Effect of Estradiol Benzoate on Cholesterol and Vitamin A Metabolism.* (30439)

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Work in this and other laboratories suggests an interrelationship between vitamin A and cholesterol metabolism(1-4). However, the effect of estrogens upon this interrelationship is not clearly understood, although certain relations have been observed. A review by Moore(5) suggests that under the same conditions female rats seem to store more vit. A in their livers than do males, while male rats seem to have higher blood levels of this vitamin than females. Male rats have also been shown to store significantly more cholesterol in their livers than females(6). Extradiol has been reported to increase the serum vit. A level in sexually immature pullets(7) and liver vit. A concentration in male rats(5), and to increase the serum and liver cholesterol concentration in male rats(8,9). Castration of male rats has been shown to decrease the amount of vit. A circulating in the blood and stored in the liver when no dietary cholesterol was fed or when cholesterol and vit. A were given together in the diet(2). Castration of male rats also has been shown to decrease the liver cholesterol deposits(10) and serum cholesterol levels(11).

This study reports the effect of one level of estradiol benzoate on the interrelationship between vit. A and cholesterol metabolism.

Procedure. Weanling male rats of the Sprague-Dawley strain† were depleted of their vit. A stores on purified diets,‡ previously described(4), complete in all requirements except vit. A. As each animal manifested vit. A deficiency symptoms *and* gained no more than 0.5 g within 3 consecutive days, it was assigned at random to 1 of 8 groups. One-half of the animals in each group were castrated at ages 27 to 28 days. Each of the 16 subgroups was composed of 6 rats and received for the 21-day experimental period either 1) 0 or 0.5% cholesterol, 2) 150 or 1000 IU of vit. A acetate daily in 0.1 ml of cottonseed oil, and 3) subcutaneous injections 3 times a week of 0.1 ml of cottonseed oil or 4 μg of estradiol benzoate in 0.1 ml of cottonseed oil. The purified basal diets containing 5% fat as cottonseed oil§ with and without cholesterol were kept frozen. Approximately a weekly supply was thawed and kept refrigerated and fed *ad libitum* daily. The vit. A supplements, protected with 1.5 mg of α-tocopherol per daily dosage, were administered orally by medicine dropper.

Feed was removed about 12 to 15 hours and vit. A supplements were given about 16 to 18 hours before sacrifice. At the end of the experimental period each rat was anesthetized with nitrous oxide by a technique already described(12). Serum was prepared from the greatest amount of blood obtainable from the posterior vena cava. Livers were weighed, homogenized, and sealed in 4-oz polyethylene bags. Both serums and livers were quick-frozen and held at —17°C until assayed. Five vit. A-depleted animals were sacrificed at the end of the depletion period to serve as negative controls.

Methods of analysis for vit. A and total cholesterol in serums and livers, and for moisture in livers were the same as used in a former study(4). Liver total lipids were analyzed by the Sperry method(13).

Results. The mean serum and liver values are summarized in Table I. The effects of dietary cholesterol level, dietary vit. A level,

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† Northwest Rodent Co., Pullman, Wash.

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§ Wesson oil purchased in one lot.

TABLE I. Mean Liver and Serum Values.

Injections, subcutaneous		Cottonseed oil*				Estradiol benzoate†			
Dietary cholesterol, %		0		0.5		0		0.5	
Dietary vit. A, IU/day		150	1000	150	1000	150	1000	150	1000
Wt gain/wk, g	M‡	27	24	24	22	23	25	18	15
	MC§	20	21	28	24	16	18	16	12
Liver									
Wt, g	M	10.3	10.5	11.0	11.0	9.4	8.6	9.2	9.2
	MC	8.9	8.4	9.8	9.8	8.3	8.5	8.3	8.5
Moisture, %	M	70.2	68.5	69.5	68.3	70.5	69.3	70.6	68.2
	MC	70.2	69.5	68.6	68.3	69.2	68.7	69.3	68.4
Total lipids, % moist wt	M	2.6	2.4	2.7	2.8	2.3	2.6	3.2	3.3
	MC	2.7	2.7	3.2	3.2	2.9	3.5	2.6	3.6
Total cholesterol, % moist wt	M	.31	.27	.89	.70	.31	.30	.99	.74
	MC	.27	.26	.78	1.03	.26	.27	.88	.63
Vit. A, μg/g moist wt	M	179	651	211	603	187	808	109	872
	MC	195	870	88	636	191	845	102	1054
Serum									
Total cholesterol, mg/100 ml	M	97	104	80	73	110	76	109	116
	MC	104	98	91	66	136	133	122	104
Vit. A, μg/100 ml	M	83	91	78	87	76	71	80	88
	MC	61	81	74	78	69	98	68	80

