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Testosterone and "5α-Dihydrotestosterone" Levels in Peripheral Plasma of Male and Female Ring Doves (Streptopelia risoria) During the Reproductive Cycle[1]

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ABSTRACT

Testosterone (T), 5α-dihydrotestosterone (DHT), and androstenedione (A) were measured by radioimmunoassay in peripheral plasma of paired male and female ring doves throughout the reproductive cycle. Before the males were moved to the breeding cage for pairing, their T levels were 183.4 ± 28.1 pg/ml and their DHT levels were 129.5 ± 16.3 pg/ml. Within 4 h after pairing with a female, male androgen levels rose (T = 322.8 ± 136.5 pg/ml, DHT = 1043.8 ± 247.3 pg/ml) and by three days after pairing T and DHT had reached peak levels (701.4 ± 116.8 pg/ml and 1279.5 ± 241.1 pg/ml, respectively). By the time the female laid her first egg, marking the end of the courtship phase and the beginning of the incubation phase, T and DHT levels had declined to 189.6 ± 26.1 and 200.0 ± 41.9. Androgen remained at approximately these low baseline concentrations throughout incubation and squab rearing, and showed a second elevation only at the beginning (courtship phase) of the next reproductive cycle. Androstenedione levels never exceeded 10 pg/ml.

In the absence of females, neither removal of a male from his home cage to a novel cage, nor pairing of a male with another male resulted in elevation of T or DHT above baseline values. Castrated males paired with females also exhibited no rise in T or DHT concentrations above baseline values. It is concluded that female doves induce increased androgen secretion in males during the coutship phase of the reproductive cycle, that the major source of the androgen surge is testicular, and that the predominant circulating androgens in male doves are T and DHT.

T and DHT were also detected in females, with somewhat higher concentrations tending to occur during courtship than at other phases of the cycle. However, androgen concentrations in females were lower than those of males at all stages of the reproductive cycle.

INTRODUCTION

During the courtship phase of a reproductive cycle male and female ring doves display a predictable sequence of behaviors. The male chases the female (his body and head held horizontally, and the rump and tail feathers ruffled) and bows and coos; these movements are often accompanied by the "kah" call (Miller and Miller, 1958; Nottebohm and Nottebohm, 1971). Taken together, these responses have been termed aggressive courtship (Lovari and Hutchison, 1975). Aggressive courtship declines rapidly after the first day of pairing. The male also engages in nest soliciting behaviors, including assumption of the *nest-coo posture* (head held down and the tail held upwards), wing flipping, and a nest-coo vocalization. At first the male displays nest soliciting behaviors in many places in the cage, but eventually these behavior patterns are performed primarily in the nest bowl (Silver, unpublished observations). The frequency of nest soliciting is high initially and declines by about three days after pairing (Lovari and Hutchison, 1975). The male then gathers straw and brings it to the female who remains in the nest bowl, and she builds the nest. Copulation occurs daily during courtship, usually in the late afternoon or early

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evening (Martinez-Vargas and Erickson, 1973). Several days (median = 7 days) after pairing the female lays the first egg and this marks the end of what we term the "courtship phase" (a period in which nest-building also occurs) and the beginning of the incubation period. A second egg is laid 40 h later and both the male and female incubate the eggs, which hatch after about 14 days (Lehrman, 1965). Both parents then feed the young by regurgitating material from the crop (Lehrman, 1965). During the incubation and brooding stages the male partner does not display bow-coo responses, unless he is exposed to a new, courtship phase female (Silver and Barbiere, 1976). After incubation and brooding are completed the pair of ring doves may undergo a new reproductive cycle.

Endocrine correlates of reproductive cycles of male and female doves have been studied by means of gonadectomy, replacement therapy, and radioimmunoassay of steroid hormones. After gonadectomy, bow-cooing and nest-soliciting diminish in males but these behaviors can be reinstated by treatment with testosterone propionate (Erickson, 1970). Radioimmunoassays of peripheral plasma estradiol (Korenbrot et al., 1974) and progesterone (Silver et al., 1974) indicate undetectable levels of estrogen and constant, low levels of progesterone throughout the reproductive cycle in the male.

The purposes of this study were to measure levels of testosterone (T), 5α-androstan-17β-ol-3-one (5α-dihydrotestosterone, DHT), and 4-androstene-3,17-dione (androstenedione, A) in the peripheral plasma of male and female ring doves during their reproductive cycles, and to correlate these findings with the expression of various behavioral patterns including bow-cooing, nest-cooing, nest-building, and incubation. The first question we asked was whether there were increases during the cycle in the plasma concentrations of androgen in the male ring dove when exposed to an intact stimulus female. Instances of transitory increases in androgen levels in males exposed to females have been reported for a variety of mammals including rabbits (Saginor and Horton, 1968), rats (Purvis and Haynes, 1974), mice (Macrides et al., 1975), guinea pigs (Harding and Feder, 1976), bulls (Katongole et al., 1971), rhesus monkeys (Bernstein et al., 1975), and men (Fox et al., 1972). If such an increase also occurred in ring doves it would be of interest to know whether a) T, DHT and A were all involved, b) such increases in plasma androgen would occur again if a male were allowed to undergo a second consecutive reproductive cycle with the same female, c) the source of increased plasma androgen was testicular or adrenal and d) pairing with another male, rather than with a female, would induce increases in plasma androgen concentrations in males.

