A Comparison of the Lympholytic Effects of Corticosterone and Testosterone Propionate in Immature Cockerels[1] (34474)

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In Aves, the principal steroid hormone of the adrenal is corticosterone (1), and for the testis it is testosterone (2). Evidence indicates that the cockerel adrenal is capable of a high level of corticosterone secretion in response to ACTH and stress as early as 4 weeks of age (1, 3–6). This is an age of lymphoid organ growth and development (7). A high level of testosterone secretion appears later, and seems to coincide with lymphoid organ involution (7, 8). Both of these hormones are recognized pharmacologically as lympholytic agents, but indirect evidence suggests a possible difference in the nature of their lympholytic action. Preliminary work in our laboratory suggested that a difference existed in the permanence of their effects. As a result this study was designed to evaluate the nature and extent of the differences using the criteria of lymphoid organ growth, morphology, and oxygen consumption in response to selected levels of corticosterone or testosterone propionate administered to immature cockerels.

Materials and Methods. One-day-old single-comb White Leghorn cockerels were obtained from the Poultry Husbandry Department of the University of Missouri. They were placed in brooder cages in an air-conditioned animal room on a 12:12 light cycle, with free access to Purina chick starter and water. At 20 days of age 160 cockerels were weighed and divided into 16 groups of 10. Each group was assigned birds from the high- and low-weight range in order to make their mean weights comparable. The cockerels were given injections at 21 to 35 days of age. Graded doses of testosterone propionate (TP), corticosterone (Comp. B), or the vehicle (20% ethanol–cottonseed oil) were given im in 0.1-ml volume. TP was injected at 0.05, 0.50, or 5.0 mg/bird/day, and corticosterone was injected at 0.05, 0.50, or 1.0 mg/bird/day.

At 5 weeks of age, one-half of each experimental and control group were killed with sodium nembutal, and pertinent data gathered. At 7 weeks of age, 2 weeks after withdrawal from the steroid hormone treatment, the rest of the birds were killed, and the same variables were again measured.

The bursa of Fabricius, left thymus, spleen, testis, and adrenals were weighed, and the organ weights were calculated as a percentage of the body weight. Portions of the bursa, thymus, spleen, and liver were weighed and ground with all glass Potter-Elvejehm homogenizers (2% w/v) in Krebs-Ringer phosphate buffer, pH 7.35. Two milligrams of

TABLE I. Body Weight after Steroid Administration.[a]

Dose (mg/day)	Immediate effects for 5-week-old birds (g body wt)[b]
Testosterone propionate	
0.0	438.8 ± 11.1
0.05	413.9 ± 9.5
0.5	425.9 ± 19.3
5.0	383.9 ± 8.9
Corticosterone (Comp. B)	
0.0	461.5 ± 13.4
0.05	434.4 ± 9.6
0.5	392.8 ± 12.4
1.0	402.0 ± 7.9

[a] Means ± SE; $N = 10$.

[b] Immediately after 14 days of treatment.

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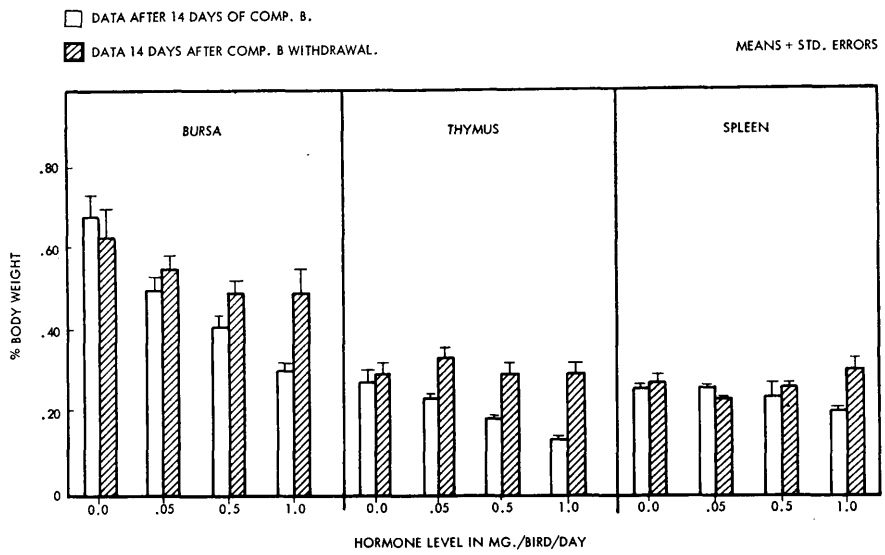

