Vitamin C in Lymphoid Organs of Rats and Cockerels Treated with Corticosterone or Testosterone[1] (35573)

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The lympholytic steroid hormones, testosterone and corticosterone, have been shown to be important factors responsible for changes in growth rate and intermediary metabolism in the developing lymphoid organs (1, 2). Recently there have been repeated demonstrations that a humoral factor elaborated by the mammalian thymus, a central lymphoid organ, influences the growth, metabolism, and immunologic function of the lymph nodes and spleen—peripheral lymphoid organs (3–7). It is probable that these hormones act in harmony to affect the physiological changes seen during ontogeny of the lymphoid organs.

Previously we have determined the concentration and oxidation state of vitamin C in the developing lymphoid organs of intact cockerels and found that the concentration of ascorbate relative to that of dehydroascorbate increased during most rapid growth, whereas during cessation of growth and ensuing involution, vitamin C concentration in the lymphoid organs decreased and the remaining portion of the vitamin was predominantly in the oxidized form (dehydroascorbate). The latter changes occurred at approximately the same period as maximum increases in comb and testis weights, and at the time of adrenal ascorbic acid depletion, suggesting that changes in testicular and/or adrenal function could have influenced the oxidation state of vitamin C in the lymphoid organs (8).

Subsequent investigations have shown an inverse correlation between the percentage of dehydroascorbate in the total vitamin C concentration of thymic preparations and the ability of thymic humoral factor to promote growth and direct glucose oxidation in the lymphoid organs (6). Additional work suggested that vitamin C was not required for the expression of thymic humoral factor *per se,* but that it was needed during ontogeny for the optimal production of the humoral factor (7).

Accordingly we examined the possibility that the lympholytic steroid hormones not only affect the growth and metabolism of the lymphoid organs directly, but may also change the oxidation state of vitamin C in the lymphoid organs, particularly in the thymus, which could indirectly affect the peripheral lymphoid organs by inhibiting the optimal production of thymic humoral factor.

Materials and Methods. Intact male Sprague-Dawley pathogen-free rats and single comb white leghorn cockerels were injected im for 14 days with 0.1 ml of testosterone propionate, corticosterone or vehicle (20% ethanol–cottonseed oil). Testosterone propionate was administered at 0.05, 0.50, and 5.0 mg/day and corticosterone at 0.05, 0.50, and 1.0 mg/day. The dose levels employed were those found in a previous study to cause near linear decreases in lymphoid organ weights; the highest dose level of corticosterone was reduced to 1.0 mg because cockerels were unable to tolerate 5.0 mg (9). Injections were begun in cockerels at 3 weeks and in rats at 5 weeks of age, because of the differences in rate of lymphoid organ development in the two species. Twenty-four hr after the last injection the cockerels were killed with an overdose of sodium Nembutal and the rats with chloroform. Body weights and weights of the lymphoid organs were recorded. Vitamin C was extracted from the lymphoid organs by homogenization in 3% metaphosphoric acid; ascorbate, de-

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hydroascorbate, and total vitamin C were determined on the cleared supernate by the indophenol method (10). The data were analyzed using Student's t test with the level of probability set at 0.05.

Results. Figures 1 and 2 illustrate the decreases in relative lymphoid organ weight (closed circles) and total vitamin C concentration (ascorbate + dehydroascorbate) in the lymphoid organs (open circles) after lympholytic steroid hormone treatment. The relative weights of the central lymphoid organs in the rats (thymus) and cockerels (bursa and thymus) decreased after hormone treatment (Figs. 1 and 2, closed circles). The central lymphoid organs were more sensitive to corticosterone than to testosterone proprionate treatment and showed a linear decrease in relative weight in response to dose of hormone employed. The relative weights of the peripheral lymphoid organs in the rats (lymph nodes and spleen) and cockerels (spleen) varied in their response to hormone treatment. The maximum decline in relative lymph node weight after testosterone propionate treatment was at the 0.05-mg dose level, but at higher dose levels relative lymph node weights increased. Corticosterone treatment resulted in decreases in relative lymph node weights that were proportional to the levels of hormone administered. In rats the decreases in relative spleen weights were not proportional to dose of hormone administered as either of the lympholytic steroid hormones, at any of the dose levels employed, resulted in equivalent decreases in spleen weight. In birds there was a proportional decrease in relative spleen weight after testosterone propionate administration, whereas corticosterone treatment caused only a slight decline in relative spleen weight.

