Action of Parathyroid Extract on Bone Phosphorus and Calcium in the Rat.* (20717)

R. V. TALMAGE,† W. E. LOTZ, AND C. L. COMAR.
U. T.-A. E. C. Agricultural Research Program, Oak Ridge, Tenn.

Recent studies have confirmed the ability of parathyroid extract to produce a marked phosphuria both in the dog(1) and in the rat (2). The ability of the extract to raise serum calcium has also been reemphasized(3,4). However, while Talmage *et al.*(5) reported a slight but significant difference in the rate of uptake of radiocalcium by the bones of normal and parathyroidectomized rats, they were unable to demonstrate by autoradiographs any difference in the distribution of radiocalcium in the bones of normal, parathyroidectomized, or rats administered parathyroid extract. Tweedy *et al.*(6,7) noted some decreased uptake of radiophosphorus in the femurs of rats under parathyroid extract administration as compared to controls. However, localization of these differences to specific sites within the bone was not done.

The following work was undertaken to determine whether the marked phosphuria produced by injection of parathyroid extract was accompanied by any significant changes in the distribution of radiophosphorus and radiocalcium in the long bones of the rat; and to compare calcium and phosphate changes produced by the extract in bone, serum, and urine.

Materials and methods. Young male mature Wistar rats, weighing between 160 and 200 g were used in these experiments. All animals were given intraperitoneal injections of either 5 microcuries of P^{32} or 10 microcuries of Ca^{45}. They were then placed in metabolism cages for urine collection. Parathyroid extract‡ was administered subcutaneously in repeated doses over the experimental period. Urine collections were made at intervals during the experiment and blood was drawn for calcium and phosphate determinations immediately prior to sacrifice. The femurs used for autoradiographs were removed immediately after sacrifice. The following three experiments were run, each consisting of a duplicate series utilizing radiophosphorus (P^{32}) in one, and radiocalcium (Ca^{45}) in the other. *A.* The radioisotope was administered two hours before the injection of parathyroid extract. 500 i.u. of the extract were administered in three equal doses given at hourly intervals. All animals were killed ten hours after the first injection of the extract. *B.* The radioisotope was given 24 hours before the first injection of parathyroid extract. 300 i.u. of the extract were given over a seven hour period and the experiment was terminated 24 hours after the first injection of the extract. *C.* The radioisotope was given 24 hours before the first injection of parathyroid extract. Each experimental animal received 300 i.u. daily for two days (total 600 i.u.) and was killed 48 hours after the first injection of the extract. Radioactivity determinations were made by standard procedures with a Geiger-Mueller counter. Serum calcium determinations were made using Clark-Collip's Modification of the Kramer-Tisdall Method. Urinary and serum phosphate determinations were made by the method of Fiske and Subbarow.

For autoradiographs, the bones were cut longitudinally (medial sagittal section) into approximately equal halves. The bones were then placed in an X-ray cassette and exposed on Kodak, type A X-ray film. The cassettes were placed in presses to insure uniform contact between the bones and the film(8). Since the bones from a given experiment were exposed simultaneously to the same sheet of film, quantitative comparisons were readily obtained. The densitometric measurements were recorded from a Welsh Densichron.

Experimental data. The data from these experiments are tabulated in Table I. The various changes produced by parathyroid ex-

* Published with permission of the Director of the University of Tenn. Agric. Sta.

† Oak Ridge Institute of Nuclear Studies (Research Participant from Rice Institute, Houston, Texas).

‡ Supplied through the courtesy of Eli Lilly and Co., Indianapolis.

TABLE I. Effect in Rats of Parathyroid Extract on Blood Levels, Excretion and Removal from Bone of Calcium and Phosphorus.

Description		Terminal serum values, mg/100 ml Ca	PO_4	Urinary excretion† of radioisotope Ca^{45}	P^{32}	Urinary excretion of PO_4, mg	Optical densities† of bone radioautographs Ca^{45}	P^{32}
A. 500 i.u. PTH ext. given 2 hr after isotope dosage, animals sacrificed 10 hr later.	Controls*	12.1	10.2	100	100		100	100
	Exp.*	14.3	7.1	25	2900		100	84
B. 300 i.u. PTH ext. given 24 hr after isotope dosage, animals sacrificed 24 hr later.	Controls*	10.5	8.3	100	100	.79	100	100
	Exp.*	14.4	7.2	160	4620	17.5	87	78
C. 600 i.u. PTH ext. given 24 hr after isotope dosage, animals sacrificed 48 hr later.	Controls*	11.9	8.3	100	100	1.68	100	100
	Exp.*	13.6	12.9	220	3523	33.1	77	41

* 4 rats used for calcium and 4 for phosphorus.

