Effects of Simultaneous Administration of Estrogen and Parathyroid Extract upon Teeth, Periodontium, and Long Bones of Growing Albino Mice

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Numerous studies have been carried out concerning the effect of parathyroid extract upon the calcified dental structures and adjacent alveolar bone.[1–7] Most of these studies utilized the rat, pigeon, or dog rather than the mouse. This latter animal was employed by Silberberg and Silberberg,[8, 9] but the calcified dental structures and the alveolar process were not evaluated in their studies.

In contrast, extensive work has been reported on the effect of estrogens upon the growth of long bones in numerous species of animals. The mouse has been studied probably more extensively than any other animal owing, in part, to the fact that it responds so readily and remarkably to this hormone.[10] However, studies of the effects of estrogens upon tooth structure and associated alveolar bone have not been as extensive or shared the popularity of the corresponding observations upon the long bones.[11–19] Even less work has been carried out on either long bones or dental apparatus in regard to the effect of parathyroid extract administered preceding, simultaneous with, or following estrogen administration.[20–24] Recently, Ranney[23] studied the action of these two hormones administered simultaneously to the mouse, but his work was confined chiefly to the effects on physiologic blood changes rather than on histologic interpretations of alterations in long bones or jaws.

The purpose of this study has been twofold: (1) to reinvestigate the effect of estrogen upon long bones and dental structures; and (2) to study the effect of subsequent parathyroid-extract administration upon long bones and dental structures previously treated with estrogen, as evaluated by histologic and radiographic techniques.

Materials and Methods

Standard Swiss albino male mice, approximately 4 weeks old and weighing between 14 and 18 gm., were used. The male sex was used in preference to the female so that the variable of the animal's own sex hormone influencing the results might be minimized.[25]

Forty-eight mice were divided into four groups of 12 each according to their initial body weight and housed in an air-conditioned room (Table 1). Their diet consisted of a standard commercial preparation† and tap water provided ad libitum. The animals

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in Group I acted as the controls. Those in Group II were treated as those in Group I, except that after 6 weeks they received a daily injection of parathyroid extract for 6 days. The animals in Group III received a weekly injection of estrogen for 6 weeks. Group IV received the weekly injection of estrogen for 6 weeks and then received a daily injection of parathyroid extract for 6 days. At the end of 7 weeks, all animals were weighed and sacrificed. Tissues were taken for histologic and radiographic study.

The estrogenic hormone utilized in this study was Progynon® (Estradiol Benzoate U.S.P.*) solution in sesame oil administered subcutaneously at a dosage of 0.5 mg. (0.5 ml.) per animal/week. The parathyroid extract† (100 U.S.P. units per ml.) was administered subcutaneously at a level of 20 units (0.2 ml.) per day.

Observations from a pilot study suggested that the optimum histologic response to estrogen was observed between the 4th and 6th week of administration. Therefore, this study was designed to extend administration of estrogen over a six-week period.

Tissue for histologic examination was fixed in Bouin's fluid, imbedded in paraffin, cut at a thickness of 8 μ, and stained routinely with hematoxylin and eosin. Sections from kidneys and salivary glands were stained also by Von Kossa's method against a known

TABLE 1

BASIC DESIGN OF THE STUDY AND WEIGHT DIFFERENCES

Group	No. of Animals	Estrogen Adm. (Weeks)	Parathormone Adm. (Days)	Initial M Body Weight (Gm.)	Final M Body Weight (Gm.)
I	12			15.5	34.0
II	12		6	15.4	35.6
III	12	6		15.1	25.6
IV	12	6	6	15.2	27.6

control to demonstrate the presence of ectopic calcification. After a hemijaw and femur from each animal had been radiographed, ground sections were made of one femur from each animal, stained with silver nitrate, and exposed to fluorescent light to develop color. Gross observation on the size of the marrow cavity and amount of trabeculation was noted on the ground sections. Unfortunately, the small size of the hemijaws negated this technique in their case. A jaw and femur radiograph from each animal was utilized to compare radiodensities of the respective groups.

Results

GROSS OBSERVATIONS OF ALL GROUPS.—The differences in final body weight and growth rates are seen in Table 1 and Figure 1. Note that those animals that received estrogen had a mean final body weight that was much less than either of the non-estrogen groups. The animals of all groups appeared quite healthy grossly.

RADIOGRAPHIC OBSERVATIONS OF ALL GROUPS.—The radiographic findings within each group were consistently similar. In Groups I and II a small irregular radiolucent area immediately below the molar teeth was noted; this area represented normal marrow

* Progynon Benzoate was generously supplied by George Babcock, Jr., M.D., Director, Medical Research Division, Schering Corp., Bloomfield, N.J.

† Eli Lilly and Company, Indianapolis, Indiana.

cavity for an animal of this age. Jaw radiographs from Groups III and IV consistently displayed sclerosis in the interradicular areas of the molars with a loss of the radiolucent area noted in Groups I and II. There was, also, a slight increased density of the incisor teeth in Groups III and IV (Fig. 2). No radiographic effects of parathyroid-extract administration were noted in the jaws.

The radiographic differences of the long bones were markedly more pronounced than

