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Role of parathyroid hormone in the phosphaturia of extracellular fluid volume expansion

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Role of parathyroid hormone in the phosphaturia of extracellular fluid volume expansion. Acute expansion of the extracellular fluid volume increases the urinary excretion of phosphate. The present study examined the importance of increased plasma parathyroid hormone concentration in the phosphaturia accompanying acute extracellular fluid volume expansion (ECVE). Infusion of a calcium-free Ringer's solution into dogs was associated with increased urinary phosphate excretion and serum immunoreactive parathyroid hormone concentration (iPTH), the latter being significantly correlated with a decrease in plasma ionized calcium concentration. Prevention of the fall in plasma ionized calcium concentration by infusion of a calcium containing Ringer's solution prevented the increase in serum iPTH but the magnitude of the phosphaturia was not affected. The phosphaturia associated with ECVE was also not affected in thyroparathyroidectomized (TPTX) dogs which received a maintenance infusion of bovine PTH. In contrast, in acutely TPTX dogs which did not receive a maintenance infusion of PTH, the phosphaturic response to ECVE was significantly depressed. These data indicate that *1*) the increase in serum iPTH concentration following ECVE is the result of a fall in plasma ionized calcium concentration, *2*) the increase in phosphate excretion accompanying ECVE is not dependent on an increase in serum iPTH concentration and *3*) in the presence of a low or falling serum PTH concentration, the increase in phosphate excretion can be significantly blunted.

Rôle de l'hormone parathyroïdienne dans la phosphaturie consécutive à l'expansion extracellulaire. L'expansion aiguë du volume extracellulaire augmente l'excrètion urinaire du phosphate. Ce travail examine le rôle de l'augmentation de la concentration d'hormone parathyroïdienne dans la phosphaturie qui accompagne l'expansion du volume extracellulaire (ECVE). L'administration à des chiens d'une solution de Ringer sans calcium est associée à une augmentation de l'excrétion urinaire de phosphate et à une augmentation de la concentration d'hormone parathyroïdienne immunoréactive du plasma (iPTH). Cette dernière est significativement corrélée à la diminution de la concentration plasmatique du calcium ionisé. L'empêchement de la diminution de la concentration plasmatique du calcium ionisé par l'administration d'une solution de Ringer contenant du calcium évite l'augmentation de iPTH dans le plasma mais n'affecte pas l'importance de la phosphaturie. La phosphaturie associée à ECVE n'est pas non plus modifiée chez des chiens thyroparathyroïdectomisés (TPTX) qui reçoivent une perfusion de PTH bovine. Au contraire chez des chiens TPTX aigus qui ne reçoivent pas de perfusion de PTX la réponse à ECVE est significativement diminuée. Ces résultats indiquent que *1*) L'augmentation de iPTH du plasma après ECVE est la conséquence d'une diminution de la concentration plasmatique du calcium ionisé, *2*) l'augmentation de l'excrétion du phosphate qui accompagne ECVE ne dépend pas de l'augmentation de iPTH du plasma et *3*) quand la concentration plasmatique de PTH est faible ou en voie de diminution, l'augmentation de l'excrétion de phosphate peut être significativement masquée.

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Frick [1] demonstrated that expansion of the extracellular fluid volume by the infusion of isotonic saline caused an increase in phosphate excretion in the rat. Similar studies in dogs by Suki et al [2] and in man by Steele [3] have also shown that the infusion of isotonic saline produces a phosphaturia. There has been, however, considerable controversy over the mechanism producing this phosphaturia. Frick initially suggested that the phosphaturia may be related to the inhibition of sodium reabsorption which is known to occur following volume expansion. Massry, Coburn and Kleeman [4] and Suki et al [2] demonstrated that the phosphaturia following the infusion of isotonic saline occurred in chronically thyroparathyroidectomized dogs suggesting that an increase in parathyroid hormone (PTH) concentration was not responsible for the phosphaturia.

