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Reduction in Hepatic Lipid and Plasma Estradiol in Estrogenized Chicks Injected with Ascorbic Acid[1] (41998)

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Abstract. Injecting White Leghorn chicks every other day with 20 mg ascorbic acid significantly reduced the increase in liver weight and lipids caused by feeding a diet with 0.1% dienestrol diacetate. In chicks fed two different basal diets containing 0.1% dienestrol diacetate, injecting chicks every other day with 20 mg α-tocopherol did not significantly reduce liver weight or lipids while the ascorbic acid injections did. Injecting meat-type chicks implanted with estradiol with 10 mg ascorbic acid daily significantly reduced liver weight, liver lipids, and plasma estradiol, but injecting with 8 mg α-tocopherol daily had no significant effect.

In female chickens in a reproductive state, feeding a nutritionally balanced, simplified corn–soybean meal diet (CS) results in fatty livers which can be prevented by including various crude feedstuffs in the CS diet (1). The excess hepatic lipid deposition is correlated with increased plasma levels of estrogens (2–4) or estrogen administration (5–8). Nutritional factors modify the effects of estrogen treatment (1, 2, 4). The specific nutritional factor(s) involved in maintaining normal estrogen and lipid metabolism remains to be identified but some evidence has been obtained that dietary fat (9) or supplementary selenium or vitamin E (1) in combination with an unidentified factor reduced hepatic lipid deposition and hemorrhages in laying hens.

A significant negative correlation of plasma ascorbic acid and iron levels, and higher hepatic lipid accumulation was observed in laying hens fed diets of different compositions (10). Recently we have found that a diet that reduces hepatic lipid deposition and plasma estradiol levels in estrogenized chicks also activates the microsomal mixed function oxidase (MFO) system as indicated by increased cytochrome *P*-450 content and increased activity of aniline hydroxylase (11). Administration of ascorbic acid and/or vitamin E modified lipid metabolism in chicks (12, 13). Furthermore, these vitamins increased MFO activity in mammals (14–16).

These experiments were conducted to determine if injection of chicks with ascorbic acid and/or vitamin E would affect hepatic lipid deposition and plasma estradiol in response to estrogen treatment.

Materials and Methods. The chicks used in these experiments were housed in electrically heated battery brooders with wire screen floors and were subjected to continuous illumination. Feed and water were provided *ad libitum*. The chicks were individually weighed at the beginning and end of each experiment to determine mean weight gain for each treatment. Chicks from each treatment were fed by groups and individual feed intake was estimated by dividing the total feed consumed by the number of chicks per treatment. At the end of each experiment chicks were killed by cervical dislocation and the livers were removed and weighed. After determination of the dry matter of the livers, lipid content was estimated by the method of Mendońca and Jensen (17). In Experiment 3 blood was obtained by heart puncture and the plasma estradiol content was determined by the method of Tojo and Huston (18). Data were analyzed using the General Linear Model procedure of Statistical Analysis System (SAS) in conjunction with the Duncan multiple range test (19).

Experiment 1. White Leghorn male chicks (14 days of age) were fed a CS diet with and

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without 0.1% dienestrol diacetate for 14 days. Ten chicks on each diet were each injected subcutaneously every other day with 20 mg L-ascorbic acid in saline while another 10 chicks from each diet were injected with saline to serve as controls.

Experiment 2. White Leghorn male chicks (11 days of age) were fed either a CS diet or one containing fish meal, alfalfa meal, and torula yeast (FAY) for 12 days. [For composition of diets see Akiba *et al.* (7).] Both diets contained 0.1% dienestrol diacetate. Eight chicks were injected subcutaneously every other day with 20 mg L-ascorbic acid in saline, 20 mg α-tocopherol in corn oil, or a combination of 20 mg ascorbic acid and 20 mg tocopherol. Control chicks were injected with a combination of the carriers.

Experiment 3. Male broiler chicks (Hubbard strain) (14 days of age) were fed a CS diet for 14 days. All chicks were implanted in the neck with silastic tubes containing estradiol prepared as described by Akiba *et al.* (7). Release rate of estradiol was estimated to be 8.5 μg/day (20). Seven chicks each were injected daily with 10 mg L-ascorbic acid, 8 mg α-tocopherol, or 10 mg L-ascorbic acid plus 8 mg α-tocopherol. Control chicks were injected with a combination of the carriers.

Results. In Experiment 1 none of the treatments affected body weight gain but dienestrol diacetate significantly increased liver weight and lipid content (Table I). Injecting ascorbic acid had no significant effect on liver weight or lipids in chicks not receiving the synthetic estrogen compound but significantly reduced both liver weight and lipids in chicks fed dienestrol diacetate.

In Experiment 2 in which all chicks received dienestrol diacetate body weight gain was again not significantly affected by injection treatments or diet (Table II). Injecting the chicks with ascorbic acid again significantly reduced liver weight and lipids but injecting with tocopherol had no significant effect on these parameters. There was a significant interaction, however, between diet and tocopherol with the injections resulting in higher liver weights and lipids in chicks fed the CS diet and lower values in chicks fed the FAY diet.

In Experiment 3 body weight gain was not significantly affected by treatments (Table III). Ascorbic acid significantly reduced liver weight, liver lipids, and plasma estradiol but tocopherol had no significant effect on these parameters.