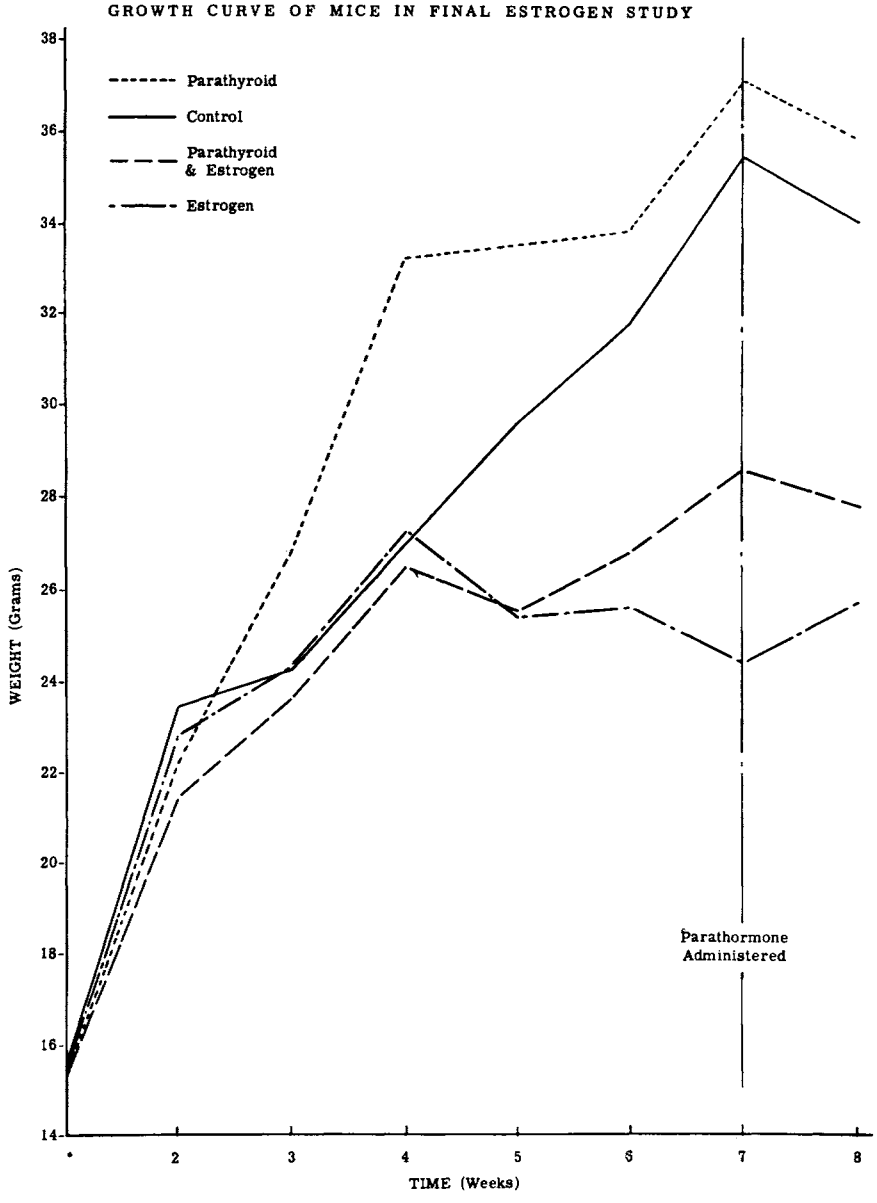

FIG. 1.—Growth curve shows the marked difference between weight gain of estrogen- and non-estrogen-treated mice.

those in the jaws (Fig. 3). Section A (Group I) demonstrated clearly that the thickness of cortical plates was quite uniform, the marrow cavity was regular and occupied the entire length of bone, while there was only the usual amount of sclerosis around the epiphysis and metaphysis. However, Section B (Group II) displayed a decidedly fuzzy, moth-eaten appearance without any obvious change in the size of the marrow cavity. Section C (Group III), on the other hand, displayed a marked reduction in the size of the marrow cavity with a concomitant sclerosis of the proximal and distal metaphysis. The D section (Group IV) showed the same sclerotic changes noted in the C section, but it

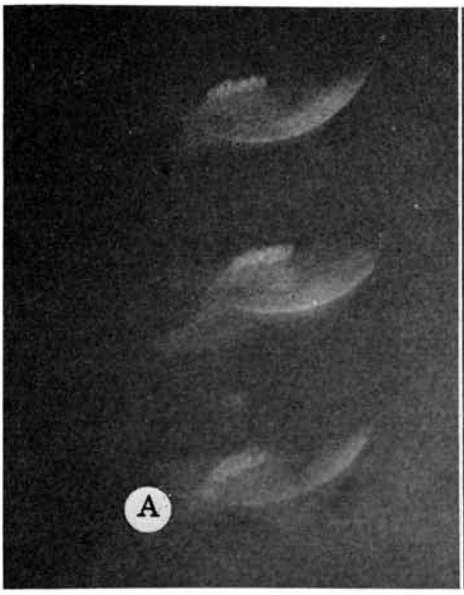

FIG. 2

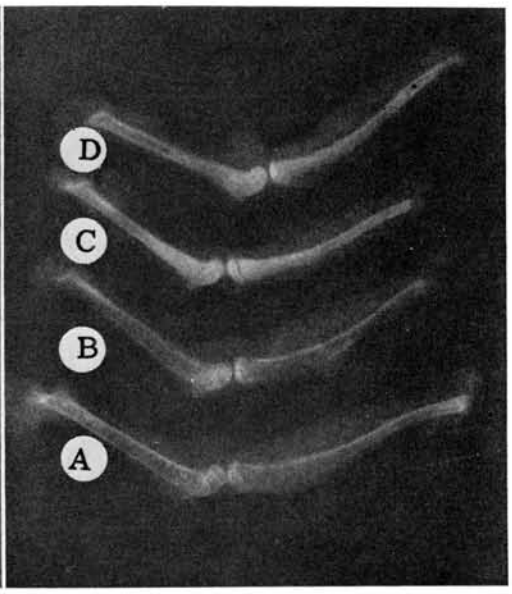

FIG. 3

FIG. 2.—Hemijaws of estrogen-treated mice—Section A is control. Radiograph displays a moderate increase in radiodensity of the incisor teeth and the interradicular area below the molars.

FIG. 3.—Long bone specimens from each group. Radiograph shows sclerosis in estrogen-treated mice (Sections *C* and *D*) and osteoporosis in parathyroid extract-treated mice (Sections *B* and *D*). Section *A*, Group I. Section *B*, Group II. Section *C*, Group III, Section *D*, Group IV. Particularly note the distal metaphysis of the femora.

also had the same fuzzy, moth-eaten appearance as Section B with a suggestion of a larger marrow cavity than in the other estrogen group (C section).

GROUND SECTION OBSERVATIONS OF FEMORA FROM ALL GROUPS.—The bones illustrated in Figure 4 were each representative of their respective groups. Section A (Group I) showed a normal thickness of the cortical plate, normal-sized marrow cavity, and a normal amount and pattern of metaphyseal trabeculation. Section B (Group II) displayed an apparent lack of rigidity to the cortical plate and a lack of normal trabeculation around the metaphysis. This accounts for the gross distortion of this section, and since the radiograph was taken of the intact femur, this artefact did not appear in Figure 2. Section C (Group III) appeared shorter than the control and displayed increased metaphyseal trabeculation with an associated proliferation of endosteal bone. These phenomena have caused a marked decrease in the size of the marrow cavity. Interestingly, Section D (Group IV) had the same gross characteristics except that the

marrow cavity appeared to be somewhat enlarged. The endosteal bone also seemed to have a friable consistency when compared with Section C.

