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EFFECT OF ADRENAL CORTICOIDS ON CHONDRO-OSSEOUS COMPLEX OSTEOCLASIA

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Introduction

Among skeletal physiologists no topic is subject to more disagreement than the action of cortisone on bone physiology. While much excellent and thorough research work is published and there need be little quarrel with the basic data of numerous authors interested in this field, their data may legitimately be interpreted in diametrically opposed ways. The problem in other words is not "what are the figures"; it is instead "what do they mean".[1,9,10,12,13]

In an effort to determine experimentally the absolute sign of the effect of hormonal agents on bone formation and resorption a study of the effect of the adrenal corticoids was done. This work is reported in summary fashion here. A clear answer was obtained which is contained in the summary. By absolute sign we mean whether the agent accelerates, retards or exerts no effect. The problem of quantitation of any observed effect is left to later work.

Although the conclusions in this and other studies of similar nature reported by us may be considered controversial, we report the work simply because it is what we have repeatedly observed: these are the facts we found. With the aid of some histological insight gained from work with undecalcified sections, these are also the interpretations which we find fit the facts best.

Materials

A pure strain of Norwegian white rat obtained from Holtzman Rat Co., Wisconsin, was studied. The present study consumed over 200 of them. The animals were kept in an air conditioned animal room with controlled humidity, temperature and cycling of light and darkness. They were fed water and complete rat diet ad libitum.

Cortisone acetate and hydrocortisone acetate were the drugs studied. These were obtained from the hospital pharmacy as sterile, injectable suspensions intended for parenteral human use.

Figure 1a

Hypex 80 gm. rat, Experiment 11. Control animal given no hormone during the experiment. Longitudinal section, hematoxylin and eosin, through proximal tibial epiphysis. The alteration in the epiphyseal plate following hypophysectomy may be seen by comparison with figures 2 and 3. Mid-diaphasis lies below. The chondro-osseous trabeculae resulting from the basal rate of growth present in the hypophysectomized animal occupy the middle third of the figure, the plate being in the upper, and hematopoietic marrow in the lower thirds.

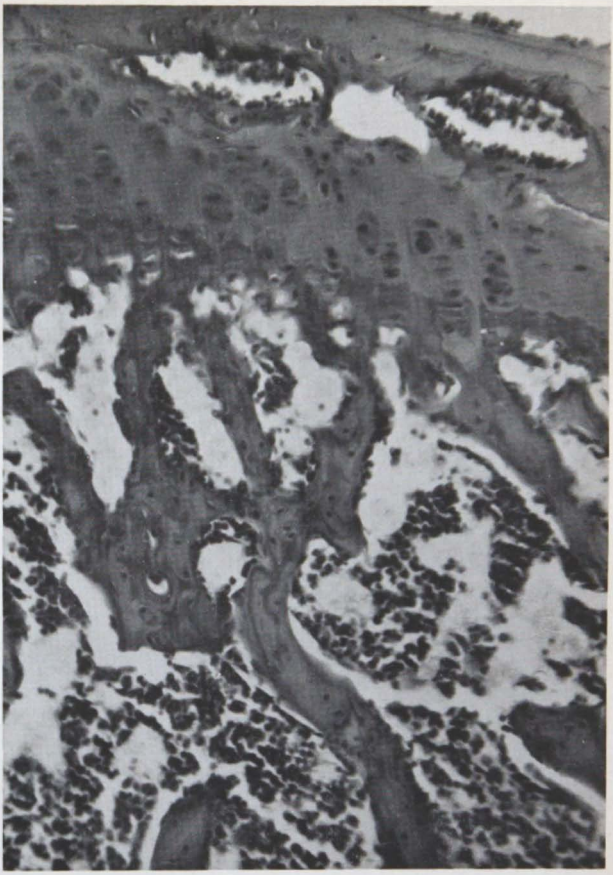

Figure 1b

Similar animal, section and experiment as in 1A but given 1 mg. cortisone acetate IP daily for 7 days before sacrifice. The growth rate of this animal was depressed about 20% compared to hypexcontrols. In spite of this, the trabeculae of the chondro-osseous complex are wider and more numerous. There are no unusual numbers of osteoblasts or osteoclasts. The epiphyseal plate is thinner than in the control and chondrocyte proliferation is obviously depressed.