In the *female* ring dove, plasma estradiol levels significantly increase during the mid-courtship phase of the reproductive cycle (Korenbrot et al., 1974), and progesterone levels also rise prior to oviposition (Silver et al., 1974). A second question approached in the present study was whether female ring doves had detectable levels of androgens in their peripheral circulation, and whether such levels varied during the course of a reproductive cycle. Androgen has been detected in the plasma of laying hens, with peak values reported to occur several hours before ovulation (Peterson et al., 1973; Shahabi et al., 1975). In view of recent evidence in female rats suggesting that T facilitates the release of FSH prior to ovulation (Gay and Tomacari, 1974), we sought to determine whether T or another androgen increases in concentration in ring doves before the expected time of ovulation. However, our experimental design did not permit exact determination of the timing of ovulation and our primary emphasis was on the possible correlations between various behaviors displayed and levels of circulating androgens.

MATERIALS AND METHODS

Subjects

Previous experience. Male and female ring doves were separated from their parents at the age of 21 days and placed in stock cages in groups of 6 to 10 birds. At 4 months of age, the sex of each bird was determined by exploratory laparotomy and the birds were placed in individual isolation cages in which they were visually isolated from other birds. When the birds were sexually mature (5 to 6 months old) they were placed in pairs in breeding cages and permitted to proceed through a complete reproductive cycle. This consisted of courtship, nest-building, laying and incubating eggs, and rearing squab to 21 days of age. At the end of the breeding cycle, doves were placed in isolated cages for a period of at least 3 weeks.

Cages and Rearing Conditions

Housing. Breeding and testing cages were of wood with wire mesh front doors, measuring 80 cm wide, 45 cm deep and 35 cm high. Stock cages were 87.5 cm cubical cages with wooden frames and wire mesh sides. Isolation cages were rack mounted, metal cages measuring 41.2 cm wide, 23.8 cm deep and 17.5 cm high.

Water, food and grit were available *ad lib* in all

experimental cages. A 11.2 cm diameter glass bowl in which the birds built their nest was placed in the right front corner of the breeding cage.

Breeding cages, stock cages and isolation cages were kept in separate rooms. Constant day length of 14 h light, 10 h dark (on 0600, off 2000 h) was maintained. Temperature was kept at 21–22°C.

Methods: Experiment 1

Treatment groups. Doves with one prior breeding experience were placed in breeding cages and permitted to proceed through a cycle. Blood (ca. 4 ml) was drawn from the heart (without anesthesia) between 1300 and 1600 h at selected stages of the reproductive cycle. A maximum of two min elapsed between taking the animal from its cage and the completion of blood withdrawal. During the courtship phase blood was drawn from the male of the pair at 1, 3 and 5 days (C1, C3, C5) after introduction to the breeding cage. Incubation-phase animals were sampled on incubation Days 1, 3, 5, 7, 9, and 13 (I1, I3, . . . I13). Incubation Day 1 was defined as the first day the egg was present in the nest at 0900 h. Brooding animals were sampled on the 2nd and 10th day after the squabs hatched. Only one sample was taken from each animal. In addition, three other groups of animals were sampled. In order to test whether exposure to a novel cage alone induced changes in androgens, males were bled 24 h after being placed, individually, in breeding cages. To determine whether exposure to another male had any effect, pairs of males were placed in breeding cages and bled 24 h later. One group of castrated males (2–3 weeks after surgery) was bled 24 h after exposure to intact females. Another group of castrated males, housed in isolation, was sampled as well. At autopsy the castrated males were laparotomized to check for completeness of gonadectomy.

Experiment 2

Treatment groups. In this experiment, the male was introduced into the breeding cage two days before observations were begun. On the first day of observations the females, nest bowls, and straw for nest material were placed in the breeding cages. Two ml blood samples were obtained (between 1300 and 1600 h) from both male and female birds by cardiac puncture without anesthesia. Baseline androgen levels were obtained by bleeding the birds two days before pairing. The pairs were then bled once during the first reproductive cycle period on day CO (4 h after pairing) or on day C1, C3, C5, I1, or I3. Some of these pairs were observed during a second courtship period, and were bled on the eighth day after the squabs (S8) hatched (second baseline). Ten days after hatching, the squabs were removed from the cage and observations for the second courtship period were begun. Since the two baseline androgen levels were uniformly low, no baseline data were obtained for the remaining pairs. These remaining birds were bled twice during the first reproductive cycle and the survivors were observed and bled twice more during the second courtship period. The minimal interval between bleedings was two days.

Behavioral observations. Pairs were observed for 30 min on each day of courtship (6 to 15 days was the variation in egg-laying latency) and on the first three days of incubation. Observations were made between 0700 and 1100 EST.

The occurrence of a particular type of behavior was recorded for each 30 second interval in the 30 min observation period. The number of 30 second intervals in which each type of behavior occurred was totalled for each pair. The male courtship behaviors were: bow-coo, nest soliciting (oblique stance, wing flip, nest coo), hop charge, picking up nest material, gathering nest material, nest building, allopreening, in nest, and copulation. The female courtship behaviors were: oblique stance, wing flip, nest coo, picking up nest material, gathering nest material, nest building, self-preening, allopreening and copulation. Instances of non-courtship behavior such as self-preening and time spent on the feeder were also recorded. All of these behaviors have been described previously (Miller and Miller, 1958; Chang, 1972; Lovari and Hutchison, 1975).

