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*J Clin Invest.* 1976;**57**(2):256-263. <https://doi.org/10.1172/JCI108276>.

### Research Article

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# Evidence for a Parathyroid Hormone-Independent Calcium Modulation of Phosphate Transport along the Nephron

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**ABSTRACT** To disclose a parathyroid-independent calcium modulation of phosphate transport along the nephron, the effect of increasing plasma calcium concentration to subnormal levels in rats 6 days after parathyroidectomy (chronic PTX) was studied. Fractional phosphate reabsorption was significantly increased. The whole kidney response to calcium infusion was similar whether or not the thyroid gland was removed, which suggests that calcitonin is not involved. The micropuncture study indicated an increase in the reabsorptive capacity for phosphate (absolute reabsorption/absolute delivered phosphate per nephron segment) in the proximal tubule, the loop, and the terminal nephron when calcium was infused. Thus, the level of plasma calcium or some related factor affects the phosphate transport by the tubule independently of parathyroid hormone.

With calcium infusion, the profile of phosphate reabsorption along the nephron became close to that of acutely parathyroidectomized rats, but with persisting differences. The level of plasma calcium concentration may partly account for the differences between the acute and the chronic steps of parathyroidectomy.

The role of possible interferences between alterations of extracellular calcium concentration or some related factor and the adenylate cyclase-cyclic AMP system in such an action of calcium was evaluated. Cyclic AMP was infused so as to achieve a  $10^{-6}$  M plasma concentration. Combined infusions of calcium and cyclic AMP were also performed. The results are compatible with calcium inhibition of adenylate cyclase, although they do not rule out a direct action of calcium.

Part of this work was accepted as a free communication at the XXVI International Congress of Physiological Sciences, New Delhi, 20-26 October 1974.

Received for publication 31 December 1974 and in revised form 6 October 1975.

## INTRODUCTION

Renal tubular transport of phosphate is modulated by parathyroid hormone (PTH),<sup>1</sup> which likely acts through the adenylate cyclase (Ac)-cyclic AMP (cAMP) system (1). Extracellular fluid calcium concentration does regulate parathyroid hormone secretion (2) and therefore indirectly influences tubular phosphate transport. Moreover, a direct, non-PTH-mediated action of calcium on tubular phosphate reabsorption has been reported. A decrease of phosphate reabsorption as a consequence of increased plasma calcium concentration has been described (3-5). On the other hand, an increase in phosphate reabsorption has been observed as a consequence of increased plasma calcium concentration (6, 7) and a decrease of phosphate reabsorption when plasma calcium concentration is lowered has been reported (8).

In line with this latter relationship between plasma calcium level and phosphate reabsorption is the observation that fractional phosphate reabsorption and plasma calcium concentration are both lower in animals studied several days after parathyroidectomy (chronic PTX) than in animals studied several hours after surgery (acute PTX) (9, 10).

Extracellular calcium concentration may act on phosphate transport through the same system as PTH, namely the Ac-cAMP one. Indeed there are several evidences of an influence of calcium on Ac activity (11-14). Conversely, calcium may act independently of this system, through a pathway insensitive to PTH (7).

<sup>1</sup>Abbreviations used in this paper: Ac, adenylate cyclase; cAMP, cyclic AMP; GFR, glomerular filtration rate; PTH, parathyroid hormone; PTX, parathyroidectomy; SNGFR, single nephron glomerular filtration rate; (TF/P), tubular fluid over plasma ratio; (U/P), urine over plasma ratio.

The present work was designed (a) to evaluate and localize the possible effect on phosphate transport along the nephron of increasing plasma calcium concentration to subnormal values; (b) to investigate possible interferences between alterations of plasma calcium concentration and the Ac-cAMP system. To avoid any interference with PTH secretion, parathyroidectomized rats were studied, and to obtain a steady state of both plasma calcium concentration and phosphate reabsorption, experiments were performed 6 days after parathyroidectomy (chronic PTX). These animals were studied either at endogenous levels of calcium and cAMP plasma concentration, or at exogenous levels of calcium and cAMP, during infusion of either or both substances.

## METHODS

Experiments were performed on 33 PTX male rats (Sprague-Dawley, Centre National de la Recherche Scientifique, Orléans La Source, France), average weight  $191 \pm 3$  g (SEM). Laboratory chow of constant composition (calcium 230 mmol/kg; magnesium 64 mmol/kg; phosphorus 297 mmol/kg; vitamin D 4,000 IU/kg; sodium 130 mmol/kg; potassium 173 mmol/kg) was made available to all rats until 13–15 h before an experiment. Free access to water was allowed.

