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Restoration of Phosphaturia in Phosphate-Deprived Rats in the Presence of Both Parathyroid Hormone and Phosphate Infusion (41540)

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Abstract. The present study tested the hypothesis that the combination of parathyroid hormone and phosphate infusion would be phosphaturic in phosphate-deprived rats. Clearance experiments were performed in Sprague–Dawley rats fed a low-phosphate diet for 4 days. The animals were first given a phosphate infusion and then administered parathyroid hormone. The reverse experiment was also performed in a second group of rats by giving the hormone first followed by a phosphate infusion. There was no increase in phosphate excretion in response to phosphate infusion or parathyroid hormone given alone. However, the combination of parathyroid hormone and phosphate infusions, given in either order, was phosphaturic in phosphate-deprived rats.

Phosphate deprivation is characterized by avid phosphate reabsorption (1, 2) and a resistance to the phosphaturic effects of parathyroid hormone (3–5) and phosphate infusions (1, 2, 6). However, whether phosphate-deprived animals are resistant to the simultaneous infusion of phosphate and parathyroid hormone is controversial.

In a recent study, Moser *et al.* (7) demonstrated that both acute and chronic phosphate-deprived rats which were supplemented with intraperitoneal injections of phosphate to maintain serum phosphate near normal, did not develop a hypophosphaturia. These investigators attributed a critical role for serum phosphate in determining urinary phosphate excretion during phosphate deprivation. However, the administration of phosphate was also associated with a significant decrease in serum calcium levels which could have stimulated the release of parathyroid hormone, thereby producing a phosphaturia. Consequently, the authors stated that although the administration of parathyroid hormone to phosphate-deprived rats is not associated with a phosphaturia, they speculated that perhaps elevation of serum phosphate by phosphate infusion may render the renal tubule responsive to parathyroid hormone.

Previous studies in rats, however, have not supported such a notion. Bonjour *et al.* (8) observed a reduced phosphaturic response to parathyroid hormone over a wide range of plasma phosphate concentrations in conscious rats fed a low-phosphate diet. On the other hand, in a study of long-term phosphate-deprived dogs, Wong *et al.* (9) found a normal phosphaturic response with the infusion of both parathyroid hormone and phosphate.

Therefore, the present study was performed to evaluate whether parathyroid hormone is phosphaturic in phosphate-deprived rats in the presence of a phosphate infusion. We also performed the reverse experiment by giving the hormone first, followed by a phosphate infusion.

Methods. Experiments were performed on male Sprague–Dawley rats weighing 212–343 g. The animals were stabilized on a low-phosphate diet (0.07%) (ICN Pharmaceuticals, Inc., Cleveland, Ohio) for 4 days prior to the experiment. Sodium and potassium content was supplemented with NaCl and KCl as previously described (5). All animals were given food and water *ad libitum*.

On the day of the experiment, the rats were anesthetized with inactin (100 mg/kg) and prepared for clearance experiments. The animals were placed on a heated table, and body temperature was monitored with a rectal probe. A tracheotomy was performed to clear the airway and animals were allowed to breathe spontaneously. Catheters were placed in jugular veins for infusions, in the carotid

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artery for blood pressure measurement and blood sampling, and in the bladder for urine collections.

All rats underwent acute thyroparathyroidectomy (TPTX) by heat cautery followed by a 2-hr recovery period to allow for attainment of a steady state. During the second hour of recovery, the infusion of a 5% inulin solution was begun at 1.2 ml/hr for the duration of the experiment. At this point one of four protocols was performed:

Group 1: Control, P_i, P_i. The purpose of this protocol was to evaluate the effects of phosphate infusions alone within the same time frame as the group given both P_i and PTH. Following the recovery period, a 30-min clearance period was taken during which saline was also infused at 2 ml/hr. After the control clearance, the saline was subsequently replaced with a phosphate solution calculated to deliver phosphate at 2 μmole/min for the duration of the experiment. The phosphate solution was made with a 4:1 mixture of dibasic:monobasic sodium phosphate salts and adjusted to pH 7.4. Phosphate infusion proceeded for 15 min before two 30-min clearance periods were obtained. An hour was allowed to elapse followed by two additional clearance periods.