FIG. 1. Relative lymphoid tissue weights of cockerels after corticosterone administration. $N = 10$.

glucose per milliliter of homogenate were added as substrate, and oxygen uptake was determined every 10 min for 1 hr, on a constant-volume Warburg apparatus. Q_{CO_2} was expressed as cubic millimeters of oxygen uptake per milligrams of dry weight per hour.

Other portions of the bursa, thymus, and spleen were prepared for histological examination. Tissues were sectioned at 6 μ and stained with a modified Schorr stain. The sections were examined for details of cellular size and morphology, relative amounts of lymphoidal and nonlymphoidal tissue, and relative amounts of connective tissue.

Results. Body weights. Both hormones had some effect on the rate of body weight gain. These effects for 5-week-old birds are illustrated in Table I. However, since the data are presented in terms of percentage of body weight, the effect on weight *per se* should not be a direct factor.

Lymphoid organ weights. The dose-response curve for corticosterone and TP showed that the bursa was the most steroid-sensitive lymphoid organ in the cockerel and that TP caused the greater involution of this organ (open bars. Figs. 1 and 2). A relatively linear lympholytic response occurred in the bursae and thymi of corticosterone-treated birds using a 1.0-mg high dose, whereas 5.0 mg of TP were required for a comparable linear response. The spleen responded differently to the two steroid hormones. TP caused a relatively linear involution, but only the 1.0-mg dose of corticosterone caused any significant decrease in spleen weight.

A general rebound in all lymphoid organ weights had occurred by 2 weeks after the withdrawal of corticosterone treatment (Fig. 1). At 7 weeks there were no significant differences between lymphoid organ weights of hormone-pretreated birds and their controls. This is in sharp contrast to the data from birds 5 weeks of age, immediately after steroid treatment, when weights were significantly different at most dose levels.

A general rebound also occurred after withdrawal of TP (see Fig. 2). However, 2 weeks after withdrawal of TP, the bursae of 5.0-mg-treated cockerels weighed significantly less than those of 7-week-old controls. In contrast, there was an unexpected significant increase in the weights of the thymi in the 0.05-mg group.

Adrenals. The data for adrenal weights are presented in Fig. 3. TP did not alter relative adrenal weights at 5 weeks of age, and withdrawal from TP treatment also had no effect. However, corticosterone caused significant

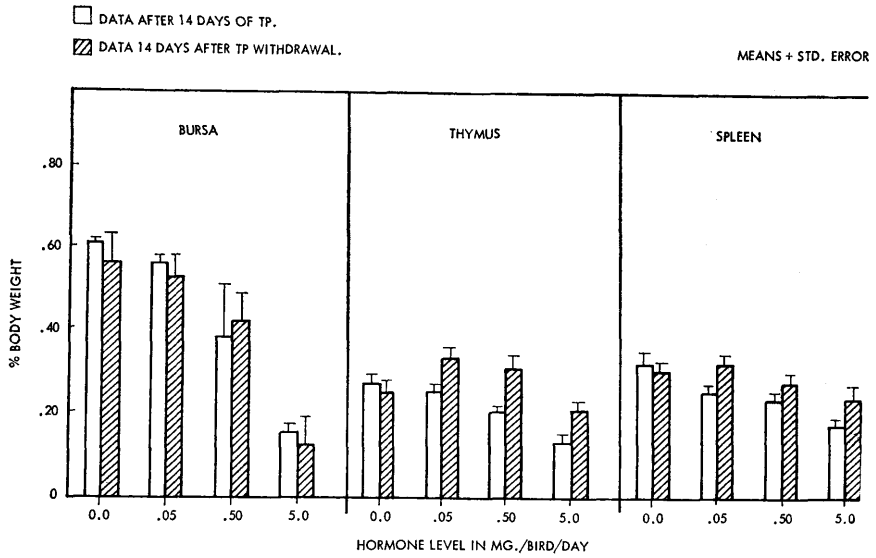

FIG. 2. Relative lymphoid tissue weights of cockerels after testosterone propionate administration. $N = 10$.

