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# INHIBITION BY VOLUME EXPANSION OF PHOSPHATE UPTAKE BY THE RENAL PROXIMAL TUBULE BRUSH BORDER MEMBRANE

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**Abstract**—Clearance studies and examination of brush border membrane (BBM) vesicle transport were performed in rats that had been volume expanded by 10% of body weight. The results were compared to those obtained in control animals. The data indicate that the phosphaturia which resulted from the expansion procedure was accompanied by an inhibition of proximal BBM phosphate uptake. The BBM uptake of proline and glucose was unchanged. Furthermore, since plasma calcium did not change, the findings are compatible with the view that the membrane transport changes resulted from alterations induced by the saline loading itself, rather than (or in addition to) any changes caused by parathyroid hormone excretion.

Expansion of the extracellular fluid volume routinely leads to the development of a natriuresis usually [1–7], but not always [8–10] associated with a phosphaturia. With regard to the mechanisms of the natriuresis, three principal theses have been advanced. First, "glomerular" factors may play a role. Thus, if glomerular filtration rate (GFR) is increased, then the filtered load of sodium will rise and urinary excretion would be expected to increase. Furthermore, if there is an increment in renal blood flow which exceeds that in GFR, filtration fraction will fall and excretion will be favored over reabsorption. Second, there may be a reduction in tubular reabsorption, independent of changes in renal hemodynamics, resulting from alterations in physical (Starling's) forces across the post-glomerular peritubular capillary. Thus, a reduction in plasma oncotic pressure due to dilution of the plasma protein concentration by saline infusion would lead to an inhibition of (proximal) tubular reabsorption [11]. Third, evidence has accumulated in support of the view that volume expansion leads to the elaboration of a natriuretic humoral substance which causes the inhibition of tubular ionic transport [12–14]. Because of the sodium dependency of its reabsorption [15], phosphate transport is thought to be altered by volume expansion by similar mechanisms.

The proximal nephron appears to be the major locus of the expansion effect since: (1) the bulk of phosphate reabsorption appears to be carried out proximally [16, 17]; (2) volume expansion can be shown to inhibit proximal tubular reabsorption in a major way [6–8, 10] and; (3) the majority of filtered sodium is reabsorbed proximally [18]. Several workers have demonstrated significant phosphate reabsorption beyond that portion of the superficial proximal convoluted tubule which is accessible to micropuncture when the parathyroid glands are removed [16, 19, 20], but whether this occurs in the late proximal tubule (i.e. the straight portion) or in the more distal nephron is not clear [21].

The initial and possibly rate-limiting step in the uptake of proximal phosphate reabsorption appears to be its transfer from the tubular lumen into the proximal tubular cell, across the luminal brush border membrane (BBM) [15]. With the development of a technique for the measurement of phosphate uptake across the BBM [22], it has become possible to study the luminal versus the transepithelial transport of this ion. In addition, previous studies performed in this laboratory have demonstrated that the *in vivo* infusion of modifiers of phosphate reabsorption leads to alterations in BBM transport which can later be detected *in vitro* [23]. Accordingly, the studies presented in this report were performed: (1) to determine if the proximal luminal BBM transport of phosphate is involved in the inhibition of phosphate reabsorption induced by volume expansion, and (2) to examine whether or not the epithelial transport alterations caused by volume expansion could later be identified *in vitro*, in tissue taken from expanded animals.

## METHODS

*Clearance experiments.* Studies were performed in three groups of rats. In each group, control and expanded animals were paired. In the first group, the kidneys were later harvested to examine the BBM vesicle transport of phosphate. In group II (N = 7 pairs), L-proline transport was studied, and in group III (N = 7 pairs), the transport of D-glucose was evaluated. Female Sprague–Dawley rats (150–250 g) were used. On the morning of each experiment, two rats were selected and anesthetized with intraperitoneal pentobarbital. A bladder catheter was placed through a suprapubic incision, and a

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carotid catheter was inserted for blood sampling. In both control and experimental groups, 2 g/100 ml inulin was infused at 0.04 $cm^3$/min and continued throughout the study. After a 60- to 90-min equilibration period, blood was drawn and two 30-min urine collections were taken. These and subsequent blood samples were analyzed for inulin, sodium, phosphate and calcium. Blood withdrawn was replaced by an equal volume of 0.154 M NaCl. The experimental group was expanded 10% of body weight with 0.154 M NaCl over 30 min. A second blood sample was drawn and three 30-min post-expansion urine collections were taken. At the end of the third post-expansion urine collection, the final blood sample was drawn and the kidneys were harvested.

