

# Effects of Experimentally Elevated Testosterone on Plasma Corticosterone and Corticosteroid-Binding Globulin in Dark-Eyed Juncos (*Junco hyemalis*)

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An earlier study of free-living male dark-eyed juncos found an increase in plasma corticosterone (B) in response to experimental elevation of plasma testosterone (T) (E. D. Ketterson *et al.*, 1991, *Horm. Behav.* 25, 489–503). To investigate whether the increase was caused by enhanced secretion of corticosterone or by a slower clearance rate, or both, we exposed 52 captive yearling male dark-eyed juncos (*Junco hyemalis*) to day lengths corresponding to those of spring and implanted them with one or two testosterone-filled or sham implants (10 T-I, 22 T-II, and 20 C-males). We then examined the effect of experimentally elevated testosterone on plasma corticosterone and on corticosteroid-binding globulin (CBG), as measured by the ability of steroid-stripped plasma to bind labeled corticosterone. Plasma samples were taken five times, 2 weeks before experimental prolongation of day length and approximately every 3 weeks thereafter. Treatment with testosterone increased both plasma testosterone and plasma corticosterone two to three times above control levels, and the degree of elevation was dose-dependent. Only when all treatment groups were pooled, however, were plasma testosterone and corticosterone significantly correlated. The relationship between plasma corticosterone and time required to

bleed the birds was similar for all three treatment groups, suggesting that there was no effect of treatment on the stress response. Testosterone significantly increased the capacity of the plasma to bind corticosterone, presumably because it contained more CBG, when compared to the plasma of controls. However, treatment with testosterone did not affect the affinity of the plasma for corticosterone. It seems likely that exogenous testosterone elevated corticosterone by slowing the corticosterone clearance rate via an increase in CBG. It is not clear what the net effect of chronic elevation of testosterone would be on the availability of corticosterone to target tissues. © 1997 Academic Press

In general, in animals that are under stress plasma corticosterone (B) levels are high and plasma testosterone (T) levels are low (Moore *et al.*, 1991). Several studies have shown that exogenous corticosterone depresses plasma testosterone levels as well as sexual and aggressive behavior (Tokarz, 1987; DeNardo and Licht, 1993; DeNardo and Sinervo, 1994). But little is known about the effect of experimentally administered testosterone on corticosterone (Wingfield and Silverin, 1986; Silverin, 1986; DeNardo and Sinervo, 1994).

Although corticosterone generally has a suppressive effect on testosterone, increased plasma levels of testosterone appear to have a variable effect on corticosterone levels. In some bird species, testosterone de-

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creases plasma corticosterone levels (Péczy, 1979). In others, exogenous testosterone has no effect on plasma corticosterone levels (Hegner and Wingfield, 1987; Wingfield, 1984). In free-living male dark-eyed juncos implanted with testosterone (T-males), corticosterone is higher than it is in controls (C-males) (Ketterson *et al.*, 1991). However, the interactions between corticosterone and testosterone levels are complex and could be obscured by other factors. For instance, in the study by Ketterson *et al.* (1991), plasma corticosterone concentration was measured by radioimmunoassay (RIA), a method that cannot distinguish between corticosterone resulting from increased secretion of corticosterone and corticosterone that remains in the plasma because it is bound to corticosteroid-binding globulin (CBG) (Wingfield *et al.*, 1992, 1984). Therefore, an increase in plasma corticosterone as measured by RIA may indicate either an increase in secretion or an increase in CBG levels.

Since its discovery (for review see Rosner, 1990; Westphal, 1971), CBG has been found in all vertebrate classes (Seal and Doe, 1966) but has been little studied except in humans and rats. In rats, CBG is a glycoprotein (50–60 kDa), produced by the liver, that preferentially binds to glucocorticoids and progesterone (Smith and Hammond, 1992; Hammond, 1990). Its plasma concentration appears to increase in response to thyroxine and estrogen, and the anterior pituitary may be involved in its regulation via the production of thyroxine-stimulating hormone (Smith and Hammond, 1992; Gala and Westphal, 1966b). Dexamethasone and corticosterone decrease serum CBG levels (Smith and Hammond, 1992; Hammond, 1990; Gala and Westphal, 1966a,b).

Avian CBG has physiologic properties similar to those of mammalian CBG and has been observed in the 23 avian species, representing eight orders, whose CBG has been examined (Wingfield *et al.*, 1984; Wingfield, 1980). In some bird species, testosterone increases the plasma's binding capacity for corticosterone (Silverin, 1986; Daniel and Assenmacher, 1974). However, in others (Péczy, 1979; Péczy and Daniel, 1979), and in several mammals, testosterone decreases this capacity (Bradley, 1987; McDonald *et al.*, 1981; Bradley *et al.*, 1980; Kley *et al.*, 1973; Gala and Westphal, 1965, 1966b), and thus, by implication, the plasma concentration of CBG.