Assay Procedure

Plasma androgens were measured by a modification of the method described by Coyotupa et al. (1972).

Plasma Extraction

Two hundred to 500 μl aliquots of plasma (they yielded the same results when expressed on a per ml basis) were pipetted into a 13 ml centrifuge tube immediately after thawing. Then 50 μl of assay buffer containing ca. 2500 cpm of ^{3}H-T and ca. 2500 cpm of ^{3}H-DHT was added to each tube and used as an internal standard to estimate recoveries. Extractions were carried out by shaking for 30 seconds with 5 ml of redistilled ether (Mallinkrodt, Nanograde). The two phases were separated by centrifugation and the ether extracts were transferred to corresponding disposable tubes and dried under nitrogen.

Column Chromatography

A modification of the celite column technique described by Abraham et al. (1970) was used. The stationary phase used in this experiment was 25 percent ethanediol in propanediol-1,2 (Matheson, Coleman and Bell, Chromatography grade). This was mixed with the celite before packing the column in a 1:2 ratio (stationary phase/celite w/w). After packing to a height of 5 cm in Kimble disposable Pasteur pipets, the celite was washed with 7 ml of 2,2,4-trimethylpentane (Fisher Sci., Spectranalyzed). Elution of the steroids was carried out stepwise. Four and a half ml of 10 percent ethyl acetate (Fisher Sci., Spectranalyzed) in 2,4,4-trimethylpentane eluted DHT (fraction X) and A and 4.5 ml of 25 percent ethyl acetate in 2,2,4-trimethylpentane eluted T (fraction Y). Ninety-seven per cent of DHT and T were eluted in fractions X and Y, respectively.

Radioimmunoassay

The dried residue was redissolved in 2 ml of assay buffer and vortex mixed for 15 seconds. An aliquot of 250 μl was taken from each sample to calculate recovery and triplicate aliquots of 500 μl were added to 15 × 85 mm disposable glass tubes. One hundred μl of antibody (S-741 #2 purchased from Dr. G. E.

Abraham) and 50 μl of assay buffer containing 30,000 dpm of ^{3}H-DHT (50 Ci/mmole, N.E.N.) or 50 μl of assay buffer containing 30,000 dpm of ^{3}H-T (50 Ci/mmole, N.E.N.) was added to the DHT or T fractions, respectively, Standard curves were constructed by drying, under nitrogen 25, 50, 100, 200, 400, 600, 800 μl of a 1 ng/ml solution of DHT (Schwartz, Mann) in absolute ethanol and 10, 25, 50, 100, 200, 400, 600 μl of a 1 ng/ml solution of T (Schwartz, Mann) in absolute ethanol. The dried residues were redissolved in 2 ml of assay buffer. Triplicate aliquots were taken from each tube and treated as above. The standard curve samples were incubated simultaneously with the plasma samples at 4°C for 4 h. A suspension containing 0.625 percent of Norit A and 0.0625 percent Dextran (Pharmacia) in assay buffer (200 μl) was added to each tube. This was mixed, incubated in an ice bath for 5 min and centrifuged for 2 min at 3000 R.P.M. at 4°C. A 500 μl aliquot of the supernatant was transferred to a scintillation vial containing 10 ml of Toluene Triton X 100 scintillation fluid (Packard). The vials were counted for 20 min each on a Packard Tri Carb 3375 with a counting efficiency of 55 percent for Tritium in the particular system used.

Specificity

No significant cross reaction with steroids other than T and DHT is observed with antibody S-741 #2 (G. E. Abraham, data supplied with antibody shipment). In several instances we separated A from DHT by modifying the column chromatography system. This was done by using the same stationary phase but eluting A in fraction #1 with 5 ml of 2,2,4-trimethylpentane, then DHT in fraction #2 with 3.5 ml of 10 percent ethyl acetate in 2,2,4-trimethylpentane, and T in fraction #3 with 4.5 ml of 25 percent ethyl acetate in 2,2,4, trimethylpentane. Androstenedione was measured in fraction #1 using the same radioimmunoassay procedure described above, but then using antibody S-1557 #2 (for assaying A) purchased from Dr. G. E. Abraham. In more than 30 samples run with S-1557 #2 at all stages of courtship and incubation, no significant amounts of A were found (all values less than 10 pg/ml). This indicates that A is not present in our samples and does not interfere with the assay procedure, thereby allowing us to collect it in the DHT fraction.

Sensitivity

The logit transformation of the percent cpm bound was plotted against the ln amount of T and DHT added to the standard curve. The coefficient of variation at each point of the standard curve was less than 5 percent and the sensitivity was 5 to 10 pg for T, and the sensitivity for DHT was 15–20 pg.

