Effect of Oral Contraceptive Hormones on Zinc Metabolism in the Rat (35617)

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(Introduced by M. R. S. Fox)

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In 1961 Johnson (1) reported that plasma zinc is depressed by pregnancy. In 1968 results were published from this laboratory which indicated that both in pregnancy and in women taking oral contraceptives low plasma zinc concentrations occurred (2). Ten women receiving oral contraceptives for 1–48 months had a plasma zinc concentration of 69 ± 8 μg/100 ml[1] compared to 96 ± 13 μg/100 ml for control subjects. This is statistically significant ($p<0.001$). A preliminary study conducted in this laboratory showed that the combination of an estrogen, mestranol[2] (ethinyl estradiol 3-methyl ether), and a progestational agent, norethindrone[3] (17α-ethinyl-17-hydroxy-4-estren-3-one), depressed plasma zinc in rats. Eight female 200-g rats consuming a control egg white diet containing 20 ppm zinc had a mean plasma zinc content of 179 ± 32 μg/100 ml; whereas 8 other rats consuming a combination of 0.07 mg of norethindrone and 0.003 mg of mestranol in the control diet showed a mean plasma zinc content of 139 ± 15 μg/ml. This is statistically significant ($p<0.01$).

There are no other published reports to our knowledge concerning the effect of oral contraceptive hormones on zinc metabolism. Which hormone(s) is responsible for the lowered plasma zinc concentration and in what other tissue(s) there is an alteration in zinc concentration are questions which we have attempted to answer in this study. We report here the effects of an estrogen (mestranol), a progestational agent (norethindrone), and a combination of these hormones on plasma zinc concentration, growth, zinc-65 distribution, and tissue weights in female rats.

Materials and Methods. Twenty female Sprague-Dawley rats 21 days of age were maintained for a 7-day adjustment period on a control basal diet (Table I) containing 25 ppm zinc. Extreme precautions were taken to eliminate zinc contamination. The rats were housed individually in suspended stainless steel cages and offered deionized water *ad libitum*. Body weights were obtained every 4 days and food intake was determined daily throughout the study.

The rats were then allotted by weight into four groups of five rats each. They were fed the following diets: (a) Control diet, (b) Control diet containing norethindrone, (c) Control diet containing mestranol, and (d) Control diet containing norethindrone and mestranol simulating Ortho-Novum.[4] The animals in group b received 0.5 μg norethindrone/g body wt/day; those in group c received 0.02 μg mestranol/g of body wt/day, and those in group d received a combination of the two hormones at these doses. On a comparative body weight basis,[5] the quantity of norethindrone and mestranol consumed by the rats was, respectively, 12.5 and 10 times the amount in the 2.0-mg daily dose of Ortho-Novum taken by a 55-kg woman. As the weight of the animals and the food consumption increased the quantity of hormone(s) in the diet increased in order to

[1] Mean ± standard deviation.

[2] Mestranol, Syntex, Palo Alta, Calif.

[3] Norlutin, Parke Davis, Detroit, Mich.

[4] Two milligrams norethindrone and 0.10 mg mestranol, Ortho Pharmaceutical Division, Raritan, N.J.

[5] $$\frac{\text{55 kg woman}}{\text{2.0 mg norethindrone}} = \frac{\text{rat wt (g)}}{X \text{ mg norethindrone}}.$$
The daily quantity of diet consumed by the rats contained 12 X mg of norethindrone and 10 X mg mestranol.

TABLE I. Composition of Basal Diet.[a]

Ingredient	%
Dried egg white[b]	20.0
Casein hydrolysate (acid, salt free)[b]	3.0
Sucrose	64.9
Coconut oil[c]	5.0
Salts (Fox-Briggs, zinc free)[d]	6.0
Trace element supplement[e]	0.1
Vitamins[f]	1.0
Ethoxyquin 4%[g]	0.5

[a] 24.8 ± 0.8 ppm zinc (mean ± SD) as determined by analyses on several aliquots.