Group 2: Control, Control, PTH. The purpose of this protocol was to evaluate the effects of PTH alone within the same time frame as the group given both P_i and PTH. Following the 2-hr recovery period, a 30-min clearance period was obtained. In this group, two additional 30-min control clearance periods were taken before PTH (Synthetic, 1-34, Beckman Instruments, Palo Alto, Calif.) was administered. The hormone was given as a bolus injection (33 units/kg) followed by a maintenance infusion (60 units/kg · hr). The infusion continued for 1 hr before two 30-min clearance periods were obtained.

Group 3: Control, P_i, P_i + PTH. The purpose of this protocol was to evaluate the renal effects of parathyroid hormone given in the presence of an ongoing phosphate infusion. The protocol was similar to Group 1 with the exception that PTH was now administered during the second hour of phosphate infusion. The doses of the hormone are similar to those already described in Group 2.

Group 4: Control, PTH, PTH + P_i. The purpose of this protocol was to assess the renal effects of phosphate infusion given in the presence of parathyroid hormone. In this group the order was reversed, with parathyroid hormone being given first, followed by the infusion of phosphate.

Inulin concentrations in plasma and urine were measured by the anthrone method (10). Glomerular filtration rate (GFR) was equated with the inulin clearance. Phosphate was measured using the Chen method (11) and calcium was determined by atomic absorption spectrometry. Sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory, Inc.).

All values are expressed as mean ± SE. Statistical comparisons were made with paired and unpaired t tests.

Results. *Control Experiments: Either P_i or PTH alone.* The effects of phosphate infusion alone in phosphate-deprived TPTX rats (Group 1) are presented in Table I. Plasma phosphate concentration increased significantly in response to the infusion of phosphate; however, no increase occurred in phosphate excretion. The high variability in the last period was due to results in one animal where phosphate excretion did increase. Plasma calcium concentration did not change

TABLE I. EFFECTS OF PHOSPHATE INFUSION ALONE IN PHOSPHATE-DEPRIVED TPTX RATS

	P_{P_i} (m*M*)	FE_{P_i} (%)	P_{Ca} (m*M*)	GFR (ml/min)	FE_{Na} (%)	BP (mm Hg)
Control	2.63 ± 0.22	0.13 ± 0.06	2.20 ± 0.29	2.1 ± 0.5	0.29 ± 0.18	129 ± 13
P_i	3.24 ± 0.30*	0.27 ± 0.19	1.98 ± 0.19	1.6 ± 0.5	0.45 ± 0.17	127 ± 14
P_i	3.81 ± 0.37*	4.13 ± 3.58	1.58 ± 0.18*	2.0 ± 0.5	1.06 ± 0.31*	111 ± 9

Note. Values are means ± SE in five animals. P_{P_i}, plasma phosphate; FE_{P_i}, fractional excretion of phosphate; P_{Ca}, plasma calcium; GFR, glomerular filtration rate; FE_{Na}, fractional excretion of sodium; BP, mean arterial blood pressure.

* Significantly different from preceding period, $p < 0.05$.

TABLE II. EFFECTS OF PARATHYROID HORMONE ALONE IN PHOSPHATE-DEPRIVED TPTX RATS

	P_{Pi} (mM)	FE_{Pi} (%)	P_{Ca} (mM)	GFR (ml/min)	FE_{Na} (%)	BP (mm Hg)
Control	2.34 ± 0.18	0.09 ± 0.01	2.45 ± 0.08	2.0 ± 0.2	0.55 ± 0.41	134 ± 3
Control	2.70 ± 0.10	0.07 ± 0.01	2.16 ± 0.04*	2.0 ± 0.2	0.99 ± 0.65	127 ± 4
PTH	3.23 ± 0.10*	1.90 ± 0.72	2.05 ± 0.05	2.1 ± 0.2	0.53 ± 0.10	112 ± 4*

Note. Values are means ± SE in six animals. PTH, parathyroid hormone. Other abbreviations as described in Table I.