Although chronic parathyroidectomy does not appear to alter the phosphaturia following the infusion of saline, Frick [1, 5–7] and Maesaka, Levitt and Abramson [8] demonstrated that acute parathyroidectomy in rats abolished the phosphaturia following saline infusion. Similar observations have recently been reported for acutely parathyroidectomized dogs. [9]. The latter investigators have suggested that

an increase in parathyroid hormone secretion, presumably due to the fall in plasma ionized calcium which accompanies the infusion of isotonic saline produces the phosphaturia following saline infusion. Support for this hypothesis was recently obtained by Spornitz and Frick [10], who showed a decrease in plasma ultrafilterable calcium concentration and a degranulation of parathyroid cells following the infusion of saline in the rat.

The explanation for the discrepancy in the data obtained from chronic parathyroidectomized animals and from acutely parathyroidectomized animals is not apparent. However, Hebert et al [11] have demonstrated that in phosphate-loaded dogs which have been acutely thyroparathyroidectomized, a normal phosphaturic response was obtained following the infusion of isotonic saline. These authors concluded that parathyroid hormone was not necessary for the phosphaturia following volume expansion. Rather, they suggested that the reason previous investigators failed to observe a phosphaturia in acutely parathyroidectomized animals was due to a low filtered load of phosphate in the presence of an increased nephron reabsorptive capacity for phosphate. However, since Hebert et al [11] could only demonstrate a phosphaturia in acutely parathyroidectomized dogs at elevated plasma phosphate concentration, their data do not demonstrate that an acute elevation of PTH is not an important mechanism in the phosphaturia following volume expansion.

The present series of experiments were designed to answer the following questions: First, is there an increase in serum PTH concentration following acute extracellular fluid volume expansion? Second, if serum PTH concentration is increased, what is the mechanism of this increase? Third, what role does an increase in serum PTH concentration have in the phosphaturia of acute extracellular fluid volume expansion?

Methods

Dogs used in these experiments were anesthetized with sodium pentobarbital (30 mg/kg of body wt) and the trachea was intubated. Catheters were placed in both jugular veins and in a femoral artery and vein for infusions and blood sampling. Both ureters were exposed through a suprapubic incision and cannulated. Sixty minutes before starting the initial clearance collections, all dogs received a priming dose of inulin and a maintenance infusion was administered at 1 ml/min throughout the experiments to maintain an inulin concentration of 0.25 mg/ml. Five groups of dogs were studied.

Group I. In six dogs, after three 15-min control clearance periods were obtained, an infusion of isotonic Ca-free Ringer's solution[1] at 1 ml/min/kg for 20 min was initiated. This infusion rate was then reduced to 0.5 ml/min/kg for the remainder of the experiment. One hour after starting the infusion, two additional 15-min clearance periods were obtained.

Group II. Six dogs were treated in an identical fashion to group I except that 6 mg/100 ml of $CaCl_2$ was added to the Ca-free Ringer's solution. Groups I and II experiments were conducted so that the persons performing analysis did not know which solution had been infused.

Group III. An acute thyroparathyroidectomy was performed in nine dogs and an infusion of parathyroid hormone (0.01 U/min/kg at 1 ml/min) was immediately started. Two hours after commencement of the parathyroid hormone infusion, three control clearance periods were obtained. An infusion identical to that given in group II was then administered and two additional 15-min clearance periods were obtained.

Group IV. Six dogs were treated in an identical manner to group III except that the Ringer's infusion was not given. These dogs served as a control for the effect of parathyroidectomy and the infusion of bovine parathyroid hormone.

Group V. In eight dogs thyroparathyroidectomy was performed two to four hours before commencement of the initial urine collections. The dogs were then treated in a similar fashion to group I.

Blood samples for clearance determinations were obtained at the midpoint of each urine collection. In both groups I and II, arterial blood samples for measurement of serum immunoreactive PTH (iPTH) and plasma ionized calcium concentrations were obtained during the controls periods and at 10, 20, 40, 60 and 90 min following the start of the Ringer's solution. Inulin in plasma and urine was analyzed by the anthrone method. Sodium was analyzed by flame photometry. Phosphate was analyzed by the method of Young [12]. Total plasma calcium concentration was analyzed by atomic absorption spectroscopy. Blood for ionized calcium was collected into a syringe and then injected into 5-ml Vacutainers (Becton-Dickinson & Co., Rutherford, NJ) containing 143 μ of heparin. Plasma was withdrawn anaerobically into a tuberculin syringe through the rubber stopper of the Vacutainer after centrifugation at 3000 rpm for ten minutes. For