reductions (Fig. 3, open bars) in relative adrenal weights at 0.05 and 1.0 mg. Two weeks' withdrawal from corticosterone, at all dose levels, resulted in significantly greater relative adrenal weights in comparison to the 7-week controls (Fig. 3, hatched bars).

Testes. At 5 weeks of age the decrease in testis weight was inversely proportional to the log dose of TP employed (Fig. 3, open bars). However, corticosterone had little effect on testis weight except at 0.5 mg, where it caused a significant weight increase above the 5-week-old controls. Two weeks after withdrawal from TP only the testes of the 5.0-mg group weighed significantly less than their controls (Fig. 3, hatched bars). Two weeks after corticosterone withdrawal the testes of the 0.05- and 1.0-mg-treated birds weighed significantly more than their controls.

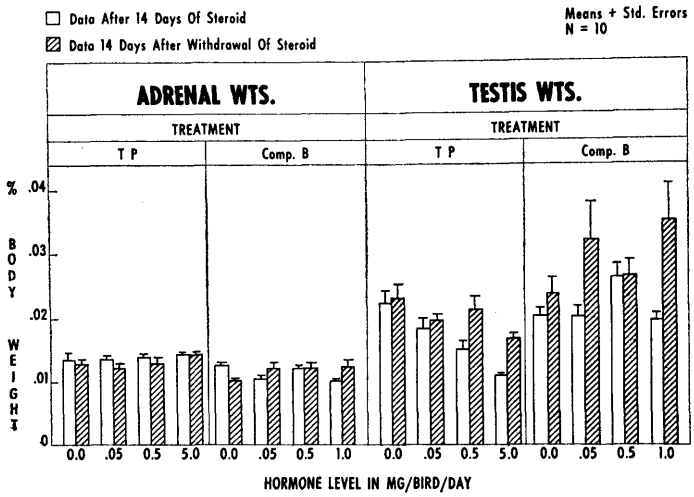

FIG. 3. Relative paired adrenal and testis weights of cockerels after testosterone propionate and corticosterone administration. $N = 10$.

TABLE II. Control Values for Treatment with TP and Corticosterone (Comp. B).[a]

Tissue	Control Q_{O_2} at end of hormone treatment ($mm^3 O_2$/mg dry wt/hr) $\bar{x}$	SE	Control Q_{O_2} 14 days after withdrawing hormone ($mm^3 O_2$/mg dry wt/hr) $\bar{x}$	SE
TP treatment				
Bursa	2.47	± 0.25	2.39	± 0.24
Thymus	3.62	± 0.17	3.15	± 0.26
Spleen	3.22	± 0.18	2.77	± 0.17
Liver	0.97	± 0.10	0.95	± 0.10
Corticosterone (Comp. B) treatment				
Bursa	2.48	± 0.21	2.09	± 0.15
Thymus	3.30	± 0.22	3.04	± 0.32
Spleen	3.06	± 0.13	2.64	± 0.12
Liver	1.10	± 0.13	0.74	± 0.08

[a] $N = 10$.

Lymphoid tissue oxygen consumption. Data for the oxygen uptake of the tissue from lymphoid organs and for comparative purposes, the liver, are presented by difference from the controls in Figs. 4 and 5. The absolute control values are listed in Table II. It shows that oxygen consumption of tissue homogenates in the presence of glucose was greatest in thymus, followed by spleen, bursa, and liver. The separate control estimates (four organs and four groups) were quite consistent, and statistical analysis shows that they may be regarded as identical for their respective age groups (5- and 7-week-old birds).