The only conclusion compatible with these facts is that resorption is depressed, but depressed less than formation.

METHODS

It was decided that two things were of paramount importance.

The first was reproductibility, the second was the necessity of producing an obvious drug effect in the sense that anyone with histological background must agree with our interpretation of a photomicrograph. We know full well that a biased investigator may, by proper selection of fields, find an area in nearly any section which illustrates, and perfectly illustrates, contradictory points of view.

To ensure reproducibility we broke our experiments up into a large number of separate ones, each run with from 8 to 20 animals. These experiments were repeated at differing times on differing lots of animals, some obtained from the supplier and some raised by us from his stock. Differing personnel were involved in the experiments.

To obtain clear drug effects it was necessary to experiment with drug dosages, times, and with routes of administration. Early experiments in which we were feeling our way are not included.

All nonhypophysectomized animals studied were postpubertal. Hypophysectomized animals weighed an average of 70 gm. on receipt. About half of these animals were donated to us, courtesy of Upjohn Co., Kalamazoo, Michigan.

The adrenal corticoids were given in dosages of 1.0 mg./day in some experiments and 2.0 mg./day in others. In the majority of experiments they were given I.P. (intraperitoneally) but in six experiments they were given S.C. (subcutaneously). The duration of administration was five days in eight experiments, seven days in six experiments and ten days in six experiments. An equilibrium period of at least seven days was established in all animals before experiments were begun.

Each experimental rat was paired with a control rat. In some cases control rats were given dummy saline injections along with the experimental animals, and in others they were not. There was no noticeable difference between these two control groups.

The following methods of evaluating changes in bone resorption activity were tried:

1. Multiband tetracycline labelling technique, reported by the writer and coworkers.[6] In this method, described briefly by Roth[11] and by Stanisavljevic[14] in companion papers, tetracycline bands were deposited in the animal's bones in vivo at three moments equidistant in time, thus delineating two bands of intervening bone. The first band is the control band during which control conditions obtain for the animal. The second band is the experimental one during which the experimental variable is introduced.

For the present purpose this technique proved worthless. It is at its best when used as described by Roth and by Stanisavljevic for the measurement of lamellar bone formation.

2. Change in chondro-osseous complex.[2] This proved the proper method and has been used extensively by us for the histological and x-ray evaluation of resorption rate changes.

The chondro-osseous complex is the trabeculae of calcified cartilage covered over with layers of fibrous bone, normally found under the epiphyseal plates in growing animals. This complex is produced at a rate dependent on the growth rate of the germinal layer of the epiphyseal plate, on the rate at which this cartilage subsequently degenerates and becomes calcified, and on the rate at which, following resorption of the floor and roof of the resulting "honeycomb", fibrous bone is deposited on the resulting exposed surfaces.

The chondro-osseous complex is also removed at a given rate, this rate being the result solely of resorption by cells termed osteoclasts which are indistinguishable functionally and morphologically from osteoclasts found in the remainder of the skeleton. It is a curious fact that the cells forming hyaline cartilage, fibrous bone and lamellar bone are different and respond differently to injury, to local controlling factors and to hormonal control. In spite of these differences, the cells resorbing these tissues when calcified appear to be one cell reacting uniformly to local and systemic controlling factors. As pointed out before, the formation of fibrous bone is not significantly impaired by the presence of adrenal corticoids.[3] Accordingly, the elaboration of chondro-osseous complex in the sense that fibrous bone continues to be formed during the presence of large amounts of corticoids affords an opportunity to study resorption in a locale where the formation process is little affected by the drug.

