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EFFECT OF ADRENAL CORTICOIDS ON LAMELLAR BONE FORMATION RATE IN RAT DIAPHYSIS

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Introduction

It is well known that high dosage of corticoids over long times lead to osteoporosis in man. [15] There is little indisputable factual basis for the beliefs that this osteoporosis develops as the result of an absolute increase in the rate of resorption or as the result of an absolute decrease in the rate of new lamellar bone formation. The reason for this deficiency is twofold.

First, methods of direct observation of the amounts of bone formed or resorbed in unit time were not formerly available. The available methods all involved one or more assumptions which rest often on uncertain ground. This lack has been corrected by the publication of a multiband tetracycline labelling method.[9]

Second, a change in skeletal balance and a change in an absolute rate of formation or resorption are not identities, so that no fixed relationship of the one to the other may be assumed.[7] This assumption is nevertheless widely made.

Although it is obvious that the absolute sign of the effect of a hormonal agent on lamellar bone formation rate is an essential item of information in formulating skeletal physiological dynamic concepts, this information is not currently available in undisputable form. A study in which decreases in osteoid seams were measured in patients receiving corticoids[8] has the potential flaw that the activity of individual seams was not measured, merely their number in a unit volume of bone. In a companion paper, it was noted that considerable change in activity of a single seam does occur.[6] Although an absolute decrease in lamellar bone formation as a result of cortisone administration to man may be inferred from these studies, this is not conclusively established.

Another study by Garrett, Collins and Johnston[10] with isotopic calcium in dogs clearly reveals the nature of the problem; the data of these authors may be explained with equal facility by assuming increased or by assuming decreased rate of formation of lamellar bone, as long as suitable assumptions about resorption rates are also made. There is nothing in their data which conclusively establishes the nature of the absolute rate change of either formation or resorption.

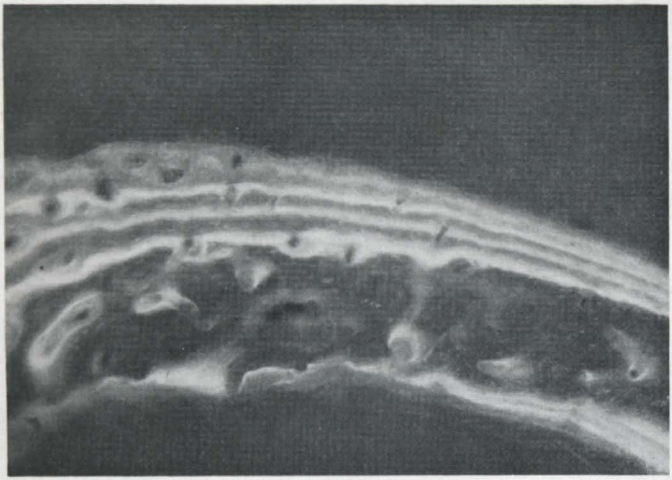

Figure 1

Experiment 4, control rat, tetracycline-triple-banded. Undecalcified cross section femur, periosteal surface above and endosteal below. The two unlabelled bands separating the three bright lines of tetracycline are the control (lower) and experimental (upper) bands. Each bright line is termed a label.

Our study was undertaken to determine, if possible, the absolute sign of the effect of cortisone on lamellar bone formation rate. By absolute sign, we mean acceleration, retardation or no effect. Quantitation of the effect was left for later work. By rate, we mean the absolute volume of new bone formed in unit time in a unit absolute volume of the skeleton. By absolute volume, we mean the volume of bone remaining after all of its marrow, vascular, lacunar and canalicular spaces have been subtracted from the macroscopically observed whole. Reasons for these definitions have been discussed in detail by Robinson and Elliott,[13] Robinson,[12] and Frost.[2,5,7]

We believe our studies have yielded a clear solution to the above problem. The results are presented in summary for the benefit of others, particularly those who wish to duplicate the work.

Materials

White, Norwegian rats of pure strain obtained from Holtzman Rat Company, Wisconsin, were studied. They were fed Rockland complete rat diet and water ad libitum. They were kept in cages in an animal room with controlled humidity, temperature and cycling of light and darkness.