There was a general decrease in the oxygen consumption of the thymus and spleen homogenates immediately after pretreatment with corticosterone (Fig. 4). This change was significant in spleen homogenates of 1.0-mg

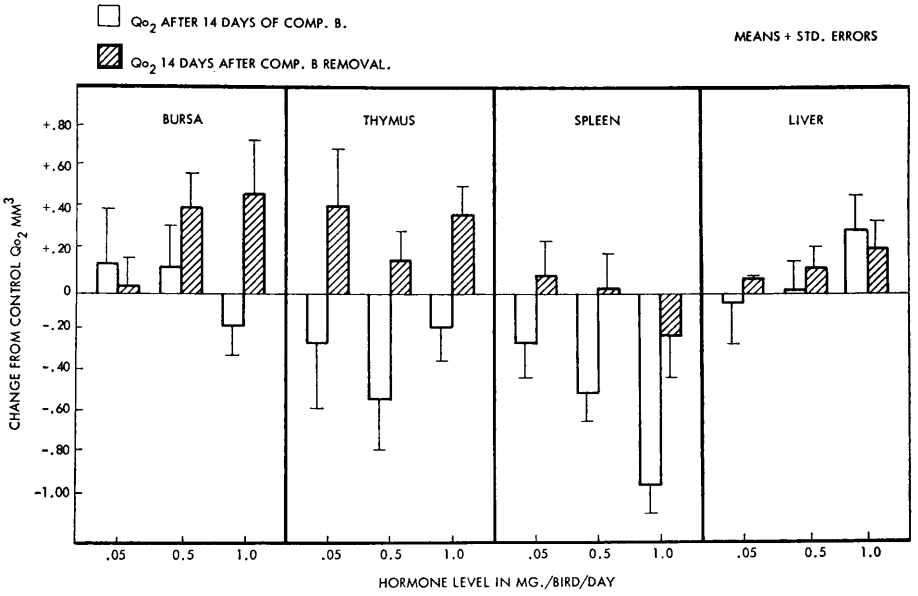

FIG. 4. Oxygen consumption of tissue homogenates after corticosterone (Comp. B) administration. Data presented as a difference from the absolute values listed in Table II. Two milligrams of glucose per milliliter of homogenate added as substrate. $N = 10$.

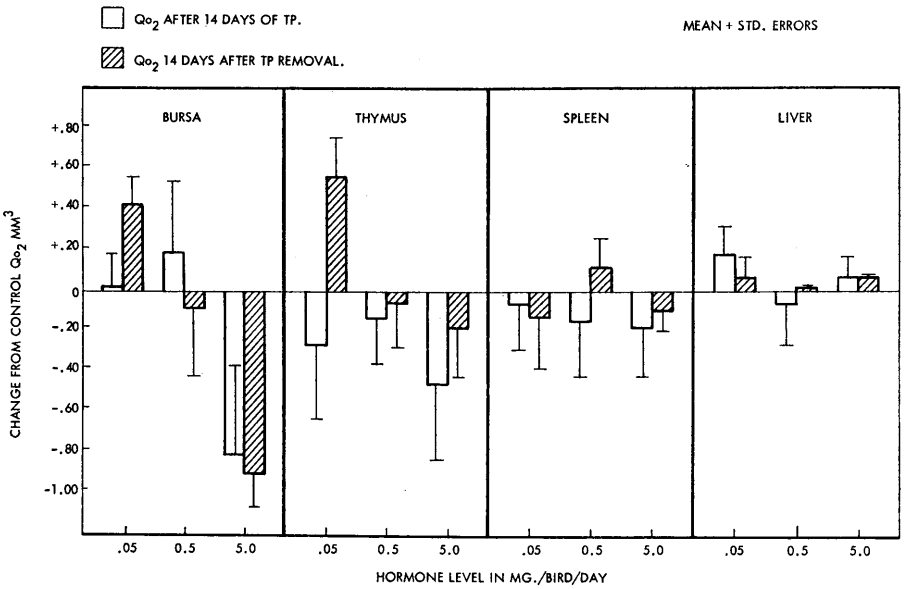

FIG. 5. Oxygen consumption of tissue homogenates after testosterone propionate administration. Data presented as a difference from the absolute values listed in Table II. Two milligrams of glucose per milliliter of homogenate added as substrate $N = 10$.

treated cockerels. There was little effect of corticosterone on oxygen consumption of bursal homogenates. In contrast, 5.0 mg of TP caused a significant decrease in oxygen consumption of bursal homogenates (Fig. 5), a slight decrease in the thymus homogenates, and had no effect on spleen homogenate oxygen consumption.