HISTOLOGIC OBSERVATIONS OF THE JAWS FROM ALL GROUPS.—In Group I the interradicular and interdental septa consisted of normal compact and cancellous bone. Associated within the septa and immediately below the apices of the teeth were irregularly shaped marrow cavities containing the usual hemopoietic tissue. The size and shape of the marrow cavities varied according to the plane of section. Owing to the small size of the mouse jaw, we found that getting a section cut on the perpendicular was more luck

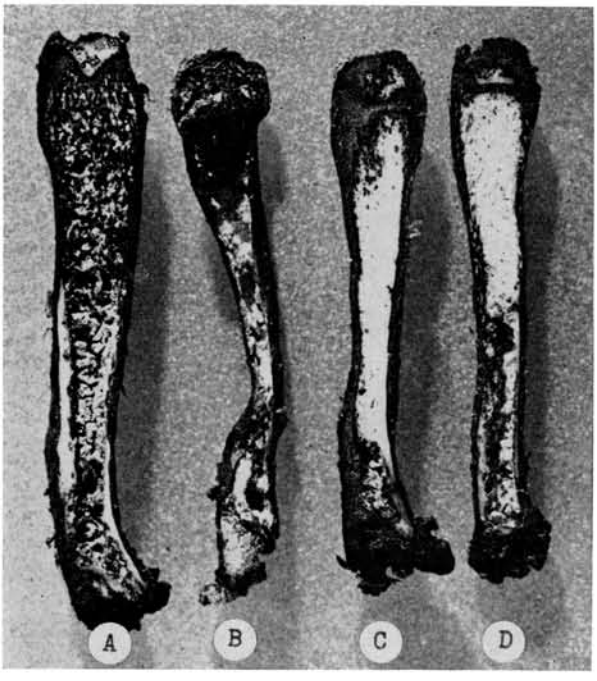

FIG. 4.—Ground femur sections stained with silver nitrate. Enlarged photograph illustrates diminution of marrow-cavity space in estrogen-treated mice (Sections *C* and *D*) and apparent osteoporosis in specimens that received parathyroid extract (Sections *B* and *D*). All femur preparations from Group II lacked rigidity and were thus distorted as Section *B* in preparation. Section *A*, Group I. Section *B*, Group II. Section *C*, Group III. Section *D*, Group IV.

than skill. Hence, most sections were cut somewhat obliquely. Nevertheless, marrow-cavity spaces were always noted somewhere in the jaw sections from those animals that did not receive estrogen (Fig. 5). After estrogen administration, these marrow cavities were not noted (Fig. 6). It was also noted that the distal borders of these septa were irregular in outline, and there was an occasional Howship's lacuna housing osteoclasts noted. The mesial borders were comparatively smooth in outline and were lined by a faint band of osteoid, indicating the natural physiologic drift of these teeth toward the distal. This histologic finding was present in all the subsequent groups. The epithelial interdental papillae were cone-shaped with the point of the cone oriented toward the oral-cavity surface (Fig. 7). There was a slight sprinkling of inflammatory cells, chiefly lymphocytes, noted in the epithelium and subjacent connective tissue of the papilla. The periodontal membrane varied in cellularity and direction of orientation according

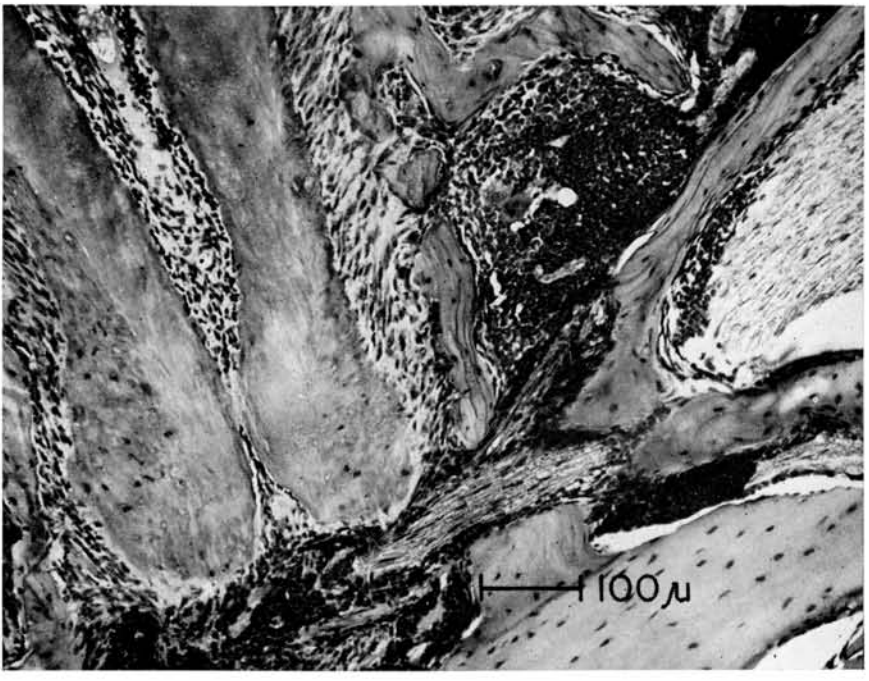

FIG. 5.—Control jaw, 7 weeks. Photomicrograph shows the numerous irregular marrow-cavity spaces that were noted in the non-estrogen-treated mice. (Hematoxylin and eosin.)

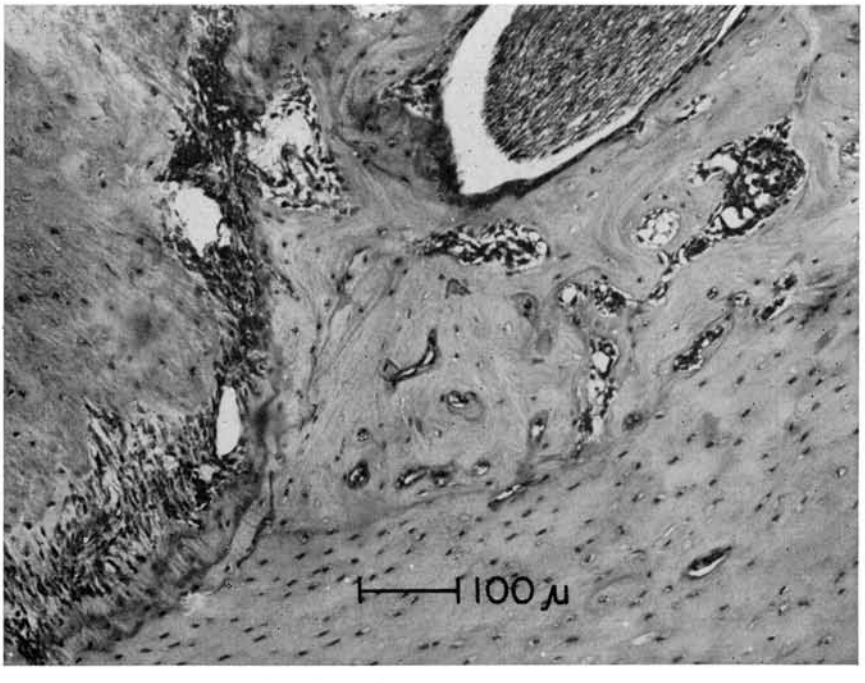

FIG. 6.—Estrogen jaw, 6 weeks. Photomicrograph shows an area normally occupied by marrow cavity that is now filling in with estrogen-stimulated endosteal bone. (Hematoxylin and eosin.)