Both cortisone acetate (Upjohn) and hydrocortisone acetate (Upjohn) were used in this study. About one-half of our hypophysectomized rats were donated, courtesy of Upjohn Company, Kalamazoo, Michigan.

METHODS

This work was designed to yield one item of information: The absolute sign of the effect of hormonal agents on rates of lamellar bone formation and resorption. Validation of experimental results by statistical methods contains an element of uncertainty which might devalue conclusions so derived. We elected to seek and accept as valid only obvious effects that a photograph or photomicrograph would reveal at a glance. It may be appreciated that potentially useful observations had to be discarded following adoption of this policy. We also found that methodology became sharply restricted, there being few techniques which yield consistent and unequivocal results in a field such as bone physiology.

One of the troublesome sources of error in experimental work on living animals is the existence of undetected — and undetectable — variables in single experiments. To avoid this, we performed large numbers of experiments on differing small lots of animals at different times, using differing personnel, using minor variations in experimental procedure and using a large total number of animals. In this way, reproducibility was assured. The experiments reported were done over an 18 month period, consumed over 200 rats and comprised four (4) basic experiments which were repeated several times with variations each time. All non-hypophysectomized rats were postpubertal.

The production of lamellar bone was observed by several means, all but one of them eventually being discarded because it seemed to us they lacked the one factor we wished most: unequivocal and direct demonstration of corticoid effect. The method on which the conclusions of this paper are drawn is the multiband tetracycline labelling method reported previously by Frost, Villaneuva and Stanisavljevic.[9]

The methods used were:

A). Multiband tetracycline labelling techniques already mentioned.[9] In this method, three bands of tetracycline are deposited in forming circumferential lamellae by I.P. administration of one of the tetracycline antibiotics at three equally separated moments during the experimental period. These labels appear by fluorescence microscopy on especially prepared undecalcified sections[4] much like growth rings in a cross section of a tree stump. The distance between bands is a measure of the rate of new bone formation under these circumstances. This method permits direct observation, direct and quantitative measurement of new lamellar bone formation rate under controlled circumstances. The method is unequivocal and effects observed may be accepted as conclusive. Interpretation may be otherwise. Tibias and femurs were studied with this method.

B). Measurement of diaphyseal outside diameter on undecalcified cross sections cut accurately perpendicular to the long axis of the bone, and on x-rays taken at various times during the experiments. There was too much individual viariation with this method to make it conclusive.

C). Measurement of the numbers of osteoblasts lining lamellar bone surfaces in decalcified, haematoxylin and eosin stained sections cut both longitudinally and transversely through the diaphysis. This proved a misleading method and was discarded.

D). Ash analyses of the diaphysis were not done for reasons discussed by Roth.[14] In brief, these analyses are frequently misleading indices of new bone formation rate due to the fact that ash and average bone age, in the sense of the days a given moiety of bone has been in existence, are interrelated so that correlation with bone formation rate cannot be done without correlation with absolute bone volume and remodelling rate. In addition, bone mass as opposed to ash is related as much to the balance between formation and resorption as it is to the amount of new bone formed in unit time. Therefore, mass of bone is not an acceptable index of new bone formation rate.

Measurements of the metaphyseal and enchondral ossification areas were not done because these areas are associated with fibrous bone formation as pointed out by Roth[11], Cretin[1] and by Frost.[5,7] Incorporation rates of isotopes were not considered because the diffuse uptake of isotopes interferes with the measurement of that portion of the isotope fixed in newly mineralizing bone matrix. Also, it is difficult to separate that portion of isotope fixed in fibrous bone from that fixed in lamellar bone. Finally, diffuse uptake in growing animals is considerably larger in quantity than the isotopic uptake due to new bone formation. These matters are related to remodelling rate, to diffusion impedence and to average bone age in the sense previously given, and are discussed in detail elsewhere.[2,5,7]

The following type-experiments were done: (1). Normal female rats compared to normal female controls. (2). Normal male rats compared to normal male controls. (3). Castrated female rats compared to castrated female controls. (4). Castrated male rats compared to castrated male controls. (5). Hypex (hypophysectomized) rats compared to hypex controls regardless of sex. These rats were commercially hypophysectomized at 50 gm. weight level and 80 of them were consumed.

