changes. 5. No ill effects occur in men when $B_6 \cdot HCl$ is administered orally or intravenously in the dosage of 100 to 200 mg. Pain occurs when the drug is injected intramuscularly. 6. In the concentration of 1:8000, $B_6 \cdot HCl$ inhibits isolated rabbit's small intestines, but contracts the isolated guinea pig's uterus.

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Effects of Subcutaneous Injection of Estrogen upon Skeleton in Immature Mice.

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It is known that the subcutaneous administration of estrogen produces osteosclerosis in immature mice.[1] Hitherto, however, detailed roentgenographic and histologic studies explaining the mode of development of the osteosclerosis have been lacking. This paper reports such an investigation.

The study was conducted upon 66 immature mice, some of the C_3H strain and some from stock colony of the Rockland Farms. The experimental subjects received subcutaneous injections of estradiol benzoate in sesame oil* each week for a certain number of weeks. The control animals received injections of sesame oil for corresponding periods of time.

At the expiration of the total period allotted for the experiment, the animals were roentgenographed and autopsied. The skeletal tissues [femur, tibia, humerus, vertebral column, calvarium, ribs, pelvis, foot and jaw (including incisors)] were fixed in Helly's fluid, decalcified in 5% nitric acid, embedded in paraffin and stained with hematoxylin and eosin. The soft tissues were likewise prepared for histologic examination.

Table I summarizes the organization of the experiments.

The control mice received weekly injections of sesame oil.

Roentgenographic and Gross Pathologic Findings: Roentgenographic examination reveals that in immature mice osteosclerosis, caused by the formation of new bone in the medullary cavity, especially in the lower end of the femur and upper end of the tibia,

1 Gardner, W. U., and Pfeiffer, C. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 678; *ibid.*, 1938, **38**, 599.

* Progynon-B was generously supplied by Schering Corporation.

TABLE I.

Series	Strain	No. of immature mice		Sex	Weekly dose of estrogen R.U.	No. of weeks treated
		Exp.	Control			
1	R. Farms	6	6	Female	1000	2- 5
30	" "	6	6	Male	500	5-10
21	C_3H	7	7	Female	500	6-12
16	R. Farms	5	5	"	500	8-20
3	" "	3	3	"	300	4-14
2	" "	6	6	"	150	8-22

follows upon subcutaneous administrations of estradiol benzoate in sesame oil. When 1000 rat units of estrogen have been given for 3 or 4 successive weeks, an area of increased density is already observed in the sub-epiphysial plate regions of the bones contributing to the knee joint. When the estradiol is given over the longer periods of time and in the larger doses, the dense shadow advances into, and may obliterate the medullary cavity. After administering 500 rat units of estradiol benzoate each week for 2 or 3 months, one notes an osteosclerosis of the distal third of the femur as well as of the proximal third of the tibia. This change occurs in both the male and female mice, but is somewhat less marked in the former.

The administration of 500 rat units of the estrogen per week for 4 or 5 months provokes a more generalized osteosclerosis. It may obliterate a considerable part of the medullary cavity and also cause a pronounced thickening of the cortices of all the long bones. The epiphyses, especially of the femur and tibia in the knee joint, are similarly transformed. The vertebral column also undergoes such changes. In the vertebrae, the sclerosis makes its first appearance in the zone of provisional calcification and then advances from the caudal and cephalad surfaces toward the middle of the vertebral body.

It is interesting that at no time were isolated foci of osteosclerosis observed. Furthermore, where cortical thickening is present, it seems to be the result of partial obliteration of the metaphysial or diaphysial medullary cavity. No periostitis is evident. The calvarium in general tends to become sclerotic, and usually does so after 3 or more months of administration of the estrogen in doses of 500 rat units per week. The density of the incisor teeth is somewhat increased. From some of the roentgenographs one gains the impression that the pulp spaces of the incisors and especially of the lower ones, are diminished in size.

Injections of 150 rat units of estradiol benzoate per week for 2 or 3 months, likewise causes a productive osteosclerosis, but to a lesser degree.

In general, the length of the skeleton, including that of the calvarium, is somewhat less in the experimental animals than in the controls.[2] This is especially true of the animals treated for 3 or more months.

