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Phosphorus and Calcium Homeostasis in Chronic Subtotally Nephrectomized Parathyroidectomized Rats

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Calcium and phosphorus balance studies were carried out in subtotally nephrectomized rats (NX), with or without parathyroidectomy (NX-PTX; NX sham-PTX) in order to determine the ability of the remnant kidney to regulate excretion of these elements in the absence of parathyroid hormone. The rats were fed three different phosphorus diets, and calcium intake was also varied. We found that the NX-PTX rats adapted to the three different phosphorus diets in a manner indistinguishable from the NX sham-PTX rats. The per cent of ingested phosphorus excreted in the urine increased as dietary phosphorus increased. When supplementary calcium was added to the diet, urinary phosphorus excretion fell and fecal phosphorus increased in an identical fashion in the NX-PTX and NX sham-PTX animals. Urinary calcium excretion decreased as dietary phosphorus increased, and $U_{Ca}V$ increased when supplementary calcium was provided in the diet. Total body calcium and phosphorus balance (intake-(urine+feces)) varied with intake, but was not significantly different between the NX-PTX and NX sham-PTX rats. These experiments demonstrate that subtotally nephrectomized rats have a parathyroid-independent mechanism(s) for regulating both urinary and fecal calcium and phosphorus excretion. The mechanism is not revealed by the present study, but may relate to changes in serum calcium and/or phosphorus which occur following parathyroidectomy.

Key Words: Chronic renal failure, parathyroid hormone, phosphorus homeostasis

Renal excretion of phosphorus is known to remain essentially constant in the presence of progressive renal insufficiency as a result of a decrease in tubular reabsorption proportional to the decrease in glomerular filtration rate (GFR) (Goldmanetal, 1954; Reiss *et al.*, 1961; Slatopolslay *et al.*, 1966; 1968). A substantial body of evidence indicates that hyperparathyroidism secondary to chronic renal failure plays a key role in the reduction in tubular reabsorption of phosphate, since acute parathyroidectomy results in a marked increase in phosphate reabsorption by the surviving nephrons and a decrease in urinary phosphate excretion (Bank *et al.*, 1978; Slatopolsky *et al.*, 1966).

A number of studies have shown that in normal animals and man the kidney is able to regulate phosphate excretion in the chronic absence of parathyroid hormone (PTH) (Amiel *et al.*, 1976; Bonjour *et al.*, 1977; Crawford *et al.*, 1955; Eisenberg, 1965; Sanderson *et al.*, 1960; Steel *et al.*, 1976; Trohler *et al.*, 1976). Although the precise mechanism for this PTH-independent regulation is unknown, it has become apparent that the normal kidney can maintain phosphate homeostasis in the absence of the hormone. In view of the evidence that hyperparathyroidism plays a critical role in renal phosphate excretion in chronic renal failure, it seemed of interest to evaluate the ability of the uremic animal to regulate phosphate homeostasis in the chronic absence of PTH.

In the present study, long-term balance measurements were made in rats with subtotal nephrectomy (NX). Measurements of urinary and fecal phosphorus excretion were made of different dietary intakes of phosphorus, before and after parathyroidectomy (PTX), and during manipulations of dietary calcium. It was found that within 4-5 days after PTX, phosphate excretion in uremic rats equalled that prior to PTX. Urinary phosphorus excretion varied with phosphorus intake in both NX-PTX rats and NX sham-PTX rats in a comparable manner. When dietary

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calcium was increased, urinary phosphorus excretion fell markedly in both groups, associated with an increase in fecal phosphorus. The observations demonstrate that rats with markedly reduced renal mass are able to regulate both urinary and fecal phosphorus and calcium excretion with the same degree of precision in the absence of PTH as they can when the hormone is present. The mechanism of this PTH-independent control is not clear, but might be related to changes in serum calcium or phosphate following PTX.

METHODS

White male Sprague-Dawley rats, weighing 200-300 gms initially were used in these experiments. Animals were fed a regular rat pellet diet (Lab Blox, Wayne Lab Animal Diets, Allied Mills, Chicago, Ill.) containing: Na 9.8 mEq/100 gm; K 26.5 mEq/100 gm; Ca 1.53 gm/100 gm; and phosphorus 1.03 gm/100 gm. A 5% sucrose drinking solution was provided ad lib in graduated J-shaped tubes.

All animals underwent subtotal nephrectomy by excision of the right kidney and ligation of the upper pole and a portion of the dorsal side of the left kidney, as described previously (Bank *et al.*, 1978). Three days were allowed for recovery from surgery. During this period, the rats were given 2 mg/day erythrocin orally. Following the recovery period, the rats were placed on a calcium-deficient test diet (ICN Pharmaceuticals, Inc., Cleveland, Ohio) containing: Na 21.5 mEq/100 gm; K 14.0 mEq/100 gm; Ca 0.0087 gm/100 gm; and phosphorus 0.413 gm/100 gm; supplemented with vitamins. Their intake of phosphorus was supplemented by providing 67.5 mg/day as buffered sodium phosphate solution in their drinking water, sweetened with 10% sucrose. This dietary regimen in uremic rats has been shown by Kaye (Kaye, 1974) to result in marked hypertrophy of the parathyroid glands which contained increased amounts of radioimmunoassayable PTH. Rats were kept in individual metabolic cages and were maintained on the above diet for 3-4 weeks. Following this period, they were weighed and then entered one of three different dietary phosphorus groups:

Group I.

