JCI The Journal of Clinical Investigation

Evidence for an Intrinsic Renal Tubular Defect in Mice with Genetic Hypophosphatemic Rickets

Larry D. Cowgill, … , Eduardo Slatopolsky, Zalman S. Agus

J Clin Invest. 1979;63(6):1203-1210. https://doi.org/10.1172/JCI109415.

Research Article

To investigate the role of parathyroid hormone (PTH) and(or) an intrinsic renal tubular reabsorptive defect for phosphate in mice with hereditary hypophosphatemic rickets, we performed clearance and micropuncture studies in hypophosphatemic mutants and nonaffected littermate controls. Increased fractional excretion of phosphate in mutants (47.2±4 vs. 30.8±2% in controls) was associated with reduced fractional and absolute reabsorption in the proximal convoluted tubule and more distal sites. Acute thyropara-thyroidectomy (TPTX) increased phosphate reabsorption in both mutants and controls with a fall in fractional phosphate excretion to ≅ 7.5% in both groups indicating that PTH modified the degree of phosphaturia in the intact mutants. Absolute reabsorption in the proximal tubule and beyond remained reduced in the mutants, however, possibly because of the reduced filtered load. Serum PTH levels were the same in intact mutants and normals as was renal cortical adenylate cyclase activity both before and after PTH stimulation.

To evaluate the possibility that the phosphate wasting was caused by an intrinsic tubular defect that was masked by TPTX, glomerular fluid phosphate concentration was raised by phosphate infusion in TPTX mutants to levels approaching those of control mice. Phosphate excretion rose markedly and fractional reabsorption fell, but there was no change in absolute phosphate reabsorption in either the proximal tubule or beyond, indicating a persistent reabsorptive defect in the absence of PTH.

We conclude […]

Evidence for an Intrinsic Renal Tubular Defect in Mice with Genetic Hypophosphatemic Rickets

LARRY D. COWGILL, STANLEY GOLDFARB, KAI LAU, EDUARDO SLATOPOLSKY, and ZALMAN S. AGUS, *Renal Electrolyte Section, Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104; and Renal Division, Department of Medicine, Washington University, St. Louis, Missouri 63110*

ABSTRACT To investigate the role of parathyroid hormone (PTH) and(or) an intrinsic renal tubular reabsorptive defect for phosphate in mice with hereditary hypophosphatemic rickets, we performed clearance and micropuncture studies in hypophosphatemic mutants and nonaffected littermate controls. Increased fractional excretion of phosphate in mutants (47.2±4 vs. 30.8±2% in controls) was associated with reduced fractional and absolute reabsorption in the proximal convoluted tubule and more distal sites. Acute thyroparathyroidectomy (TPTX) increased phosphate reabsorption in both mutants and controls with a fall in fractional phosphate excretion to ≅7.5% in both groups indicating that PTH modified the degree of phosphaturia in the intact mutants. Absolute reabsorption in the proximal tubule and beyond remained reduced in the mutants, however, possibly because of the reduced filtered load. Serum PTH levels were the same in intact mutants and normals as was renal cortical adenylate cyclase activity both before and after PTH stimulation.

To evaluate the possibility that the phosphate wasting was caused by an intrinsic tubular defect that was masked by TPTX, glomerular fluid phosphate concentration was raised by phosphate infusion in TPTX mutants to levels approaching those of control mice. Phosphate excretion rose markedly and fractional reabsorption fell, but there was no change in absolute phosphate reabsorption in either the proximal tubule or beyond, indicating a persistent reabsorptive defect in the absence of PTH.

We conclude that hereditary hypophosphatemia in the mouse is associated with a renal tubular defect in phosphate reabsorption, which is independent of PTH and therefore represents a specific intrinsic abnormality of phosphate transport.

INTRODUCTION

Familial hypophosphatemia or vitamin D-resistant rickets (VDRR)[1] is characterized by hypophosphatemia and inappropriately increased urinary excretion of phosphate. Whereas previous studies have demonstrated a defect in intestinal phosphate absorption (1, 2), excessive urinary phosphate excretion is generally considered to be the basis for the hypophosphatemia (3). Two hypotheses have been suggested to explain the renal phosphate wasting. First, Albright et al. (4) proposed that decreased gut absorption of calcium initiates a form of secondary hyperparathyroidism. Evidence in favor of this concept includes principally the demonstration of decreased gut calcium absorption (5) and the normalization of phosphate excretion in patients with VDRR when parathyroid hormone (PTH) is suppressed by calcium infusion (6–8). Unfortunately, recent measurements of circulating PTH levels in untreated patients with this disorder have been conflicting, and mildly elevated (9–11), normal (12–15), and low (16) values have been reported. Alternatively, VDRR may result from an intrinsic phosphate transport defect in the kidney (17, 18). This hypothesis does not easily explain the fall in phosphate excretion after PTH suppression. More recently, these two hypotheses have

This work was presented in part at the National Meeting of the American Society for Clinical Investigation, Washington, D. C., May 1977 and was submitted by Dr. Cowgill as partial fulfillment of the requirements for the Ph.D. degree, University of Pennsylvania.