[b] Obtained from General Biochemicals, Chagrin Falls, Ohio.

[c] Obtained from Meade Johnson Laboratories, Evansville, Ind. Lipid fraction consisting primarily of the triglycerides of the C_8 and C_{10} saturated fatty acids.

[d] Provided in diet (mg/kg): $CaCO_3$, 10,000; $CaHPO_4$, 28,400; $CuSO_4$, 7; $FeC_6H_5O_7 \cdot 5H_2O$, 200; $MgSO_4$, 3000; $MnSO_4 \cdot H_2O$, 250; KCl, 7000; KIO_3, 10; NaCl, 4000; Na_2HPO_4, 7000.

[e] Provided in diet (mg/kg): $Co(C_2H_3O_2)_2 \cdot 4H_2O$, 21.2; $Na_2MoO_4 \cdot 2H_2O$, 5.0; Na_2SeO_3, 0.3; NaF, 44.2; $CrCl_3 \cdot 6H_2O$, 10.2; $NiCl_2 \cdot 6H_2O$, 4.0; $Na_2B_4O_7$, 23.3; $Na_2HAsO_4 \cdot 7H_2O$, 1.3; $ZnCO_3$, 34.4.

[f] Provided in diet (mg/kg): thiamin · HCl, 50; riboflavin, 25; pyridoxine · HCl, 30; calcium pantothenate, 100; niacin, 200; Menadione, 50; folic acid, 20; biotin, 5; vitamin B_{12}, .05; choline dihydrogen citrate, 5000; *i*-inositol, 1000; α-tocopherol acetate, 200; ascorbic acid, 1000; *p*-aminobenzoic acid, 100; and (in micrograms) vitamin A, 6900; and vitamin D_2, 100.

[g] Dissolved in ethanol.

maintain the same hormone dosage per day per gram of body weight throughout the study.

After 110 days on this dietary regimen, the rats were lightly anesthetized with ether and were injected via the saphenous vein with 10 μCi $^{65}ZnCl_2$[6] in 0.5 ml physiological saline. They were placed in stainless steel metabolic cages for a 24-hr collection of urine and feces. The animals were then sacrificed by exsanguination under ether anesthesia. Gross observations were made at autopsy and the liver, spleen, adrenals, ovaries, uterus, pancreas, and femur were removed.

[6] New England Nuclear Corp., Boston, Mass. $ZnCl_2$ in 2 *N* HCl, 2.47 mCi/mg.

The uptake of zinc-65 in each organ and the femur was determined by counting the samples in a well-type crystal scintillation counter. The injection standards were counted along with the samples to eliminate the necessity for decay calculations. The zinc-65 uptake was calculated as the percentage of dose per gram of fresh tissue. Zinc concentrations in the plasma were determined by atomic absorption spectrophotometry (3). Differences in means were tested for significance by Student's *t* test (4).

Results. Gross observations at autopsy appeared normal except for the accumulation of adipose tissue in those rats given mestranol.

Mestranol affected the growth of the rats. After 110 days the control animals and those consuming norethindrone attained a mean net gain of 213 ± 38 and 218 ± 27 g, respectively. Those consuming mestranol and the combination of hormones gained 168 ± 11 and 165 ± 24 g, respectively (Fig. 1). The decreased growth in the latter two groups was accompanied by a decreased food consumption. The mean daily food intake for those rats in the control and norethindrone groups was 15 g; whereas for those fed mestranol and the combination of hormones it was 12 g. The mean food efficiency ratio[7] from Day 1 until Day 35, the major growth period for the rats, was not significantly different among groups. For this 35-day period a daily intake of 3.6 ± 2.4 g of diet resulted in a daily weight gain of 1.0 g in those rats in the control group. The mean food efficiency ratio for the norethindrone, mestranol, and norethindrone–mestranol groups was 3.3 ± 1.7, 3.3 ± 1.8, and 3.8 ± 2.7, respectively.