Parathyroidectomy was performed surgically under light ether anesthesia 6 days before the experiments. Its efficiency was controlled by plasma calcium determination at the beginning of each experiment. In five rats (see below) a thyroidectomy was performed at the same time as the parathyroidectomy.

Anesthesia was achieved by the i.p. administration of sodium 5 ethyl-5 (1'-methyl-propyl)-2-thiobarbiturate (Inactin, Promonta, Hamburg, W. Germany) 10 mg/100 g body wt. The animals were placed on a heated table and the rectal temperature was maintained between 36.5 and 37.5°C. Isotonic saline was infused via a tail vein at a constant rate of 50  $\mu$ l min/100 g body wt. The arterial blood pressure was monitored and blood samples were obtained through a catheter inserted into the right common carotid artery. A tracheotomy was performed. Both ureters were catheterized to establish that urine flow from both kidneys was similar. The experimental kidney was always the left one. During surgery estimated fluid losses were replaced by a single i.v. supplemental injection of 0.15 M NaCl (0.5 ml/100 g body wt). A priming dose of radioisotopes was given at the end of the surgery: tritiated inulin ( $[methoxy-^3H]inulin$ , New England Nuclear, Boston, Mass.) and  $^{32}P$ -radiophosphate ( $^{32}P$ -neutral sodium phosphate, Commissariat à l'Énergie Atomique, Saclay, France). The priming dose was followed immediately by a sustaining dose of isotopes via an NaCl infusion (5% of the priming dose of tritiated inulin and 1% of the priming dose of radiophosphate per minute). The experiments began after a 90-min equilibration period.

Three series of experiments were performed: micropuncture experiments (20 rats), clearance studies (5 rats), and plasma ultrafiltration controls (8 rats). In micropuncture experiments the left kidney was exposed, immobilized, covered with mineral oil, and illuminated as described by Lechêne, et al. (15). The early distal and the late accessible proximal convolutions of the same superficial nephron were located by stereomicroscopic observation after an injection

of about 1–2 nl of lissamine green (0.4% wt:vol in 0.15 M NaCl) in the proximal tubule via a micropipette whose external tip diameter was 3–4  $\mu$ m. The accessible early distal and late proximal convolutions of each nephron studied were subsequently micropunctured. Samples of tubular fluid were obtained during the distal immobilization of a previously injected oil column. The duration of the collection was 3 min for distal samples and 2 min for proximal ones. A blood sample was obtained every 30 min on dried sodium heparinate. Urine from the experimental kidney was collected in a weighed tube during sequential 30-min periods. Overall, three to six clearance periods were performed and five to ten nephrons were sampled in each rat (136 nephrons in 20 rats).

The animals undergoing micropunctures were divided into four groups of five rats each. One group did not receive either calcium or cAMP (group I: PTX). Another group was infused with  $CaCl_2$ , 2.25  $\mu$ mol min/100 g body wt after a priming dose of 1 nmol/100 g body wt, administered at the same moment as the isotopes (group II: PTX + Ca). The third group was infused with 3'-5' cAMP (Sigma Chemical Co., Inc., St. Louis, Mo.; A 9501), 4 nmol min/100 g body wt after a priming dose of 1 nmol/100 g body wt, administered at the same moment as the isotopes (group III: PTX + cAMP). The animals of the fourth group received both the  $CaCl_2$  and the cAMP infusions at the same rates as above (group IV: PTX + Ca + cAMP).

Clearance experiments were performed on five rats thyroparathyroidectomized 6 days before the experiment. All those rats were infused with  $CaCl_2$  like the animals of group II.

Calcium concentration in plasma ultrafiltrate was measured in eight rats divided into four groups (two rats in each, infused as groups I–IV).

*Analytical procedures.* Determination of inorganic phosphate, sodium, calcium, and magnesium concentrations, and  $^{32}P$  and  $[^3H]inulin$  radioactivities were performed as described earlier (16). Ultrafiltration of plasma was made as by Morel et al. (17). Calcium determination in plasma ultrafiltrate was performed by ultramicro-atomic absorption spectrometry, as described previously (18).

The plasma data were interpolated, between the two consecutive samples withdrawn at a 30-min interval, at the mid-time of tubular fluid or urinary collections. The single glomerular filtration rate (SNGFR) was calculated twice for each nephron. The values yielded by the distal collection were correlated to those obtained from the proximal one:  $SNGFR_{distal} = 0.58 \times SNGFR_{proximal} + 15.9$  (nl/min);  $r = 0.64$ ;  $n = 136$ ;  $P < 0.001$ . A small but significant difference appeared between proximal and distal determination, the latter being lower. For the calculation of absolute reabsorptions in the different segments of each nephron, the value of SNGFR obtained from the distal tubule was used.