Dr. Agus is the recipient of a Career Development Award 1-KO4-AM-00258 from the National Institutes of Health and Dr. Goldfarb was a Research and Education Associate of the Veterans Administration. Dr. Cowgill's present address is the Department of Medicine, School of Veterinary Medicine, University of California, Davis, Calif. 95616.

Received for publication 27 February 1978 and in revised form 14 February 1979.

[1] *Abbreviations used in this paper*: CAMP, cyclic AMP; GF, glomerular fluid; GFR, glomerular filtration rate; PTH, parathyroid hormone; TF, tubular fluid; TPTX, thyroparathyroidectomy(ized); VDRR, vitamin D-resistant rickets.

been synthesized to suggest that VDRR may be caused by a renal tubular hypersensitivity to normal levels of PTH (14).

Eicher et al. (19) have recently described a new mutation in the laboratory mouse (C57 BL/6J) that is characterized by hypophosphatemia, dwarfism, bone disease-resembling rickets, and renal phosphate wasting. The mutant gene maps at the distal end of the X chromosome and the disorder is transmitted by sex-linked dominant inheritance. The availability of an animal model closely resembling the human disease provides a unique opportunity to characterize the nature of the phosphate-wasting defect and the role of PTH in this disorder. The present series of experiments were designed to investigate renal tubular phosphate transport by clearance and micropuncture techniques in both normal and mutant mice in the presence and absence of PTH. The data clearly demonstrate the presence of an intrinsic tubular reabsorptive defect for phosphate in a mouse model of VDRR.

METHODS

Animals

Male C57BL/6J +/Y (normal controls) and C57BL/6J *Hyp*/Y (hemizygous mutants) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine at 50–60 d of age. Affected and nonaffected animals of a single litter were housed together in plastic and stainless steel cages in a single room of a standard animal facility. All animals were allowed free access to tap water and a standard rodent diet (Wayne Lab-Blox, Allied Mills, Inc., Chicago) that contained 1.2% calcium and 0.99% phosphorus for at least 2 wk before study. When studied, animals ranged from 70–160 d of age with a mean of 98±4.8 (SE) d.

Surgical preparation

Fasted animals, allowed ad libitum water intake, were anaesthetized with Inactin (Byk Golden-Lomberg Chemische Fabrik Gmblt, Konstanz, West Germany), 120 μg/mg body wt, after ether induction. Additional doses of Inactin were given subcutaneously as needed throughout the experiment. Animals were then secured to a heated table that maintained rectal temperature between 38° and 39°C. A tracheostomy was performed and the airway maintained with PE 100 tubing. Both external jugular veins and one common carotid artery were cannulated with PE 10 tubing for infusions and blood pressure recording.

After the venous catheterization, a priming infusion of 0.32 μCi [^{3}H]methoxyinulin (New England Nuclear, Boston, Mass.) was given in 80 μl of 0.9% saline, followed by a sustaining infusion that contained 0.4 μCi of [^{3}H]methoxyinulin/μl saline at a rate of 3.4 μl/min. A 2.5-cm segment of PE 10 tubing was secured in the neck of the bladder for urine collection. The left kidney was exposed through a paracostal incision and supported in a stainless steel holder; care was taken not to stretch the renal vasculature. The exposed kidney was continuously bathed with paraffin oil warmed to 37°C. All incisions were closed with nylon sutures or metal clips to prevent fluid losses. Blood pressure was measured continuously with a Statham P23-Db pressure transducer (Statham Instruments, Inc., Oxnard, Calif.) and recorded on a Sanborn chart recorder (Sanborn Mfg. Co., Springfield, Minn.). Animals were considered suitable for micropuncture if the initial blood pressure was >100 mm Hg, and experiments were terminated and discarded if the mean blood pressure fell to <85 mm Hg during the course of the experiment.