Accuracy

The accuracy of the assay was assessed by adding 10, 50 and 200 pg of T and 25, 50 and 200 pg of DHT to assay buffer (Table 1). Accuracy of the assay was also assessed for T by measurements taken from 5 samples of plasma from ovariectomized ring doves. The values obtained were 20.1, 14.6, 12.3, 25.8 and 25.1 pg/ml ($\bar{X}$ = 19.6). When 25 pg of T was added to plasma from six ovariectomized subjects, the values obtained were 40.0, 24.8, 31.1, 43.4, 44.2 and 40.8 pg/ml ($\bar{X}$ = 37.4). When 100 pg of T was added to six samples of plasma from ovariectomized doves the values obtained were 120.2, 94.1, 178.6, 150.7, 133.7 and 119.1 pg/ml ($\bar{X}$ = 131.1). These data indicate a "blank" of about 20–30 pg in gonadectomized doves. A portion of this may be attributable to adrenal secretion of androgen but it is not feasible to adrenalectomize ring doves. In another series of tests, we added 25 pg of T to water and obtained values of 20.3, 27.6, 28.1, 26.3, 25.6 and 26.6 pg by assay. Addition of 100 pg T to water resulted in values of 140.9, 146.3, 99.1, 113.3, 79.1 and 111.9 pg.

TABLE 1. Assessment of assay accuracy by addition of unlabelled Testosterone (T) and 5α-Dihydrotestosterone (DHT) to assay buffer.

Amount added (pg)	DHT found	% deviation	T found	% deviation
10	...	...	12	+20
			10	0
			10	0
25	28	+12		
	22	-12		
	25	0		
50	63	+26	68	+36
	50	0	60	+20
	31	-38	51	+2
200	141	-29	193	-3
	161	-19	177	-12
	139	-31		

Recovery

After ether extraction and celite chromatography the mean recovery for DHT was 81.55 percent ± 2.56 (X ± SEM) and for T, 89.89 percent ± 1.72 (x ± SEM).

Precision

Precision between assays was evaluated by duplicate measurements of the same plasma sample. The coefficient of variation was computed by Snedecor's formula C.V. $=\sqrt{\frac{Ed^2}{2n}}$ where d = [highest value between duplicates/lowest value between duplicates − 1] × 100. For 12 duplicates considered, the coefficient of variation between assays was 15.79 percent for DHT and 11.20 percent for T.

RESULTS

Because the plasma androgen levels measured in Exp. 1 (no behavioral observations and only one plasma sample taken from each animal) and Exp. 2 (behavioral observations recorded and each animal bled 2 to 4 times) showed similar patterns, the cycle results have been pooled. Figure 1 shows T and DHT levels in males kept in isolation and in males paired with intact females at representative stages of the reproductive cycle. In males housed in isolation cages (N=10), plasma T levels were 183.4 ± 28.1 pg/ml ($\bar{x}$ ± SEM). When the males were placed with females in breeding cages, there was a slight rise (to 322.8 ± 136.5 pg/ml) in plasma T at CO (4 h after pairing) and this increased to 654.1 ± 148.5 pg/ml at C1 and 701.4 ± 116.8 pg/ml at C3. By C5, a decline in plasma T had begun (552.3 ± 101.1 pg/ml) and levels characteristic of isolated males were reached by day I1 (189.6 ± 26.1 pg/ml). Levels of T remained low throughout incubation and the post-hatching period (I1 through S10). In some birds the squabs were removed at S8 and the pair was permitted to court again. In males of these pairs, another rise in plasma T occurred during the second courtship period, the increase in T being similar to that seen in the first courtship phase. By I1 of the second cycle, T levels had declined to low values as in the first cycle.

