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A Rat Model for Hormone and Mineral Changes in Chronic Renal Failure[1] (41724)

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Abstract. Plasma parathyroid hormone (PTH) and calcitonin (CT) in rats with surgically induced chronic renal failure were measured by radioimmunoassay. Plasma PTH levels in uremic rats (2897 ± 693 pg/ml) were significantly ($P < 0.001$) higher than those in nonuremic rats (286 ± 18 pg/ml). A low-phosphorus diet for 4 and 8 days increased serum calcium and decreased the elevated PTH level to the level of sham-operated rats. Plasma CT levels in uremic rats (60.1 ± 7.8 pg/ml) were significantly ($P < 0.05$) higher than those in nonuremic rats (37.6 ± 2.4 pg/ml). The low-phosphorus diet for 4 days, but not 8 days, increased the CT levels in uremic rats. Our results demonstrate the importance of phosphate and calcium in influencing the secretion of PTH and CT in uremia. The rat, along with appropriate radioimmunoassays, appears to be a useful model for studying bone mineral metabolism in chronic renal failure.

Chronic renal failure is accompanied by dramatic changes in calcium and phosphorus and the hormones which regulate skeletal metabolism (1). Most experimental studies of hormonal and mineral changes in chronic renal failure have been performed in large animals like the dog (2, 3). Despite the widespread use of the rat in studies of calcium and skeletal metabolism, this animal has been sparingly used for such studies in renal failure (4–6). This has been largely due to the difficulties associated with measurements of the calcitropic hormones in the rat. We have developed in our laboratory the procedures needed for systematic studies of calcium and skeletal metabolism in the rat. This is a report of those procedures and their beginning application to studies of chronic renal failure. Specifically, the present experiments were undertaken to study plasma parathyroid hormone (PTH) and calcitonin (CT) in rats with chronic renal failure and to examine the effect of varying dietary phosphorus (P) and calcium (Ca) intake on the secretion of these hormones.

Methods. Adult male Sprague–Dawley rats (Hilltop, Calif.) weighing approximately 250 g were employed in this study. The modified method of Platt *et al.* (7) was used to create chronic renal failure rats. One week after the removal of the left kidney cortex by scissors, total right nephrectomy was performed under nembutal anesthesia. Prior to manipulation, the rats had been on a standard rat diet containing 1.2% calcium, 0.86% phosphorus, and 530 U vitamin D/100 g. After surgery the animals were divided into five groups of 11 or 12 animals each. Group I continued the standard diet; Group II received the standard diet and water containing 1% calcium lactate (0.18% calcium solution) for 4 days prior to sacrifice; Group III received a low-phosphorus diet (BioServe, Inc., N.J.) containing 1% calcium, 0.004% phosphorus, and 300 IU vitamin D/100 g, and distilled water for 4 days; and Group IV received the low-phosphorus diet and water containing the 1% calcium lactate solution for 4 days. Group V was given the low-phosphorus diet for 8 days. In addition, 16 rats were sham-operated and divided into two further groups (Groups VI and VII). Group VI received the standard diet and group VII was given the low-phorphorus diet for 4 days before sacrifice.

Twenty-five days after the second operation (nephrectomy), 5–7 ml of blood was taken from the abdominal aorta of anesthetized rats for measuring plasma PTH and CT, serum Ca and P, and creatinine (Cr). In the partially nephrectomized groups tail blood was collected for plasma PTH 4 days before sacrifice just before starting the low-phosphorus diet.

Plasma PTH was measured by a modifi-

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TABLE I. BIOCHEMICAL AND IMMUNOASSAY VALUES IN NORMAL AND PARTIALLY NEPHRECTOMIZED RATS AND THE EFFECT OF DIETARY PHOSPHORUS AND CALCIUM

	Chronic renal failure rats					Sham rats	
	I[a]	II	III	IV	V	VI	VII
Number of rats	12	11	12	11	12	8	8
Serum creatinine (mg/dl)	1.57 ± 0.15†	1.69 ± 0.20†	1.67 ± 0.17†	1.78 ± 0.27†	1.59 ± 0.11†	0.42 ± 0.02	0.50 ± 0.005
Serum Ca (mg/dl)	9.70 ± 0.22	9.62 ± 0.24	12.70 ± 0.41†	12.14 ± 0.35†	12.40 ± 0.30†	9.57 ± 0.17	11.02 ± 0.28*
Serum P (mg/dl)	6.14 ± 0.60	5.68 ± 0.32	1.97 ± 0.26†	2.22 ± 0.33†	3.14 ± 0.45†	6.12 ± 0.27	4.66 ± 0.48
Plasma PTH (pg/ml)	2897 ± 693†	2350 ± 579†	365 ± 77††	257 ± 55††	209 ± 25††	286 ± 18††	240 ± 31
Plasma CT (pg/ml)	60.1 ± 7.8	63.4 ± 4.5	131.4 ± 25.7†††	121.8 ± 16.8†††	30.6 ± 17.2	37.6 ± 2.4	44.8 ± 3.6
Weight (g)	299 ± 5†	295 ±6 †	297 ± 9†	291 ± 7†	269 ± 10†	401 ± 8	369 ± 15