to the height and plane of section. Group II differed from Group I slightly; there was an increased number of osteoclasts and bone resorption noted along the periphery of both the mesial and distal surfaces of the septa. Within the area of resorption, a fibrous connective-tissue stroma was laid down in and about the trabeculae of bone. The marrow spaces were still evident, and the epithelial interdental papillae were cone-shaped with the point of the cone oriented away from the underlying connective tissue.

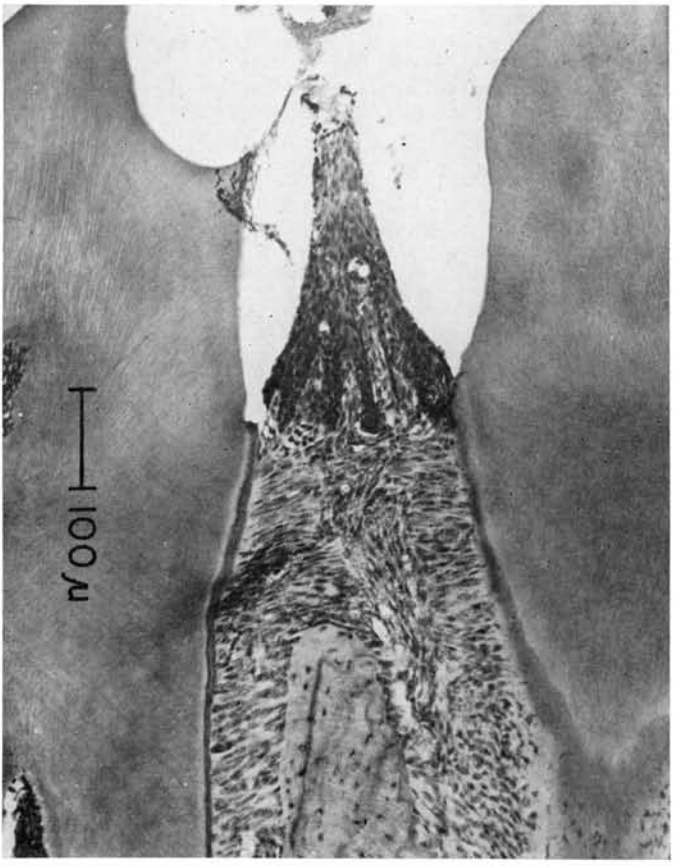

FIG. 7.—Jaw, 6 weeks. Photomicrograph demonstrates a typical cone-shaped interdental papilla. (Hematoxylin and eosin.)

The histologic picture of Group III differed considerably from Groups I and II. Although the interdental and interradicular septa were still composed of normal compact and cancellous bone, the medullary cavities containing hemopoietic tissue, as noted in Groups I and II, were absent in Group III. In lieu of the cavities, only an area of newly formed bone could be noted (Fig. 6). This bone differed from the adjacent interradicular bone in that the inclosed osteocytes were more polyhedral and/or spherical in shape. They were also less uniform in appearance. This bone also had a more homogeneous appearance owing to the decreased number of incremental lines.

The epithelial interdental papillae were decidedly different from the papillae in the non-estrogen groups; most of the papillae were quite blunt, if not concave (Fig. 8), and

displayed a moderate amount of materia alba on the surface. The spinous cell layer of the oral mucosa was moderately increased in thickness, while the subjacent connective tissue was infiltrated by a mild to severe inflammatory cell infiltrate consisting of lymphocytes and a few plasma cells. No consistent histologic changes were noted in either the teeth or the periodontal membrane.

Group IV manifested the same histologic features as Group III regarding the loss of the marrow cavities and their replacement by newly formed endosteal bone. There was,

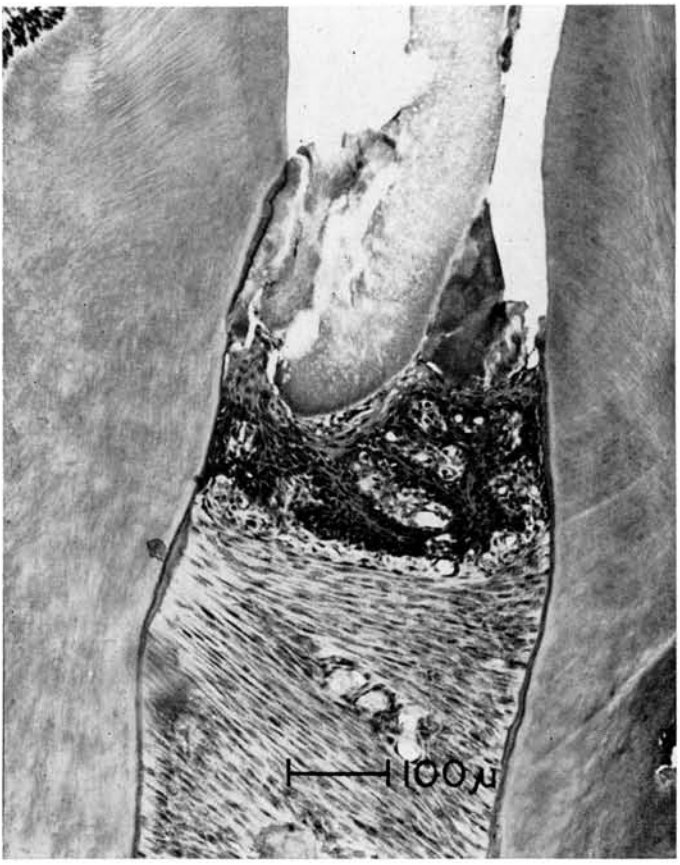

FIG. 8.—Estrogen jaw, 6 weeks. Photomicrograph demonstrates a typical bunted papilla with materia alba and inflammatory cell infiltrate. (Hematoxylin and eosin.)

however, an increased number of osteoclasts on the distal surfaces of the interradicular septa. Moreover, there were Howship's lacunae and osteoclasts noted on the mesial surfaces of these septa. The morphologic and inflammatory changes of the interdental papillae were no different from the changes noted in Group III.