[1] Calcium-free Ringer's solution contained Na (138 mEq/liter), K (3.7 mEq/liter), Mg (2.2 mg/100 ml), PO_4 (6 mg/100 ml), HCO_3 (25 mmoles/liter), Cl (113 mEq/liter) and glucose (100 mg/100 ml). The solution was bubbled with 95% O_2 and 5% CO_2.

measurements of ionized calcium in plasma, a flow-through calcium activity electrode (Orion, Orion Research, Cambridge, MA) [13] was used with the following modifications. *1*) Standards were prepared in Vacutainers containing the same amount of heparin as did samples. 2) No trypsin or triethanolamide was added to the standards, which were prepared weekly. *3*) The membrane was primed by pooled normal plasma before the daily standard curve was run. Plasma pH was measured by a microelectrode and a standard pH meter.

The techniques of measurement of serum iPTH were the same as those previously reported [14]. The antiserum used in the present studies was termed CH12M (chicken antibovine PTH) and was described in the previous report. In the present studies, a crude saline extract of pooled normal dog parathyroid glands was used as a standard in all radioimmunoassays. It was assigned an arbitrary value of 1000 μEq/ml and immunoassay curves produced with this standard superimposed on curves produced with multiple dilutions of a serum obtained from a dog made chronically hypocalcemic with citrate infusions. ^{131}I-bovine PTH was used in assays as the labeled hormone species. Antiserum CH12M reacts with synthetic bovine PTH 1–34 (Beckman Bioproducts) almost as well as with bovine PTH 1–84 and therefore recognizes the biologically active region of the PTH molecule. Sera from normal dogs consistently decreased the ratio of antibody bound to free ^{131}I-bovine PTH (B/F ratio) by 30 to 50% whereas sera from hypoparathyroid dogs did not alter this ratio significantly indicating that iPTH was being measured and not some nonspecific effect of serum on the immune system. In order that this potential problem could be circumvented, we used hypoparathyroid dog serum in assays as a blank and made corrections for small nonspecific changes in the B/F ratio as has been described [15]. All measurements of serum iPTH concentration in the present study were done in duplicate in three different serum dilutions. Intra-assay and interassay variations were 12 and 15%, respectively.

The average for a variable during the initial clearance periods was compared by Student's *t* test for paired comparison to the average obtained for that variable during the experimental clearance periods. For statistical analysis, changes in the various plasma variables for groups I and II are based on the mean of the control samples and the mean of the 60- and 90-min postinfusion samples. This procedure was chosen since it corresponds to the time periods during which the urinary excretion data were collected. Student's *t* test for group comparison was used to analyze the differences between the various groups. The data is expressed as the mean value ± 1 SEM.

Results

Groups I and II. Following the infusion of calcium-free Ringer's solution (Group I), plasma ionized calcium concentration decreased in all six dogs (Table 1 and Fig. 1). The decrease occurred within 10 min of starting the infusion, and by 60 to 90 min following the start of the infusion, ionized calcium had decreased -0.55 ± 0.05 mg/100 ml ($P < 0.001$). The immunoreactive PTH activity increased in five of the six dogs within 10 min of starting the infusion, and by 60 to 90 min following the start of the infusion, iPTH activity had increased 54 ± 16 μEq of PTH/ml ($P < 0.025$). In the five dogs in which iPTH activity increased, there was a highly significant correlation between the fall in plasma ionized calcium concentration and the increase in iPTH. In dog six, although ionized calcium decreased, for some unknown reason, no increase in immunoreactive PTH was observed. Nevertheless, the mean values for r (-0.68 ± 0.25), for the Y intercept (491 ± 141 μEq of PTH/ml) and for the slope ([-99 ± 31 μEq of PTH/ml]/[mg of ionized calcium/100 ml]) were significantly different from zero.