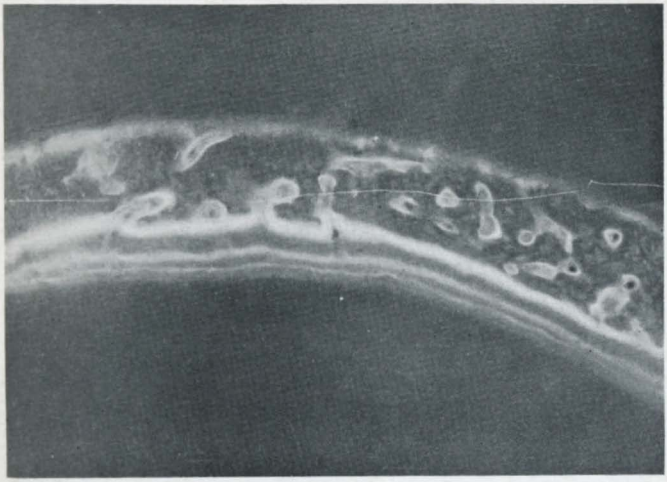

Figure 2

Experiment 4, cross section femur, rat given 1.0 mg. cortisone acetate daily during time the experimental band of bone was being formed. Periosteal surface above, endosteal below. Lack of periosteal lamellar bone formation is not due to medication, but to the part of the section illustrated. There is pronounced inhibition of lamellar bone formation on the endosteal surface during the experimental period.

Drugs were administered as follows: (a). 1.0 or 2.0 mg. of cortisone acetate I.P. daily during the experimental period. Four experiments, 90 rats. (b). 2.0 mg. cortisone acetate daily S.C. during the experimental period. Four experiments, 40 rats. (c). 1.0 or 2.0 mg. hydrocortisone acetate daily during the experimental period. Some experiments administered I.P. and some S.C. Five experiments, 91 rats. (d). 1.0 mg. cortisone acetate daily plus 1.0 mg. estradiol benzoate daily in castrated female rats. Two experiments, 18 rats.

The timing of experiments was 7 day control and experimental bands in most and 10 day bands in 15 per cent of the experiments. These periods are brief in comparison to periods adopted by other authors.

The standard bone studied was the tibia. In some experiments, additional bones were studied, these bones being the parietal bone of the skull, humerus, femur and vertebra.

Results

In brief form, the following were the reproducibly observed facts: (a). Cortison acetate retards the rate of lamellar bone formation (Figures 1, 2, 3). (b). Hydrocortisone acetate also retards lamellar bone formation, but to greater degree than cortisone acetate. (c). Absolute retardation of lamellar bone formation by corticoids is greatest in the non-castrated female and least in the non-castrated male. Relative retardation is greatest in the castrated female. (d). Retardation of the basal rate of lamellar bone formation present in hypex rats is minimal, and is obvious in only one-half of the animals studied. (e). The number of osteoblasts

lining various bone surfaces is a poor index of the rate of new lamellar bone formation, there often being an increase in the number of osteoblasts in the presence of decrease in the total amount of new bone elaborated in unit time. (f). As many others have noted, cortisone administration was associated with retardation in weight gain, or with loss of weight, with retardation in longitudinal bone growth, with thinning of the germinal layer of the epiphyseal plates. (g). The adverse effects of the corticoids were more evident in our animals when given S.C. than when given I.P.

Discussion

The importance of the present study, whose findings are illustrated in Figures 2 and 3, lies in direct demonstration of the sign of the effect of corticoids on lamellar bone formation. It is understood that the nature of any adjustments of the endocrine milieu to our experimental procedure, and the nature of these effects on bone, are not considered here.

