</td>
<td>Replicate × Amplitude₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>19</td>
<td>Replicate × Phase₃</td>
<td>-</td>
<td>-</td>
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<tr>
<td>20</td>
<td>Replicate × Amplitude₄</td>
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<td>-</td>
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<tr>
<td>21</td>
<td>Replicate × Phase₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Mean square error.
FIGURE TITLES

Fig. 1.- Deep body temperature transmitter approximately 1.8 cm in diameter. (A) transmitter before placement; (B) transmitter in position.

Fig. 2.- The data acquisition system constructed to collect continuous data on activity, heart rate and deep body temperature. All components except the transmitter are commercially available (Fryer and Deboo, 1964).

Fig. 3.- Daily temperature variation of the female bird for 14 consecutive days. Time of oviposition is marked with a star.

Fig. 4.- Daily variations in body temperature of a male bird maintained at 27°C.

Fig. 5.- Example of expanded elevated portion of deep body temperature curve. Inset is the 24-hr curve from which data were taken. Shaded area is time of oviposition established with photography.

Fig. 6.- Example of a single temperature peak during the period of maximum body temperature. The single peak indicates only oviposition since the bird did not lay the next day.

Fig. 7.- Correlogram of female deep body temperature collected every 0.1 hr where $r_k$ is the value of the correlation coefficient for lag k (i.e., when $k = 1$, lag = 0.1 hr); therefore, $k = 275$ represents a lag of 27.5 hr. If $\mu_j$ is the jth observation in the series ($j = 1, 2, \ldots, 3360$) then $r_k = \frac{\text{Cov}(\mu_j, \mu_{j+k})}{(\text{Var} \mu_j \text{Var} \mu_{j+k})^{1/2}}$. 

-15-
Fig. 8.- Graph of autocovariance function of female hourly means plotted against lag \( k \) hr \((k = 1, 2, \ldots, 190)\).

\[
R_x(k) = \frac{1}{n-k} \sum_{i=1}^{n-k} x_i x_{i+k} \quad k = 0, 1, 2, \ldots, 190
\]

where

\[
x_i = \mu_i - m
\]

where

\[
m = \frac{1}{n} \sum_{i=1}^{n} \mu_i
\]

where \( \mu_i \) are the hourly means, \( i = 1, 2, \ldots, 336 \).

Fig. 9.- Graph of the power spectrum estimates of female hourly means against frequency (cycles/hr).

Fig. 10.- Harmonic regression curves fitted to collected female data. Upper curve (C) is the estimated harmonic regression fitted to 9 cycles of 28-hr duration each. Lower left (A) is the estimated harmonic regression fitted to the first 14-hr cycle. Lower right (B) is the estimated regression fitted to the second 14-hr period of cycle C. This cycle (B) contains the ovulatory time.