Twelve rats were placed on a sodium-deficient diet (ICN Pharmaceuticals, Inc. Cleveland, Ohio), which contained: Na 0.92 mEq/100 gm; K 23.5 mEq/100 gm; Ca 0.82 gm/100 gm; and phosphorus 0.413 gm/100 gm. Seven meq NaCl per day was provided in their drinking water, which was sweetened with 10% sucrose. After 3-4 days on this new dietary regimen, balance measurements were begun (period 1). Food intake was measured daily and 24-hr urine output was collected for volume, Na, K, Ca, and P determinations. Collection of urine samples was made under oil in glass flasks which separated feces from urine (Misco Microchemical Specialties, Co., Berkeley, Calif.). The collectors and the steel funnels of the metabolic cages were siliconized with Disicote (Beckman Instruments, Inc., Mountainside, N. J.). The urine was transferred into graduated cylinders, volume measured, and 9-10 drops of concentrated HCl were added. After mixing, urine aliquots were filtered (Whatman filter paper No. 3) and stored at 4°C for analysis. Stools were collected daily and refrigerated. Following this, 6 rats were parathyroidectomized (PTX), and the other 6 underwent neck surgery without removal of the parathyroid gland (sham-PTX). The parathyroidectomy was achieved by either electrical cauterization or surgical ablation of the gland under direct microscopic visualization (Bank *et al.*, 1978). The glands were enlarged and easily identified. The thyroid glands were left intact. Following surgery, the PTX rats required 2-3 days before food and fluid intake reached the pre-PTX level, whereas sham-PTX animals achieved this state in a day or two. Food intake and urine and stool output were then measured starting immediately after neck surgery. The data for the first four days were analyzed separately from days 5-10, which constitute balance period 2. At the end of period 2, the rats were given supplementary calcium (50 mg/day) as calcium gluconate dissolved in the drinking solution for 5 days. Their diet and drinking solution otherwise remained unchanged. Food intake and urine and stool collections were measured as above (period 3). Weight of the animal was measured at the end of each dietary period.

Group II.

Fifteen subtotally nephrectomized rats, prepared by the same protocol described above, were placed on the sodium-deficient diet after 3-4 weeks on the low calcium, high phosphorus intake. A buffered sodium phosphate solution containing 34 mg phosphorus in 50 ml, 10% sucrose and 140 mEq/L Na was given as drinking solution (50 ml/day). After 3-4 days, urine and stool collections were begun, as described above (period 1). Four to five days later, 8 rats underwent PTX and 7 sham-PTX, as in the Group I experiments. Food and liquid intake and urine and stool output were measured for an additional 8-10 days (period 2). At the end of this period, intake of calcium was increased by a supplement of 50 mg calcium/day as calcium gluconate solution (25 ml/day).

They continued to receive 34 mg P/day in 25 ml drinking solution. Both Ca and P solutions contained 140 mEq/L NaCl. Urine and stool samples were collected while on this dietary intake (period 3). In a final balance period, the calcium gluconate solution was withheld from animals but all other electrolyte and water intake was continued as before. Urine and stool collections were obtained on this intake for an additional 4-5 days (period 4). Weight was measured at the end of each balance period.

Group III.

A third group of 13 subtotally nephrectomized rats was prepared in the same manner as described for Groups I and II. During the four experimental balance periods described above, these rats were placed on a supplement of 68 mg P/day in their drinking solution. In order to study the possible role of thyroxine and calcitonin in phosphorus homeostasis, three rats in this group underwent surgical removal of both thyroid and parathyroid glands (TPTX), while four others underwent parathyroid-ectomy only. The other 6 rats in this group underwent sham-PTX, as described above. Balance measurements were carried out for 10 days following neck surgery (period 2). The intake of calcium was varied in the 3rd and 4th balance periods as in the preceding two groups, whereas sodium and water intake were kept constant. Urine collections were obtained during the 4 different dietary periods, as in the preceding two grous of animals. Stool collections were technically unsatisfactory in this Group because diarrhea developed in most animals due to the high phosphorus intake. However, urine samples were apparently not contaminated by feces, since urinary electrolytes were comparable in animals with and without diarrhea.

Blood was drawn under light anesthesia by direct cardiac puncture or from the tail vein at the end of each experimental period in all three groups of animals.

At the end of the balance studies, rats were anesthetized with i.p. Inactin, a jugular vein and carotid artery catheterized, and the urinary bladder exposed and catheterized as previously described (Bank *et al.*, 1978). Ringer's lactate solution containing (methoxy-^{3}H)-inulin was infused at a rate of 2.3 ml/h. Blood pressure was monitored by a strain gauge via the femoral artery catheter. Timed urine collections and mid-point arterial blood were obtained for measurement of (^{3}H) by liquid scintillation counting and glomerular filtration (GFR) was calculated as inulin clearance (Bank *et al.*, 1978). We found that the NX-PTX rats were hypotensive under anesthesia (mean BP 90 mmHg) and often expired before adequate urine samples could be obtained, whereas the NX sham-PTX rats tolerated anesthesia and surgery well. Data for inulin clearance are presented only for the NX sham-PTX rats therefore.