Sixty minutes following the infusion of 6 mg/100 ml of Ca Ringer's solution, ionized calcium concentration had decreased slightly (-0.13 ± 0.03; $P < 0.025$); however, this decrease was significantly smaller (-0.42 ± 0.06 mg/100 ml; $P < 0.001$) than the decrease

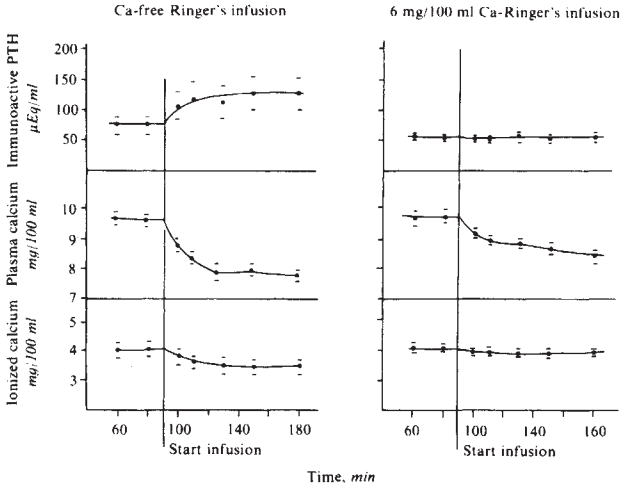

Fig. 1. *Effect of the infusion of calcium-free* (left) *and 6 mg/100 ml calcium containing* (right) *Ringer's solution on serum immunoreactive parathyroid hormone, plasma calcium and plasma ionized calcium.* Each *point is the mean* ± 1 SEM for the six dogs in each group.

Table 1. Effect of calcium-free Ringer's infusion on immunoactive parathyroid hormone activity and plasma ionized calcium

Dog No.	iPTH μEq/ml								Ionized calcium mg/100 ml							
	60 min	80 min	90 min[a]	100 min	110 min	130 min	150 min	180 min	60 min	80 min	90 min[a]	100 min	110 min	130 min	150 min	180 min
1	63	78	—	100	145	132	155	160	3.09	2.96	—	2.71	2.58	2.43	2.33	2.33
2	135	125	—	160	195	190	200	176	3.68	3.76	—	3.52	3.45	3.57	3.37	3.32
3	82	80	—	165	—	160	180	—	4.52	4.58	—	4.18	3.97	4.14	4.06	4.02
4	83	96	—	125	120	130	135	150	4.79	4.79	—	4.39	4.23	4.10	4.06	4.11
5	31	29	—	42	46	36	59	63	3.71	3.90	—	3.68	3.65	—	3.36	3.32
6	50	44	—	44	44	38	38	45	4.10	4.33	—	3.93	3.78	3.74	3.61	3.71
Mean	74	75		106	119	114	128	129	3.98	4.05		3.74	3.61	3.50	3.47	3.47
± SEM	15	14		22	25	26	27	24	0.25	0.27		0.24	0.23	0.31	0.26	0.27
			Δ = 54 ± 16; $P < 0.025$								Δ = −0.55 ± 0.05; $P < 0.001$					

[a] Start Ca^{++}-free infusion.

Table 2. Effect of calcium containing Ringer's infusion on immunological parathyroid hormone activity and plasma ionized calcium

Dog No.	iPTH μEq/ml								Ionized calcium mg/100 ml							
	60 min	80 min	90 min[a]	100 min	110 min	130 min	150 min	180 min	60 min	80 min	90 min[a]	100 min	110 min	130 min	150 min	180 min
7	60	60	—	56	53	74	80	80	4.70	4.58	—	4.54	4.60	4.54	4.67	4.55
8	64	64	—	65	58	75	47	63	4.44	4.33	—	4.23	4.36	4.25	4.14	4.35
9	60	43	—	50	54	42	55	70	3.92	4.00	—	3.89	3.85	3.88	3.73	3.94
10	56	—	—	46	47	41	44	44	3.77	3.88	—	3.78	3.73	3.64	3.67	3.63
11	42	38	—	36	32	20	25	24	3.74	4.01	—	3.85	3.81	3.94	3.78	3.85
12	48	44	—	49	51	48	45	43	3.94	3.89	—	3.66	3.63	3.52	3.68	3.60
Mean	55	51		50	49	52	49	53	4.09	4.12		3.99	4.00	3.96	3.95	3.99
± SEM	3	4		4	4	8	7	9	0.16	0.11		0.13	0.16	0.16	0.16	0.16
			Δ = −1 ± 6; $P > 0.5$								Δ = −0.13 ± 0.03; $P < 0.025$					

[a] Start Ca^{++}-Ringer's infusion.

which occurred following the infusion of calcium-free Ringer's solution. Although ionized calcium concentration decreased slightly, no significant increase in serum iPTH concentration was observed (Table 2 and Fig. 1). The serum iPTH concentration obtained during the control periods of 55 ± 3 μEq of PTH/ml was not significantly different from the serum iPTH concentration of 51 ± 7 μEq of PTH/ml obtained 60 to 90 min after the start of the infusion.