*Preparation of the brush border membranes.* The proximal tubule brush border membrane vesicles were prepared using a previously described calcium precipitation method [23, 24]. In brief, renal medulla was removed carefully and discarded. The remaining tissue was suspended in 50 mM mannitol and homogenized using a Polytron homogenizer. To this homogenate was added $CaCl_2$ such that the final calcium concentration was 10 mM. After 20 min of stirring on ice, the homogenate was centrifuged at 4000 rpm for 10 min. The resulting supernatant fraction was spun at 18,000 rpm for 20 min. The pellet from this spin was suspended in 300 mM mannitol and homogenized using a Dounce homogenizer. The resultant suspension was centrifuged at 18,000 rpm for 15 min and resulted in a pellet with a red inner portion surrounded by a white halo. The halo was suspended carefully in 300 mM mannitol leaving the red portion, and another spin was performed at 18,000 rpm for 15 min. The white halo obtained from the pellet which resulted from this spin was resuspended carefully and was utilized as the final brush border membrane fraction. Purity of the preparation was measured using a gamma-glutamyl transpeptidase assay [23, 24]. Experiments were excluded if enzyme enrichment was less than a factor of 8. There was no significant difference in enzyme activity between control and experimental preparations.

*Brush border membrane phosphate transport.* Measurement of BBM vesicle transport was accomplished using a previously described technique [23, 24]. Briefly, 5-$\mu$l aliquots of the vesicle preparation were incubated at 20° with 45 $\mu$l of a solution, the final concentration of which was: 100 mM NaCl, 100 mM mannitol, 5 mM Tris–HEPES* (pH 8.5), and either 0.1 mM $K_2H^{32}PO_4$ (New England Nuclear, Boston, MA), or 0.025 mM L-[$^3$H]proline or 0.05 mM D-[$^3$H]glucose. Both of the latter isotopes were obtained from Amersham, Arlington Heights, IL. Transport was terminated at predetermined times by addition of an ice-cold arsenate solution. Vesicles were collected on filter paper of 0.65 $\mu$m pore size (Millipore Corp., Bedford, MA). The activity of $^{32}$P or $^3$H in the vesicles was then measured in the scintillation counter. The sodium-independent transport was measured by replacing NaCl in the incubation medium with 100 mM KCl.

* HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

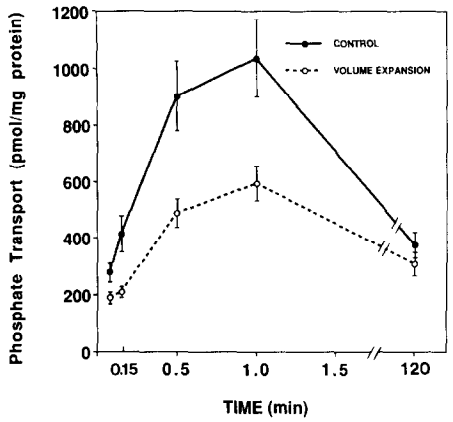


Fig. 1. Comparison of the brush border membrane vesicle uptake of inorganic phosphate (ordinate) in volume-expanded rats (○) with the values obtained in control animals (●). Data are the mean (±SE) uptake values for nine paired studies at the times specified on the abscissa.

Protein concentration was determined by the method of Bradford [25] using crystalline bovine serum albumin as a standard.

## RESULTS

In Fig. 1 are provided the BBM vesicle transport data obtained from the group I expanded animals compared to the observations provided by the control rats. Vesicle uptake of phosphate was reduced significantly at the 0.5- and 1.0-min time points, representing the peak of the initial uphill phase of transport [24]. At 30 sec, the mean value (±SE) for the expanded animals was 448 ± 51 pmol/mg protein, and in the controls it was 902 ± 123 ($P < 0.01$). At 60 sec, it was 593 ± 62 pmol/mg protein vs 1036 ± 136 in controls ($P < 0.025$). At the equilibrium point (120 min), the two values did not differ: expanded rats: 309 ± 42 pmol/mg protein; controls: 375 ± 45 ($P > 0.10$). The latter observations suggest equality of vesicle size in the two groups of animals and verify the stability of the vesicle preparation.

