DIURNAL GAMETIC PERIODICITY IN AVIAN ISOSPORA.*

BY
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Introduction.

Many activities of protoplasm are so adjusted in relation to environmental influences as to recur at intervals of greater or less extent and of different degrees of regularity. To a great variety of phenomena of living organisms we apply some such terms as "rhythms," "cycles," "periodicity." If we look for the causes underlying such phenomena, we find that they lie in the interaction of the intrinsic potentialities of the species with the environmental conditions under which activity occurs. To differentiate completely between these two sets of factors is probably impossible, since the very units of inheritance themselves cannot exist without some sort of medium. However, that many activities of protoplasm exhibiting a "rhythm" or "periodicity" may have as an immediate controlling factor some regular sequence of external environmental changes is a thesis not difficult to establish. One needs only to mention the seasonal cycles of flowering plants or the diurnal habits of many mammals.

If we consider only the phenomena showing a diurnal regularity, the controlling environment appears to be, either directly or indirectly, the alternation of periods of lightness and darkness. Photosynthesis and associated processes in pelagic algae are controlled directly by the sun, which supplies the necessary energy and stimuli at regular intervals. The physiological processes within the body of a higher

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161
animal are controlled indirectly by the regular alternation of light and
dark periods, which, in this case, may be the principle factors in the
formation of various habits involving nutrition, muscular and nervous
activity, rest, and sleep. The complex animal body, then, when con-
sidered in relation to its own tissues or its parasites, commensals, or
symbionts, becomes an intricate environment more or less complete
within itself, which, nevertheless, often exhibits a regularity in activ-
ity brought about by such external factors as the diurnal revolution
of the earth. Hence in studying the tissues of an organism or the
species living within it, one must consider the inherent potentialities
of cell or parasite as they are allowed expression by the regulated
physiological processes of the organism itself.

In this paper the writer proposes to define the term *periodicity* to
mean an ordered and rhythmic appearance of the individuals of a
particular stage at definite intervals during the extent of a given
phase. Thus merozoite-production in the malaria parasites would be
considered as displaying periodicity, since merozoites are produced in
large numbers at definite periods (and not at others) during the
asexual phase. Or, as in the present case, oocyst-production in the
eocidial would exhibit periodicity if oocysts were produced in large
numbers at definite periods (being very scarce or absent at others)
during the sexual phase.

The problems of periodicity in host-parasite relationships are dis-

cussed in a later section of this paper, following the presentation of the
observations on oocyst-production in avian *Isospora*. However, a
brief consideration of the bird as a host may be inserted to advantage
at this point. The high metabolic rate of birds requires strict habits
of feeding, activity, and rest, which produce a marked regularity in
certain fundamental physiological functions. This seems a desirable
condition for studies on the host-environment of parasites. The work
of Rowan and of Bissonnette (for references to their several papers
see Rowan (1929) and Bissonnette (1932)) has shown that the sea-
sonal changes in birds, especially those related to gonadal activity, are
evidently dependent upon the shifting of the relative lengths of day
and night during the course of the year. Certain wild birds were
forced into gonadal activity during the winter months by the use of
artificial light to supplement the short winter daylight period. Rowan
considers the additional exercise permitted under this con-
dition to be the important factor, while Bissonnette emphasizes the
effect of the light itself and finds different degrees of effectiveness for
various colored lights. To whatever immediate cause the specific
action may be due, it is apparent that the organism has responded to
the alteration of the relative lengths of the light and dark periods by
establishing a modified internal environment in which the gonadal
activity can subsist. Foley (1929) found that the first and second
maturation divisions in spermatogenesis of the English sparrow take
place between 7 and 8 a.m. He states: "There are indications that the
rhythmic waves of division are correlated with the degree of activity
of the bird; that they occur between the beginning of the rest period
and the beginning of pronounced physical activity." Prof. M. F.
Guyer informs the writer that mitoses in the testes of the domestic
fowl are very numerous during and practically confined to the period
around 5 a.m., during the summer months (data unpublished). Such
facts as these designate the birds as logical hosts for studies on para-
site-periodicity.

Normal periodicity in oocyst-production.

During the course of studies on daily number and size of the
Isospora of the English sparrow, it became apparent that large num-
ers per day were not indicative of constant oocyst-production through-
out the day. On several occasions the total fecal count for a twenty-
four hour period was high, but a number of individual droppings
passed during the same period were devoid of oocysts. Likewise,
random examinations of various sparrows retained in the laboratory
for short intervals indicated that oocysts might be quite abundant at
one time, only to be extremely difficult to find at others. Evidently
this situation had been encountered but not recognized in a previous
study (Boughton, 1930) where an attempt was made to record meas-
urements of the daily output of oocysts for a series of consecutive days.
Two to five days sometimes elapsed during which no oocysts were ob-
served. This failure to find oocysts regularly was due apparently, in
the light of the present study, to the fact that on such occasions smears
were made during hours between the periods of oocyst-production.

In order to test for a possible regularity in oocyst-production, the
droppings of three sparrows were examined hourly for twenty-four
hours. The droppings of each bird showed oocysts for certain hours
only; what seemed of particular interest was the fact that the three
birds showed oocysts during approximately the same hours. This
observation led to the further investigations presented in this paper,
preliminary reports of which have appeared elsewhere (Boughton,
1932 and 1932 a).