Analytic Methods:

Phosphorus was measured in blood, urine and stool samples by the method of Gomori (Gomori, 1953), and total calcium was measured by the method of Meites (Meites, 1965). Urinary sodium and potassium were measured by flame photometry. Blood urea nitrogen (BUN) was measured by the method of Kaplan (Kaplan, 1965). In order to minimize stool collection errors, all stools for an entire experimental period were pooled and analyzed as a single sample. Each batch was homogenized, using a pestle tissue grinder (Arthur H. Thomas Co., Philadelphia, Pa.) and 2-3 ml portions of homogenates were prepared in duplicate; one aliquot for phosphorus analysis was made alkaline with 50% KOH, while the other sample for Ca measurement was made acid with 0.25 N H_2SO_4. Samples were dried at 110°C for 6-8 hours and ashed at 570°-590°C for 16 hours (Electric Multiple Furnace Unit, Hevi Duty Electric Co., Milwaukee, Wis.). The alkaline ash was then dissolved in 10 ml 1N HCl, and the acid ash was dissolved in 10 ml 2N HNO_3. Stool phosphorus and calcium were measured in both the alkaline and acid ashes.

Statistical analysis was carried out by Student's test. In period 1 (before PTX or sham-PTX), the data for all animals in each group were considered together, as preparation up to this point was identical. In periods 2-4, the data for each animal were compared with its own values in the preceding period by paired test analysis.

RESULTS

In Table 1 are shown the dietary intake of calcium, phosphorus, sodium and potassium for the NX-PTX rats (E) and the NX sham-PTX rats (S) during the various balance periods. As can be seen, food intake fell somewhat in period 2 following PTX in all 3 groups, whereas sham-PTX rats usually maintained a constant food intake. In the NX-PTX rats, phosphorus intake average 57.1 mg/day in group I, 75.6 mg/day in group II and 101.2 mg/day in group III. Phosphorus intake was not significantly different in the NX sham-PTX rats. Intake of calcium varied according to the protocol, but there were no significant differences between NX-PTX and NX sham-PTX animals. The sham-PTX rats gained weight consistently whereas the NX-PTX rats

Table 1. Dietary electrolyte and food intake, and wt. changes

	Period	Ca mg/day E	Ca mg/day S	P mg/day E	P mg/day S	Na meq/day E	Na meq/day S	K meq/day E	K meq/day S	Food Intake gm/day E	Food Intake gm/day S	Wt. Changes gm/day E	Wt. Changes gm/day S
Group I	1	123.3±15.0	97.5± 5.2	60.0± 7.3	48.0± 2.7	7.1±0.03	7.1±0.01	3.5±0.4	2.8±0.2	14.9±1.5	11.9±0.6	+2.7±1.3	+0.8±0.7
	2	94.8±15.3	94.4± 5.4	50.9± 7.3	47.8± 2.8	6.7±0.2	7.1±0.1	2.7±0.4	2.8±0.2	11.6±1.9	11.9±0.7	−1.4±0.7	+2.5±0.7
	3	173.8±14.1	141.2± 8.4*	60.5± 6.5	45.0± 4.3	7.0±0.1	7.1±0.01	3.5±0.4	2.6±0.2	15.1±1.7	11.1±1.0	−0.4±0.9	+2.0±0.7
Group II	1	90.0±13.4	96.4± 8.7	78.4± 6.6	81.0± 4.3	7.1±0.02	7.1±0.01	2.8±0.4	2.8±0.3	11.0±1.6	11.8±1.1	−0.8±1.5	+0.5±1.1
	2	84.0±10.7	100.0± 9.6	71.1± 8.9	80.0± 3.5	7.0±0.1	7.1±0.1	2.7±0.1	2.9±0.3	10.2±1.3	12.2±1.2	+0.8±1.0	+2.1±1.1
	3	137.7± 7.0	132.6±10.0	78.0± 3.8	73.5± 5.9	7.1±0.1	7.1±0.2	2.4±0.3	2.5±0.4	10.7±0.9	10.7±1.5	+2.8±0.8	+1.8±1.1
	4	86.0± 7.4	115.9± 3.1	74.8± 4.1*	90.8± 1.6*	6.8±0.3	7.1±0.0	2.5±0.2	3.3±0.1*	10.5±0.9	14.1±0.4*	+1.5±1.0	+2.4±0.7
Group III	1	103.3± 9.3	124.1±11.6	118.4± 4.6	129.8± 6.3	7.1±0.01	7.1±0.02	3.0±0.3	3.6±0.3	12.6±1.1	15.1±1.4	−1.2±0.4	+2.1±0.6
	2	74.1± 7.6	108.6±17.2*	92.1± 6.9	122.6± 9.0*	5.8±0.5	7.1±0.02	2.1±0.2	3.1±0.5	9.0±0.9	13.3±2.1*	−1.4±0.6	+1.4±1.3
	3	155.8±18.2	146.5± 8.8*	104.0± 6.9	114.8± 5.7*	5.8±0.5	7.0±0.2*	3.0±0.5	2.8±0.3	12.9±2.2	11.8±1.1	−0.1±0.9	+0.4±0.9
	4	88.8±13.8	106.6±23.4	90.3±13.9	115.7±17.5	4.9±1.2	6.6±0.6	2.5+0.4	3.1+0.7	10.8+1.7	13.0+2.8	+2.1+0.6	0