In this study our objectives were to determine

whether experimentally elevated testosterone in captive male juncos increases plasma corticosterone levels as it does in free-living males (Ketterson *et al.*, 1991) and, if so, whether it also increases the plasma's binding capacity for corticosterone, presumably by increasing CBG. If the previously observed testosterone-related increase in plasma corticosterone of free-living juncos were repeated, we expected as one alternative that there would be no change in the plasma's capacity to bind to corticosterone. We could then tentatively attribute the increased plasma corticosterone to increased secretion. Alternatively, an increase in plasma corticosterone could reflect a related increase in CBG, which in binding corticosterone would decrease its metabolic clearance rate. Thus, for a given rate of secretion, a greater concentration of CBG would cause an increase in plasma corticosterone concentration. To discriminate between these two possibilities we again used testosterone to elevate corticosterone, and we measured associated changes in CBG.

## METHODS

### *Species and Treatment*

We studied individuals of the subspecies *Junco hyemalis carolinensis*, which we caught at Mountain Lake Biological Station, in the Allegheny Mountains of southwestern Virginia (Giles County 37,22' N, 80,32' W) in October 1991. Fifty-two yearling males were transported to Bloomington, Indiana, housed in large outdoor aviaries until 21 November 1991, and then transferred to indoor quarters where they were housed individually in small cages (29 × 22.5 × 26 cm) at a temperature of approximately 16°. The cages were divided among three similar rooms; two contained 15 birds (rooms A and B) and the third contained 22 birds (room C). The juncos were visually, but not acoustically, isolated. The light:dark cycle was 8.5L:15.5D (lights on at 12:30) until 28 December 1991, when we began to lengthen the days to simulate day length at the start of the breeding season. Day length was increased by 1 hr every other day until 7 January 1992, when the cycle reached 14.5L:9.5D (lights on at 12:30). This regimen was maintained until the end of the experiment, on 16 March 1992.

The juncos were implanted on 23 January 1992. After being anesthetized with methoxyflurane, some experimental males (T-II males) were implanted subcutaneously along the flank with two 10-mm lengths of Silastic tubing (Dow Corning; 1.47 mm i.d., 1.96 mm o.d.) packed with crystalline testosterone (Sigma Chemical) and sealed with Silastic glue (Dow Corning) (see Ketterson *et al.*, 1991, for details). Other experimental males (T-I males) received one 10-mm tube packed with testosterone and one empty 10-mm tube. C-males were treated identically, except that their two implants were empty. All males were randomly assigned to treatment groups. In each of rooms A and B there were 15 birds, 5 C-males, 5 T-II males, and 5 T-I males. Within each treatment group, birds were randomly assigned to room A or B. In room C there were 10 C-males and 12 T-II males. It was not possible to obtain sufficient plasma from an individual male to permit both hormone analysis and CBG analysis. Therefore, the birds in rooms A and B were bled only for analysis of testosterone and corticosterone. The birds in room C were bled only for CBG. Because all rooms were identical in dimensions, light regime, and climate control, we assume that hormone levels of birds in room C were similar to hormone levels of birds in rooms A and B and that CBG levels found in room C were similar to those in rooms A and B.

### Blood Sampling

We took plasma samples from the left wing vein of each male at intervals of approximately every 3 weeks beginning 20 December 1991. Blood sampling Week 1 occurred 2 weeks before experimental prolongation of day length. Sampling Week 2 occurred 4 days after prolongation of day length. Sampling Weeks 3, 4, and 5 occurred 1.5, 4, and 6.5 weeks, respectively, after implanting. In order to minimize the effects that stress might have on baseline hormonal states, we kept disturbance time, i.e., time spent removing a bird from its cage and room and bleeding it, to a minimum. All birds were captured and removed within 45 sec of entry into the room by one of us. Time elapsed between capture and the completion of bleeding for hormones was  $4.81 \pm 0.13$  min ( $N = 85$ ). There was no significant difference in bleeding time for hormones among the treatment groups ( $N = 85$ ,  $df = 2$ ,  $P = 0.235$ , Kruskal-Wallis). When bleeding for CBG, the mean

bleeding time was  $5.45 \pm 0.21$  minutes ( $N = 101$ ). Blood was drawn into heparinized capillary tubes, which were then centrifuged for 5 min in a clinical centrifuge. The volume of each plasma sample was measured in microliters with a Hamilton syringe, and plasma was transferred to a 0.5-ml Eppendorf tube and stored at  $-20^\circ$ .

