Temporal Synergism of Corticosterone and Prolactin Controlling Gonadal Growth in Sparrows

Abstract. *Gonadal growth was controlled in two avian species by corticosterone and prolactin injected daily at various times. Testicular growth was induced in photorefractory house sparrows* (Passer domesticus) *kept in continuous light by prolactin injected 4 or 8 hours after administration of corticosterone. Other temporal patterns were ineffective. Gonadal growth was also stimulated in photosensitive white-throated sparrows* (Zonotrichia albicollis) *kept in continuous light by prolactin injected 12 hours after administration of corticosterone. Daily injections of prolactin 8 hours after injection of corticosterone inhibited gonadal growth. The seasonal cycle of reproductive photorefractoriness and photosensitivity is controlled by a changing relation between the daily rhythms of plasma concentrations of corticosterone and prolactin.*

Since Rowan's initial discoveries (*1*), many workers have demonstrated that the daily photoperiod has an important function in regulating the annual cycle of reproduction in birds of the temperate zone (*2*). In the spring, the reproductive system is stimulated by the increasing day length and the gonads enlarge. In many birds, the reproductive system eventually becomes refractory to long photoperiods so that in late summer, after the breeding season, the gonads regress. They remain small until the next spring when the reproductive system is again photosensitive. The physiological bases for reproductive photosensitivity and photorefractoriness are unknown.

In migratory birds, the urge to migrate and the accompanying physiological conditions, such as heavy fat stores, are also induced in spring by the increasing photoperiod (*3*). The annual cycle of metabolic events can be correlated with the annual cycle of reproduction. With respect to fattening, many birds are photosensitive in the spring and photorefractory later in the summer. A principal difference between the annual cycles of reproduction and fattening is that gonadal growth does not usually occur along with fattening during the autumn migratory period.

Our laboratory has been studying the role of circadian systems in the endocrine control of fattening. In the migratory white-throated sparrow (*Zonotrichia albicollis*) prolactin stimulates large increases in the amount of body fat that are comparable to those found in feral birds during the migratory seasons. However, the time of day when the injections are made is critical (*4*). On a 16-hour photoperiod, injections of prolactin at midday cause fattening whereas injections given early in the day reduce the amounts of body fat. The photoperiodic synchronization of the daily rhythms of the fattening response appears to be mediated by the interrenal system (*5*). In the white-throated sparrow kept in continuous light, injections of corticosterone entrain a bimodal daily rhythm of responsiveness to prolactin. The maximum responsiveness for fattening occurs 12 hours after administration of corticosterone, and the primary minimum responsiveness occurs 8 hours after administration of corticosterone. The amounts of fat in the birds receiving prolactin after a 12-hour interval are as high as those found in feral birds during the migratory seasons, and the amounts in the birds receiving prolactin after an 8-hour interval are as low as those found during the summer photorefractory period, a time of year when the white-throated sparrows are leanest. These differences in fat content were obtained after only 5 days of prolactin injections.

In addition to a role in regulating stores of body fat, prolactin also influences the avian reproductive system. It reduces the weights of enlarged gonads and partially inhibits gonadal growth in several birds stimulated with light (*6*). Prolactin may, on the other hand, augment as well as inhibit gonadal responses to the gonadotropic hormones (*7, 8*), depending on the time of day when the injections are made (*8*).

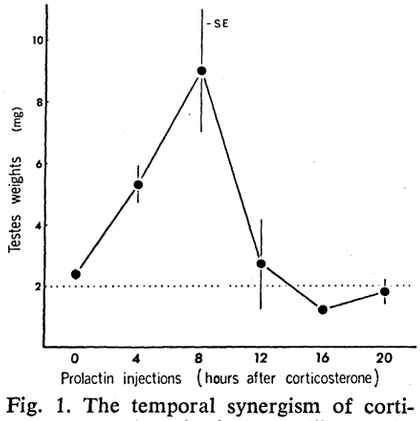

Fig. 1. The temporal synergism of corticosterone and prolactin controlling testicular growth in photorefractory house sparrows maintained in continuous light. The dotted line represents the testes weights of untreated controls.

