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EFFECT OF ESTROGEN ON RESORPTION OF CHONDRO-OSSEOUS COMPLEX IN THE RAT

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INTRODUCTION

The idea that estrogen stimulated the formation of new bone was a brilliant inference but was based on incomplete knowledge of the histological physiology of the epiphyseal growth mechanism. Many observers have confirmed the observation by Day and Follis that the observed increase of calcified material under the epiphyseal plate in growing animals following administration of large doses of estrogen was the result of inhibition of resorption rather than stimulation of formation. It has been pointed out that bone formation in this area is largely fibrous in type and not subject to the inhibitions affecting lamellar bone.

In a study of the effect of estrogen on new lamellar bone formation in rat diaphysis the opportunity was afforded us to observe in the same animals the effect of the drug on the chondroosseous complex. These observations are reported in summary fashion here and confirm inferences made by Day and Follis. Follis, in a subsequent study of the effect of cortisone on the chondroosseous complex, observed that the effects of this drug were similar to the effects of estrogen and speculated about the possible inter-relationship between the two. As Turner notes, adrenal hyperplasia following estrogen therapy is well known, and the work of Bernstein suggests that this result is not entirely mediated through the hypophysis. A paper currently in press makes extensive use of these possibilities in synthesizing a model of the endocrine control of bone remodelling.

MATERIALS

A pure strain of Norwegian white rat obtained from Holtzman Rat Co., Wisconsin, was studied. All rats were postpubertal. They were kept under control conditions for at least a week prior to institution of an experiment in an animal room which is air conditioned and has controlled humidity, temperature and cycling of light and darkness.

The estrogen tested was estradiol benzoate a sesame oil base suitable for injection. Rats were given complete rat diet and water ad libitum.
Figure 1a

Low power view, longitudinal section through distal epiphysis of femur of normal, control rat. Experiment 2, hematoxylin and eosin. The epiphyseal plate lies in the middle of the figure, the epiphysis in the upper third, the metaphysis in the lower third, most of the metaphysis is filled with hematopoietic marrow, the chondro-osseous complex being only slightly, but variably, thicker than the plate itself.
METHODS

A companion paper discusses some of the physiology of the chondro-osseous complex. Briefly, this complex lies under the epiphysial plates and is produced at a given rate. It is also absorbed at a given rate. The balance between the rate of formation and the rate of resorption leads to an observed thickness of individual trabeculae and to an observed length of individual trabeculae of the complex. The rate of formation may be estimated by observing and correlating with the rate of growth in length of a standard bone such as the tibia, it being immaterial whether the length is measured by x-ray or by undecalcified longitudinal section. Decalcified sections are not suitable for length measurements because of their variable shrinkage during paraffin embedding, a phenomenon primarily due to the necessary heating, as noted by Villanueva.

When rate of growth in length is correlated with density and length of chondro-osseous complex valid conclusions about alterations in rate of resorption of the complex, if any, may be made.

The length of the chondro-osseous complex may be observed on decalcified sections, on undecalcified sections or on x-rays, it again being immaterial which if the alterations produced by the experimental design are major in nature. We have been wary of alterations which are subtle, preferring to ignore them rather than risk making inferences from them which might later prove erroneous.

In this study observations of alterations in the length and density of the chondro-osseous complex were correlated with observations of the growth in length of tibias to yield information about the alterations in resorption rate produced by S.C. (subcutaneous) injection of 1.0 or 2.0 mg. of estradiol in oil. The animals so injected were sacrificed at periods varying from 5 to 21 days afterwards. In some of them x-ray determinations of length were made, in others length was observed in undecalcified sections and in still others we did not bother to measure length.
Figure 2a
Low power view, similar section and source as in Figure 1A but from castrated female given estrogen three weeks before sacrifice. Note the thinning of the epiphyseal plate.

Most of the proximal tibial epiphyseal mechanisms were studied by means of decalcified hematoxylin and eosin stained sections. Some were studied by means of undecalcified sections and some by means of x-rays combined with the above two methods.

The following experiments are reported: (a). Uncastrated female rats compared to uncastrated female controls. (b). Castrated female rats compared to castrated female controls. (c). Uncstrated male rats compared to uncastrated male controls. (d). Castrated male rats compared to similar controls.

Experiment types were performed at least twice at different times. The experiments extended over a 12 month period and involved different lots of animals, some raised by us from the supplier's stock. Eight to 16 rats were run at each experiment, over 110 rats being consumed in this phase of our investigations.

In summary form we observed the following:

a. Estrogen retards the resorption of chondro-osseous complex, the effect being most obvious in animals given the larger doses of estrogen and in those in which the longest interval elapsed between injection and sacrifice.

b. Estrogen in the larger dosage produced thinning of the epiphyseal plate in most but not all of the animals tested.

c. Uncastrated males were less susceptible to the estrogen effect on chondro-osseous resorption.

d. Castrated males exhibited inhibition of chondro-osseous complex resorption but to a lesser degree than female animals.
Figure 2b

High power of the area between the India ink bars in Figure 2A. There is much more chondro-osseous complex than in control animals. There was not an increase in growth rate, but a slight inhibition of growth was present in this animal.
Figure 2c

Higher power view of chondro-osseous complex from another estrogen treated rat. The illustration clearly reveals the bars of calcified cartilage, surrounded by fibrous bone. In the face of normal or decreased linear growth this proves conclusively that inhibition of resorption is present and is the cause of the increased metaphyseal density.
EFFECTS OF ESTROGEN ON RESORPTION

e. Maximum relative effect was observed in uncastrated female rats.

f. The larger dosage of estrogen produced retardation of growth in length in most animals. The smaller dose and the smaller time interval between injection and sacrifice were associated with inconstant alteration in longitudinal growth rate.

g. See Figures 1, 2 for illustrations of the above events.

DISCUSSION

As Follis remarked, the effect of estrogen is similar in some ways to that of cortisone. It cannot be said from this work whether this is a chance similarity or whether it is based on a relationship between adrenal and gonadal function which has not been given much attention. Our impression from our own material is that the cortisone effect is much more pronounced than the estrogen effect, particularly on growth in length and on inhibition of lamellar bone formation, while the effect on resorption of the two agents appears to be of similar magnitude. We suspect the two effects are related.

One might theorize on the basis of these remarks that while estrogen might not be a good bone formation stimulator, it may be to some degree an inhibitor of the resorptive phase of an osteoporosis and thus retard its development, although not preventing its development.

Adjustments of the animal's endocrine milieu to our treatment has not been considered but might play an important part in the events observed.

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REFERENCES


