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MODULATION OF GLUCOCORTICOID HORMONE RECEPTOR LEVELS IN CHICKEN LYMPHOID TISSUE FOLLOWING TREATMENT WITH ANDROGENS *IN VIVO**

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SUMMARY

Lymphatic tissues are highly sensitive to androgens and androgens are thought to contribute to sex differences in the immune response. In this study we have examined the effects of androgens on cytosolic glucocorticoid receptor levels in lymphoid tissues. The immature chick was chosen for our experimental model because it allows the separate evaluation of the bursa of Fabricius (primarily B-cells) compared to the thymus (primary T-cells). Treatment with dihydrotestosterone (a potent androgen in chicks) for 3–12 days *in vivo* reduced the cytosolic glucocorticoid (triamcinolone acetonide-[^{3}H]) receptors in the bursa tissue to ~42% of control levels after 5 days and ≤5% of control levels after 7 days of treatment. The chick thymus tissues were still ~92% of control triamcinolone acetonide receptor levels after 5 days of androgen treatments. However, the thymus levels had dropped to ≤5% of control values after 12 treatment days. Thus a difference in the rate of decrease in the bursa of Fabricius compared to the thymus was indicated. The blastogenesis index (BI), a measurement of the percentage of cells progressing through the cell cycle, was figured using fluorescent DNA staining with diamidino phenylindole followed by flow cytometry analysis. After 3, 5, or 7 days of androgen treatment, the bursa of Fabricius from dihydrotestosterone treated chicks (2 mg/day/chick) had a mean BI = 11.17 (±3.07 SD) which was significantly lower than the bursa of Fabricius from control chicks which showed a mean BI = 27.33 (±3.42 SD). The thymus from dihydrotestosterone treated chicks had a mean BI = 19.57 (±2.19 SD) which was slightly but not significantly higher than the control thymus BI = 17.38 (±0.89 SD). In summary, androgen treatment *in vivo* induced a decrease in the cytosolic glucocorticoid hormone receptor levels in both the chick thymus and bursa of Fabricius tissues while decreasing the blastogenesis index in the bursa cells but not in the thymus cells.

INTRODUCTION

Involution of the lymphatic tissues accelerates as plasma sex steroid concentrations increase during the normal sexual maturation seen in birds and mammals at puberty [1]. As early as 1898, Calzolari noted that in mammals, thymus size decreased concomitant with an increase in either adrenal secretions or sex gland activity [2]. Exogenous treatment of rodents with either purified cortisol or dihydrotestosterone leads to a decrease in thymus weight [3]. In the chicken, treatment *in ovo* with as little as 5 μg of androgen or glucocorticoid hormones results in complete "chemical" bursectomy and the loss of mature differentiated B-cells [4–6]. Results from other studies indicate that the thymus in the adult female chicken is larger than in males. This may allow the female to mount a more vigorous (or at least a different type of) immune response compared to the male [7–10]. Females in general appear to have a heightened humoral immunity (B-cell) and a depressed cellular immunity (T-cell) which may be mediated by sex hormones. IgM is consistently higher in females than in males and is highest in females with three "X" chromosomes and lowest in "XY" or "XO" genotypes [11]. Prepuberty castration of either sex results in decreased thymus atrophy compared to the uncastrated controls [12]. In adult male rodents, orchiectomy leads to increased immune function [13–15] and larger thymus gland size [3].

Whether these sexual differences are due to higher androgen plasma levels in males compared to females or whether both estrogens and androgens can directly influence specific immunologic tissues is not well understood.

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The chick offers a distinct advantage as a model system to study the action of sex steroids on lymphatic cells because the effects on B and T cells can be evaluated separately. Indirect immunofluorescent staining with antiglobulins to B or T cells have shown that the chick bursa of Fabricius from 2 month old chicks contains approximately 85% B cells and 5% T cells while the thymus contains 90% T cells and 9% B cells [5, 16, 17].