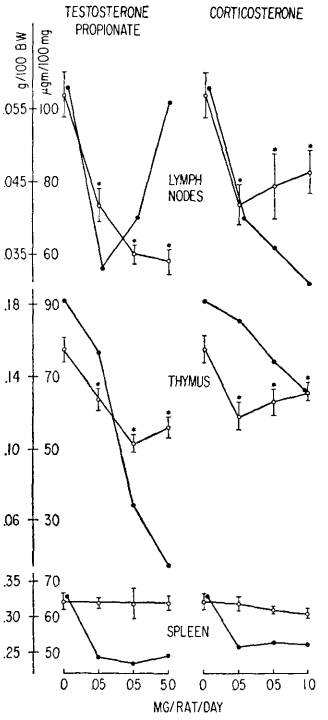

FIG. 1. The concentration of total vitamin C (ascorbate + dehydroascorbate) (μg/100 mg of tissue wt) (○); and relative weights (g/100 g of body wt) (●) of rat lymphoid organs after 14-days steroid hormone administration. Mean values for 7–10 rats, with standard errors of the mean indicated for total vitamin C concentration. *Significantly different concentration from controls, $p<0.05$, Student's t test.

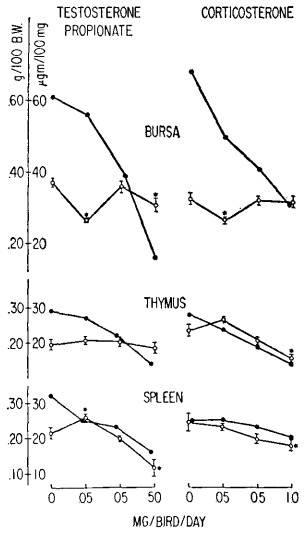

FIG. 2. The concentration of total vitamin C (ascorbate + dehydroascorbate) (μg/100 mg of tissue wt) (○); and relative weights (g/100 g of body wt) (●) of chicken lymphoid organs after 14-days steroid hormone administration. Relative weights are replotted from data submitted in a previous publication (9). Mean values for 8–16 cockerels, with standard errors of the mean indicated for total vitamin C concentration. * Significantly different concentration from controls, $p<0.05$, Student's t test.

In comparable lymphoid organs of control animals the concentration of total vitamin C was two- to threefold higher in rats than in cockerels (Figs. 1 and 2, open circles). However the content of total vitamin C (μg/organ) was 400 μg in the thymus and in the spleen of both rats and cockerels, 200 μg in the lymph nodes of rats, and 1600 μg in the bursa of cockerels.

Lympholytic steroid hormone treatment, at any dose level, caused a significant decrease in the concentration of total vitamin C in the lymph nodes and thymi of rats, but no change in the concentration in the spleens (Fig. 1). Decreases in the concentration of total vitamin C in the lymphoid organs of hormone-treated cockerels were not as marked (Fig. 2). There was a significant decrease of vitamin C in the bursa and the spleen after 5.0 mg testosterone propionate treatment, and in the thymus and spleen after 1.0 mg corticosterone treatment. In addition 0.05 mg of either hormone resulted in a significant decrease in vitamin C in the bursa, but in the spleen this level of testosterone propionate treatment significantly increased the concentration of total vitamin C.

In Tables I and II, the concentrations of oxidized (dehydroascorbate) and reduced (ascorbate) vitamin C have been expressed as percentages of the concentration of total vitamin C in the lymphoid organs of hormone-treated rats and cockerels. We presented these data in terms of concentration rather than content because of the decreases in organ weight induced by hormone treatment.

In the lymphoid organs of rats and cockerels ascorbate comprised 80–90% of the total vitamin C in the lymph nodes, bursa, and thymus, and 50–70% in the spleen. In all of the lymphoid organs except for the bursa and lymph nodes, lympholytic steroid hormone treatment decreased the percentage of ascorbate and increased the percentage of dehydroascorbate. Corticosterone treatment did not affect the oxidation state of vitamin C in the bursa, nor did testosterone propionate treatment in the lymph nodes. The greatest percentage change from controls after hormone treatment occurred in the dehydroascorbate moiety of the central lymphoid organs of the rat (thymus) and cockerel (bursa and thymus), where greater than 100% increases were seen. The highest dose level of testosterone propionate appeared to cause a slightly greater change in the percentage dehydroascorbate of the thymus than the highest dose level of corticosterone, although the thymus was much more sensitive to corticosterone treatment, since only 0.05 mg was needed to cause a significant change in the oxidation state of vitamin C. Testosterone propionate was the only hormone to affect the oxidation state of vitamin C in the bursa.