Cyclic AMP determination was performed by Gilman's technique (19) as modified by Rabinowitz and Katz (20).

Micropuncture results were analyzed according to Snedecor and Cochran (21) and Lellouch and Lazar (22) as a  $2^2$  factorial experiment. Plasma calcium concentration and plasma cAMP concentration were considered as each of the two factors. The low level of each factor was the endogenous one. The high level of each factor resulted from infusion of calcium or cAMP. The relationships between the absolute phosphate reabsorption versus the absolute phosphate delivery to each tubular segment were treated by covariance analysis (21, 22).

## RESULTS

### Results of micropuncture experiments

**Plasma and clearance data (Table I).** Plasma calcium concentration reached subnormal level in the groups infused with  $\text{CaCl}_2$  (plasma calcium level of intact rats, similarly fed and prepared for experimentation was  $2.43 \pm 0.075$  mM,  $n = 14$ , in the same laboratory). Plasma cAMP concentration was  $36 \pm 8$  nM (SEM),  $n = 10$ , in groups I and II and  $2.5 \pm 0.3$   $\mu\text{M}$ ,  $n = 10$ , in groups III and IV. Fractional phosphate excretion calculated as:  $(\text{U/P}^{32}\text{P}/\text{U/P}^{3}\text{H}]\text{In}) \times 100$ , in ureteral urine was significantly decreased by calcium infusion and significantly increased by cAMP infusion. Fractional sodium excretion, fractional water excretion, and magnesium urinary excretion were not significantly influenced either by calcium or by cAMP infusions; calcium urinary excretion was significantly increased by calcium infusion. The whole kidney glomerular filtration rate (GFR) was decreased by calcium infusion. There was a significant correlation between mean GFR and mean SNGFR of each rat:  $\text{GFR (ml/min)} = 0.017 \text{ distal SNGFR (nl/min)} + 0.302$ ,  $n = 20$ ,  $r = 0.75$ ,  $P < 0.001$ . The apparent number of nephrons, calculated as the ratio of mean GFR to mean distal SNGFR for each rat, was not significantly different in the four situations. Thus, an intrarenal redistribution of glomerular filtrate is unlikely. Still, these calculations cannot exclude that the nephron population under study may not necessarily be representative of all the nephrons in the kidney. Calcium had no significant effect on plasma phosphate concentration, which was significantly lowered by cAMP infusion.

**Late accessible proximal tubule results (Table II).** The inulin tubular fluid over plasma ratio, (TF/P)- $^{3}\text{H}]\text{In}$ , at the late proximal tubule was not affected by either calcium or cAMP. Neither was SNGFR.

The  $^{32}\text{P}$  tubular fluid over plasma ratio, (TF/P) $^{32}\text{P}$ , and the fractional delivery of phosphate to late proximal tubule were significantly decreased by calcium and not significantly increased by cAMP.

**Early accessible distal tubule results (Table II).** At the early accessible tubule neither (TF/P) $^{3}\text{H}]\text{In}$  nor SNGFR were affected by calcium or cAMP. Both (TF/P)  $^{32}\text{P}$  and (TF/P)  $^{32}\text{P}/^{3}\text{H}]\text{In}$  were significantly decreased by calcium and significantly increased by cAMP.

**Results from the loops (arbitrarily defined as the nephron segments located between late accessible proximal and early accessible distal tubule, Table II).** The percentage of filtered water reabsorbed in the loop was similar in the four groups. The contribution of the loop to the overall nephron phosphate reabsorption (loop reabsorption expressed in percentage of filtered load) was unaffected during calcium infusion and significantly decreased during cAMP infusion. Phosphate reabsorption was also calculated as the percentage of the phosphate load delivered to the loop. This figure was increased during calcium infusion and decreased during cAMP infusion.

In all the four groups significant positive correlations appeared between fractional reabsorption of water ( $x$ ) and fractional reabsorption of phosphate in the same loop ( $y$ );  $y = 0.46x + 6.1$ ,  $r = 0.48$ ,  $n = 33$ ,  $P < 0.01$ ;  $y = 0.45x - 3.8$ ,  $r = 0.62$ ,  $n = 39$ ,  $P < 0.001$ ;  $y =$

