12. Dobson, E. L., Jones, H. B., *Acta Med. Scand.*, 1951, v144, Suppl. 273.

13. Everett, N. B., Simmons, B., Lasher, E. P., *Circulation Res.*, 1956, v4, 419.

14. Hsieh, A. C. L., Carlson, L. D., *Am. J. Physiol.*, 1957, v188, 40.

15. Carlson, L. D., Hsieh, A. C. L., *ibid.*, 1957, v190, 243.

16. Depocas, F., *Canad. J. Biochem. Physiol.*, 1958, v361, 691.

17. Lundholm, L., *Acta Physiol. Scand.*, 1956, v39, Suppl. 133.

18. Freund, H., Jansen, S., *Pflüger's Arch. Physiol.*, 1923, v200, 96.

19. Donhoffer, Sz., Szeguari, Gy., Varga-Nagy, I., Jarai, I., *Pflüger's Arch. Physiol.*, 1957, v265, 104.

Received March 16, 1959. P.S.E.B.M., 1959, v101.

Renal Excretion of Calcium and Phosphate in the Mouse as Influenced by Parathyroids.* (24920)

G. D. BUCHANAN,† F. W. KRAINTZ AND ROY V. TALMAGE

Dept. of Biology, Rice Inst., Houston, Texas

Over recent years, influence of the hormone of parathyroids on bone metabolism has been reemphasized. McLean(1) described parathyroid effect on bone as a "feedback" mechanism for maintaining serum calcium levels. Our reports(2,3) have also emphasized importance of the action of parathyroids on bone. However, for complete picture of parathyroid action, influence of the hormone on kidney function cannot be disregarded. Greenwald's classic observation(4) that parathyroids directly influence renal phosphate excretion has been amply confirmed by many workers, and formed the basis for widely held theory of parathyroid function(5). That parathyroids might also exert a direct influence on renal calcium excretion has been for the most part ignored. Nevertheless Jahan and Pitts(6) reported increased tubular reabsorption of calcium following parathyroid administration in dogs; Talbot *et al.*(7) suggested that parathyroid extract raised renal calcium excretion thresholds. Talmage *et al.* (8,9) studying influence of parathyroidectomy on parathyroid extract administration on calcium excretion interpreted their data as changes in renal threshold for calcium, which caused altered excretion rates until plasma levels equilibrated with new thresholds established by changes in circulating parathyroid hormone concentrations. The work here presented was undertaken to study this effect in yet another species with hope of further emphasizing the overall role of parathyroids on renal excretion.

Methods and materials. Approximately 240 Rockland mice weighing 20-25 g were used. In most experiments, mice were placed on calcium and phosphate free diet with 14 mg Ca/100 ml in drinking water for 2 to 3 days prior to use. In a few parathyroid extract experiments, animals were maintained on stock diet. Parathyroids were removed individually under Nembutal anesthesia, using jewelers' forceps and dissecting microscope. A midline incision was made just posterior to larynx and thyroids exposed. The single pair of parathyroids lie on lateral border of thyroids, usually midway of length of the gland. Occasionally glands are slightly dorsal to thyroids. Parathyroid extract when given was injected subcutaneously in 6 doses at 1 hour intervals. Radioisotopes were injected intraperitoneally. In a typical experiment, animals were given radioisotope and 1 ml water intraperitoneally at beginning of experiment. Mice, in groups of 4 to 6, were placed on metabolism funnel and urine collected for 2 hour periods. Additional doses of 1 ml water were given at start of each collection period. Parathyroidectomy or parathyroid extract administration was performed as indicated in re-

* Aided in part by grant from Atomic Energy Comm.

† Present address: Division of Anatomy, Univ. of Tennessee, Memphis.

TABLE I. Parathyroid Influences on Renal Excretion of Ca^{45} and Phosphate in Mouse.

	No. of animals (groups)	Periods I	II	III	IV	V	VI	VII
Ca^{45}, total radioactivity/hr/mouse	PTH 65 (17)	868 ± 153	520 ± 82	485 ± 71	523 ± 100	308 ± 63	475 ± 115	382 ± 156
	CON 71 (17)	1000 ± 151	1000 ± 159	1000 ± 110	1000 ± 90	1000 ± 115	1000 ± 91	1000 ± 143
	PTX 33 (8)	3158 ± 1800	4302 ± 1774	7018 ± 1480	3175 ± 952	2458 ± 240	1341 ± 598	902 (1351-451)
Ca, mg/hr/mouse	PTH 65 (17)	.0071 ±.0010	.0053 ±.0006	.0057 ±.0005	.0033 ±.0005	.0033 ±.0005	.0031 ±.0004	.0047 ±.0004
	CON 71 (17)	.0094 ±.0013	.0056 ±.0006	.0055 ±.0009	.0067 ±.0010	.0050 ±.0008	.0055 ±.0008	.0043 ±.0008
	PTX 33 (8)	.0080 ±.0016	.0068 ±.0010	.0097 ±.0016	.0137 ±.0026	.0153 ±.0014	.0086 ±.0016	.0050 ±.0005
PO^4, mg/hr/mouse	PTH 65 (17)	.14 ±.03	.18 ±.04	.21 ±.02	.21 ±.02	.22 ±.03	.19 ±.02	.19 ±.04
	CON 71 (17)	.12 ±.02	.09 ±.02	.11 ±.01	.14 ±.02	.12 ±.02	.17 ±.02	.17 ±.02
	PTX 33 (8)	.04 ±.01	.03 ±.01	.03 ±.01	.04 ±.01	.04 ±.02	.07 ±.04	.07 (.13-.01)