### Hormone Assays

Circulating levels of plasma testosterone and corticosterone were determined by specific radioimmunoassay following procedures of Wingfield and Farner (1975), Ball and Wingfield (1987), and Ketterson *et al.* (1991). Briefly, plasma samples were equilibrated overnight with 2000 cpm of labeled testosterone and corticosterone, extracted with 4.5 ml dichloromethane, and separated on celite/glycol columns using 20% and 50% ethyl acetate in isooctane, respectively, after DHT was removed. The expected value for the testosterone standards was 250 pg, and the observed average was 222 pg ( $N = 4$ ,  $SE = 18.84$ ,  $cv = 17.0\%$ ). Recoveries for testosterone averaged 0.56 ( $N = 85$ ,  $SE = 0.008$ ). The expected value for the corticosterone standards was 1000 pg and observed values averaged 758.48 pg ( $N = 4$ ,  $SE = 86.05$ ,  $cv = 23.0\%$ ). Recoveries for corticosterone averaged 0.78 ( $N = 85$ ,  $SE = 0.004$ ). All samples were run in one assay.

### Corticosteroid-Binding Globulin Assays

Binding affinity and capacity (the number of binding sites available) were assessed according to a procedure described by Wingfield *et al.* (1984). In order to obtain enough plasma for CBG assays, we treated pairs of individuals as sets and pooled equal volumes of plasma taken from each member of a set; each individual contributed at least 0.1 ml. Sets were paired according to treatment, and, considering samples from all dates of bleeding, we obtained 44 paired samples, 22 T and 22 C. To strip CBG of endogenous steroids, we treated each paired sample with an equal volume of dextran-coated charcoal (250 mg charcoal-NoritA and 25 mg Dextran T-70) suspended in 100 ml phosphate-buffered saline plus 0.1% gelatin (PBSG) and incubated the mixture at  $30^\circ$  for 30 min. The sample was then centrifuged at 2000 rpm for 10 min. The charcoal absorbed all of the steroids, including any corticoste-

rone bound to CBG, and it formed a pellet after centrifugation. The supernatant was essentially steroid-free plasma and contained the unbound CBG. The supernatant was then diluted to 2% in PBSG. To determine binding capacity of the stripped plasma, duplicate 0.5-ml aliquots of diluted plasma were incubated (2 hr at 4°) with increasing concentrations of unlabeled corticosterone (range 0.2–100 ng/100  $\mu$ l) and a constant amount (100  $\mu$ l or approximately 10,000 cpm) of tritiated corticosterone. Bound and unbound corticosterone were then separated by adding dextran-coated charcoal suspended in PBSG and incubating the mixture at 4° for 10 min, then centrifuging the sample at 2000 rpm for 10 min. The supernatant, which contained the bound hormone, was decanted into a scintillation vial; 5 ml of scintillation liquid was added and activity counted to 2% accuracy.

The bound/unbound ratio ( $B/F$ ) was calculated, and this ratio was used to determine the total mass of bound hormone (Scatchard, 1949). The bound/unbound ratio was plotted against the total mass of bound hormone, and the slope of the line was fitted to the curve by least-squares regression, which produced the association constant ( $K_a$ ) and its reciprocal, the dissociation constant ( $K_d$ ). The intersection of the line with the abscissa was the binding capacity in nanomoles per liter (see sample curve B, Fig. 5). All the lines fitted to curves were corrected for nonspecific binding, as suggested by Chamness and McGuire (1975).

### Statistics

Statistical analyses were performed using SYSTAT and SPSS. Plasma concentrations of testosterone and preimplant values for corticosterone and CBG capacity and affinity were compared according to treatment using a 1-way analysis of variance (ANOVA). Postimplant data for corticosterone, CBG capacity, and CBG affinity were compared using a repeated measures 2-way ANOVA. Because bleeding time was significantly correlated with postimplant plasma corticosterone levels ( $N = 55$ ,  $r_s = 0.43$ ,  $P = 0.002$ , Spearman's), it was entered as a covariate in analysis of postimplant corticosterone. When bleeding time was plotted against plasma corticosterone levels, the homogeneity of slopes for each treatment group was tested using an ANOVA. Correlations between testosterone and corticosterone

were tested using Spearman's rank-order correlation coefficient.

## RESULTS

### Plasma Testosterone Concentrations

During sampling Week 3 (1.5 weeks after implanting), plasma levels of testosterone differed among treatment groups ( $N = 19$ ,  $df = 2, 16$ ,  $F = 9.26$ ,  $P = 0.002$ , ANOVA; Fig. 1). T-II males had plasma testosterone levels higher than those of T-I males, whose testosterone in turn was higher than that of controls (Fig. 1; 13.27 vs 5.81 vs 1.23 ng/ml, respectively). T-II males had significantly higher testosterone than controls (Scheffe's,  $P = 0.0023$ ), but there was not a significant difference between T-II and T-I males (Scheffe's,  $P = 0.1141$ ) or between C-males and T-I males (Scheffe's,  $P = 0.4287$ ). Plasma T levels of T-II males were well below the physiological maximum attainable by juncos in the wild during early spring (19 ng/ml), although somewhat greater than the early