Plasma calcium concentration decreased significantly from 9.7 ± 0.2 to 7.9 ± 0.2 and 8.5 ± 0.2 mg/100 ml following the infusion of the calcium-free and 6 mg/100 ml Ca Ringer's solution, respectively (Fig. 1). The decrease in plasma calcium concentration was significantly greater following the infusion of the calcium-free solution. The plasma protein concentration decreased a similar amount following both infusions (−1.5 g/100 ml—calcium-free, $P < 0.001$; and −1.7 g/100 ml—6 mg/100 ml of Ca; $P < 0.001$).

Following the infusion of the Ca-free Ringer's solution, glomerular filtration rate (+13 ± 6 ml/min), FC_{PO_4} (+29 ± 6 ml/min/100 ml GFR) and FC_{Na} (+5.2 ± 1.4 ml/min/100 ml GFR) increased significantly (Table 3). Similarly, following the infusion of the 6 mg/100 ml calcium Ringer's solution, glomerular filtration rate (+11 ± 5 ml/min), FC_{PO_4} (39 ± 9 ml/min/100 ml GFR) and FC_{Na} (+9.5 ± 1.7 ml/min/100 ml GFR) increased significantly. The $\Delta FC_{PO_4}/\Delta FC_{Na}$ ratio was similar for the two groups. There were no statistically significant differences between any of the clearance variables or in any of the changes in these variables between group I and group II. There was no significant change in plasma phosphate concentration following volume expansion in either

Correlation coefficient for (iPTH = a + b [−Ca]$^{++}$)			
a	b	r	P<
444	−123	−0.96	0.025
531	−101	−0.80	0.005
1005	−203	−0.99	0.01
411	−67	−0.92	0.025
263	−60	−0.96	0.005
7	9	0.57	NS
444	−91	−0.68	
135	30	0.25	
$P < 0.05$	$P < 0.05$	$P < 0.05$	

group of dogs (Table 3). However, due to the increase in glomerular filtration rate following the infusions of Ringer's solutions, the filtered load of phosphate was increased in both groups. In Fig. 2 the relationship between changes in filtered load of phosphate and changes in reabsorption of phosphate is presented. There was a significant correlation between the change in filtered load of phosphate and the change in phosphate reabsorption. The values for the Y intercept of −753 μg/min and −715 μg/min for groups I and II, respectively, are significantly different from zero ($P<0.01$). There was, however, no significant difference between the Y intercepts or between the slopes for these two groups.

Groups III and IV. Glomerular filtration rate did not change significantly in either group of thyroparathyroidectomized (TPTX) dogs receiving a maintenance infusion of bovine PTH. Following extracellular fluid volume expansion produced by infusion of the 6 mg/100 ml Ca Ringer's solution (group III), both the fractional clearance of phosphate (+13.6 ± 3.0 ml/min/100 ml GFR) and the fractional clearance of sodium (+3.3 ± 0.8 ml/min/100 ml GFR) increased significantly (Table 3). Neither the plasma phosphate concentration nor the filtered load of phosphate was significantly increased following the infusion of 6 mg/100 ml Ca Ringer's solution (1973 ± 215 and 2101 ± 240 ng/min). The $\Delta FC_{PO_4}/\Delta FC_{Na}$ ratio (4.9 ± 1.2) was not statistically different from the ratios obtained for groups I and II. Plasma calcium decreased from 9.7 ± 0.1 mg/100 ml to 8.9 ± 0.2 mg/100 ml following the infusion of 6 mg/100 ml Ca Ringer's solution which was similar to the change in plasma calcium obtained in group II.