After withdrawal of corticosterone administration, oxygen consumptions of the thymus and spleen homogenates were higher at all dose levels than those measured at 5 weeks of age, immediately after 2 weeks of hormone administration (Fig. 4). At the two higher dose levels oxygen consumptions of bursal homogenates was also increased.

Withdrawal from TP treatment did not result in broad functional recovery, as measured by tissue oxygen consumption (Fig. 5). Only the homogenates of the bursa and thymus in the 0.05-mg group showed a positive response.

Steroid hormone treatment did not significantly alter the oxygen consumption of any liver homogenates.

Histology. Histological examination of the lymphoid organs of 5-week-old birds revealed that TP caused a massive proliferation of connective tissue in the bursa and thymus. There was no evidence of such a response in the bursa and thymus of corticosterone-treated birds. Corticosterone seemed to stimulate the reticular network in bursa and thymus. In the bursa the cortical/medullary ratio of the follicles was greatly reduced, but it was greatly increased in the lobules of the thymus.

The bursa and thymus of birds allowed 2 weeks recovery from TP treatment showed continued and progressively greater connective tissue infiltration, although there was some evidence of recovery from the lowest level of treatment (0.05 mg). Recovery was indicated by finger-like outgrowths of cortical lymphoid areas into the medullary regions.

Bursae from birds allowed 2 weeks withdrawal from corticosterone treatment contained fewer medullary lymphocytes in comparison to controls, but the cortical regions were densely packed with lymphocytes. A 2-week withdrawal from corticosterone did not permit the thymus to recover to a pretreatment condition. The major effects retained were an increase in medullary vascularization and reduction in medullary area and numbers of medullary lymphocytes. As

in the bursa, after corticosterone withdrawal there was an increase in proliferative activity in the cortical region of the follicles.

The spleens of a randomly selected group of cockerels were examined to determine the percentages of red and white pulp. Quantitative estimates of the areas occupied by these components were obtained by using an ocular micrometer. At least four separate sections of five spleens were evaluated for each group. Spleens from 5-week-old control birds contained 95% red pulp and 5% white pulp. Corticosterone treatment at 0.50 mg changed this ratio to 90 and 10%, and 1.0-mg treatment to 85 and 15%. TP treatment had no effect on the red and white pulp ratio except at the 5.0-mg level, where it changed the ratio to 97% red pulp and 3% white pulp. After withdrawal from steroid hormone treatment there were no differences between control and experimental spleens, in regard to the red and white pulp ratio.

Discussion. Treatment with either steroid hormone affected the rate of lymphoid organ development; however, the lymphoid organs were more sensitive to low levels of corticosterone than to low levels of TP. It is important to note that in all instances the bursa was the more hormone-sensitive organ. The thymus and spleen showed similar but less intense patterns of response.

A striking difference between the effects of these two lympholytic steroids was noted after their withdrawal. There was no apparent correlation between tissue weight loss and tissue metabolism immediately after hormone treatment; after withdrawal of the hormone tissue oxygen consumption increased greatly in corticosterone-pretreated birds, but remained depressed in TP-pretreated ones. A related difference occurred in the recovery of lymphoid organs.

It appears that the thymus and spleen are able to tolerate elevated corticosteroid or TP levels during juvenile development. However, androgens permanently impair normal developmental progress in the bursa. This greater sensitivity of the bursa is probably mediated through an effect on differentiation of bursal lymphocyte precursors. This result is consistent with the embryonic effects of testosterones on the bursa (10–12), and early post hatching effects (13). It suggests that testosterone is capable of permanently blocking or redirecting the differentiation of stem cells which are necessary to the development of bursal lymphoid cells or that testosterone can permanently modify the environment necessary for differentiation and development of circulating lymphoid cells.