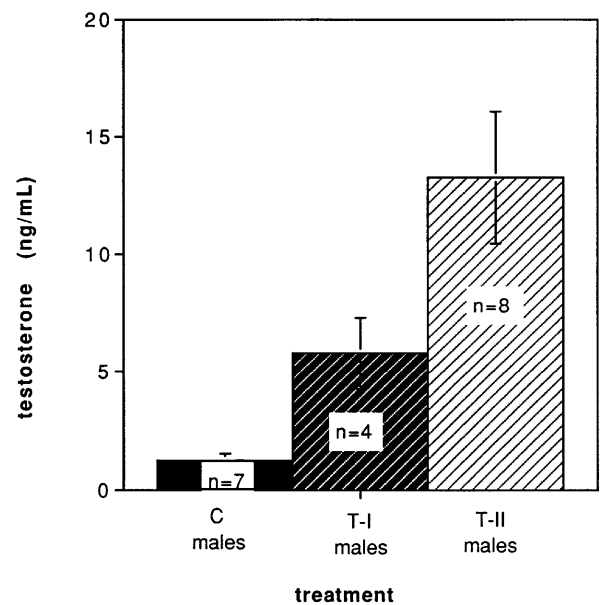


FIG. 1. Plasma testosterone levels after implanting; means  $\pm$  1 SE. Levels of T-II males were significantly higher than those of T-I males and controls; levels of T-I males were significantly higher than those of controls (analysis by 1-way ANOVA).

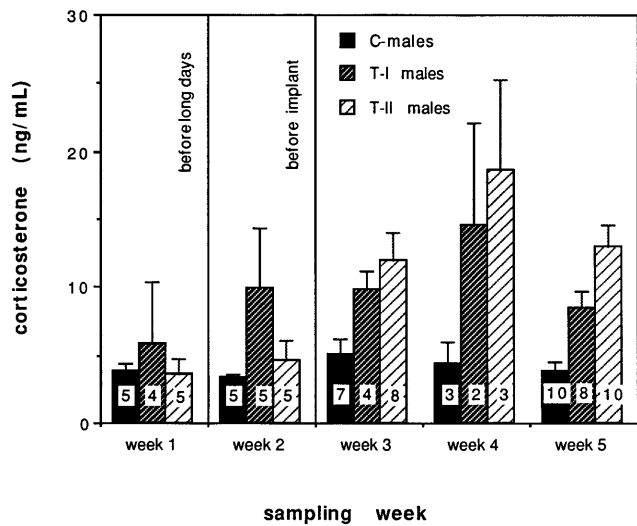


FIG. 2. Effect of treatment with testosterone on plasma levels of corticosterone; means  $\pm$  1 SE. Prior to implanting, there were no significant differences among treatment groups (Weeks 1 and 2 were each analyzed using a 1-way ANOVA). After implanting, T-II males had the highest amounts of corticosterone, followed by T-I males, and controls (Weeks 3 and 4 were combined for analysis and compared to Week 5; analysis by repeated measures 2-way ANOVA; bleeding time entered as a covariate).

spring average for free-living adult males (10 ng/ml) (Ketterson and Nolan, 1992).

**Plasma Corticosterone Concentrations**

Prior to experimental prolongation of day length and therefore also prior to implanting (sampling Week 1), corticosterone levels of treatment groups did not differ significantly ( $N = 14$ ,  $df = 2, 11$ ,  $F = 2.36$ ,  $P = 0.14$ , ANOVA; Fig. 2). The average plasma corticosterone levels, all in nanograms per milliliter, were 3.78 for C-males, 5.87 for T-I males, and 3.65 for T-II males. There were also no significant differences among treatment groups when they were sampled after days were lengthened but prior to implanting (sampling Week 2) ( $N = 15$ ,  $df = 2, 12$ ,  $F = 1.394$ ,  $P = 0.289$ , ANOVA; Fig. 2). Average plasma corticosterone levels for C-males, T-I males, and T-II males were 3.41, 9.90, and 4.66 ng/ml, respectively.