Parathyroid extract (PTH) administered in 6 hourly doses beginning with 3rd hour.
Parathyroidectomy (PTX) performed prior to first collection period.

Values given with stand. error $= \pm\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

Collection each 2 hr.

sults. Calcium determinations were made with flame spectrophotometer by procedure involving precipitation with ammonium oxalate and redissolution with perchloric acid. Phosphate determinations were made by the method described by Allport and Keyser(10). Ca^{45} determinations were made on dried aliquots of urine, using a flow counter. A small amount of sucrose solution was added to samples before drying to bring total sample weights into a range where sample variation in self-absorption was less than overall experimental error. Sr^{85} determinations were made with NaI scintillation counter and pulse height analyser.

Results. Phosphate excretion changes. Expected changes in phosphate excretion, noted for other mammalian species following parathyroid extract administration or parathyroidectomy, were seen also in the mouse. Shortly after parathyroidectomy renal excretion values dropped to approximately one-third of control values, while administration of 60 to 90 I.U. of parathyroid extract doubled phosphate excretion rates (Table I). Length of experimental period was 12-14 hours. By end of this period excretion values in both experimental groups appeared to return toward normal values. This can be noted in the large standard error seen in parathyroidectomized groups after 10th hour. The magnitude of this error was due to some groups showing a complete return to normal excretion values.

Calcium excretion changes. Changes in calcium excretion rates in the experimental groups were in the opposite direction to phosphate changes. These changes agree with data previously reported for the rat(9,10). The increase in calcium excretion following parathyroidectomy is interpreted as due to decreased tubular reabsorption for calcium ions causing a temporary increase in urinary calcium values. For groups receiving parathyroid extract, the reverse was true, calcium excretion dropping temporarily (Table I). By end of experimental period all calcium excretion values were within control range.

To indicate further these calcium changes, radiocalcium (Ca^{45}) was administered at be-

ginning of experimental period. Though values for radiocalcium excretion were not directly proportional to total calcium excretion throughout experimental period, radioactivity changes paralleled those for total calcium (Table I). Since injected radioactivity was far from equilibrium with calcium in the body, radiocalcium changes can only be used as a further indication of direction of change in renal calcium excretion and not for degree of change. A few experiments were run using Sr^{85} as indicator of calcium changes. Changes in radiostrontium excretion followed the same pattern as for radiocalcium. The reason for use of this isotope has been previously explained(2).

Discussion. Indications that the hormone of parathyroids directly influences renal calcium excretion have now been given for man (7), dog(6), rat(9), and in our studies for the mouse. Results in regard to renal calcium effects are never as spectacular or "clear-cut" as are effects on phosphate excretion. Reasons for this are 2-fold. Calcium excretion values are in the order of magnitude of 10% of that for phosphate. Calcium excretion rates are, therefore, normally very low, and because of excretion variability, changes are difficult to establish. The second reason concerns the relationship of handling of these 2 ions by kidney and bone. With higher hormone titers, increased phosphate release from bone is coupled with decreased renal phosphate reabsorption. Thus excretion changes are exaggerated. Conversely, an outpouring of calcium from bone is coupled with presumably simultaneous increased tubular reabsorption and therefore excretion values may change little or not at all despite marked changes in handling of this ion by the kidney. In dogs actual excretion rate changes for calcium under varying parathyroid states have not as yet been shown though increased tubular reabsorption following parathyroid administration has been claimed(6). In both rat and mouse temporary changes in renal excretion rates can be demonstrated in both hypo- and hyperparathyroid states but are seen only until bone-plasma equilibrium adjusts to the new tubular reabsorption rate in the kidney.

If the generally assumed function of parathyroid hormone, namely maintenance of normal plasma calcium values, is correct, this certainly would be done most efficiently by the hormone if, with removal of calcium from bone, a simultaneous increase in renal tubular reabsorption occurred to reduce loss through the kidney. The renal phosphate effect, however, being in the opposite direction, not only permits loss *via* the kidney of phosphate removed from bone with the calcium, but also (by excretion of additional phosphate) prevents blood from reaching saturation point for these 2 ions.