The length of chondro-osseous complex, that is, the distance it extends from the epiphyseal plate towards the diaphysis, is accordingly the result of a state of balance

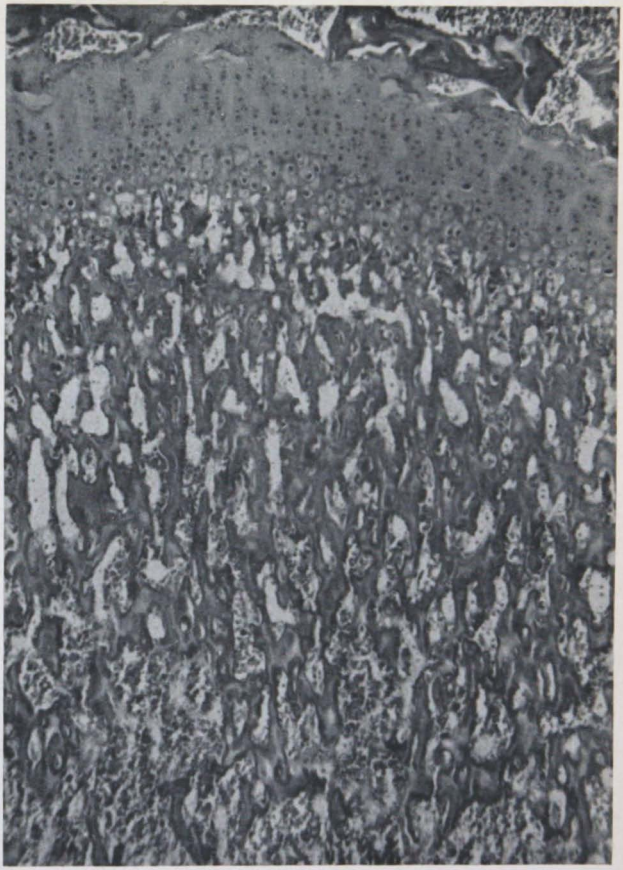

Figure 2

Control, normal animal — longitudinal section through proximal tibial epiphysis. Experiment 6. The epiphyseal plate lies at the top. The bulk of the figure below the plate is filled with chondro-osseous complex and marrow. Note the relative thickness of individual traceculae and the thinning out of the complex near the bottom of the figure.

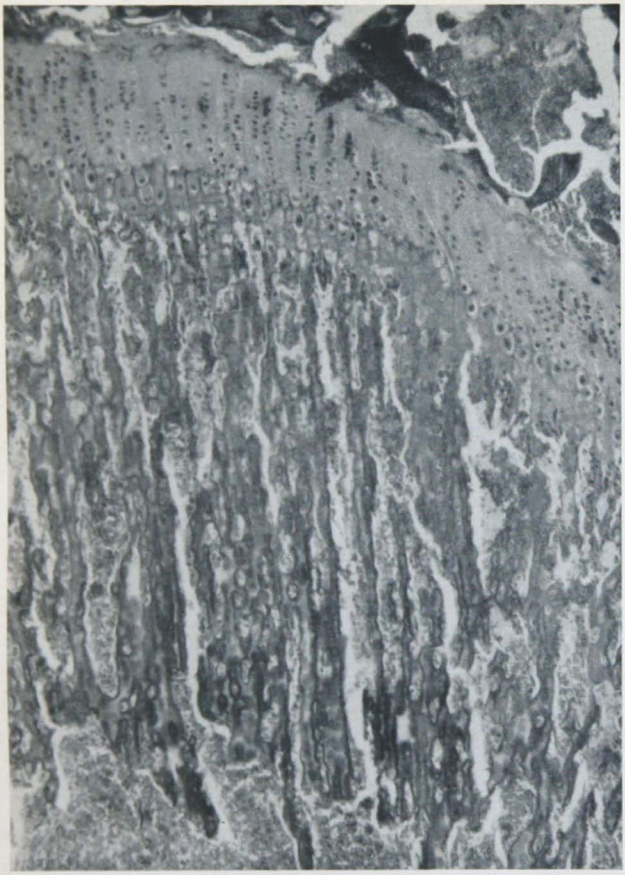

Figure 3

Experiment 6, section and stain as in Figure 2. This animal was given cortisone acetate I.P. daily for 7 days, 1 mg. Note the increased length, width of chondro-osseous trabeculae and decreased thinning out (compared to Figure 2) at the bottom of the figure. This bone was shorter than in controls, and grew 30% less during the period the drug was given than during an equal period of absention before starting the drug. The epiphyseal plate is not particularly thinned, however. Follis' illustrations[8] resemble this figure.

between formation rate and resorption rate during the time before the moment of observation. If formation exceeds resorption the complex will become longer and longer. If formation is less than resorption the complex will become shorter and shorter.