Because the sample size for sampling Week 4 was small ( $N = 8$ ), we combined those data with data collected during sampling Week 3. Only two individuals were bled in both weeks. Both were T-II males, and we chose the smaller of the corticosterone values to

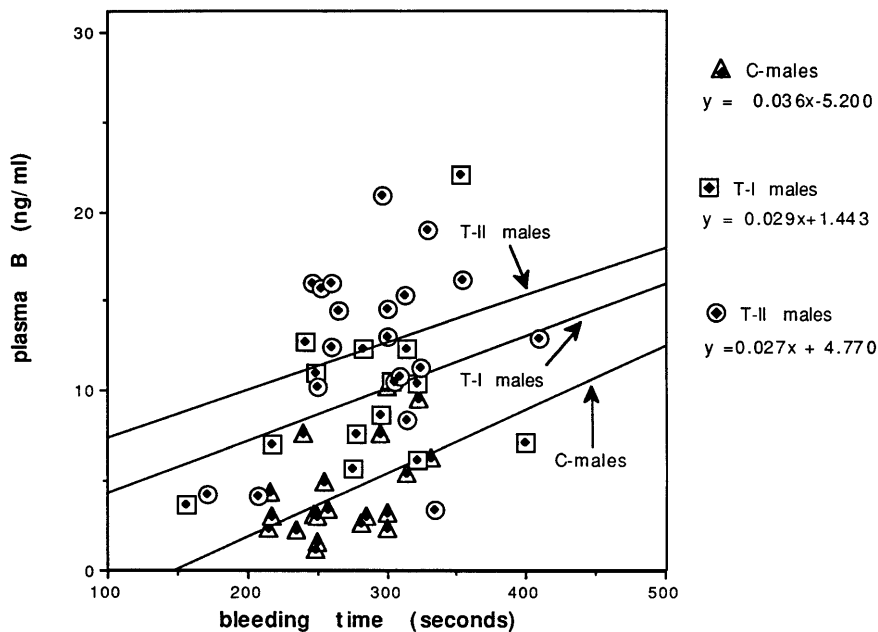


FIG. 3. Correlation between plasma levels of corticosterone and bleeding time. All three treatment groups showed a similar rate of increase of corticosterone with bleeding time.

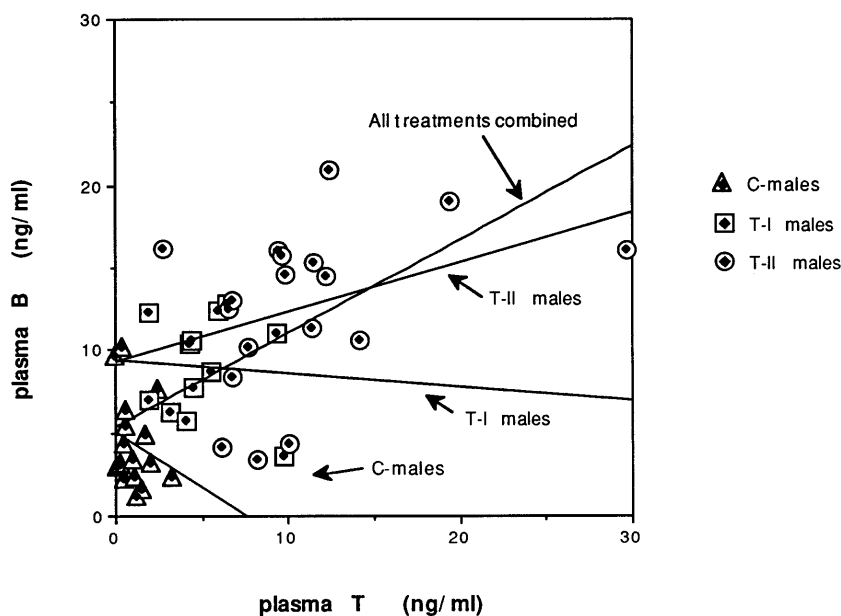


FIG. 4. Correlation between plasma levels of corticosterone and testosterone. Only when all treatment groups were combined were testosterone and corticosterone levels significantly positively correlated.

include in the analysis. After implanting, when comparing Week 3/4 and 5 according to treatment, plasma levels of corticosterone differed among treatment groups ( $N = 25$ ,  $df = 2, 21$ ,  $F = 23.06$ ,  $P < 0.01$ , repeated measures 2-way ANOVA; Fig. 2). T-II males had plasma corticosterone levels higher than those of T-I males, whose corticosterone was in turn higher than that of controls. Sampling week had no significant effect on corticosterone ( $F = 0.65$ ,  $P = 0.43$ ), and there was no significant interaction between treatment and sampling week.

To determine whether testosterone-implanted males responded differently than controls to the stress of being handled and bled, we plotted corticosterone levels against bleeding time (Fig. 3). The slopes were homogeneous for all three treatment groups, indicating no effect of treatment on the stress response ( $N = 55$ ,  $df = 2, 49$ ,  $F = 0.13$ ,  $P = 0.88$ , ANOVA; Fig. 3).

We also asked whether levels of testosterone and corticosterone were correlated. Analyzed according to treatment groups, they were not: C-males ( $N = 20$ ,  $r_s = -0.268$ ,  $P > 0.05$ ), T-I males ( $N = 14$ ,  $r_s = 0.213$ ,  $P > 0.05$ ), T-II males ( $N = 21$ ,  $r_s = 0.247$ ,  $P > 0.05$ ). When individuals of all treatment groups were pooled, however, testosterone and corticosterone levels were

significantly positively correlated ( $N = 55$ ,  $r_s = 0.646$ ,  $P < 0.002$ ; Fig. 4).

