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Changes in the Daily Rhythm of Plasma Corticosterone Concentration Related to Seasonal Conditions in the White-Throated Sparrow, *Zonotrichia albicollis*[1] (39035)

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Although circadian rhythms in concentrations of plasma corticosteroids have been reported in many mammals as well as in several nonmammalian species, few studies have been made of the corticosteroid rhythm with respect to varying environmental and physiological conditions. The effect of length of the daily photoperiod on the corticosteroid rhythm has been investigated in only two avian species, a *Coturnix* quail (1, 2) and the common pigeon, *Columba livia* (3). In addition, only one systematic study has been made of the daily corticosteroid rhythm of an animal (the white-throated sparrow) in several different physiological conditions during the year (4).

Recent studies have indicated that the daily rhythms of plasma corticosteroids may have important roles in regulating metabolic, reproductive, and behavioral conditions (reviews, 5, 6). In the white-throated sparrow, the temporal relations of the circadian rhythms of corticosterone and prolactin are thought to be components of the mechanism that controls the annual cycle of reproductive (7) and migratory (8, 9) conditions. In addition, the results of experiments utilizing injections of corticosterone suggest that the daily rhythm of the endogenous hormone may be involved in the photostimulatory effect of long daily photoperiods on the reproductive system (10).

The validity of these hypotheses depends on whether the phases of the daily rhythms of plasma corticosterone are compatible with the model based experiments utilizing exogenous hormones. This study examines the concentrations of plasma corticosterone at various times of day in the white-throated sparrow maintained on short (10-hr) and long (16-hr) daily photoperiods and when the birds are in several different conditions of reproductive and migratory readiness.

Materials and methods. The white-throated sparrows, *Zonotrichia albicollis*, were collected from wintering flocks near Baton Rouge, Louisiana, and maintained in large outdoor holding aviaries until they were transferred indoors. The birds were given grower poultry feed supplemented with grain.

The birds were brought indoors on 10 February, 1973, and placed into large cages (25 birds per cage: 76 cm × 100 cm × 70 cm high). The cages were constructed so that individual birds could be directed into a smaller capture cage with a minimum of disturbance. The birds were kept on a 10-hr daily photoperiod (0800–1800), supplied by "daylight" fluorescent bulbs, 1000 lux at mid-cage perch level, until 12 March, 1973, when the photoperiod was adjusted to 16 hr (0500–2100). Blood samples were collected in heparinized capillary tubes from the wing veins on March 7, May 16, June 22, and September 17 beginning at the onset of light and at 4-hr intervals for 24 hr. Six or seven birds were bled during each sampling time. Each bird was bled once only during the day of sampling.

The plasma concentrations of corticosterone were estimated by a corticosteroid binding globulin (CBG) method patterned after Murphy (11). The sample steroids were extracted with ethanol; human male plasma was the source of binding globulin for the tritiated corticosterone and sample corticosterone; and florisil was employed as the adsorbent. The plasma concentrations of corticosterone in the white-throated sparrow estimated by the CBG method are very close to those obtained by a fluorometric method (4). The plasma corticosterone level from each bird was determined in triplicate and a

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TABLE I. REPRODUCTIVE, METABOLIC, AND BEHAVIORAL INDEXES IN WHITE-THROATED SPARROWS MAINTAINED ON 10-hr AND 16-hr DAILY PHOTOPERIODS.

Date (photoperiod)	Physiological condition	Body weights (g)	Body lipid (% dry b.w.)	Migratory restlessness	Weights (mg)		
					Testes	Ovaries	Oviducts
March 7 (0800–1800)	Photosensitive-non-stimulated	23.1	19.8	Absent	1	8	5
May 16 (0500–2100)	Photosentitive-photo-stimulated	26.8	55.0	Present	440	46	38
June 22 (0500–2100)	Photorefractory	23.6	13.2	Absent	2	8	8
September 17 (0500–2100)	Fall-migratory	(near May levels)[a]		Present	(near June levels)[b]		

[a] The levels are visual approximations.

[b] Based on previous experiences of birds treated in similar manner.

sample from a plasma pool was assayed to verify the accuracy of each separate determination of plasma levels from six different birds. The data was analysed statistically by an analysis of variance and by orthogonal polynomials (12).

