all. Normally nipples are never found in the males of our colony and do not appear in the female until the 2nd to 4th day post partum.

In the female offspring the uteri are large and distended, the ovarian capsule does not develop and the gonads are bare.

Examination of 6 animals by serial sections has revealed changes similar to those obtained with the natural estrogens. In the males, prostates are absent, seminal vesicles, epididymides and vasa deferentia are inhibited and portions of the uteri are present. The upper (Müllerian) vagina is well developed; the lower vagina (that part which is derived from the urogenital sinus) is partially developed. In the females there is some persistence of the Wolffian ducts (cranial and caudal remnants) and some inhibition of the lower vagina.

From these results it is apparent that compounds other than the natural estrogens are capable of modifying embryonic sexual development in the rat.

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Growth-Stimulating Action of Ferric Chloride-Treated Wheat Germ Oil.*

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Several investigators have reported the existence of a growth-promoting factor, necessary for rats, that was either vitamin E or a substance closely related to it.[1-4] The pure substance, α-tocopherol, stimulates growth in rats which have plateaued in weight on a vitamin E-low diet.[5] The possibility that more than one factor might possess this growth-stimulating action was shown by Martin,[6] who

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1 Evans, H. M., *J. Nutr.*, 1928, **1**, 23.

2 Blumberg, H., *J. Biol. Chem.*, 1935, **108**, 227.

3 Olcott, H. S., and Mattill, H. A., *J. Biol. Chem.*, 1936, **114**, lxxvii.

4 Olcott, H. S., and Mattill, H. A., *J. Nutr.*, 1937, **14**, 305.

5 Emerson, G. A., and Evans, H. M., *J. Nutr.*, 1937, **14**, 169.

6 Martin, G., *J. Nutr.*, 1937, **13**, 679.

was apparently able to separate the growth-promoting factor from the fertility-restoring factor. He suggested, however, that the apparent difference might be quantitative.

Young rats, suckled by mothers with minimal stores of vitamin E, usually develop a characteristic muscular dystrophy at the end of the lactation period. Goettsch and Ritzmann[7] have found that protection against the dystrophy was afforded by wheat germ oil extracted from a wheat germ from which the antisterility vitamin had been inactivated by $FeCl_3$, and by α-tocopherol. They concluded that the activity of a preparation in preventing muscular dystrophy did not necessarily correspond to its antisterility potency.

Experimental. The results reported deal with the growth-stimulating action of $FeCl_3$ treated wheat germ oil as compared with the original oil. The oils were fed as supplements to female rats of the Long-Evans stock that had plateaued in weight on a vitamin E-free regimen.

The wheat germ oil was treated with a 1% ether solution of $FeCl_3$, according to the method of Waddell and Steenbock.[8] One hundred cc of the oil was shaken for 1 hour in an open flask with 100 cc of a 1% ether solution of $FeCl_3$ at about 50°C; the 2 solutions were thenceforth left together. Residual traces of solvent were removed *in vacuo*. The treated oil was less viscous than the original oil and had a sharp odor. It was kept at room temperature.

Forty-two female rats were placed at 21 days of age on the standard E-low diet 427.[5] They plateaued at an average weight of 232 g after approximately 120 days on the diet. At 145 days of age they were divided into 3 groups of like average weights, as follows:

Group 1—no supplement (controls).

Group 2—80 mg wheat germ oil 6 times weekly.

Group 3—80 mg $FeCl_3$ treated wheat germ oil 6 times weekly.

After receiving the supplements for 50 days the average gains in the wheat germ oil and the $FeCl_3$ treated wheat germ oil groups were approximately the same (Table I).

TABLE I.

Growth Responses of Rats Maintained on a Vitamin E-Low Diet to Supplements of Wheat Germ Oil and $FeCl_3$ Treated Wheat Germ Oil.

Treatment 6 times weekly	No. of rats	Days Supplemented	Avg gain in wt, g
None (controls)	12	145-195	2
80 mg wheat germ oil	15	"	30
80 mg $FeCl_3$ treated wheat germ oil	15	"	28

[7] Goettsch, M., and Ritzmann, J., *J. Nutr.*, in press.

[8] Waddell, J., and Steenbock, H., *J. Biol. Chem.*, 1928, **80**, 431.

TABLE II.
Vitamin E Activity of Wheat Germ Oil and $FeCl_3$ Treated Wheat Germ Oil.

Material	Level fed, g	No. of rats fed	No. of litters	% littering	Avg No. living young per litter	Avg wt, g	Dead young
Wheat germ oil	0.5	6	6	100	8.0	5.6	1
Same oil treated with $FeCl_3$	2.0	4	0	0	0	0	0
Same oil treated with $FeCl_3$	4.5	7	2	29	3.0	4.2	2

These findings have 2 possible interpretations: that more than one substance possesses growth-stimulating activity or that an amount of vitamin E sufficient for growth in the quantity fed, remained in the oil. If the latter were the case, the amount of the untreated oil fed was then in excess of the requirement.

The $FeCl_3$ treated wheat germ oil had been assayed for vitamin E activity in a single dose of 2.0 g and was found completely inactive although the original oil enabled 6 out of 6 rats to bear living young when fed at a single dose of 0.5 g. It appeared advisable to assay the $FeCl_3$ treated wheat germ oil at an even higher level, namely, at 4.5 g. One-half the oil was given on the first day of the test gestation and the remainder on the second day. Undersized litters of underweight young resulted in 2 out of 7 pregnancies (Table II).

Thus it would appear that the treated oil retained a small percentage of its original vitamin E activity. The results obtained do not permit a quantitative comparison, but it would appear that about one-tenth of the original vitamin E activity of the oil remained in the treated preparation. The rats tested for the growth-promoting properties of the $FeCl_3$ treated oil were therefore receiving only the equivalent of approximately 8 mg of the original oil in terms of its vitamin E activity. If the assumption is made that 500 mg of wheat germ oil is equivalent to 3 mg of α-tocopherol, then the quantity of vitamin E fed in 80 mg of the treated oil would be the equivalent of 0.05 mg of α-tocopherol.

The possibility that more than one substance possesses growth-stimulating activity is not ruled out. It would appear that the feeding of low levels of α-tocopherol will either enable us to establish a low quantitative need for this substance for normal late growth or let us refer the favorable effects of $FeCl_3$ treated wheat germ oil to some constituent other than vitamin E.

Summary. $FeCl_3$ treated wheat germ oil containing approximately one-tenth of the E activity of the original oil stimulated growth when fed at a level of 80 mg six times weekly to female rats which had plateaued in weight on a vitamin E-low diet.