### Corticosteroid-Binding Globulin

Prior to our prolonging days (and therefore before implanting), approximately half of the paired samples ( $N = 3$  of 7, 43%) showed a nonspecific, high-capacity binding pattern that indicated binding by albumin only (Silverin, 1986) (Fig. 5; curve C). In addition, one paired sample (C-male, Week 5) also showed this nonspecific, high-capacity binding pattern after implant. These four samples were not included in Fig. 6. The remainder of the paired samples prior to photostimulation ( $N = 4$ ) showed a biphasic binding pattern, indicating the existence of two types of binding systems (Fig. 5, curve A). Birds that were destined to be C-males and T-II males had an average capacity of 78.5 and 35.2 nmol/L, respectively (Fig. 6).

After the days were prolonged, all samples of both treatments showed a biphasic binding pattern (Fig. 5, curve A). One binding system, presumably CBG, had high-affinity, low-capacity binding; the other, presumably albumin, had low affinity and a very high capacity (lower, flat part of curve A). Prior to implanting

(sampling Week 2), there was no significant difference between treatment groups in the plasma's capacity to bind corticosterone ( $P = 0.51$ ; Fig. 6). Birds that were destined to be C-males and T-II males had an average capacity of 76.6 and 70.4 nmol/L, respectively.

After implanting, the average capacity of T-II birds to bind corticosterone was greater than that of controls ( $N = 6$ ,  $df = 1, 4$ ,  $F = 12.88$ ,  $P = 0.023$ , repeated measures 2-way ANOVA; Fig. 6). Sampling week did not have a significant effect on the capacity of the plasma to bind corticosterone, and there was no significant interaction between treatment and sampling week. There were no significant differences in the affinity of CBG for corticosterone between the treatment groups

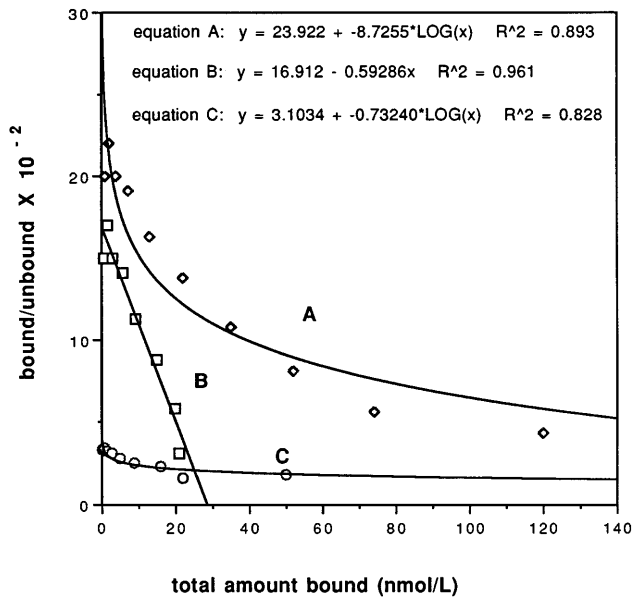


FIG. 5. Scatchard plot of protein binding of corticosterone in junco plasma. Each plasma sample was produced by combining equal volumes from paired individuals. The steep portion of curve A indicates a high-affinity, low-capacity binding system that is specific for corticosterone. The shallow, linear portion of the curve indicates a low-affinity, high-capacity binding system that is nonspecific for corticosterone. Nonspecific binding was corrected for (subtracted) according to the method of Chamness and McGuire (1975), and the corrected result is line B. The slope of this line is the association constant ( $K_a$ ), and its reciprocal is the dissociation constant ( $K_d$ ).  $K_d$  is a measure of the affinity that CBG has for corticosterone. The intersection of the line with the abscissa is the binding capacity in nanomoles per liter. Line C is an example of the nonspecific binding pattern that was found in birds prior to their being subjected to long days. It indicates that only a low-affinity, high-capacity binding system is present, probably attributable to albumin.