TABLE I  
Plasma Data and Left Kidney Function in Micropuncture Experiments

	Group I Ca = 0* cAMP = 0*	Group II Ca = +* cAMP = 0*	Group III Ca = 0* cAMP = +*	Group IV Ca = +* cAMP = +*	Ca effect‡	cAMP effect‡	Inter- action‡
Ca plasma, mM	0.99 ± 0.05	2.11 ± 0.11	1.18 ± 0.04	2.34 ± 0.16	$P < 0.001$	NS	NS
Pi plasma, mM	3.49 ± 0.17	3.22 ± 0.09	2.52 ± 0.07	2.65 ± 0.14	NS	$P < 0.001$	NS
GFR, ml · min <sup>-1</sup>	1.100 ± 0.102	0.999 ± 0.037	1.137 ± 0.097	0.888 ± 0.047	$P < 0.05$	NS	NS
Number of nephrons§	25,379 ± 1,041	23,479 ± 1,282	26,087 ± 1,856	24,106 ± 1,888	NS	NS	NS
$\dot{V}_u$ , $\mu\text{l} \cdot \text{min}^{-1}$	13.51 ± 2.04	13.65 ± 4.96	13.25 ± 1.24	13.05 ± 6.44	NS	NS	NS
(U/P) $^{3}\text{H}]\text{In}$	87.16 ± 11.78	109.65 ± 25.36	92.34 ± 6.41	127.18 ± 32.96	NS	NS	NS
FE Pi	8.44 ± 1.86	3.01 ± 0.68	19.21 ± 0.66	15.33 ± 2.13	$P < 0.01$	$P < 0.001$	NS
FE Na	2.97 ± 0.50	2.32 ± 0.41	2.31 ± 0.23	1.91 ± 0.73	NS	NS	NS
[Ca] <sub>u</sub> · $\dot{V}_u$	5.31 ± 1.03	62.65 ± 19.35	7.39 ± 2.59	91.24 ± 20.26	$P < 0.001$	NS	NS
[Mg] <sub>u</sub> · $\dot{V}_u$	61.83 ± 12.01	70.03 ± 12.84	77.11 ± 20.05	108.97 ± 3.48	NS	NS	NS

Data are means ± SEM. Each value is the mean of the five mean values from the five rats of one group. Abbreviations: NS, not significant ( $P > 0.05$ );  $\dot{V}_u$ , rate of ureteral urinary flow; (U/P) $^{3}\text{H}]\text{In}$  urine to plasma inulin ratio; FE, fractional excretion as percent of filtered load; [ ]<sub>u</sub>, concentration in ureteral urine.

\* 0, no infusion; +, infusion.

‡ From factorial analysis.

§ Apparent number of nephrons calculated as GFR/distal SNGFR.

TABLE II  
Results from Micropuncture Experiments

	Group I Ca = 0* cAMP = 0*	Group II Ca = +* cAMP = 0*	Group III Ca = 0* cAMP = +*	Group IV Ca = +* cAMP = +*	Ca effect‡	cAMP effect‡	Inter- action‡
Late proximal							
SNGFR, $nl \cdot min^{-1}$	45 ± 3	47 ± 5	49 ± 5	43 ± 3	NS	NS	NS
(TF/P)[ <sup>3</sup> H]In	1.94 ± 0.17	1.93 ± 0.04	1.96 ± 0.12	1.96 ± 0.07	NS	NS	NS
(TF/P) <sup>32</sup> P	0.78 ± 0.01	0.55 ± 0.04	0.83 ± 0.05	0.65 ± 0.04	<i>P</i> < 0.001	NS	NS
(TF/P) <sup>32</sup> P/[ <sup>3</sup> H]In × 100	42.52 ± 3.79	28.90 ± 1.91	44.02 ± 2.22	33.58 ± 2.78	<i>P</i> < 0.001	NS	NS
Pi reabsorption, $\mu mol \cdot min^{-1} nephron$	89.55 ± 14.37	98.48 ± 8.51	62.17 ± 7.90	66.41 ± 6.75	NS	<i>P</i> < 0.01	NS
Early distal							
SNGFR, $nl \cdot min^{-1}$	43 ± 4	43 ± 3	44 ± 5	37 ± 3	NS	NS	NS
(TF/P)In- <sup>3</sup> H	3.81 ± 0.34	4.53 ± 0.44	4.04 ± 0.35	4.40 ± 0.22	NS	NS	NS
(TF/P) <sup>32</sup> P	0.91 ± 0.06	0.34 ± 0.07	1.28 ± 0.15	0.87 ± 0.13	<i>P</i> < 0.001	<i>P</i> < 0.001	NS
(TF/P) <sup>32</sup> P/[ <sup>3</sup> H]In × 100	25.60 ± 3.58	8.82 ± 2.07	32.62 ± 3.09	20.27 ± 3.52	<i>P</i> < 0.001	<i>P</i> < 0.001	NS
Loop							
H <sub>2</sub> O reabsorbed, % of filtered	26.79 ± 2.73	27.71 ± 2.01	26.54 ± 2.01	28.31 ± 1.57	NS	NS	NS
Pi reabsorbed: % of filtered	16.77 ± 2.15	20.08 ± 1.27	11.40 ± 2.93	13.54 ± 1.24	NS	<i>P</i> < 0.01	NS
% of delivered to the loop	39.44 ± 5.15	70.60 ± 5.28	23.24 ± 6.26	40.53 ± 6.28	<i>P</i> < 0.001	<i>P</i> = 0.001	NS
Pi reabsorption, $\mu mol \cdot min^{-1} nephron$	26.48 ± 4.74	27.31 ± 2.39	14.84 ± 5.35	13.53 ± 1.58	NS	<i>P</i> < 0.005	NS
Terminal nephron							
Pi reabsorbed: % of filtered	16.96 ± 2.26	5.93 ± 1.55	13.43 ± 2.70	4.98 ± 1.65	<i>P</i> < 0.001	NS	NS
% of delivered	62.27 ± 6.01	56.79 ± 9.38	37.55 ± 5.52	18.41 ± 6.13	NS	<i>P</i> < 0.001	NS
Pi reabsorption, $\mu mol \cdot min^{-1} nephron$	25.05 ± 1.07	8.70 ± 2.97	14.34 ± 1.61	5.29 ± 1.87	<i>P</i> < 0.001	<i>P</i> < 0.005	NS