Note. I and VI: Standard diet (Std); II: standard diet + 1% Ca lactate solution (Ca); III and VII: Low P diet for 4 days (low P); IV: low P diet and Ca solution for 4 days; V: low P diet for 8 days. Values are means ± SE. †$P < 0.01$ vs VI, *$P < 0.05$ vs VI, ††$P < 0.01$ vs I.

[a] Group number.

cation for rat PTH (8–10) of previously described procedures for human and bovine PTH (11, 12). A chicken antiserum, designated C-7, raised against bovine PTH (Inolex) and acetic acid extracts of rat parathyroid glands, was used at a final dilution of 1:4000. Radioiodinated bovine PTH was used as tracer and the assay conditions were otherwise as previously described (8–12). In this assay system the mean (±SE) concentration of PTH in normal adult rats such as those used in this study is 300 ± 50 pg/ml. Induced hypercalcemia and hypocalcemia produce a respective decrease and increase in plasma PTH and the hormone in undetectable in parathyroidectomized rats. Preliminary details about this procedure have been presented (8–10) and a more detailed report shall be published separately.

Plasma CT was measured by a radioimmunoassay based on human calcitonin which was adapted to the measurement of rat calcitonin (14, 15). Plasma calcium, phosphorus, and creatinine were measured by autoanalyzer. Statistical analysis was done using the student's *t* test.

The data were analyzed by a one-way ANOVA and for each measurement a significant ($P < 0.0001$) F ratio was achieved. The individual measurements were then evaluated for significance by the method of Tukey for making pair-wise comparisons among means (13).

Results. The results of this study are summarized in Table I and Fig. 1. The surgery effectively created renal failure in all rats, of which 85% survived for the duration of the experiment. The plasma creatinine levels of the partially nephrectomized rats with a standard diet (Group I) were significantly higher than those of sham-operated rats with the standard diet (Group VI). Although there was not a significant difference in plasma Ca or P between these two groups, plasma PTH and CT levels were significantly higher in uremic rats. A positive correlation was present between plasma PTH and creatinine levels in the uremic rats of Group I ($r = 0.970$, $P < 0.001$). No significant differences were observed in the serum creatinine levels of the uremic groups (Groups I–V).

Effect of dietary calcium. The daily addition

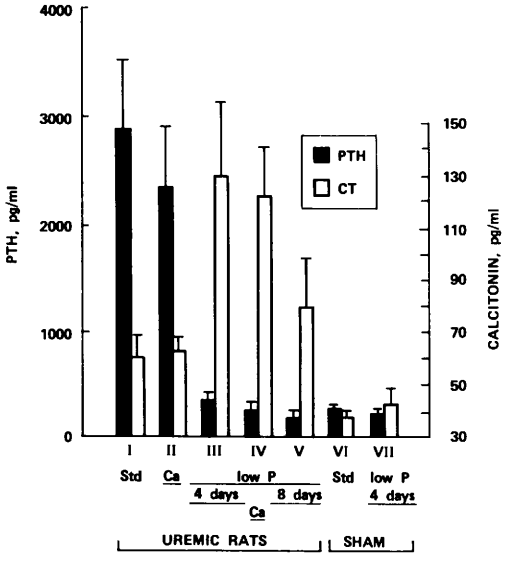

FIG. 1. Effect of calcium supplementation and phosphorus depletion on plasma PTH and CT levels in rats with surgically induced chronic renal failure and sham-operated controls.

of extra calcium to the diet of the rats in Group II (367 mg of calcium compared to 211 mg in Group I) had no significant effect on any of the measurements although there was a slight decrease in PTH.

Effect of low-phosphorus diet. The low-phosphorus diet produced a significant decrease in serum P, an increase in serum Ca, and a decrease in plasma PTH in both the uremic (Groups III–V) and sham rats (Group VII). Plasma CT was increased in these same groups, significantly so in all but Group V. The rats with the lower P levels had generally higher Ca levels, lower PTH levels, and higher CT levels, although these differences were not always significant.