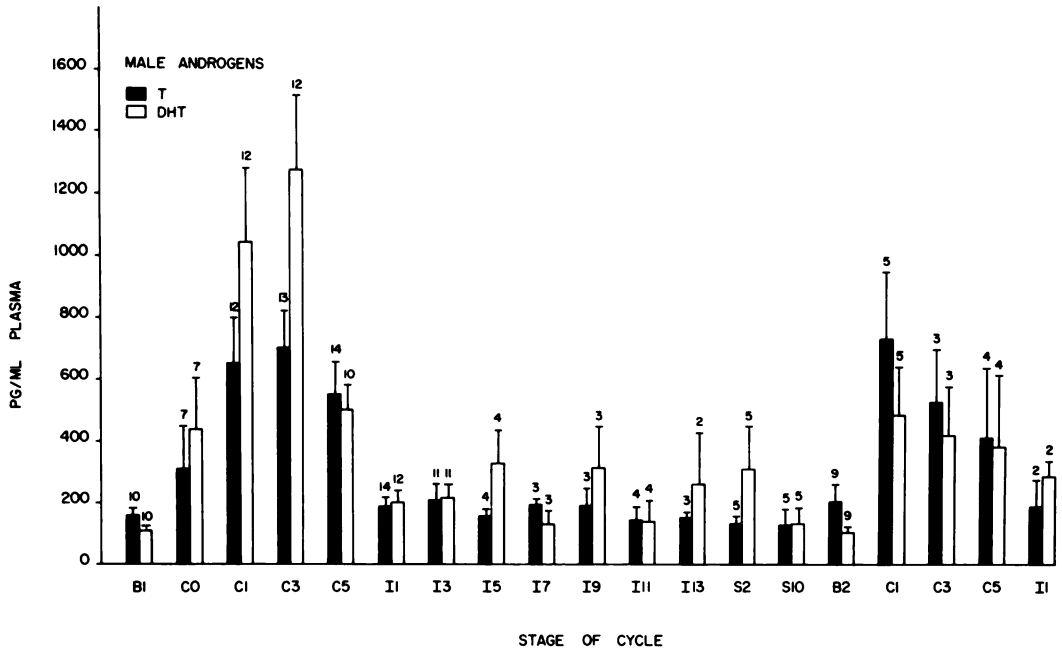

FIG. 1. Plasma androgen levels in male ring doves throughout the reproductive cycle (B = baseline levels). Testosterone levels showed significant variations over the first cycle (Analysis of Variance, F = 6.22) with C3>B_1, B_2, I_1, I_3, I_{5-7}, I_{9-13}, S_2 and S_{10} (P<0.01, Newman-Keuls) and C5>B_1, B_2, I_1, I_3, I_{5-7}, I_{9-13}, S_2 and S_{10} (P<0.05, Newman-Keuls). Dihydrotestosterone levels also varied significantly over the first cycle (Analysis of Variance, F = 106). Specific statistically significant differences were as follows (Newman-Keuls test): all C days>all I days (P<0.01), all C days and all I days>both B values (P<0.01), first cycle C_1, C_3, C5>second cycle C_1, C_3, C_5 (P<0.01), first cycle C_1, C_3, C_5>first cycle C_0 (P<.01), first cycle C_1>first cycle C_5 (P<0.01), first cycle C_3>first cycle C_1, C_5 (P<0.01), and second cycle C_3, C_5>second cycle C_0, C_1 (P<0.05). Measure of central tendency was mean ± S.E.M.

The pattern of DHT levels in males was similar to that of T at each stage of the cycle, with highest values occurring at C1 and C3 (Fig. 1). Androstenedione levels, in contrast, were low throughout the reproductive cycle and never exceeded 10 pg/ml. For this reason, androstenedione values are not shown in Fig. 1 and were not measured in subsequent experiments with males.

Figure 2 shows T and DHT levels in females housed in isolation and at representative stages of the reproductive cycle. Both T and DHT were detectable in peripheral plasma of females, while A levels were lower and remained below 10 pg/ml. Generally, T and DHT levels were higher during courtship phases than isolation, incubation and squab rearing. However, in females elevations in T and DHT during courtship were not as dramatic or consistent as those seen during comparable periods in the male. Results of statistical tests for both males and females during the cycle are indicated in Figs. 1 and 2, respectively.

As part of Exp. 1 males (N = 4) were placed individually in breeding cages and plasma samples taken 24 h later. Animals exposed to this novel cage situation did not exhibit plasma levels of T (266.6 ± 21.4 pg/ml) or DHT (140.0 ± 17.3 pg/ml) significantly in excess of those measured in males housed in isolation in their home cages (T = 183.4 ± 28.1 pg/ml and DHT = 129.5 ± 16.3 pg/ml for 10 and 9 animals, respectively). Thus, the stress of being moved to a new cage does not induce increased T or DHT titers in plasma of males.

Also in Exp. 1, males were paired with males for 24 h prior to plasma sampling (N = 17 pairs). T levels were 193.0 ± 29.5 pg/ml and DHT levels were 89.2 ± 16.6 in these males. These values are obviously lower than those seen in males paired with females for 24 h (T = 654.1 ± 148.5 pg/ml, DHT = 1043.8 ± 24.73 pg/ml, N = 12). No systematic relationship was noted between androgen level and dominance status in the pairs of males.

Finally, in Exp. 1 castrated males (N = 6) exposed to females for 24 h had T levels of 159.2 ± 25.7 pg/ml and DHT levels of 224.8 ± 54.6, while their female partners had comparable levels of androgen (T = 127.3 ± 25.6

