Effect of Expansion of Extracellular Fluid Volume on Renal Phosphate Handling

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ABSTRACT To examine the specific effect of extracellular fluid (ECF) volume expansion on phosphate excretion studies were performed in thyroparathyroidectomized dogs receiving saline solution intravenously. The natriuresis resulting from ECF volume expansion was consistently accompanied by an increase in phosphate excretion. The possible role of increased filtered load of phosphate was eliminated in experiments in which the filtered load of phosphate was reduced by acute reduction in the glomerular filtration rate. Despite considerable reductions in filtered phosphate, ECF volume expansion resulted in a consistent increase in phosphate excretion. Furthermore, the possible contribution of alteration in blood composition was investigated in experiments in which saline was infused during thoracic inferior vena cava constriction. In these experiments saline infusion failed to increase sodium or phosphate excretion. Cessation of saline infusion and release of caval constriction resulted in a prompt natriuresis and increased phosphate excretion. It is concluded from these studies that extracellular fluid volume expansion results in an increased phosphate excretion in the parathyroidectomized dog. This effect is the specific consequence of ECF volume expansion and is not due to increase in the filtered load of phosphate or alterations in blood composition.

INTRODUCTION

The acute expansion of the extracellular fluid (ECF) volume by the infusion of saline solutions has been demonstrated to result in an increase in the excretion of calcium (1–3) and magnesium (3) in addition to sodium. Chronic expansion of the ECF volume has also been shown to increase the excretion of calcium (4, 5) and magnesium (5). Futhermore, sodium diuresis induced by osmotic agents such as urea and mannitol is accompanied by an increased excretion of calcium (1, 6). An increase in phosphate excretion after ECF volume expansion or osmotic diuresis, however, has not been consistently demonstrated (2, 6–11). In those experiments where phosphate excretion rose it is difficult to attribute this rise to a primary effect of volume expansion because concurrent dilution of the serum calcium (2, 3) may have stimulated parathormone secretion and secondarily inhibited phosphate reabsorption by the kidney. In addition, changes in blood composition induced by intravenous infusions may have contributed to alterations in phosphate excretion.

The purpose of this investigation is to reexamine the relationship between expansion of ECF volume and phosphate excretion in the absence of parathormone and to evaluate the role of alterations in blood composition in this relationship. Studies were performed in thyroparathyroidectomized dogs receiving large infusions of saline. The specific effects of ECF volume expansion were further examined under circumstances where the filtered load of phosphate was reduced and in conditions where the infusion of saline did not expand the "effective" ECF volume but induced identical changes in blood composition.

The results of these studies demonstrate that expansion of ECF volume in the absence of parathormone results in a clear-cut increase in phosphate excretion. The increase in phosphate excretion was the specific consequence of expanded ECF volume and not due to changes in blood composition resulting from saline infusion or the increase in filtered load of phosphate.

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METHODS

Studies were performed on 20 mongrel dogs weighing between 10 and 20 kg and subjected to thyroparathyroidectomy 48–72 hr before study. Through a midline neck incision extending from the level of the cricothyroid cartilage to the suprasternal notch the trachea was exposed by separation of the muscles. The thyroid and parathyroid glands were identified on the posteromedial aspect of the trachea and excised *in toto*. The completeness of parathyroidectomy was checked in most animals by measurement of the serum calcium. The animals were starved overnight and the experiments begun in the morning and concluded in the early afternoon. 15 animals were studied in the hydropenic state; the remaining five were investigated after water diuresis had been induced by the administration of 300–500 ml water by gastric tube. Anesthesia was induced by the intravenous administration of sodium pentobarbital in a dose of 25 mg/kg body weight; the trachea was then intubated and the animal ventilated with a Harvard respirator. A polyethylene catheter was placed in the femoral vein and another in the femoral artery in each animal. Through a small midline suprapubic incision both ureters were cannulated with polyethylene catheters. In four animals a snare, consisting of a polyethylene tubing and umbilical tape threaded through it, was placed around the left renal artery through a left subcostal incision. In another six animals, a right thoracotomy was performed and a snare placed around the thoracic inferior vena cava above the level of the diaphragm. At the conclusion of the surgical procedures a loading dose of 0.5 μc/kg body weight of iothalamate-^{125}I (Glofil) was given intravenously followed by an equivalent dose given hourly dissolved in isotonic saline and infused at the rate of 1 ml/min. After an equilibration period of at least 45 min, control urine collection periods were begun. After the collection of one to three control periods isotonic or hypertonic (2%) saline solution was infused at a gradually increasing rate (4–20 ml/min) to all 15 hydropenic dogs and two dogs undergoing water diuresis and several more urine collections made. In six experiments, after a significant increase in the rate of urine flow was accomplished by volume expansion, urine flow rate was acutely reduced by the constriction of the left renal artery (four experiments) or the thoracic inferior vena cava (two experiments) and several more urine collections made subsequently. Constriction of the thoracic inferior vena cava at the height of saline diuresis has been shown by Blythe and Welt to reduce the GFR but not to abolish the natriuresis (12). In another four experiments the thoracic inferior vena cava was constricted sufficiently to raise femoral venous pressure by 10 cm of water before the infusion of saline. Isotonic saline was then infused at the rate of 20 ml/min until 1 liter had been infused and two to four urines collected. The vena cava constriction was then released and urine collection continued for several more periods. Thoracic caval constriction before the infusion of saline has been shown by Cirksena, Dirks, and Berliner to prevent or severely blunt the development of natriuresis (13).