* Significantly different from preceding period, $p < 0.05$.

during the first period of phosphate infusion but declined significantly with continued infusion. Glomerular filtration rate and mean arterial blood pressure were not significantly changed, although blood pressure tended to fall during the later stages of the experiment.

The results of Group 2 rats which were given only parathyroid hormone appear in Table II. Plasma phosphate was significantly increased during infusion of the hormone, but no increase in phosphate excretion was observed. Plasma calcium decreased significantly in the second control period but upon PTH infusion did not decline further. Glomerular filtration rate was not affected by parathyroid hormone. Blood pressure was significantly decreased, yet remained within an acceptable range (i.e., >100 mm Hg).

Experiments in the presence of both P_i and PTH. In Table III, data are presented from Group 3 wherein phosphate was infused first, followed by the additional infusion of parathyroid hormone. In response to the infusion of phosphate, there was no change in phosphate excretion. The rise in plasma phosphate was not statistically significant. There were no significant changes in blood pressure or glomerular filtration rate. Upon the addition of parathyroid hormone to the infusion of phosphate there was a marked increase in fractional phosphate excretion from 0.09 to 22.5%. The increase in plasma phosphate was variable and did not attain statistical significance. However, plasma calcium decreased progressively. Mean arterial blood pressure was significantly lower than in the previous period, but glomerular filtration rate remained stable.

In Group 4, the reverse experiment was performed in which parathyroid hormone was administered first, followed by the combination of parathyroid hormone and phosphate infusion. The results appear in Table IV. The administration of parathyroid hormone alone did not elicit any significant changes in the parameters evaluated. Fractional phosphate excretion was not significantly increased and the value of approximately 2% was similar to that observed in the time control group (Table II). The initiation of phosphate infusion in the presence of an ongoing infusion of parathyroid hormone resulted in increases in fractional excretion of phosphate from 2.4 to 26.4%. Plasma phosphate also increased significantly; however, in this group there were no significant changes in plasma calcium. Mean arterial blood pressure was significantly lower in this period, as had been observed in the previous group. Nonetheless, glomerular filtration rate remained stable.

In these experiments, volume expansion

TABLE III. EFFECTS OF PARATHYROID HORMONE IN THE PRESENCE OF PHOSPHATE INFUSION IN PHOSPHATE-DEPRIVED TPTX RATS

	P_{Pi} (mM)	FE_{Pi} (%)	P_{Ca} (mM)	GFR (ml/min)	FE_{Na} (%)	BP (mm Hg)
Control	2.40 ± 0.13	0.09 ± 0.01	1.91 ± 0.12	2.0 ± 0.2	0.09 ± 0.03	140 ± 9
P_i	2.65 ± 0.17	0.09 ± 0.01	1.59 ± 0.09*	2.0 ± 0.1	0.36 ± 0.09*	131 ± 9
P_i + PTH	3.01 ± 0.37	22.5 ± 6.4*	1.21 ± 0.10*	2.2 ± 0.1	0.75 ± 0.11*	110 ± 9*

Note. Values are means ± SE in eight animals. Other abbreviations as described in Table I.

* Significantly different from preceding period, $p < 0.05$.

TABLE IV. EFFECTS OF PHOSPHATE INFUSION IN THE PRESENCE OF PARATHYROID HORMONE IN PHOSPHATE-DEPRIVED TPTX RATS

	P_{P_i} (m*M*)	FE_{P_i} (%)	P_{Ca} (m*M*)	GFR (ml/min)	FE_{Na} (%)	BP (mm Hg)
Control	2.80 ± 0.25	0.78 ± 0.35	1.95 ± 0.02	1.7 ± 0.1	0.47 ± 0.36	139 ± 3
PTH	2.96 ± 0.13	2.35 ± 1.79	2.00 ± 0.08	2.3 ± 0.1	0.31 ± 0.12	138 ± 5
PTH + P_i	3.72 ± 0.20*	26.4 ± 9.9*	1.87 ± 0.03	2.3 ± 0.08	0.52 ± 0.23	121 ± 6*

Note. Values are means ± SE in six animals. Other abbreviations as described in Table I.
* Significantly different from preceding period, $p < 0.05$.

was minimized, as evident from the fractional excretion of sodium which was less than 1% in all groups.