Discussion. A number of experimental models have been developed to study the mineral and hormonal perturbations of chronic renal failure. These animal models along with clinical studies have established that in the blood of patients with renal failure there is an increase in phosphorus (P), parathyroid hormone (PTH), and calcitonin (CT), and a decrease in calcium (Ca) (1–4, 16). The increases in PTH and CT are thought to reflect changes in both secretion and renal metabolism of the hormones (1, 16, 17). Multiple factors contribute to the secondary hyperparathyroidism, including phosphate retention (18, 19), hypocalcemia (19), abnormal vitamin D metabolism (20, 21), impaired calcium absorption (22), and skeletal resistance to PTH (23). The abnormal parathyroid function can be attenuated by increasing calcium intake and decreasing phosphorus intake; the latter effect is especially dramatic (2, 4, 24).

We have demonstrated that P restriction also dramatically lowers plasma PTH in the uremic rat (Table I). This decrease, also present in normal rats, is accompanied by an increase in serum Ca and it is likely that the increased Ca suppresses parathyroid gland function. The addition of calcium to the low-phosphorus diets caused only a slight decrease in PTH, probably because there was no increase in serum Ca. Calcium intake may suppress the increased PTH of uremic rats when supplemented to a low-calcium diet (4). Furthermore, a longer duration of calcium supplementation may be necessary to show any effect on plasma PTH (17).

An increased plasma CT is also present in uremic rats, corresponding to the increased levels reported in humans with uremia (16, 25). The significance of these high concentrations of CT has not been completely clarified, although Heynen *et al.* suggested the high concentrations of CT in some patients might protect against renal bone disease (25). There has been no report concerning CT secretion in rats with chronic renal failure. Since these animals are not hypercalcemic, the increased CT must largely represent decreased metabolism of the hormone (26). However, like PTH, CT exhibited the appropriate relationship to changes in serum calcium (Table I).

Plasma CT levels were higher in the uremic rat with the low-phosphorus diet for 4 days than in the uremic rats with the standard diet. But the CT levels in uremic rats with the low-phosphorus diet for 8 days were not significantly higher than the levels in uremic rats with standard diet, although the former had hypercalcemia. This might be due to depletion of CT in the thyroid gland as a result of chronic hypercalcemia (27).

Our studies further demonstrate that radioimmunoassays based on bovine PTH can be used to measure serum rat PTH (28–31). In addition, we also measured rat CT and

demonstrated here the simultaneous measurement of both peptides in rat serum. We have also developed a new assay for the measurement of $1,25(OH)_2D$ in available volumes of rat blood (32, 33). Thus, it is now possible to perform systematic studies of the interrelationships among the three calcitropic hormones in rat models of normal and abnormal states of calcium and skeletal metabolism.