In the middle of each urine collection period of every experiment a blood sample was obtained from the femoral artery. Blood and urine samples from each experiment were analyzed for sodium and potassium by internal standard flame photometry using an IL flame photometer, for phosphate using the method of Fiske and SubbaRow (14), and for their gamma emission by the counting of equal aliquots in a gamma ray scintillation counter. The dose of iothalamate administered resulted in counts per minute always in excess of tenfold background in both plasma and urine. Glomerular filtration rate (GFR) was calculated from the clearance of iothalamate-^{125}I (15).[1] GFR measured in this manner yielded a small error of ±2.6%. Clearance of sodium (C_{Na}) and clearance of phosphate (C_P) were calculated in the conventional manner and corrected to 100 ml of GFR.

RESULTS

The effects of infusion of isotonic or hypertonic saline on the excretion of sodium, potassium, and phosphate during hydropenia and water diuresis are demonstrated in Table I. It can be seen from the first experiment (dogs 7–25) that the expansion of ECF volume during hydropenia by the infusion of 2% saline solution resulted in a prompt rise in the rate of sodium and potassium excretion. These changes in the excretion of sodium and potassium were associated with a concomitant increase in the excretion of phosphate. Although in the early periods after the infusion of 2% saline the glomerular filtration rate also rose, the increase in excretion of sodium, potassium, and phosphate could not be solely due to the increased filtration rate since the fractional excretion of sodium ($C_{Na} \times 100/GFR$) and phosphate ($C_P \times 100/GFR$) continued to rise, despite return of the filtration rate towards normal. In the second experiment (dogs 3–24) the effects of volume expansion during water diuresis were identical with those of the previous experiment.

The results from all experiments are summarized in Table II. In this table the minimum fractional clearance of phosphate in the control period and the maximum fractional clearance of phosphate accomplished with ECF volume expansion are listed and compared with the fractional clearances of sodium in these two respective periods. Two observations may be made from these data. First, the fractional clearance of phosphate was less than 11% in every experiment, confirming the completeness of parathyroidectomy. The very low rates of phosphate excretion and fractional phosphate clearance observed in the control periods of the first 11 experiments in Table II are not due to hydropenia and very low urine flow rates. When urine flow rate was increased by water diuresis in five experiments fractional phosphate clearance ranged between 0.1 and 10.8%. The second observation is that the increased excretion of sodium induced by ECF volume expansion was accompanied by a clear-cut elevation in the fractional clearance of phosphate in every experiment.

[1] In about fifty separate observations the ratio of iothalamate clearance to the inulin clearance averaged 0.86 ±0.01 (SEM) at urine flow rates below 1 ml/min and 0.85 ±0.03 (SEM) at flow rates in excess of 1 ml/min. The clearance of iothalamate, therefore, is a very close and stable estimate of GFR in the dog at all levels of urine flow.

