EFFECTS OF OVARIAN HORMONES UPON UTERINE PIGMENTATION IN VITAMIN E-DEFICIENT RATS*

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Acid-fast pigmentation of the skeletal and cardiac musculature and the smooth muscle of the reproductive tract is now recognized as being characteristic of vitamin E deficiency in the rat. The chocolate-brown discoloration of the uterus was first noted in the E-deficient rat by Martin and Moore.[1] Subsequent histological studies demonstrated that pigment was deposited in fine granules in the cells of the uterine musculature.[2, 3] Mason and Emmel[4] further noted the presence of numerous pigment-laden macrophages scattered throughout the uterus of the E-deficient rat. Their findings indicated that the pigment was deposited first in the muscle cells and later transferred to the macrophages.

The rôle of the gonads in the regulation of the functional activity of the musculature of the reproductive tract, particularly that of the uterus, has suggested the possibility of a physiological relationship between the gonadal hormones and vitamin E. Mason and Emmel[4] did not observe any diminution in muscle pigmentation in prepuberally ovariectomized animals as compared with intact controls. Ovariectomy, however, was followed by a decrease in the number of pigment-containing macrophages appearing during the course of the avitaminosis. More recently,[5] it has been shown that a definite decrease in muscle pigment occurs when ovariectomized rats are maintained on a diet containing a lower percentage of fat than the ration used by Mason and Emmel.

The present experiments were undertaken to ascertain the effect of ovarian hormone treatment upon the deposition of uterine pigment in ovariectomized E-deficient rats maintained on a relatively low unsaturated fat intake.

Materials and Methods

Fifty-two female rats of the "Sherman" strain were used in these experiments. Most of the animals were born of mothers maintained on a vitamin-E-deficient simplified diet (TABLE 1) supplemented with 3 mg. of dl-alpha-tocopherol acetate per 100 gm. of ration.§ A few of the animals were born of mothers maintained on the deficient diet alone. The experimental rats were weaned at 3 to 4 weeks of age. The majority were immediately placed on the E-deficient diet. A few animals, however, were not placed on the diet until several weeks after weaning. The E-deficient ration limited the daily intake of alpha-tocopherol to approximately 30 μg. per rat.

The young females were divided into several groups for subsequent study.

* Aided by grants from the Williams-Waterman Fund of the Research Corporation and the United States Public Health Service.

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‡ With the technical assistance of R. E. Johnson and A. Fuhr.

§ The alpha tocopherol acetate and other synthetic vitamins were supplied through the courtesy of Dr. Leo A. Pirk of Hoffman-La Roche, Inc.

Forty-one were bilaterally ovariectomized at 19 to 34 days of age. Weekly subcutaneous injections of the following substances were begun immediately: (a) 0.2 cc. of sesame oil—7 rats; (b) 5 μg. of estradiol*—7 rats, 10 μg. of estradiol—7 rats; (c) 5 μg. of estradiol and 4 mg. of progesterone—2 rats, 10 μg. of estradiol and 4 mg. of progesterone—9 rats; (d) 4 mg. of progesterone—9 rats. Crystalline hormones* were used, the weekly dose being dissolved in 0.2 cc. of sesame oil. The remaining 11 rats were not ovariectomized, but were injected with 0.2 cc. of sesame oil weekly. All animals were maintained on the E-deficient diet and the weekly injections were continued for a period of from 5 to 10 months, at which time the animals were sacrificed.

TABLE 1

COMPOSITION OF THE TOCOPHEROL-DEFICIENT DIET USED

Basal Mixture	*Per Cent*
Lard	10
Casein (Bordon's crude #453)	30
Cerelose	54
Celluration	2
Salt Mixture (Hawk Oser)	4
Supplements to Basal Mixture	*mg./kg.*
Thiamine Chloride	2
Riboflavin	4
Pyridoxine	4
Nicotinic Acid	100
Choline	1000
Vitamin K	4
p-amino-benzoic Acid	300
Ca Pantothenate	10
Oleum Percomorphum (ml./kg.)	0.2

The rats were autopsied promptly after sacrifice, and the uteri were fixed in Bouin's fluid. Tissue specimens were dehydrated in ethyl alcohol, cleared in xylene, and embedded in paraffin. Sections were cut 7 microns in thickness and were stained with hematoxylin and the Kinyoun modification of the Ziehl-Nelsen carbol fuchsin method to ascertain the presence and distribution of acid-fast pigment.