Data are means ± SEM. Each value is the mean of the five mean values from the five rats of one group. Abbreviations: NS, not significant (*P* > 0.05); (TF/P), tubular fluid over plasma ratio.

\* 0, no infusion; +, infusion.

‡ From factorial analysis.

$0.29x + 19.3$ ,  $r = 0.42$ ,  $n = 35$ ,  $P < 0.05$ ;  $y = 0.76x - 5$ ,  $r = 0.78$ ,  $n = 29$ ,  $P < 0.001$  in groups I to IV, respectively.

Comparison of early distal fluid and ureteral urine composition (Table II). Phosphate reabsorption from early distal tubule to ureteral urine was calculated without regarding the heterogeneity of the nephron population. The contribution of the terminal nephron to the overall phosphate reabsorption (terminal reabsorption expressed in percentage of filtered load) was significantly lower when calcium was administered and not significantly altered by cAMP. Phosphate reabsorption was also calculated as the percentage of the phosphate load delivered to the early distal. This figure was significantly decreased during cAMP infusion. The decrease by calcium did not achieve significance.

In all four groups significant positive correlations appeared between phosphate fractional delivery to the early distal ( $x$ ) and phosphate fractional delivery in ureteral urine ( $y$ ):  $y = 0.25x + 2.0$ ,  $r = 0.67$ ,  $n = 33$ ,  $P < 0.001$ ;  $y = 0.16x + 1.7$ ,  $r = 0.63$ ,  $n = 39$ ,  $P < 0.001$ ;  $y = 0.12x + 15.3$ ,  $r = 0.48$ ,  $n = 35$ ,  $P < 0.01$ ;  $y = 0.48x + 5.59$ ,  $r = 0.86$ ,  $n = 29$ ,  $P < 0.001$  in groups I to IV, respectively.

Relationship between phosphate load and phosphate reabsorption in each segment. Since the phosphate load to each segment studied was not similar in the four experimental groups, the relationship between the absolute phosphate load delivered to each of the three segments (proximal, loop, and terminal nephron) and the absolute reabsorption in the same segment was studied. A significant linear correlation appeared in the four experi-

TABLE III  
Results from Covariance Analysis of the Linear Relationships between Absolute Phosphate Reabsorption by and Absolute Phosphate Delivery to Each Segment (Figs. 1-3)

		Proximal tubule	Loop	Terminal nephron
Ca effect	Group II vs. I	$P < 0.001$	$P < 0.001$	$P < 0.001$
cAMP effect	Group III vs. I	NS	NS	$P < 0.001$
Ca + cAMP effect	Group IV vs. I	$P < 0.005$	NS	$P < 0.001$
	Group IV vs. II	$P = 0.01$	$P < 0.001$	$P < 0.001$
	Group IV vs. III	$P < 0.001$	$P < 0.001$	NS

NS, not significant ( $P > 0.05$ ).

mental situations and for each of the three segments. These correlations are depicted in Figs. 1-3.

