

**Skeletal Changes in Mice Receiving Estrogens.\***

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In recent studies of the influence of estrogens on the incidence of mammary tumors in mice, comparatively large amounts of hormones have been injected over long periods of time. The appearance of lymphoid tumors in some of the experimental animals led to study of the reticulo-endothelial tissues and the discovery of extensive changes in the bones. Previous studies on the pelvis of mice receiving estrogens<sup>1</sup> had revealed an extensive resorption of the medial and ventral parts of the pubic and ischial bones and the replacement of the pubic symphysis by an interpubic ligament.<sup>1</sup> Changes in other parts of the skeleton contrast markedly with those occurring in the pelvis.

Gross observations and histological studies have been made on the femurs of 34 male and female mice which had received from 100 to 1000 I.U. of hydroxy-estrin benzoate† or 0.1 mg. of equilin benzoate‡ weekly for periods up to 348 days. The remainder of the skeleton was studied by X-ray photographs. Controls consisted of 3 male and 4 female mice (uninjected) of comparable ages and one male and 2 females which had received 1/20 cc. of oil of benne (the solvent for the estrogens) weekly. The mice, which were from 4 different inbred strains,§ were all maintained under similar conditions and had access to food (Fox Chow, Ralston-Purina Co.) and water at all times.

The femurs of untreated mice and those receiving the injections of oil were a reddish color, the marrow being visible through the comparatively thin translucent compact bone of the diaphyses. Osseous spicules were found only near the epiphyses. The femurs of the animals which had received 250 I.U. or more of hydroxy-estrin benzoate or 0.1 mg. of equilin benzoate weekly for 56 or more

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<sup>1</sup> Gardner, W. U., *Am. J. Anat.*, 1936, **50**, 459.

† The estrogens used were "Benzo-gynoestryl" obtained from the Laboratoire Français de Chimiothérapie (Paris), and Progynon-B, generously supplied by Schering Corporation through the courtesy of Drs. E. Schwenk and G. Stragnell.

‡ The equilin benzoate was obtained through Dr. G. M. Smith from Professor Girard (Paris).

§ The mice were supplied by Dr. L. C. Strong from 4 strains in his colony.

days were white, opaque, and very hard and brittle. However, unlike those of the normal mice, they were resistant to fracture at the epiphyseal lines. The marrow cavities were almost completely replaced by compact bones or by coarse bone spicules, making it impossible to insert a probe or to obtain bone marrow. Histological preparations showed that the spicules of bone filled the epiphysis, leaving only small, widely separated island of marrow. The marrow cavity of the diaphysis consisted only of a small central canal or several minute, irregular cavities containing blood vessels and a few marrow cells. The new bone seemed to be entirely endosteal in origin.

These changes are apparently reversible since in 2 cases partial recovery was observed within 2 months after the cessation of injections.

X-ray photographs indicate that the increased calcification which occurs in the femur also takes place throughout the entire skeleton, with the exception of the bones which unite at the pubic symphysis, and that certain epiphyses are closed which do not normally unite in the mouse.

Extensive studies of the bones of animals receiving estrogens have not been previously undertaken. More attention has been devoted to studies of the blood calcium, phosphorus, and phosphatase (see <sup>2</sup> and <sup>3</sup>), with somewhat variable results. It has, however, been reported that the epiphyses unite more rapidly in dogs receiving estrogen.<sup>4</sup>

*Summary.* Endosteal bone nearly or completely replaces the marrow cavities of the femurs of mice which have received estrogen (250 I.U. or more weekly) for long periods. Direct examination of the bones and X-ray photographs show that similar changes occur throughout the skeleton except in the immediate vicinity of the pubic symphysis.

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<sup>2</sup> Folley, S. J., *Biochem. J.*, 1936, **30**, 2262.

<sup>3</sup> Riddle, O., and Dotti, L. B., *Science*, 1936, **84**, 557.

<sup>4</sup> Tausk, M., and de Fremery, P., *Acta Brevia Neerl.*, 1935, **5**, 19.