To determine whether the increase in GFR consequent to ECF volume expansion may have contributed to the observed rise in phosphate excretion by raising the filtered load of phosphate, studies were performed where the filtration rate was reduced at the height of saline diuresis either by acute constriction of the renal artery or of the thoracic inferior vena cava. The results of one such experiment are recorded in Table III. It can be seen from this experiment that the infusion of isotonic and then hypertonic saline solution resulted in an increase in the excretion of sodium, potassium, and phosphate and a modest increase in GFR. With the continued infusion of hypertonic saline there was a further rise in the fractional clearance of phosphate despite a reduction in the glomerular filtration rate by as much as 55%. An observation of note is the fact that at fractional clearances of sodium during vena cava constriction that are comparable with the clearances before this maneuver the fractional clearance of phosphate was significantly higher. This change in the relationship between sodium and phosphate clearance may be attributed to enhanced distal reabsorption of sodium with a limited or nonexistent distal reabsorption of phosphate. The results from all experiments during which the filtered

TABLE I

*Effect of Saline Infusion on the Excretion of Sodium, Potassium, and Phosphate in Thyroparathyroidectomized Dogs during Hydropenia (Dogs 7–25) and Water Diuresis (Dogs 3–24)**

	GFR		V		$U_{Na}V$		U_KV		U_PV		$C_{Na} \times 100/GFR$		$C_P \times 100/GFR$		P_P
Time	L	R	L	R	L	R	L	R	L	R	L	R	L	R	
	ml/min		*ml/min*		*μEq/min*		*μEq/min*		*mmoles/min*		%		%		*mmoles/liter*
Dog No. 7–25															
−45	^{125}I-iothalamate in isotonic saline at 1 ml/min														
0–31	19	16	0.1	0.1	10	9	15	13	0.5	0.5	0.4	0.4	1.3	1.8	1.9
31–61	25	24	0.1	0.1	17	17	16	16	0.3	0.6	0.5	0.5	0.7	1.3	1.9
61	2% saline solution started at 4 ml/min														
61–76	35	34	0.3	0.2	53	46	27	25	0.9	1.3	1.0	0.9	1.3	2.0	1.9
76–91	30	29	0.9	0.7	157	123	32	28	1.3	1.5	3.5	2.9	2.4	2.9	1.8
91	2% saline solution increased to 6 ml/min														
91–106	30	25	1.6	1.0	300	200	42	32	2.5	2.4	6.5	5.2	4.4	5.2	1.9
106–121	31	28	1.8	1.2	364	250	47	39	4.0	3.8	7.7	5.9	6.7	7.1	1.9
121–136	31	27	2.3	1.6	480	331	51	42	5.3	4.7	9.9	7.8	9.0	9.0	1.9
136	2% saline solution increased to 12 ml/min														
136–151	29	25	2.7	2.1	555	429	45	38	5.6	5.2	11.9	10.7	10.5	11.4	1.9
151–166	25	20	3.3	2.4	665	481	43	34	5.7	4.9	16.4	14.3	13.0	13.3	1.8
166–181	20	13	3.6	2.0	724	393	43	26	5.9	3.6	21.3	17.8	16.0	15.0	1.8
Dog No. 3–24															
−60	500 ml distilled water intragastric														
−45	^{125}I-iothalamate in isotonic saline at 1 ml/min														
0–20	36		1.5		5		12		2.8		0.1		6.9		1.1
20–40	38		2.2		8		20		3.5		0.2		7.6		1.2
40–60	36		2.7		3		24		4.3		0.3		9.7		1.2
60	2% saline solution started at 4 ml/min and increased to 10 ml/min														
60– 80	34		3.7		116		26		4.7		2.6		12.3		1.1
80–100	42		12.3		1591		37		11.5		24.7		22.4		1.2
100–120	45		16.9		2721		42		17.8		36.0		30.0		1.3
120–130	47		21.3		3653		64		24.1		44.3		38.3		1.3
130–140	38		14.0		2569		49		18.2		35.9		34.0		1.4

* Data from both the left (L) and right (R) kidney are recorded for dogs 7–25. GFR = glomerular filtration rate; V = urine flow rate; $U_{Na}V$ = sodium excretion rate; U_KV = potassium excretion rate; U_PV = phosphate excretion rate; $C_{Na} \times 100/GFR$ = clearance of sodium as per cent of GFR; $C_P \times 100/GFR$ = clearance of phosphate as per cent of GFR; P_P = plasma phosphate.