HISTOLOGIC OBSERVATIONS OF THE FEMORA FROM ALL GROUPS.—The femora from Group I displayed the usual thickness of cortical plate and a normal-sized marrow cavity. There were regular, slender trabeculae of bone extending from the epiphyseal plate and projecting down into the metaphysis. The marrow cavity contained the usual amount of hemopoietic tissue (Fig. 9). The femora of Group II resembled those of

Group I. The cortical plate was of the usual thickness, and the marrow cavity was normal sized; however, multinucleated giant cells, interpreted to be osteoclasts, were prominently scattered about the trabeculae of the metaphyseal area. The histologic picture was markedly different in Group III. These femora resembled their corresponding jaw sections. Due to the marked endosteal bone proliferation, there was a loss of marrow cavity space with an associated decrease in hemopoietic tissue. This proliferation began at the metaphysis, particularly the proximal, and progressed toward the center of the bone shaft. Through apposition of endosteal bone and anastomosing of enlarging trabeculae, this new bone replaced the marrow cavity (Fig. 10). The histologic

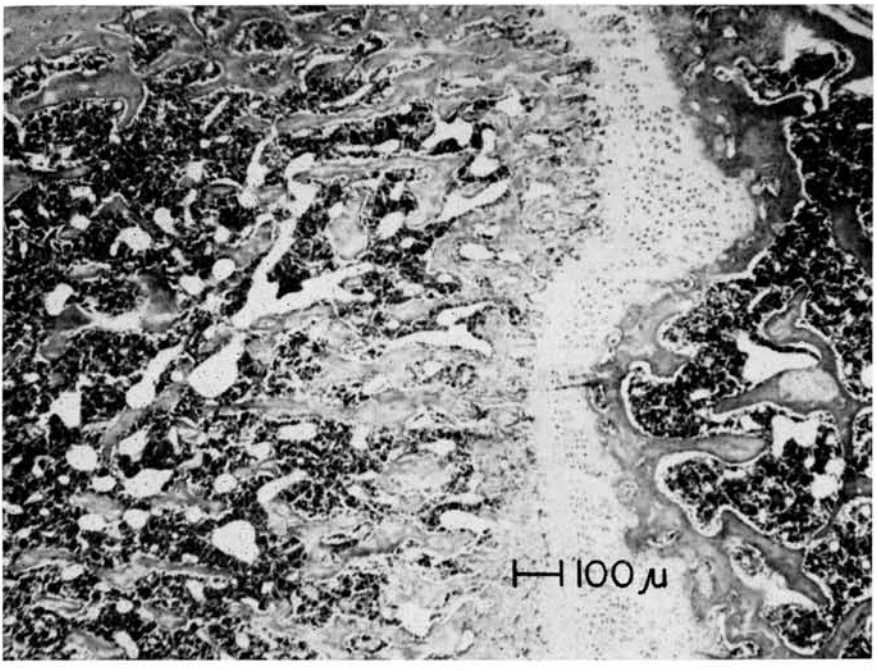

FIG. 9.—Control femur, 7 weeks. Head of femur shows a normal trabecular pattern with associated hemopoietic tissue. (Hematoxylin and eosin.)

sections of Group IV were similar to those in Group III in that there was endosteal bone formation with consequent narrowing of the marrow cavity. This group was similar to group II in that there was increased bone resorption and an increased number of osteoclasts, which were not confined to any one area but were generalized in distribution (Figs. 11 and 12). The femora displayed both increased vascularity and fibrous replacement within the area previously occupied by the hemopoietic tissues.

HISTOLOGIC OBSERVATIONS OF THE SOFT TISSUES FROM ALL GROUPS.—No histologic differences were noted between the kidney and salivary glands of the control and experimental groups on the routine hematoxylin- and eosin-section preparations. Histologic evidence of ectopic calcification within these organs by Von Kossa's stain proved to be negative in all groups. There were no histologic changes indicating either inflammation or degeneration noted in any of these sections.

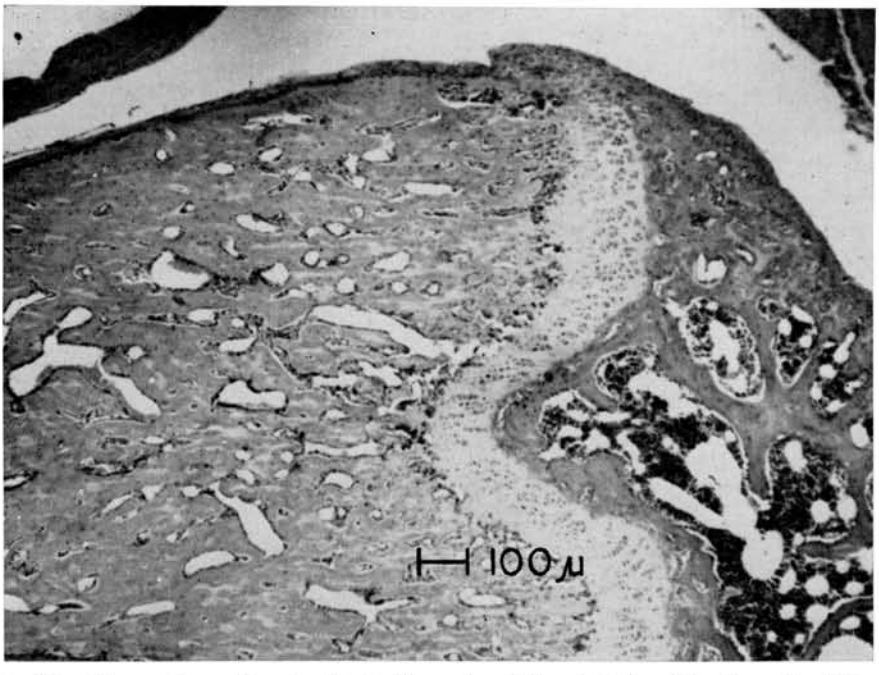

FIG. 10.—Estrogen femur, 6 weeks. Head of femur in which endosteal proliferation with obliteration of marrow cavity is seen. (Hematoxylin and eosin.)