Both avian and mammalian systems have been described where glucocorticoid hormones directly inhibit or stimulate specific metabolic responses in lymphoid tissues [18–20]. The ability of glucocorticoids to influence growth and metabolism in lymphoid cells correlates directly with the presence of specific glucocorticoid hormone receptors in these cells [21–23]. Sullivan and Wira in 1979 [24], our laboratory, and other investigators have measured the presence of cytosolic glucocorticoid steroid receptors in the lymphoid or reproductive organs of untreated chickens [24, 25] and mammals [3, 26] but much remains to be learned about the factors that modulate steroid receptor levels in lymphatic tissues.

This study was designed to determine whether androgens can "regulate" the levels of cytosolic glucocorticoid hormone receptors in a dynamic manner in either the chick thymus or the chick bursa of Fabricius, thereby influencing the relative responsiveness of these lymphoid tissues to glucocorticoids.

MATERIALS AND METHODS

Animals and tissue

White female Leghorn chicks (Hy-Line Strain W36) were injected in the leg muscle [27] daily for up to 12 days with sesame oil (controls) or 2 mg androgen (testosterone or dihydrotestosterone). Young chicks were used because the thymus reaches its greatest weight by the 3rd to 4th week of age which is prior to sexual maturation [4, 5, 28]. The chicks were killed by cervical dislocation and bled for 1–2 min. Bursa of Fabricius (or cloacal bursa), a dorsal median diverticulum attached to the cloaca and the multi-lobed thymus tissue lying along each jugular vein [29], were removed rapidly, rinsed, and frozen immediately on dry ice. Samples were stored at −80°C until analyzed. In some experiments, the chicks were implanted in the neck with a diethylstilbestrol (DES) pellet for 12 days because they were being used also in another unrelated experiment [27]. The pellets were removed 1–2 weeks prior to beginning the experiment. Chicks which had received this primary estrogen exposure showed no apparent difference in response to later androgen treatment than that seen in otherwise "untreated" chicks.

Steroid hormone receptor assay

Several different laboratories have used dextran coated charcoal assays to measure glucocorticoid hormone cytosolic receptors in chick reproductive [27, 30] and lymphatic tissues [24, 31]. In our study, [^{3}H]triamcinolone acetonide (New England Nuclear, NET-470, SA = 37 Ci/mmol) was used as the isotopic probe to avoid problems with uptake by transcortin since plasma glucocorticoid binding globulins do not bind synthetic glucocorticoids carrying a 9-alpha-fluoro atom [32]. Cold triamcinolone acetonide competitor, at 200-fold higher concentration than the labeled ligand, was used to identify specific saturable binding sites for glucocorticoids. In preliminary studies, most of the glucocorticoid receptors in immature chicks were localized in the cytosolic fraction. Therefore, we elected to concentrate on the cytosolic triamcinolone receptor levels.

The frozen lymphatic samples were fractured by pounding and 0.5 g of tissue were homogenized with a polytron sonicator (Brinkman) in 2 ml of TSMM buffer (10 mM Tris, pH 7.4 at 0°C; 0.25 M sucrose; 3.0 mM $MgCl_2$; 10 mM sodium molybdate). The homogenate was centrifuged (800 rev./min × 20 min, 0°C) to yield a supernatant and low speed pellet [25]. The supernatant was recentrifuged at 100,000 ***g*** for 60 min in a preparative ultracentrifuge (L5-75B, Beckman). The high speed supernatant was pretreated for one minute with a low concentration of dextran coated charcoal (final concentration DCC = 0.05% charcoal, 0.1% dextran) which did not result in loss of receptor. The samples were rapidly vortexed and the charcoal-bound endogenous steroids discarded after centrifugation at 2000 rev./min × 10 min × 0°C.

The cytosolic receptor fraction obtained was diluted to 4 ml with TSMMG buffer (10 mM Tris, pH 7.4 at 0°C; 0.25 M sucrose; 15 mM monothioglycerol; 10 mM sodium molybdate; and 10% glycerol). Tubes containing isotope ± cold competitor were dried before 100 μl cytosolic receptor preparations were added to each tube. Receptor preparations were exchanged (incubated) for 1 h at 25°C, then vortexed and the incubation continued at 0°C for 16 h [24, 27, 33].