To determine whether there has been an absolute change in resorption rate, in addition to a change relative to formation rate, it is necessary to measure the length of the bone studied between the proximal and distal epiphyseal plates. If growth in length were retarded while at the same time the length of chondro-osseous complex were increased, the necessary conclusion is that resorption has been slowed down. If resorption were accelerated by the corticoids the chondro-osseous complex would regularly become shorter on administration of the drug. We point out here that numerous observers have noted in animals and in children that large doses of corticoids regularly retard growth in length of the long bones, an observation originally made by Follis.[6]

For the above reasons, which we feel are compelling, we interpret increased length of the chondro-osseous complex in the face of decreased growth in length of the bone as evidence of retardation of resorption. Similar arguments apply to the density and trabecular thickness of the chondro-osseous complex as well as to its length. These arguments need not be elaborated further.

We observed alterations in the length and density of chondro-osseous complex and of intact bones by means of: (a.) Decalcified, haematoxylin and eosin stained longitudinal sections, (b.) Undecalcified, fresh, longitudinal, fuchsin stained sections,[4,5] (c.) By serial x-rays of the animals with the bones held in standard position, using the same x-ray machine, film and exposure factors.

The following experiment-types were run: (a.) Normal females compared to normal female controls, (b.) Normal males compared to normal male controls, (c.) Castrated females compared to castrated female controls, (d.) Castrated males compared to castrated male controls, (e.) Hypex (hypophysectomized) rats compared to Hypex controls.

All of these type experiments were run at least twice and some were run five times at different times using different lots of animals. Dosages and route of administration of drug were varied at different times but in any single experiment were held constant. A total of 25 experiments was run.

The standard bone studied in all animals was the tibia; the standard epiphyseal growth mechanism was the proximal. In some animals, skull, vertebra, femur and humerus were also studied.

Results

In summary form, the following are our reproducibly observed results:

a. The adrenal corticoids regularly inhibit resorption of bone and longitudinal bone growth in the rat.

b. Hydrocortisone is more effective an osteoclasia-inhibiting agent than cortisone in the rat, milligram for milligram.

c. Castrated females exhibited the greatest proportional inhibition of resorption.

d. Normal female rats exhibited the greatest absolute inhibition of resorption.

e. Hypex rats exhibited the least inhibition of resorption. It was as though the corticoids are far less able to inhibit or "dampen" biochemical reactions proceeding at basal rates in Hypex rats than the relatively active reactions occurring in animals with intact hypophysis.

f. Many uncastrated male rats and about 10 percent of the uncastrated female rats *appeared* to be immune to the resorption inhibiting effect of the corticoids, no alteration in the pattern of the chondro-osseous complex being discernable. However, since there was a retardation of growth in bone length in these animals especially from the larger dosage of corticoids, the apparently normal

Figure 4

Experiment 8, section and stain as in Figure 2. Animal given 2.0 mg. hydrocortisone S.C. daily for five days. Marked thickening of the trabeculae of the chondro-osseous complex has occurred as a result of inhibition of resorption.