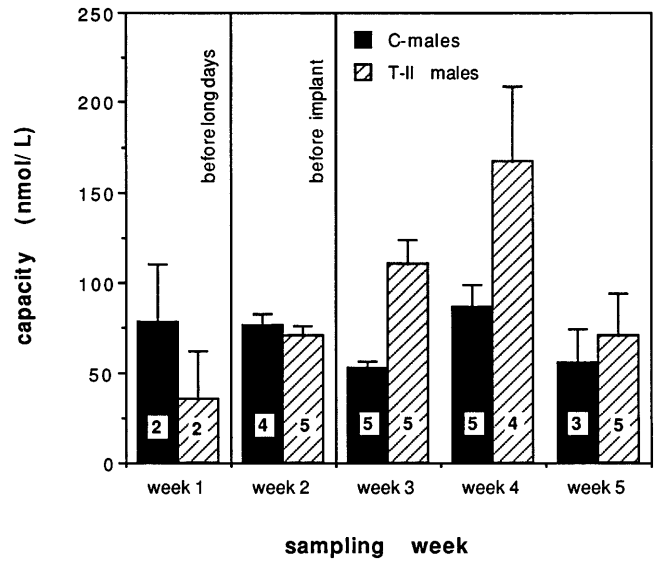


FIG. 6. Effect of treatment with testosterone on the capacity of CBG; means  $\pm$  1 SE. Prior to implanting, there was no significant difference between treatment groups (analysis by 1-way ANOVA). After implanting, the plasma of T-implanted birds had a greater capacity to bind corticosterone (analysis by repeated measures 2-way ANOVA).

for any of the weeks sampled ( $N = 6$ ,  $df = 1, 4$ ,  $F = 0.95$ ,  $P = 0.385$ , repeated measures 2-way ANOVA; Fig. 7).

## DISCUSSION

Treatment with experimentally elevated testosterone significantly increased plasma levels of corticosterone in captive juncos. Birds that received a dose of testosterone equivalent to the spring peak in free-living juncos (T-II implants) had plasma corticosterone levels two to three times those of controls (see Fig. 2). Plasma corticosterone of individuals that were given an intermediate dose of testosterone (T-I implants) fell between that of controls and T-II individuals. Ketterson *et al.* (1991) also found that testosterone implants increased plasma corticosterone levels in free-living and captive juncos. In pied flycatchers, *Ficedula hypoleuca*, Silverin and Wingfield (1982) reported that plasma levels of corticosterone were elevated when the birds arrived in the breeding area, a time when plasma levels of testosterone were also elevated, and remained

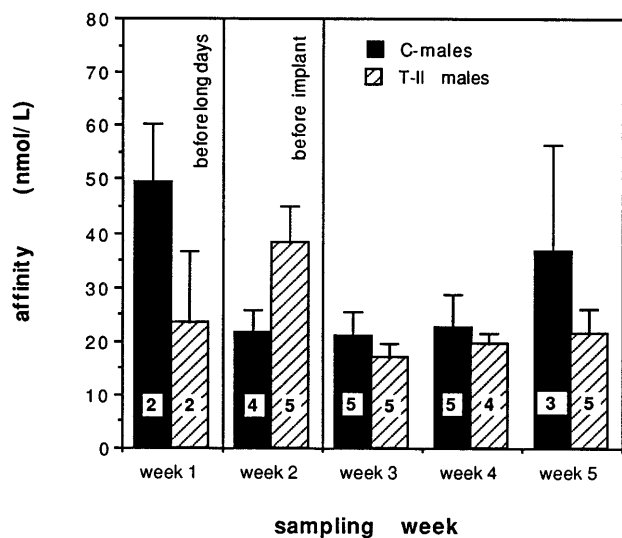


FIG. 7. Effect of treatment with testosterone on the affinity of CBG; means  $\pm$  1 SE. There were no significant differences in affinity between treatment groups (preimplant analysis by 1-way ANOVA; postimplant analysis by repeated measures 2-way ANOVA).

high until the end of the nest-building period. Similarly, the plasma concentration of corticosterone in male white-crowned sparrows, *Zonotrichia leucophrys gambelii*, increased dramatically during vernal migration and remained high throughout the breeding season, concurrently with high testosterone levels (Wingfield and Farner, 1978).

Other studies, however, have found no effect of testosterone on plasma corticosterone levels. In testosterone-implanted house sparrows (*Passer domesticus*; Hegner and Wingfield, 1987) and song sparrows (*Melospiza melodia*; Wingfield, 1984), plasma corticosterone was slightly, but not significantly, elevated. In still other studies, testosterone appears to suppress plasma corticosterone levels. Péczy and Daniel (1979) found that the plasma corticosterone of photostimulated Japanese quail (*Coturnix coturnix*) was significantly increased by castration and thus the removal of gonadal testosterone. However, the effect may have been owing to castration per se, because photostimulation alone, accompanied by gonadal recrudescence and thus by increased testosterone, also significantly increased plasma levels of corticosterone (Péczy and Daniel, 1979). But in photostimulated Peking ducks, domestic pigeons (*Columbia livia*), and Japanese quail, castration also significantly increased corticosterone;

moreover, the levels of plasma corticosterone fell to control levels when castrated birds were given testosterone (Péczy, 1979).

Was the testosterone-induced increase in plasma corticosterone in juncos indicative of increased secretion? It is possible for radioimmunoassay to reveal a higher concentration of corticosterone even in the absence of elevated secretion, if much of the corticosterone is bound to CBG (Wingfield *et al.*, 1992, 1984). Therefore, we asked whether the increase in plasma corticosterone levels detected by RIA could be attributed to an increase in corticosterone secreted by the adrenal cortex.