E = NX-PTX rats; S = NX sham-PTX rats
Data presented as mean ± S.E.M.
*p<0.05 E vs S.

either lost weight or gained less than the sham-PTX rats. Sodium and potassium intake in Groups I and II were not significantly different (NX-PTX vs NX sham-PTX) and remained relatively constant throughout all periods of balance. Some fall in Na intake occurred in the Group III NX-PTX rats, associated with diarrhea and weight los.

In Table 2 are shown the serum calcium and phosphorus values, measured at the end of each balance period. The data for period 1 in each group (before PTX or sham-PTX) were pooled, as the animals were treated identically up to this point. The data shown for period 2, 3, and 4 are mean ± S.E.M. in which paired T test analysis was performed for individual animals. Following PTX, serum calcium fell significantly in all 3 groups, the magnitude of the fall being proportional to the amount of phosphorus in the diet. Serum calcium in period 2 was significantly lower in the NX-PTX rats than in the NX sham-PTX rats in all three groups ($p<0.05$). When supplementary calcium was fed in period 3, serum calcium rose significantly in Groups II and III, although it was still significantly lower than in the NX sham-PTX rats ($p<0.025$). When the supplementary calcium was removed from the drinking solution, serum calcium fell again. Serum phosphorus rose significantly after PTX only in the Group III rats. When supplementary calcium was fed, serum phosphorus fell significantly in Group II and III. It rose again in Group III after the supplementary calcium was discontinued. In the NX sham-PTX rats, serum calcium and phosphorus tended to remain more stable throughout the different dietary periods, although serum calcium did fall somewhat in period 4 in Groups II and III. Serum phosphorus remained relatively stable in the NX sham-PTX rats with variations in dietary phosphorus and calcium. Mean serum phosphorus values were slightly higher in NX-PTX rats than in NX sham-PTX in all periods, although the differences reached statistical significance ($p<0.05$) only in period 2 of the Group III animals.

In Fig. 1 are presented data on 24 h urinary phosphate excretion for all 3 groups of rats, expressed as a per cent of ingested phosphorus (mean±S.E.M.). It can be seen that following sham-PTX (right side of Fig.), a small fall in % phosphorus excretion occurred in Groups I and II, even though phosphorus intake was unchanged (Table 1). When extra calcium was added to the drinking solution (period 3), urinary phosphorus excretion fell significantly ($p<0.001$ paired T test) in all 3 NX sham-PTX groups and when the extra calcium was withheld (period 4) phosphorus excretion rose again. On the left half of

Table 2. Serum calcium and phosphorus in subtotally nephrectomized rats before and after PTX and dietary manipulations

	Period	Calcium		Phosphorus	
		E	S	E	S
		mg%		mg%	
Group I	1	9.8±0.5*		7.2±0.6*	
	2	7.8±1.2+	10.9±0.3	6.9±2.1	5.5±2.3+
	3	8.8±0.2	11.0±1.5	6.0±0.3	5.3±1.3
Group II	1	10.7±0.3*		6.9±0.3*	
	2	8.0±2.2+	10.8±0.8	7.3±2.4	5.6±0.4+
	3	9.5±1.3+	10.9±0.5	6.3±0.9+	5.9±1.1
	4	8.5±1.0+	8.8±1.4+	6.2±0.9	5.3±1.6
Group III	1	10.9±0.2*		6.0±0.2*	
	2	6.2±1.5+	10.4±0.8	11.0±3.3+	5.8±0.9
	3	8.6±1.3+	10.5±0.5	5.6±2.4+	5.4±4.0
	4	7.6±1.4+	9.6±0.5+	7.5±0.7+	5.8±1.4

* Mean±S.E.M. for entire group before PTX or sham neck surgery
+P<0.05, paired t test