Reflections on the relation of fattening and reproduction and on the dual role of prolactin in controlling amounts of body fat and gonadal weights led us to believe that the temporal synergism of corticosterone and prolactin might be involved in the responsiveness of the reproductive system as well as in lipid metabolism. Accordingly, we performed two experiments, one with the house sparrow (*Passer domesticus*) and another with the white-throated sparrow, to test whether or not a temporal synergism of corticosterone and prolactin could control gonadal growth in birds maintained in continuous light.

Male house sparrows were collected in Baton Rouge, Louisiana, during September when the reproductive mechanisms become refractory to long photoperiods. The birds were brought indoors on 10 October 1970 and maintained at room temperature (23° to 25°C) in continuous light (300 lumen/m^2 at cage level) supplied by "daylight" fluorescent lamps. The birds were divided into two groups. One received injections of corticosterone (25 μg per injection) at 0900; the other received corticosterone at 2100 on alternate days beginning 19 October. Each group was then subdivided into three groups that received daily injections of prolactin (25 μg per injection) at 0900, 1300, or 1700 beginning on 21 October. Thus, there were six groups that received prolactin at different times after administration of corticosterone (0, 4, 8, 12, 16, and 20 hours). This program was similar to that used with photorefractory white-throated sparrows (*5*) except that the injections were given over a longer period (14 daily injections of prolactin). Controls were injected with saline at the same times of day that experimental birds received injections of prolactin and corticosterone (two or three birds in a group).

The house sparrows were killed on 5 November, and the testes were removed and weighed immediately. The weights of paired testes for an uninjected group (six birds) were low (2.0 mg) and confirmed the photorefractory condition of the birds during the experimental period. The weights of testes for the controls were also low in

each of the six groups (combined mean of 2.2 mg). The weights of testes for the treated birds varied according to the times at which corticosterone and prolactin were given (Fig. 1). No testicular growth occurred in the groups (six birds per group except the 16-hour group in which four died) that received prolactin at 0, 12, 16, or 20 hours after injection of corticosterone. However, the weights of testes were higher for all individuals in the groups that received prolactin at 4 or 8 hours after injection of corticosterone, with the peak (9.0 mg) occurring in the 8-hour group.

Both groups that received prolactin at 0 or 12 hours after injections of corticosterone were injected with prolactin at 0900. Similarly, the 4- and 16-hour groups were injected at 1300 and the 8- and 20-hour groups were injected at 1700. A comparison of the results of the latter two sets reveals that the differences noted in gonadal weights are not functions of the actual time of day when the injections were made. In addition, the time of day when prolactin and adrenal steroids are given appears to be irrelevant for a variety of vertebrates in which a temporal synergism of hormones controlling fattening has been demonstrated (*9*). The steroids entrain similar daily rhythms of fattening responsiveness to prolactin regardless of when they are given.

A least-squares analysis of variance was applied to the variations in testes weights. The time differences are highly significant ($P < .01$). The daily pattern of testes weights form a quadratic curve ($P < .01$). There were no significant changes in body weight in the house sparrow.

The second experiment was performed with white-throated sparrows of both sexes collected from wintering flocks near Baton Rouge, Louisiana. The principal breeding range of this nocturnal migrant is in Canada. The regimen followed in this experiment is similar to the one used with the house sparrow. The birds were brought indoors to continuous light on 9 January 1971; corticosterone was injected at 0900 or 2100 on alternate days beginning 19 January, and prolactin was injected daily beginning on 19 January at 0900, 1300, or 1700. Another group received injections of corticosterone at 2100 and injections of saline at 0900 to serve as a control for the experimental group that received prolactin at 0900, 12 hours after the injection of corticosterone. The sparrows were killed 1 February 1971, and the testes or ovaries and oviducts were removed and weighed. The carcasses were dried, and the fat was extracted with diethyl ether by use of a Soxhlet apparatus.