One week before blood sampling several of the birds from each cage were placed individually into activity recording cages (8) to determine the level of nocturnal restlessness (an index of readiness to migrate). After the March, May and June samplings several birds were killed to determine the lipid index (% of fat of the dry body weight) and the weights of the testes, ovaries, and oviducts.

Results. When the birds were examined in March, they were in a photosensitive but not in a photostimulated condition. The reproductive organs of both sexes were undeveloped, the amount of body fat was low, and nocturnal migratory restlessness was absent (Table I). There was a unimodal daily rhythm of plasma corticosterone concentration ($P < .05$). A sharp increase in corticosterone concentration occurred between 0400 and 0800; and the daily peak occurred near the onset of the daily photoperiod (0800–1800). There was also a sharp decline in corticosterone concentrations after 0800 so that relatively low concentrations were present during the major portion of the day (Fig. 1).

When the birds were examined in May, the reproductive and migratory indexes were fully developed. The reproductive organs of both sexes were near the maximal sizes generally observed in photostimulated white-throated sparrows, the body fat stores were very great, and there was intense nocturnal activity (Table I). There was also a unimodal daily rhythm of plasma corticosterone concentration in these photostimulated birds ($P < .02$) although the changes during the day were more gradual than those noted in the March nonstimulated birds. The daily rise occurred between the trough at 0500 (onset of the 16-hr daily photoperiod: 0500–2100) and the peak at 1300 and was followed by a gradual decline during the remainder of the day (Fig. 1).

When the birds were examined in June, they were in a photorefractory condition. The reproductive organs were fully regressed to weights comparable to those observed in the photosensitive birds on a nonstimulatory daily photoperiod in March (Table I). In addition, the body fat stores were low, there was no nocturnal restlessness, and the birds were undergoing a complete exchange of feathers characteristic of post nuptial molt. Contrary to the finding with respect to the photosensitive birds, there was no evidence of a daily rhythm in plasma concentration in the photorefractory birds in June (Fig. 1). Further, the concentrations were lower throughout the day than those noted in the photosensitive birds ($P < .02$: Student's t).

When the birds were examined in September they appeared to be in a condition of readiness for fall migration. There were heavy fat stores and considerable nocturnal restlessness (Table I). The concentrations of

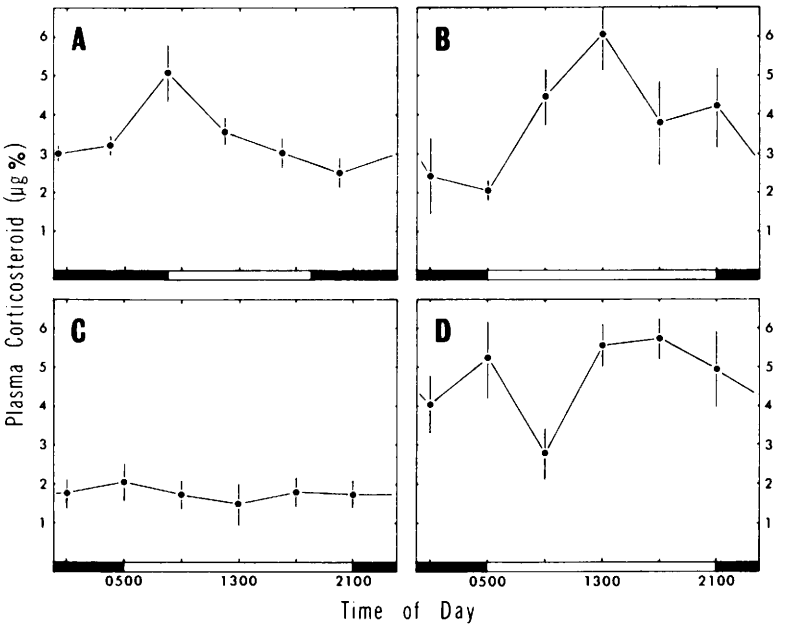

FIG. 1. Plasma corticosterone concentrations in the white-throated sparrow during different physiological conditions. A, Reproductively photosensitive, non-photostimulated birds on a 10-hr daily photoperiod. B, Reproductively photosensitive, photostimulated birds on a 16-hr daily photoperiod. C, Photorefractory birds on a 16-hr daily photoperiod. D, Birds in a Fall migratory condition on a 16-hr daily photoperiod.

plasma corticosterone were approximately twice as great as those observed in the photorefractory birds in June and comparable to those found in birds in the vernal migratory condition in May. The daily rhythm ($P < .05$) in September, however, differed from that found in May in that it appeared to be a bimodal rhythm with peaks at 0500 and again at 1700.