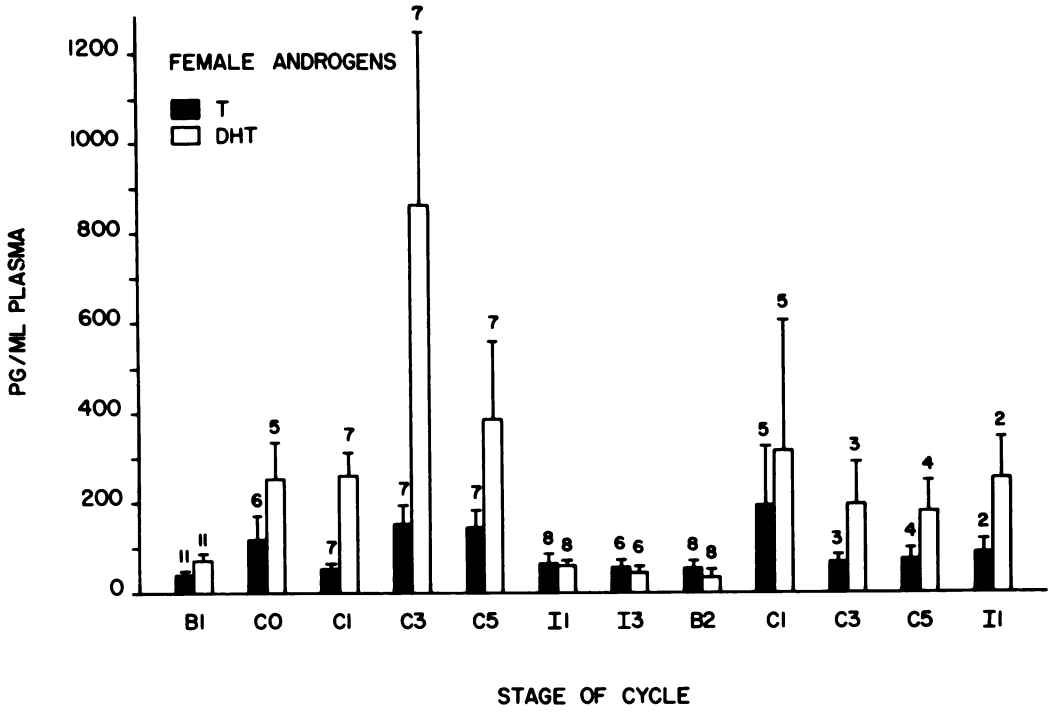

FIG. 2. Plasma androgen levels in female ring doves throughout the reproductive cycle (B = baseline levels). No significant variation in testosterone occurred over the cycle (Analysis of Variance). A significant overall difference in dihydrotestosterone was found (Analysis of Variance, F = 2.94), but the only specific difference was first cycle C_3>all other values except first cycle C_5. Measure of central tendency was mean ± S.E.M.

pg/ml, DHT = 410.1 ± 145.1 pg/ml, N = 6). Intact males exposed to females for 24 h had T levels of 654.1 ± 148.5 pg/ml and DHT levels of 1043.8 ± 247.4 pg/ml (N = 12). Castrates not exposed to females but kept in isolation in their home cages were assayed for T only (N = 3) and had a mean level of 143.2 ± 16.0 pg/ml, while two sham castrated males kept in isolation had a mean T level of 230.9. In the same run of this assay a water blank taken through the entire procedure yielded a value of 65.0 ± 5.2 pg/ml (3 samples). This high "water blank" was not a consistent finding (see section on Accuracy in Methods). The blank was not subtracted from the plasma values, even though to do so would have emphasized the difference between intacts and castrates. To sum up, the work with castrated males indicates that the source of the female-induced androgen increase in males is testicular rather than adrenal. A slight difference in T levels between castrates and baseline levels in intacts may exist, but is probably somewhat obscured by the method blank and by individual variations in T secretion.

In Exp. 2, attempts were made to determine whether there were correlations between behaviors of T in males and females. Figures 3 and 4 show the frequency of a number of behaviors demonstrated by both sexes during the courtship phase of the reproductive cycle. Table 2 shows that the only behavior significantly correlated with T levels in both courtship periods was wing-flipping by the male. It remains to be established whether this relationship is causal or coincidental.

DISCUSSION

This study demonstrates that male ring doves exhibit significantly increased levels of plasma T during the courtship phase of the reproductive cycle. Three lines of evidence

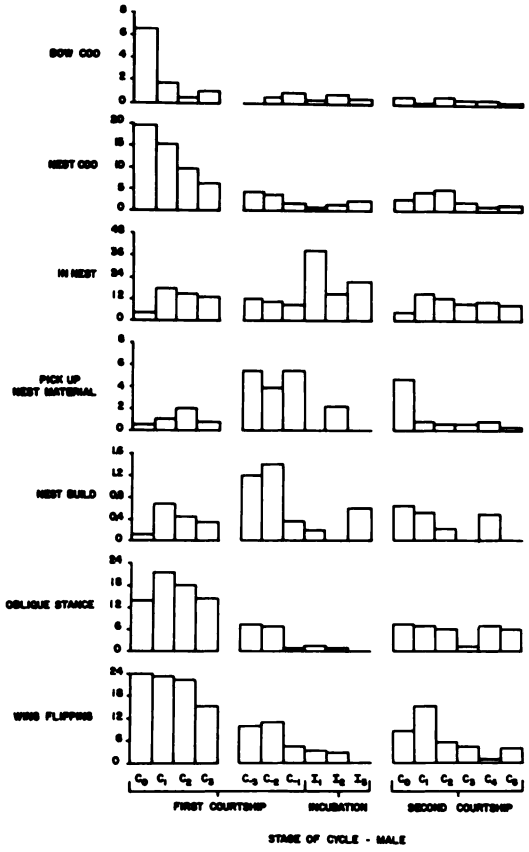

FIG. 3. Mean frequencies of occurrence of various behaviors in male ring doves during the reproductive cycle. The ordinates are the numbers of behavioral occurrences. Table 2 should be consulted for correlations between androgen levels and behaviors.

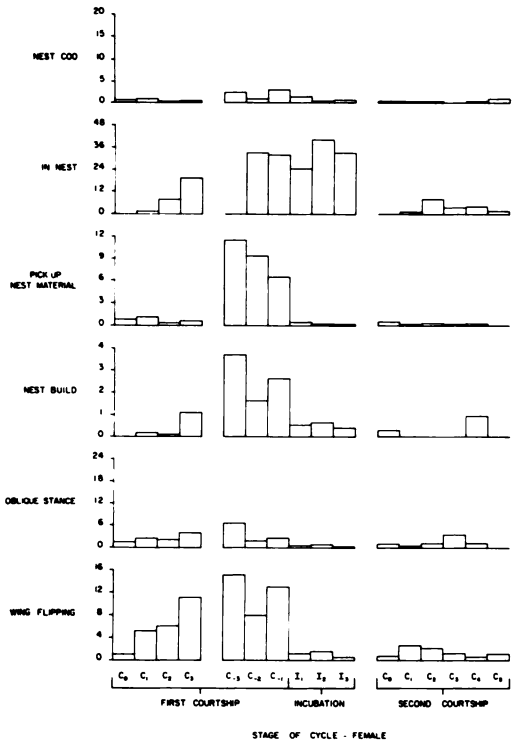

FIG. 4. Mean frequencies of occurrence of various behaviors in female ring doves during the reproductive cycle. The ordinates are the numbers of behavioral occurrences. Table 2 should be consulted for correlations between androgen levels and behaviors.

TABLE 2. Spearman rank-order correlations between testosterone level and frequency of behaviors.

		Bow cow	Hop charge	Nest coo	Oblique stance	Wing flip	Pick up	Gather	Build	Self preening	Allo-preen	In nest
Male	1st courtship	.11	.10	.22	.31*	.37*	-.62*	-.51*	.12	.18	.28	.31*
	2nd courtship	.16	.16	.73**	.18	.45*	.13	.44	.58*	.21	-.10	.12
Female	1st courtship	...	...	.15	.41*	.16	-.32*	.29	.47**	.16	-.61	.18
	2nd courtship	...	...	.23	.31	.39	.15	.16	.16	.34	-.41	.31

*P<.05.
**P<.01.

indicate that the increased T levels can not be attributed to non-specific factors such as stress. First, placement of a male alone in a novel (breeding) cage does not result in elevated T titers. Second, an increase in T levels is seen in the second courtship period of two consecutive breeding cycles even though the male is left in the same breeding cage with the same mate. Third, increased T levels become evident in males within 24 h after pairing with a female but not within 24 h pairing with other males. Because the male shows aggressive behaviors (bow-cooing, charging, cackling) towards both males and females, it is apparent that the performance of these behaviors does not by itself lead to increased levels of T. In some respects, this finding is analogous to the report of Fox et al. (1972) in which ejaculation produced by masturbation was not accompanied by increases in plasma T or accompanied by only a small increase in plasma T (Purvis et al., 1976) in men, while ejaculation as a result of heterosexual intercourse was accompanied by larger increases in plasma T. The characteristics of the female dove responsible for producing the increases in T in male doves have not yet been identified, but it seems reasonably clear that the rise in plasma T is of gonadal rather than adrenal origin. This is shown by the fact that castrated males paired with females for 24 h have low plasma T values. Although there is a circumscribed period during courtship in which a rise in plasma T levels occurs in intact males, T is present at levels of about 180 pg/ml throughout the remainder of the reproductive cycle. Basal levels of this magnitude are also found in castrates and intact males in isolation. No decision can be made as to whether the basal levels of T in males are of adrenal or testicular origin, but it should be noted that basal levels of T are lower in females than in males, and that direct comparison of intact vs. castrated males within one run of an assay shows somewhat higher levels of T in the intacts.

Aside from a study of a relatively small number of animals by Hutchison and Katongole (1975) this is the only study in which plasma T has been measured in ring doves during the reproductive cycle. The ranges of T values reported in both studies are similar and Hutchison and Katongole found increased T during courtship in males showing nest-oriented behaviors, a result consistent with our findings. However, these investigators also report that

the highest T values occur during the incubation phase of the cycle, and this result is not consistent with our findings. Procedural differences may account for the discrepancy between our results and theirs.

The functional significance in the males of the basal levels of T and of the increased T seen upon pairing with a female can be considered separately. The behavioral function of basal levels of T may be assessed by determining which behaviors appear before T levels rise. Frequent bow-cooing, chasing and cackling occur within seconds or minutes after introduction to a female or male, and therefore may have a low threshold for activation by T. Collectively, these behaviors can be termed "aggressive courtship" patterns (Lovari and Hutchison, 1975). Because these behaviors may have to be shown at any point of the reproductive cycle a low threshold of activation would be of adaptive advantage. In fact, such aggressive courtship behaviors are shown by males taken from the incubation and brooding phase and tested with stimulus females (Silver and Barbiere, 1976) and by incubation phase feral ring doves observed in Florida (Rae Silver, personal observation). One puzzling aspect of the present study is the finding that castrated males had approximately the same levels of plasma T as intact isolated males. Yet such castrates do not show aggressive courtship behaviors (Hutchison, 1970). At present, we cannot account for this apparent paradox. Perhaps differences in basal T levels exist between castrates and intacts, but such differences are too small to be reliably demonstrated by the methods used in this study.

The functional value of the rise in plasma T titers during courtship in males is problematic. The increased T levels are consistently associated with an increased frequency of wing-flipping behavior (Table 2) and are sometimes associated with an increased frequency of other nest-oriented behaviors such as nest-cooing (Table 2). One might suppose that these behaviors, which need be shown only at a particular phase of the reproductive cycle, have a higher threshold to androgenic activation than aggressive behavior patterns. However, this is unlikely because Hutchison (1974) and Cheng and Lehrman (1975) have presented data showing that the bow-coo component of aggressive courtship behavior has a higher threshold to androgen activation than nest-oriented behaviors (e.g., wing-flipping, nest-cooing). At this point no causal, but only correlative, evidence links elevated endogenous T levels to the expression of nest-oriented behaviors in males. Another possible function of increased T at courtship is regulation of the duration of the courtship phase and timing of the onset of incubation behavior. This is indirectly suggested by the fact that there was a statistically significant negative correlation between males' T levels at C0–C1 and the length of time required for female partners to ovulate (Spearman rank order correlation coefficient $-.835$, $P<0.01$). However, it is unclear whether high T levels in males accelerated ovulation or whether females that were likely to ovulate relatively soon after pairing induced higher T levels in males than did females destined to ovulate at a longer interval after pairing. In any event, it is unlikely that an increase followed by a decline in plasma T levels is a requirement for onset of incubation behavior. Male doves treated with testosterone propionate daily did not exhibit alterations in average duration of incubation (Silver and Feder, 1973). It may be that the stimulatory effects of the female on male testicular function are important in timing the beginning of each season's breeding cycle, as suggested by Burger's (1953) work with starlings, but not the timing of events within a breeding cycle. Another possible function of increased plasma T during courtship is to affect testicular and/or epididymal morphology. Testicular morphology and weight do not change significantly during the course of the ring dove reproductive cycle (Lehrman et al., 1961; Silver and Barbiere, 1976), but in a recent study, Murton and Westwood (1975) found that the numbers of spermatozoa, the diameter of the epididymis and the height of epithelium lining the epididymis increased in feral pigeons during nest-building and before the onset of incubation.

In addition to measurement of T, DHT levels were determined in male ring doves. In general the pattern of plasma levels of DHT paralleled that of T. Surprisingly, the plasma concentration of DHT was as high or sometimes even higher than that of T. Although DHT is an important androgen in mammals, it is generally not found in high concentrations in the peripheral plasma (Wilson, 1975). Wingfeld and Farner (1975) have also reported substantial quantities of a substance they could not distinguish from DHT in a variety of avian species. Neither out study, nor that of Wingfeld and Farner provides definitive identification of DHT in avian

plasma. Our use of the term "DHT" throughout this study merely connotes the existence of a substance(s) that has the mobility of DHT in our column chromatography system and reacts with the antibody we used. The functional significance of large quantities of circulating DHT is unknown. DHT may be a potent activator of avian peripheral tissues as it is in the prostate and seminal vesicle of mammals (Wilson, 1975). Alternatively, DHT may be a relatively ineffective metabolite of T, and the conversion of T to DHT may constitute a protective mechanism for reducing the plasma concentrations of potent androgens. This latter possibility is suggested by the fact that large subcutaneous silastic implants of DHT do not activate male behavior in male doves while implants of T are effective (Saad and Silver, unpublished data). A similar protective mechanism exists in the case of progesterone in rats. Progesterone, a highly active steroid, is rapidly and extensively metabolized to the less potent compound 20α-dihydroprogesterone, and this catabolic process may serve to reduce the progestational potency of ovarian secretions during the estrous cycle or during pregnancy in rats (Wiest et al., 1968).

The present study also demonstrated that detectable levels of T and DHT occur in female ring doves. There appeared to be higher concentrations of these steroids during courtship than during incubation or squab rearing in females, but the results were too variable to make a definitive statement. This variability probably arose from the circumstance that female plasma data were arranged according to number of days of pairing with males and were not arranged so as to take into account individual differences in follicular development after a particular number of days of pairing. A more extensive study of this problem is now underway. If female doves have increased androgen levels during courtship, the increase may be of shorter duration than that seen in the male and therefore more difficult to detect. The function of androgen in females of avian species may be tied in a specific temporal pattern, to stimulation of LH secretion (Fraps, 1955; Furr and Smith, 1975; Wilson and Sharp, 1976). Curiously, however, there was a positive correlation between T (but not DHT) levels in females at C0–C1 and interval between pairing and ovulation (Spearman rank order correlation coefficient +.625, $P<0.05$). The only other significant positive correlations between testosterone and behaviors of females occurred during the first of two courtship phases, and involved nest-oriented behaviors (oblique stance, gathering nest material, building nest) (Table 2). Despite the occurrence of fairly substantial quantities of T and DHT in some courtship phase females, no aggressive behaviors (bowcoo, charge) were shown by the females. This is consistent with data showing that exogenous T propionate is relatively ineffective in activating such behaviors in female doves (Cheng and Lehrman, 1975).

In conclusion, Lehrman and his colleagues have demonstrated that male ring doves stimulate release of ovarian hormones by their female partners during courtship (Lehrman et al., 1961). The present results extend the notion that male-female interactions during courtship are mutually stimulatory, by demonstrating that females stimulate release of androgens from the testes of their male partners. The factors responsible for the rise, the maintenance, and the fall of androgen levels during courtship, and the function these hormonal changes might serve, remain to be explored. Presumably, the increase in androgens in males exposed to females is mediated by secretion of LH in the males. An additional question is whether an increase in male LH, induced by a female, plays some role in regulating behavior, either by itself or in synergy with androgen.

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