TABLE II

*Effect of Saline Infusion on the Fractional Clearances of Sodium and Phosphate in Thyroparathyroidectomized Dogs**

Experiment No.	Side	$C_{Na} \times 100/GFR$		$C_P \times 100/GFR$	
		Control	Experimental	Control	Experimental
		%		%	
7–11	L	0.5	3.2	0.8	9.8
	R	0.5	5.1	0.7	6.2
7–22	L	0.1	9.2	8.7	20.8
	R	0.1	10.3	8.8	22.0
7–23	L	0.1	12.1	5.6	18.7
	R	0.2	11.8	9.9	22.4
7–25	L	0.4	21.3	1.3	16.0
	R	0.4	17.8	1.8	15.0
8–5	L	1.9	19.6	6.1	13.8
	R	2.9	20.1	6.7	13.7
8–8	L	0.8	6.1	9.8	21.5
	R	0.8	2.5	7.4	13.5
8–9	L	0.3	7.6	7.6	22.0
	R	0.2	6.9	7.9	21.4
8–15	L	0.8	6.1	10.3	18.4
	R	0.5	2.8	10.5	18.6
8–20	L	0.2	5.2	7.4	18.8
	R	0.2	7.0	7.6	24.5
8–22	L	2.3	9.4	1.2	4.7
	R	2.3	10.3	1.2	4.7
9–5	L	0.8	6.8	8.3	27.1
	R	0.1	5.8	4.8	27.0
3–21	B	0.1	26.7	2.1	23.9
3–42	B	0.1	44.3	6.9	38.3

* For explanation of symbols, see Table I. B = urine from both kidneys collected from the bladder. The last two experiments were performed during water diuresis; all others were performed during hydropenia.

load of phosphate was reduced are plotted in Figs. 1 and 2. In Fig. 1 the change from control values in phosphate excretion induced by volume expansion is plotted against the reduction below control levels in the filtered load of phosphate (Fig. 1 A) and the per cent decrease in filtered phosphate (Fig. 1 B). It can be seen from this figure that despite reduction of filtered phosphate by as much as 30 μmoles/min or 60% extracellular fluid volume expansion increased phosphate excretion significantly in 17 collection periods. Only with greater falls of GFR and filtered phosphate did phosphate excretion actually fall. To correct for these drastic reductions in the filtered load of phosphate the excreted phosphate was expressed as a fraction of the filtered load. These data are presented in Fig. 2. It is evident from this figure that despite the reduction in filtered load of phosphate by 2–60 μmoles/min below control level the expansion of extracellular fluid volume was attended by an increase in the fractional clearance of phosphate in every instance. There was no difference between the effect of constriction of the thoracic inferior vena cava and that induced by constriction of the renal artery.

To further examine the specific effect of ECF volume expansion on phosphate excretion studies were performed in which 1 liter of saline was infused at a rate of 20 ml/min during constriction of the thoracic inferior vena cava; the saline infusion was then stopped and the vena cava constriction released. The results of these studies are summarized in Table IV. In this table the changes, above or below control values, in fractional clearances of sodium and phosphate are shown. It can be seen that the infusion of saline during cava constriction resulted in no (experiment 1) or moderate (experiment 3) changes in fractional sodium and phosphate clearance. Despite the discontinuation of saline infusion release of caval constriction resulted in more marked increase in fractional sodium and phosphate clearance.

DISCUSSION

The present studies clearly demonstrate that, in the thyroparathyroidectomized dog, the expansion of ECF volume results in an increase in the clearance of phosphate. Several explanations may be proposed to account for this observation. It is possible that expansion of ECF volume by causing an elevation in the glomerular filtration rate and thereby in the filtered load of phosphate exceeds the tubular capacity for phosphate reabsorption and results in the observed increase in phosphate excretion. Reduction in the filtered load of phosphate to far below control levels, however, failed to abolish the increased clearance of phosphate that followed ECF volume expansion. Another possibility is that saline infusion, by altering blood composition, may have secondarily resulted in the observed increase in phosphate clearance. This explanation may be readily discarded since the infusion of saline during constriction of the thoracic inferior vena cava failed to result in an increased clearance of phosphate despite the induction of similar compositional changes in blood. Furthermore, cessation of saline infusion and release of vena cava constriction resulted in a prompt diuresis of both sodium and phosphate. It appears from these observations, therefore, that the increase in phosphate clearance is the specific outcome of ECF volume expansion.