A number of observations were made on several groups of birds.
Since the methods vary somewhat, these observations are best described for (1) sparrows and (2) other birds, consideration being given in each case to (a) methods of observation and (b) results of observations.

1. In the English Sparrow.

Methods of observation. Young English sparrows (Passer domesticus) were trapped or caught by other methods during July and August, 1931, in Madison, Wisconsin. They were retained in cages within the laboratory and fed on bread and grain; some were trapped within an hour or two of the observational period. Practically 100 per cent were naturally infected with the typical Isospora of sparrows, the criterion for a positive diagnosis being the presence of oocysts in at least one dropping during a twenty-four hour period.* The natural infections were utilized in the work reported here.

For each 24-hour observation records were obtained for the general diurnal activities of the host, i.e., (1) rest (in darkness) and (2) nutritional activity, as well as for (3) oocyst output. The methods of recording these items require brief explanations.

Rest. While in the dark, the birds remain relatively quiet and do not feed; much of the time they sleep. In this study the hours of darkness are simply recorded and represented in the various diagrams as bold bars.

Nutritional activity. This was recorded indirectly by means of the fecal output. Birds were numbered and isolated in separate cages of one-half inch wire mesh measuring approximately $8 \times 8 \times 8$ inches. The wire floor permitted the passage of droppings. Each bird was supplied with moistened bread and water. A sheet of thick wrapping paper, placed about 4 inches below the cage floor, served to catch the droppings. Such a collection paper was removed and replaced by a clean one at the end of each hour (2-hour periods were employed in certain experiments).

The fecal mass for a given bird for a given hour was determined in the following manner: the number of intestinal droppings were counted directly and this number multiplied by a size value chosen as typical for the hour's sample. The size values ranged from 1 to 4, which represented respectively the smallest and largest normal types observed in sparrows; size values of 2 and 3 represented correspond-

*Presumably the lower figure (66 per cent) of Boughton (1929) and those of other writers, determined without regard to periodicity, resulted from listing as negative many birds which quite probably would have shown oocysts had they been examined during appropriate hours.
ingly intermediate sizes. "Chalky droppings," composed for the most part of kidney excretion, were not included in the count or size determination. Thus, if a bird showed 5 intestinal droppings the typical size of which was determined by inspection as 2, the fecal mass was recorded as 10. The average fecal masses for a group of birds for a series of consecutive hours served as an index of nutritional (intestinal) activity.

Oöcyst output. This factor was recorded in oöcyst-quantities, which were determined by the inspection of a fresh smear made from a random sampling of the various droppings on a collection paper. With fine forceps a small amount of material from most of the droppings of a given hour was mixed in a drop of tap-water and covered with a cover-slip. Such a preparation was examined under low power. The oöcyst-quantities were recorded as 0, 1, 2, 3, 4, or 5. If short searching (crossing back and forth across the field several times) failed to reveal any oöcysts, the oöcyst-quantity was recorded as 0. This does not imply that the sample was always devoid of oöcysts, but, as shown below, that there were at most less than 12,500 per gram of dried feces. When only one, two, or three oöcysts were found in a smear the value was recorded as 1. The oöcyst-quantities 2, 3, and 4 represented respectively two, four, and eight times as many oöcysts as the quantity 1. The highest value, 5, represented great masses of oöcysts, often clumped together like erythroplastids in a blood smear. It was found that with very little practice one could distinguish these six values by the smear method without resorting to actual dilution counts. However, for convenience in making comparisons with fecal counts of other types, the numbers of oöcysts per gram of dried feces were determined as follows: a number of droppings showing, by inspection of a smear, the same oöcyst-quantity were collected, dried, weighed, and emulsified in tap water according to the proportion of 50 cc. of water for each gram of dried feces. The oöcysts in a .05 cc. sample were counted directly and the number per gram of feces estimated from the count. The results follow:

<table>
<thead>
<tr>
<th>Oöcyst-quantities as determined by smear</th>
<th>Oöcysts per gram of dried feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>less than 12,500</td>
</tr>
<tr>
<td>1</td>
<td>25,000</td>
</tr>
<tr>
<td>2</td>
<td>50,000</td>
</tr>
<tr>
<td>3</td>
<td>100,000</td>
</tr>
<tr>
<td>4</td>
<td>200,000</td>
</tr>
<tr>
<td>5</td>
<td>2,000,000</td>
</tr>
</tbody>
</table>
TABLE 1.