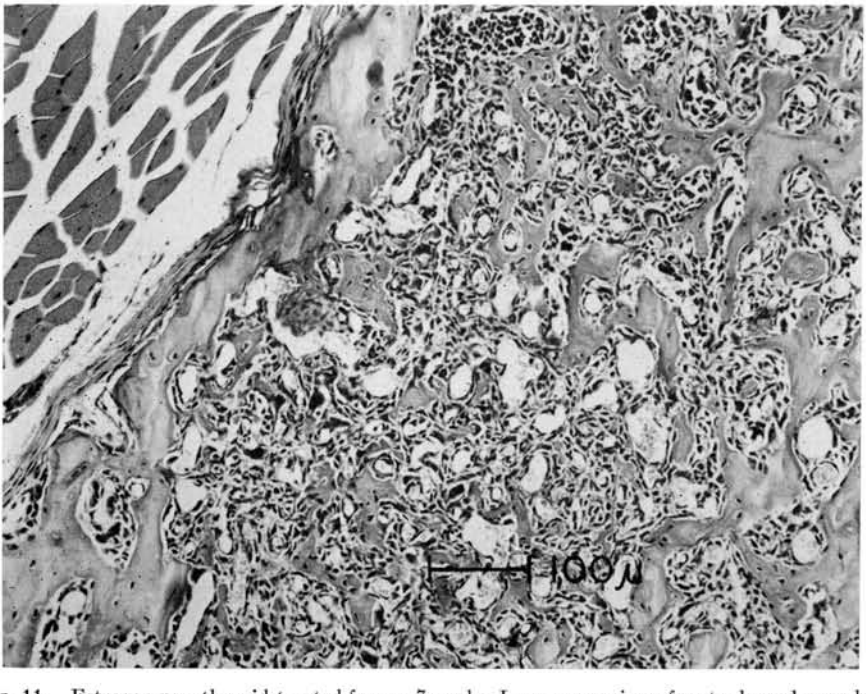

FIG. 11.—Estrogen-parathyroid-treated femur, 7 weeks. Low power view of metaphyseal area denoting numerous multinucleated giant cells. (Hematoxylin and eosin.)

Discussion

Examination of the results shows a significant difference between the estrogen- and non-estrogen-treated animals in regard to weight gain (Fig. 1 and Table 1). The animals in Group I did not receive estrogen and had a mean final body weight of 34.0 gm. The Group II animals were also non-estrogen animals, and they had a mean final body weight of 35.6 gm. Groups III and IV were both administered estrogen, and their respective mean final body weights were 25.6 and 27.6 gm. This substantiated the findings of previous workers, who reported that estrogen administration did stunt the growth of mice. The effect of parathyroid-extract administration on weight gain or loss is equivocal, and the experimental design of this study does not provide adequate appraisal of this effect.

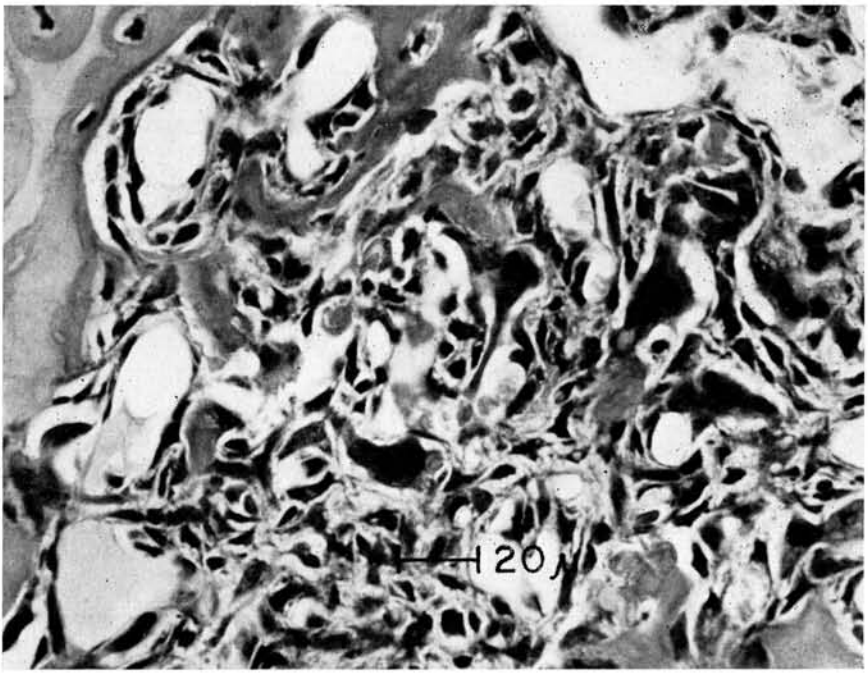

FIG. 12.—Estrogen-parathyroid-treated femur, 7 weeks. High power view of Figure 10 demonstrating the multinucleated giant cells. (Hematoxylin and eosin.)

Several studies have reported that estrogen affects the periodontal-membrane fibers by initially increasing the amount of collagen and number of fibroblasts then ultimately producing irregular, less dense collagen fibers with fewer fibroblasts.[16, 18, 19, 26, 27] The present investigation did not disclose any histologic difference in the periodontal membrane that (in this study) could not be attributed specifically to the plane of section. In contrast to the findings of Glickman and others,[16, 18, 19, 26, 27] Stahl, Weinmann, Schour, and Budy,[14] and Nutlay, Bhaskar, Weinmann, and Budy[15] did not report similar changes in their investigation on the periodontium of estrogen-treated mice. However, oral epithelium in this study did manifest some obvious changes in the estrogen-treated animals. Specifically, there was flattening and moderate proliferation of the interdental epithelial papillae with associated subjacent chronic inflammation. These findings are comparable

to those of other investigators.[14, 15] Stahl *et al.*[14] reported that they did not notice any changes in the epithelial attachment as reported by Nutlay and his co-workers.[15] The present study, too, did not demonstrate consistent proliferation of the epithelial attachment; this may be due to non-consistency in the preparation of the sections. Nevertheless flattening and proliferation of the interdental epithelial papillae was apparent in all histologic sections from estrogen-treated animals; this feature was not seen in the non-estrogen group.

It might be assumed that the change in the interdental papillae was due to local irritation. Yet, these local irritating factors were apparently, only present in the groups receiving estrogen. It is well recognized that soft diets can accumulate around the teeth of animals and act as a local irritant in initiating gingivitis and periodontal disease. However, the animals in this study all received the same diet. Therefore, it seems safe to assume that estrogen must have acted either directly or indirectly regarding the morphologic and inflammatory changes noted in the interdental papillae. Estrogen may affect the epithelium in such a way as to render it less resistant physiologically to other unknown factors that may be directly responsible for the inflammation noted in the papillae. It is interesting to recall that Ziskin, Rosenstein, Drucker, Blackberg, and Slanetz,[28–30] reported hyperplasia and increased keratinization in the oral epithelium of women and monkeys who received estrogens, but they did not report an associated gingivitis and hyperplasia of the subjacent fibrous connective tissue.

