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Species Differences in the Reaction of the Mammalian Skeleton to Estrogens.*

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In recent years it has been observed that the bones increase in density in immature mice and rats treated with large doses of estrogen. It has not been adequately emphasized, however, that the bone changes in mice described by Gardner and Pfeiffer,[1] are very different from the skeletal reaction in rats described by Follis and Day.[2] Nor is it generally appreciated that many other mammals, including man, react differently from either the rat or mouse.[3-5] This communication is the introduction to an extensive survey of the reactions of the skeleton in mice, rats, rabbits, hamsters, guinea pigs, cats, and dogs treated with 7 different natural estrogens and 4 different synthetic estrogens. For the sake of brevity, only the conclusions related to the above title will be stated from gross, x-ray, and histological examinations of the bones. Undecalcified sections, prepared according to technics previously described,[6,7] were used in addition to standard decalcified histological sections.

Mice. Large doses of α-estradiol benzoate or diethylstilbestrol, approximately 0.5 mg per week, produced endosteal bone formation in mice somewhat like that observed in estrogen-treated birds.[6] This reaction was produced

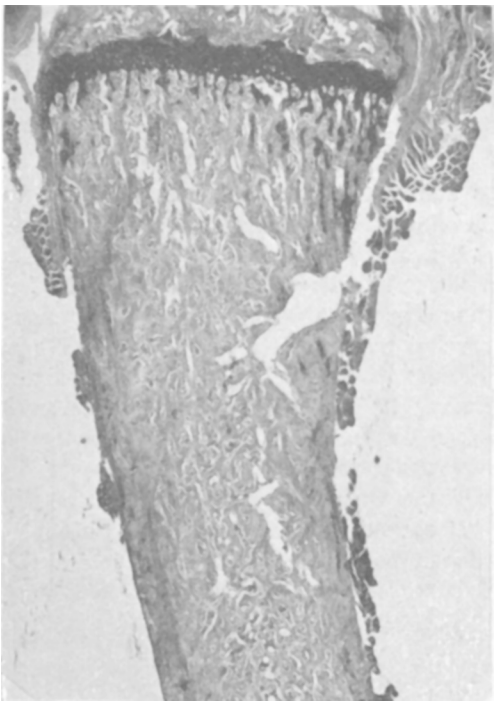

FIG. 1.
Photomicrograph of the upper end of the tibia of an immature mouse, approximately 7 weeks of age, treated with 1.0 mg of alpha-estradiol benzoate in 2 weeks and sacrificed 1 week later. The metaphysis is increased in density and the marrow cavity is filled with a network of new bone. This is the result of two processes, stimulation of endosteal or intramedullary new bone formation, and inhibition of bone resorption. Hematoxylin-eosin and azure-II stain.

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

2 Day, H. G., and Follis, R. H., *Endocrinol.*, 1941, **28**, 83.

3 Sutro, C. J., and Pomerantz, B. S., *Arch. Path.*, 1942, **33**, 305.

4 Koneff, A. A., Simpson, M. E., and Evans, H. M., *Anat. Rec.*, 1946, **94**, 169.

5 Urist, M. R., Bell, R., Jr., and Voshell, A. F., unpublished studies on children with Osteogenesis Imperfecta.

6 Bloom, M. A., McLean, F. C., and Bloom, W., *Anat. Rec.*, 1942, **83**, 99.

7 Bloom, W., Bloom, M. A., and McLean, F. C., *Anat. Rec.*, 1941, **81**, 443.

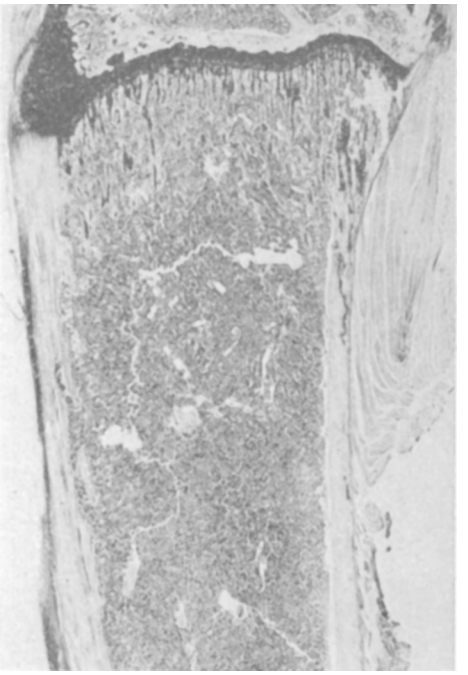

FIG. 2.
Photomicrograph of the upper end of the tibia of a rat, 12 weeks of age. Note the wide intervals between bone trabeculae in the area below the hypertrophic cells of the epiphyseal plate. Note the length of the new bone trabeculae of the primary spongiosa. The new bone formed in endochondral ossification contains a core of calcified cartilage matrix. Hematoxylin-eosin and azure-II stain.