Covariance analysis (Table III) showed that, in the calcium-infused animals of group II, the phosphate reabsorptive capacity for an equal load was increased in each of the three segments, as compared to animals of group I. The combined infusions of calcium and cAMP yielded results intermediate between those of group II, on the one hand, and of group III, on the other hand, in both the proximal and the loop. In the terminal nephron, however, combined infusions of calcium and cAMP yielded results not significantly different from those of cAMP infusion alone.

#### Results of clearance experiments (TPTX + Ca animals)

Fractional phosphate excretion in ureteral urine was  $3.27 \pm 1.53\%$  ( $n = 5$ ), not significantly different from the  $3.01 \pm 0.68\%$  obtained in PTX + Ca animals of group II.

#### Results of ultrafiltration experiments

No significant difference appeared in the ratio of ultrafiltrable calcium to total calcium between the four groups. The mean ratio was:  $0.535 \pm 0.024$ ,  $n = 8$ . It was also verified that the ratio  $^{32}\text{P}$  in ultrafiltrate to  $^{32}\text{P}$  in plasma was not altered in animals receiving a  $\text{CaCl}_2$  infusion. Values of 1.10 and 1.09 were obtained from two such animals.

### DISCUSSION

*Effects of increasing plasma calcium concentration to subnormal levels in chronic PTX rats.* Raising plasma calcium concentration to subnormal level in chronic PTX rats resulted in a significant increase in phosphate reabsorptive capacity in proximal tubule, loop, and terminal nephron. The increase in reabsorptive capacity of the terminal nephron was reflected neither in the fractional reabsorption of the filtered load, nor in the fractional reabsorption of the load delivered to the terminal nephron. This apparent discrepancy is accounted for by the

magnitude of the variations in the phosphate load delivered to the terminal nephron in the four experimental groups. Covariance analysis (Table III) of the relation between absolute delivery to and absolute reabsorption by the terminal nephron shows, at equal loads, a significantly higher reabsorptive capacity in calcium-loaded animals.

The overall calcium effect was observed in rats with intact thyroid glands. It is likely that calcium infusion stimulated calcitonin release from the thyroid gland (23). Accordingly, the role of this calcitonin secretion in the observed increase in phosphate reabsorption may be questioned. The comparison of the clearance results from group II (PTX + Ca) and the TPTX + Ca group showed no significant difference in fractional phosphate reabsorption. Thus one may conclude that the increase in phosphate reabsorption induced by calcium infusion is not mediated by a likely calcitonin release.

The discrepancies reported in the literature about the effects of calcium infusion on phosphate reabsorption may be accounted for by several factors: (a) species differences between man, dog, and rat; (b) duration of calcium administration; (c) method of calcium elevation (diet versus infusion); (d) calcium concentration achieved (6) (and it is likely that hypercalcemic situations may yield results different from those where a normal plasma calcium concentration, in previously hypocalcemic animals, is obtained, as in the present work); (e) effects that alterations in extracellular fluid

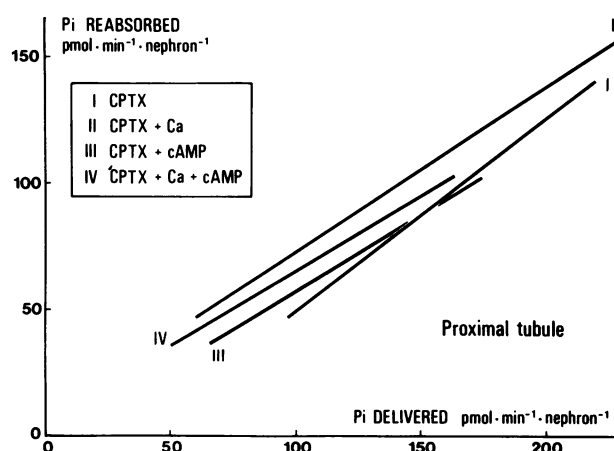


FIGURE 1 Linear relationships between absolute reabsorption by (ordinate) and absolute delivery to (abscissa) proximal tubule in each of the four experimental groups. Individual points were omitted for clarity. Regression lines were drawn in the interval between the lowest and the highest value obtained for the abscissa.  $n = 33, 39, 35,$  and  $29$  in groups I-IV, respectively. Equations of regression lines for proximal tubule: group I:  $y = 0.76x - 26.1$ ,  $r = 0.89$ ,  $P < 0.001$ ; group II:  $y = 0.65x + 8.31$ ,  $r = 0.90$ ,  $P < 0.001$ ; group III:  $y = 0.60x - 2.90$ ,  $r = 0.83$ ,  $P < 0.001$ ; group IV:  $y = 0.59x + 6.61$ ,  $r = 0.87$ ,  $P < 0.001$ .

volume might have; (f) composition of the diet previously administered. It seems reasonable to postulate that some of the factors mentioned above explain the differences between the results reported here and those obtained by Wen (5), who studied chronic thyroparathyroidectomized dogs fed a high-calcium diet and infused with  $\text{CaCl}_2$  up to a hypercalcemic level while undergoing extracellular fluid expansion.