During the experimental period, it was obvious that the individuals in certain groups were gaining weight and body fat, whereas those in other groups were not. The effects of the temporal synergism of corticosterone and prolactin on the body weights and fat contents (Fig. 2) were very much like those obtained previously in August on photorefractory white-throated sparrows subjected to similar hormone treatment (*5*). A daily rhythm of fattening resulted from the temporal pattern of hormone treatment. The peak of fat content (44 percent of the dry body weight) was found in the group that received prolactin 12 hours after administration of corticosterone and the lowest fat content (20 percent of the dry body weight) was found in the group that received prolactin 8 hours after the injection of corticosterone. The fat contents were higher in the males than in the females in the 0-, 4-, and 12-hour groups. In untreated birds, the amount of fat was 15 percent of the dry body weight before the experimental period and 25 percent of the dry body weight at the end. In the group that received saline injections 12 hours after the time of corticosterone treatment, fat made up 20 percent of the dry body weight. This value differs from that for the experimental group that received prolactin 12 hours after corticosterone ($P < .001$, Student's t-test) but not from the values of the untreated controls.

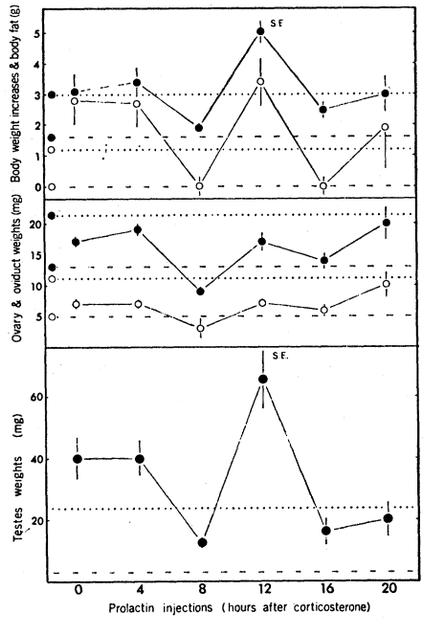

Fig. 2. The temporal synergism of corticosterone and prolactin in controlling fattening and reproductive development in photosensitive white-throated sparrows maintained in continuous light. The dashed line represents the weights of testes, ovaries, oviducts, body fat, or the total body of control birds before experimental treatment; the dotted line represents those weights of untreated controls at the end of the experiment. The open circles represent ovary weights or increases in total body weight, and the closed circles represent paired testes, oviduct, or body fat weights.

The testes weights averaged 2.2 mg in a group of birds killed at the time when the experimental birds were placed in continuous light. When the birds were killed (1 February), the testes weights of untreated control birds averaged 22.8 mg, indicating that the white-throated sparrows were photosensitive. In general, the patterns of administration of corticosterone and prolactin that stimulate fattening also favor gonadal growth, and the temporal hormonal synergisms that cause losses in body fat also suppress the weights of testes, ovaries, and oviducts (Fig. 2). The results with the males were most distinct. The heaviest testes were found in the 12-hour group (65.5 mg), the lightest testes in the 8-hour group (13.0 mg). Heavy testes were also found in the 0- and 4-hour groups, and light testes were found in the 16- and 20-hour groups. There were at least three birds of each sex in each group of nine birds.

According to a least-squares analysis of variance, the temporal patterns are highly significant ($P < .01$) for body weight, body fat, testis weight, and oviduct weights. The temporal variations also form cubic curves ($P < .01$). The testes weights (30.2 mg) of the control groups that received saline injections 12 hours after the time of corticosterone treatment were lower ($P < .01$, Student's t-test) than those of the experimental group (65.5 mg) injected with prolactin 12 hours after corticosterone treatment, but they did not differ from those of the untreated controls (22.8 mg) at the end of the experiment.

Although complete repression of gonadal growth was not brought about with prolactin, we feel that a complete block may be possible under natural conditions. The interpretations of experiments in which exogenous hormones are used should take into account possible contributions of endogenous

hormones. For example, the temporal pattern of the rhythms of endogenous corticosterone (*10*) and prolactin (*11*) in photosensitive birds could be expected to favor gonadal growth. Consequently, there would be competition between the endogenous system and one applied by exogenous hormones in which the temporal pattern inhibits gonadal growth. Similarly, the rhythms of the hormones in photorefractory birds (*10, 11*) would inhibit gonadal growth and allow for a limited rate of increase in gonadal size even when the exogenous hormones are supplied in a temporal pattern favoring rapid gonadal development.