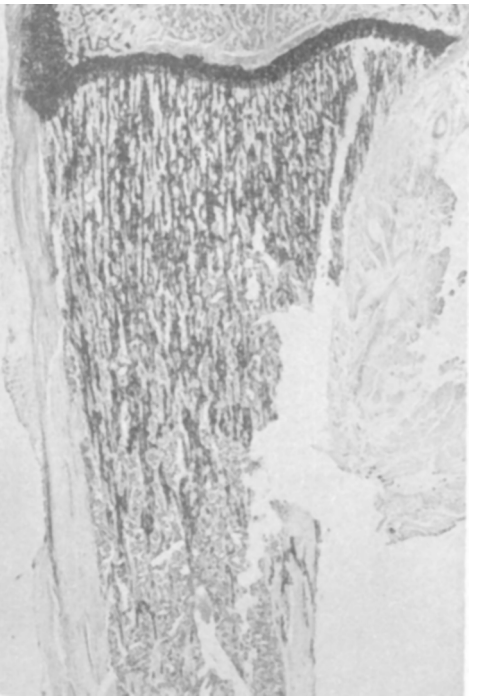

FIG. 3.
Photomicrograph of the upper end of the tibia of a litter-mate of the rat shown in Fig. 2, after 0.56 mg of alpha-estradiol benzoate in a period of 8 weeks. Note the metaphysis is approximately 5 times longer than normal. Because of the failure of resorption of most of the bone formed by endochondral ossification, the trabeculae extend down into the medulla and each one contains a core of deeply-staining calcified cartilage matrix. There is no endosteal new bone, as found in the reaction of the skeleton of the mouse, shown in Fig. 1. Hematoxylin-eosin and azure II.

consistently in animals between 1 and 6 months of age. The process began in the metaphyses of the rapidly growing long bones at 10 days and extended along the shaft until, at 10 to 12 weeks, the entire medulla was filled with new bone (Fig. 1). The intramedullary or endosteal new bone formation was not as widespread in treated mice as in birds[7] during the preovulatory phase of the egg-laying cycle. In birds, osteogenesis was rapidly initiated in the endosteum all along the inner shaft. In mice, osteogenesis progressed only by extension from the metaphyses, but the new bone formed similarly at the lines of apposition of osteoblasts and transforming bone marrow cells. The histological picture suggested that the reticular cells of the marrow stroma are drawn into the osteogenic process and become osteoblasts. Bone resorption was markedly inhibited, osteoclasts were rarely formed, and all the medullary new bone appeared to remain as long as the injections of estrogen were continued.

Rats. Large doses of α-estradiol benzoate or diethylstilbestrol (0.14 to 0.5 mg per week) repressed the resorption of the new bone produced in endochondral ossification. There was no appreciable effect upon bone formed in intramembranous ossification, except perhaps a non-specific stunting of the growth of the whole animal.

The effect of estrogen on the skeleton of the rat is specifically upon the process of resorption of cartilage matrix and new bone formed at the epiphyseal lines. Two effects are seen in normal bone resorption: the "thinning out" of the spongiosa and the control of the normal

length of the trabeculae. Estrogen prevented both the normal thinning and shortening of the metaphysis which is seen in young growing animals with rapid turnover of the body calcium stores. The metaphyses increased in length in young rats because there was no bone resorption at the ends of the trabeculae. Osteoclasts were almost completely absent from the scene. After 8 weeks of treatment, the bone trabeculae were everywhere filled with a core of unresorbed cartilage matrix, both in the secondary spongiosa near the shaft and in the new cancellous bone at the epiphyseal lines.

The overall increase in the length of the spongiosa was proportional to the period of treatment. Growth of the bones in length was retarded by estrogen, particularly when large doses were given to weanlings for 5 weeks. With moderate doses there was less inhibition of growth and the length of the metaphyses was greater than in animals treated with large doses for the same period of time.

The increase in density of the spongiosa, or the number of bone trabeculae in the metaphysis, was directly proportional to the dose of estrogen. On large doses, the cancellous bone became so dense that it was indistinguishable from the compact bone of the shaft in the roentgenograms. In rats these effects were produced only between 1 and 6 months of age and were most pronounced during the period of rapid growth between 1 and 3 months of age.

Other Species. Previous experimental investigations dealing with the effects of estrogen upon the skeleton of other mammals have been limited to the treatment of hamsters,[4] guinea pigs,[8] and puppies[3] with relatively small doses of estradiol benzoate or diethylstilbestrol. We have treated these and other species with doses of estrogen as large as the animal would tolerate. The bone changes were quite different from those described above in mice and rats. In hamsters, guinea pigs, rabbits, kittens, and puppies, proliferation of epiphyseal cartilage and new bone formation were suppressed. The overall result of treatment was that the growth of the long bones was stunted and the metaphyses were reduced to one-half or one-fourth the normal length. There was also an appreciable reduction in the number of osteoblasts and bone trabeculae in some species, *i.e.*, immature rabbits treated with 30 to 60 mg of diethylstilbestrol within 4 weeks. In many species the marrow showed widespread degenerative changes and formation of colloid cysts. The histological picture in general suggested nonspecific repression of all cellular activity with none of the stimulating action of estrogen upon osteogenesis as seen in the mouse or none of the inhibiting effect of estrogen on cartilage resorption in the rat.

Summary. Estrogenic steroid hormones stimulate endosteal bone formation and inhibit bone resorption in mice. In young growing rats, the same substances do not produce endosteal bone but interfere with endochondral ossification by inhibiting resorption of cartilage matrix and new bone. In other rodents, such as hamsters, guinea pigs, and rabbits, and also in cats and dogs, there is no apparent specific effect of estrogens upon formation of bone.

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[8] Sutro, C. J., and Pomerantz, L., *Arch. Surg.*, 1939, **39**, 992.