† For ease of comparison, values for control animals have been set at 100 and experimental corrected to this.

tract in bone, serum and urine are summarized below.

Phosphate and P^{32} changes. It may be noted that an immediate and very marked increase in phosphate excretion by the kidneys occurred in all animals receiving hormonal treatment. The average daily excretion of $PO_4^{\equiv}$ by the control rats used in these experiments was approximately 1 mg. The average serum inorganic phosphate value was determined to be about 8.5 mg %. Rats, of the weight used, have a total serum volume of less than 10 ml. Therefore, under these experimental conditions it appears that the control rats excreted daily, through the kidneys, an amount of $PO_4^{\equiv}$ equivalent to the total amount of inorganic phosphate present in the serum. With the addition of parathyroid extract, this excretion increased to between 15 and 25 mg during the first 24 hours of treatment. It is obvious that this amount of phosphate must have come from sources other than inorganic serum phosphate.

Following radiophosphorus administration, the major portion of the radioactivity taken up by the long bones is found to be deposited immediately below the epiphyseal plate, that is in the region of the primary spongiosa. This distribution can be seen as early as one hour after injection of the radiophosphorus and changes thereafter in intensity only, reaching its peak in approximately 24 hours. Following parathyroid extract administration there is a comparatively rapid removal of this radioactivity from the epiphyseal region of the long bones. This loss is significant by 10 hours after the start of parathyroid extract administration (Table I). After 48 hours of treatment, the loss of radiophosphorus from the epiphyseal region is of such magnitude that densitometric measurements show a loss of over 50%, and examination of the radioautographs shows the radioactivity in sections of the epiphyseal region to be reduced to the level of the remainder of the bone (Table I, Plate I). Since the radiophosphorus is diluted considerably with stable phosphorus upon reaching the bone, the removal of the activity indicates the removal from the bone of a considerable amount of phosphate.

When given in sufficient quantity, parathyroid extract will produce in most animals a rise in serum calcium values and a corresponding decrease in serum phosphate levels. This was found to be true here with one important difference. In all determinations made, the serum phosphate value fell during the first 24 hours, being significantly lower by 10 hours. However, as can be seen in Table I, Experiment C, a majority of the animals carried through two days of high level parathyroid extract treatment, showed a terminal serum

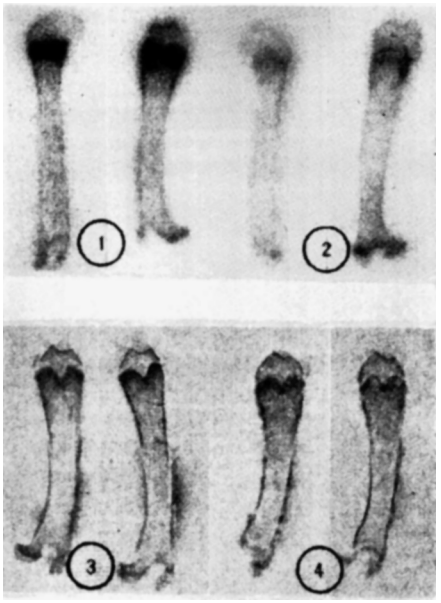

PLATE I. Removal of radiophosphorus and radiocalcium from the femurs of rats by parathyroid extract. Radioactivity given 24 hr before beginning of parathyroid extract administration. Animals killed 48 hr after first inj. of extract.

FIG. 1. Femurs of control rats showing P^{32} distribution.

FIG. 2. Experimental animals showing the marked removal of P^{32} from the subepiphyseal region.

FIG. 3. Control rats showing Ca^{45} distribution.

FIG. 4. Experimental animals showing some removal of Ca^{45} from the subepiphyseal region.

phosphate level which was well above normal (average 12.9 mg %). These animals also showed an increasing antidiuresis and a decreasing output of phosphate in the urine during the second 24-hour period. These effects were probably due to calcium precipitation in the kidney, interfering with its function, a point which will be discussed in more detail below. It is important to note that the output of phosphates during the second 24-hour period was still at least ten times that of the controls, showing that despite the increased (over normal) rate of $PO_4^{\equiv}$ excretion, this ion was entering the serum faster than it was being eliminated, causing an elevation in serum phosphate levels.