Hourly records of fecal masses (f) and oocyst-quantities (oq) for various sparrows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Bird</th>
<th>Hours exposed to light</th>
<th>Date</th>
<th>A.M.</th>
<th>P.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
</tr>
<tr>
<td>1</td>
<td>161</td>
<td>5 A.M.-7:30 P.M.</td>
<td>8/24-25/31</td>
<td>f</td>
<td>oq</td>
</tr>
<tr>
<td>2</td>
<td>169</td>
<td>5 A.M.-7:30 P.M.</td>
<td>8/24-25/31</td>
<td>0 2 0 0 0 5 4 10 6 4 2 0</td>
<td>0 2 6 8 6 2 2 0 4 2 0 0</td>
</tr>
<tr>
<td>3</td>
<td>179</td>
<td>5 A.M.-7:30 P.M.</td>
<td>8/29-30/31</td>
<td>f</td>
<td>oq</td>
</tr>
<tr>
<td>4</td>
<td>203</td>
<td>5 A.M.-7:30 P.M.</td>
<td>8/29-30/31</td>
<td>1 7 8 10</td>
<td>10 6 4 8 3 0 0 3 2</td>
</tr>
<tr>
<td>5</td>
<td>161</td>
<td>6 P.M.-8:30 A.M.</td>
<td>8/29-30/31</td>
<td>f</td>
<td>oq</td>
</tr>
<tr>
<td>6</td>
<td>203</td>
<td>6 P.M.-8:30 A.M.</td>
<td>8/24-25/31</td>
<td>1 0 2 0 0 3 2 1 3 3 1</td>
<td>0 0 0 0 0 0 0 0 6 12 6 0 8</td>
</tr>
<tr>
<td>7</td>
<td>186</td>
<td>6 P.M.-8:30 A.M.</td>
<td>8/29-30/31</td>
<td>f</td>
<td>oq</td>
</tr>
<tr>
<td>8</td>
<td>196</td>
<td>6 P.M.-8:30 A.M.</td>
<td>8/24-25/31</td>
<td>0 1 2 0 0 6 4 14 2 4 0 4</td>
<td>0 1 0 2 3 4 0 0 2 1 3 0</td>
</tr>
<tr>
<td>9</td>
<td>169</td>
<td>12 noon-12 midn.</td>
<td>8/29-30/31</td>
<td>f</td>
<td>oq</td>
</tr>
<tr>
<td>10</td>
<td>179</td>
<td>12 noon-12 midn.</td>
<td>8/24-25/31</td>
<td>0 3 3 0 0 0 6 0 0 0 0 0 0</td>
<td>4 6 4 4 6 4 6 4 4 6 4 2 2 0</td>
</tr>
<tr>
<td>11</td>
<td>186</td>
<td>12 noon-12 midn.</td>
<td>8/24-25/31</td>
<td>f</td>
<td>oq</td>
</tr>
<tr>
<td>12</td>
<td>196</td>
<td>12 noon-12 midn.</td>
<td>8/29-30/31</td>
<td>0 4 2 2 0 0 3 0 3 0 2 1 0</td>
<td>2 2 12 6 8 6 8 6 10 3 10 8 2</td>
</tr>
</tbody>
</table>
In the analysis of the observed oocyst-quantities, it was found convenient to group these values as follows: negative by smear (oocyst-quantity = 0), low oocyst-output (oocyst-quantities of 1 and 2), and high oocyst-output (oocyst-quantities of 3–5 inclusive).

Results of observations. The largest quantities of oocysts were found in the droppings from 2 p.m. to 8 p.m. Relatively few oocysts were found at other times in the twenty-four hour period. The individual records of each of the 61 birds observed, as well as the graphs composed from the hourly values for all 61 birds, demonstrate this condition.

The records shown in items 1 to 4 of table 1 are characteristic of the 61 individual records. The large numbers of oocysts are found, for the most part, between 2 p.m. and 8 p.m. High oocyst-quantities (3 to 5 inclusive) were preceded by low oocyst-quantities (1 to 2 inclusive) for at least one hour in 81.4 per cent of the individual records and were followed by these low values in 74.6 per cent of the cases. This shows that a peak of oocyst-quantities is usually preceded and followed by periods in which fewer oocysts are freed, the extent of such periods depending apparently upon the degree of infection and the activity of the intestine. In cases where heavy oocyst-production and small numbers of droppings occurred simultaneously, several "drag hours" were required to flush the intestine. The oocysts in such cases showed the protoplasm contracted into a sphere, indicating a slightly prolonged stay within the host. Such a condition was found for sparrow 203 (item 4, table 1) in the droppings for 10–11 p.m. and for 11–12 p.m. which were released following an hour (9–10 p.m.) in which no droppings had been passed. Only two birds (nos. 144 and 156) out of the 61 failed to show oocyst-quantities above 2. Each however, passed a few droppings in which one or two oocysts could be found in a smear; in all cases this occurred between 2 p.m. and 8 p.m.

Consideration of the 61 birds as a group permits the graphic representation (diagram 1) of the presence of oocysts in relation to the periods of rest and nutritional activity of the host under ordinary summer daylight conditions. The upper panel of diagram 1 shows the mean fecal masses, representing the intestinal activity throughout the 24-hour period. There is a rapid rise for the first five hours after daylight, a flattened peak about mid-day, and a decline toward evening. Fecal masses are relatively small during the night. In the second panel are shown the percentages of birds showing large oocyst-quantities (i.e., over 50,000 oocysts per gram of dried feces).
ENGLISH SPARROWS UNDER DAYLIGHT CONDITIONS.

From 3 a.m. until noon there are practically no birds showing large numbers of oöcysts. The percentage increases from noon until 6 p.m., after which there is an irregular falling off. In the third panel are plotted the percentages of birds showing only a few oöcysts (i.e., 25,000 to 50,000 per gram of dried feces). These increase gradually from 9 a.m. to 3 p.m., at which time there is a sudden drop correlated with the great increase in the number of birds passing large oöcyst-quantities, remain low during the hours of high oöcyst-output, and then increase abruptly to fall off irregularly following the period in which the birds are releasing many oöcysts. Thus, the hours during which birds show a maximum number of oöcysts are preceded and followed by periods characterized by fewer oöcysts. This is borne out by records of individual birds, as pointed out above, in which a few oöcysts are often observed an hour or two before the large numbers are found. The bottom panel shows the percentages of birds at the height of oöcyst-production as determined by an arbi-
trary scheme. It was decided to include five consecutive hours from each individual record as representing the height of oocyst-production for the bird involved; the first hour showing the highest oocyst-quantity exhibited by a given bird was taken as a ‘key-hour,’ the other four including one immediately preceding and three consecutively succeeding this ‘key-hour.’ Such a scheme does away with the irregularity in the percentages due to the intermittent passing of droppings after 8 p.m. (as exhibited in the second panel of diagram 1). The percentages of birds at the height of oocyst-production form a symmetrical polygon embracing the hours from 12 noon to 10 p.m.; from 3 p.m. to 6 p.m. over 90 per cent of the birds were at the height.