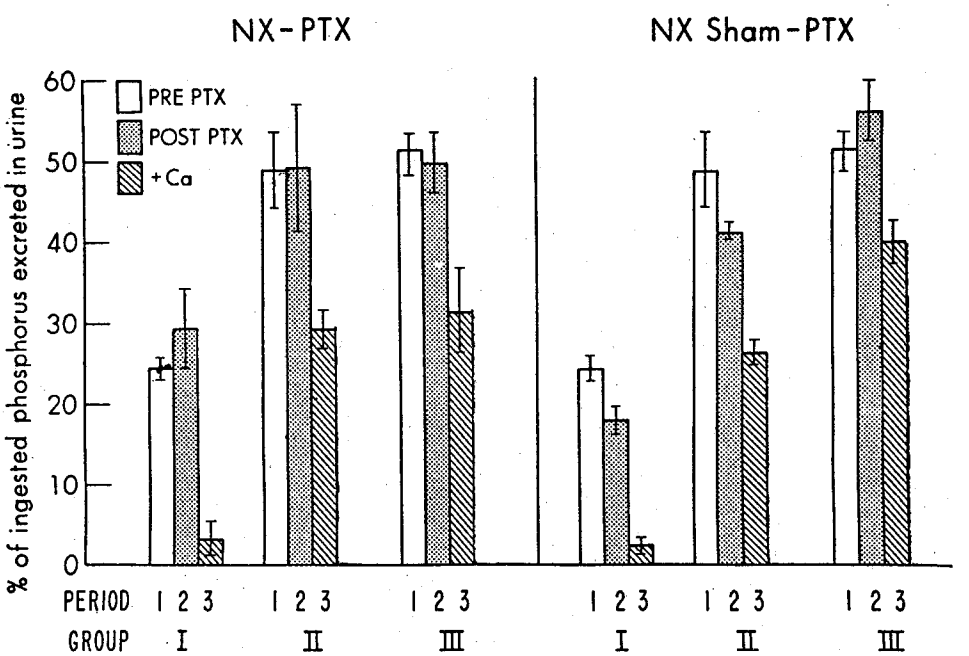

Fig. 1. *Per cent of ingested phosphorus excreted in urine of NX-PTX and NX sham-PTX rats. See Table 1 for diets of different groups and periods. Data presented as mean ± S.E.M.*

the Fig. are shown the data for the rats that underwent PTX. Phosphorus excretion measured on days 5-10 after PTX are shown in the Figure. It can be seen that per cent excretion was not significantly different after PTX than before PTX on each of the three different intakes of phosphorus ($p>0.5$ paired T test). When extra calcium was provided in the diet (period 3), urinary phosphorus excretion fell markedly ($p<0.001$ paired T test) and rose again after the extra calcium was withheld (period 4). Thus, the NX-PTX rats responded in a qualitatively similar fashion to the NX sham-PTX rats with regard to urinary phosphorus ex-

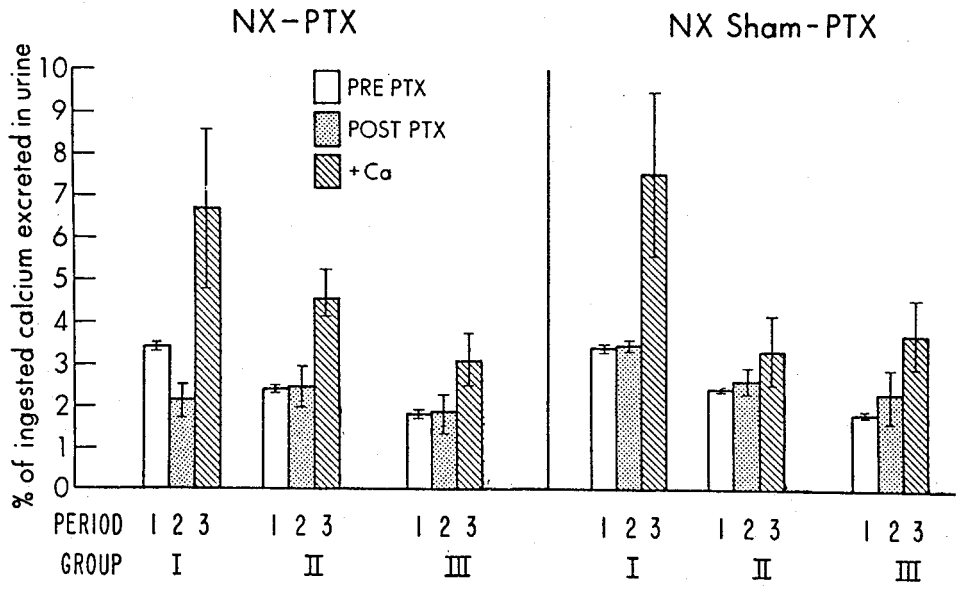

Fig. 2. Per cent of ingested calcium excreted in urine of NX-PTX and NX sham-PTX rats. See Table 1 for diets of different groups and periods. Data presented as mean ± S.E.M.

cretion. Urinary phosphorus excretion seemed to reach a plateau of 50-60% of ingested phosphorus on the two higher dietary intakes in both the NX-PTX and NX sham-PTX rats, and responded to changes in calcium intake in a similar manner whether the parathyroid glands were present or not. Statistical analysis of the NX-PTX vs NX sham-PTX data by unpaired T test revealed no significant differencs in per cent phosphorus excretion except for period 4 in Groups II and III. However, the differences in period 4 were inconsistent and probably represent biological variation rather than true experimental results.