Summary. Changes in renal excretion rates of calcium and phosphate, as affected by parathyroidectomy and parathyroid extract administration, were studied in mice. Parathyroidectomy temporarily increased urinary calcium values to 3 times control values, while decreasing phosphate excretion to ⅓ normal values. Urinary excretion of both ions returned to within normal range by 14 hours after start of experiment. Parathyroid extract administration produced opposite changes of about the same magnitude. Urinary calcium values decreased while those for phosphate rose. These changes were also temporary. Results are interpreted as due to changes in renal tubular reabsorption rates for the 2 ions, causing altered excretion rates until plasma levels adjust to the new renal thresholds. Particular emphasis is placed on changes in renal calcium excretion. These studies demonstrate parathyroid influences on handling of calcium by the kidney for a fourth mammalian species, suggesting the importance of this little considered aspect of the action of parathyroid hormone in overall parathyroid physiology.

1. McLean, F. C., Ch. XXII, *Biochemistry and Physiology of Bone,* edited by G. H. Bourne, Acad. Press, 1956.

2. Talmage, Roy V., Elliott, J. R., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 306.

3. ———, *Endocrinology,* 1958, v62, 717.

4. Greenwald, G. S., *Am. J. Physiol.,* 1911, v28, 103.

5. Albright, F., Reifenstein, E. C., *Parathyroid Glands and Metabolic Bone Disease,* Williams and Wilkins, 1948.

6. Jahan, I., Pitts, R. F., *Am. J. Physiol.*, 1948, v155, 42.

7. Talbot, N. B., Sobel, E. H., McArthur, J. W., Crawford, J. D., *Functional Endocrinology,* Harvard Univ. Press, 1952.

8. Talmage, Roy V., Kraintz, F. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 263.

9. Talmage, Roy V., Kraintz, F. W., Buchanan, G. D., *ibid.*, 1955, v88, 600.

10. Allport, N. L., Keyser, J. W., *Colorimetric Analysis,* v1, Chapman and Hall, 1957.

Received March 17, 1959. P.S.E.B.M., 1959, v101.

Effects of Extremely High X-Ray Intensities and Dosages on Mice. (24921)

W. S. MOOS, H. C. MASON,* M. COUNELIS AND R. A. HARVEY
(Introduced by J. P. Marbarger)
Dept. of Radiology, College of Medicine, University of Illinois, and Ill. State Psychopathic Inst., Chicago

Considerable information has been collected on effects of low and medium high dosages of ionizing radiation upon living organisms. Recently, interest has developed in delivering large amounts of such radiant energy to animals, due in part to ability to produce sufficiently high energy flux, and to increasing recognition that interactions between living organisms and ionizing radiation can now be investigated which might not be observable at lower radiation dosages and dose rates(1-6). However, data obtained with extremely high dosages and/or high intensity x-rays is rather scarce(7). Extremely high x-ray dosages and intensities referred to in this paper connote dosages of 10^5 r or more, and intensities of 10^5 r/minute or greater.

Methods. The x-ray tube was placed inside a lead lined cabinet with lead glass window. Temperature in cabinet stayed reasonably constant over considerable time. A strong fan served to remove ozone and stabilize temperature. High intensity radiation was produced by Machlett AEG 50 Beryllium window tube at 45 KVp and 40 mA. No filtration was added. Distance from target to animal's skin was 3.6 cm with dose rate of 2.1 x 10^5 r/minute. X-ray output of tube was repeatedly checked with chemical dosimeter (8). For this purpose ferrous sulfate reagent (0.5 ml, 5 mN) was placed in small glass cups fitting snugly into lucite holder and irradiated for various time intervals† (distance from target to level of liquid = 3.6 cm). Depth of fluid was 2-3 mm. Following irradiation, 0.3 ml of ferrous sulfate reagent was removed and diluted with 3 ml of 0.8 N sulfuric acid. Optical density after irradiation was compared to that of non-irradiated samples of solution in Beckman spectrophotometer at wave length setting of 3020 Å. The dose of 2.1 x 10^5 r/min thus obtained is representative of energy absorbed in first few mm of skin over area of 2.25 cm^2. This procedure compares quite well with mesh ionization chamber measurements performed earlier at same x-ray energies and similar exposure dose rates. X-ray absorption measurements were carried out with same type of dosimeter by placing shallow containers of liquid inside a lucite holder and covering holder with pieces of mylar or lucite varying in thickness 12 μ to 2.45 cm. In this particular instance, distance from tube window to liquid level was 5.5 cm and remained constant throughout. The resulting absorption curve (Fig. 1) shows that approximately 85% of x-rays are absorbed in the first cm of tissue. Output constancy measurements with a CdS crystal dosimeter were $\pm$ 5%; however, after tube had been in operation for about 150 to 200 hours a gradual reduction in output was observed to as low as 1.3 to 1.1 x 10^5 r/min. Some of our repeat

* Present address: State of Washington Health Dept., Seattle.

† Amount of x-rays delivered to ferrous sulfate solution did not exceed 5 x 10^4 r and therefore should be within useful range of this dosimetric method. This was confirmed by 2 measurements performed with Ceric-Cerous dosimetry system.