The possibility that the stresses incurred by handling may serve as *Zeitgebers*, and produce responses, appears unlikely. There were no responses among controls injected with saline. In addition, adrenal steroids entrain rhythms of fattening responses to prolactin in a fish, a lizard, and the common pigeon, and they entrain a daily rhythm of pigeon crop sac response to prolactin whereas saline injections do not (*9*). Our experience supports the evidence (*12*) that the adrenal steroids are important synchronizers of daily rhythms, including the daily rhythms of responses to prolactin.

The daily rhythms in the amounts of corticosterone in the plasma (*10*) and prolactin in the pituitary (*11*) of the white-throated sparrow are interesting in light of its rhythms of fattening and gonadal response. In May when birds are photosensitive, there is a 12-hour interval between the rise of plasma corticosterone and the release of pituitary prolactin; whereas in August when the birds are photorefractory, there is an interval of about 6 hours between the increase in plasma corticosterone and the release of prolactin. These findings corroborate our conclusion that the relations between the daily rhythms in plasma concentrations of corticosterone and prolactin have a role in organizing metabolic and reproductive conditions in some birds. Changes in the relations of the two hormone rhythms may account for the seasonal conditions of photosensitivity and photorefractoriness.

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Thyroxine: Conversion to Triiodothyronine by Isolated Perfused Rat Heart

Abstract. *Thyroxine labeled with carbon-14 and iodine-125 was perfused through surviving rat hearts. Only when unlabeled triiodothyronine was added as a carrier could the newly formed doubly labeled triiodothyronine be isolated. The fact that this triiodothyronine was labeled with the correct ratio of carbon-14 to iodine-125 indicated that it originated from thyroxine. Approximately 5 percent of the initial carbon-14 radioactivity was found in the recovered triiodothyronine.*

The conversion of thyroxine (T-4) to triiodothyronine (T-3) occurs in vitro (*1*) by removal of iodine from the β-ring. Triiodothyronine has been isolated from human plasma (*2, 3*). Since only part of the T-3 found in serum originates in the thyroid, the T-3 formed elsewhere becomes important for the evaluation of the total metabolic picture.

Some patients experience tachycardia when treated with T-4. Since T-3 is physiologically four to five times more potent than T-4 (*4*), it is possible that tachycardia may occur as a result of formation of T-3 by the heart. There has been no evidence for the direct conversion of T-4 to T-3 by the heart although evidence exists for such conversion in some tissues (*5*). Sterling and others have identified the presence of T-3 in human serum after T-4 therapy (*6–8*).

We have perfused surviving isolated rat hearts (*9, 10*) in investigating the possibility of this conversion. Perfusions were carried out at 37°C with 36 ml of Krebs Ringer buffer (*9*) containing [2-^{14}C; 3′,5′-^{125}I]thyroxine (50 μg/ml; 1 μc of ^{14}C and 1 μc of ^{125}I per milliliter). Attempts to isolate T-3 from samples of the circulating fluid obtained at regular intervals throughout the duration of the perfusion (90 minutes) yielded T-3 in only trace amounts. We used the analytical techniques of Sterling *et al.* (*3*). Preliminary chromatography on Dowex AG50 WX-2 was followed by paper chromatography with a mixture of hexane, *tert*-amyl alcohol, 2*N* NH_4OH (1 : 5 : 6 by volume). The procedures for protecting T-4, suggested by Kobayashi *et al.* (*11*), were used throughout this work.

There is evidence (*12*) that T-3 is rapidly metabolized by some tissues. Thus, it is possible that newly formed T-3 could be rapidly absorbed or degraded by the heart tissues (or both). This could account for the fact that only trace amounts of T-3 were isolated from the circulating fluid. To saturate the heart and thus reduce the chances of labeled T-3 being metabolized, we added unlabeled T-3 (1.0 mg) to the circulating perfusion medium to trap the labeled T-3 that might be formed. The hearts maintained approximately the same heartbeat [185 ± 30 (S.D.) beats per minute] when the new perfusion medium containing carrier T-3 was used.

When the modified perfusion solution containing carrier T-3 was used, it became possible to repeatedly isolate labeled T-3. This T-3 exhibited a ratio of ^{14}C to ^{125}I double that in the T-4 used initially; this finding conforms