Observations

Histological examination of the uteri from the unspayed rats treated with sesame oil alone revealed the presence of numerous strongly acid-fast granules throughout the cytoplasm of the myometrial cells. There was also a considerable number of pigment-containing macrophages scattered throughout the intramuscular connective tissue and, to a lesser extent, the endometrial stroma. No evidence of pigment deposition in the epithelial elements

* The alpha estradiol was supplied through the courtesy of Dr. Kenneth W. Thompson of Organon, Inc., the progesterone by Dr. F. E. Houghton of Ciba Pharmaceutical Products, Inc.

of the endometrium was discernible. These findings are characteristic of the intact E-deficient rat.

In the ovariectomized animals injected with sesame oil, on the other hand, there was complete absence of pigment in the longitudinal layer of the myometrium. The circular layer of muscle was peculiar in that the cells were filled with small granules which, unlike those in the intact animal, possessed but negligible to weak acid-fastness. In addition to these changes in muscle pigmentation, there was also a great reduction in the number of pigment-containing macrophages.

In 13 of the 16 animals treated with estrogen, acid-fast pigment was present in the same distribution seen in the uteri of the unoperated controls. The intensity of staining, however, was somewhat diminished. There was no discernible difference between the animals which had received 5 μg. of estradiol and those which had received 10 μg. of the hormone weekly. In 3 rats, there was but negligible pigment deposition. It is interesting to note that 2 of these animals had not been placed on the E-deficient diet until 2 to 3 weeks after weaning.

In the rats treated with progesterone, the amount and distribution of acid-fast pigment was not materially different from that in the castrates injected with sesame oil.

The results of treatment with estrogen and progesterone together are less clear-cut than in the preceding groups. In general, the distribution of pigment is similar to that seen in the animals receiving estrogen alone. However, in about half the animals receiving both hormones, there is a considerable reduction in the pigment of the longitudinal musculature and a decrease in the number of pigment-containing macrophages.

Discussion

The present observations clearly demonstrate that prepuberal ovariectomy results in a decreased accumulation of acid-fast pigment in the uterus of vitamin E-deficient rats maintained on a diet relatively low in unsaturated fats. Prolonged treatment with estrogen promotes uterine pigmentation in the ovariectomized animal, whereas progesterone alone does not have this effect. In fact, progesterone given concurrently with estrogen seems partially to neutralize the effect elicited by estrogen alone.

Evidence has been presented which indicates that the deposition of pigment in the E-deficient animal represents the peroxidation and polymerization of unsaturated fatty acids due to the decrease in tissue tocopherols which act as antioxidants.[6, 7] Whether or not this represents the complete mechanism of pigment accumulation, it seems inescapable, from the present observations, that the effects of ovarian hormones on uterine pigmentation are mediated through their regulatory function in the metabolic processes of the tissues of the reproductive tract.

The well-known effects of the ovarian hormones on the morphology, contractility, and respiration of the myometrium indicate a profound metabolic influence. It may be surmised that the diminution of various physiological processes which accompanied ovariectomy is reflected in the decreased rate

of pigment formation. Conversely, the increased pigmentation in the uterus of the estrogen-treated castrate may be related to its concommitant increase in metabolic activity. Progesterone alone has but negligible effects on uterine activity and, under certain conditions, may act as an antagonist to estrogen. Here, again, the parallelism between the effects of hormonal influence on uterine metabolism and pigmentation is apparent.

An alternate explanation of the present observations might lie in the hypothesis that estrogen plays a direct rôle in the lipid metabolism of the myometrium. In any case, there is a paucity of information relating to the interaction of ovarian hormones and tocopherol in tissue metabolism. Further studies must be made before a definitive solution to the problem can be attained.

Summary

1. Rats ovariectomized at weaning and maintained 5 to 10 months on a vitamin E-deficient diet, relatively low in unsaturated fat, did not develop the acid-fast pigmentation of the uterine muscle characteristic of the intact E-deficient controls.

2. Ovariectomized rats treated with estrogen during the course of the avitaminosis developed uterine pigmentation similar to that seen in intact E-deficient animals.

3. Treatment of ovariectomized E-deficient rats with progesterone did not promote pigment deposition.

4. Progesterone given to E-deficient ovariectomized rats concurrently with estrogen may partially neutralize the pigmentation-promoting effect of the latter hormone.

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