Our results indicate that there was little CBG activity before the juncos were exposed to a light-dark regime that simulated the breeding season, and that the blood's capacity to bind corticosterone, i.e., its CBG-binding capacity, increased significantly in response to treatment with testosterone. Prior to photostimulation, the plasma of approximately half of the birds (3 of 7) showed only a nonspecific, high-capacity binding pattern (Fig. 5, curve C) that indicates binding by albumin only (Silverin, 1986). Natural testosterone levels were also extremely low prior to photostimulation (Cawthorn *et al.*, in preparation). After photostimulation, there were no significant differences between the treatment groups in the affinity of CBG for corticosterone for any of the weeks sampled (Fig. 7), suggesting that the increased capacity of the plasma to bind corticosterone should be attributed to an increase in the concentration of CBG and not to any change in the affinity of CBG for corticosterone.

Our results are not consistent with those of Péczy and Daniel (1979), who found that the concentration of CBG was significantly higher in Japanese quail (*C. coturnix*) held on short days than it was in those held on long days. Also, Péczy (1979) and Péczy and Daniel (1979) reported that castration led to increased levels of CBG and that subsequent administration of testosterone to castrates decreased CBG to control levels. However, like us, Silverin (1986) found that CBG levels were higher in the breeding season. The CBG concentration in the plasma of male pied flycatchers (*F. hypoleuca*) decreased dramatically from prenest building, when testosterone levels were high, to the nestling stage, when levels of testosterone had returned to basal (Silverin, 1986). Likewise, Daniel and



Assenmacher (1974) reported that CBG levels in male Peking ducks increased after testosterone injections. Unlike the findings by Péczely and Daniel (1979), CBG levels decreased when the ducks were castrated (Daniel and Assenmacher, 1974).

Because CBG in juncos also increases in response to testosterone, the testosterone-induced increase in corticosterone detected by RIA may reflect only an increase in CBG levels, not an increase in secretion of corticosterone; or it is possible that both CBG production and corticosterone secretion increase in response to elevated testosterone. However, Silverin (1979) found that the cortical tissues of pied flycatchers (*F. hypoleuca*) show little activity during the breeding season when testosterone levels are high. White-crowned sparrows (*Z. leucophrys*) also show low cortical activity during the time when they show maximal sexual activities (Lorenzen and Farner, 1964). Yet, plasma corticosterone of both these species is quite high during sexual activity (Silverin and Wingfield, 1982; Wingfield and Farner, 1978). Perhaps the apparent inconsistency between cortical activity and actual plasma corticosterone is due to CBG (Silverin, 1986). Breeding passerine birds may suppress secretion of corticosterone while keeping a store of it available in the circulation by means of CBG. This pattern of low cortical activity during sexual activity, however, is not seen in all bird species (e.g., *P. domesticus*, Moens and Coessens, 1970; *Parus major*, Silverin, 1978). Obviously more studies are needed on the seasonal fluctuations of testosterone, corticosterone (including cortical activity), and CBG.

At this time, we can only speculate about the function of avian CBG. There is still considerable debate about its role in the mammalian endocrine system (for review see Rosner, 1990). CBG may provide a reservoir for glucocorticoids that can readily be made available to the free pool of hormone by dissociation (Mendel, 1989). For example, in humans, CBG slows the rate of cortisol disappearance (Bright, 1995). CBG may provide breeding male juncos with a rapidly available quantity of corticosterone in times of stress, such as during inclement weather or injury. Another hypothesis is that mammalian CBG acts as an agent to deliver glucocorticoids directly to lymphocytes (Hammond *et al.*, 1990a,b). CBG is a member of the serpin (serine protease inhibitor) family and is cleaved by neutrophil elastase, and it has been suggested that

CBG delivers bound cortisol to sites of inflammation (Pemberton *et al.*, 1988; Hammond *et al.*, 1990a,b). Therefore, at a time when male juncos are engaging in territorial defense, it may be advantageous to have increased levels of CBG in the event of injuries. Finally, it is possible that CBG is a prohormone that is activated by cortisol and increases cAMP to some unknown biological end (Nakhla *et al.*, 1988).

In sum, among the few passerine birds studied, testosterone, corticosterone, and CBG rise naturally at the start of the breeding season. This increase may be achieved not by increased secretion, but rather by employing CBG to extend the half-life of available corticosterone and allow for its rapid delivery in response to environmental stressors. Experimental treatment with testosterone enhances both corticosterone and CBG and suggests that testosterone may play a regulatory role in this coordinated response to breeding. Understanding the complexity of the relationship between testosterone, corticosterone, and CBG will require much further work.

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