Calcium and Ca^{45} changes. Following parathyroid extract administration, radiocalcium excretion values by the kidney dropped over the first 10 hours, followed by a gradual increase, so that at both the 24-hour and 48-hour period, the extract-treated animals showed a slightly increased urinary excretion of Ca^{45} (Table I). This eventual increase in the rate of excretion of calcium by the kidney has been explained in the past as resulting from the elevation of the serum calcium level.

Radiocalcium, once deposited in the bone, was not so easily removed as was the radiophosphorus. The distribution of this radioelement in the bone is essentially the same as that of radiophosphorus. It can be seen from Table I that no evidence of removal was apparent by 10 hours after parathyroid administration, but that by 24 hours there appeared to be sufficient removal to be detected by examination of the autoradiographs. During the second 24 hours, the removal of radiocalcium from the epiphyseal region became more noticeable, but did not begin to compare with the removal of radiophosphorus. The radioautographs of the femurs of animals treated for 48 hours are shown in Plate I, Fig. 3 and 4. During the second 24-hour period calcium was deposited in the kidney cortex. The deposition of calcium was determined on removal of the kidney, both by chemical and radiochemical means. The radioactivity measurements showed an activity in the kidneys of the experimental animals of 365 times that of the controls. Total calcium determinations showed that approximately 12 mg of calcium had been deposited in the kidneys. Similar measurements taken after the first twenty-four hours showed no significant difference between the two groups.

Discussion. It is apparent that parathyroid extract, when given in comparatively large doses is able to remove considerable amounts of calcium and phosphate from the bone. Based on the removal of radioactivity, it would appear that phosphate is removed to a greater extent than is calcium. This is further indicated by the fact that for the first 24 hours almost 20 mg of $PO_4^{\equiv}$ were lost through the kidney, while the corresponding loss of calcium through the kidney or by deposition in the kidney was negligible. However, much of the apparent difference in the movement of these two ions from the bone may possibly be explained by the phenomenon of exchange. If it

is supposed that a function of parathyroid extract is to dissolve bone or at least to place these two ions in a chemical condition where they are more readily exchangeable, then the greater removal of radiophosphorus may be due to the fact that there is a large pool of phosphate outside of the bone compared to little or no calcium, allowing much of the radiophosphate to exchange with stable ions. This is further indicated by the fact that the extract increased the excretion of inorganic phosphate by a factor of 20, while the increase in radiophosphate excreted was by a factor of 40. The differences in the increase in urinary excretion of calcium and phosphate may be partially explained by the removal of phosphate from sources other than bone and by possible changes in intestinal excretion not yet studied. However, the possibility that phosphate can be moved in and out of bone at a rate different than that of calcium is a point that cannot be dismissed lightly and deserves further study.

Of prime interest is the bearing of this study on the site of action of the hormone of the parathyroids. Since this study utilized an extract of the parathyroids which admittedly is far from pure, caution must be taken in assuming that all the results attained are normally a function of the parathyroid hormone. However, Kraintz *et al.*(9) have reported a similar study with radiophosphorus using normal and parathyroidectomized rats in which the conditions presented here are essentially reversed, indicating that these results can basically be attributed to the hormone of the parathyroid gland.

With the reservation of possible side effects from the use of a crude extract, the results presented here may be interpreted to show that the parathyroids exert a *direct* effect on bone metabolism as well as an *indirect* effect produced by the increased excretion of phosphate through the kidney. Evidence for this hypothesis is the terminal high serum phosphate values. It would seem that should the effect be on the kidney alone, the serum phosphate level would be expected to remain at normal level or lower as long as the phosphate excretion by the kidney was elevated above normal. In these experiments, during the second day of prolonged high level administration of parathyroid extract, the phosphate excretion was restricted due to a rather severe anti-diuresis probably due to deposition of calcium. The excretion of phosphate was, during this period, still about 10 times normal. The serum phosphate levels, however, rose considerably above normal, indicating that inorganic phosphate was pouring into the serum at a faster rate than it was being excreted. It seems plausible to conclude, therefore, that parathyroid extract directly affects phosphate metabolism both in the kidney and the bone, possibly through a similar enzyme system.