In other birds.

Methods of observation. Thirty-five groups of birds in the Milwaukee Zoological Gardens were examined for Isospora and for periodicity in the oocyst-production.* Seventeen of these groups were positive for Isosporan oocysts and are included in this paper in table 2. Most of these groups were checked on November 28-29, 1931, and again on December 22-23, 1931; when available, both observations are included in table 2. In addition to the birds in the Zoological Garden, a robin and a cowbird were held in small cages in the laboratory for 48 hours; a typical 24-hour observation-record is recorded for each (see items 29 and 30, table 2).

Birds in the Zoological Gardens were examined while in their large cages, papers being spread on the cage floor to catch the droppings. As the birds could not be examined individually, it was only possible in some cases to demonstrate the presence of oocysts for a cage, without regard to the number or species infected. Smears made from random samples at three-hour intervals were classified as negative or positive (— or + in table 2). Additional + signs indicated relatively larger numbers of oocysts. Droppings were not secured for all observational periods, such periods being designated in table 2 by blank spaces.

Results of observations. The oocysts are most abundant during the afternoon and evening hours. Of the 69 observations for which oocysts are recorded (the + entries of table 2), 54 or 78.3 per cent fell in the three observation-periods between 3 p.m. and 12 midnight.

Inspection of the percentages of bird-groups showing oocysts for the series of 8 observation periods reveals the close parallelism be-

* An account of the species of birds involved is given in a forthcoming paper by the present writer, ‘Avian Hosts of the Genus Isospora.’
<table>
<thead>
<tr>
<th>Item</th>
<th>Bird-Group</th>
<th>Date</th>
<th>A.M.</th>
<th>P.M.</th>
<th>Periods of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 Pope and 8 Crested Cardinals</td>
<td>11/28-29/31</td>
<td>12-3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Same as 1</td>
<td>12/22-23/31</td>
<td>0-9</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 Jackdaws and 2 Crested Jays</td>
<td>11/28-29/31</td>
<td>3-6</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Several Java Sparrows</td>
<td>11/28-29/31</td>
<td>3-6</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1 Turtled Thrush</td>
<td>12/22-23/31</td>
<td>6-9</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Same as 4</td>
<td>12/22-23/31</td>
<td>6-9</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10 Calises and 5 Grosbeaks</td>
<td>11/28-29/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Same as 8</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1 Elfed Bird of Paradise</td>
<td>11/28-29/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Same as 10</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Several Scarlet Tanagers</td>
<td>11/28-29/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Same as 12</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>5 Green Glossy Starlings</td>
<td>11/28-29/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Same as 14</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1 Hill Mynah</td>
<td>12/28-29/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Same as 16</td>
<td>12/28-29/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>6 Spec. of S. A. Tanagers</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1 Japanese Robin</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Same as 18</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>16 African Weavers (3 spec.)</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>20 Finches (4 spec.)</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Same as 22</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Same as 24</td>
<td>12/28-29/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>2 Mexican Blue Thrushes</td>
<td>12/28-29/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>16 Sparrows (7 spec.)</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>1 Robin</td>
<td>12/22-23/31</td>
<td>9-12</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>1 Cowbird</td>
<td>12/28-29/31</td>
<td>9-12</td>
<td>+</td>
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</tr>
</tbody>
</table>

Twenty-four hour records of the presence of Isospora oocysts in the droppings of miscellaneous birds.
between the results of the observations on these miscellaneous bird-groups and those on the English sparrow. For convenience of comparison, the data on the 61 sparrows discussed above are arranged in 8 three-hour periods and placed in table 3 along with those on the

**Table 3.**
The numbers and percentages of sparrows and bird-groups positive for oocysts recorded for eight 3-hour periods throughout the day.

<table>
<thead>
<tr>
<th>Periods of observation</th>
<th>Sparrows</th>
<th>Bird-Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number individuals observed</td>
<td>Number showing oocysts</td>
</tr>
<tr>
<td>12-3 A.M.</td>
<td>48</td>
<td>25</td>
</tr>
<tr>
<td>3-6 A.M.</td>
<td>57</td>
<td>10</td>
</tr>
<tr>
<td>6-9 A.M.</td>
<td>61</td>
<td>5</td>
</tr>
<tr>
<td>9-12 Noon</td>
<td>61</td>
<td>15</td>
</tr>
<tr>
<td>12-3 P.M.</td>
<td>60</td>
<td>49</td>
</tr>
<tr>
<td>3-6 P.M.</td>
<td>61</td>
<td>60</td>
</tr>
<tr>
<td>6-9 P.M.</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>9-12 Midnight</td>
<td>56</td>
<td>51</td>
</tr>
</tbody>
</table>

bird-groups. In both sets of data the large percentages are found from 12 noon to 12 midnight; the three largest figures in each set are confined to the three consecutive periods from 3 p.m. to 12 midnight.