In 1961 Svoboda[31] reported that estrogenic substances enhance the induction of skin tumors in mice by the fact that constant estrogen absorption and the associated constant estrus prevents normal postovulatory decline in mitosis and hence maintains abnormal mitotic activity. If mitosis is activated in epithelium by estrogen, then one might suggest that estrogen also excites the osteoblasts, a connective-tissue cell, in a similar manner. Even though this investigation only displayed epithelial changes in the interdental papillae and no connective-tissue changes in the periodontal membrane, there still remains the question of increased endosteal proliferation of bone, inasmuch as bone is connective tissue. Gardner,[32] in 1942, concluded that estrogen does stimulate osteoblasts to produce bone, and some workers[33–35] have shown that parathyroid-extract administration can cause osteoblasts to differentiate into osteoclasts and revert ultimately to spindle or reticulum cells (fibrous marrow). Therefore, it seems possible that estrogen does affect connective tissue, but the mechanism of action remains unknown. The administration of parathyroid extract did not affect the epithelium or the teeth in the animals in this study. There was no observable histologic difference in the degree of dentinal calcification. Since osteoclasts were more prominent and distributed on both the mesial and distal surfaces of the interradicular and interdental septa, an increase in osteoclastic activity was suggested in those animals that received parathyroid extract. The long bones also displayed an increased number of osteoclasts, which were generalized in distribution throughout the metaphysis. There appeared to be more osteoclasts per unit area in the group that received estrogen than in the group that did not. This may be owing to the fact that there was more bone to be resorbed in the estrogen group, or it may be because there were more osteoblasts to act as precursors for the formation of the osteoclasts. Moreover, it is commonly accepted that the osteoblast may act as a precursor to the osteoclast.[36]

The apparent increased radiographic density of the incisor teeth of animals receiving

estrogen is not an original finding.[11, 14] Although the present study lends credence to the fact that estrogen administration does affect tooth calcification, it should be noted that Pindborg[12] and Bauer[13] concluded in their reports that tooth calcification was apparently unaffected by estrogen administration. The increased density of the incisors could be accepted as a possibility since these teeth are continually developing and erupting in rodents; however, the molar teeth do not share this unique growth feature, and it therefore seems difficult to imagine or explain how anything could increase their radiographic density after they have once formed.

The mechanism whereby the incisor teeth become radiographically more dense or increase in degree of calcification is unknown. One possible mechanism is suggested by the work of Ranney.[23] In 1959 he reported that estrogen accelerated the rate of calcium accretion and enlarged the size of the labile-bone mineral pool. Thus, it seems plausible to suggest that this increase in available calcium is equally shared with the developing teeth as well as with the newly formed endosteal bone.

The administration of parathyroid extract appeared, grossly, to cause extreme demineralization of long bones. This was particularly noted in Group II. Evidence of a demineralization process was noted also in the respective radiograph.

Although a definitive attempt to measure the thickness of cementum was not undertaken in this study, it was observed that hypercementosis was evident histologically on several occasions. This condition was not related specifically to estrogen administration, inasmuch as it was noted in jaw sections of animals from all four groups. It may be that the hypercementosis in this case is more aptly related to the age or diet of the animals rather than to estrogen administration. No differences were noted between the estrogen- and non-estrogen-treated animals in regard to the amount of collagen, number of fibroblasts, or direction of the fibers in the periodontal membrane.

Summary

Based upon the findings of a pilot study, this study employed 12 animals per group and was continued for 7 weeks. The estrogen-treated animals received weekly injections for 6 weeks while the parathyroid extract-treated animals were injected daily for 1 week.

It was concluded that estrogen and parathyroid extract, administered individually or in combination, could produce changes in the jaws, periodontium, and long bones, which may be noted and evaluated by histologic and radiographic techniques in the growing, male, albino mouse.

This study indicated that estrogen administration markedly affects the growth of the animals as evaluated by weight gain. However, any appreciable effect of parathyroid-extract administration upon weight gain was not discernible in this study.

Estrogen and parathyroid-extract administration did not produce any obvious histologic or radiographic changes in the periodontal membrane or teeth, except that the incisors of those animals receiving estrogen exhibited an increased radiodensity.

Estrogen produced endosteal bone proliferation in the marrow spaces of the jaws as well as in the long bones of these animals, and there was an associated decrease in the size of the marrow cavity. The proliferation of endosteal bone was accompanied by a fibrous replacement of the hemopoietic tissue, and this phenomenon appeared most prominently in those animals that also received parathyroid extract.

Estrogen administration alone or with parathyroid extract was associated with a

marked change in the morphology of the epithelial interdental papillae and chronic gingivitis. This morphologic change was characterized by a flattening of the normal pointed papillae and a thickening of the spinous cell layer.

Parathyroid extract produced or was associated with an increased osteoclastic activity in both jaws and long bones, but this finding was definitely more pronounced in the estrogen-treated animals.