In Fig. 2 are shown the data on 24 h urinary calcium excretion, also expressed as a per cent of ingested calcium (mean±S.E.M). In the NX sham-PTX rats, urinary calcium was inversely proportional to the amount of dietary phosphorus, in agreement with the observations of Kaye (Kaye, 1974). When supplementary calcium was given in the drinking solution (period 3), urinary calcium excretion increased,the increase being much grater in the lower phosphorus group (I) than in the two higher phosphorus group (II and III). In the NX-PTX rats, the per cent of ingested calcium excreted in the urine was almost identical to that observed in the NX sham-PTX rats. Following PTX, the per cent of calcium excreted in the urine fell in Group I, but remained constant in Groups II and III, just as in the NX sham-PTX animals. Addition of calcium to drinking water caused a rise in per cent of ingested calcium excreted in the urine (period 3) and again the magnitude of the rise in urinary calcium was inversely proportional to the amount of phosphorus in the diet. Thus, as in the case of urinary phosphorus, urinary calcium excretion responded in a qualitatively similar fashion in NX-PTX and NX sham-PTX rats. Statistical analysis by unpaired T test revealed no significant difference between NX-PTX and NX sham-PTX rats, except for period 2 of group I rats, in which calcium excretion was lower in NX-PTX animals than in NX sham-PTX animals ($p<0.05$).

In Table 3 are shown the urine and stool balance data for Groups I and II, expressed as a per cent of ingested phosphorus or calcium appearing in the urine and stool respectively. There were no significant differences between the NX-PTX and NX sham-PTX rats with regard to urinary phosphorus, fecal phosphorus or total body phosphorus balance. The per cent of ingested phosphorus excreted in the urine was higher on the higher phosphorus diet, and the per cent appearing in the feces was lower. When supplementary calcium was given (period 3), urinary phosphorus fell and fecal phosphorus rose in a comparable manner in the NX-PTX and NX sham-PTX animals. Calcium excretion in the urine was lower on the higher phosphorus diet in both NX-PTX and NX sham-PTX rats, and increased comparably when supplementary calcium was given. Total body phosphorus and calcium balance was positive in all animals, and no

Table 3. Phosphorus and Calcium Balance in NX-PTX and NX sham-PTX rats.

		Phosphorus						Calcium					
		Urine		Feces		Balance		Urine		Feces		Balance	
		%		%		%		%		%		%	
	Period	E	S	E	S	E	S	E	S	E	S	E	S
Group I	1	17.2	21.0	56.9	34.5	+26.1	+44.5	4.8	3.3	68.2	46.5	+27.0	+50.3
		±3.6	±0.7	±0.3	±8.9	± 3.0	± 9.6	±2.4	±0.4	±1.2	±10.6	± 1.2	±10.6
	2	19.8	17.5	48.1	41.9	+32.3	+40.7	2.3	3.4	56.0	55.3	+34.4	+41.8
		±6.7	±1.9	±0.2	±4.4	± 7.0	± 4.0	±0.95	±0.4	±0.8	±3.4	± 4.4	± 3.0
	3	3.1	2.6	58.0	56.5	+39.0	+41.0	6.8	7.6	56.4	54.9	+36.9	+37.6
		±2.2	±1.2	±3.4	±4.7	± 5.6	± 3.8	±1.9	±1.9	±2.8	±1.5	± 0.94	± 2.7
Group II	1	42.7	43.1	24.7	17.7	+32.6	+39.3	2.4	1.9	48.2	41.5	+48.4	+65.6
		±4.0	±2.2	±6.7	±7.1	± 5.4	± 5.2	±0.1	±0.2	±8.1	±10.9	± 7.6	±11.8
	2	42.9	42.9	21.5	24.6	+35.6	+32.5	1.7	2.5	39.8	45.7	+58.6	+51.8
		±4.0	±2.0	±2.0	±4.3	± 4.5	± 4.8	±0.4	±0.3	±5.4	±8.0	± 5.2	± 7.8
	3	32.0	28.8	33.1	40.0	+34.9	+31.0	4.3	3.5	50.0	53.5	+45.7	+43.0
		±3.2	±4.3	±8.1	±4.2	±8.6	± 7.7	±1.1	±0.8	±14.1	± 6.0	±13.1	± 5.5
	4	44.7	34.4*	24.2	35.0*	+32.8	+31.6	5.0	2.4	47.8	57.7	+47.1	+39.9
		±4.7	±2.1	±3.8	±6.0	± 5.4	± 6.0	±3.6	±0.3	±10.4	±10.0	± 7.3	± 9.9

Data are expressed as per cent of dietary intake excreted in urine or feces, mean ± S.E.M.
* <0.05 E vs S.

significant difference in balance was found between NX-PTX and NX sham-PTX rats. The only statistically significant differences found were in Group II, period 4 animals in which urinary phosphorus was lower in the sham animals than in the PTX, and fecal phosphorus was higher.

Urinary phosphorus excretion during the first 4 postoperative days after PTX or sham-PTX is shown in Table 4 for Group II and III animals. As indicated above, the PTX rats often had decreased food and fluid intake for several days after surgery. Urinary phosphorus excretion often equalled or exceeded intake during this period, in spite of the absence of PTH. Thus, some renal phosphorus wasting occurred in the NX-PTX rats for 2 days after surgery. In the NX sham-PTX rats, food and fluid intake was only slightly decreased. They also manifested some exaggerated urinary phosphorus excretion after sham-PTX, but this was not as marked as in the NX-PTX rats. Although the mechanism of this relative phosphaturia is not clear, it is apparent that the NX-PTX rats were able to excrete considerable amounts of phosphorus in the urine within the first 24 h after PTX.