Clearance and micropuncture studies

At least 40 min were allowed between completion of surgical manipulations and the initiation of clearance and micropuncture procedures. Blood for inulin determinations was collected from the tail in heparinized microhematocrit tubes at the beginning and end of urine collections. Timed (30–40 min) urine collections were made under oil in calibrated micropipettes (microcaps, Drummond Scientific Co., Clifton, N. J.). At the beginning and end of each urine collection, one to three surface glomeruli were punctured with sharpened, oil-filled micropipettes, 6–8 μm in diameter and glomerular fluid obtained for calcium, phosphate, sodium, potassium, and inulin determinations. Glomerular collections were initiated and maintained entirely by spontaneous flow without suction. After the glomerular collections, one to four late proximal tubular punctures were performed during each clearance period with sharpened micropipettes filled with water-equilibrated mineral oil, colored with Sudan black. Late proximal tubules were identified as the last visible surface convolution of a tubule randomly punctured with a 2–3 μm micropipette and infused with a 0.2% solution of lissamine green in 0.9% saline. Tubules with visible leaks or lack of lissamine green flow in distal nephron segments were discarded.

Experimental groups

Intact mice. 12 normal mice and 10 mutant littermates were studied with the techniques described above.

Thyroparathyroidectomized (TPTX) mice. In 10 normal mice and 7 mutants, the parathyroid glands were surgically excised. To insure complete parathyroidectomy, the thyroid gland was also removed and the surrounding tissues cauterized with electrocautery. TPTX was performed at the beginning of the experiment and clearance and micropuncture studies as described above were begun ≅60–90 min later.

Phosphate infusion. To evaluate the role of the filtered load of phosphate, an additional five mutant mice were subjected to TPTX and maintained with a sustaining [^{3}H]inulin infusion that contained 0.29% calcium chloride infused at 1.53 μl/min. In addition, a neutral sodium phosphate solution (Na_2HPO_4:NaH_2PO_4, pH 7.4, 300 mosmol/kg) was infused in a different vein at a rate of 1.53 μl/min continuously throughout the experiment. Clearance and micropuncture studies were performed as described above.

To provide a control for the effects of volume expansion and hypocalcemia, a separate group of nine normal mice were subjected to acute TPTX, phosphate infused, and expanded with isotonic saline to 5% of body wt. Clearance studies only were performed in this group.

Analytical procedures

After collection, glomerular and tubular fluid samples were transferred under oil to quartz tubing and aliquots removed for measurement of [^{3}H]inulin activity by liquid scintillation spectrometry (20). Calcium, phosphate, sodium, and potassium concentrations were determined by electron microprobe analysis as described (21). Urine calcium was measured by an automated fluorometric method involving titration of a cal-

cium-calcein complex with EGTA, phosphate with an Auto-Analyzer (Technicon Instruments Corp., Tarrytown, N. Y.), and sodium and potassium by flame photometry as described (21).

Calculations

The clearance of phosphate was calculated in the usual manner with the glomerular filtrate concentration as the ultrafilterable concentration of plasma. The clearance of inulin was determined with plasma inulin concentration and the percentage of fractional excretion of phosphate was calculated as $CPO_4 \div C_{In} \times 100$, where CPO_4 is clearance of phosphate and C_{In} is clearance of inulin. Proximal tubule phosphate reabsorption was calculated for individual mice from the mean glomerular filtrate phosphate concentration and the fraction of filtered phosphate reabsorbed by the late proximal puncture site $(1 - [(TF/GF)PO_4/(TF/P)In]$, where TF is tubular fluid, GF is glomerular fluid, P is plasma, and In is inulin) and corrected to 100 μl/min glomerular filtration rate (GFR, whole kidney) (22). The "distal nephron" is defined as consisting of those tubular segments distal to the point of proximal puncture and therefore includes the last segments of the proximal pars convoluta, pars recta, loop of Henle, distal pars convoluta, and collecting system as well as subsurface nephrons whose reabsorptive capacity is unknown. The fraction of filtered phosphate reabsorbed in this distal nephron segment was taken as the difference between whole kidney fractional reabsorption and fractional reabsorption in the superficial proximal tubule during the same experimental periods. Absolute reabsorption in this segment was determined as above with glomerular fluid phosphate concentration and distal nephron fractional reabsorption corrected to 100 μl/min × GFR.

For statistical analysis, data from individual tubules were averaged to provide a mean value. The means for each group of mice were compared by using the *t* test for independent variables (23). Results are expressed as the mean±SEM.