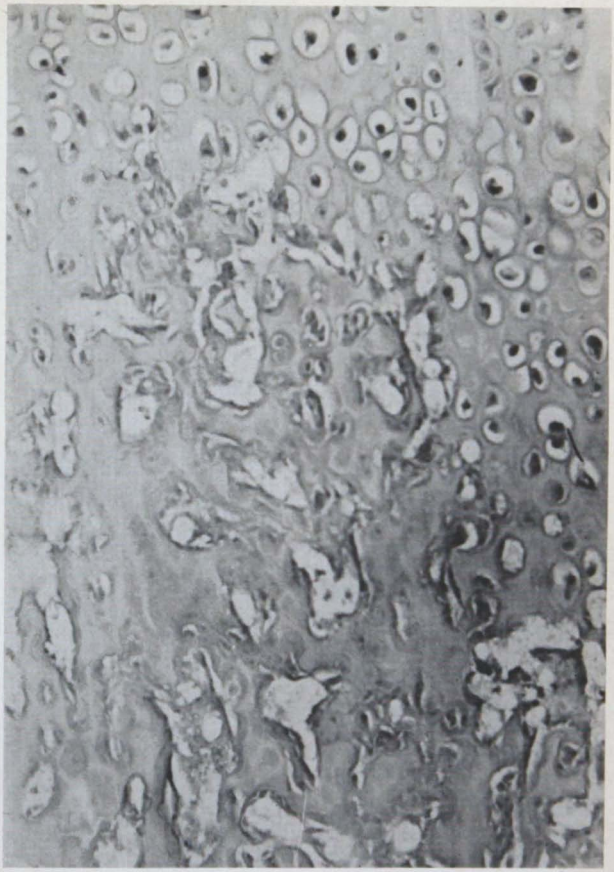

Figure 5

Experiment 13, otherwise identical to the animal in Figure 4. Again increased lateral width of trabeculae is noted as the result of hydrocortisone injection. There is more disorganization of the cartilage lacunae than in Figures 3 and 4.

proportions of chondro-osseous complex to bone length indicated that resorption was retarded in these animals too, but the retardation was equal to the retardation affecting formation. If resorption had not been retarded the chondro-osseous complex would have become thinner — which it did not.

g. The S.C. route led to greater inhibition of osteoclasia than the I.P. route.

h. See Figures 1-8. These figures illustrate the effects listed above.

i. We have made no effort in this study to observe the effects of internal adjustments in the endocrine milieu to the drugs given. The dosage of experiments were selected to minimize these adjustments.

Discussion

Unless there are other effects of the adrenal corticoids on osteoclasia than we have observed with our experimental design, it seems logical to adopt the working hypothesis that corticoids inhibit resorption of bone. There is additional evidence from human material which makes this hypothesis more acceptable but which is part of another study to be presented separately.

Assuming this hypothesis is correct, certain pathophysiological states in man may be interpreted tentatively.

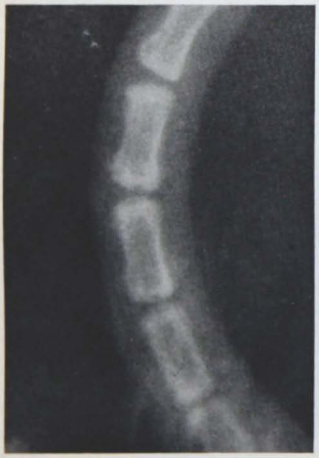

Figure 6a

Experiment 5. X-ray of tail of a rat taken the day daily injection of cortisone was begun. Note the extent of end-plate density. Cephalad is down.

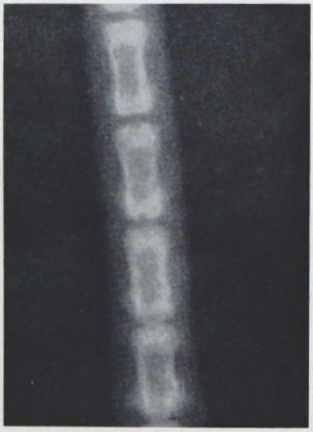

Figure 6b

X-ray of tail of same animal six days later after daily I.P. injections of 2-0 mg. cortisone acetate for six days. There is increased density and increased length of the distal chondro-osseous complex in each segment. Cephalad is down.