Dextran coated charcoal was added to the cytosolic receptor tubes as a suspension (final concentration DCC = 0.25% charcoal, 0.5% dextran), vortexed, incubated for 10 min, and then pelleted by centrifugation at 2000 ***g*** × 10 min. Duplicate aliquots (50 μl) from the DCC treated supernatants were counted using a scintillation cocktail containing Omnifluor (4 g/l; New England Nuclear) in toluene plus 15% Triton X-100 (Sigma) which gave approximately 67% counting efficiency. The non-specific steroid binding was subtracted from the total [^{3}H]-triamcinolone binding to obtain fmol of receptor and then normalized per mg cytosolic protein or per mg DNA. Statistical analysis of the data was assessed for significance by Student's *t*-test for paired comparisons [34].

Aliquots of the cytosolic receptor fraction were assayed for protein using a modified Lowry assay [35] with purified bovine gamma globulin as the protein standard. Protein values ranged from 3–5 mg/ml of cytosolic receptor. The carboxymethyla-

tion treatment procedure of Ross and Schatz [36] was used to avoid interference from high levels of reducing agents and glycerol. The nuclear pellet was quantitated for DNA content using the diaminobutyric acid procedure of Setaro and Morley [37].

Mitotic analysis by DNA flow cytometry

For automated flow cytometry, nuclear isolation and DNA fluorochrome staining were combined in a rapid one step procedure [38]. The frozen lymphoid tissues were thawed at room temperature in phosphate buffered saline. A small fragment of tissue (~50 mg) was minced and teased apart with a scalpel in 3 ml of the DNA staining solution containing the fluorochrome, 4',6-diamidino-2-phenylindole-2HCl (DAPI, 10 μg/ml Sigma), in a nuclear isolation media containing 0.6% Nonidet P-40 (NP40, Accurate Scientific Co) and 0.2% bovine serum albumin (Fraction V, Gibco). The suspension was incubated approximately 3 min at room temperature, then filtered through a 50 micron nylon mesh, aspirated twice through the 23-gauge, 1 inch needle with a 5 ml syringe to eliminate clumping and the solution of nuclei refiltered through a clean mesh. This particular preparation is stable for up to 2 h at room temperature. Samples not analyzed immediately could be stored at 4°C for up to 24 h with no change in the DNA staining distribution [38].

The stained suspensions of nuclei were analyzed for DNA content by employing flow cytometry with a Phywe, ICP-21 flow cytometer (Ortho Instruments) interfaced to a SUE Lockheed computer (Lockheed Electronics). A HBO W/2 mercury lamp, a BG1 excitation filter, a TK 405 dichroic, and a LP 395 barrier filter were used to quantitate the DAPI blue-green fluorescence. The intensity of the fluorescent DNA stain (DAPI) in linearly proportional to the DNA concentration inside the cell [38].

The intensity of the DNA stain per cell was recorded and the results stored in computer memory and graphed as the number of cells versus the DNA content per cell. Data from approximately 10,000 cells (counted at a rate of 500 cells/s) was summarized via computer programming. The number of cells in G1, S and G2 + M were obtained by integrating under the curve, utilizing an assembler program and a Tektronic 4010 terminal display to set the limits of integration. The coefficients of variation obtained ranged from 1.0 to 3.0, thus indicating a high resolution for the various amounts of DNA/nuclei graphed over a distribution of 2,000 channels (abscissa, Fig. 4).

RESULTS

Androgen effects on glucocorticoid receptors

Our first experiments were designed to evaluate the short term effects of sex steroids on glucocorticoid hormone receptor levels in chick lymphatic tissue. Immature chicks were treated for 24 h with estradiol-17β (2 mg in sesame oil); with testosterone plus estradiol (2 mg each in sesame oil); or with vehicle alone (sesame oil) as a control. Glucocorticoid hormone receptors were measured using triamcinolone acetonide-[^{3}H] as the labeled ligand. The results, shown in Fig. 1, indicate that the combination of estradiol plus testosterone (24E + T) inhibited triamcinolone receptor levels in both cytosolic (Rc, top) and nuclear (Rn, bottom) in the bursa of Fabricius but had variable effects in the thymus. Under the assay conditions described, approximately 75% of the glucocorticoid receptors were located in the cytosolic fraction (data not shown); for this reason, efforts to elucidate the effects of androgens were then directed towards evaluating the cytosolic glucocorticoid receptors.