The clearance data for these experiments are given in Table 1. There was a modest but consistent increase in GFR in the control rats from 0.83 ± 0.17 to 1.20 ± 0.13 ml/min ($P < 0.025$). However, GFR in the expanded rats increased by better than 2-fold, from 0.93 ± 0.12 to 2.43 ± 0.20 ml/min ($P < 0.001$). The experimental procedure resulted in no change in electrolyte excretion in control rats, while both a major natriuresis and a brisk phosphaturia occurred in the expanded animals. Absolute sodium excretion ($U_{Na}V$) increased from 0.84 ± 0.26 to 47.0 ± 7.3 $\mu$Eq/min, and the percentage of filtered sodium excreted (%ENa) rose from less than 1% to better than 14% ($P < 0.001$). Absolute phosphate excretion ($U_pV$) almost quadrupled and the percentage of filtered phosphate excreted (%EP) rose from a baseline of 27.8 ± 4.5 to 38.3 ± 4.7% ($P < 0.005$). Plasma calcium did not change in the expanded animals (4.22 ± 0.06 and 4.28 ± 0.06,

Table 1. Clearance data in control and volume-expanded rats

| | $C_{In}$ (ml/min) | | $U_{Na}V$ (μEq/min) | | %ENa | | $U_pV$ (μmol/min) | | %EP | | $P_{Ca}$ (mEq/L) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | E | C | E | C | E | C | E | C | E | C | E |
| Control | 0.83 ± 0.17 | 1.20 ± 0.13 | 0.83 ± 0.16 | 1.75 ± 0.68 | 0.87 ± 0.18 | 1.07 ± 0.43 | 0.55 ± 0.08 | 0.68 ± 0.11 | 28.8 ± 4.3 | 21.2 ± 2.9 | 4.32 ± 0.09 | 4.66 ± 0.09 |
| P | <0.025 | | NS | | NS | | NS | | NS | | <0.01 | |
| Volume expanded | 0.93 ± 0.12 | 2.43 ± 0.20 | 0.84 ± 0.26 | 47.0 ± 7.3 | 0.75 ± 0.22 | 14.41 ± 2.15 | 0.50 ± 0.05 | 1.84 ± 0.18 | 27.8 ± 4.5 | 38.3 ± 4.7 | 4.22 ± 0.06 | 4.28 ± 0.06 |
| P | <0.001 | | <0.001 | | <0.001 | | <0.001 | | <0.005 | | NS | |

Abbreviations: $C_{In}$ = clearance of inulin; $U_{Na}V$, $U_pV$ = absolute excretion rates of sodium and phosphate respectively; %ENa, %EP = percentage excretion rates of sodium and phosphate respectively; $P_{Ca}$ = plasma calcium concentration; C, E = control and experimental phases of the studies respectively; NS = not statistically significant. Data (means ± SE) were obtained from nine pairs of rats; statistical analyses were performed utilizing Student's *t*-test for paired variables.

$P > 0.30$, in the control and the experimental phases of the study respectively). However, a modest but statistically significant increase occurred in the control animals.

In the group II studies, those in which the kidneys were harvested for the examination of BBM vesicle proline transport, clearance results similar to those obtained in the group I animals were observed. Thus, $U_{Na}V$ rose from 2.9 ± 0.7 to 46.2 ± 9.3 μEq/min ($P < 0.005$) and %ENa increased from 0.7 ± 0.1 to 12.4 ± 1.9% ($P < 0.001$) in the expanded animals. The natriuresis was associated with a phosphaturia: %EP increased from 12.5 ± 2.9 to 32.3 ± 6.9% ($P < 0.01$). The value for $U_pV$ trended upward (from 0.6 ± 0.1 to 1.2 ± 0.4 μmol/min), but the value did not reach statistical significance ($P > 0.05$). There were no changes in absolute or percent phosphate excretion in the control rats, and glomerular filtration rate ($C_{In}$) did not change in either group of animals. The values for plasma calcium fell slightly in the control rats (4.5 ± 0.2 → 4.3 ± 0.2 mEq/L, $P < 0.01$) and in the expanded animals: 4.6 ± 0.2 → 4.2 ± 0.2 mEq/L ($P < 0.05$). There were no differences in proline transport between the expanded and control rats at any time point (Table 2).

