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Influence of estradiol on apparent phosphatidyl choline synthesis in rats*

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SUMMARY

Oophorectomized Sprague-Dawley rats, 28 to 30 days old, were maintained for 31 days on either a normal diet or a high fat, low protein, low choline diet, with or without vitamin B_{12}. Twice weekly injections of sesame oil or estradiol in sesame oil were administered subcutaneously. The animals kept on the high fat, low choline diet showed severe fatty metamorphosis of the liver, a decreased hepatic lecithin concentration, and a twofold increase in the relative specific activity of lecithin. Estradiol inhibited fatty metamorphosis and increased the lecithin concentration slightly. The animals receiving a vitamin B_{12}-supplemented diet and estradiol showed the greatest lipotropic effect and an increased liver lecithin concentration. Estradiol alone, or combined with vitamin B_{12}, did not correct the apparent choline deficiency, as measued by the abnormally high rate of P^{32} incorporation into lecithin. The results suggest, therefore, that the prevention of fatty livers by estradiol depends on mechanisms other than those involved in the lipotropic properties of choline or vitamin B_{12}.

Previous studies by Gyorgy and Rose (1) and Emerson *et al.* (2) have shown that the administration of an estrogenic hormone prevents the development of fatty livers and cirrhosis in animals receiving a diet deficient in lipotropic agents. Studies in this laboratory have yielded similar results. Plagge *et al.* (3) found that the lipotropic effect of administered estradiol differed from that of methionine. Estradiol decreased the degree of fatty metamorphosis produced by a high fat, low choline diet, but did not increase growth. Methionine, on the other hand, not only inhibited the development of fatty livers, but also improved the growth rate. Nevertheless, the effect of estradiol on nutritional fatty livers might be related to an enhancement of the synthesis of choline or its precursors. The beneficial effect of vitamin B_{12} in experimental nutritional fatty livers has been related to the effect of this substance on the biosynthesis of methyl groups (4), and therefore vitamin B_{12} has been considered to possess choline-sparing action.

This study was designed to determine whether estradiol has a choline-sparing action (5). Choline availability was measured indirectly by the following changes in the liver: the severity of fatty metamorphosis, the concentration of lecithin, and the rate of P^{32} incorporation into lecithin of rats kept on a normal diet or a high fat, low choline diet, with and without added vitamin B_{12}.

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METHODS

Ninety castrated female Sprague-Dawley rats, weighing 75 to 95 g (28 to 30 days old), were divided into the following groups: I. a control group fed Rockland rat chow (C.D.); II. a group fed a high fat, low protein, low choline (HFLC) diet (3); and III. a group fed the above high fat, low protein, low choline diet but supplemented with 30 μg of vitamin B_{12} per kilogram of diet (HFLC + B_{12}). In each of the above groups approximately one-half were injected subcutaneously with sesame oil (0.1 ml) and the other half with estradiol propionate (0.1 mg in 0.1 ml sesame oil).

The rats were maintained for 31 days. Radioactive phosphate was injected intraperitoneally in a dosage of 1 μc/g of body weight 4 to 6 hours prior to sacrifice.

TABLE 1. EFFECT OF ESTRADIOL ON LIVER LIPID AND LECITHIN CONCENTRATION*

Treatment	No. of Rats	Avg. Initial Weight	Avg. Final Weight	Liver		
				Weight	Lipid	Lecithin
		g	*g*	*g*	*per cent*	*per cent*
Sesame						
C.D.	18	89.2 ± 5	212 ± 12	6.70 ± 0.85	4.47 ± 0.47	1.48 ± 0.23
HFLC	15	91.5 ± 6	79.6 ± 5	4.78 ± 1.05	24.1 ± 7.7	0.81 ± 0.19
HFLC + B_{12}	16	91.5 ± 4	74.6 ± 5	2.88 ± 1.00	20.2 ± 12.3	1.18 ± 0.24
Estradiol						
C.D.	16	86.2 ± 10	138 ± 6	5.06 ± 0.43	4.27 ± 0.78	1.40 ± 0.28
HFLC	12	82.9 ± 5	70.9 ± 5	3.12 ± 0.36	9.08 ± 3.2	1.05 ± 0.13
HFLC + B_{12}	12	83.2 ± 9	63.0 ± 7	2.42 ± 0.31	4.77 ± 1.43	1.30 ± 0.33

* Means ± standard deviation.

Liver tissue was extracted (6) and the total lipids weighed. The phospholipids were isolated (7) and lecithin separated (8). Total phosphorus (9) and inorganic phosphorus (10) were determined. Radioactivity was determined with a thin window G.M. tube to a counting error less than 2%. The relative specific activity (RSA) is defined as the specific activity of lecithin divided by the specific activity of inorganic phosphorus present in the tissue at the time of sacrifice. The RSA of lecithin at 4 and 6 hours following injection of inorganic P^{32} was determined as a measure of lecithin turnover and P^{32} incorporation.

RESULTS

The data on the lipid analysis of all groups studied are summarized in Tables 1 and 2. The livers removed from group I, oophorectomized rats, were found to have a normal lipid content even with the administration of estradiol. At autopsy, the livers from animals in group II, without estradiol, showed an approximately twofold enlargement relative to body weight, and exhibited severe fatty metamorphosis. The concentration of lecithin was found to be 33% less than that of the control rats. The RSA of lecithin was almost doubled for the 4- and 6-hour intervals studied.

