Transport of Phosphate by Plasma Membranes of the Jejunum and Kidney of the Mouse Model of Hypophosphatemic Vitamin D-Resistant Rickets (43607)

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Abstract. **The hypophosphatemic mouse is a useful model for the study of hypophosphatemic vitamin D-resistant rickets in humans. Hypophosphatemia and hyperphosphaturia are the main biochemical findings in the patients and in mice with the disorder. The exact membrane localization of the site of the defect in phosphate transport in humans is not known. We utilized a well-validated technique of brush border and basolateral membrane vesicles to investigate phosphate transport across the enterocyte and the renal tubule cells of the hypophosphatemic (Hyp) mice model. Phosphate uptake by brush border membranes of jejunal enterocytes revealed similar initial rates (slopes were 0.007 and 0.006 for Hyp and control mice, respectively). Kinetics of jejunal Na^+-dependent phosphate uptake showed a V_{max} of 0.21 ± 0.03 and 0.19 ± 0.02 nmol/mg protein/15 sec, and K_m of 0.12 ± 0.07 and 0.09 ± 0.02 m*M* in the Hyp and control mice, respectively. Kinetics of basolateral uptake of phosphate were also similar (V_{max} of 0.05 ± 0.01 and 0.06 ± 0.02 nmol/mg protein/10 sec and K_m of 0.013 ± 0.004 and 0.028 ± 0.022 m*M*, respectively). On the other hand, kinetics of Na^+-dependent phosphate uptake by renal brush border membrane vesicles (BBMV) were markedly decreased (V_{max} of 0.42 ± 0.03 and 1.09 ± 0.06 nmol/mg protein/15 sec, $P < 0.01$, and K_m of 0.01 ± 0.003 and 0.05 ± 0.02 m*M*, $P < 0.02$, in the Hyp and control mice, respectively). Kinetics of Na^+-dependent phosphate uptake by renal basolateral membrane were not decreased (V_{max} of 0.19 ± 0.02 and 0.21 ± 0.02 nmol/mg protein/10 sec and K_m of 0.012 ± 0.003 and 0.012 ± 0.004 m*M* for Hyp and control mice, respectively). To determine whether the decrease in renal BBMV is secondary to alteration in the Na^+-dependent phosphate transporter or due to changes in the Na^+ gradient, two studies were conducted: first, a tracer exchange study in renal BBMV which showed a decrease in phosphate uptake in Hyp BBMV compared with controls, confirming the kinetic studies; and second, an Na^+ permeability study in renal BBMV of Hyp and control mice which showed no differences in Na^+ permeability across the renal BBMV. These findings suggest that the defect in the hypophosphatemic mice is localized only to the brush border membranes of the kidney and is not due to alteration in the driving forces across the membranes.**

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Hypophosphatemic vitamin D-resistant rickets is the most common form of hypophosphatemic rickets in humans (1). It is characterized by low renal tubular reabsorption of phosphate, low plasma phosphate, absence of elevated 1,25-dihydroxyvitamin D despite the presence of hypophosphatemia, and osteomalacic bone disease (1).

The hypophosphatemic (Hyp) mouse model appears to be an animal model of X-linked hypophosphatemic rickets in humans, because the Hyp mutation in mice produces the same abnormalities and both disorders are inherited as X-linked dominant traits and loci on the X-chromosome of different mammals are highly conserved (2).

Phosphate transport across the intestinal and renal epithelium occurs by similar mechanisms. This process includes a secondary active uptake across the brush

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border membrane, movement of phosphate across the cytosol or into the metabolic phosphate pool, and finally exit at the basolateral membrane. Studies utilizing renal membrane vesicles in the Hyp mice revealed a defect in the transport of phosphate (3, 4). The mechanisms underlying this defect are not known. Moreover, the exit of phosphate across the renal basolateral membrane in the Hyp mice has not been determined. Furthermore, the studies on the jejunal phosphate transport across the brush border membrane are in apparent conflict (5–8), and the study of the exit of phosphate across the jejunal basolateral membrane in the Hyp mice has not been undertaken.

The current studies were designed to extend previous studies on renal phosphate transport in the Hyp mice, and to resolve the controversy regarding the intestinal transport of phosphate. The studies utilized well-validated brush border and basolateral membrane vesicles across renal and intestinal epithelium of Hyp and control mice.

Experimental Procedures

C57BL/6J +/Y males (control) and C57BL/GJ Hyp/Y males (genetically hypophosphatemic) were obtained from the Jackson Laboratory (Bar Harbor, ME) at 3 months of age. The animals were maintained in overhanging cages, with food and water supplied *ad libitum*, until their sacrifice by cervical dislocation.

Materials. $KH_2[^{32}P]O_4$ (1 Ci/nmole) was purchased from Du Pont-New England Nuclear (Boston, MA). Enzymes and substrates for leucine aminopeptidase were obtained from Sigma Chemical Co. (St. Louis, MO). Cellulose nitrate filters, 0.45-μm pore size, were obtained from Sartorius Filter, Inc. (Hayward, CA). All other chemicals were of the highest purity available.

