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Lack of Effect of Growth Hormone on Deposition of Radiostrontium in Bone.*

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The pituitary growth hormone is known to play an important role in the regulation of processes concerned with bone formation. Deposition of minerals in the skeletal system represents one phase of this complex phenomenon. The experiments reported here were carried out in order to determine whether mineral deposition in bone is influenced by growth hormone. Pecher, in experiments on the deposition of radioactive isotopes of calcium and strontium, has shown that the distribution of these two elements in body tissues is similar.[1] In the present experiments, radiostrontium was employed, since it is more easily available and the methods for its measurement are more practical.

In order to exclude possible effects of other pituitary factors, hypophysectomized rats were used as test animals. Female rats were hypophysectomized at 26-28 days of age. In each experiment, the rats, after a post-operative interval that varied in different experiments from 10-20 days, were divided into two groups, one of which received daily intraperitoneal injections of growth hormone, the other group serving as control. 2-5 days after onset of the growth hormone treatment, radioactive strontium was given intraperitoneally to both the experimental and control groups. At varying intervals (8-48 hr) after the strontium administration, the animals were autopsied. Details of the experimental procedures are given in Table I. At autopsy, femurs and mandibles (including teeth) were freed from soft tissues, weighed and ashed (with HNO_3, followed by dry ashing in a muffle furnace). The radioactivity of each specimen was determined using the Lauritzen electroscope. Appropriate standards of radiostrontium were used for estimating the quantities of strontium deposited. Correction for self-absorption did not affect the results within the limits of error of the method.

The growth hormone preparations were "cysteine treated globulin fractions."[2] The preparations used in Experiments I, II, IV and V were contaminated with less than 1% of any of the 5 other "accepted" pituitary hormones; in the preparation used in Experiments III and VI no interstitial cell stimulating or adrenocorticotropic hormones were detected at a total dose of 3.0 mg, whereas a trace of thyrotropic activity was demonstrable at this level (4-day test in hypophysectomized rats). In none of the preparations used was lactogenic hormone found at a level of 10 mg (systemic crop test in squabs). The strontium lactate preparations employed contained radioactive strontium (Sr^{89}, half life 55 days).[4] Radioactivity of the preparations varied from 3 to 7 microcuries per mg (1 to 6 microcuries per total dose). The quantities of growth hormone and strontium given, and the duration of the injections are indicated in Table I.

Table II summarizes 6 experiments in which a total of 43 rats was used. In 5 out of 6 experiments, there was practically no

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1 Pecher, C., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 86.

2 Fraenkel-Conrat, H. L., Meamber, D. L., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1940, **27**, 605. (A modification of the procedure will be published in the near future.)

3 Marx, W., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1942, **30**, 1.

4 Stewart, D. W., Lawson, J. L., and Cork, J. M., *Phys. Rev.*, 1937, **52**, 901.

TABLE I.
Experimental Conditions.

Exper.	No. of hyp. rats		Growth hormone		Radiostrontium	
	Exp.	Contr.	Total dose, mg*	Period of injection, days	Total dose, mg	Period of injection, days
I	3	3	0.6	3	1.0	1
II	3	3	1.2	4	1.0	1
III	4	4	1.5	5	0.5	1
IV	3	4	1.8	3	0.3	1
V	4	4	2.0	8	0.5	4
VI	4	4	3.25	15	2.25	10

*The average potency of the growth hormone preparations varied from 30-50 growth hormone units per mg (10-day test in hypophysectomized rats).[3]

TABLE II.
Effect of Growth Hormone on Deposition of Radioactive Strontium in Bone.