The rats consuming mestranol had a significantly lower plasma zinc concentration ($p<0.02$) compared to the control group (Table II). In contrast, norethindrone did not significantly decrease plasma zinc. The combination of the hormones resulted in somewhat lowered mean plasma zinc levels; however, this value was not significantly different from the control group. It is doubtful that the lowered daily food intake in the case of

[7] Food efficiency ratio: Average daily food consumption (g) divided by average daily weight gain of the animals (g).

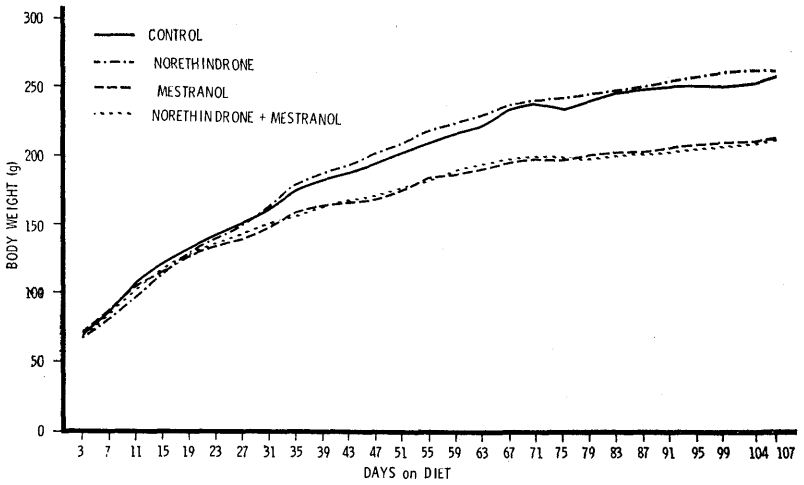

FIG. 1. Growth curves of female rats fed oral contraceptive hormones showing a growth depression in those animals consuming mestranol.

the mestranol group contributes to the lowered plasma zinc as 12 g is not a limiting dietary intake but rather the normal average daily food intake for mature female rats (5) and 25 ppm of dietary zinc is more than sufficient for optimal growth (6).

A fresh weight was determined on the selected tissues. There was no significant difference in the mean fresh weights as related to body weight among groups except for the femur. The rats receiving mestranol had significantly ($p<0.01$) heavier femurs (0.32 ± 0.02% of body wt and 0.32 ± 0.01% for groups c and d, respectively, compared with 0.28 ± 0.02% for the control group). Norethindrone had no significant effect on the weight of the femur.

TABLE II. Effect of Oral Contraceptive Hormones on Plasma Zinc Concentration in Female Rats.

Hormone	Plasma zinc (μg/100 ml)
None	181 ± 23[a]
Norethindrone[b]	169 ± 19[c]
Mestranol[d]	149 ± 7.5[e]
Norethindrone[b] plus mestranol[d]	157 ± 21[e]

[a] Values represent means ± SD of groups of five rats.

[b] 0.50 μg/g body wt/day.

[c] Not significant in comparison to the control group.

[d] 0.02 μg/g body wt/day.

[e] $p < 0.02$ in comparison to the control group.

Zinc-65 retention in the tissues studied is presented in Table III. There was a significantly greater uptake of zinc-65 in the liver of rats fed mestranol ($p<0.02$) and the combination of hormones ($p<0.01$). Mestranol-treated rats also had a significantly increased uptake of zinc-65 in the spleen, adrenals and uterus. There was no effect of the hormones on zinc-65 retention in any of the other tissues. Hormone supplementation did not significantly affect the excretion of zinc-65 in the feces and urine.