Summary. 1. Effect of parathyroid extract upon removal of calcium and phosphate from the long bones of rats was studied by the use of radiocalcium and radiophosphorus. Autoradiographs showed that significant amounts of both phosphate and calcium could be removed from the subepiphyseal plate region of the long bones of rats by parathyroid extract administration; the amount of radiophosphorus removed was greater than the radiocalcium. 2. These experiments indicate further that parathyroids act directly on bone to produce this removal of calcium and phosphate, and that this effect is enhanced by an additional effect on the kidney whereby the excretion of phosphate is increased. This assumption is based on the fact that serum phosphate values, which are initially reduced by parathyroid extract can be raised above normal by extended treatment of the extract. This increase in serum phosphate levels was accomplished while kidney excretion of phosphate was still approximately ten times the normal daily output, indicating that phosphate was entering the blood at a faster rate than its withdrawal, presumably primarily from the bone.

1. Talmage, Roy V., Buchanan, G. D., and Kraintz, F. W., XIX International Physiological Congress, 1953, p. 820.

2. Rubin, Betty L., and Dorfman, R. I., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 223.

3. Munson, P. L., Kenny, A. D., and Ison, O. A., *Fed. Proc.*, 1953, v12, 249.

4. Talmage, R. V., Kraintz, F. W., Frost, R. C, and Kraintz, L., *Endocrinology,* 1953, v52, 318.

5. Talmage, R. V., Kraintz, F. W., and Kraintz, L., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 553.

6. Tweedy, W. R., and Campbell, W. W., *J. Biol.*

Chem., 1944, v154, 339.
7. Tweedy, W. R., Chilcote, M. E., and Patras, Mary C., *ibid.*, 1947, v168, 597.
8. Lotz, W. E., Gallimore, J. C., and Boyd, G. A., *Nucleonics*, 1952, v10, 28.
9. Kraintz, F. W., Kraintz, L., and Talmage, R. V., in preparation.

Received October 28, 1953. P.S.E.B.M., 1953, v84.

Bioassay of Mouse Pituitary Gonadotropin by Induced Ovulation in Pregnant Mice. Evaluation and Biologic Application.* (20718)

AARON J. LADMAN,† JOY E. PALM,‡ AND MEREDITH N. RUNNER.
(Introduced by D. W. Fawcett.)

From the Department of Anatomy, Harvard Medical School, Boston, Mass. and the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

The induced ovulatory reaction in pregnant mice has been suggested by Burdick and Whitney(1) as a bioassay for gonadotropins. We have recently shown this reaction to be a sensitive assay for gonadotropin content of the mouse pituitary(2), and have used it to show changes in synthesis and release of gonadotropin by the anterior pituitary during gestation(3). This report presents data on the responsiveness of the ovaries of pregnant mice to injections of a commercially standardized preparation of human chorionic gonadotropin. In addition, the differences in the responsiveness of the ovaries to injected homogenates of mouse pituitaries obtained at parturition and at the first postpartum ovulation are shown.

Materials and methods. Swiss female mice, 3 to 9 months of age and approximately 14 to 19 days pregnant, were used for assay animals. The care of animals and the procedure of bioassay of mouse pituitary for gonadotropins has been described earlier(2), so that they need only brief mention here. Commercially standardized human chorionic gonadotropin (HCG)§ was employed for the determination of the responsiveness of the pregnant animals' ovaries. The gonadotropin was made up in the desired concentrations in physiological saline solution and injected intraperitoneally in a uniform volume. Pituitary glands to be assayed were collected from animals which had been used in a previous study to determine the relationship between the time of parturition and ovulation(4). These glands were dehydrated in acetone, desiccated in vacuum and stored. Preparatory to assay they were homogenized, suspended in saline and serially diluted. The homogenates were injected intraperitoneally. The frequency with which ovulation was observed in groups of pregnant assay animals was adjusted by the statistical procedure recommended by Reed and Muench(5). This technic has the effect of augmenting the number of responses at the dosages on either side of the 50% end point. Since this design partially offsets chance variation, the method defines the end point more precisely than would otherwise be possible.

Results. The ovulatory responses of 144 pregnant female mice which received HCG in varying amounts are summarized in Table I. The dose of HCG that would have been required to produce a response in 50% of the animals has been interpolated from the adjusted values. This calculated 50% end

* This work was done at the Roscoe B. Jackson Memorial Laboratory and was supported by a grant-in-aid to that Institute from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council.

† Fellow of the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, 1952-54.

‡ Present address: Department of Genetics, University of Wisconsin, Madison, Wisc.

§ We are indebted to Drs. A. P. Grieve and A. S. Cook of Ayerst, McKenna and Harrison for generous supplies of human chorionic hormone.