Along with the rather general sclerotic changes and inhibition of growth, there is evidence of resorption in the pubic bones. This commences in the body of these bones and advances along the superior and inferior ramus. These transformations may be observed after 4 or more weekly injections of 500 rat units of estrogen. If such injections are continued for 8 weeks, the superior ramus of the pubic bone becomes very short, thin and dense. In many cases in which the administration of estrogen is continued for 12 or more weeks, the entire pubic bone undergoes resorption.

Histologic examination of the skeletal tissues of representative animals in each group reveals that the epiphysial plates—especially of the bones of the knee joint—are quickly affected. The columns of hypertrophic cells are diminished in height and their matrices show premature calcification. The activity of the resting and proliferating cells is likewise inhibited, in that their rate of division is retarded. The number of cartilage cells in the epiphysial plate is diminished to a slight degree. A striking finding is the absence of the columnar arrangement of newly formed trabeculae in the zone of provisional calcification. Instead of this arrangement, one finds a disorderly agglomeration of blood vessels which have erupted into the growth plate and which may have reached the level of the proliferating cells. Around the numerous blood vessels, new bone has formed by means of osteoblastic activity in the connective tissue in the region of the zone of provisional calcification.

The new trabeculae of bone contain very small amounts of cartilaginous matrix. Instead of growing in a normal strictly vertical plane, the sheets of new bone develop in a disorderly fashion; a good many are in a horizontal plane. Most of the trabeculae fuse to form an almost solid block of bone.

The new bone rapidly fills up the medullary cavity. It advances into the diaphysial marrow cavity by spreading along the endosteal surfaces of the cortices.

It is interesting that in a group of female mice (Series 3) new bone formation was associated with resorptive phenomena. Specifically, the vessel canals were found enlarged in the cortices of the long bones and in the tables of the calvarium. Furthermore, in many places, the linings of these canals showed occasional osteoclasts.

[2] Zondek, B., *Folia Clin. Orient*, 1937, **1**, 1.

The region of the zone of provisional calcification of the bones comprising the knee joint presented a very loose vascular osteogenic tissue in which areas of new bone were forming. The connective tissue spread for a considerable distance into the diaphysial portion of the marrow cavity. In the calvarium, the newly formed intramedullary connective tissue appeared to arise from the stroma of suture lines.

In the other series of animals, the bone-forming elements were predominant. In fact, the new bone may fill a large portion of the medullary cavity and replace a considerable amount of the lymphoid marrow. Although the most obvious effects occur in the metaphysial region of the bones of the knee joint, the upper end of the femur and the humerus, and the vertebral bodies become similarly involved, though to a lesser degree. Replacement of the lymphoid marrow by new bone occurs in the epiphyses also, and especially in those of the bones comprising the knee joint region. In these epiphyses, the subchondral areas become sclerotic.

In the articular cartilages, as in the growth plates, the cells are retarded in their activity. The cartilage cell outlines in the columnar regions are much more distinct than they would normally be. In the zone of provisional calcification, the blood vessels may advance into the calcified cartilage beyond the normal level. Interestingly enough, however, the matrix of the articular cartilage above the zone of provisional calcification does not undergo premature fibrillization or degeneration.

In general, the extent and severity of the changes in the bone and cartilage vary in accordance with the duration of the experiment and the dosage of estrogen. In the course of 4 to 5 months, the femur, tibia, and other long bones and the vertebral column, may come to show extensive replacement of the lymphoid elements in the metaphysial and diaphysial portions of the medullary cavity by newly formed bone. There is no increase in periosteal activity.

Conclusion. Injections of estradiol benzoate into immature female and male mice in the dosages listed in the table, stimulate the proliferation of new bone in the medullary cavities of certain bones and especially of the lower end of the femur and upper end of the tibia. Other bones, including the calvarium, are also affected, but to a lesser degree. The proliferation begins around the zone of provisional calcification and advances into the diaphysial portion of the bone. The changes in question can easily be recognized on roentgenographic examination. When the pubic bones are involved, they undergo in addition, more or less resorption, the extent of which depends upon the dosage of estrogen and the duration of the experiment.