Discussion. In a previous study we observed an age-dependent decrease in the concentration of vitamin C in chicken lymphoid organs, and suggested that it could be due to steroid hormone output from the adrenals and/or testes (8). Present results support that hypothesis and show that exogenous administration of either lympholytic steroid hormone, testosterone or corticosterone, results in the reduction of vitamin C in chicken lymphoid organs. The lymphoid organs of the rat, which develop at a different rate (11), also respond to lympholytic steroid hormone treatment with vitamin C depletion. The rate of synthesis of vitamin C in these species is also affected by steroid hormones. Renal vitamin C synthesis in the chicken is inhibited by androgens (12); whereas hepatic vitamin C synthesis in the rat is augmented by androgens (13, 14). Yet corticosteroids, at least in chickens, have been shown to have no effect on vitamin C synthesis (12). The decreases in vitamin C concentration observed in the lymphoid organs of androgen-treated cockerels could thus be due to an androgenic inhibition of vitamin synthesis; however, the decreases in lymphoid organs of rats and in corticosterone-treated cockerels tend to indicate that hormone-induced vitamin depletion of the lymphoid organs is a response common to all types of lymphoid organs that is unrelated to hormonal effects on vitamin C biosynthesis.

The changes in the oxidation state of vitamin C evoked in the lymphoid organs of the hormone-treated animals were of particular interest. The potential for reversible oxidation–reduction of ascorbate and dehydroascorbate, like that of reduced and oxidized glutathione (15), could assume importance in var-

TABLE I. Amounts of Dehydroascorbate (DHA) and Ascorbate (AsA) Expressed as a Percentage of Total Vitamin C in the Lymphoid Organs of Rats Injected 14 Days with Steroid Hormones.

Seven to 10 rats/group.

	Testosterone propionate			Corticosterone		
Organ	Dose (mg/day)	% DHA	% AsA	Dose (mg/day)	% DHA	% AsA
Lymph nodes	0	19.8 ± 2.1[a]	80.2	0	19.8 ± 2.1	80.2
	0.05	25.2 ± 3.3	74.8	0.05	27.8 ± 4.2	72.2
	0.5	20.9 ± 3.0	79.1	0.5	31.8 ± 5.1[b]	68.2[b]
	5.0	20.0 ± 3.1	80.0	1.0	30.7 ± 3.5[b]	69.3[b]
Change from control (%)		None	None		+55.1	−13.6
Thymus	0	11.5 ± 1.0	88.5	0	11.5 ± 1.0	88.5
	0.05	10.3 ± 1.4	90.7	0.05	19.1 ± 3.0[b]	80.9[b]
	0.5	11.9 ± 2.3	88.1	0.5	19.8 ± 3.1[b]	80.2[b]
	5.0	26.4 ± 5.4[b]	73.6[b]	1.0	23.6 ± 2.4[b]	76.4[b]
Change from control (%)		+129.6	−16.8		+105.2	−13.7
Spleen	0	29.2 ± 1.2	70.8	0	29.2 ± 1.2	70.8
	0.05	37.5 ± 2.5[b]	62.5[b]	0.05	35.8 ± 2.3[b]	64.2[b]
	0.5	39.4 ± 1.6[b]	60.6[b]	0.5	37.2 ± 2.3[b]	62.8[b]
	5.0	43.0 ± 1.7[b]	57.0[b]	1.0	40.4 ± 1.5[b]	59.6[b]
Change from control (%)		+47.3	−19.5		+38.4	−15.8

[a] Means ± SEM, standard errors for AsA same as those for corresponding DHA.
[b] Significantly different from controls at $p<0.05$, Student's t test.

TABLE II. Amounts of Dehydroascorbate (DHA) and Ascorbate (AsA) Expressed as a Percentage of Total Vitamin C in the Lymphoid Organs of Chickens Injected 14 Days with Steroid Hormones.

Eight to 16 chickens/group.