In the group III experiments, those in which the transport of D-glucose across the BBM was evaluated, there was again a natriuresis and phosphaturia in the expanded animals but no change in the control rats. Thus, $U_{Na}V$ rose from 3.7 ± 1.0 to 54.6 ± 4.4 μEq/min in the expanded rats ($P < 0.001$) and did not change in the controls (3.8 ± 0.7 → 2.7 ± 0.6 μEq/min, $P > 0.10$). %ENa increased from 0.9 ± 0.2 to 14.0 ± 1.2% in the expanded rats ($P < 0.001$) and was unchanged in the controls (0.9 ± 0.2 → 0.9 ± 0.2%, $P > 0.60$). %EP doubled in the expanded animals (from 5.9 ± 2.0 to 12.5 ± 2.8%, $P < 0.02$) and did not change in the control rats (6.5 ± 1.3 → 5.3 ± 2.0%, $P > 0.30$). Qualitatively similar results were obtained with regard to $U_pV$: controls, 0.3 ± 0.1 → 0.2 ± 0.1 μmol/min ($P > 0.05$); expanded rats, 0.3 ± 0.1 → 0.7 ± 0.2 μmol/min ($P < 0.025$). There were no changes in $C_{In}$ in either group. The mean values for $P_{Ca}$ did not change in either the expanded animals (5.3 ± 0.1 → 5.2 ± 0.3 mEq/L, $P > 0.50$) or the controls (5.2 ± 0.1 → 5.2 ± 0.2 mEq/L, $P > 0.50$). As was the case for proline, there were no differences in BBM vesicle glucose uptake for any of the time points (Table 2).

## DISCUSSION

The data presented in this paper reveal that the phosphaturia and natriuresis induced by volume expansion in the rats in which BBM vesicle phosphate uptake was studied resulted from the combined effects of the procedure on glomerular and tubular factors (Table 1 and Fig. 1). Thus, GFR increased substantially, but BBM transport was also inhibited. These observations have the following important implications. First, they document the fact that a maneuver performed *in vivo* can result in an alteration in membrane transport that can later be detected by *in vitro* methodology. These findings are

Table 2. Effect of volume expansion on the BBM vesicle transport of L-proline and D-glucose*

| | Uptake (pmol/mg protein) | | | | |
|---|---|---|---|---|---|
| | 0.08 min | 0.15 min | 0.5 min | 1.0 min | 120 min |
| L-Proline | | | | | |
| Control rats | 382 ± 62 | 564 ± 78 | 901 ± 91 | 898 ± 111 | 192 ± 37 |
| Expanded rats | 460 ± 59 | 607 ± 36 | 1005 ± 80 | 978 ± 89 | 186 ± 36 |
| P | >0.30 | >0.60 | >0.40 | >0.50 | >0.90 |
| D-Glucose | | | | | |
| Control rats | 504 ± 68 | 817 ± 108 | 1431 ± 204 | 893 ± 83 | 322 ± 81 |
| Expanded rats | 681 ± 115 | 836 ± 166 | 1415 ± 245 | 1056 ± 131 | 416 ± 115 |
| P | >0.20 | >0.90 | >0.90 | >0.30 | >0.50 |

* Values are means ± SE, (N = 7 pairs in each case).

consistent with the results of recent studies performed in this laboratory in which the isolated perfused tubular transport method was employed [26]. In the latter experiments, proximal straight tubules removed from the kidneys of volume-expanded animals demonstrated inhibited fluid and phosphate transport when compared with lumen-to-bath fluxes in paired control animals. These studies [26], taken together with the experimental findings reported here, suggest that the proximal tubule demonstrates "memory" for the expansion procedure.

Second, the data verify the proximal tubule as a major locus of the phosphate transport inhibition which results in a subsequent phosphaturia. In addition, they demonstrate that the luminal uptake of phosphate was affected by volume expansion to a significant degree. Third, because volume expansion did not affect proline or glucose uptake, it is clear that the tubular effects of volume expansion were not merely the result of nonspecific generalized changes in proximal tubular permeability or transport mechanisms.

At first inspection, our data would appear to be at variance with previous micropuncture and clearance experiments that have reported reduced proximal tubular glucose transport following volume expansion [27–29]. However, there are two important, and perhaps crucial, differences between these studies and our own. First, glucose loading was performed prior to expansion in the studies cited, but not in our experiments. When the former maneuver is either omitted [30], or is performed in a subthreshold manner [31], glycosuria does not occur. Second, because our glucose transport studies were performed *in vitro*, those factors (alterations in renal hemodynamics or in physical forces) that have a major impact on glucose reabsorption *in vivo* were obviated.