Discussion. The daily patterns in plasma concentrations of corticosterone were distinctive at each of the 4 months tested. Unimodal rhythms were found in photosensitive birds maintained on both a nonstimulatory 10-hr daily photoperiod (March) as well as on a photostimulatory 16-hr daily photoperiod (May). No discernible rhythm was found in photorefractory birds (June). A bimodal rhythm different from those observed in photosensitive birds was found in birds in the fall migratory condition (September).

When differences in the lengths of daily photoperiods and intervals of blood sampling are taken into account, these results for photosensitive birds maintained indoors on artificially supplied daily L:D schedules are comparable with those reported previously for photosensitive white-throated sparrows maintained in outdoor aviaries and tested in the winter and spring (4). The results of the two studies differ, however, for photorefractory birds. Dusseau and Meier found a distinct rhythm in photorefractory birds whereas one was not discernible in the present study. A difference in the studies that might be relevant is that Dusseau and Meier tested the birds during the initial stages of photorefractoriness whereas the blood samples were collected about 2 wk later in the present study. Perhaps photorefractoriness should be considered a dynamic rather than a static condition in so far as corticosterone rhythms are concerned. After photorefractoriness has been initiated by specific temporal relations of corticosterone and prolactin rhythms (6, 7), progressive changes may include a complete loss of the corticosterone rhythm. The very low concentrations of plasma corticosterone found in photorefractory white-throated sparrows is consistent with the histological evidence of low

adrenal activity reported in photorefractory white-crowned sparrow, *Zonotrichia leucophrys gambelii* (13).

Very few studies have explored the possibility of seasonal changes in the circadian rhythms of hormones. However, most of the studies of hormone rhythms have been performed with laboratory rodents which do not undergo the marked seasonal changes in physiology and behavior that occur in many feral vertebrates. Seasonal changes in the phases of plasma corticosterone rhythms with respect to the daily photoperiod have now been observed in an iguanid lizard, *Anolis carolinensis*, (T. N. Trobec, Ph.D. Dissertation, Louisiana State University, 1974) as well as in the white-throated sparrow (4). Seasonal changes in prolactin rhythms have also been found in the white-throated sparrow (14) and in a teleost fish, *Mugil cephalus* (unpublished). In the white-throated sparrow, the seasonal changes in the temporal relation of the corticosteroid and prolactin rhythms are thought to have important roles in controlling seasonal conditions including migratory fattening, restlessness, and directional orientation as well as photosensitivity and photorefractoriness of the reproductive system (reviews, 6, 15). The differences in the daily patterns of plasma corticosterone concentration noted in photosensitive and photorefractory birds maintained on constant 16-hr daily photoperiods further suggest that the hormonal alterations are consequences primarily of physiological (circannual mechanism?) rather than environmental changes.

The daily rise in concentration of plasma corticosterone is perhaps the most significant aspect of the circadian rhythm. The daily rise is triggered by cholinergic neural elements in the domestic cat (16) and in a teleost fish (17) and can be blocked by prior injections of atropine. In photosensitive white-throated sparrows the daily rise occurred about 12 hr after the onset of darkness whether the birds were kept on 10-hr or 16-hr daily photoperiods. These findings suggest that the onset of darkness may be the most important entraining element in the daily L:D schedule for the corticosteroid rhythm.