	Testosterone propionate			Corticosterone		
Organ	Dose (mg/day)	% DHA	% AsA	Dose (mg/day)	% DHA	% AsA
Bursa	0	12.9 ± 1.6[a]	87.1	0	12.9 ± 1.6	87.1
	0.05	12.7 ± 1.6	87.3	0.05	14.3 ± 2.1	85.7
	0.5	17.7 ± 2.8	82.2	0.5	14.8 ± 3.1	85.2
	5.0	32.4 ± 3.5[b]	67.6[b]	1.0	14.3 ± 2.0	85.7
Change from control (%)		+151.2	−22.4		None	None
Thymus	0	15.2 ± 2.3	84.8	0	15.2 ± 2.3	84.8
	0.05	14.8 ± 2.2	85.2	0.05	22.9 ± 1.7[b]	77.1[b]
	0.5	31.3 ± 3.6[b]	68.7[b]	0.5	35.0 ± 2.1[b]	65.0[b]
	5.0	55.6 ± 4.1[b]	44.4[b]	1.0	34.5 ± 5.0[b]	65.5[b]
Change from control (%)		+265.8	−47.6		+127.0	−22.8
Spleen	0	49.5 ± 3.2	50.5	0	49.5 ± 3.2	50.5
	0.05	39.7 ± 3.3	60.3	0.05	58.6 ± 3.7	41.4
	0.5	55.4 ± 6.3	44.6	0.5	68.8 ± 7.3[b]	31.2[b]
	5.0	77.9 ± 10.7[b]	32.1[b]	1.0	68.3 ± 6.9[b]	31.7[b]
Change from control (%)		+57.4	−36.4		+38.0	−37.2

[a] Means ± SEM, standard errors for AsA same as those for corresponding DHA.
[b] Significantly different from controls at $p<0.05$, Student's t test.

ious dynamic processes that occur during lymphoid organogenesis. We found that lymphoid organ involution after hormone treatment was accompanied by a change in the oxidation state of vitamin C, such that the percentage of dehydroascorbate increased and that of ascorbate decreased. In other tissues, dehydroascorbate has also been associated with degenerative processes such as mitotic inhibition, activation of lysosomal enzymes, lipid peroxidation, and loss of control of membrane permeability (16); whereas regenerative processes such as ribonucleic acid synthesis (17) and collagen formation (18) are stimulated by ascorbate. The changes in the oxidation state of vitamin C found in the lymphoid organs of hormone-treated animals obviously must be of physiological significance. Note that the decrease in total vitamin C was paralleled by a decrease in ascorbate, and in terms of percentage change from controls, hormone treatment in some instances increased the percentage of dehydroascorbate more than 100%. Thus it could potentially reflect a greater than 2-fold change in effectiveness.

In earlier studies (6, 7) indirect evidence was obtained which indicated that dehydroascorbate inhibited the optimal production of a nonsteroidal humoral factor from the thymus. Thymic humoral factor has been shown to stimulate the growth (3, 5), metabolism (6, 7), and immunologic function (4) of the peripheral lymphoid organs. Present results demonstrate that lympholytic steroid hormone treatment affected a much greater change in the oxidation state of vitamin C in the central lymphoid organs (the thymus and bursa) than in the lymph nodes or spleen. These data collectively support the hypothesis that lympholytic steroid hormones cause an inhibition of the production of thymic humoral factor by oxidizing thymic ascorbate to dehydroascorbate, and thus affect the growth and function of the peripheral lymphoid organs.

Corticosterone was found to be far more effective in this regard than testosterone propionate, as only 0.05 mg was needed to significantly alter the oxidation state of vitamin C in the thymus. This difference in response should prove to be of value in future experiments regarding vitamin C–hormone interrelationships, since we found earlier that androgen effects on lymphoid organ growth and metabolism were more permanent than those of corticosterone (9). Also it would appear to be important in the immune development of animals that inhibitory changes evoked by adrenocortical stressor hormones, such as oxidation of ascorbate in the lymphoid organs, would be of a transient rather than a permanent nature.

Summary. Immature rats and cockerels were injected for 14 days with 0.05–5.0 mg of testosterone propionate or corticosterone, and the concentration and oxidation state of vitamin C in their lymphoid organs was measured. The total vitamin C concentration (ascorbate + dehydroascorbate) in the lymphoid organs of both animals decreased after hormone administration, but to a greater extent in rats than in cockerels. These changes appear to be independent of any direct hormonal influence on vitamin C biosynthesis. Significant and marked changes in the oxidation state of lymphoid organ vitamin C occurred after lympholytic steroid hormone treatment; the percentage of dehydroascorbate increased and that of ascorbate decreased. After hormone treatment the largest percentage change from controls (more than 100% increases in percentage dehydroascorbate) occurred in the central lymphoid organs, the bursa and thymus, rather than in the spleen or lymph nodes. Much lower dose levels of corticosterone (0.05 mg) than of testosterone propionate (5.0 mg) were required to produce this shift in the oxidation state of lymphoid organ vitamin C. We suggest the ascorbate–dehydroascorbate system in the lymphoid organs is an integral portion of the physiological mechanisms operative during hormonal modulation of the growth, metabolism, and function of the lymphoid organs.

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