Because these studies were performed in animals with intact parathyroid glands, no definitive statement can be made about the participation or lack thereof of parathyroid hormone in the phosphaturia which resulted, following volume expansion. Participation of the parathyroids could have occurred in one or both of two ways. First, parathyroid secretion could have been stimulated if dilution of plasma contents with saline reduced the level of ionized calcium. Since the latter determination was not performed, no conclusive statement can be made. However, the absence of any fall in the total plasma calcium militates against this possibility (Table 1). Furthermore, studies in rabbits recently reported from this laboratory [26] indicate that volume expansion alters phosphate transport measured *in vitro* in both intact and thyroparathyroidectomized animals. Second, the intactness of the parathyroid glands provides for a more complete delivery of proximally rejected phosphate ions into the final urine [19, 20]. It is therefore entirely possible that some of the phosphate which appeared in the urine resulted from transport inhibition at more "distal" nephron loci [21].

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## REFERENCES

1. S. G. Massry, J. W. Coburn and C. R. Kleeman, *J. clin. Invest.* **48**, 1237 (1969).
2. W. N. Suki, M. Martinez-Maldonado, D. Rouse and A. Terry, *J. clin. Invest.* **48**, 1888 (1969).
3. J. B. Puschett and M. Goldberg, *J. Lab. clin. Med.* **73**, 956 (1969).
4. T. H. Steele, *Metabolism* **19**, 129 (1970).
5. J. B. Puschett, Z. S. Agus, D. Senesky and M. Goldberg, *Am. J. Physiol.* **223**, 851 (1972).
6. P. Poujeol, D. Chabardes, N. Roinel and C. De Rouffignac, *Pflügers Archs* **365**, 203 (1976).
7. A. Frick and I. Durasin, *Pflügers Archs* **370**, 115 (1977).
8. J. B. Puschett, H. A. Feldman and D. B. Sylk, *Mineral Electrolyte Metab.* **2**, 7 (1979).
9. H. Kuntziger, C. Amiel and G. Gaudebout, *Kidney Int.* **2**, 318 (1972).
10. A. Frick, *Am. J. Physiol.* **223**, 1034 (1972).
11. B. M. Brenner, J. L. Troy and T. M. Daugharty, *J. clin. Invest.* **50**, 1596 (1971).
12. H. D. De Wardener, I. H. Mills, W. F. Clapham and C. J. Hayter, *Clin. Sci.* **21**, 249 (1961).
13. V. M. Buckalew, Jr., F. J. Martinez and W. E. Green, *J. clin. Invest.* **49**, 926 (1970).
14. M. A. Kaplan, J. J. Bourgoignie, J. Rosecan and N. S. Bricker, *J. clin. Invest.* **53**, 1568 (1974).

15. F. G. Knox, A. Hoppe, S. A. Kempson, S. V. Shah and T. P. Dousa, in *Renal Handling of Phosphate* (Eds. S. G. Massry and H. Fleisch), pp. 79–114. Plenum, New York (1980).
16. C. Amiel, H. Kuntziger and G. Richet, *Pflügers Archs* **317**, 93 (1970).
17. J. C. Strickler, C. C. Thompson, R. M. Klose and G. Giebisch, *J. clin. Invest.* **43**, 1596 (1964).
18. G. Giebisch, R. M. Klose and E. E. Windhager, *Am. J. Physiol.* **206**, 687 (1964).
19. P. C. Fernandez and J. B. Puschett, *Am. J. Physiol.* **225**, 954 (1973).
20. L. H. Beck and M. Goldberg, *Kidney Int.* **6**, 18 (1974).
21. C. Amiel, in *Renal Handling of Phosphate* (Eds. S. G. Massry and H. Fleisch), pp. 39–58. Plenum, New York (1980).
22. L. Cheng and B. Sacktor, *J. biol. Chem.* **256**, 1556 (1981).
23. S. A. Kempson, J. C. Kowalski and J. B. Puschett, *Biochem. Pharmac.* **32**, 1533 (1983).
24. S. A. Kempson, J. C. Kowalski and J. B. Puschett, *J. Lab. clin. Med.* **101**, 308 (1983).
25. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
26. T. O. Pitts, J. A. McGowan, T. C. Chen, M. Silverman, M. E. Rose and J. B. Puschett, *J. clin. Invest.* **81**, 997 (1988).
27. A. M. Robson, P. L. Srivastava and N. S. Bricker, *J. clin. Invest.* **47**, 329 (1968).
28. A. D. Baines and J. H. V. Bishop, *J. clin. Invest.* **50**, 2414 (1971).
29. S. Boonjarern, M. E. Laski and N. A. Kurtzman, *Pflügers Archs* **366**, 67 (1976).
30. S. F. Wen, *Clin. Res.* **22**, 550A (1974).
31. S. F. Wen, J. W. Boynar and R. W. Stoll, *J. Lab. clin. Med.* **101**, 708 (1983).