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Estrogen Response and Pigmentation of the Uterus in Vit. E-Deficient Rat.*

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Several investigations have been made concerning the possibility that a relationship exists between the biological action of estrogen and vitamin E.[1-3] Although some of these experiments have suggested an "activating" effect of tocopherol on estrogen, the data have not been conclusive. A related problem, that of the role of the ovaries in the development of the characteristic pigmentation of the uterus during vitamin E deficiency, also remains unsettled. Therefore the present experiments were designed to determine the effect of alpha-tocopherol deficiency on the uterine response to estrogen and the effect of ovariectomy on the development of uterine pigmentation in vitamin E-deficient rats.

Materials. The animals used in the present experiments were obtained in the following manner. Female rats of the "Sherman" strain were reared from weaning on Rockland rat diet. Upon reaching 2-3 months of age they were placed on a vitamin E-deficient diet (Table I) which limited the daily intake of alpha tocopherol to approximately 30 μg per rat.† Within one week after they had been placed on the deficient diet, the animals were mated with non-deficient males. The tocopherol-deficient offspring were used in the present experiments as indicated below. After weaning of the young, the mothers were returned to the Rockland diet for 3 weeks and the same breeding procedure was then repeated.

Within 3 days after birth the young were pooled and 6 females were given to each of several mothers. These young were thereafter considered as "litter-mates". Between 3 and 4 weeks of age they were weaned and ovariectomized. Thirty-nine animals were continued on the vitamin E-deficient diet and 48 were placed on the same ration supplemented with 3 mg of synthetic dl alpha-tocopherol acetate per 100 g diet.‡ Eight unspayed animals were kept on the deficient diet as controls.

The uterine response to estrogen was determined with crystalline alpha-estradiol benzoate as indicated below.§ The hormone was dissolved in sesame oil which was freed of tocopherol by previous treatment with ferric chloride.

For histological examination the uteri were fixed in Bouin's fluid within 10 minutes after the animals were killed. Tissue specimens were dehydrated in ethyl alcohol, embedded in paraffin and sections were cut 7 microns in thickness. Parallel sections were stained with hematoxylin and eosin and with carbol-fuchsin to determine the presence and dis-

* Aided by a grant from the Williams-Waterman Fund of the Research Corporation.

1 Spoto, Pompeo, *Z. Vitaminforschg.*, 1940, **10**, 235.

2 Beerstecher, E., Jr., *Endocrinology*, 1941, **28**, 344.

3 Tobin, Charles E., and Birnbaum, Jean P., *Arch. Pathol.*, 1947, **44**, 269.

† About one-half of the determinations of the tocopherol content of the basic diet were carried out according to the method of Kaunitz and Beaver.[6] For the remaining tests we are greatly indebted to Drs. Philip L. Harris and Mary L. Quaife of the Distillation Products, Inc.

6 Kaunitz, H., and Beaver, J. J., *J. Biol. Chem.*, 1944, **156**, 653.

‡ The alpha tocopherol acetate and the other synthetic vitamins were supplied through the courtesy of Dr. Leo A. Pirk of Hoffmann-LaRoche, Inc.

§ The alpha estradiol benzoate was supplied through the courtesy of Dr. Kenneth W. Thompson of Roche-Organon, Inc.

TABLE I.
Composition of the Tocopherol-deficient Diet Used.

Basal mixture	%	Supplements to basal mixture	mg/kg
Lard	10	Thiamine chloride	2
Casein (Borden's crude) No. 453	30	Riboflavin	4
Cerelose	54	Pyridoxine	4
Celluration	2	Nicotinic acid	100
Salt mixture (Hawk Oser)	4	Choline	1000
		Vit. K	4
		Para-amino-benzoic acid	300
		Ca pantothenate	10
		Percomorphum oil (ml/kg)	0.2

tribution of acidfast pigment.

Experiments and observations. 1. Uterine response to estrogen. The uterine weights of deficient and non-deficient ovariectomized animals after the administration of alpha-estradiol benzoate are given in Fig. 1. In

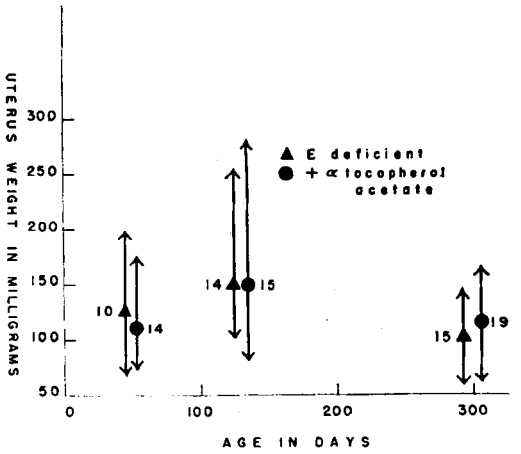

FIG. 1.