Because a one day (24 h) treatment may not have allowed sufficient time to realize slower long term changes in cytosolic triamcinolone acetonide receptor (TA-Rc) levels, other experiments were conducted to evaluate changes over a 12 day treatment period. Chicks were injected intramuscularly daily for 3–12 days with 2 mg dihydrotestosterone (DHT) in sesame oil or with the oil vehicle alone. Sacrifice of the chicks occurred on the days indicated, after which the thymus and the bursa of Fabricius tissues were removed and assayed separately for triamcinolone acetonide-[^{3}H] binding (fmol/mg cytosolic protein). Chicks treated with dihydrotestosterone showed a slight decrease (to 94% of controls) in triamcinolone acetonide binding in the bursa of Fabricius after 3

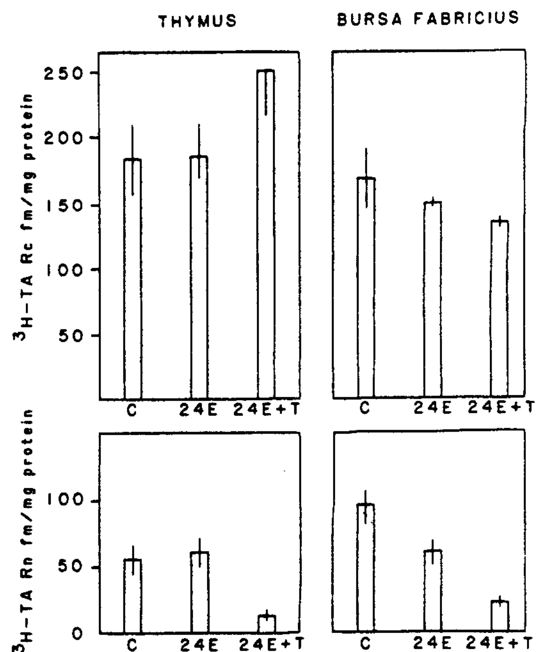

Fig. 1. Triamcinolone acetonide cytosolic receptors (TA-Rc = fmol/mg protein) in chick thymus tissue (Left side) and in bursa of Fabricius tissue (Right side) normalized to cytosolic protein. Chicks were injected intramuscularly (IM) for 24 hours with sesame oil as control, 0.2 ml, (C); estradiol 17β, 2 mg/0.2 ml oil, IM, (24 E); or testosterone, 2 mg/0.1 ml oil, plus estradiol 17β, 2 mg/0.1 ml oil (24 E + T). The graph represents the mean $\pm$ SD ($n = 4$). Experiment was repeated two times.

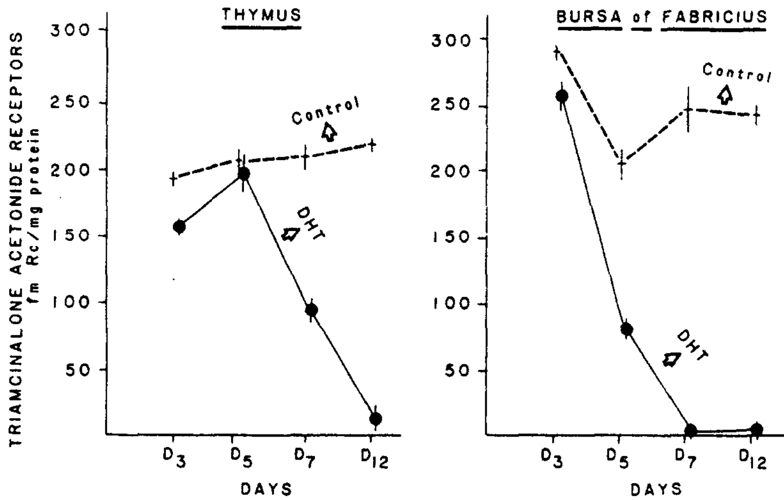

Fig. 2. Quantitation of cytosolic glucocorticoid receptors (triamcinolone acetonide-^{3}H = Ta-Rc) from chick thymus (Left graph) or bursa of Fabricius tissue (Right graph) were normalized per mg cytosolic protein. Chicks were treated *in vivo* with dihydrotestosterone (DHT) in oil for 3 (D_3), 5 (D_5), 7 (D_7) or 12 (D_{12}) days, or with vehicle for controls (C). Tissues from day 0 (D_0) birds were unfortunately lost for this particular experiment. Brackets represent mean ± SD (n = 4 chicks per point).

days (Fig. 2, right graph). The cytosolic triamcinolone receptor levels in the bursa continued to drop, reaching ~42% by 5 days and ≤5% of controls after 7 days of treatment.