*Comparison of calcium-infused chronic PTX rats and acute PTX rats.* During calcium infusion in chronic PTX rats, the overall handling of phosphate by the kidney becomes close to that of acute PTX animals previously reported (16). However, a fractional phosphate excretion in ureteral urine as low as that observed in acute PTX was not achieved. In the late accessible proximal tubule a  $(\text{TF}/\text{P})^{32}\text{P}$  ratio as low as, or even lower than, that of acute PTX is observed. Fractional phosphate delivery was similar. However the figure is quite different for fractional water, and hence sodium, reabsorption. In the loop, the relationship between fractional phosphate delivery to the early distal and fractional phosphate delivery to the late proximal was maintained during calcium infusion. In acute PTX rats such a relationship is abolished (16). At the distal level  $(\text{TF}/\text{P})^{32}\text{P}$  does not achieve as low values in group II as in acute PTX. Thus, it appears that if plasma calcium level may have a role in generating some of the differences in tubular phosphate handling between chronic and acute PTX, still this cannot be the unique parameter involved. Indeed, among several other factors, 25- and

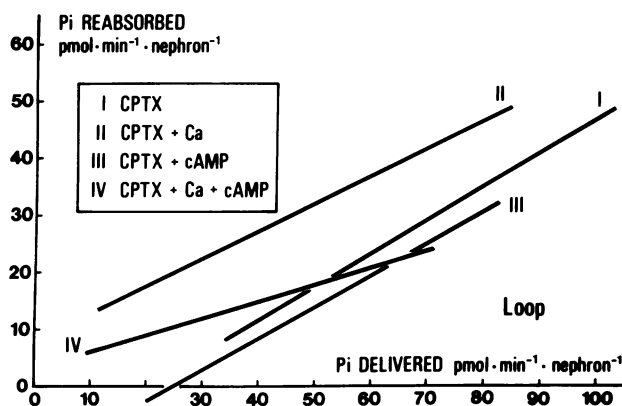


FIGURE 2 Linear relationships between absolute reabsorption by (ordinate) and absolute delivery to (abscissa) loop in each of the four experimental groups. Individual points were omitted for clarity. Regression lines were drawn in the interval between the lowest and the highest value obtained for the abscissa.  $n = 33, 39, 35,$  and  $29$  in groups I to IV, respectively. Equations of regression lines for loop: group I:  $y = 0.60x - 12.0, r = 0.65, P < 0.001$ ; group II:  $y = 0.47x + 8.09, r = 0.77, P < 0.001$ ; group III:  $y = 0.57x - 14.4, r = 0.81, P < 0.001$ ; group IV:  $y = 0.29x + 3.39, r = 0.58, P = 0.001$ .

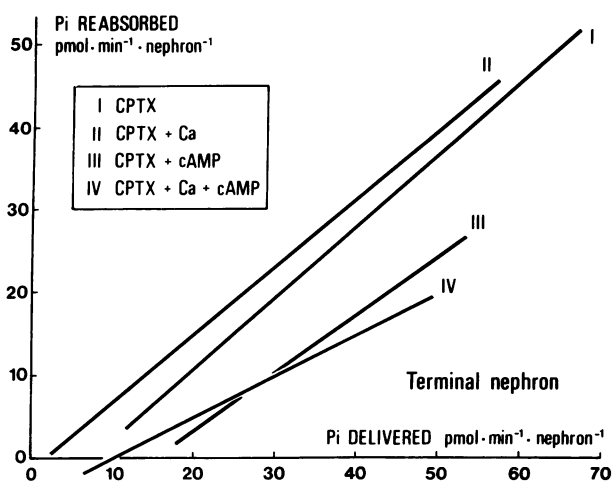


FIGURE 3 Linear relationships between absolute reabsorption by (ordinate) and absolute delivery to (abscissa) terminal nephron in each of the four experimental groups. Individual points were omitted for clarity. Regression lines were drawn in the interval between the lowest and the highest value obtained for the abscissa.  $n = 33, 39, 35,$  and  $29$  in groups I-IV, respectively. Equations of regression lines for terminal nephron: group I:  $y = 0.87x - 6.50, r = 0.96, P < 0.001$ ; group II:  $y = 0.82x - 2.06, r = 0.98, P < 0.001$ ; group III:  $y = 0.71x - 11.0, r = 0.85, P < 0.001$ ; group IV:  $y = 0.50x - 5.07, r = 0.90, P < 0.001$ .