Effect of alpha estradiol benzoate upon the uterine weight of spayed rats on a vitamin E-deficient and a tocopherol-supplemented purified diet. The triangles and circles indicate average uterine weights; the figures adjacent to the triangles and circles indicate the number of animals used in the respective series.

the age group of 40-50 days, 1 μg of hormone dissolved in 0.2 cc of sesame oil was injected subcutaneously 48 hours before sacrifice. In the groups 120-137 and 280-309 days of age, a total of 4 μg of the estrogen, divided in successive daily doses of 1 μg in 0.2 cc of oil, was injected. The final dose was given 48 hours before sacrifice. Several of the deficient and tocopherol-supplemented animals in the 120 day group had received a total of 11 μg of estradiol benzoate between the 43rd and 78th day. Since their uterine response was similar to that of the other animals in this age group, they have been included in the final observations.

There is no significant difference in the uterine response to estrogen between the tocopherol deficient and tocopherol-supplemented animals in the same age group. There is, however, a difference in the response between animals in the 120-137 day and the 280-309 day groups, irrespective to tocopherol administration. Statistical evaluation of the mean uterine weights of these two groups yielded a probability factor of 3.7 indicating that the response of the 4-month-old rat to estrogen is significantly greater than that of the 9-10-month-old animal.

2. Uterine pigmentation. As reported by previous investigators, a brownish pigmentation of the uterus was macroscopically discernible in the non-spayed rats maintained on the tocopherol-deficient diet for approximately 300 days. In sharp contrast, there was no evidence of pigment deposition in the majority of the spayed animals fed the same diet for the same period of time. As would be expected, no pigmentation occurred in either spayed or non-spayed animals receiving the tocopherol-supplemented diet.

Histological examination of the uteri of 8 unspayed deficient animals revealed that the muscle cells of the myometrium were filled with numerous uniformly small acid-fast pigment granules. In addition, a considerable number of pigment-containing cells were scattered throughout the intermuscular connective tissue and, to a lesser extent, the endometrial stroma. These cells, presumably macrophages, varied in size and shape, but

were generally irregularly rounded. The larger were often multinucleate. Unlike in the myometrium, the pigment inclusions in these connective tissue cells varied considerably in size. No pigment was present in the surface and glandular epithelium of the endometrium. These findings are in good agreement with previous histological studies of the uterus in the vitamin E-deficient rat.

The uteri from 8 of 9 spayed animals maintained on the tocopherol-deficient diet exhibited little or no pigmentation of the myometrial cells and contained but few macrophages with pigment inclusions. The remaining animal in the group resembled the unspayed deficient animals in amount and distribution of pigmentation. Neither the unspayed nor spayed animals maintained on the tocopherol-supplemented diet showed microscopic evidence of abnormal uterine pigmentation.

Discussion. The present observations clearly indicate the uterine response to estrogen in vitamin E-deficient ovariectomized rats does not differ significantly from that of tocopherol-supplemented controls. This finding suggests that an intimate physiological relationship between the two substances is not very probable. It is possible, however, that conditions in the unovariectomized animal may be materially different in regard to the metabolism of estrogen and tocopherol.

Studies concerning the influence of vitamin E deficiency upon uterine pigmentation have been made by Mason and Emmel.[4] Unlike the present experiments, these authors did not find unequivocable evidence concerning the influence of ovariectomy upon the development of uterine pigment in their vitamin E-deficient rats. These contrary results may have been caused by the different experimental diets used.‖ The fat content of the diet used by Mason and Emmel was double that of our diet and, in addition, their diet contained 2% cod liver oil. This is of particular significance since it has been shown that pigmentation in vitamin E-deficiency is intimately related to the intake of fat, especially if it contains large amounts of highly unsaturated compounds as does cod liver oil.[5] However, the apparent non-essentiality of the ovary in uterine pigment deposition in rats maintained on a high fat intake as compared with rats on low fat intake must remain unexplained at the present time.

The question may arise as to whether the absence of pigment in our vitamin E-deficient spayed animals might be due to the estrogen treatment just preceding sacrifice. This is not the case since, in other work not reported here, tocopherol-deficient spayed animals which had not received estrogen exhibited no macroscopic evidence of uterine pigmentation.

Summary. (1) Rats ovariectomized at weaning were maintained on a vitamin E-deficient diet for from 6 weeks to 10 months. No difference in the uterine weight response to injected estradiol benzoate was discernible between these animals and control animals of the same age maintained on the same diet supplemented by alpha-tocopherol acetate

(2) The uterine weight response to alpha-estradiol benzoate was significantly greater at 3 months of age than at 10 months regardless of tocopherol administration.

(3) Accumulation of acid-fast pigment in the uterus is characteristic of intact rats maintained on a vitamin E-deficient diet. Ovariectomy at weaning prevented the appearance of uterine pigmentation in animals maintained on the deficient diet for as long as 10 months.

[4] Mason, Karl E., Dam, Henrik, and Granados, Humberto, *Anat. Rec.*, 1946, **94**, 265.

‖ It has been suggested by Dr. Karl E. Mason that the differences in the results may have been due partly to their use of Zenker's fixative whereas Bouin's fixative has been used in the present studies. For this and many other creative criticisms we are highly indebted to Dr. Mason.

[5] Mason, Karl E., and Emmel, Anna F., *Anat. Rec.*, 1945, **92**, 1945.