*Oocyst periodicity and host metabolism.*

A cyclic occurrence of oocysts at any given time of day might be explained by supposing an infection to have been set up at the appropriate time, the parasites requiring a given period of growth and development before the appearance of oocysts. However, since the vast majority of hosts showed oocysts during the same specific hours regardless of the long periods during which infection might have occurred, one is led to look for the controlling factors in the activities of the host. It seems quite probable that the production or release of oocysts may be due to the interplay of factors intrinsic in the parasites with those of a regulatory nature in the host-environment. The experiments described below deal with the production of oocysts in hosts treated to a variety of conditions.

The methods of examination and of recording the data are essentially the same as described above; minor modifications were required in some cases. Young sparrows were employed in most experiments,
although adults used as checks gave the same results. The experiments have been named in a manner to suggest the treatment of the host. The procedures and results are given for each experiment, discussion of the results being postponed to a later section.

Exp. a. Reversed light treatment. In August, 1931, about forty sparrows were confined in a large cage under controlled artificial light. Ordinary electric bulbs used for illumination of the room were employed. The lights were on from 6-7 p.m. to 8-9 a.m., food and water being available during these hours. After at least four days under these conditions, 28 birds were isolated and checked hourly for oocysts. Records of the mean fecal masses and the percentages of birds showing over 50,000 oocysts per gram of dried feces (condensed to 2-hour periods) are shown in the top graph of diagram 2. The high percentages of birds showing oocysts are found during the morning hours, as opposed to the condition in birds under daylight treatment (see second panel of diagram 1).

Four of the 28 birds had been treated to daylight previously, and when checked at that time showed oocysts during the afternoon hours. After four days under reversed treatment, all showed oocysts during the forenoon hours. The records of sparrow no. 161 for 8/24-25/31 and 8/29-30/31 (items 1 and 5 of table 1) illustrate the characteristic pictures of daylight treatment and reversed light treatment respectively.

Five of the 28 birds were subsequently returned to daylight conditions, and, after at least four days, showed oocysts during the afternoon hours. The records of sparrow no. 203 (items 6 and 4 of table 1) are typical for these five birds.

The process of reversal was followed for four days in sparrow no. 300, the record of which is given in five graphs in diagram 2. In this case the lights were on from 6 p.m. until 6 a.m. for all periods except the third. In the latter the lights were kept on until 8 a.m. since the food supply had been allowed, accidentally, to become low during the period 4-6 a.m. and did not seem adequate for maintaining the bird throughout the forthcoming 12-hour dark period. The oocysts are found during the earlier afternoon hours for the first two 24-hour periods, occur irregularly throughout the third, are abundant throughout most of the fourth, and become concentrated in and confined to the morning hours during the fifth.

Exp. b. P.M. light treatment. During August, 1931, twenty-four sparrows were confined in a large cage under controlled light conditions such that the lights were on from 12 noon until 12 midnight.
Diagram 2.

Fecal output (dotted lines) and presence of oocysts (solid line and solid areas) for sparrows under reversed light.
Food and water were available during the light period. The mean fecal masses and the percentages of birds showing over 50,000 oocysts per gram of dried feces (condensed to 2-hour periods) are shown in the second graph of diagram 3. The high percentages of birds showing oocysts are found during the late evening and early morning hours. The peak comes between that of the birds under daylight treatment (see upper panel of diagram 3) and that of the birds under reversed light treatment (see top graph of diagram 2).

Four of the 24 birds, kept for at least four days under daylight conditions, had been checked prior to the p.m. light treatment and showed oocysts during the afternoon hours. After four days under p.m. light treatment, all showed a shifting of the oocysts to the later hours. The records of sparrow no. 169 (items 2 and 9 of table 1) illustrate this condition.
Four of the 24 birds which were subsequently returned to daylight conditions showed oocysts during the afternoon hours after a 4-day period had elapsed. The records of sparrow no. 179 (items 10 and 3 of table 1) are typical for these five birds.

Three of the 24 birds of this experiment had been kept under reversed light conditions prior to the p.m. light treatment. When first checked they showed oocysts during hours characteristic for reversed birds, but later, after the 4-day period under p.m. light, exhibited oocysts during hours characteristic of the latter. The records of sparrow no. 196 (items 8 and 12 of table 1) are typical for such birds.

Exp. c. A.M. starvation treatment. On June 24, 1932, about 20 sparrows were placed in an outdoor cage and deprived of food until 12 noon. The grain was removed from the cage floor at night so that the birds could not feed during any of the forenoon hours. Water was available at all times. Plenty of grain was supplied at noon. This treatment proved to be a severe one, as approximately 50 per cent of the animals died during the first five days.

Eight birds were isolated and individual records of fecal masses and oocyst-quantities obtained for twelve 2-hour periods for the fifth day of the treatment. When one of the birds under examination died, it was replaced by another of the original lot which had been kept under the experimental conditions in the meantime. Thus eight records were available for each observation period. The mean fecal masses and the percentages of birds showing over 50,000 oocysts per gram of dried feces are shown in the third graph of diagram 3. Naturally the fecal masses for the forenoon hours are quite low. The hours during which the birds show many oocysts correspond closely to those of the birds under the ordinary daylight treatment.