Discussion. Hormones, other than mestranol and norethindrone, have been studied in relation to zinc metabolism. Human chorionic gonadotrophin, luteinizing hormone, prolactin in the presence of follicle-stimulating hormone (FSH), and FSH alone have been reported to increase the zinc-65 uptake in specific male reproductive and non-reproductive tissues (7). Plasma zinc concentration was not reported. It is beyond the scope of this investigation to determine the mechanism of the effect of mestranol on zinc metabolism. However, it has been reported that mestranol in animal studies inhibits pituitary gonadotrophins (8). Gunn and Gould (9) reported a decreased zinc concentration and a decreased zinc-65 uptake in the dorsolateral prostate, a high zinc-containing tissue, following hypophysectomy in rats. Treat-

TABLE III. Effect of Oral Contraceptive Hormones on Zinc-65 Uptake in Female Rat Tissues.

Hormone	Mean values for % of dose/g of fresh tissue						
	Liver	Spleen	Adrenals	Ovaries	Uterus	Pancreas	Femur
None	.4725 ± .0663[a]	.5588 ± .0565	.4395 ± .0346	.5663 ± .0994	.3525 ± .0264	.6104 ± .1705	.2504 ± .0648
Norethindrone	.5539 ± .0678	.5862 ± .0734	.4798 ± .0435	.5086 ± .0670	.4092 ± .0678	.5395 ± .0100	.2510 ± .0173
Mestranol	.5876 ± .0556[b]	.6653 ± .0458[c]	.5441 ± .0927[d]	.6400 ± .1276	.4547 ± .0538[c]	.6334 ± .0812	.2906 ± .0447
Norethindrone and mestranol	.6612 ± .0921[c]	.7217 ± .1545	.6299 ± .2551	.6231 ± .0916	.4327 ± .0830	.7578 ± .1072	.2648 ± .0173

[a] Values represent means ± SD of groups of five rats.
[b] $p < 0.02$.
[c] $p < 0.01$.
[d] $p < 0.05$.

ment with gonadotrophin increased the uptake of zinc-65 in the dorsolateral prostate. Based on the above information one may speculate that the lowered plasma zinc concentration and the change in zinc-65 tissue distribution in those animals receiving mestranol might be in response to a decrease in pituitary gonadotrophin. The increased zinc-65 uptake in selective tissues in this study where pituitary gonadotropic hormones are suppressed (8) does not agree with Rosoff *et al.* (6) who found an increased tissue uptake of zinc-65 in the presence, not absence, of pituitary gonadotropic hormones.

It is of interest that mestranol, the estrogen component of the oral contraceptive, is associated with the change in plasma zinc concentration and distribution of zinc-65 in various tissues since it has also been implicated in many of the side effects and metabolic changes in women taking oral contraceptives (10). Further work on the effects of specific estrogen and progestational agents given singly is required to determine if estrogen alone is influential in the many metabolic changes which have been reported to occur with the intake of oral contraceptives. Increased serum concentrations of iron, thyroxin, growth hormone and insulin, raised serum transaminase levels, decreased serum and urine magnesium, increased levels of specific blood coagulation factors, and a disturbance of tryptophan metabolism (10) have all been implicated as effects of the oral contraceptives.

Summary. The effects of an estrogen (mestranol), a progestational agent (norethindrone), and a combination of the two hormones on plasma zinc concentration, growth, and zinc-65 uptake in selected tissues were investigated in female rats fed a zinc-sufficient diet. Mestranol significantly ($p<0.02$) lowered plasma zinc concentration whereas norethindrone had no effect. Mestranol caused a significant increase in the uptake of a single injected dose of zinc-65 in the liver, spleen, adrenals, and uterus. Mestranol also caused a significant decrease in the growth.

The authors gratefully acknowledge Dr. M. R. Spivey Fox, Food and Drug Administration, for the

critical evaluation of this manuscript, Dr. J. Taylor, National Institutes of Health, for contributing the hormones, and Mr. Frank Sasse for his technical assistance.

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Received Dec. 8, 1970. P.S.E.B.M., 1971, Vol. 137.