The major site of phosphate reabsorption is the proximal convoluted tubule (16). Volume expansion is known to depress proximal tubular reabsorption of sodium and water (17). It is reasonable to assume, therefore, that this portion of the nephron is the site where ECF volume expansion exerts its inhibitory effect on phosphate reabsorption. It is generally accepted that phosphate reabsorption in the dog exhibits saturation kinetics (7). Inhibition of phosphate reabsorption must be medi-

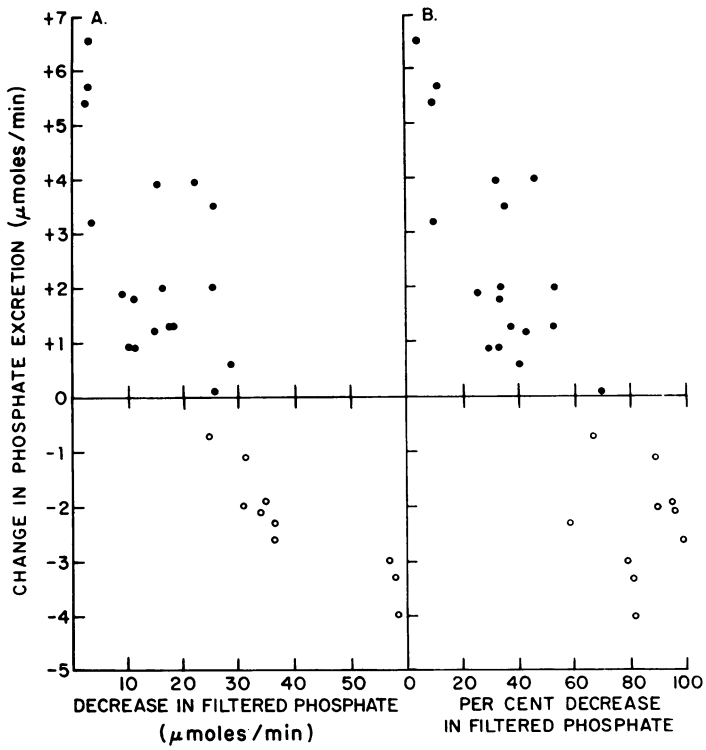

FIGURE 1 The effect of volume expansion on phosphate excretion during reduction in the filtered load of phosphate. The change, from the mean of the control periods, in phosphate excretion is plotted on the vertical axis against the absolute decrease in filtered phosphate (Fig. 1 A) and the per cent decrease in filtered phosphate (Fig. 1 B). The closed circles represent periods in which phosphate excretion increased, and open circle periods in which it decreased. Note that despite a decrease in filtered phosphate by 30 μmoles/min or 60% of control, volume expansion resulted in significant increase in phosphate excretion.

ated, therefore, by a reduction in either the threshold or the tubular maximum (Tm). The mechanism of this inhibition, however, is not known. Several possibilities are worthy of consideration. One possibility is that ECF volume expansion results in the elaboration of a humoral factor which inhibits phosphate transport. Evidence for a factor that inhibits sodium reabsorption has been advanced by several groups (18–20); although this evidence is now in question (21). If present, this factor might inhibit phosphate transport as well. In addition to sodium and phosphate, ECF volume expansion also inhibits the reabsorption of such other substances as calcium, magnesium, and uric acid (22). That one humoral agent is responsible for the inhibition of reabsorption of such varied substances may seem untenable. This possibility, however, is not unlikely since a substance present in plasma from volume expanded animals has been found to inhibit *p*-aminohippuric acid (PAH) transport by renal cortical slices in vitro (23). A second possible explanation for the increased clearance of phosphate after ECF volume expansion is that volume expansion results in increased back-diffusion of reabsorbate into the proximal tubule lumen. It has been suggested that ECF volume expansion exerts its inhibitory effect on sodium and water reabsorption by the proximal tubule through an increase in interstitial volume or pressure (24) and an increase in the back-diffusion of reabsorbate into the tubular lumen. If this were, indeed, the case, the increased back-diffusion of phosphate into the tubular lumen by increasing the load of the phosphate transport mechanism exceeds its capacity and results in the increased excretion of phosphate. Finally, it is possible that a fraction of the filtered phosphate is reabsorbed passively in the proximal tubule secondary to sodium and water reabsorption. Inhibition of sodium reabsorption would result, therefore, in an increased phosphate excretion. It is impossible from the present studies to determine which of these possibilities

TABLE III

*Effect of Reduction of Glomerular Filtration Rate on the High Rate of Sodium, Potassium, and Phosphate Excretion Induced by Saline Infusion.**