The role of the gonad in development of lymphoid tissues has also been indicated in mammals (14). They studied the effect of gonadectomy on peripheral lymphoid tissue development of thymectomized mice and found that it enhanced lymphoid development.

Immunological evidence has suggested that the bursa begins functional differentiation prior to the thymus or spleen (9). The hormone sensitivity may be related to functional differentiation since it seems well established that differentiating cells are more susceptible to hormonal effects than nondifferentiating ones.

Histological examination of the bursa and thymus indicated that the prophlogistic effects of TP were intense, of a permanent nature, and were in large part related to the permanent cessation of lymphoid tissue growth and metabolism. The major effect of corticosterone appeared to be an alteration in the type or quantity of tissue lymphocytes. However, some areas of cortical proliferation were evident after withdrawal. It is well established that corticosterone treatment will deplete the thymus of lymphocytes, and it is now know that repopulation of the short-lived and eventually the long-lived lymphocytes occurs (15, 16). A similar replacement series, albeit of a different nature, also appears to take place in the bursa. The enhancement of oxidative metabolism noted in this study after corticosterone withdrawal may reflect the gross metabolic dynamics of such a replacement series.

Histological results in the spleen conflicted with the classical concept of a lympholytic response since the relative amount of splenic lymphoid tissue seemed to be increased rather than decreased by corticosterone. However, if corticosterone prompted a release of

medullary cells from primary lymphoid organs (bursa and thymus), one might expect the observed increase in splenic white pulp; the spleen can be regarded as an immunological staging area for cells which are seeded from primary lymphoid centers (17). It is also possible that the data reflect a disproportionate decrease in red pulp giving the impression of an increase in white pulp.

The changes in adrenal and testis weights show that the administered hormone levels were sufficient to block tropic hormone output. The increased growth rate of these organs after hormone withdrawal also tends to support this interpretation. These indices also suggest that the level of steroid required to inhibit tropic hormone output was much lower for corticosterone than for TP. Maximum adrenal weight reduction occurred at the 0.05-mg level. With TP the maximum reduction of testicular size didn't occur until the 5.0-mg level. These findings would indicate that the hypothalamic and hypophyseal receptors of the adrenal–pituitary axis in birds are more sensitive than the testicular–pituitary axis receptors, and perhaps are functional at an earlier age. This is in agreement with recent mammalian work (18, 19) and most avian work (7, 20, 21).

Summary. Intact cockerels were injected with selected levels of corticosterone or testosterone propionate (TP) for 2 weeks. At 5 weeks of age, (immediately after hormone treatment), and at 7 weeks (2 weeks after hormone withdrawal), the weights, histology, and tissue oxygen consumptions of the bursa Fabricii, thymi, and spleens were determined. TP caused a greater involution of lymphoid organs than corticosterone, but tissue sensitivity to corticosterone was greater. Tissue size and tissue oxygen consumption were not directly correlated immediately after hormone treatment. Two weeks after hormone withdrawal the weights and oxygen consumptions of lymphoid organs in corticosterone-treated birds were increased and those in TP-treated birds decreased. This result suggests that lymphoid organs are less impaired by elevated corticosteroid levels during early developmental stages, than by elevated TP levels. For the bursa the data suggest that the mechanisms by which the two steroid hormones cause their lympholytic effect are different. The corticoids cause a transient, and androgens a permanent, effect. This contention was corroborated by histological examination which showed that the major effect of TP on the bursa is permanent and virtual replacement of lymphoid tissue by collagen, while with corticosterone there is a transient loss of lymphocytes, mainly in medullary regions.

It is postulated that the effects of TP are on the differentiation and development of stem cells necessary to the propagation of bursal lymphocytes. Corticosterone's effect is apparently more directly metabolic or lymphocytolytic, and the withdrawal of corticosterone treatment permits the lymphoid tissues to rebound.

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