Preparation of Brush Border Membrane Vesicles in the Hyp and Control Mice. Six mice were used for each preparation. After sacrifice, the jejunal segments and kidneys were removed from each mouse. The kidneys were decapsulated and kept in 0.9% NaCl at 4°C. Cortical tissue was removed by slicing the cortex, using a sharp blade. The jejunum extended from the ligament of Treitz to approximately 15 cm aborally. The jejunal segments were washed with ice-cold 0.9% NaCl and everted on a glass rod. The mucosa was scraped from each segment, and brush border membrane vesicles (BBMV) were prepared by using a modified divalent cation precipitation method originally described for renal BBMV and used extensively in our laboratory (9, 10). Preparations were maintained at approximately 4°C at all times. Mucosal scrapings or cortical slices were placed in 60 ml of mannitol buffer (300 m*M* mannitol, 5 m*M* EGTA, and 12 m*M* Tris/HCl, pH 7.1) and 240 ml of ice-cold distilled water and then homogenized with a Waring blender-type homogenizer at maximum speed. The homogenate was treated with 3 ml of 1 *M* $MgCl_2$ and centrifuged at 3,000*g* for 15 min. The supernatant was then centrifuged at 27,000*g* for 30 min. The resulting pellet was resuspended in 60 ml of 60 m*M* mannitol, 5 m*M* EGTA, and 12 m*M* Tris/HCl (pH 7.1) and homogenized in a Potter-Elvehjem apparatus for 10 strokes at the highest speed. The homogenate was treated with 0.6 ml of 1 *M* $MgCl_2$ and centrifuged at 3,000*g* for 15 min. The supernatant was spun at 27,000*g* for 30 min. The pellet was resuspended in 30 ml of 250 m*M* mannitol and 20 m*M* Hepes/Tris (pH 7.4) and homogenized with the Potter-Elvehjem apparatus for 10 strokes at the highest speed. The suspension was centrifuged at 48,400*g* for 30 min. With a 25-gauge needle and syringe, the pellet was resuspended in the desired volume of transport buffer, as described in the Figure Legends. All centrifugation steps were carried out in a Beckman J2–21 rotor. A sample of each final BBMV preparation was removed for protein determination by the method of Lowry *et al.* (11) using bovine serum albumin as a standard.

Preparation of Basolateral Membrane Vesicles in the Hyp and Control Mice. Jejunal and renal basolateral membrane vesicles (BLMV) were prepared by using a modified centrifugation technique, followed by separation on a Percoll gradient (12). Six mice were used for each preparation. After sacrifice, the jejunal segments and kidneys were removed from each mouse. The kidneys were decapsulated and kept in 0.9%NaCl at 4°C. Cortical tissue was removed by slicing the cortex, using a sharp blade. The jejunums were removed, flushed with ice-cold Ringer lactate solution, and then filled with warmed buffer solution (37°C) containing 1.5 m*M* KCl, 96 m*M* NaCl, 8 m*M* KH_2PO_4, 5.6 m*M* Na_2HPO_4, 27 m*M* sodium citrate, and 2 m*M* dithiothreitol (pH 7.4). The jejunums were then clamped and incubated for 15 min in a shaking water bath at 37°C. The clamps were then removed and the contents were emptied. The jejunums were then filled with ice-cold buffer containing 250 m*M* mannitol and 12 m*M* Hepes/Tris buffer, pH 7.4 (Buffer 1), and gently palpated with fingers for 5 min to release epithelial cells. The contents were then drained into a beaker on ice, and the volume was made up to 150 ml with Buffer 1. The cells were then centrifuged at 200*g* for 5 min, and the cell pellet or renal cortical slices were homogenized in 150 ml of Buffer 1 in a Waring blender-type homogenizer for 3 min. The homogenate was then centrifuged at 2,500*g* for 20 min. The supernatant was then collected and centrifuged at 22,000*g* for 25 min. The supernatant was discarded, and the resulting fluffy layer of the pellet was resuspended in 90 ml of Buffer 1 and homogenized in a Potter-Elvehjem apparatus (20 strokes). The resultant homogenate was mixed with Percoll (Pharmacia, 15%) and then centrifuged at

48,000*g* for 75 min. A distinct band of basolateral membrane was seen at the upper one third of the Percoll gradient. The band was aspirated with a needle and diluted with buffer containing 100 m*M* mannitol, 100 m*M* KCl, and 12 m*M*-Hepes/Tris, pH 7.4. The diluted basolateral membrane was then centrifuged at 48,000*g* for 20 min twice, and finally suspended in 100 m*M* KCl, 100 m*M* mannitol, and 20 m*M* Hepes/Tris buffer (pH 7.4).

Purity of the Membrane Vesicle Preparation. The purity of the membranes was assessed by the measurement of leucine aminopeptidase, an enzyme marker for intestinal brush border membranes, with Sigma kit 251. The procedure is based on the principle that leucine aminopeptidase cleaves a substrate L-leucyl-β-naphthylamide to leucine + β-nahthylamin, which can be measured spectrophotometrically. (Na^+K^+)-ATPase was measured according to the method of Scharschmidt *et al.* (13). Cytochrome *c* oxidase and NADPH-cytochrome *c* reductase were assayed as described by Beaufay *et al.* (14).

Transport Measurements. Uptake of substrates (phosphate, sodium) was measured by rapid-filtration technique (15). All experiments were performed at 25°C. Transport was initiated by adding 20 μl of the final vesicle suspension to the desired incubation medium containing labeled substrate. The composition of the incubation medium for each individual experiment is described in the Figure Legends. At the destined time intervals, the reaction was stopped by the addition of ice-cold stop solution, consisting of 100 m*M* mannitol, 100 m*M* NaCl, 20 m*M* Hepes/Tris (pH 7.4), and 10 m*M* K_2PO_4. The vesicles were immediately collected on a cellulose nitrate filter (0.45-μm pore size; Sartorius Filters) and kept under suction while they were washed with ice-cold stop solution. The amount of radioactive substrate remaining on the filter was determined in a Beckman liquid scintillation counter, with Bray's solution (New England Nuclear) as the liquid scintillant. Radioactivity remaining in the filters after pipetting incubation medium into the radioactive substrate in the absence of vesicles was used as background and was considered in the calculations. All transport experiments in control and Hyp mice were carried out in the same day.