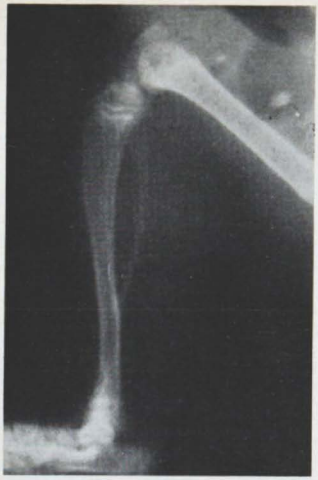

Figure 7a

Experiment 18, hypophysectomized animal. X-ray of tibia in standard position on the day when cortisone injections were begun. Note apparent width of epiphyseal plate and particularly the density just distal to the proximal tibial epiphyseal plate and proximal to the distal plate. Cephalad is up.

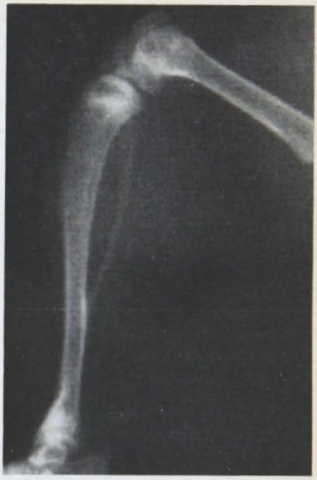

Figure 7b

Same tibia after seven daily injections of 1.0 mg. cortisone acetate I.P. Note that the chondro-osseous complex at both ends of the tibia is noticeably longer and denser. Growth in length of tibia in hypex animals is little to moderately depressed in comparison to the depression seen in normal animals given comparable doses of corticoids.

a. Osteoporosis of Cushing's disease: The basic resorption effect of the high level of endogenous corticoids in this disease should be inhibition of resorption. We have shown that lamellar bone formation is also inhibited by these agents.[14] Relatively speaking, inhibition of formation is greater than inhibition of resorption, with the result that a negative skeletal balance ensues which leads to osteoporosis. The osteoporosis occurs in the face of decreased resorption, an apparent contradiction which leads to the following paragraph. Although this interpretation may startle some readers, it has already been sugegsted by Sissons.[13]

b. An osteoporosis is the result of a negative skeletal balance. That is, there is is more bone being resorbed than is being formed. However, a state of balance between two reaction rates is not the same thing as an absolute change in either rate. In other words, a state of balance and an absolute rate are not identities in the mathematical sense. It is no more justifiable to deduce the nature of an absolute change in rate from a balance in the absence of other data than it is to deduce the total national income from the face of the moon; these two things are not identities either.

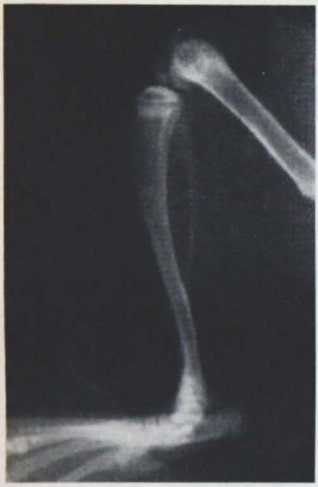

Figure 8a

Normal female rat, x-ray of tibia in standard position. Taken at beginning of observation period. Note the density and length of the chondro-osseous complex at the proximal tibia. Cephalad is up.

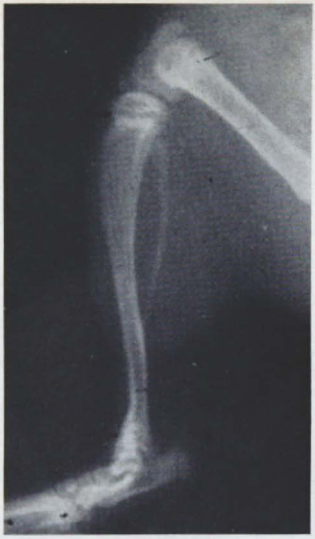

Figure 8b

Same animal as in 8A, after one week of observation but no cortisone. The x-ray technique is slightly "softer". There is no essential difference in 8A and 8B. The animal was started on cortisone the same day this x-ray was taken.