* Injected 0.1 ml 3 times a week.
† Injected 4 μg in 0.1 ml cottonseed oil 3 times a week.
‡ Male rats.
§ Castrated male rats.

estrogenic treatment, and castration were determined by an analysis of variance. Only those differences that were statistically significant ($P<0.01$) are discussed unless otherwise indicated.

In general those rats injected with estradiol benzoate (EB) had significantly lower mean weekly weight gains and food intakes than those injected with the oil carrier only, irrespective of other treatment.

The mean liver weights of the castrated male animals were significantly lower than those of the intact males. Estrogenic treatment significantly reduced the liver weights of all animals except the castrates given 1000 IU of vit. A daily and no cholesterol. This latter group had little change in their liver weights due to EB.

As previously reported(4), the intact male rats showed an inverse relationship between dietary vit. A level and liver total cholesterol. This was significant on a concentration per dry weight basis. However, the castrated males showed little change in their liver total cholesterol concentration due to different dietary vit. A level without dietary cholesterol whether receiving EB or not. When castrates received estrogenic injections and dietary cholesterol, the inverse relationship between dietary vit. A and liver cholesterol deposition was reestablished. Liver cholesterol deposition increased as the dietary vit. A level increased in castrated rats fed cholesterol not modified in EB.

Estrogenic treatment significantly depressed the cholesterol content per liver in the intact rats when cholesterol was fed, and in the castrates when cholesterol was fed with the high level of vit. A. However, this may only be a reflection of the depressive effect of castration upon liver weight.

Injections of EB increased the liver vit. A concentration in the castrates fed cholesterol and in the intact rats not fed cholesterol. In the intact males fed cholesterol the effect of EB on the liver vit. A concentration depended on the level of dietary vit. A. At the 150 IU level of vit. A, administration of EB decreased the liver concentration, while at the 1000 IU level, it increased it.

Estradiol benzoate injections also significantly increased the serum cholesterol level in all animals except for the intact rats fed no cholesterol and 1000 IU of vit. A daily.

These latter rats showed a significant decrease in their serum cholesterol level due to EB.

When corrections were made for other treatment effects, castrated animals had a significant ($P<0.01$) negative correlation ($r = -0.46$) between liver content of cholesterol and concentration of vit. A on a dry weight basis. This relationship also existed between the concentration of liver cholesterol and the log concentration of liver vitamin A ($r = -0.36$) to a lesser degree ($P<0.05$). The animals receiving estrogenic injections had no significant correlation between their liver deposits of vit. A and cholesterol. However, the rats receiving only comparable injections of the carrier oil had negative correlations ($P<0.05$) between liver cholesterol content and vit. A concentration on a dry weight basis ($r = -0.34$).

Discussion. These results suggest that either estradiol benzoate (EB) administered to castrates fed dietary cholesterol or the natural testicular hormones in the intact rats decreased the liver total cholesterol deposition as the dietary vit. A level increased. It appears that both male and female sex hormones are involved in bringing about this relationship. Administration of EB to the intact male rats seems to exaggerate the increase in liver cholesterol deposition with increased dietary vit. A level. The fact that this relationship exists only when cholesterol is fed suggests that it is the exogenous cholesterol that is more involved than the endogenous.

It also seems that EB specifically depresses liver vit. A concentration in the castrates when exogenous cholesterol is absent, but increases it when exogenous cholesterol is present. In intact rats EB increased the concentration of liver vit. A when no cholesterol was fed. These differences imposed by estrogenic treatment on intact and castrated male rats not fed cholesterol suggests that the testicular hormones also enter into the regulation of liver vit. A deposition either directly or indirectly. When exogenous cholesterol was present, the effect of EB on the deposition of liver vit. A on these intact rats seemed to depend on the amount of dietary vit. A fed. At the moderate level of 150 IU daily, the EB significantly decreased their vit. A deposition; at the very high level of 1000 IU daily, EB significantly increased their vit. A deposition.