In vitro procedures and PTH measurement

In five normal and five mutant littermates, blood was collected from the carotid artery, rapidly centrifuged, and the plasma quickly frozen. Plasma levels of mouse PTH were determined by radioimmunoassay as described (24) with an antiserum (CHAl) obtained from a cockerel immunized with bovine PTH (25). Parathyroid hormone was detectable in 90% of normal mice with a lower limit of detectability for the antiserum of 20 pg/ml of bovine PTH.

In separate experiments, cortical tissue of four normal mice and four mutant littermates was studied in vitro to determine adenylate cyclase activity and cyclic AMP production in response to synthetic 1-34,tetratricontapeptide PTH (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) as described (26).

In an additional series of studies cyclic AMP (CAMP) production in response to two doses of highly purified bovine PTH, 1-84 was determined by equilibrium radioimmunoassay (27) in serial cortical slices from six control and six mutant mice. The slices were preincubated for 10 min at 37°C in a buffered balanced salt solution that contained bovine serum albumin (1 mg/ml). After addition of PTH, incubations were continued for 20 min and then terminated by chilling in ice water at 4°C. The medium was aspirated and adjusted to a concentration of 5% trichloroacetic acid, and then centrifuged kidney slices were homogenized in 5% trichloroacetic acid, centrifuged and the supernates of tissue and medium were combined with ether and lyophilized before radioimmunoassay. Recovery was monitored by the addition of 1 pmol of [^{3}H]-CAMP. The tissue pellet was dissolved in 1 N NaOH for protein determinations and results expressed as total CAMP in tissue and medium in pmol/mg protein per 20-min incubation. The antibody was kindly supplied by Dr. Charles Parker (Washington University, St. Louis, Mo.).

TABLE I
*GF Electrolyte Concentrations in Intact Mice**

	Phosphate	Calcium	Sodium	Potassium
	mg/dl	*meq/liter*	*meq/liter*	*meq/liter*
Controls, n = 10				
Mean	6.0	2.7	143	4.8
SEM	0.2	0.05	1.5	0.1
Mutants, n= 12				
Mean	3.9	2.6	138	4.9
SEM	0.2	0.06	2.2	0.2
P value	<0.001	NS	NS	NS

* n, number of animals. P value refers to significance of the difference of the mean NS, P value >0.05.

RESULTS

Intact mice. The electrolyte concentrations in the glomerular fluid[2] (GF) are shown in Table I. GF phosphate concentration was significantly lower in the mutant mice than in normal controls (3.9±0.2 vs. 6.0±0.2 mg/dl, $P < 0.001$). There were no differences between the two groups in the concentration of the other electrolytes.

The clearance and micropuncture data are summarized in Table II. Inulin clearance was significantly lower in mutants than control mice and this difference was not related to differences in age, body weight, or surface area. Fractional excretion of phosphate in the final urine was significantly elevated in the mutant mice (47.2±4.1%) compared to controls (30.8±2.2%), $P < 0.001$, despite comparable fractional excretions of sodium (0.20±0.02 vs. 0.31±0.05%) and potassium (17.9±1.7 vs. 17.6±0.9%), which are not shown in the table. In the proximal tubule, there was no difference in tubular fluid (TF):plasma inulin, whereas the ratio of tubular fluid/glomerular fluid phosphate concentration (TF:GF PO_4) was significantly higher in the mutant mice (1.12±0.05 vs. 0.9±0.03, $P < 0.001$), resulting in a decreased fractional reabsorption to the point of puncture of 38.8±3.0% compared to 50.2±2.7% in controls.

Fig. 1 depicts the calculated rates of phosphate transport in the proximal tubule and in the distal nephron, defined above as the segments between the point of proximal puncture and the final urine. Phosphate reab-

[2] The mean glomerular fluid:plasma inulin concentration for all glomerular punctures was 1.05±0.01 (n = 128) and there were no differences in the ratio in any of the five groups of mice studied. Assuming a plasma protein concentration of 5.7 g/dl, as is characteristic of the rat (28), the ratio of glomerular fluid:plasma water inulin would be 0.99, a value similar to unity and typical of capsular collections (29, 30).