In the PTX dogs receiving a maintenance infusion of bovine PTH without extracellular volume expansion (group IV), the fractional clearance of phosphate decreased slightly (−2 ± 0.6 ml/min/100 ml GFR; $P<0.05$), while there was no significant change in the fractional clearance of sodium (Table 3). The plasma

Table 3. Summary of clearance data

	Dogs N	Glomerular filtration rate ml/min		Plasma phosphate mg/100 ml		Fractional clearance ml/min/100 ml GFR				$\Delta FC_{PO_4}/\Delta FC_{Na}$
						PO_4		Na		
		C[a]	E[b]	C	E	C	E	C	E	
Group I	6	33	46[c]	49	50	15.3	44.5[c]	0.46	5.7[d]	5.3
Ca-free Ringer's		±5	±7	±2	±3	±4.3	±3.7	±0.21	±1.4	±0.7
Group II	6	34	45[c]	56	52	14.5	53.9	0.48	9.9[d]	5.6
6 mg/100 ml Ca Ringer's		±4	±6	±5	±4	±4.2	±9.2	±0.18	±1.9	±1.0
Group III	9	37	39	55	54	12.5	26.1[c]	0.71	4.0[d]	4.9
PTX & PTH 6 mg/100 ml Ca Ringer's		±4	±4	±4	±3	±3.5	±4.3	±0.19	±0.9	±1.2
Group IV	6	23	27	71	73	12.3	10.6[c]	0.75	0.64	—
PTX & PTH		±3	±4	±6	±7	±4.2	±4.3	±0.24	±0.21	—
Group V	8	38	40	61	58	5.2	9.4[c]	0.78	7.8[d]	0.61
PTX Ca-free Ringer's		±6	±7	±4	±4	±1.8	±2.8	±0.24	±1.1	±0.27

[a] C = control clearance periods.
[b] E = experimental clearance periods.
[c,d] Statistical significance ([c] $P<0.05$; [d] $P<0.001$) is based on the *t* test for paired comparisons.

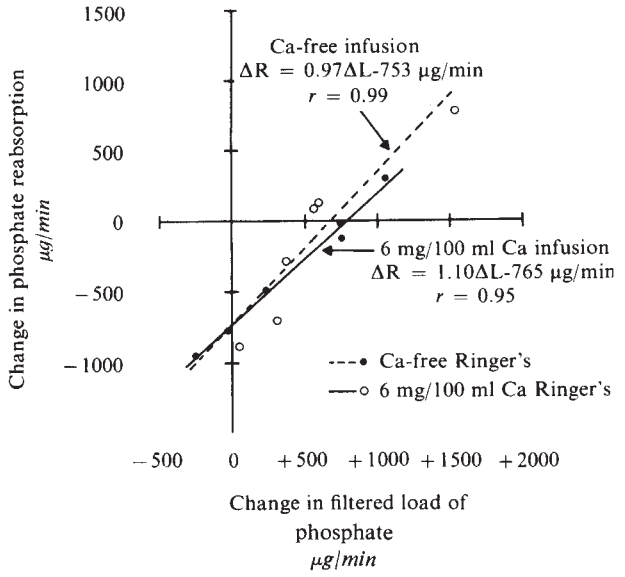

Fig. 2. *The relationship between changes in filtered load of phosphate and phosphate reabsorption following the infusion of calcium-free* (○ - - - - ○) *and 6 mg/100 ml calcium containing* (○——○) *Ringer's solutions.*

calcium concentration obtained during the initial clearance periods (9.8 ± 2 mg/100 ml) was not significantly different from the 9.4 ± 0.3 mg/100 ml obtained during the second set of clearance periods. Similarly, the plasma phosphate concentration was not significantly changed during these experiments.

Group V. Within two hours of the acute TPTX, plasma calcium concentration had decreased from 10.1 ± 0.2 mg/100 ml to 8.9 ± 0.2 mg/100 ml ($P < 0.001$). There was a further decrease in plasma calcium concentration to 7.5 ± 0.2 mg/100 ml following the infusion of the Ca-free Ringer's solution. There was no significant change in glomerular filtration rate (2 ± 2 ml/min) or in the plasma phosphate concentration (Table 3). The fractional clearance of sodium increased $+7.0 \pm 1.0$ ml/min/100 ml GFR ($P < 0.001$) and the fractional clearance of phosphate increased $+4.2 \pm 1.8$ ml/min/100 ml GFR ($P < 0.05$). The increase in the FC_{PO_4}, however, was significantly less than that obtained in groups I through III ($P < 0.05$). The $\Delta FC_{PO_4}/\Delta FC_{Na}$ ratio of 0.61 ± 0.27 was also significantly lower than that obtained in groups I through III.