The significant negative correlations between liver deposition of vit. A and cholesterol among the castrates and rats injected with carrier oil only suggest that these components in some way may compete for storage in the liver of these animals. This is not consistently evident, however, in a comparison of means in Table I. A previous study(4) has revealed a significant positive correlation between liver vit. A and cholesterol deposition in intact male rats. This was not demonstrated in the intact animals of the present study, however.

Summary. Weanling male rats depleted of their liver vit. A stores were given the following for 21 days: 0.5% or no cholesterol, 150 or 1000 IU of vit. A acetate daily, and subcutaneous injections of 4 μg of estradiol benzoate 3 times a week in 0.1 ml of cottonseed oil or the carrier oil only. One-half of the rats were castrated at about 4 weeks of age. Castrates showed an inverse relationship between liver vit. A and cholesterol deposition. Estrogenic-treated castrates fed cholesterol responded like the intact rats with an inverse relationship between liver cholesterol deposition in dietary vit. A level. Estradiol benzoate 1) depressed the cholesterol/liver content in the intact rats fed cholesterol and the castrates fed cholesterol with the high vit. A level which may be a reflection of the effect of EB on liver weight only; 2) increased the liver vit. A concentration in castrates fed cholesterol and in intact rats fed cholesterol and the high vit. A level; 3) increased the serum cholesterol level in all groups except the intact rats fed no cholesterol and the high vit. A level.

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Growth of Rats Fed Bile Salts, Urea and Chlortetracycline.*. (30440)

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Bile acids are secreted into the gastrointestinal lumen in conjugated form and may undergo hydrolysis by microorganisms but not by endogenously produced enzymes(1,2). Feces of germfree and antibiotic treated animals have been found to contain no detectable deoxycholic acid(3,4), often the most prominent bile acid in feces of animals and man(5,6). *In vitro* incubation with deoxycholic acid causes marked damage to the intestinal villi(7) and extensive histological changes in the small intestinal mucosa of mice have been reported following 2 days of feeding on a diet containing 2% sodium deoxycholate(8). Microorganisms in the intestine liberate ammonia by urea hydrolysis and strains known to hydrolyze bile acids also produce urease(9,10). Ammonia production in the gastrointestinal tract by microorganisms may lead to toxic manifestations and is reduced with oral administration of antibacterial substances(11). The effects of varying the type or quantity of bile acids in the diet of growing animals with antibiotics in the diet have been studied(12,13). However, the authors have not found published data for growing animals following simultaneous feeding of bile salts, urea and antibacterial substances.

Methods. Weanling male rats of the Holtzman strain having initial weights of 48-60 g were fed and housed in individual cages with wire floors. Their purified basal diet calculated to meet the dietary requirements of growing albino rats(14) contained the following ingredients in per cent: vitamin free casein 14; sucrose 75; stripped lard† 5; glucose monohydrate 1.8075; dl methionine 0.18; minerals 3‡; vitamins in glucose 1§; antioxidant 0.0125.‖ Additions were made

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† Most volatile fraction from prime steam lard obtained by molecular distillation. Contains less than 5 ug of tocopherols per g of fat and fulfills fatty acid requirements of the rat. Distillation Products Industries, Rochester, N. Y.

‡ Minerals per kg of diet: $Ca(PO_4)_2$ 11.1 g; $Ca(H_2PO_4)_2$ H_2O 11.1 g; $KHCO_3$ 4.67 g; $MgCO_3$ 1.42 g; NaCl 834 g; Na_2HPO_4 549 mg; $MnSO_4$ H_2O 156 mg; $FeSO_4$ $7H_2O$ 126 mg; $ZnCO_3$ 23 ug; $CuSO_4$ 5HO 20 ug; KIO_3 253 mg; Na_2SeO_3 87.8 ug.

§ Crystalline vitamins provided per kg of diet: thiamine HCl 1.25 mg; riboflavin 2.5 mg; pyridoxine HCl 1.2 mg; niacinamide 15 mg; calcium pantothenate 8 mg; choline dihydrogen citrate 1582 mg; vit. B_{12} 5 ug; vit. A 2000 I.U.; vit E 60 I.U.

‖ Santoquin, Monsanto Chemical Co., St. Louis, Mo.