Discussion. The present study tested the hypothesis that the combination of parathyroid hormone and phosphate infusion would be phosphaturic in rats acutely phosphate deprived. There was no increase in phosphate excretion in response to phosphate infusion or parathyroid hormone given alone. However, in the presence of both factors given in either order, a marked phosphaturia was evident.

Our findings confirm the results in dogs, but differ from previous studies in rats. Wong *et al.* (9) reported, in a recent study involving long-term (30–60 day) phosphate-deprived dogs, that a normal phosphaturic response was observed with the infusion of both parathyroid hormone and phosphate. On the other hand, Bonjour *et al.* (8) observed a reduced phosphaturic response to parathyroid hormone over a wide range of plasma phosphate concentrations, induced by phosphate infusions, in rats fed a low-phosphate diet.

There are, however, several fundamental differences between the present study and the study of Bonjour *et al.* (8) in rats which should be highlighted. Although the diet utilized by Bonjour *et al.* contained more phosphate (0.2%) than the one used in the present study (0.07%), the rats were phosphate-deprived for 7 days as compared to our 4-day phosphate deprivation. Troehler *et al.* (2) have shown that the effects of phosphate-deprivation are evident within 3 days. However, it is possible that the slightly longer period of phosphate deprivation could have resulted in a more severe condition of phosphate depletion, which may explain their observation of a persistent resistance to parathyroid hormone. Second, the previous experiments were performed in conscious, chronic (48-hr) TPTX rats, whereas the present studies were in anesthetized, acute TPTX rats. Finally, different forms of parathyroid hormone were utilized in both studies. Bonjour *et al.* used a TCA extract (190–250 IU/mg from Wilson Laboratories) and infused 2.5 IU/rat · hr, whereas we infused a synthetic hormone (1-34, Beckman Instruments, 6000 IU/mg) in both a bolus (33 IU/kg) and a sustaining (60 IU/kg · hr) infusion. It is possible that the different hormone preparations and doses utilized contributed to the differences in response.

Wong *et al.* (9) suggested that the combination of phosphate and parathyroid hormone infusions restored intracellular phosphate levels and hence led to a phosphaturia. The relationship of phosphate infusion to plasma phosphate and the intracellular phosphate pools is not clear. We observed a phosphaturia in the presence of modest infusions of phosphate (2 μmole/min). Indeed, in the group given phosphate first (Group 3) a phosphaturia ensued in the absence of significant increases in plasma phosphate (Table III). Moreover, the effect was evident even when parathyroid hormone was administered first followed by phosphate (Group 4), a situation where the extent of intracellular phosphate pool repletion is not presumably as large. Clearly, the interrelationships between intracellular phosphate pools and the renal response to phosphaturic stimuli remain to be elucidated.

Phosphate infusions may have altered the pattern of segmental phosphate reabsorption along the nephron. The nephron sites of action of parathyroid hormone have been localized to the proximal convoluted and straight segments and along the accessible dis-

tal convoluted tubule (12, 13). A preliminary study (14) recently reported that phosphate uptake into brush border membrane vesicles was decreased following phosphate infusions in phosphate-deprived rats. Taken together, it is conceivable that phosphate infusions decrease phosphate reabsorption in early sites along the nephron but, in the absence of parathyroid hormone, the increased delivery of phosphate is reabsorbed in later sites, such as the pars recta or distal tubule, resulting in no increase in urinary phosphate excretion. Therefore, in the presence of parathyroid hormone, the later sites would not reabsorb the increased delivery of phosphate leading to a phosphaturia.

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