The change in glucocorticoid receptor levels in chick thymus following androgen treatment occurred more slowly than in the bursa of Fabricius. After 5 days of dihydrotestosterone treatment, triamcinolone acetonide cytosolic receptors in the thymus were still at 92% of control levels. However, triamcinolone acetonide receptors continued to decrease, reaching ~43% after 7 days and ≤5% of controls after 12 days of dihydrotestosterone treatment (Fig. 2, left graph).

Protein content in different cell types can vary widely in amount. In lymphoid tissue, bursa cells are almost twice as large as thymus cells. We tested the possibility that different results would be found if the data were expressed per unit of DNA. However, the thymus and the bursa of Fabricius tissues both showed equally dramatic decreases in receptor concentrations after they were normalized to DNA Fig. 3).

Androgen effects on the blastogenesis index

Glucocorticoid hormone treatment *in vivo* at high dosages is known to decrease uridine incorporation into RNA, depress lymphoid cell division, and eventually cause lymphoid cell lysis. These effects can be blocked *in vitro* by 11-deoxy steroids (such as cortexolone or progesterone) which compete with glucocorticoids for binding to their receptors [39].

To evaluate the effects of androgen treatment on kinetic parameters (such as cell cycle traverse or mitotic index) in thymus and bursa tissues, we utilized

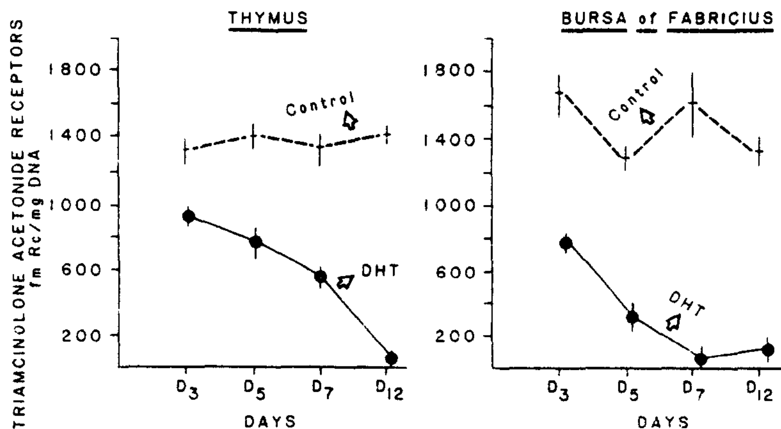

Fig. 3. Cytosolic glucocorticoid receptors (triamcinolone acetonide-[^{3}H] = TA-Rc) normalized per mg cellular DNA. Chick thymus (Left graph) or bursa of Fabricius (Right graph) were treated (IM) with dihydrotestosterone (DHT) in oil for 3 (D_3), 5 (D_5), 7 (D_7) or 12 (D_{12}) days. Brackets represent mean values ± SD (n = 4 chicks per point).