Discussion

The present findings support the previous suggestions that following acute extracellular fluid volume expansion there is an increase in serum PTH concentration caused by a decrease in plasma ionized calcium concentration. The infusion of Ca-free Ringer's solution caused an increase in serum iPTH concentration ($+54 \pm 16$ μEq/ml) and a large fall in plasma ionized calcium concentration (-0.55 ± 0.5 mg/100 ml). The increase in serum iPTH was significantly correlated with the fall in plasma ionized calcium concentration as shown in Table 1. The addition of 6 mg/100 ml) of calcium to the Ringer's solution prevented the increase in serum iPTH concentration (-2 ± 6 μEq/ml) and caused only a slight decrease in plasma ionized calcium concentration (-0.13 ± 0.3 mg/100 ml) following volume expansion. If the increase in serum iPTH concentration following volume expansion was the result of a decrease in plasma ionized calcium concentration, then a small increase in serum iPTH might have been expected following the infusion of the calcium containing Ringer's solution. Several possibilities may account for this lack of an increase in serum iPTH. First, the small decrease in plasma ionized calcium may not have been sufficient to stimulate the release of PTH. In support of this possibility is the fact that in only two of the six dogs were the postinfusion plasma ionized calcium concentrations consistently below the range of the control values. Furthermore, in a previous study [16], no significant change in plasma ionized calcium concentration ($+0.08 \pm 0.08$ mg/100 ml; unpublished observation) occurred following the infusion of a 6 mg/100 ml Ca containing Ringer's solution which was identical to the one used in the present study. A second possibility is that the serum iPTH concentration may have been decreased because of dilution subsequent to the plasma and extracellular fluid expansion. Thus, a normal serum iPTH concentration in the presence of a larger volume of distribution suggests that an increase in PTH release occurred following the infusion of the Ca containing Ringer's solution. Third, since plasma proteins have been shown to cause an inhibition of the binding of PTH to the antibody [14] the immunoassay for PTH is dependent on the plasma protein concentration of the sample. Thus, because volume expansion produced a fall in plasma protein concentration, the serum iPTH concentration may have been slightly underestimated in both groups following volume expansion. Fourth, volume expansion may have altered some other factor(s) which opposed the stimulating effect of a small decrease in plasma ionized calcium concentration on PTH secretion. Nevertheless, it is clear that the presence of 6 mg/100 ml of Ca in the Ringer's solution prevented the marked increase in serum iPTH concentration following acute extracellular volume expansion.

Although the addition of 6 mg/100 ml of Ca to the Ringer's solution prevented the increase in serum

iPTH concentration, the phosphaturic effect of extracellular fluid volume expansion was not affected. The increased serum iPTH concentration following the Ca-free infusion did not produce a greater increase in the fractional clearance of phosphate than that obtained when no increase in serum iPTH concentration was detected. In both groups there were significant increases in the filtered load of phosphate caused primarily by increases in glomerular filtration rate. However, the similarity in the relationship between changes in filtered load of phosphate and changes in phosphate reabsorption (Fig. 2) suggests an inhibition of phosphate reabsorption occurred following the infusion of either the Ca-free or Ca containing Ringer's solution. Thus, an increase in plasma PTH concentration does not appear to detectably contribute to the phosphaturic effect of extracellular fluid volume expansion.

The addition of Ca to the volume expansion solution has previously been reported to prevent the phosphaturia accompanying volume expansion, presumably by preventing an increase in PTH secretion [6, 9]. However, these investigators maintained total plasma calcium concentration at or above the control levels. Since volume expansion dilutes the plasma protein concentration, the percentage of plasma calcium in the ionized form is increased, as demonstrated by a 5% increase in the percentage of plasma calcium in the ionized form following expansion in group II. Thus, maintaining a constant total plasma calcium concentration would actually result in an increase in plasma ionized calcium which should inhibit PTH release. A falling plasma PTH concentration may have counteracted the effect of volume expansion on phosphate reabsorption resulting in no apparent change in phosphate excretion. Since experiments to control for the increase in plasma ionized calcium concentration in the absence of volume expansion were not conducted by these investigators, it is difficult to interpret their phosphate excretion data. The results from this study demonstrate that if the plasma ionized calcium concentration is prevented from decreasing, a normal phosphaturic response to volume expansion is obtained without any detectable increase in serum iPTH concentration.