Statistical Evaluation. All values were expressed graphically as the mean ± 1 SE. Student's *t* test was used to evaluate the statistical significance of differences between the groups. A probability value of $P < 0.05$ was considered statistically significant.

Results

Marker Enzyme Studies. *BBMV.* Leucine aminopeptidase, a marker for brush border enzymes, was enriched 10 ± 12-fold compared with crude homogenate in both jejunal and renal membranes of Hyp and control mice. The activities of (Na^+K^+)-ATPase, cytochrome *c* oxidase, and NADPH-cytochrome *c* reductase were depleted to 0.6 ± 0.1-fold, 0.9 ± 0.1-fold, and 0.8 ± 0.1-fold, respectively.

BLMV. The activity of (Na^+K^+)-ATPase, a marker for basolateral membranes, was enriched 9 ± 2-fold as compared with crude homogenate in both jejunal and renal membranes of Hyp and control mice. The activities of leucine aminopeptidase, cytochrome *c* oxidase, and NADPH-cytochrome *c* reductase were depleted to 0.8 ± 0.2-fold, 0.6 ± 0.1-fold, and 0.7 ± 0.1-fold, respectively.

Functional Studies. Previously, we have shown that jejunal basolateral membranes prepared with Percoll density gradient lack Na^+ gradient-dependent glucose uptake and possess an ATP-dependent calcium uptake (16, 17). To provide functional evidence for the renal basolateral membranes in the Hyp and control mice, we carried out two experiments. First, D-glucose uptake was measured in the presence of Na^+ gradient. Second, calcium uptake was measured in the presence of ATP. As seen in Figure 1, glucose uptake did not show an overshoot phenomena, whereas ATP stimulated calcium uptake compared with no ATP conditions, in both Hyp and control mice.

Initial Rate of Phosphate Uptake by Renal and Jejunal BBMV in the Hyp and Control Mice. To be able to determine the kinetics of phosphate uptake, it was essential to investigate the linearity of uptake with time. Figure 2 depicts the initial rate of phosphate uptake by renal and jejunal BBMV. The initial rate of renal and jejunal phosphate uptake was linear in both membranes up to 15 s. The slopes representing initial rates of jejunal phosphate uptake were similar in the Hyp and control mice. On the other hand, the slopes representing the initial rate of renal phosphate uptake were significantly decreased in the Hyp mice compared with control mice ($P < 0.01$).

Kinetics of Na^+-Dependent Phosphate Uptake by Renal and Jejunal BBMV in the Hyp and Control Mice. In an effort to define the kinetics of Na^+-dependent phosphate transport, vesicles from Hyp and control mice were incubated in Na^+-containing and Na^+-free buffers with varying phosphate concentration. Transport was measured at 15 s during the linear phase of uptake. When Na^+ in the incubation media was replaced with K^+, uptake became linear. Figure 3a depicts kinetic parameters for Na^+-dependent phosphate uptake (Na^+-dependent minus Na^+-independent uptake) by renal BBMV in the Hyp and control mice. V_{max} values by renal BBMV were significantly lower in the Hyp mice compared with corresponding values in control mice (0.42 ± 0.03 compared with 1.09 ± 0.06 nmol/mg protein/15 sec, $P < 0.01$). K_m values were also significantly different (0.01 ± 0.003 compared with 1.09 ± 0.06 nmol/mg protein, $P < 0.01$). K_m values

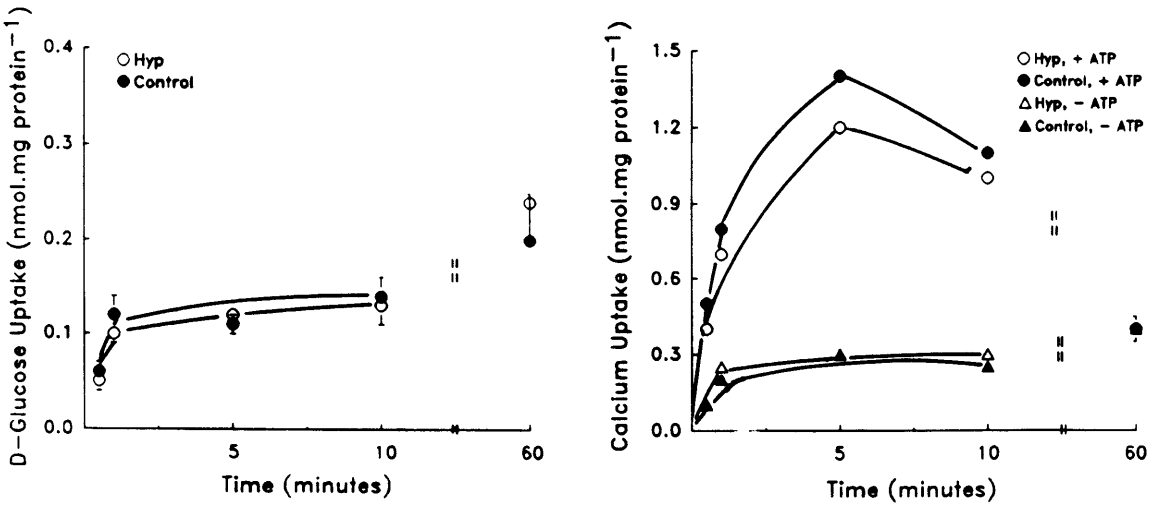

Figure 1. D-Glucose and calcium uptake by renal basolateral membrane vesicles of the Hyp and control mice. Renal BLMV from Hyp and control mice were prepared in 100 m*M* KCl, 100 m*M* mannitol, and 20 m*M* Hepes/Tris buffer (pH 7.4). Reaction was started by incubating the vesicles with 100 m*M* NaCl, 100 m*M* mannitol, 20 m*M* Hepes/Tris (pH 7.4), 0.1 m*M* D-glucose, and [^{3}H]glucose. The incubation media for the calcium studies were 100 m*M* mannitol, 100 m*M* KCl, 5 m*M* $MgCl_2$, 20 m*M* Hepes/Tris, 0.1 m*M* $CaCl_2$, and tracer ^{45}Ca in the presence or absence of 5 m*M* ATP Tris salt. Reaction was stopped at desired time points. Values are mean ± SE of three separate experiments run in triplicate.