TABLE II
*Summary of Clearance and Micropuncture Data in Intact Mice**

	Whole kidney			Proximal tubule		
	C_{In}	C_{PO_4}	C_{PO_4}/C_{In}	$TF:P_{In}$	$TF:GF\ PO_4$	Fractional reabsorption
	μl/min	*μl/min*	%			%
Normal controls, n = 10						
Mean	181	54.7	30.8	1.85	0.90	50.2
SEM	11	5	2.2	0.06	0.03	2.7
Mutants, n = 12						
Mean	119	56.6	47.2	1.86	1.12	38.8
SEM	6	6.3	4.1	0.06	0.05	3.0
P value	<0.05	NS	<0.001	NS	<0.001	<0.01

* C_{In} and C_{PO_4}, clearance of inulin and phosphate, respectively; C_{PO_4}/C_{In}, fractional excretion of phosphate; $TF:P_{In}$ and $TF:GF\ PO_4$ refer to ratio of tubular fluid to plasma inulin and tubular fluid to glomerular fluid phosphate concentration. Fractional reabsorption (to the point of puncture in the proximal tubule) was calculated as $1 - (TF/GF)PO_4/(TF/P)In$. P value refers to the significance of the difference of the mean of the two groups. n, number of animals.

sorption in the mutants was reduced compared to controls in the proximal tubule (48.2±5.5 vs. 92.2±8.6 nmol/min per 100 μl GFR, $P < 0.001$) and in the distal nephron (19.8±6.5 vs. 40.9±6.7 nmol/min per 100 μl GFR, $P < 0.05$).

TPTX mice. With TPTX, GF PO_4 rose significantly in both mutants and controls ($P < 0.01$ and $P < 0.001$ compared to intact mice, respectively) but remained markedly lower in the mutants compared to controls (Table III). The TPTX mutants also had slightly but significantly lower GF calcium concentrations than TPTX controls (2.45±0.06 vs. 2.74±0.11 meq/liter, $P < 0.025$).

Fractional excretion of phosphate was markedly reduced compared to intact animals in both mutants and control mice to similar levels (7.4±2.2 and 7.6±0.9%). In the proximal tubule, TF:plasma inulin was similar, and TF/GF PO_4 fell in both groups with TPTX but was reduced to significantly lower levels in the mutants (0.24±0.05 vs. 0.44±0.05, $P < 0.005$). As a result, fractional phosphate reabsorption to the point of puncture in the proximal tubule, although enhanced in both groups compared to intact animals, was then significantly greater in the mutants (86.6±2.8 vs. 76.4±3.0%, $P < 0.025$).

Absolute phosphate reabsorption, shown in Fig. 2, was increased in the proximal tubule in both mutants and controls with TPTX and was unchanged in the distal nephron. In both segments, absolute reabsorption remained lower in mutants than in controls despite opposite changes in fractional reabsorption. This is

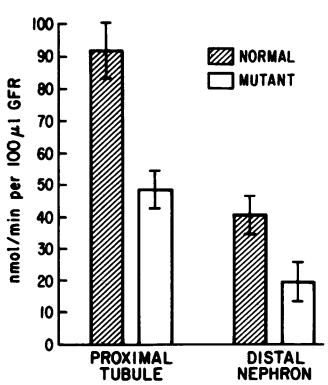

FIGURE 1 Absolute phosphate reabsorption in the proximal tubule and distal nephron of normal (shaded bar) and mutant (open bar) mice. Each bar represents the mean±SEM of 12 normal and 10 mutant mice.

TABLE III
*GF Electrolyte Concentration in TPTX Mice**

	Phosphate	Calcium	Sodium	Potassium
	mg/dl	*meq/liter*	*meq/liter*	*meq/liter*
Controls, n = 7				
Mean	8.1	2.74	138	4.7
SEM	0.3	0.11	1.4	0.2
Mutants, n = 10				
Mean	4.7	2.45	145	4.6
SEM	0.3	0.06	4.2	0.1
P value	<0.001	<0.025	NS	NS
Phosphate-infused mutants, n = 5				
Mean	7.4	2.16	142	4.1
SEM	0.5	0.14	3.5	0.2
P value	<0.001	<0.005	NS	NS

* P value refers to the significance of the difference of the means between controls and mutants and between mutants and phosphate infused mutants. NS, P value >0.05.

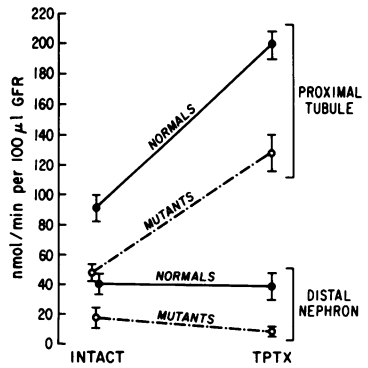

FIGURE 2 The effect of acute TPTX on absolute phosphate reabsorption in the proximal tubule and distal nephron of normal and mutant mice. The intact data are plotted from Fig. 1 and the TPTX data are presented as the mean±SEM of 10 normal and 7 mutant TPTX mice.