Exper.	Group	Avg body wt gain in g	Strontium content in μg/100 mg ash			
			Femur		Mandible	
			Avg values	Range of values individual rats	Avg values	Range of values individual rats
I	Exper.*	5	32	27-38	21	17-25
	Contr.	0	31	30-31	18	17-19
II	Exper.*	9	40	37-42	25	24-27
	Contr.	—2	29	23-36	17	12-23
III	Exper.*	15	18	16-22	12	10-13
	Contr.	4	20	19-22	12	12-13
IV	Exper.*	4	14	12-14	—	—
	Contr.	—2	13	12-14	—	—
V	Exper.*	16	20	17-25	14	12-16
	Contr.	1	19	17-22	13	11-14
VI	Exper.*	20	75	65-85	—	—
	Contr.	—3	73	60-82	—	—

*Experimental groups received growth hormone in the amounts specified in Table I.

difference between growth hormone treated and untreated animals; the average values for the strontium content of femur and mandible were essentially the same, as were the ranges covered by the values for individual animals. The results of one of the 6 experiments might indicate some difference in the strontium depositions of treated and of control animals (Experiment II). This difference, however, was not considered significant, since a repetition of this experiment did not confirm these results (Experiment III). The quantity of strontium deposited in femur or mandible in the various groups was quite constant when calculated as per cent of the total amount injected, regardless of growth hormone administration, the amount of strontium injected, or within the limits of these experiments, the duration of treatment. The average values for the percentage deposited of the total strontium injected were femurs, 2.6% (range 2.0-2.9%); mandibles, 2.3% (range 1.8-2.7%).

It appears, therefore, in experiments of relatively short duration, that untreated hypophysectomized rats, in which growth stasis had been reached, deposited essentially the same amount of strontium in bone, as did rapidly growing animals treated with growth hormone. These results would appear to indicate that mineral deposition in bone as indicated by strontium metabolism is a reaction which, in these acute experiments, can proceed independently of the action of the

pituitary growth hormone, a principle which is generally assumed to control somatic growth. In agreement with this finding are observations by various authors that mineral exchange in the skeletal system may continue even after practical cessation of growth, such as is the case in old age, or, in young animals, after ablation of the pituitary.

Summary. Hypophysectomized rats treated with growth hormone and consequently in a state of rapid growth, deposited in femur and mandible, when injected with radioactive strontium, essentially the same amounts of strontium, as did untreated hypophysectomized control rats injected simultaneously with the same amount of radioactive strontium.

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Purification of Antigonadotropic Sera by Enzymatic Digestion.*

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It has been shown by Coghill *et al.*[1] that antitoxin in horse serum can be purified to a considerable extent without much loss of activity by digestion with the enzyme Taka-Diastase. Since the antitoxins as well as the antigonadotropic substances produced in the blood of animals over a long period with gonadotropic extracts are found in the globulin fraction of the serum, it was considered probable that the antigonadotropic serum could be purified by the same method. The results of our preliminary work support this view.

Antigonadotropic serum of the horse and the goat were used in this study. The horse serum was obtained from a mare pony which had been injected 3 to 6 times a week for 3 years with a crude sheep pituitary extract (SAP). The goat serum was obtained from an adult female goat which had been injected 5 to 6 times a week for 2 years with a purified pregnancy urine preparation (PU).

Both sera were digested with Taka-Diastase (obtained from Parke, Davis and Company, Detroit, Michigan) in a manner similar to that employed by Coghill *et al.*[1] One volume of serum was diluted with 2 volumes of distilled water and the pH adjusted to 4 by the addition of crystalline citric acid. Taka-Diastase in the proportion of 5 mg to each ml of undiluted serum was added together with a few drops of toluene. The mixture was shaken well and incubated for 96 hr at 37°C. During the digestion a large, flocculent precipitate was formed. At the end of 96 hr the mixture was filtered. The precipitate was removed from the filter paper, taken up in a volume of distilled water equal to that of the undiluted serum, and shaken well with glass beads to make a homogeneous suspension. This was filtered as before. The combined filtrates were dialyzed first against running tap water and then against several changes of distilled water.

This practically salt-free solution was made 40% saturated with ammonium sulfate and allowed to stand in the refrigerator for several hours. The precipitate (Fraction A) was removed by filtration through a hard filter paper (S&S 575). The filtrate was made 50% saturated with ammonium sulfate and, after standing, the precipitate (Fraction B) was removed as before. Assays showed that the antigonadotropic substance was present in fraction A of the goat serum, and

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1 Coghill, R. D., Fell, N., Creighton, M., and Brown, G., *J. Immun.*, 1939, **39**, 207.