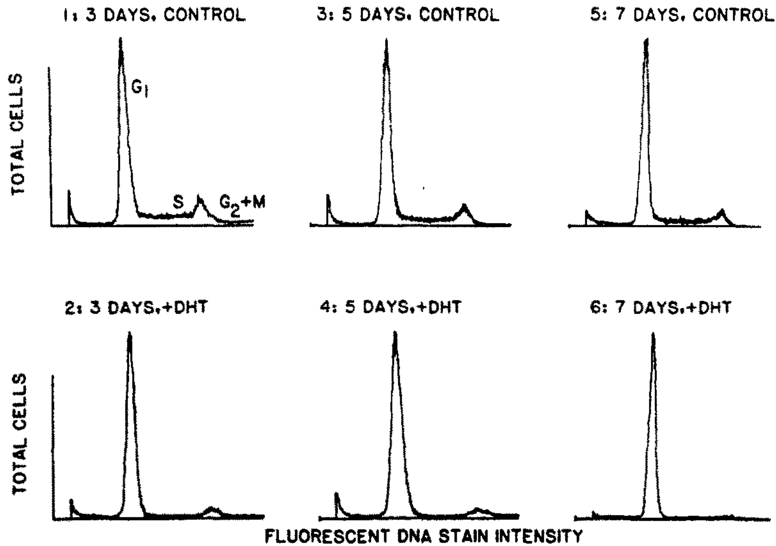

Fig. 4. Nuclear DNA levels in the cells from bursa of Fabricius tissues were quantitated by fluorescent staining with DAPI as described in Methods and analyzed by flow cytometry. Chicks treated for 3, 5, and 7 days with sesame oil (panels No. 1, 3, 5; top row) were compared to birds treated with daily injections of dihydrotestosterone (panels No. 2, 4, 6; bottom row) for the same period of time (2 mg per day, i.m.). The major peak in each graph represents the non-dividing G1 population of cells which contain a 2C complement of DNA. The smaller right hand peak represents the cells in G2 and M which have doubled their DNA but have not completed cytokinesis. The section S between the G1 peak and the G2 + M peak represents those cells which are in various levels of DNA replication.

flow cytometry to analyze chick cells for cell division. Following hormone treatment chick lymphatic tissues were stored frozen at −80°C until assayed. Isolated nuclei were prepared in 0.6% Nonidet P-40 solution containing the DNA fluorochrome DAPI (4', 6-diamidino-2-phenylindole-2HCl, 10 μg/ml) and measured by means of flow cytometry as described previously. The dihydrotestosterone treatment caused a decrease in the number of bursa of Fabricius cells in the S phase and in the G2 + M phase of the cell cycle (Fig. 4: 1, 3, and 5 compared to Fig. 4: 2, 4, and 6).

The DNA content of the cells contained in the G1 peak, the S phase, and the G2 + M portions of the cell cycle was integrated and expressed as the percent of total cells counted [38]. The chick thymus showed no statistically significant change in the pattern of G or G2 + M or S cells in the various portions of the cell cycle over the entire treatment period (Fig. 5). After the bursa of Fabricius tissue was treated with dihydrotestosterone, the percentage of cells in the S phase was decreased as were also the number of cells in the G2 + M phase; cells in the G1 stage showed

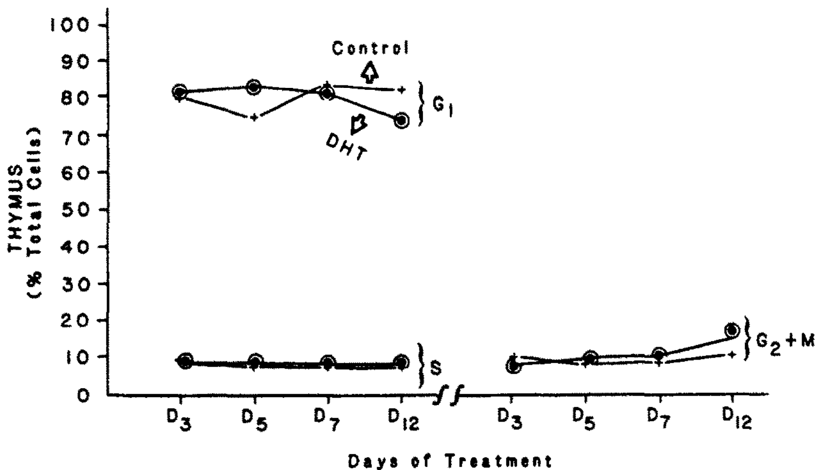

Fig. 5. Analysis of DNA levels in chick thymus cell nuclei stained with the fluorochrome DAPI. Chicks were treated with dihydrotestosterone (+ = DHT, 1 mg/day) for 3–12 days or with sesame oil (⊙ = control). The amount of DNA per cell was evaluated by flow cytometry and graphed as the percentage of the total cells in the G1 stage, the S stage or the G2 + M stage of the cell cycle. Each point is the mean of 3 determinations.