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1. Avioli LV. In Avioli LV, Krane SM, eds. Metabolic Bone Disease. New York, Academic Press, Vol. 2, 1978.
2. Hruska KA, Kopelman R, Rutherford WE, Klahr S, Slatopolsky E. Metabolism of immunoreactive parathyroid hormone in dog role of kidney and effects of chronic renal disease. J Clin Invest **56**:39–48, 1975.
3. Slatopolsky E, Caglar S, Pennel JP, Taggart DD, Canterbury JM, Reiss E, Bricker NS. On the pathogenesis of hyperparathyroidism in chronic experimental renal insufficiency in the dog. J Clin Invest **50**:492–499, 1971.
4. Grubenmann W, Binswanger U, Hunziker W, Fischer JA. Effects of calcium intake and renal function on plasma immunoreactive parathyroid hormone levels in rats. Metab Res **10**:438–443, 1978.
5. Kawaguchi Y, Kawahima H, Ueno K, Isawa Y, Makita T, Kurozumi S, Hashimoto Y, Yamamoto M, Kimura Y, Imamura N, Oda Y, Sakai S, Ogura Y, Kushida K, Inoue K. Effect of 1-alpha-hydroxyvitamin D_3 in rats with experimental renal osteodystrophy. Endocrinol Japon **1**:73–80, 1979.
6. Kaye M. The effects in the rat of varying intakes of dietary calcium, phosphorous, and hydrogen ion on hyperparathyroidism due to chronic renal failure. J Clin Invest **53**:256–269, 1974.
7. Platt R, Roscoe MN, Smith W. Experimental renal failure. Clin Sci **11**:217, 1952.
8. Berger ME, Golub MS, Sower JR, Brickman AS, Nyby M, Troyer H, Rude RK, Singer FR, Horst R, Deftos LJ. Hypercalcemia in association with a Leydig cell tumor in the rat: a model for tumor-induced hypercalcemia in man. Life Sci **30**:1509–1515, 1982.
9. Bone HG, Onishi T, Miller M, Deftos LJ. Acute subacute and chronic hormone levels: Application and validation of a radioimmunoassay. Proc 2nd Annual Meeting American Soc Bone and Mineral Res. Abstr 27, 1980.
10. Spiegel AM, Saxe AW, Deftos LJ, Brennan MF. Humoral hypercalcemia caused by a rat Leydig-cell tumor is associated with suppressed parathyroid hormone secretion and increased urinary cAMP excretion. Horm Metab Res, in press.
11. Deftos LJ. Parathyroid hormone. In: Jaffe BM, Behrman HR, eds. Methods of Hormone Radioimmunoassay. Academic Press, New York, 1974, pp231–247.
12. Stewart AF, Horst R, Deftos LJ, Cadman ED, Lang R, Rasmussen H, Broadus AE. Biochemical evaluation of patients with malignancy-associated hypercalcemia: Evidence for humoral and non-humoral groups, New Engl J Med **303**:1377–1383, 1980.
13. Kirk RE. Experimental Design: Procedures for the Behavioral Sciences, Belmont, Calif., Brooks/Cole, pp88–91, 1968.
14. Deftos LJ, Boorman GA, Roos BA. Immunoassay of calcitonin in rat medullary thyroid carcinoma. Horm Metab Res **8**:83–84, 1976.
15. Roos BA, Deftos LJ. Radioimmunoassay of calcitonin in plasma, normal thyroid, and medullary thyroid carcinoma of the rat. J Lab Clin Med **88**:173–182, 1976.
16. Lee JC, Parthemore JG, Deftos LJ. Immunochemical heterogeneity of calcitonin in renal failure. J Clin endocrinol Metab **45**:528–533, 1977.
17. Arnaud CD. Hyperparathyroidism and renal failure. Kidney Int **4**:89–95, 1973.
18. Goldman R, Bassestt SH. Phosphorus excretion in renal failure. J Clin Invest **33**:1623, 1954.
19. Hebert LA, Leman J, Petersen JR, Lennon EJ. Studies of mechanisms by which phosphate infusion lowers serum calcium concentration, J Clin Invest **45**:1886, 1966.
20. Brumbaugh PF, Haussler DH, Bressler R, Haussler MR. Radioreceptor assay for 1-alpha, 25-dihydroxyvitamin D_3. Science **183**:1089–1091, 1974.
21. Fraser DR, Kodicek E. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. Nature (London) **228**:764, 1970.
22. Baerg RD, Kimberg DV, Gershon E. Effect of renal insufficience on active-transplant of calcium by small intestine. J Clin Invest **49**:1288, 1970.
23. Massry SG, Coburn JW, Lee DBN, Jowsey J, Kleeman CR. Skeletal resistance to parathyroid-hormone in renal failure—studies in 105 human subjects. Ann Intern Med **78**:357–364, 1973.
24. Kaplan MA, Canterbury JM, Bourgoignie JJ, Veliz G, Gavellas G, Reiss E, Bricker NS. Reversal of hyperparathyroidism in response to dietary phosphorous restriction in the uremic dog. Kidney Int **15**:43–48, 1979.
25. Heynen G, Kanis JA, Oliver D, Ledingham JFF, Russell RGG. Evidence that endogenous calcitonin protects against renal bone disease. Lancet **2**:1322–1325, 1976.
26. Ardillou R, Sizonenko P, Mayrier A, Vallee G, Beaugas C. Metabolic clearance rate of radiotadinated human calcitonin in man. J Clin Invest **49**:2345–2362, 1970.

27. Yamada Y, Ito S, Kayamori R, Shibata A. Effects of vitamin D_2 on the somatostatin and calcitonin concentration in the rat thyroid. Horm Metab Res **11**:184–185, 1979.
28. Chanard J, Black R, Purkerson M, Lewis J, Klahr S, Slatopolsky E. The effect of colchicine and vinblastine on parathyroid hormone secretion in the rat. Endocrinology **101**:1792–1800, 1977.
29. Fumita T, Ikano K, Ohata M, Yoshikawa M. A radioimmunoassay for rat serum parathyroid hormone using N-terminal of PTH. Endocrinol Japon **22**:159–162, 1975.
30. Hargis GK, Bowser EN, Henderson WJ, Williams GA. Radioimmunoassay of rat parathyroid hormone in serum and tissue extracts. Endocrinology **94**:1644–1649, 1974.
31. Tanaka Y, DeLuca HF, Ghazarian JF, Hargis GK, Williams GA. Effect of vitamin D and its metabolities on serum parathyroid hormone levels in the rat. Miner Electrolyte Metab **2**:20–25, 1979.
32. Monolagas SM, Deftos LJ. Cytoreceptor assay for 1,25-dihydroxy vitamin D_3: A noval radiometric method based on binding of the hormone to intracellular receptors in vitro. Lancet **2**:401, 1980.
33. Manolagas SM, Howard JE, Culler FL, Brickman AS, Deftos LJ. Cytoreceptor assay for 1,25-dihydroxyvitamin D and its application to clinical studies. J Clin Endocrinol Metab **56**:751, 1983.

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