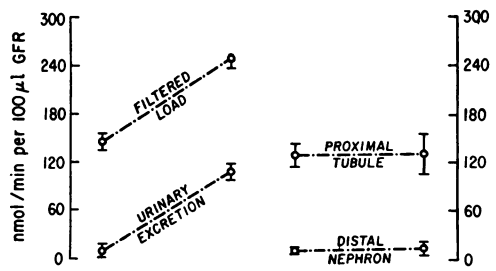

FIGURE 3 The effect of phosphate infusion of filtered phosphate load, urinary phosphate excretion and absolute reabsorption in the proximal tubule and distal nephron. Open circles represent mean±SEM for 7 noninfused (left side of each panel) and 5 infused (right side of each panel) TPTX mutant mice. Note that phosphate infusion increased filtered load in mutant TPTX mice to levels comparable to those obtained in TPTX (see text) controls. Urinary excretion rose in parallel with the change in filtered load and there was no change in absolute reabsorption in either proximal tubule or distal nephron.

presumably a result of the lower filtered load of phosphate in the mutants.

To evaluate the role of filtered load, TPTX mutant mice were infused with phosphate. GF phosphate rose to 7.4±0.5 mg/dl, a concentration comparable to that observed in TPTX control mice (Table III). Fractional phosphate excretion rose markedly with phosphate infusion to 43.8±3.6%. This value is significantly greater than in TPTX control mice and comparable to values observed in intact mutants (Table II).

In the control TPTX, phosphate-loaded, saline-loaded mice, plasma calcium was 5.4±0.6 mg/dl, plasma phosphate 12.1±1.6 mg/dl, fractional phosphate excretion was 19.5% (range 7.8–32.3). Thus, phosphaturia was lower than that observed in the mutants despite volume expansion, hypocalcemia, and hyperphosphatemia. In the proximal tubule, TF:GF PO_4 rose and fractional phosphate reabsorption fell, both values being similar to intact mutant mice.

Fig. 3 depicts the filtered load, urinary phosphate excretion, and absolute phosphate reabsorption in the proximal tubule and distal nephron in TPTX mutants with and without phosphate infusion. It is apparent that the increased filtered load produced by the phosphate infusion was excreted in the final urine. There was no difference in absolute phosphate reabsorption either in the proximal tubule or in more distal segments between infused and noninfused TPTX mutants. Thus despite normalization of the serum phosphate and the absence of PTH, phosphate reabsorption remained significantly lower than in TPTX controls (Fig. 3, solid circles) in the proximal tubule (129.1±24 vs. 198±9.2 nmol/min per 100 μl GFR, $P < 0.001$) and distal nephron (12.0±8.1 vs. 39.9±10.1 nmol/min per 100 μl GFR, $P < 0.025$).

In vitro studies and PTH measurements. Serum PTH concentrations were comparable in mutants (43.6 ±4.8 pg equivalents of bovine PTH/ml) and controls (46.0±4.8 pg equivalents of bovine PTH/ml). Renal cortical CAMP production was similar in the two groups before (42±5 and 32±4 pmol/mg protein per 20-min incubation in controls and mutants, respectively) and after PTH stimulation (83±8 and 87±8 pmol/mg protein per 20 min, respectively). The base-line adenylate cyclase activity was also not different in the two groups (181±8.0 vs. 197±23 pmol CAMP/mg protein per 10-min incubation). After PTH stimulation, adenylate cyclase activity increased in both groups and the value in the mutants was slightly but significantly reduced compared to littermate controls (1,068±19 vs. 1,429±144, $P < 0.05$).

The data from the radioimmunoassay for CAMP were similar. In control mice, CAMP production was 6.8 ±0.35, 15.5±2.1, and 20.7±1.35 pmol/mg protein per 20-min incubation during base-line and after bovine PTH, 1–84 at 10^{-8} and 10^{-6}, respectively. In the mutants the corresponding values were 7.2±0.64, 16.2 ±2.3, and 19.3±1.2. There were no differences between control and mutant mice in either base-line or after PTH.