Exp. d. P.M. starvation treatment. On June 24, 1932, about 20 sparrows were placed in an outdoor cage and deprived of food after 12 noon. The grain was placed on the cage floor late at night so that feeding could begin early; large quantities were available until noon. Approximately 30 per cent of the animals died during the first five days of the treatment.

Five birds were isolated and their twelve 2-hour records successfully completed. The mean fecal masses and the percentages of birds showing over 50,000 oocysts per gram of dried feces are shown in the fourth graph of diagram 3. The fecal masses increase rapidly during the forenoon and remain low after noon. The hours during which the birds show many oocysts correspond closely to those of birds under the ordinary daylight treatment and also to those of birds under the
a.m. starvation treatment. (Compare with first and third graphs of diagram 3).

Exp. e. 6–12 light treatment. On June 24, 1932, twenty-three sparrows were confined in a large cage under controlled artificial light. The light was on from 6 a.m. to 12 noon and from 6 p.m. to 12 midnight. Food and water were available during the light periods.

After five days of this treatment, eight birds were isolated and records obtained for each bird for twelve 2-hour observation periods.

The mean fecal masses and the percentages of birds showing over 50,000 oocysts per gram of dried feces are plotted in the first graph of diagram 4. Seven out of the eight birds showed large oocyst-
quantities from 6 p.m. to 10 p.m. Apparently the hours of high oocyst-production form a consecutive group as is the case under various treatments involving only one dark and one light period for the twenty-four hours.

Exp. f. 12–6 light treatment. On February 23, 1932, twenty sparrows were confined in a large cage under artificial light regulated in such a way that the light was on from 12 midnight to 6 a.m. and from 12 noon to 6 p.m. Food and water were available during the light periods.

After seventeen days of this treatment, 16 birds were isolated and individual records obtained for twelve 2-hour periods. The mean fecal masses and the percentages of birds showing over 50,000 oocysts per gram of dried feces are plotted in the second graph of diagram 4. As in the case of the 6–12 treatment, the hours of oocyst-production form a single group, which, however, assumes a position different from that of the 6–12 birds. The hours of heavy oocyst-production for the 12–6 birds precede those of the 6–12 birds by approximately six hours. (Compare first two graphs of diagram 4.)

Exp. g. Reversed birds under 6–12 treatment. On June 24, 1932, thirty-two birds were placed under reversed light. The lights were on from 6 p.m. to 6 a.m. Food and water were available during the light period. On June 29th four of these birds were isolated and checked for twelve 2-hour periods. All gave the picture typical of reversed birds, the large oocyst-quantities being confined to the periods 2–4 a.m. to 8–10 a.m.

Immediately following this preliminary check, fifteen birds were started (June 30th) on the 6–12 schedule. On July 4th, after four days of 6–12 treatment, eight birds were isolated and records obtained for twelve 2-hour periods. Over 50 per cent of the birds showed high oocyst-quantities from 6 a.m. to 4 p.m. Also 40 per cent of the birds were releasing large oocyst-quantities from 12 midnight to 6 a.m. From the results of the second test a few days later (see succeeding paragraph) this latter condition apparently was due to a gradual shifting of the hours of large oocyst-quantities from the periods characteristic for reversed birds (2–4 a.m. to 10–12 noon) to later periods corresponding to the 6–12 condition.

On July 8th, eight days after the beginning of the 6–12 treatment, six sparrows (including some of those checked July 4th) were isolated and records were kept of fecal masses and oocyst output. The results were similar to those of the first set of birds. Large oocyst-quantities were found from 6 a.m. to 6 p.m., but in this case the hours from 12
midnight to 6 a.m. showed very low percentages of birds releasing large oocyst-quantities, suggesting that the adjustment had been more complete.

The results of the two sets of observations are combined in the third graph of diagram 4, where the mean fecal masses are based upon 14 birds and the “percentages of birds” represent the average of two percentages (one from each set of birds) for each 2-hour period.

*Exp. h.* Reversed birds under 12-6 treatment. On June 30th, eight of the reversed birds, described under experiment g as having been treated for four days, were started on the 12-6 schedule. On July 4th, after four days of 12-6 treatment, eight birds were isolated and records obtained for twelve 2-hour periods. A similar set (including some of the individuals of the first test) was examined on July 8th, after eight days under 12-6 treatment. In both cases large oocyst-quantities were found from 2 a.m. to 2 p.m., and distributions of the percentages of birds showing high oocyst-quantities throughout the 24-hour period were essentially parallel in the two sets.

The results of the two observations are combined in the fourth graph of diagram 4, where the mean fecal masses are based upon 16 birds and the “percentages of birds” represents the average of the two percentages (one from each set of birds) for each 2-hour period.

**Discussion of results.**

The results of the observations of oocyst-production under normal conditions and under various experimental conditions demonstrate that the occurrence of Isosporan oocysts in the droppings of passerine birds is a periodic phenomenon more or less dependent upon the environmental conditions to which the hosts are subjected.