Inulin clearance in the NX sham-PTX rats was 1.80±0.31 ml/min/kg, a value approximately 19% of

Table 4. Urinary phosphorus excretion in Group II and III NX rats during the first 4 days after PTX or sham-PTX

Day	Group II		Group III	
	NX-PTX	NX sham-PTX	NX-PTX	NX sham-PTX
	%	%	%	%
1	99±44	63±10	106±14	59±2.5
2	103±57	57± 6	157±19	56±1.0
3	62±14	45± 5	109±33	55±3.0
4	57±16	41± 2	78±17	52±2.0

Data expressed as per cent of ingested phosphorus, mean±S.E.M.

that in normal rats in our laboratory (Bank *et al.*, 1978). Blood urea nitrogen was 27.2±1.7 mg% in NX-PTX rats and was 32.0±1.8 mg% in NX sham-PTX rats (p NS).

DISCUSSION

The initial diet of all the NX rats in this study, before PTX or sham-PTX was performed, was designed to

produce hypertrophy of the parathyroid glands. Dietary calcium was very low and phosphorus intake was high, conditions shown by Kaye (Kaye, 1974) to result in maked parathyroid hypertrophy and increased hormone activity in NX rats. Subsequent to this initial period, the basic diet contained a moderate amount of calcium (0.82 gm%) which might have resulted in some involution of the parathyroid hypertrophy in the sham-PTX animals. However, uremic rats receiving a similar amount of calcium in Kaye's study showed significant evidence of parathyroid gland enlargement and excessive homonal effects on renal phosphate reabsorption and bone trabecular resorption (Kaye, 1974). Thus, the NX sham-PTX rats in our study most likely continued to have a considerable degree of hyperparathyroidism during the balance periods. The rats that underwent PTX demonstrated convincing evidence of hypoparathyroidism, in that serum calcium fell significantly in all instances, the degree of fall being proportional to the amount of phosphorus in the diet. Thus our study compares phosphorus and calcium balance in subtotally nephrectomized rats, one group with hyperparathyroidism and the other with hypoparathyroidism.

The results show that NX rats are able to vary urinary phosphorus excretion in response to changes in dietary phosphorus equally well in the absence of parathyroid glands as in their presence. Thus, on a low phosphorus diet, urinary phosphorus excretion was low in both NX-PTX and NX sham-PTX animals, and as dietary phosphorus was increased, urinary phosphorus excretion rose to an equivalent degree in both groups. Furthermore, both PTX and sham-PTX rats responded to changes in dietary calcium in a similar manner, ie. urinary phosphorus excretion fell markedly when calcium intake was increased and rose again when calcium intake was decreased (Fig. 1). Balance studies of stool and urine demonstrated that fecal phosphorus excretion was also similar in NX-PTX and NX sham-PTX rats (Table 3), as was total body balance of phosphorus. Varying the calcium in the diet resulted in a redistribution of phosphorus excretion between urine and stool, total balance remaining unchanged, and this redistribution appeared to be the same whether the parathyroid glands were present or not (Table 3). In the Group III studies, 3 out of 7 parathyroidectomized rats also underwent thyroidectomy. The urinary excretion results in these 3 rats were indistinguishable from those in the other 4, and therefore have been combined in the data shown in Figs. 1 and 2. These observations suggest that thyroxine and calcitionin are not necessary for the regulation of phosphorus and calcium homeostasis observed in the NX-PTX rats.

In uremic animals and man with hypertrophied parathyroid glands, parathyroid hormone plays a critical role in regulating urinary phosphorus excretion (Bank *et al.*, 1978; Goldman *et al.*, 1954; Reiss *et al.*, 1961; Slatopolsky *et al.*, 1966, 1978). It has been shown that renal handling of phosphate correlates with serum PTH levels (Madsen *et al.*, 1976) and acute PTX increases renal tubular phosphorus reabsorption markedly (Bank *et al.*, 1978; Slatopolsky *et al.*, 1966). In balance studies of uremic rats with hyperparathyroidism, Kaye (Kaye, 1974) found that the response of urinary phosphorus excretion to changes in dietary intake of phosphorus or calcium could be explained by alterations of serum ionized calcium and the resultant effect on PTH secretion. Increasing phosphorus in the diet caused a decrease in serum ionized calcium and stimulated parathyroid hormone secretion, which in turn inhibited renal tubular reabsorption of phosphorus. Adding supplementary calcium to the diet raised serum ionized calcium, suppressed parathyroid hormone secretion, which in turn led to increased renal tubular phosphorus reabsorption. Changes in parathyroid gland weight, assayable PTH, and bone resorption measurements were consistent with this interpretation. Thus, the regulation of renal phosphorus excretion in our NX sham-PTX rats could be explained by variations in PTH secretion.