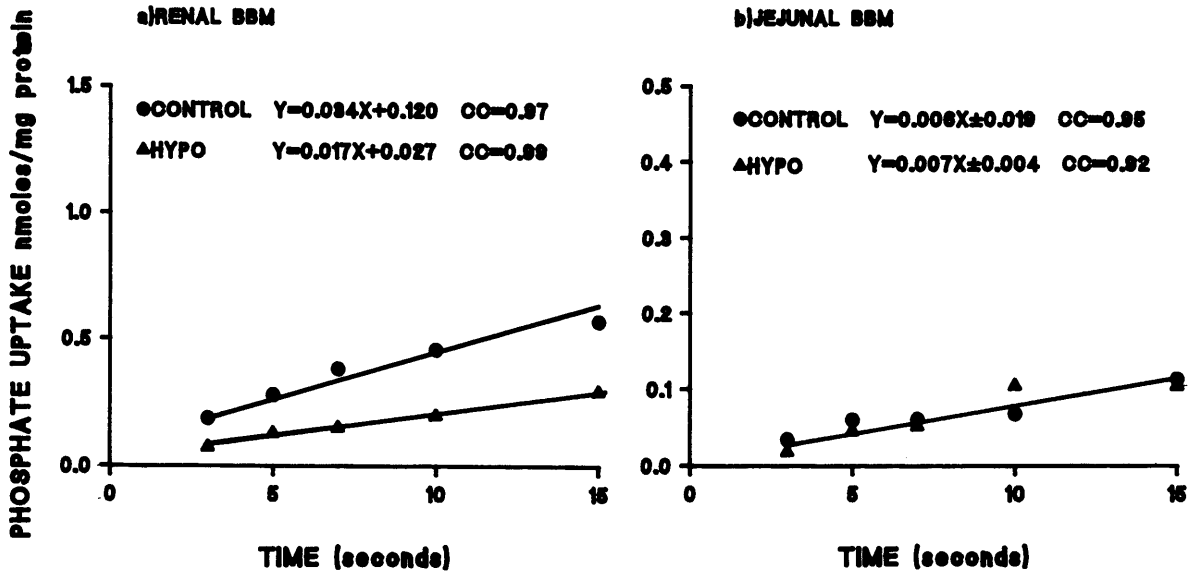

Figure 2. (a & b) Initial rate on phosphate uptake by renal and jejunal BBMV in the Hyp and control mice. BBMV were prepared in 280 m*M* mannitol and, 20 m*M* Hepes/Tris buffer (pH 7.4). Reaction was started by incubating the vesicle with 100 m*M* NaCl, 100 m*M* mannitol, 20 m*M* Hepes/Tris, and 0.1 m*M* KH_2PO_4. Reaction was stopped at desired intervals. Phosphate uptake was linear in both renal and jejunal BBMV up to 15 sec. The slopes representing initial phosphate uptake into renal BBMV were significantly different in the Hyp compared with control mice. However, the slopes were similar for jejunal BBMV. Uptake values represent the mean of three independent experiments. Each run in triplicate.

were also significantly different (0.01 ± 0.003 compared with 0.05 ± 0.02 m*M*, $P < 0.02$). Figure 3b demonstrates kinetic parameters for Na^+-dependent phosphate uptake (Na^+-dependent minus Na^+-independent uptake) by jejunal BBMV.

V_{max} and K_m values in jejunal BBMV of Hyp mice were 0.21 ± 0.03 nmol/mg protein/15 sec and 0.12 ± 0.07 m*M*, and in control mice the values were 0.19 ± 0.02 nmol/mg protein/15 sec and 0.09 ± 0.05 m*M*, respectively. There were no significant differences between V_{max} and K_m values of Hyp and control mice.

Na^+ Permeability in Renal BBMV of Hyp and Control Mice. To determine whether the differences in phosphate uptake between Hyp and control mice were related to a change in Na^+ permeability, ^{22} uptake into BBMV from Hyp and control mice was determined. Figure 4 depicts ^{22}Na uptake expressed as nmol/mg vesicle protein. At 30 sec and at 1, 2, and 60 min, $^{22}Na^+$ uptake was not significantly different between Hyp and control mice. Moreover, vesicle size as calculated from equilibrium values was similar at 1.2 ± 0.02 μl/mg protein in both Hyp and control mice.