In the estradiol-treated rats of group II, the per cent of liver lipid was 9.1 ± 0.3%. The lecithin was significantly increased ($p < 0.001$) when compared to the animals receiving HFLC diet without estradiol. The RSA value of this group, however, was abnormally high, as was that observed in animals receiving HFLC diet without estradiol.

The rats in group III, receiving HFLC + B_{12} diets, showed extreme variability in liver lipid content, which ranged from 5% to 35% with a mean of 20.2%. The lecithin concentration was similar to that of the control group.

The lipotropic effect of estradiol on rats maintained on HFLC + B_{12} was greater than that produced by the hormone in rats receiving the HFLC diet alone. The animals of group III had a normal hepatic lipid content and concentration of lecithin. The RSA values of lecithin were abnormally high in this group, as in the other groups fed the HFLC diet.

The animals in group II, estradiol-treated rats, ate less food per day than any other HFLC diet group studied (i.e., 3.8 ± 0.5 g per rat as compared to 5.3 ± 1 g).

TABLE 2. EFFECT OF ESTRADIOL ON THE RELATIVE SPECIFIC ACTIVITY OF LIVER LECITHIN AT 4 AND 6 HOURS AFTER INJECTION OF P^{32}*

Treatment	No. of Rats	4 Hours	No. of Rats	6 Hours
Sesame				
C.D.	10	0.64 ± 0.14	8	0.86 ± 0.30
HFLC	7	1.20 ± 0.08	8	1.54 ± 0.25
HFLC + B_{12}	8	1.15 ± 0.30	8	1.45 ± 0.50
Estradiol				
C.D.	9	0.67 ± 0.16	7	0.88 ± 0.36
HFLC	7	1.18 ± 0.21	5	1.38 ± 0.13
HFLC + B_{12}	5	1.13 ± 0.26	7	1.33 ± 0.32

* Means ± standard deviation.

DISCUSSION

The results of the present study indicate that the effects of a high fat, low protein, choline-deficient diet administered to the rat are similar to those in the dog, as reported recently by Di Luzio and Zilversmit (5). Both studies showed that in animals with fatty livers produced by this regimen there is an abnormally high rate of P^{32} incorporation into the phosphatidyl choline fraction, and a significant decrease in the hepatic concentration of lecithin. Artom (11) has suggested that livers of animals on diets deficient in protein and choline still have sufficient endogenous choline and

ethanolamine to show a compensatory increase in the rate of phospholipid synthesis.

Evidently the results of chronic administration of estradiol, unlike those results reported for chronic choline administration (5), produced a lipotropic effect without decreasing the high rate of phosphatide turnover. This is interpreted to mean that although estrogen appears to be lipotropic, it apparently does not influence the synthesis or availability of choline nor its methyl precursors sufficiently to correct the abnormally high rate of lecithin metabolism.

The lipotropic effect of estradiol was significantly increased by the presence of vitamin B_{12} supplement in the diet. This additive effect is analogous to that observed with the addition of small quantities of methionine to animals receiving estradiol and the HFLC diet (3).

Estradiol inhibits nutritional fatty metamorphosis as does choline (5) and vitamin B_{12} (12). The vitamin B_{12} administered to choline-deficient animals appears to reduce the requirement for choline through the synthesis of methyl groups (4). In this study the concentration of lecithin in livers was elevated toward normal by the presence of vitamin B_{12} in the diet without demonstrating a consistent effect on total lipids of the liver. The lipotropic action of vitamin B_{12} was dependent upon the fat composition of the diet, since it has been reported that on a high fat diet, vitamin B_{12} has no lipotropic action (12), whereas it is effective on a low fat diet (13). No significant effect was observed in this study using a diet containing 40% fat. It also has been demonstrated that the ability of the liver to store the vitamin is decreased in animals with nutritional liver disease (14).

Estradiol, vitamin B_{12}, and choline have been observed to increase the lecithin concentration of the liver in animals fed a HFLC diet (Table 3). Yet only choline reduces the apparent high rate of phospholipid synthesis characteristic of choline-deficient animals. In this study vitamin B_{12} and estradiol were equally effective in altering the RSA and were equally effective in increasing the lecithin content of the liver; however, B_{12} was far less effective than estradiol in "normalizing" liver lipid concentrations. Furthermore, estradiol apparently inhibited weight gain in animals consuming a normal and a HFLC diet, while choline and B_{12} have been reported to reverse the weight loss produced by diets deficient in protein and choline (15). The relationship of growth inhibition to lipotropism has been previously reviewed (16). These differences suggest that the mechanism of the lipotropic action of estradiol differs from that of choline and vitamin B_{12}.

TABLE 3. COMPARISON OF LIPOTROPIC EFFECTS OF ESTRADIOL, VITAMIN B_{12}, AND CHOLINE, BASED ON FOUR DIFFERENT PARAMETERS

Effects	Estradiol	B_{12}	Choline
Decreased total liver fat	+	+*	+
Increased lecithin content	+	+	+
Decreased abnormally high RSA	−	−	+
Reversed weight loss as produced by low protein diets†	−	+	+

* References 12, 13.
† Reference 16.

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