With this knowledge, it is possible to interpret the data of authors such as Rich, Ensinck and Fellows.[11] Using a continuous infusion technique and Ca^{45} and Sr^{85}, these authors showed that less skeleton exchanged with the blood in osteoporosis resulting from cortisone administration than in osteoporosis of the senile type. The

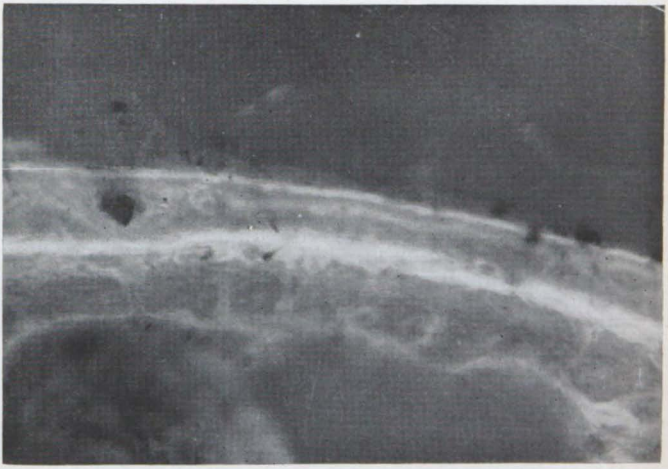

Figure 3

Experiment 14. Cross section femur, rat given 2.0 mg. hydrocortisone acetate daily for 7 days after an initial like period without medication. Periosteal surface at top.

There has been a pronounced decrease in lamellar bone formation during the experimental period. Lack of endosteal bone formation is not due to the drug, but to the part of the section illustrated. Both periosteal and endosteal lamellar bone formation are affected by cortisone, however.

inference from the present study is that cortisone retards the rate of formation and thus of mineralization of new lamellar bone matrix, leading to less skeletal isotope uptake. In addition, it appears that the increased average age and increased average diffusion impedence[2,7] accompanying suppression of lamellar bone formation similarly reduces diffuse exchange of the isotope with the skeleton.

In many types of isotopically based experiments, interpretation of the observed results has been the most difficult problem and one in which there has been the least agreement. The reason is that a reduction in skeletal uptake may be interpreted as the result of increased return of isotope from bone to blood by resorption as well as by decreased bone formation. There are several camps at present, divided over this one point. We feel our evidence makes it reasonable to incorporate a depression of lamellar bone formation induced by corticoids into skeletal physiological dynamic thought, and that the converse is illogical.

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REFERENCES

1. Cretin, A.: Les deux parties de l'os enchondral, Acta anat. 30:239, 1957.
2. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. Med. Bull. 8:36, 1960.
3. Frost, H. M.: Observations of fibrous and lamellar bone, *Ibid.* 8:199, 1960.
4. Frost, H. M.: Preparation of fresh thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.
5. Frost, H. M.: Osteoporosis; a hard look, J. Am. Geriatrics Soc. 8:568, 1960.
6. Frost, H. M.: Human osteoblastic activity. II. Measurement of biological half life with some results, Henry Ford Hosp. Med Bull. 9:87, 1961.
7. Frost, H. M.: A model of endocrine control of bone remodelling, *Ibid.* 10: 1962.
8. Frost, H. M., and Villanueva, A. R.: Human osteoblastic activity. I. Comparative method of measurement with some results, *Ibid.* 9:76, 1961.
9. Frost, H. M., Roth, H., and Stanisavljevic, S.: Experimental multiband tetracycline measurement of lamellar osteoblastic activity, *Ibid.* 9:312, 1961.
10. Garrett, E., Collins, E. J., and Johnston, R. L.: Effect of steroid regimen on Ca^{47} distribution, excretion and resorption from bone in young beagles, (Paper submitted to the Gordon Conference on Structure and Physiology of Bones and Teeth, Meriden, N. H., July 1961.)
11. Rich, C., Ensinck, J., and Fellows, H.: Use of continuous infusions of Ca^{45} and Sr^{85} to study skeletal function, J. Clin. Endocrin. & Metab. 21:611, 1961.
12. Robinson, R. A.: Chemical Analysis and Electron Microscopy of Bone, *In*, Bone as a Tissue, New York, Blakiston, 1960.
13. Robinson, R. A., and Elliott, S. R.: Water content of bone. I. Mass of water, inorganic crystals, organic matrix, and "CO_2 Space" components in a unit volume of dog bone, J. Bone & Joint Surg. 39A:167, 1957.
14. 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)
15. Turner, C. D.: General Endocrinology, ed. 3, Philadelphia, Saunders, 1960.