Yet this is a prevalent error in interpreting skeletal physiological events and has led to much erroneous formulation of mechanisms. This error occurs in other fields, particularly in biochemistry.

c. When a patient, placed on corticoids in pharmacodynamic doses, goes into negative calcium balance, this is probably the result of an *unequal* inhibition of both bone formation and bone resorption. Until some method of measuring one or the other absolute rate in vivo is developed any other conclusion is less logical than the one we outline here. In interpreting these events keep in mind that the effects of adjustments in function of other endocrines are not considered here but are probably important in the patient.

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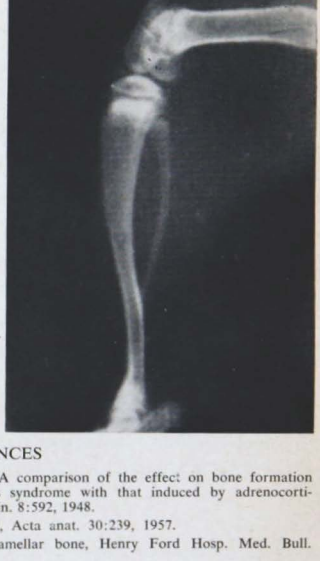

Figure 8c

Same animal as in 8A, after one week of daily I.P. injection of 2.0 mg. hydrocortisone acetate. There is now considerable increase in length and density of the chondro-osseous complex. The scale of these illustrations is not constant so that changes in length should not be estimated from these photos. There was + 50% reduction in growth in length in 8c compared to 8B, when compared to linear growth during the interval from 8A to 8B.

REFERENCES

1. Barter, F. C., Forbes, A. P., and Albright, F.: A comparison of the effect on bone formation of the hyperadrenocortisonism of Cushing's syndrome with that induced by adrenocorticotrophic hormone (ACTH), J. Clin. Endocrin. 8:592, 1948.
2. Cretin, A.: Les deux parties de l'os enchondral, Acta anat. 30:239, 1957.
3. Frost, H. M.: Observations on fibrous and lamellar bone, Henry Ford Hosp. Med. Bull. 8:199, 1960.
4. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.
5. Frost, H. M.: Staining of fresh undecalcified thin bone sections, *Ibid.* 34:135, 1959.
6. Frost, H. M., Roth, H., Villenueva, A. R., and Stanisavljevic, S.: Experimental multiband tetracycline measurement of lamellar osteoblastic activity, Henry Ford Hosp. Med. Bull. 9:313, 1961.
7. Frost, H. M., and Villanueva, A. R.: Human osteoblastic activity. I. Comparative method of measurement with some results, *Ibid.* 9:76, 1961.
8. Follis, R. H.: Effect of cortisone on growth of bones of the rat, Proc. Soc. Exper. Biol. & Med. 76:722, 1951.
9. Heaney, R. P., and Whedon, G. D.: Radiocalcium studies of bone formation rate in human metabolic bone disease, J. Clin. Endocrin. & Metab. 8:1246, 1958.
10. Nordin, B. E. C.: Side effects of systemic adrenal steroid therapy, Brit. J. Derm. 72:40, 1960.
11. Roth, H., Stanisavljevic, S., Villanueva, A. R., and Frost, H. M.: Effect of estrogen on lamellar bone formation in the rat, Henry Ford Hosp. Med. Bull. (In press)
12. Singer, L., and Armstrong, W. D.: Retention of Ca^{45} in the stable bone fraction during dietary calcium restriction, Arch. Biochem. & Biophys. 80:410, 1959.
13. Sissons, H. A.: Osteoporosis of Cushing's disease, *In*, Bone as a Tissue, ed. by K. Rodahl, J. T. Nicholson and E. M. Brown, Jr. New York, Blakiston, 1960.
14. Stanisavljevic, S., Roth, H., Villanueva, A. R., and Frost, H. M.: Effect of adrenal corticoids on lamellar bone formation in the rat, Henry Ford Hosp. Med. Bull. (In press)