The onset of darkness appears to be the most significant element in photoperiodic entrainment of the corticosteroid rhythm in two other avian species, a *Coturnix* quail (2) and the common pigeon, *Columba livia* (3). In quail, the daily increase in adrenal corticosterone occurred 8–12 hr after the onset of darkness in birds adjusted to 6-, 12-, or 18-hr daily photoperiods. The plasma rhythm had essentially the same pattern. In pigeons, the daily rise in plasma corticosterone concentration occurred 3 hr after the onset of darkness in birds maintained on either 12- or 16-hr daily photoperiods.

In addition to a role in the regulation of the annual cycle of reproduction and migration, the circadian rhythm of corticosterone has also been implicated in the mechanism of photoperiodism (6, 8, 10). Our current understanding of photoperiodism in birds is based on the hypothesis first advanced by Bunning (18). The daily photoperiod is thought to act in two capacities, as an entrainer of a photosensitive phase and as a photoinducer of hormone production if light coincides with the sensitive phase. Thus the presence of light during the photosensitive phase in any photostimulatory photoperiodic schedule (such as long daily photoperiods and interrupted night schedules or any other ahemeral regimen) results in the stimulation of the reproductive system or of migratory indexes. Several experimental studies and discussions of the photoperiodic mechanism in birds are available (6, 19, 20). In at least some species there are two photosensitive phases for photostimulation of the gonadotropic hormones, one for luteinizing hormone and the other for follicle-stimulating hormone (9, 21). In addition, there is apparently a photosensitive phase for the production of a hormone (probably prolactin) which stimulates fattening and migratory behavior in photosensitive white-throated sparrows (22). We believe that the photoperiodic entrainment of all these photosensitive phases is mediated by the circadian rhythm of plasma corticosterone concentration.

Some of the evidence of an adrenal role in photoperiodism is based on timed injections of corticosterone and timed disturbances. In white-throated sparrows maintained on a

nonstimulatory 6-hr daily photoperiod, daily injections of corticosterone elicited increases in gonad weights and fat stores and induced nocturnal restlessness when the injections were made 18 hr before the onset of light. Injections made at other times of day were ineffective (6, 8, 10). Stressful stimuli applied daily at specific times relative to a daily photoperiod can also influence reproductive and migratory indexes in the white-throated sparrow (unpublished) and reproductive indexes in the sedentary house sparrow, *Passer domesticus* (10). In birds maintained on photostimulatory 16-hr daily photoperiods, stresses of handling and sham injections may have either stimulatory or strongly inhibitory influences depending on the time of day when they are carried out. The greatest stimulatory effect in the white-throated sparrow is produced by stresses applied shortly before midday at a time that corresponds with the daily rise of plasma corticosterone concentrations in photostimulated white-throated sparrows. These results suggested that photosensitive phases for the production of the gonadotropic hormones and prolactin can be set by injections or corticosterone or by the stress-associated increases in plasma corticosterone so that they occur in the light or dark depending on the time of injection or stress.

The present study has produced data that allow us to apply a critical test for a possible adrenal cortical role in photoperiodism. This test may best be visualized by referring to Fig. 2. By adding the interval (12 hr) between the photoperiodic entrainer (onset of darkness) and the daily rise of plasma corticosterone and the interval (18 hr) between injections of corticosterone and the apparent photosensitive phases for FSH and prolactin, as determined in previous studies, we conclude that the photosensitive phases should occur 30 hr after the photoperiodic entrainer. When this interval (30 hr) is applied to 10- and 16-hr daily photoperiods, the photosensitive phases occur during the light in the longer photostimulatory photoperiodic schedule and during the dark in the shorter nonstimulatory photoperiodic regimen. Thus the temporal aspects of this mechanism are consistent with adrenal mediation of the photoperiodic entrainment of photosensitive phases.

A further test of this hypothesis is provided by an experiment involving ahemeral L:D schedules. In a study of the white-throated sparrow (Alexander, M.S. thesis, Louisiana State University, 1966; described elsewhere, 22) that was patterned after the methodology of Hamner (18), groups of birds were maintained on 72-hr cycles with schedules of either 6L:18D:6L:42D or 6L:30D:6L:30D. The latter schedule had a stimulatory influence on gonad weights, fat stores, and nocturnal activity whereas the former did not. When the 30-hr interval from onset of darkness to the onset of photosensitive phases is related to these ahemeral cycles (Fig. 2), the photosensitive phases occur during the light in the stimulatory schedule (6L:30D:6L:30D) and during the dark in the nonstimulatory schedule (6L:18D:6L:42D).