DISCUSSION

Our studies clearly indicate a decrease in both fractional and absolute tubular phosphate reabsorption in intact hypophosphatemic, mutant mice compared to intact normal controls. The data in the intact mice are qualitatively similar to those obtained by Giasson et al. (31) who also demonstrated decreased tubular phosphate reabsorption in the proximal tubule and final urine of mutant mice. TPTX in our studies resulted in a marked decrease in fractional phosphate excretion to identical levels in mutants and controls. In the proximal

tubule, fractional reabsorption was enhanced to a greater extent than in TPTX control mice, suggesting that the presence of PTH was necessary for expression of the reabsorptive defect of the intact mutants. Phosphate infusion and elevation of GF PO_4 in TPTX mutants to levels found in controls however demonstrated persistence of the proximal tubular and whole kidney phosphate reabsorption defect. Thus the defect in tubular phosphate transport was clearly present in the absence of PTH. The apparently normal tubular response to TPTX, despite continued presence of an intrinsic abnormality in phosphate transport, suggests that the physiological action of PTH is qualitatively normal although the base-line level of phosphate reabsorption is abnormally low. In intact mutant mice the presence of normal concentrations of circulating PTH may reduce phosphate reabsorption in the PTH-dependent system to extremely low levels. The enhanced fractional reabsorption in the TPTX mutant mice may be the result of the low-filtered load of phosphate presented to the normally functioning PTH-dependent system. As the reabsorptive capacity of this system is exceeded with phosphate infusion, the remainder of the tubular phosphate load is presented to the defective system and excreted in the final urine. Thus, although urinary phosphate excretion and tubular phosphate reabsorption seem to be "hypersensitive" to the removal of PTH, the data are consistent with a normal response to PTH removal in a system with a reduced base-line transport capacity. The normal levels of PTH measured in the mutant mice and the in vitro analysis of the PTH-adenylate cyclase system are also compatible with normal tubular responsiveness to PTH.

Brunette et al. (32) demonstrated increased responsiveness of the distal convoluted tubule to calcitonin induced generation of CAMP in the genetic hypophosphatemic mouse. As the defect in our studies persisted in the absence of calcitonin however, the relevance of these observations to the pathophysiology of the tubular defect remains in question.

The tubular sites of the defective transport of phosphate in hypophosphatemic mice seem to include both the proximal convoluted tubule and sites beyond the superficial proximal tubule. The precise localization of the more distal sites is not addressed by our studies. Recent data collected in isolated tubular segments from the rabbit suggest that the pars recta may be an important phosphate reabsorptive site (33) and data in the rat suggest that the distal convoluted tubule may also participate (34). Our data suggest that the convoluted tubule of the mouse is similar to that of the rat and more responsive than that of the rabbit to PTH (33). Differences between phosphate reabsorptive rates in superficial and deeper nephrons could also theroetically explain the apparent "distal" defect in our studies. If proximal tubules of deeper nephrons were characterized by a greater reduction in phosphate transport than that seen in the superficial population, the results could appear identical to those observed in our study. It should be pointed out that in these mice the influence of PTH appears limited to the proximal tubule because phosphate reabsorption beyond the site of puncture in both normal and mutant mice was not influenced by TPTX. This is in contrast to the dog (35, 36) and rat (37, 38). In the normal mice, however, it is possible that the increase in proximal tubular transport after TPTX may have reduced the delivered load to more distal sites to a degree that limited further reabsorption. Phosphate infusion, however, did not alter transport in the TPTX mutant mice in this segment and the increase in delivered load was excreted in the final urine (Fig. 3). Thus, it would seem that whereas the defect in phosphate transport is present in several segments of the nephron in hypophosphatemic mice, alterations in delivery out of the proximal tubule would be the major determinant of final urinary phosphate excretion. One would therefore predict that agents that enhance proximal tubular phosphate reabsorption through changes in PTH activity, to which sensitivity is maintained, such as reduction of PTH levels by surgical or chemical (calcium infusion) means, would markedly reduce phosphate excretion. In contrast, procedures that inhibit proximal tubular phosphate reabsorption, such as PTH infusion, would be associated with an exaggerated phosphaturia. Thus although the defect in transport is independent of PTH, expression of the defect would clearly be influenced by the level of circulating PTH.

Recent studies of phosphate transport in this mouse model of hereditary hypophosphatemic rickets have been performed by Tenenhouse et al. (39). These workers have demonstrated, by the brush border membrane vesicle technique, that phosphate uptake is reduced in the brush border of the proximal tubule of these mutant, hypophosphatemic mice. These data support the concept of an intrinsic defect in tubular phosphate transport in this disorder.