Table 1. Cell cycle analysis and blastogenesis index (BI)

Chicken tissue	Treatment (i.m.) (3, 5, and 7 days)	% G_1 ± SD	% S ± SD	% G_2 + M ± SD	BI ± SD
Thymus	Control	80.48 ± 3.88	7.89 ± 0.91	9.49 ± 0.86	17.38 ± 0.89
Thymus	DHT (2 mg/day)	80.40 ± 3.75	8.98 ± 0.63	10.59 ± 3.74	19.57 ± 2.19
Bursa Fabricius	Control	73.65 ± 5.52	17.22 ± 4.86	9.11 ± 1.96	26.33 ± 3.42
Bursa Fabricius	DHT (2 mg/day)	88.82* ± 7.52	6.74* ± 3.74	4.43* ± 2.41	11.17* ± 3.07

Evaluated Days 3, 5, and 7, $n = 9$. *Significantly different at the 1% level of confidence. DHT = dihydrotestosterone. Control = sesame oil vehicle. Blastogenesis Index = $S + G_2 + M/G_1/G_0 + S + G_2 + M \times 100$.

an increase for all days of treatment evaluated (Table 1).

The blastogenesis index (BI), as defined in Table 1, is a measure of the fraction of cells progressing through the cell cycle. Thus the BI is similar to a mitotic index which is often calculated on the basis of thymidine-[^{3}H] incorporation. However, the BI gives a more complete measurement of the number of cells outside of the G1 or resting state and is not influenced by the length of the isotopic pulse or the length of the mitotic divisions as is the case for the mitotic index. In our experiments, a statistically significant decrease at the 1% level of confidence in the blastogenesis index for the bursa of Fabricius was quite evident (Table 1). Although the thymus tissue results suggest a small increase in the BI or mitosis following androgen stimulation, the values found were not statistically significant.

DISCUSSION

Sex steroids can modulate or influence hormone receptor levels in various systems, including the mammalian uterus and the avian oviduct [27, 40]. When androgen treatment of immature chicks was carried out for 3 to 12 days with daily injections of the potent androgen, dihydrotestosterone, cytosolic triamcinolone acetonide receptor concentrations in cells from both the thymus and the bursa of Fabricius showed dramatic decreases. Control studies in this laboratory and others [16, 19, 24, 41] have shown that neither estrogens nor androgens (DHT) in physiological doses compete with glucocorticoid hormones (triamcinolone acetonide or dexamethasone) for binding to the cytosolic glucocorticoid receptors. However, as demonstrated in recent studies, high testosterone or dihydrotestosterone (concentrations = 10^3–10^4 times the labeled ligand) will begin to compete for binding to the glucocorticoid cytosolic receptor [42]. In this study competition from injected ro endogenous steroids would have influenced binding during the early treatment periods (24 h and 3 days) as well as during later periods, but this was not observed.

Triamcinolone acetonide uptake by cytosolic glucocorticoid receptors in control chicks ranged, in this study, from 150 to 350 fmol/mg cytosolic protein depending on the groups of birds used and the tissues analyzed. This range is lower than that found in the chick studies of Schaumberg and Crone (1971) who compared static cytosolic receptors for corticosterone-[^{3}H] in bursa of Fabricius to those in thymus cells from intact mature chicks [43]. Because they found corticosterone receptors of ~40,000 to ~50,000 fmol/mg total protein in both the thymus and the bursa of Fabricius, these investigators concluded that the two tissues did not differ in binding capability [43]. A decrease in the values reported in the literature for glucocorticoid cytosolic receptors can be noted in the 10 years since those early studies. When non-specific binding was taken into account by using synthetic fluoro-steroids (dexamethasone-[^{3}H] or triamcinolone acetonide-[^{3}H]), Naray *et al.*, in 1980 [31] found approximately 1,000 fmol/mg cytosolic protein when dexamethasone-[^{3}H] was applied to avian thymus and bursa cells. In agreement with our study, Sullivan and Wira [24], in 1979, found 520 fmol/mg cytosolic protein when using dexamethasone-[^{3}H] to measure binding in chick bursa cells. Synthetic steroids allow the determination of specific, saturable receptors without interference from non-specific uptake because these steroids are not bound to transcortin (corticosteroid binding globulin), alpha fetoproteins, or sex binding globulins [18, 44] Recently, the use of sulfhydryl protective agents and protease inhibitors (such as molybdate, trasylol, leupeptin, etc.) have also contributed to improved precision for the assay of glucocorticoid receptors [45–47].

Regulation of chick glucocorticoid receptor levels in lymphoid tissue by glucocorticoid steroids has been described by other workers [16]. On the other hand, the effects of androgens on receptor levels has not been previously described. When rat thymus cells in short term tissue culture are treated with cortisol, cytosolic glucocorticoid receptors peak by 2 min and then decrease; at the same time, nuclear cortisol receptor levels continue to rise for 5–10 min, thus indicating continued translocation from the cytoplasm into the nucleus. Within 20 min, a decrease shows in glucose transport and acetate incorporation into lipids and, by 2 h, protein synthesis undergoes a general inhibition which leads to cell lysis within 8–12 h. Longer term treatment with glucocorticoids causes a decrease in the thymus mitotic index, a fall in cytosolic glucocorticoid receptors, and a decrease in certain immunologic responses. Decreases in bursa of Fabricius wet weight, which became apparent after testosterone treatment (data not shown), were remin-

iscent of the changes observed by others which occurred in young chicks after they had been treated with cortisol or cortisone [4, 48–50]. Glucocorticoid hormones probably act directly on the lymphatic cells via their steroid receptors but may also modify the synthesis or the release of thymosin hormone or block the spleen production of T-cell growth factors, which will create further immunosuppression [51, 52].

When we evaluated tissues from androgen treated chicks for the distribution of cells traversing the cell cycle, we found that dihydrotestosterone had a greater percent effect on bursa of Fabricius cells than on the thymus cells (Table 1). The thymus contains distinct medulla cells, cortex cells plus lymphoid and non-lymphoid (reticuloepithelial) cells and may have only a small percent of its total cell population responsive to either sex steroids or glucocorticoid steroids. Therefore, the changes in bursa compared to thymus may not be as easy to distinguish when analyzed as a mixed tissue. The greater sensitivity of the bursa of Fabricius to androgens, measured as a decrease in blastogenesis index, agrees well with the greater decrease in weight in the bursa compared to the thymus or spleen as was reported by Dieter and Breintenbach [53] and Glick [4]. Both androgen and estrogen hormone binding to the bursa of Fabricius cells in immature chicks have been demonstrated by autoradiography methods and have been quantitated by assaying the lymphatic tissues via radioreceptor techniques. Sex steroid receptors appeared in higher concentrations in the epithelial components of the bursa of Fabricius than in the lymphoid components [24, 26, 54]. This finding agrees with those in which higher androgen receptor levels were observed in rat reticuloendothelial matrix of the thymus than in the lymphatic fraction [3, 55]. In the future studies, we will evaluate thymus medulla cells and cortex cells separately for changes that occur in response to androgen treatment.

Our present results demonstrate the dramatic effect of androgens on the cytosolic glucocorticoid hormone receptor levels in chick lymphoid tissue. Analysis of the results suggests, but does not prove, that the effect of dihydrotestosterone is direct at the lymphatic tissue level working through androgen receptors. However, sex hormones given *in vivo* in high doses may function at several different target levels. For instance, high concentrations of androgens could inhibit circulating thymic hormone levels [56–58] or interact with the adrenal system to cause changes in circulating glucocorticoid hormones or in the population of lymphatic cells moving in and out of the thymus or bursa. Future work with androgens on isolated cell types in tissue culture systems *(in vitro)* may aid us in elucidating whether the effect of androgens on the chick lymphatic B and T cells is direct or indirect. Since estrogen and androgen receptors can be demonstrated in lymphoid tissue, a direct effect of these steroids is quite plausible.

In conclusion, high doses of androgens apparently depress glucocorticoid hormone cytosolic receptor levels in both the chick thymus and the bursa of Fabricius cells. The levels of cytosolic glucocorticoid receptors continue to decrease as the period of sex steroid treatment is prolonged. The androgen treatment had a more rapid inhibitory effect on both the glucocorticoid receptors and the blastogenesis index in bursa of Fabricius cells of immature chicks than was found in thymus cells.

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