Time	GFR	V	$U_{Na}V$	U_KV	U_PV	$C_{Na} \times 100/GFR$	$C_P \times 100/GFR$	P_P
min	*ml/min*	*ml/min*	*μEq/min*	*μEq/min*	*μmoles/min*	%	%	*mmoles/liter*
−45	^{125}I-iothalamate in isotonic saline at 1 ml/min							
0–15	33	0.4	104	29	0.6	2.3	1.2	1.5
15	0.9% saline solution started at 8 ml/min							
15–30	34	0.6	138	30	1.4	2.9	2.9	1.4
30–45	37	1.0	185	35	1.8	3.6	3.3	1.5
45	2% saline solution started at 6 ml/min, 0.9% saline discontinued.							
45–60	34	1.7	277	37	1.5	5.6	3.0	1.5
60–75	34	2.9	485	46	2.4	9.4	4.7	1.5
75	Thoracic inferior vena cava constricted to raise femoral vein pressure by 10 cm water							
75–87	22	2.6	465	36	2.6	13.7	8.0	1.4
87–97	15	0.9	170	41	4.5	7.0	17.2	1.7
97–107	17	1.2	251	44	4.5	8.8	13.9	1.9
107–117	13	0.6	127	24	2.6	6.0	11.5	1.7

* For explanation of symbols, see Table I.

best explains our observations. Other studies will have to be designed to resolve this dilemma.

The physiological and clinical implications of these observations are several. Calcium and adenosine 3′,5′-monophosphate (cyclic AMP) increase phosphate excretion in parathyroidectomized animals (25, 26). This has led to the suggestion that these substances have parathormone-like properties. Calcium infusion, however, increases sodium excretion. In experiments where cyclic AMP was administered, although sodium excretion was not measured, potassium and magnesium excretion increased suggesting that a concomitant increase in sodium excretion might have also occurred. The increase in phosphate excretion with these agents, therefore, must be interpreted with caution since it may have been the simple consequence of increased sodium excretion. Furthermore, clinical studies of phosphate clearance or tubular phosphate reabsorption have been known to be complicated by such factors as dietary phosphate content and diurnal rhythm of phosphate excretion. It

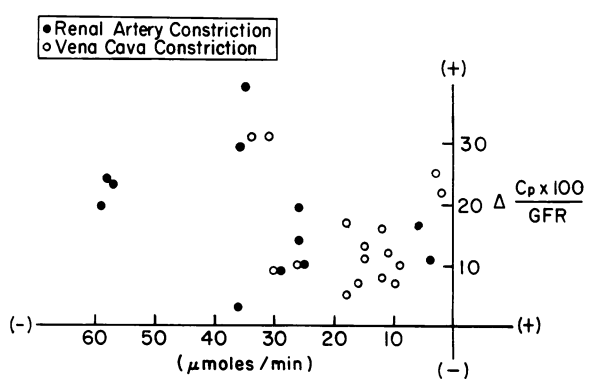

FIGURE 2 The effect of volume expansion on fractional clearance of phosphate during reduction in the filtered load of phosphate. The data plotted represent the change from the mean of the control periods. Closed circles represent results of experiments in which the filtered load was reduced by constriction of renal artery; open circles represent results of experiments in which the filtered load was reduced by constriction of the thoracic inferior vena cava. Note that despite consistent reductions in the filtered load of phosphate volume expansion always resulted in a net increase in the fractional clearance of phosphate.

TABLE IV

*Changes (from Control) in Fractional Clearance of Sodium and Phosphate Induced by Saline Infusion during Constriction of the Thoracic Inferior Vena Cava and after Cessation of Saline and Release of Constriction**

Experiment No.	Side	$\Delta C_{Na} \times 100/GFR$		$\Delta C_P \times 100/GFR$	
		Constriction	Release	Constriction	Release
1	L	0	+2.0	+0.1	+ 7.4
	R	0	+2.2	−0.6	+ 7.5
2	L	+0.1	+3.1	−4.6	+ 4.2
	R	+0.1	+3.5	+1.8	+12.5
3	L	+1.0	+4.3	+8.3	+18.6
	R	+1.2	+4.7	+9.7	+19.1
4	L	+0.7	+4.8	−0.4	+16.4
	R	+0.6	+3.8	+1.9	+14.3

* For explanation of symbols, see Table I.

now appears that, in addition to these two factors, sodium intake needs to be taken into consideration as it may complicate the interpretation of the observed results.

In conclusion, the expansion of the ECF volume results in an increased excretion of phosphate in the urine. This effect is the specific consequence of volume expansion and is not due to an increase in the filtered load of phosphate or to alterations in blood composition. The exact mechanism underlying this observation is not currently known.

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