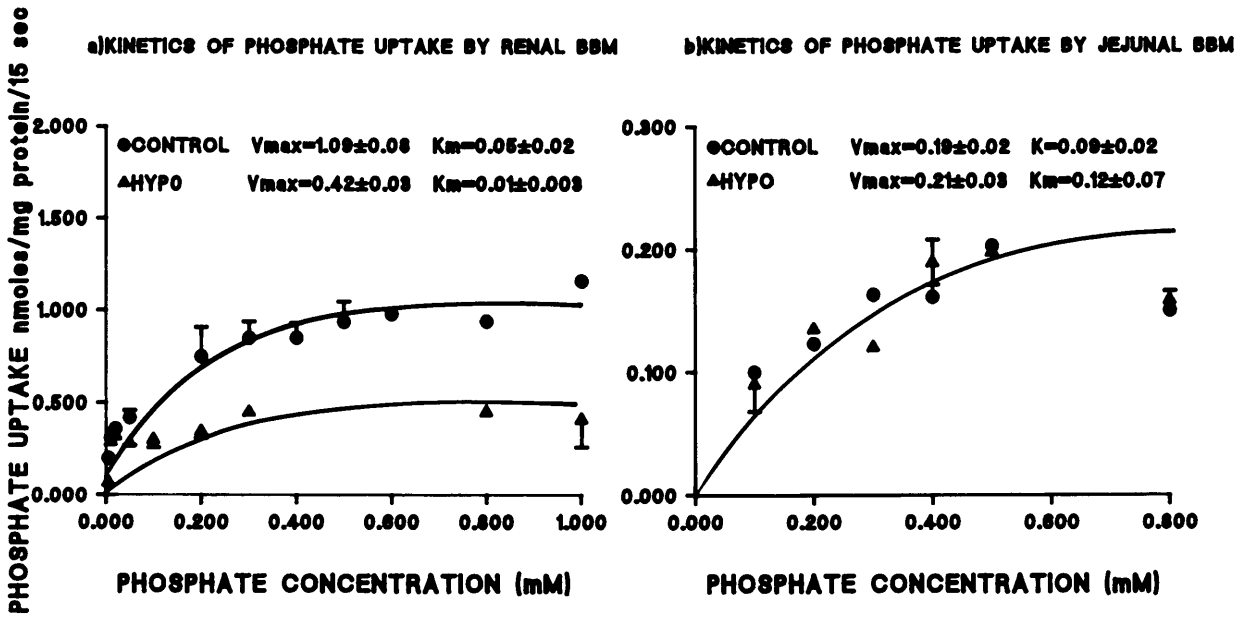

Figure 3. (a & b) Kinetics of Na^+-dependent phosphate uptake by renal and jejunal BBMV in the Hyp and control mice. BBMV were prepared in 280 m*M* mannitol and 20 m*M* Hepes/Tris buffer (pH 7.4). Reaction was started by incubating the vesicles with 100 m*M* NaCl or 100 m*M* KCl, 100 m*M* mannitol, 20 m*M* Hepes/Tris, and varying concentrations of KH_2PO_4 and tracer ^{32}P. Reaction was stopped at 14 sec. Mean uptake values in the presence of 100 m*M* KCl were subtracted from mean values in the presence of 100 m*M* NaCl. Each value depicted represents mean ± SE of three experiments run in triplicate. Kinetic analyses were done utilizing a computerized model of Michaelis-Menten kinetics.

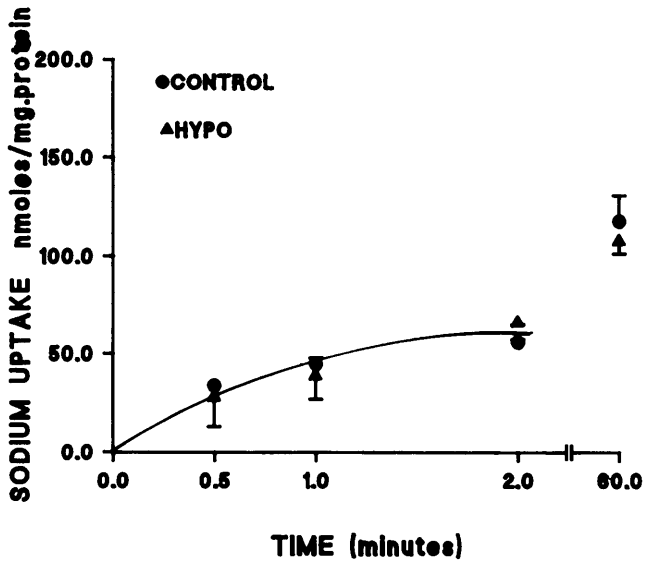

Figure 4. Na^+ permeability in renal BBMV of Hyp and control mice. Renal BBMV from the Hyp and control mice were raised in 280 m*M* mannitol and 20 m*M* Hepes/Tris buffer (pH 7.4). Membrane vesicles were then incubated in a media containing 100 m*M* NaCl, 100 m*M* mannitol, 20 m*M* Hepes/Tris, and ^{22}Na. Uptake of Na^+ at 30 sec and at 1, 2, and 60 min was similar in the Hyp and control BBMV. Each value represents mean ± SE of three experiments run in triplicate.

Tracer Exchange Studies in Renal BBMV of Hyp and Control Mice. Isotope exchange studies were performed to determine whether the observed differences in phosphate uptake between Hyp and control mice relate to a change in the activity of the Na^+-phosphate cotransporter (Fig. 5). In these studies, the ionophore gramicidin increases the cation conductance of membranes (18), nullifying all electrochemical gradients

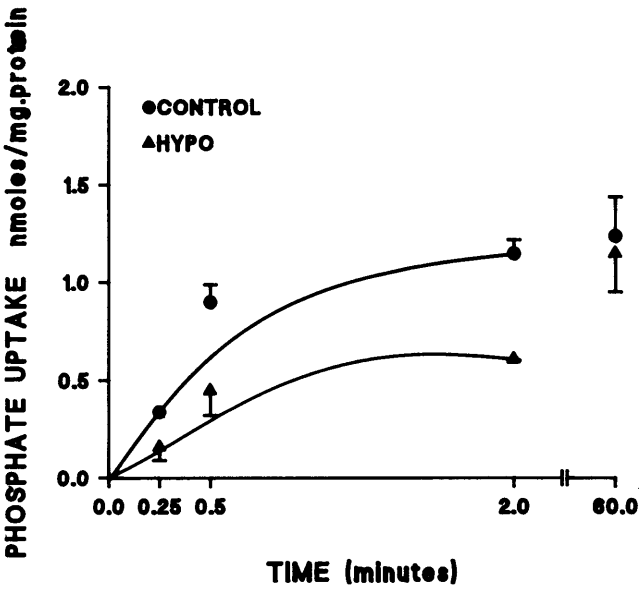

Figure 5. Tracer exchange studies in renal BBMV of Hyp and control mice. Renal BBMV from the Hyp mice or control mice were loaded with 0.1 *M* mannitol, 0.1 *M* NaCl, 0.8 m*M* KH_2PO_4, 20 m*M* Hepes/Tris (pH 7.4), and gramicidin (6 μg/ml). The vesicles were incubated in a medium containing 0.1 *M* mannitol, 0.1 m*M* NaCl, 0.8 m*M* KH_2PO_4, and 20 m*M* Hepes/Tris (pH 7.4). Each value represents mean ± SE of three experiments run in triplicate.

across the vesicle membrane. Phosphate uptake occurring under these experimental conditions is due to activity of the Na^+-dependent phosphate cotransporter. At all time points, uptake in control mice was significantly greater than uptake in the Hyp mice ($P < 0.05$–0.001), confirming the kinetic studies and suggesting a decrease in the activity of the renal BBMV Na^+-dependent phosphate cotransporter in the Hyp mice.

Initial Rate of Phosphate Uptake by Renal and Jejunal BLMV in the Hyp and Control Mice. Figure 6 depicts the initial rate of phosphate uptake by renal and jejunal BLMV. The initial rate of renal and jejunal phosphate uptake was linear in both membranes up to 15 sec. The slopes representing initial rates of renal and jejunal phosphate uptake were similar in the Hyp and control mice.

Kinetics of Na^+-Dependent Phosphate Uptake by Renal and Jejunal BLMV in the Hyp and Control Mice. Transport was measured at 10 sec during the linear phase of uptake. Figure 7a demonstrates the kinetic parameter for Na^+-dependent phosphate uptake (Na^+-dependent minus Na^+-independent uptake) by renal BLMV in the Hyp and control mice. V_{max} and K_m values in renal BLMV of the Hyp mice were 0.19 ± 0.02 nmol/mg protein/10 sec and 0.012 ± 0.003 mM, and in control mice the values were 0.21 ± 0.02 nmol/mg protein/10 sec and 0.012 ± 0.004 mM, respectively. As seen Figure 7b, V_{max} and K_m values in jejunal BLMV of the Hyp mice were 0.05 ± 0.01 nmol/mg protein/10 sec and 0.013 ± 0.004 mM, and in control mice the values were 0.06 ± 0.02 nmol/mg protein/10 sec and 0.028 ± 0.002 mM, respectively. There were no significant differences between V_{max} and K_m values of Hyp and control mice in both renal and jejunal BLMV.

Discussion

We used well-validated techniques for isolation of BBMV and BLMV from both groups of mice. The purity of the BBMV and BLMV was assessed by marker enzyme studies. These studies indicated similar enrichment in both the control and Hyp mice.

The current studies demonstrated that the slope of the initial rate of renal phosphate uptake in the Hyp mice was significantly decreased compared with control mice. Kinetic analysis of the data indicated that the V_{max} and K_m of renal phosphate uptake in the Hyp mice were significantly decreased, compared with the corresponding mean values in control mice. These results suggest that the number/activity and affinity of the renal Na^+-dependent phosphate transporter are altered in the Hyp mice compared with controls. These results are qualitatively similar to our previous observation (19). However, to determine the mechanism underlying the decrease in the V_{max} of phosphate uptake by the kidney of the Hyp mice compared with control mice, two studies were carried out. The first experiment examined the driving force for phosphate uptake by determining Na^+ permeability across renal BBMV in the Hyp and control mice. The results show no differences in Na^+ permeability between Hyp and control mice. The second experiment was an isotopic exchange study in which all electrochemical gradients across renal BBMV were nullified. Under these conditions, any

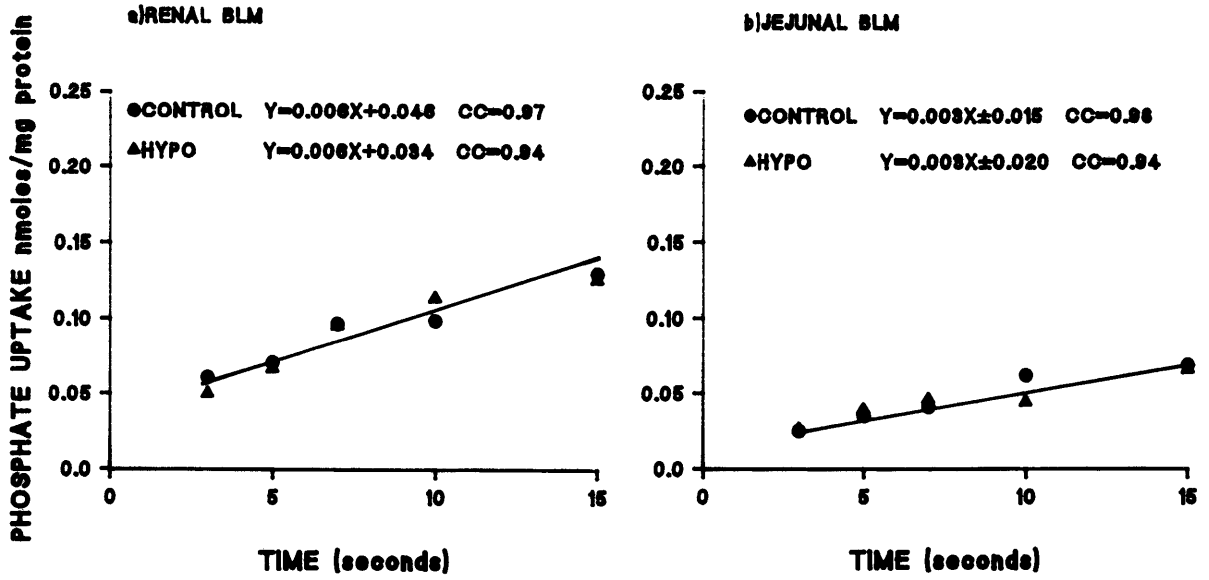

Figure 6. (a & b) Initial rate of phosphate uptake by renal and jejunal BLMV in the Hyp and control mice. BLMV were prepared in 100 mM KCl, 100 mM mannitol, and 20 mM Hepes/Tris buffer (pH 7.4). Reaction was started by incubating the vesicles with 100 mM NaCl, 100 mM mannitol, 20 mM Hepes/Tris, and 0.1 mM KH_2PO_4. Reaction was stopped at desired intervals. Phosphate uptake was linear on both renal and jejunal BLMV up to 15 sec. The slopes representing initial phosphate BLMV were similar in the Hyp compared with control mice. Each value represents mean ± SE of three experiments run in triplicate.

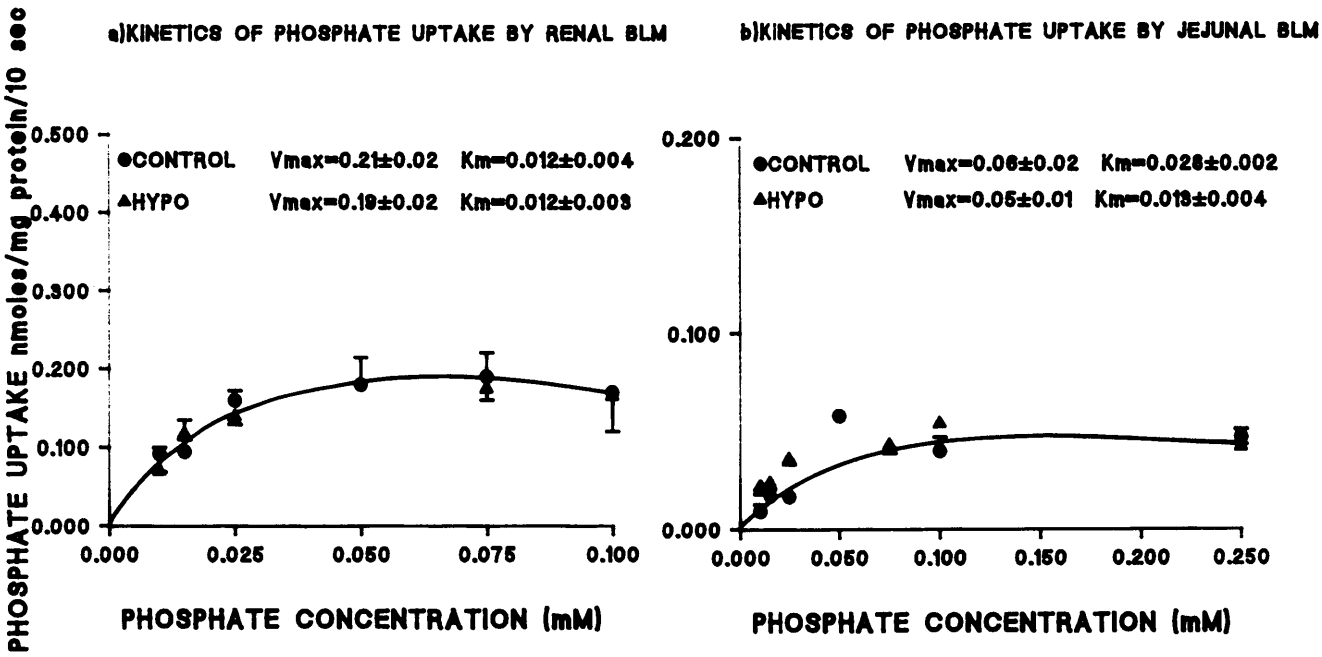

Figure 7. (a & b) Kinetics of Na^+-dependent phosphate uptake by renal and jejunal BLMV in the Hyp and control mice. BLMV were prepared in 100 m*M* KCl, 100 m*M* mannitol, and 20 m*M* Hepes/Tris buffer (pH 7.4). Reaction was started by incubating the vesicles with 100 m*M* NaCl or 100 m*M* KCl, 100 m*M* mannitol, 20 m*M* Hepes/Tris, and varying concentrations of KH_2PO_4 and tracer ^{32}P. Reaction was stopped at 10 sec. Mean uptake values in the presence of 100 m*M* KCl were subtracted from mean values in the presence of 100 m*M* NaCl. Each value represents mean ± SE of three experiments run in triplicate. Kinetic analysis were done utilizing a computerized model of Michaelis-Menten kinetics.

transport taking place must be carrier mediated (9, 10). Therefore, phosphate uptake would reflect Na^+-dependent phosphate cotransporter activity. As seen in Figure 4, phosphate uptake is significantly decreased in the Hyp mice compared with control mice, suggesting that there is a decrease in the activity of the Na^+-dependent phosphate cotransporter in the Hyp mice. On the other hand, phosphate uptake by jejunal BBMV was similar between Hyp and control mice as depicted by similar initial rates and kinetic parameters. Although we used adult Hyp mice in all experiments, Braut *et al.* (20) have found that phosphate malabsorption in the Hyp mice is an age-related phenomenon and these changes parallel the malabsorption of calcium and plasma 1,25-dihydroxyvitamin D levels of young Hyp mice.

This study also characterized for the first time phosphate transport across BLMV of Hyp and control mice in both renal and jejunal epithelium. Our basolateral membranes were prepared by an established technique utilizing a Percoll density gradient (16) with some modifications. The studies demonstrate the presence of an Na^+-dependent phosphate cotransporter in the basolateral membrane of Hyp and control mice in both renal and jejunum epithelium, which has different kinetic characteristics compared with the brush border membrane phosphate transporter. The results of BLMV studies demonstrate that the slope of initial rate of renal and jejunal phosphate uptake in the Hyp mice was similar compared with control mice, and the V_{max} and K_m of renal and jejunal Na^+-dependent phosphate uptake were also similar between Hyp and control mice. The results suggest that the defect in Hyp phenotype is not expressed in the BLMV of renal and jejunal epithelium. The presence of an Na^+-dependent process for phosphate uptake is similar to our previous studies in humans (16) and rats (17).

Tenehouse *et al.* (4) demonstrated that tissue phosphorus concentrations are similar in Hyp and control mice even when the extracellular phosphorus concentration is low in the Hyp mice. Our findings are consistent with their proposal in that an intact component of phosphate transport, which is related to phosphate fluxes across the basolateral membrane, maintains tissue concentration of phosphate in the Hyp phenotype.

We, therefore, believe that the defect in Hyp mice is located at the renal brush border membrane and that the transport process at the renal basolateral membrane and at the jejunal BBMV and BLMV are intact.

1. Rasmusseun H, Anast C. Familial hypophosphatemic rickets and vitamin D dependent rickets. In: Stanbury JB, Wyngaarden JB, Frederickson DS, Goldstein JL, Brown MS, Eds. The Metabollic Basis of Inherited Disease. New York:McGraw, pp1743–1773, 1983.
2. Eicker EM, Southard JL, Scriver CR, Glorieux FH. Hypophosphatemia: Mouse model for human familial hypophosphatemic rickets. Proc Natl Acad Sci USA 73:4667–4671, 1976.

3. Cowgill LD, Goldfrb S, Lau K, Slatopolsky E, Agus ZS. Evidence for an intrinsic renal tubular defect in mice with genetic hypophosphatemia. J Clin Invest **63**:1203–1210, 1979.
4. Tenenhouse HS, Scriver CR, McInnes RR, Glorieux FH. Renal handling of phosphate *in vivo* and *in vitro* by the X-linked hypophosphatemic male mouse: Evidence for a defect in the brush border membrane. Kidney Int **14**:236–242, 1978.
5. Beamer WG, Wilson MC, Deluca HF. Successful treatment of genetically hypophosphatemic mice by 1-hydroxyvitamin D_3 but not 1,25-dihydroxyvitamin D_3. Endocrinology **106**:1949–1955, 1980.
6. Chan JCM, Lovinger RD, Mamunes P. Renal hypophosphatemic rickets: Growth acceleration after long-term treatment with 1,25-dihydroxyvitamin D_3. Pediatrics **66**:445–454, 1980.
7. Glorieux FH, Morin CL, Travers R, Delvin EE, Poirer R. Intestinal phosphate transport in familial hypophosphatemic rickets. Pediatr Res **10**:691–696, 1976.
8. L'Doherty PJA, Deluca HF, Eicher EM. Lack of effect of vitamin D and its metabolite on intestinal phosphate transport in familial hypophosphatemia of mice. Endocrinology **101**:1325–1330, 1977.
9. Barnard JA, Ghishan FK, Wilson FA. Ontogenesis of taurocholate transport by rat ileal brush border membrane vesicles. J Clin Invest **75**:869–873, 1985.
10. Ghishan FK, Wilson FA. Develomental maturation of D-glucose transport by rat jejunal brush border membrane vesicles. Am J Physiol **248**:G87-G92, 1985.
11. Lowry DH, Rosebrogh NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
12. Scalera V, Huang YK, Hildmann B. A simple isolation method for basolateral plasma membranes from rat kidney cortex. Membr Biochem **4**:49–54, 1981.
13. Scharschmidt BF, Keeffe EB, Blankenship NM, Ockner RK. Validation of a recording spectrophotometric method for measurement of membrane-associated Mg^{++} and Na^+-K^+ATPase activity. J Lab Clin Med **93**:790–799, 1979.
14. Beaufay HA, Amas-Costesec AA, Feytmans E, Thinessempoux D, Wibo M, Robbi M, Berthet J. Analytical study of microsomes and isolated subcellular membranes from rat liver. J Cell Biol **61**:188–200, 1974.
15. Hopfer U, Nelson K, Penotto J, Isselbacker KJ. Glucose transport in isolated brush border membrane from rat small intestine. J Biol Chem **247**:25–32, 1973.
16. Kikuchi K, Ghishan FK. Phosphate transport by basolateral plasma membranes of human small intestine. Gastroenterology **93**:106–113, 1987.
17. Ghishan FK, Kikuchi K, Arab N. Phosphate transport by rat intestinal basolateral membrane vesicles. Biochem J **243**:641–646, 1987.
18. Pressman B. Inophorus antibiotics as models for biological transport. Fed Proc **27**:1238–1288, 1968.
19. Nakagawa N, Arab N, Ghishan FK. Characterization of the defect in the Na^+-phosphate transport in vitamin D-resistant hypophosphatemic mice. J Biol Chem **266**:13616–13620, 1991.
20. Braut BA, Meyer MH, Meyer RA Jr. Malabsorption of phosphate by the intestines of young X-linked hypophosphatemic mice. Calcif Tissue Int **43**:289–293, 1988.