Discussion. These results demonstrate that injecting chicks with ascorbic acid modifies their hepatic response to either synthetic or natural estrogens. This may be caused by an increased rate of metabolism of estrogens as a result of an increased activity of the MFO system. Ascorbic acid deficiency in guinea pigs results in a decreased metabolism of steroids (21) and a significant correlation between hepatic ascorbic acid concentrations and drug metabolism has been observed in these animals (22, 23). The reduced level of estradiol in plasma of chicks injected with ascorbic acid in chicks implanted with this hormone provides evidence for an increased rate of degradation. Previous studies in our laboratory have demonstrated a significant correlation between plasma estradiol levels and hepatic lipid accumulation (4) and diets

TABLE I. EFFECT OF INJECTION WITH ASCORBIC ACID ON BODY AND LIVER WEIGHTS AND LIVER LIPIDS IN CHICKS FED DIETS WITH AND WITHOUT DIENESTROL DIACETATE

Treatment					
Vitamin C	Dienestrol diacetate in diet (0.1%)	Body weight gain (g/14 days)	Feed intake (g/day)	Liver weight (g/100 g BW)	Liver lipid (%)
−	−	212 ± 4[a]	32	2.38 ± 0.04[a]	5.74 ± 0.15[a]
+	−	209 ± 6[a]	30	2.67 ± 0.06[a]	5.14 ± 0.25[a]
−	+	221 ± 10[a]	33	5.37 ± 0.16[c]	12.59 ± 2.55[c]
+	+	211 ± 8[a]	32	4.83 ± 0.22[b]	8.34 ± 0.86[b]

Note. Values (except feed intake) are expressed as means of 10 observations ± SEM. The chicks treated with ascorbic acid were injected every other day for 14 days with 20 mg. Control chicks were also injected with a solution containing no ascorbic acid.

[a,b,c] Means within each column with different superscript letters are significantly different; ($P < 0.05$).

TABLE II. EFFECTS OF ASCORBIC ACID (C) AND/OR TOCOPHEROL (E) INJECTIONS ON BODY AND LIVER WEIGHT AND LIVER LIPID IN ESTROGENIZED WHITE LEGHORN MALE CHICKS FED TWO DIFFERENT DIETS (EXPERIMENT 2)

Diet	Treatment	Body weight gain (g/12 days)	Feed intake (g/day)	Liver weight (g/100 g BW)	Liver lipid (%)
CS	Control	129 ± 9	23	4.87 ± 0.30	7.14 ± 0.72
	C	143 ± 7	24	4.81 ± 0.15	5.48 ± 0.42
	E	144 ± 6	25	5.38 ± 0.11	8.07 ± 0.87
	C + E	143 ± 7	26	4.60 ± 0.23	6.47 ± 0.70
FAY	Control	136 ± 7	24	5.20 ± 0.20	7.67 ± 0.52
	C	143 ± 5	26	4.95 ± 0.24	6.08 ± 0.35
	E	133 ± 7	24	4.59 ± 0.20	6.87 ± 0.51
	C + E	118 ± 6	23	4.44 ± 0.25	5.94 ± 0.22
		Analysis of variance (probabilities)			
Diet (D)		NS		NS	NS
C		NS		$P = 0.048$	$P = 0.002$
E		NS		NS	NS
D × C		NS		NS	NS
D × E		NS		$P = 0.026$	$P = 0.004$
D × C × E		NS		NS	NS

Note. Values (except feed intake) are expressed as means of eight observations ± SEM. The chicks were fed either CS (corn–soybean meal) or FAY (a diet containing fish meal, alfalfa meal, and torula yeast) containing 0.1% dienestrol diacetate. Chicks receiving C and/or E were injected subcutaneously every 2 days for 12 days with 20 mg of each substance.

that have been shown to reduce plasma estradiol and hepatic lipids have also been shown to stimulate MFO activity (11, 24).

Treatment of the chicks with estrogens may also have lowered tissue ascorbic acid levels because treatment of guinea pigs with estrogens significantly reduced ascorbic acid levels in both plasma and blood vessels (25). Reports have indicated that oral contraceptives affect ascorbic acid metabolism and increase its requirement but the evidence remains controversial (26).

In contrast to ascorbic acid, tocopherol administration failed to significantly reduce liver weight or lipids in the chicks. Excess vitamin E in chicks and other animals suppresses thyroid activity (27). Suppression of thyroid activity could result in increased liver lipid because feeding antithyroid agents in combination with a synthetic estrogen caused

TABLE III. EFFECT OF INJECTIONS WITH ASCORBIC ACID (C) AND/OR TOCOPHEROL (E) ON BODY AND LIVER WEIGHT, LIVER LIPID, AND PLASMA ESTRADIOL IN ESTROGENIZED CHICKS

Treatment		Body weight gain (g/14 days)	Feed intake (g/day)	Liver weight (g/100 g BW)	Liver lipid (%)	Plasma estradiol (pg/ml)
C	E					
−	−	389 ± 18	49	4.78 ± 0.19	7.98 ± 0.59	1758 ± 240
+	−	399 ± 23	49	4.25 ± 0.30	6.83 ± 0.31	1231 ± 293
−	+	408 ± 30	50	4.81 ± 0.30	9.23 ± 1.29	1758 ± 328
+	+	388 ± 21	50	4.10 ± 0.29	6.13 ± 0.36	1365 ± 247
		Analysis of variance (probabilities)				
C		NS		$P = 0.035$	$P = 0.011$	$P = 0.050$
E		NS		NS	NS	NS
C × E		NS		NS	NS	NS

Note. Values (except feed intake) are expressed as means of seven observations ± SEM. All chicks were implanted with tubes containing estradiol. Chicks were injected daily with 10 mg C, 8 mg E, a combination of 10 mg C and 8 mg E, or vehicles without C or E.

fatty livers in chicks (28). Tocopherol administration may cause opposing effects from different actions of the vitamin resulting in no significant effect on hepatic lipid deposition. A significant interaction between diet and tocopherol in Experiment 2 suggested a different response to the vitamin when chicks were fed isocaloric, isonitrogenous diets of different ingredient composition.

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