When acutely TPTX animals, given an infusion of bovine PTH to maintain a near normal serum PTH concentration, were volume expanded, the fractional clearance of phosphate increased significantly. That the increase in the fractional clearance of phosphate was caused by the volume expansion and not by the maintenance infusion of bovine PTH was indicated by the decrease in fractional clearance of phosphate obtained in identically treated animals which were not volume-expanded. Although it is impossible to prove that acute TPTX completely eliminates all parathyroid tissue, several factors suggest that the acute parathyroidectomy significantly reduced endogenous PTH secretion. First, care was taken to remove all visible thyroid and parathyroid tissue. Second, if no infusion of bovine PTH was administered, the plasma calcium concentration and the fractional clearance of phosphate were significantly decreased within two to four hours of parathyroidectomy.

The increase in the fractional clearance of phosphate was less in the TPTX animals given a maintenance infusion of PTH compared to the increase in FC_{PO_4} obtained in either group of volume-expanded animals with intact parathyroid glands. However, this difference in the change in the FC_{PO_4} was probably not due to the lack of intact parathyroid tissue. Rather, the difference in the change in the FC_{PO_4} was apparently related to the differences in the change in FC_{Na}. Thus, the change in FC_{PO_4} factored by the change in FC_{Na} was not significantly different between these groups. Several investigators have previously demonstrated a similar relationship between the FC_{PO_4} and the FC_{Na} [4]. Furthermore, in a recent study in our laboratory [16] in which animals were volume-expanded with either an infusion of a calcium-containing Ringer's solution or a calcium-free saline solution, the increases in the FC_{Na} ($+3.91 \pm 0.63$ ml/min/100 ml GFR; $+3.1 \pm 0.5$ ml/min/100 ml GFR) were similar to that obtained in the volume-expanded TPTX dogs given a maintenance infusion of PTH. The increases in the FC_{PO_4} ($+11.2+3.4$ ml/min/100 ml GFR; $+9 \pm 3.4$ ml/min/100 ml GFR) and the ratio $\Delta FC_{PO_4}/\Delta FC_{Na}$ (5.1 ± 1.5; 4.6 ± 1.2) were also similar to those obtained in the volume-expanded TPTX dogs given a maintenance infusion of PTH. Thus, the differences in the change in the FC_{PO_4} are apparently related to the differences in sodium excretion rather than to the presence of intact parathyroid tissue. No explanation can be given for the differences in sodium excretion but large differences in sodium excretion often occur between groups of volume-expanded dogs.

The finding of a normal phosphaturic response to extracellular fluid volume expansion (ECVE) without significant increases in PTH secretion clearly demonstrates that PTH does not play a predominate role in the phosphaturia accompanying ECVE as suggested by Spornitz and Frick [10] and Maesaka et al [8]. However, following ECVE in acutely TPTX dogs, the magnitude of the phosphaturia was significantly decreased. Thus, low plasma PTH concentrations can influence the magnitude of the phosphaturia accompanying ECVE. The mechanism causing depression

in the magnitude of phosphaturia has not been determined. The inhibition of phosphate reabsorption by the proximal tubule accompanying ECVE in normal animals [17] has been shown to occur in acutely TPTX animals by Maesaka et al [8] and Wen [18]. This latter observation suggests that reabsorption of phosphate in the distal nephron during ECVE may account for the blunted phosphaturia observed in TPTX animals. The presence of phosphate reabsorption by the distal nephron is controversial but Amiel [19] and Maesaka et al [8] in normal and TPTX rats and, more recently, Beck and Goldberg [20] and Wen [18] in TPTX dogs have demonstrated a significant reabsorptive mechanism for phosphate in the distal nephron segments. A phosphate reabsorptive mechanism in the distal nephron segments is consistent with the conclusions of Hebert et al [11] that in the absence of PTH an enhanced nephron reabsorptive capacity for phosphate, possibly in the distal nephron segments, accounts for the absence of a normal phosphaturia following ECVE in TPTX animals. Thus, the phosphaturia accompanying ECVE is primarily the result of volume expansion rather than an increase in plasma PTH concentration.

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