The main finding of our study is that urinary and fecal phosphorus and calcium excretion responds to dietary changes in a qualitatively similar fashion in NX-PTX rats as in NX sham-PTX rats. Swenson, Weisinger, Reggeri, and Reaven (Swenson *et al.*, 1975) found that dogs with surgical reduction of renal mass were able to maintain normal serum phosphorus levels by decreasing tubular reabsorption of phosphorus in the absence of PTH. Our findings confirm a PTH-independent mechanism of renal phosphate excretion in uremic rats, and show furthermore that urinary calcium and fecal phosphorus and calcium excretion can be regulated in the absence of PTH.

In recent years, a number of studies have shown that parathyroidectomized rats with normal renal function can vary urinary phosphorus excretion in response to different dietary intakes of phosphorus (Amiel *et al.*, 1976; Bonjour *et al.*, 1977; Crawford *et al.*, 1955; Steele *et al.*, 1976; Trohler *et al.*, 1976). We assume that our rats with markedly reduced renal mass were manifestaing a similar PTH-independent regulation. The mechanism for this regulation is knknown, but three possibilities can be considered: changes in vitamin D, serum calcium, or serum and/or in-

tracellular phosphorus.

With regard to vitamin D, PTX would be expected to decrease 1,25 $(OH)_2D_3$ synthesis by the remnant kidney (Hartenbower *et al.*, 1977; Larkins *et al.*, 1973; Van Stone *et al.*, 1977), so that the NX-PTX rats might have become more deficient in this metabolite than the NX sham-PTX rats. However, Steele and co-workers found no difference in renal phosphate response to variations in dietary phosphorus in chronic TPTX rats, whether they were vitamin D deficient or not (Steele *et al.*, 1975, 1976). It seems unlikely therefore that variations in D_3 can account for the response to changes in dietary phosphorus and calcium in our animals.

Changes in serum calcium in the NX-PTX rats might account for the regulation of phosphorus excretion in the absence of PTH. Amiel *et al.* (Amiel *et al.*, 1976) showed that calcium infusion in chronic TPTX rats, to raise serum calcium to subnormal levels, increases renal phosphorus reabsorption. Sanderson, Marshall and Wilson (Sanderson *et al.*, 1960) infused calcium into TPTX dogs to produce hypercalcemia, and found increased renal phosphorus reabsorption. Popovtzer *et al* (Popovtzer *et al.*, 1975) found a similar effect of hypercalcemia in PTX rats. However, Goldfarb *et al* (Goldfarb *et al.*, 1978) recently reported that calcium infusion in TPTX dogs does not change urinary phosphorus excretion, although inhibition of phosphorus reabsorption was found in the proximal tubule. In hypoparathyroid man, calcium infusion decreases renal reaborption of phosphorus (Eisenberg, 1965). Thus, while the data are conflicting, there is evidence that in PTX rats, hypocalcemia increases urinary phosphorus excretion and raising serum calcium decreases phosphorus excretion. In our NX-PTX rats, a direct effect of serum calcium on renal phosphorus reabsorption could account for the changes in urinary phosphorus excretion in periods 2, 3 and 4.

Finally, phosphorus excretion in the NX-PTX rats might have been regulated by changes in serum and/or intracellular phosphorus. Steele and DeLuca (Steel *et al.*, 1976) and Trohler, Bonjour, and Fleisch (Trohler *et al.*, 1976) found that urinary phosphorus excretion in chronic TPTX rats responds appropriately to variations in dietary phosphorus, and that excretion changes are accompanied by parallel changes in serum phosphorus. In the study by Trohler *et al* (Trohler *et al.*, 1976), the alterations in phosphorus excretion occurred without changes in serum calcium. Thus, their data suggest that either the filtered load of phosphorus or intracellular phosphorus affects the rate of phosphorus reabsorption by the renal tubules in the absence of PTH. It has been shown that the proximal convoluted tubule of the rabbit has a sharply-defined Tm for phosphate reabsorption which is not influenced by PTH (Dennis *et al.*, 1976, 1977). One can postulate, therefore, that in the NX-PTX rats, phosphorus excretion responded to the dietary manipulations as a result of changes in the filtered load of phosphorus. The mean serum phosphorus concentrations were slightly higher in the NX-PTX rats that in the NX sham-PTX rats, and even though the differences in most instances were not statistically significant, they may have been enough to account for differences in urinary excretion.

The present observations should not be interpreted to mean that parathyroid hormone is unimportant for phosphorus and calcium homeostasis in renal failure, but rather that another mechanism(s) can substitute in the absence of the hormone. Although phosphorus and calcium balance was maintained in the NX-PTX rats, this was at the expense of hypocalcemia and weight loss in many animals. The NX-PTX rats manifested hemodynamic instability under the stress of anesthesia and surgery. Thus, whereas the NX-PTX and NX sham-PTX rats regulated phosphorus and calcium excretion comparably, the NX sham-PTX rats maintained much more constant serum levels of calcium and phosphorus in the face of dietary manipulations. Maintenance of serum calcium and phosphorus concentrations is apparently one important role of PTH in renal failure.

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