References

1. ERDENHEIM, J. Morphologische studien über die Beziehunger der Epithelkorperchen zum Kalkstaffwechsel. *Frankfurt. Z. Path.*, **8**:175, 238, 259, 293, 1911.
2. JAFFE, H. L., and BODANSKY, A. Experimental Fibrous Osteodystrophy (ostitis fibrosa) in Hyperparathyroid Dogs. *J. exp. Med.*, **52**:669–94, 1930.
3. SCHOUR, I., and HAM, A. W. Effect of Single Dose of Ergosterol or Parathorome on Histologic Structure of Dentine of Rat Incisor, Correlated with Blood Calcium Findings. *J. dent. Res.*, **13**:194, 1933, (abstract).
4. ———. The Action of Vitamin D and of the Parathyroid Hormone on the Calcium Metabolism as Interpreted by Studying the Effect of Single Doses on the Calcification of Dentine. *Arch. Path.*, **17**: 22–34, 1934.
5. SCHOUR, I., TWEEDY, W. R., and MCJUNKIN, F. A. The Effect of Single and Multiple Doses of the Parathyroid Hormone on the Calcification of the Dentine of the Rat Incisor. *Amer. J. Path.*, **10**: 321–42, 1934.
6. SCHOUR, I., TWEEDY, W. R., CHANDLER, S. B., and ENGEL, M. B. Changes in Teeth Following Parathyroidectomy. *Amer. J. Path.*, **13**:971–84, 1937.
7. WEINMANN, J. P., and SCHOUR, I. Experimental Studies in Calcification: III. The Effect of Parathyroid Hormone on the Alveolar Bone and Teeth of the Normal and Rachitic Rat. *Amer. J. Path.*, **21**:857–75, 1945.
8. SILBERBERG, M., and SILBERBERG, R. Effect of Hormones on the Skeleton of Mice, Guinea Pigs, and Rats. *Endocrinology*, **29**:475–82, 1941.
9. ———. The Effects of Parathyroid Hormone and Calcium Gluconate on the Skeletal Tissues of Mice. *Amer. J. Path.*, **19**:839–60, 1943.
10. URIST, M. R., BUDY, A. M., and MCLEAN, F. C. Specie Differences in Reaction of Mammalian Skeleton to Estrogen. *Proc. Soc. exp. Biol. (N.Y.)*, **68**:324–26, 1948.
11. SUTRO, C. J. Effects of Subcutaneous Injections of Estrogens upon the Skeleton of Immature Mice. *Proc. Soc. exp. Biol. (N.Y.)*, **44**:151–54, 1940.
12. PINDBORG, J. J. Experimental Investigations on the Influence of Estrogen on Calcium Metabolism. *Acta path. microbiol. scand.*, **22**:290–98, 1945.
13. BAUER, W. H. Effect of Estrogen on Tooth Buds and Bone in Growing Dogs. *J. Amer. Coll. Dent.*, **12**:192–201, 1945.
14. STAHL, S. S., WEINMANN, J. P., SCHOUR, I., and BUDY, A. M. The Effect of Estrogen on the Alveolar Bone and Teeth of Mice and Rats. *Anat. Rec.*, **107**:21–42, 1950.
15. NUTLAY, A. G., BHASKAR, S. N., WEINMANN, J. P., and BUDY, A. M. The Effect of Estrogens on the Gingiva and Alveolar Bone of Molars in Rats and Mice. *J. dent. Res.*, **33**:115–27, 1954.
16. GLICKMAN, I., and SHKLAR, G. Modification of the Effect of Cortisone upon Alveolar Bone by the Systemic Administration of Estrogen. *J. Periodont.*, **25**:231–39, 1954.
17. FLEMING, H. S. Growth and Development of Tooth Germ Homotransplants in Ovariectomized Guinea Pigs. *J. dent. Res.*, **34**:686, 1955 (abstract).
18. SHKLAR, G., and GLICKMAN, I. Effect of the Systemic Administration of Estrogenic Hormones upon the Alveolar Bones of White Mice. *J. Periodont.*, **27**:16–23, 1956.
19. GLICKMAN, I., and QUINTARELLI, G. Further Observations on the Effects of Ovariectomy upon the Periodontium of Experimental Animals. *J. Periodont.*, **31**:31–36, 1960.
20. MANUNTA, G., SAROFF, J., and TURNER, C. W. Relationship between Estradiol and Parathyroid on Retention of Ca^{45} in Bone and Blood Serum of Rats. *Proc. Soc. exp. Biol. (N.Y.)*, **94**:785–87, 1957.
21. URIST, M. R., DEUTSCH, N. M., POMERANTZ, G., and MCLEAN, F. C. Interrelations between Actions of Parathyroid Hormone and Estrogen on Bone and Blood in Avian Species. *Amer. J. Physiol.*, **199**:851–55, 1960.
22. POLIN, D., and STURKIE, P. D. Parathyroid and Gonad Relationship in Regulating Blood Calcium Fraction in Chickens. *Endocrinology*, **63**:177–82, 1958.
23. RANNEY, R. E. Antagonism between Estrogen and Parathyroid Extract in Their Effects upon Bone Accretion. *Endocrinology*, **65**:594–601, 1959.

24. ———. Antagonism between Estrogen and Parathyroid Extract in Calcium Metabolism of Bone. *Nutr. Rev.*, **18**:108–10, 1960.
25. HAMMETT, F. S. A Biological Study of Bone Growth. I. Changes in the Ash, Organic Matter, and Water during Growth. *J. biol. Chem.*, **64**:409–28, 1925.
26. GLICKMAN, I., and SHKLAR, G. The Steroid Hormones and Tissues of the Periodontium. *Oral Surg.*, **8**:1179–91, 1955.
27. PIROSHAW, N. A., and GLICKMAN, I. The Effect of Ovariectomy upon the Tissues of the Periodontium and Skeletal Bones. *Oral Surg.*, **10**:133–47, 1957.
28. ZISKIN, D. E. The Effect of Hormonal Treatment on the Gums and Oral Mucosa of Women. *J. dent. Res.*, **16**:367–78, 1937.
29. ZISKIN, D. E., ROSENSTEIN, S., and DRUCKER, L. Interrelation of Large Doses of Estrogen and Vitamin A and Their Effect on the Oral Mucosa. *J. Oral Surg.*, **29**:163–77, 1943.
30. ZISKIN, D. E., BLACKBERG, S. N., and SLANETZ, G. A. Effects of Subcutaneous Injection of Estrogenic and Gonadotrophic Hormones on Gums and Oral Mucous Membrane of Normal and Castrated Rhesus Monkeys. *J. dent. Res.*, **15**:407–28, 1936.
31. SVOBODA, D. J. The Influence of Stilbesterol on Skin Tumors Induced in Mice with Tobacco Tar and Methylcholanthrene. *Cancer Res.*, **21**:993–96, 1961.
32. GARDNER, W. U. Stimulating Effect of Estrogens on Osteoblasts. In *Metabolic Aspects of Convalescence, Transactions of the First Conference 1942*, ed. EDWARD C. REIFENSTEIN, p. 23, New York: Josiah Macy, Jr., Foundation, 1942.
33. AVEY, L. B. The Origin and Fate of Osteoclasts. *Anat. Rec.*, **11**:319–22, 1917.
34. KROON, D. B. Effect of Parathyroid Extract on Osteogenic Tissue. *Acta. morph. meerl.-scand.*, **2**:38–58, 1958.
35. HELLER, M., MCLEAN, F. C., and BLOOM, W. Cellular Transformations in Mammalian Bones Induced by Parathyroid Extract. *Amer. J. Anat.*, **87**:315–48, 1950.
36. TONNA, E. A., and CRONKITE, E. D. Use of Tritiated Thymidine for the Study of the Origin of the Osteoclast. *Nature (Lond.)*, **190**:459–60, 1961.