

Endosteal Bone Deposition in Femurs of Vitamin D-Deficient Mice Treated with Estrogen.

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It has been reported by a number of investigators¹ that the treatment of mice with estrogens produces an extensive proliferation of endosteal bone in the femurs which may result in the complete obliteration of the marrow cavity of these bones. This phenomenon is usually accompanied by resorption of the pubic bones and formation of a pubic ligament. As is well known, a similar resorption of the pubic bones occurs as a normal process during the reproductive cycle in the pocket gopher.²

It was of interest to determine whether the changes in the bones observed by others in mice treated with estrogens and maintained on adequate diets could be reproduced by similar treatment of mice fed food deficient in vitamin D. It seemed possible that vitamin D might play a role in the mechanism of deposition of osteoid tissue in the femur. It was, therefore, decided to study the effect of treatment with estrogenic hormone on mice fed vitamin D-deficient diets. The absence of changes in the femurs of mice treated in this manner would implicate vitamin D in the mechanism. On the other hand, the observation that the changes occurred in the absence of vitamin D as well as in its presence would indicate that dietary vitamin D was not a major factor in this process. In an initial study the effect of estrogen treatment on two different strains of mice fed commercial food mixtures was investigated.

Experimental. Male mice of two strains, CHI and C3H, were employed in the present investigation. The animals were bred from stock animals obtained from the colony maintained by Dr. Leonell Strong of Yale University. The groups of animals fed a vitamin

D-deficient diet were protected from light by cardboard shields, and the cages were provided with wire screens to keep the animals off the droppings. Vitamin D-free diets of two different types were used. One of the diets with a normal Ca:P ratio and deficient in vitamin D was identical in composition with that employed by Sherman and Stiebeling³ in some of their studies on vitamin D. The other deficient diet, U.S.P. XII Rachitogenic Diet No. 1, possessed the abnormally high Ca:P ratio which is characteristic of many rachitogenic diets. α -Estradiol dipropionate[†] was the estrogenic agent administered in this study and the duration of treatment was 12 weeks. At the end of this time the mice were killed with ether and autopsied. One femur of each animal was removed, dried, and ground as a gross specimen. The pubic bones were inspected.

The diets fed to the several groups of experimental animals and the amounts of estrogen received by the mice in the different groups are shown in Table I. The experimental results as shown in Table I mark an interesting strain difference between the CHI and the C3H mice. The former show no obliteration of the marrow cavity of the femur when they are treated with estrogen and fed either Purina Fox Chow or Pratt's Nurishmix, although some resorption of pubic bone was observed. On the other hand, mice of the latter strain showed complete obliteration of the marrow cavity when treated with estrogen and fed a stock diet. For this reason, mice of the C3H strain were used for the vitamin D study. The treatment of the mice and the

³ Sherman, H. C., and Stiebeling, H. K., *J. Biol. Chem.*, 1929, **83**, 497.

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¹ Gardner, W. U., and Pfeiffer, C. A., *Physiol. Rev.*, 1943, **23**, 139.

² Hisaw, F. L., *J. Exp. Zool.*, 1925, **42**, 411.

TABLE I.
Effect of α -Estradiol Dipropionate on Endosteal Bone Deposition in Different Strains of Mice and in Mice Fed Diets Deficient in Vitamin D.

Strain	No. of mice completing 12-week period	Diet	Amt of estrogen inj. subcut. once a wk, ¹ γ	Obliteration of marrow cavity of femur after 12 wks, ²	Resorption of pubic bones after 12 wks ³
CHI	12	Purina Fox Chow ⁴	50	0	++
CHI	9	" " " 4	0	0	0
CHI	12	Pratt's Nurishmix ⁴	50	0	++
CHI	10	" " " 4	0	0	0
C3H	10	Purina Fox Chow ⁴	50	++++	++
C3H	11	" " " 4	0	0	0
C3H	10	Pratt's Nurishmix ⁴	50	+++ ⁵	++
C3H	8	" " " 4	0	0	0
C3H	13	Vitamin D-deficient diet ⁶	50	++++ ⁷	+
C3H	12	" " " 6	0	0	0
C3H	11	Vitamin D-deficient diet ⁶ + viosterol ⁸	50	++++	+
C3H	12	" " " 6 + " 8	0	0	0
C3H	13	Vitamin D-deficient diet ⁹	50	++++	++
C3H	12	" " " 9	0	0	0

¹ In 0.05 cc sesame oil; control animals received the oil alone.

² 0 = none; +++ = slight persistence of proximal end of marrow cavity; ++++ = complete obliteration.

³ 0 = none; + = 1 to 2 mm pubic ligament; ++ = 2.1 to 4 mm.

⁴ Supplemented semi-weekly with lettuce.

⁵ 2 of animals had ++++.

⁶ Sherman Stiebling Diet.

⁷ 3 of animals had +++.

⁸ 50 units of viosterol in 0.05 cc corn oil injected subcutaneously weekly.

⁹ U.S.P. XII Rachitogenic Diet No. 1.

results of this study are likewise shown in Table I. It is noteworthy that the mice treated with estrogen and fed a vitamin D-deficient diet exhibited the typical changes in the femur whether or not they were receiving viosterol.

Summary. Obliteration of the marrow cavity of the femur by endosteal bone and some resorption of pubic bone was observed in C3H mice fed commercial stock diets and treated with estrogen. Comparable treatment of CHI mice did not cause obliteration of the

marrow cavity of the femur although some resorption of pubic bone was noted.

C3H mice treated with estrogen and fed a vitamin D-deficient diet supplemented with viosterol showed the typical changes in the femur. This result was also obtained in animals fed the same diet unsupplemented with vitamin D. Under the conditions of these experiments dietary vitamin D did not play a major role in the mechanism of endosteal bone deposition in the femurs of mice treated with estrogen.