Periodicity has been reported for metazoan and protozoan parasites. The microfilariae of *Wuchereria bancrofti* in China, India, and North and South America are found in the peripheral blood of man from 10 p.m. to 2 a.m. and are not to be found during other periods of the day. (See Hegner, Root, and Augustine (1929) for a brief review of this subject.) This nocturnal periodicity is correlated with the night feeding habits of the intermediate host, *Culex fatigans*. However, the microfilariae of the same species in the Philippine Islands exhibit no periodicity; the intermediate host in this case is *Aedes variegatus*, which feeds only during the day. Also it is well established that asexual sporulation in the *Plasmodia* is periodic in nature, the usual explanation being that growth and maturing require
a definite length of time after penetration of the erythroplastids by sporozoites or merozoites. Sporulation in bird malaria occurs around 6 p.m. as demonstrated by the studies of Taliaferro (1925), Drensky and Hegner (1926), Hartman (1927), and Boyd (1929). As far as the present writer is aware, periodicity has not been reported previously for the coccidia. Recently Henry (1932) examined the individual droppings of a young English sparrow, and although she states (p. 294): "In some droppings no oocysts were found, although several examinations were made," she makes no mention of the periodicity reported here.

In the case of the periodicity in the production of oocysts, rest and activity of the host present themselves as the important factors. Under ordinary conditions of feeding and rest as well as under modified conditions involving one dark and one light period for each 24-hour period, the oocysts appear during the latter portion of the light period and continue into the earlier hours of the rest period. In cases where a marked change from the normal hours of light and dark is established experimentally, such as a complete reversal (Exp. a.) or a confining of the light to the p.m. hours (Exp. b.), from three to five days are required for the adjustment, the oocysts appearing finally during the hours embracing the close of the feeding period and the beginning of the rest period. A similar adjustment in the asexual sporulation of the malaria parasite in female canaries has been reported by Boyd (1929). The time of oocyst-production as established for any experimental conditions could be changed back to the normal quite readily upon returning the host to normal daylight. This phenomenon simulates the discovery of Boyd (1929) that the *Plasmodium*, induced to sporulate at 6 a.m. in canaries kept under reversed light conditions, when used to infect canaries kept under ordinary daylight conditions resumed the normal sporulation time, 6 p.m.

Such a condition is at first thought suggestive of a direct dependence of the oocysts upon the time at which the food products of the host are available to the parasite. Boyd (1931) has emphasized host feeding in relation to reproduction in avian *Plasmodium*. However, if available food (or some product of digestion) is the essential factor, it is evident from the starvation experiments reported in this paper that availability of food to the parasite need not be correlated with the time of feeding of the host. The starving of the sparrows during the a.m. hours (Exp. c) or during the p.m. hours (Exp. d) resulted in no change in the time of day at which the oocysts appeared. Here the feeding activity was confined to approximately half the number of
hours of the normal condition; the beginning of the feeding period was moved backward seven hours in the one case (a.m. starvation) and the termination of the feeding period was advanced seven hours in the other (p.m. starvation), but in neither case was the time of the appearance of oocysts changed from the normal. (See diagram 3.)

More direct evidence points to the rest period (darkness during which there is little muscular activity and no feeding) as the significant factor. Birds treated to p.m. light (Exp. b, second panel of diagram 3) and those treated to normal light but starved until noon (Exp. c, third panel of diagram 3) had the initial feeding hours at exactly the same time of day. For at least eight hours the intestinal activity, as judged by the fecal masses per 2-hour periods, was essentially the same in the two sets of birds. And yet the oocysts appeared at significantly different times under these two experimental conditions. Although the feeding hours corresponded closely, the periods of rest (darkness) occurred at different times. The time of the appearance of oocysts seemed to be adjusted to the time of the rest periods. Thus in the case of the p.m. light treatment, in which the birds were kept in darkness until noon, the oocysts appeared at a correspondingly later time of day than in the case of birds kept in darkness during the normal summer night and starved until noon.

The results of splitting the 24-hour day into two 6-hour light periods separated by two 6-hour dark periods (Exp. e, f, g, and h) have an interesting bearing upon the differentiation of the effects of feeding and rest. In the first place, under this treatment of short, double periods of light and darkness, the output of oocysts is confined to one group of hours during the 24-hour period. This is to be expected if the minimum time of development of the gametes is about twenty-four hours (or some multiple of 24). However, all birds of a given experiment release oocysts during approximately the same range of hours, a particular time of day being characteristic for each set of imposed experimental conditions. The large oocyst-quantities are found during one of the 6-hour light periods at the time when intestinal activity, as judged by the fecal output, is increasing rapidly as was the case in birds starved until noon. However, in the cases of the 6-hour periods, the hours of large oocyst-quantities are immediately preceded by six hours of darkness. Thus we have yet another combination of the factors of activity and rest in which the twelve hours preceding the release of oocysts consists of an active feeding period of six hours followed by one of darkness of similar length (see diagram 4). If we assume the significant factor for the
appearance of oocysts to be the termination of a rest period some
twelve hours previous to the hours of large oocyst-quantities, we can
explain the six hour difference in the time of oocyst-production for
birds under 6–12 light and for those under 12–6 light. In the case of
the 6–12 treatment the termination of a preceding rest period occurs
at 6 a.m. and the high percentages of birds showing many oocysts
occur twelve hours later at 6–8 p.m., which is somewhat later than
under normal conditions. Under the 12–6 treatment the termination
of a preceding rest period occurs at 12 midnight and the high per-
centages of birds showing many oocysts occur twelve hours later at
12–2 p.m., which is somewhat earlier than under normal conditions.