In conclusion, we have presented evidence consistent with an intrinsic renal tubular abnormality in phosphate transport that is independent of PTH in mice with hereditary hypophosphatemic rickets. If the disease in mutant mice is the same as that in humans, the underlying pathogenesis would appear to involve a tubular transport defect that is unrelated to abnormalities of parathyroid gland function or target tissue sensitivity to PTH, although expression of the defect would be influenced by PTH levels. The relationship of this defect to other phosphate-transport systems, which also may be independent of PTH, such as the glucose-phosphate and sodium-phosphate pathways (40–42), remains to be investigated. In this regard, it is interesting to note that Barbour et al. (18) and Baran et al. (43) have noted

decreased phosphate reabsorption with glucose infusion in subjects with VDRR, suggesting an intact glucose-phosphate reabsorptive system. Taken together, the present studies and the reported coexistence of a specific absorptive defect for phosphate in the intestine (1, 2, 44) makes attractive the hypothesis that a single gene locus codes for a transport protein in both intestine and the kidney. Further studies in the mutant mice may allow more direct investigation of this intriguing possibility and possibly provide additional insights into the management of this disease in man.

ACKNOWLEDGMENTS

We would like to thank Dorothy Senesky, Carmen D'Angelo, Marva Lee, Phyllis May, and Sandra Markus for technical assistance in performing the studies and for the laboratory analyses.

This work was supported by research grants HL-00340, AM-09976 and AM-19478, and by training grants GM 54049 and AM 07006 from the National Institutes of Health.

REFERENCES

1. Condon, J. R., J. R. Nassim, and A. Rutter. 1971. Pathogenesis of rickets and osteomalacia in familial hypophosphatemia. *Arch. Dis. Child.* **46:** 269–272.
2. Short, E. M., H. J. Binder, and L. E. Rosenberg. 1973. Familial hypophosphatemic rickets: defective transport of inorganic phosphate by intestinal mucosa. *Science (Wash. D. C.).* **179:** 700–702.
3. Williams, T. F., and R. W. Winters. 1972. Familial (hereditary) vitamin D-resistant rickets with hypophosphatemia. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Company, New York. 3rd edition. 1465–1485.
4. Albright, F., A. M. Butler, and E. Bloomberg. 1937. Rickets resistant to vitamin D therapy. *Am. J. Dis. Child.* **54:** 529–547.
5. Soergel, K. H., K. H. Mueller, R. F. Gustke, and J. E. Geenen. 1974. Jejunal calcium transport in health and metabolic bone disease: effect of vitamin D. *Gastroenterology.* **67:** 28–34.
6. Field, M. H., and E. Reiss. 1960. Vitamin D-resistant rickets: the effects of calcium infusion on phosphate reabsorption. *J. Clin. Invest.* **39:** 1807–1812.
7. Lafferty, F. W., C. H. Herndon, and O. H. Pearson. 1963. Pathogenesis of vitamin D-resistant rickets and the response to a high calcium intake. *Clin. Endocrinol.* **23:** 903–917.
8. Falls, W. F., Jr., N. W. Carter, F. C. Rector, and D. W. Seldin. 1968. Familial vitamin D-resistant rickets. Study of six cases with evaluation of the pathogenetic role of secondary hyperparathyroidism. *Ann. Intern. Med.* **68:** 553–560.
9. Levy, J. E., E. C. Cabana, H. A. Repetto, J. M. Canterbury, and E. Reiss. 1972. Serum parathyroid hormone in hypophosphatemic vitamin D-resistant rickets. *J. Pediatr.* **81:** 294–300.
10. Reitz, R. E., and R. L. Weinstein. 1973. Parathyroid hormone secretion in familial vitamin D-resistant rickets. *N. Engl. J. Med.* **289:** 941–945.
11. Hahn, T. J., C. R. Scharp, L. R. Halstead, J. Haddad, D. M. Karl, and L. V. Avioli. 1975. Parathyroid hormone status and renal responsiveness in familial hypophosphatemic rickets. *J. Clin. Endocrinol. Metab.* **41:** 926–937.
12. Arnaud, D., F. Glorieux, and C. Scriver. 1971. Serum parathyroid hormone in x-linked hypophosphatemia. *Science (Wash. D. C.).* **173:** 845–847.
13. Fanconi, A., J. A. Fischer, and A. Prader. 1974. Serum parathyroid hormone concentrations in hypophosphatemic vitamin D-resistant rickets. *Helv. Paediatr. Acta.* **29:** 187–194.
14. Short, E., R. C. Morris, Jr., A. Sebastian, and M. Spencer. 1976. Exaggerated phosphaturic response to circulating parathyroid hormone in patients with familial x-linked hypophosphatemic rickets. *J. