Hormonal Control of the Synthesis and Distribution of Ascorbic Acid in Cockerels (*Gallus domesticus*) (33523)

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Current evidence indicates that ascorbic acid synthesis in mammals is under hormonal regulation. Stubbs and McKernan found a marked sex difference in the biosynthesis and storage of ascorbate in rats (11) and indirectly demonstrated that testosterone stimulated hepatic synthesis of ascorbate and probably accounted for the sex difference in tissue and plasma levels of this metabolite (12).

Ascorbic acid studies in Aves have revealed a number of differences from the mammalian pattern in regard to tissue localization and concentration (3), hormonal sensitivity (9), and site of synthesis (10). For instance, ascorbic acid was found to be synthesized in the kidneys of galliform birds, but in most other birds and mammals the organ of synthesis was found to be the liver (2, 10). Young birds failed to respond to ACTH with adrenal ascorbic acid depletion (8, 13), but the ascorbate concentrations of the adrenals and other tissues were decreased after testosterone propionate administration (1, 3), whereas in mammals, castration lowered the tissue vitamin concentration (12).

These differences prompted an investigation of the hormonal control of ascorbic acid synthesis in galliform birds. The influence of testosterone propionate or corticosterone administration on the renal biosynthesis of ascorbic acid was examined in young and older cockerels and the evidence correlated with plasma and tissue distribution of ascorbic acid.

Materials and Methods. One-day-old white leghorn cockerels were maintained in brooder cages in an air-conditioned, light-controlled animal room. Purina chick starter and water were supplied, *ad libitum*. At 10 days of age three equal groups of chicks (60 total) were injected i.m. twice daily for 4 days with 0.1 ml of vehicle (20% ethanol–cottonseed oil), or testosterone propionate, or corticosterone at concentrations of 0.1 mg/injection. Between 9:00 a.m. and noon on the fourteenth day, one half of the chicks were killed with a cardiac overdose of sodium Nembutal. Four days later the remaining chicks were sacrificed in the same manner. The adrenals, liver, and kidney were removed, frozen in dry ice, and used for ascorbic acid determinations and enzyme studies.

In a second experiment day-old chicks were raised as described above, and transferred to large holding cages at 5 weeks of age. Between 5 and 6 weeks of age, eight cockerels were gonadectomized under sodium Nembutal anaesthesia, using a bilateral intercostal entry. Eight cockerels were laparotomized and served as controls. At 7 weeks of age, at least 1 week following the operations, the laparotomized and gonadectomized cockerels were injected i.m. daily for 2 weeks with 0.1 ml of the vehicle (propylene glycol) or with testosterone propionate (2.0 mg/injection), respectively. Between 9:00 a.m. and noon, the day following the last injections, the birds were sacrificed as before. The adrenals, liver, kidneys, and blood plasma were frozen and used for ascorbic acid determinations and enzyme study.

Ascorbate determinations. Between 300 and 500 mg of liver or kidney were homogenized in 10 ml and both adrenals (40–100 mg) in 5 ml of ice-cold 3% metaphosphoric acid. Homogenates were cleared by refrigerated centrifugation (2500 rpm) or filtration, and ascorbic acid, dehydroascorbic acid, and total vitamin C determined by the indophenol method (4) on a Bausch and Lomb Spectron-

TABLE I. Effect of Steroid Hormones on Renal Vitamin C.

Treatment[a]	Immediately after treatment (μg/100 mg of tissue; means $\pm$ SEM, $n = 10$)		
	Ascorbate	Dehydroascorbate	Total vitamin C
Controls	35.5 $\pm$ 1.2	28.9 $\pm$ 3.5	64.4 $\pm$ 3.3
Testosterone propionate	44.2 $\pm$ 1.6[b]	26.8 $\pm$ 2.6	71.0 $\pm$ 2.0
Corticosterone	31.7 $\pm$ 0.9[b]	28.2 $\pm$ 2.9	59.9 $\pm$ 2.3
	Four days following treatment		
Controls	36.3 $\pm$ 2.1	30.0 $\pm$ 2.0	66.3 $\pm$ 1.9
Testosterone propionate	27.6 $\pm$ 1.0[b]	23.4 $\pm$ 2.2[b]	51.0 $\pm$ 2.1[b]
Corticosterone	27.5 $\pm$ 1.8[b]	30.1 $\pm$ 3.5	57.6 $\pm$ 2.2[b]

[a] Vehicle (20% ethanol–cottonseed oil) or steroids (0.1 mg/inj.) administered twice daily for 4 days; see text for experimental details.

[b] Indicates means are significantly different from controls at $p < .05$.

ic 20 at 520 mμ. These values were compared to standards of *l*-ascorbic acid run through the same procedure. Three ml of plasma were extracted in an equal volume of 3% metaphosphoric acid, spun in a refrigerated centrifuge (2500 rpm), and total vitamin C was determined on the supernatant fraction as above.

Enzyme studies. Kidney tissue was homogenized (4%, w/v) for 2 min in cold 0.05 *M* Tris buffer (pH 7.4) and gulonate NADP oxidoreductase activity of the whole homogenate was assayed by the measurement of $NADPH_2$ oxidation in a Beckman model DU recording spectrophotometer at room temperature. Cuvettes contained 2.6 ml of Tris buffer (0.05 M, pH 7.4), 0.1 ml of NADPH (1.7×10^{-4} *M*), 0.2 ml of whole homogenate, and 0.1 ml of *l*-gluconate (7.7×10^{-4} *M*. It was shown that the concentration of reactants used gave maximal velocity, and that the velocity was directly proportional to the amount of tissue extract.

Protein concentration of the whole homogenates were measured by the phenol method (6, 7) and were read at 750 mμ. Values were compared to standards of crystalline bovine serum albumin run through the same procedure.

Results and Discussion. The effects of hormone treatment on the concentration of reduced, oxidized, and total vitamin C in the kidney and liver of immature cockerels are given in Tables I and II. There was a slight increase (10%) and decrease (7%), respectively, in renal and hepatic total vitamin C immediately after testosterone propionate or corticosterone treatment. Significant parallel changes which occurred in the ascorbate concentration of these organs suggested the principal effect of the steroid hormones was on the reduced component of vitamin C. *A. priori* it seemed that androgens might have stimulated and corticoids inhibited renal biosynthesis of vitamin C, but the data in Table III indicate otherwise. Testosterone propionate significantly inhibited the activity of an enzyme in the ascorbic acid biosynthetic pathway converting glucuronate $\rightarrow$ gulonate (gulonate NADP oxidoreductase) and corticosterone had no effect.

Four days following testosterone propionate treatment (Tables I and II) there was a significant decrease in all renal vitamin C components, hepatic ascorbate and total vitamin C, but no further decline in gulonate NADP oxidoreductase activity of the kidney (Table III), implying that a temporal delay may occur before tissue vitamin concentrations respond to the androgenic repression of renal biosynthetic activity. At this time the kidney and liver of corticosterone treated birds also exhibited decreases in several vitamin C components (Tables I and II). Since there was still no effect of corticosterone on renal enzyme activity (Table III), the above

TABLE II. Effect of Steroid Hormones on Hepatic Vitamin C.

Treatment[a]	Immediately after treatment (μg/100 mg of tissue; means $\pm$ SEM, $n = 10$) Ascorbate	Dehydroascorbate	Total vitamin C
Controls	50.8 $\pm$ 1.6	21.1 $\pm$ 3.2	71.9 $\pm$ 3.6
Testosterone propionate	55.4 $\pm$ 1.9	23.1 $\pm$ 1.9	78.5 $\pm$ 2.1
Corticosterone	39.1 $\pm$ 1.4[b]	28.8 $\pm$ 1.8	67.9 $\pm$ 1.6
	Four days following treatment		
Controls	51.0 $\pm$ 2.8	24.5 $\pm$ 3.2	75.5 $\pm$ 3.9
Testosterone propionate	34.2 $\pm$ 2.7[b]	24.8 $\pm$ 1.4	59.0 $\pm$ 2.1[b]
Corticosterone	35.9 $\pm$ 1.8[b]	33.1 $\pm$ 3.3	69.0 $\pm$ 3.3

[a] Dose levels as in Table I.

[b] Indicates means are significantly different from controls at $p < .05$.

decreases may be attributed to altered tissue utilization or transport of vitamin C. Earlier work in mammals, as well as birds, indicated corticosterone and, or ACTH were capable of altering plasma and tissue concentrations of vitamin C (3, 5, 8).

The second experiment on older gonadectomized birds confirmed that testosterone propionate inhibits renal gulonate NADP oxidoreductase activity. Androgen treatment resulted in a 28% reduction in kidney enzyme activity from a mean of 12.15 milliunits $\pm$ 1.0 SEM, to 8.72 $\pm$ 0.7 ($p < .02$, 14 df). Table IV shows there was no correlation between the plasma or tissue vitamin C concentration and the reduction in kidney enzyme activity. In three instances androgen treatment of the gonadectomized birds resulted in significant increases in the concentration of liver and kidney vitamin C components. This may also be due to a time factor, since in comparable mammalian work, assays of liver enzyme activity and plasma and tissue vitamin C concentration were lower at 6 than at 2 weeks after gonadectomy (12).

TABLE III. Effect of Steroid Hormones on Gulonate NADP Oxidoreductase Activity of Kidney (means $\pm$ SEM, $n = 10$).

Treatment[a]	Enzyme activity (milliunits)[b]
	Immediately after treatment
Controls	7.37 $\pm$ 0.64
Testosterone propionate	5.12 $\pm$ 0.26[c]
Corticosterone	6.20 $\pm$ 0.24
	Four days following treatment
Controls	6.40 $\pm$ 0.46
Testosterone propionate	6.10 $\pm$ 0.34
Corticosterone	6.57 $\pm$ 0.24

[a] Dose levels as in Table I.

[b] Unit of enzyme activity defined as micromoles of substrate reduced per milligram of protein per minute.

[c] Indicates means are significantly different from controls at $p < .001$.

Note that in control birds the concentration of renal and hepatic vitamin C were twice as high in younger than in older birds. The specific enzyme activity in the kidneys was only slightly greater in older than younger birds. Perhaps the decline in tissue concentration of vitamin C in older birds is simply a reflection of lower total activity in the renal enzymes associated with ascorbic acid biosynthesis. Such an explanation is also compatible with earlier results in birds which illustrated a decline in lymphatic organ vitamin C concentration during pubertal development (3).

Summary. Testosterone propionate treatment of intact 10-day-old cockerels significantly depressed renal gulonate NADP oxidoreductase activity; after a delay there was a significant decrease in kidney and liver vitamin C concentrations. Administration of corticosterone did not affect the renal biosynthesis of ascorbic acid, but did result in significant decreases in kidney and liver vitamin

TABLE IV. Effect of Testosterone Propionate Treatment on Plasma and Tissue Vitamin C Concentration (means ± SEM, $n = 8$).

	(μg/100 mg)							
	Plasma	Adrenals	Liver			Kidney		
Treatment[b]	AA[a]	AA	AsA	DHA	AA	AsA	DHA	AA
Laparotomy + propylene glycol administration	1.72 ±0.12	69.9 ±3.6	18.5 ±1.9	13.1 ±3.6	31.6 ±3.7	9.9 ±1.0	13.8 ±1.3	23.7 ±1.0
Castration + testosterone propionate administration	1.43 ±0.11	82.2 ±7.2	17.9 ±1.0	23.1[c] ±1.2	41.0[c] ±1.5	15.2[c] ±0.9	10.7 ±1.1	25.9 ±0.9

[a] AA, AsA, and DHA indicates total vitamin C, ascorbic acid, and dehydroascorbic acid, respectively.

[b] Vehicle (propylene glycol) or testosterone propionate (2.0 mg/inj.) administered daily for 2 weeks; see text for experimental details.

[c] Indicates means are significantly different from controls at $p < .05$.

C concentrations. The decline in tissue vitamin C concentration 4 days after cessation of androgen treatment may have been caused by the previous depression of renal enzyme activity, but the decrease in tissue vitamin C after corticoid treatment showed no such correlation and was interpreted as a nonenzymatic hormonal effect on vitamin C utilization or transport by the tissues/Androgen treatment of 10-week-old capons also resulted in a significant reduction of renal gulonate NADP oxidoreductase activity. However renal and hepatic vitamin C concentrations were significantly elevated in this instance, possibly because insufficient time had elapsed for a vitamin depletion response.

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