1,25-hydroxycholecalciferol may play a role in modulating tubular phosphate reabsorption. It has been suggested that both steroids increase proximal tubular phosphate reabsorption (24, 25), and that 1-25 renal synthesis is inhibited when intracellular inorganic phosphate concentration is high (26), which is likely in chronic PTX animals. It has been shown, however, that 25 and 1,25-cholecalciferol do not act on tubular phosphate reabsorption in the absence of PTH (27).

*Response to cAMP infusion.* The urinary fractional phosphate excretion in response to cAMP infusion is similar in chronic PTX of group III and acute PTX, previously reported (16). However, this result is achieved in the former by an effect on the terminal nephron and in the latter by an effect on the proximal tubule and the loop. The lack of significant inhibitory action of exogenous cAMP on proximal and loop phosphate reabsorption in chronic PTX could suggest that cAMP synthesis is already stimulated in this situation, tentatively by hypocalcemia or some related factor.

*Mode of action of calcium on tubular phosphate reabsorption.* Schematically, at least two modes of action are possible. Firstly, calcium could act through the same system as PTH, i.e. adenylate cyclase and cAMP. Secondly, calcium could modulate another component of tubular phosphate transport, independent of PTH, as suggested by Glorieux and Scriver from studies of X-linked hypophosphatemic patients (7). The experi-

mental approach of the possible modulation by calcium concentration of the Ac-cAMP system is made difficult by: (a) the ignorance of the alterations in intracellular calcium concentration in response to changes in the extracellular one, although an increase in extracellular calcium has been postulated to increase intracellular calcium, through enhanced cellular uptake of calcium (28); (b) the reported discrepancies in calcium action on Ac activity in vitro, inhibition by high calcium concentrations being observed in broken cells (11–14) but not in intact ones (29), (which suggests that the effect of calcium obtained in vitro cannot be extrapolated to the physiological mechanisms in vivo); (c) the administration of exogenous cAMP, which does not necessarily induce events identical to those determined by stimulation of intracellular cAMP synthesis. It is, however, well documented that increasing extracellular cAMP concentration decreases phosphate reabsorption, as does PTH via the Ac-cAMP system (1, 30–33). Moreover, it has been suggested that extracellular cAMP easily penetrates tubular cells in isolated perfused rat kidney (34).

The results of combined calcium and cAMP infusions may suggest an interpretation of the mode of action of calcium. In the terminal nephron combined infusions of calcium and cAMP induce a phosphate handling quite similar to that observed during cAMP infusion alone. This observation is compatible with calcium inhibition of adenylate cyclase. In both the loop and the proximal tubule, combined infusions of calcium and cAMP yield results intermediate between the "calcium alone" and the "cAMP alone" situations. This is either compatible with calcium inhibition of adenylate cyclase, if the dose of cAMP used was insufficient to counterbalance the Ac inhibition, or compatible with the existence of two effects, one through the Ac-cAMP system, and the other independent of this system. Beck et al. (14), from clearance experiments in a similar protocol, have concluded that an action of calcium at a step beyond the generation of cAMP was unlikely and that calcium is likely to inhibit the PTH stimulation of adenylate cyclase activity.

In conclusion: (a) raising plasma calcium concentration to subnormal levels in rats deprived of parathyroid hormone for 6 days induces an increase in phosphate reabsorptive capacity in the three segments studied: accessible proximal, loop, and terminal nephron. (b) The lower plasma calcium concentration in chronic PTX rats explains only partly the differences in that situation compared to the acute PTX one. (c) The main action of exogenous cAMP in chronic PTX rats is neither a proximal one nor a loop one as it is in acute PTX, but a terminal one. (d) The effects of calcium and cAMP infusions administered simultaneously suggest either a calcium inhibition of Ac or a combination of this effect

and a direct, Ac-cAMP-independent enhancement of tubular phosphate reabsorption.

It is proposed that, in the absence of PTH, extracellular fluid calcium concentration, or some related factor, participates in the modulation of tubular phosphate transport.

#### ACKNOWLEDGMENTS

The authors are indebted to Dr. C. Gaudebout for invaluable advices in the statistical analysis and to Mrs. C. Nicolas for excellent technical assistance.

This work was supported in part by a grant from the Délégation Générale à la Recherche Scientifique et Technique (N° 73 7 1237), and in part by a grant from the Institut National de la Santé et de la Recherche Médicale (N° 75 5 129 5).

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