The fact that all the birds in any one of the experiments involving
two 6-hour light periods separated by 6-hour dark periods (Exp. e,
f, g, and h) had approximately the same group of hours for their large
quantities of oocysts is of significance. Here we have two sets of light
and dark periods either one of which might be expected a priori to
assume a dominant rôle in the adjustment of the time of oocyst-produc-
tion. However, the same adjustment seems to have been made in all
the birds of the same group. Perhaps it is of significance that the
6–12 and the 12–6 treatments were both begun at midnight. At any
rate, the termination of the dark period corresponding most closely to
that of the normal (3:30 a.m. to 4:00 a.m. during June) seems to have
been established in each case, i.e. 6 a.m. for the 6–12 birds (first graph
of diagram 4) and 12 midnight for the 12–6 birds (second graph of
diagram 4). The same sort of an adjustment to the termination of
the dark period corresponding most closely to that of the reversed
treatment (6 a.m.) is made by reversed birds treated to 6–12 light and
to 12–6 light (third and fourth graphs of diagram 4). Here again it
may be significant that the 6–12 and 12–6 treatments began at mid-
night.

Of further interest is the fact that two series of reversed birds
showing oocysts during the a.m. hours retained that condition (with
but slight adjustments) when placed under 6–12 and 12–6 treatments
—experimental conditions which had maintained (with but slight
adjustments) the normal p.m. oocyst-production. Thus the conditions
of the two experiments are capable of maintaining either normal or
reversed oocyst-productions for at least eight days; introducing birds
first treated to ordinary daylight into the 6–12 and 12–6 treatments
establishes the p.m. oocyst-production, and reversed birds treated to
6–12 and 12–6 conditions continue their a.m. oocyst-production. Fur-
thermore, the possible objection that the shifts in the time of oocyst-
production reported in this paper may be due to the ingestion of infective oocysts during the light periods of the experimental treatment and the consequent development of gametes which are released at a new time in the 24-hour period can scarcely be upheld. Reversed birds under either 6–12 or 12–6 treatment had ample opportunity for the ingestion of oocysts during the same 6-hour light periods available to the normal birds. And yet reversed birds showed oocysts during the a.m. hours and birds previously treated to normal daylight showed oocysts during the p.m. hours while under the same experimental schedule.

In this work no attempt has been made to discover the more detailed factors influencing oocyst-production. The stress has been placed upon the major changes in the environment of the host. The parasites themselves probably have a minimum time for development, but they evidently take advantage of some condition of the host in its diurnal changes. The "critical point" may be the time at which some product of digestion is available in adequate amounts or the period during which some metabolic change (such as a lowering in body temperature) gives the parasites an opportunity for development. The appearance of the oocysts in the droppings very probably lags several hours behind the time of stimulation. Further work is required to determine the exact stage at which the host environment plays its important part. In the opinion of the writer, the present evidence points to the latter portion of a rest period at least three days prior to the appearance of the oocysts. It seems justifiable to predict that the production of oocysts for any given day (and perhaps the course of the infection) can be influenced by manipulations of a "critical point" at some time approximately three days antecedent to the appearance of the oocysts in question.

Summary and conclusions.

The time of day at which the Isosporan oocysts appear in the droppings of a large number of English sparrows and in seventeen groups of other passerine birds was studied by microscopic examination of the feces at short intervals throughout a 24-hour period. A normal periodicity was studied in relation to the factors of the rest (darkness) and the nutritional activity (light) of the host in a series of eight experiments. The results of the experiments are discussed in relation to the periodicities reported for other parasites. The observations and experiments seem to justify the following conclusions:
GAMETIC PERIODICITY IN AVIAN ISOSPORA 183

1. Under ordinary daylight conditions the English sparrow (Passer domesticus) and other passerine birds show Isosporan oocysts in their droppings during the p.m. hours, 3 p.m. to 8 p.m. embracing the hours of heavy oocyst-production.

2. The appearance of large quantities of oocysts is often preceded and followed by small numbers of oocysts in such a manner as to form a peak of oocyst-production for each bird.

3. Sparrows are desirable animals for the study of oocyst periodicity on account of the absence of large caeca and the rapidity with which food is passed through the intestine.

4. Infected birds may appear negative when examined for oocysts by the smear method during the forenoon.

5. Over 99 per cent of the English sparrows were found to be infected when the presence of oocysts in at least one dropping during a 24-hour period was taken as the criterion for a positive diagnosis.

6. Reversal of the time of the appearance of oocysts to the period from 3 a.m. to 8 a.m. takes place after three to five days under artificial conditions of light at night and darkness during the day.

7. The time of day at which the oocysts appear can be changed at will by the proper manipulation of the periods of light and darkness.

8. Starvation during the a.m. or p.m. hours does not change the time of oocyst-production provided the normal light and dark periods are retained.

9. When sparrows which exhibit the normal time of oocyst-production (3-8 p.m.) are treated to two 6-hour periods of light separated by 6-hour periods of darkness, oocyst-production remains in the p.m. hours.

10. When sparrows in which the reversed time of oocyst-production (3-8 a.m.) has been induced experimentally are treated to two 6-hour periods of light separated by 6-hour periods of darkness, oocyst-production remains in the a.m. hours.

11. Adjustments in the time of oocyst-production seem to be more clearly correlated with the time of the termination of an antecedent rest period than with the time of nutritional activity.

12. Diurnal periodicity in the oocyst-production of avian Isospora appears to be the first instance of periodicity described for the sexual phase of a Sporozoan. It is determined, at least in part, by changes in the general metabolism of the host, which are in turn regulated by the diurnal habits set up in response to the alternation of periods of light and darkness.
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