This study contributes additional evidence indicating that circadian hormone rhythms have primary roles in regulating physiological and behavioral conditions. Circadian rhythms of plasma corticosterone concentration appear to be involved in controlling the seasonal reproductive and migratory conditions in the white-throated sparrow. Although the phase of the corticosteroid rhythm is probably set by the daily L:D schedule and may be a significant aspect of the photoperiodic mechanism the shape of the rhythm and its phase relation with respect to the daily photoperiod and with other hormone rhythms changes in time so that the photoperiod is interpreted in different ways during the year. Further research is necessary to test these hypotheses and to elucidate other components of the proposed mechanisms.

Summary. Circadian variations in concentrations of plasma corticosterone were investigated in the white-throated sparrow maintained on short (10-hr) or long (16-hr) daily photoperiods. In addition, the plasma concentrations of corticosterone were determined throughout a day in birds that were in the reproductively photosensitive spring migratory condition, the reproductively photorefractory post nuptial molt condition,

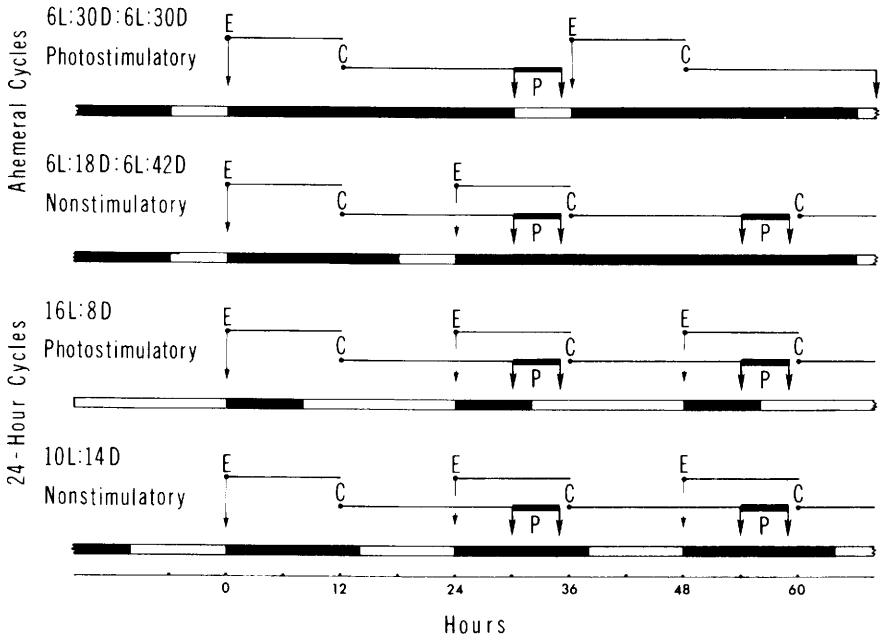

FIG. 2. Schema of the photoperiodic regulation of reproduction and migration in the white-throated sparrow. The daily rise of plasma corticosterone concentration (C) occurs 12 hr after the entraining (E) cue of the photoperiod (offset of light). The corticosterone rhythm in turn sets the photoinducible phase (P) for hormones involved in reproduction (FSH) and migration (Prolactin). In stimulatory L:D schedules the coincidence of light during the photoinducible phase, 30–36 hr after the offset of light (18–24 hr after the daily rise of corticosterone), results in hormone production.

and the fall migratory condition. Distinct unimodal rhythms were found in photosensitive birds. The daily rise occurred 12 hr after the offset of light in birds kept on both the short and the long photoperiodic regimens. There was no discernible daily variation in photorefractory birds kept on a 16 hr daily photoperiod and there was a bimodal rhythm in the birds that were in the fall migratory condition. The results are consistent with an hypothesis that assigns an important role to the circadian rhythm of corticosteroid concentration in the photoperiodic mechanism controlling seasonal reproductive and migratory conditions in the white-throated sparrow.

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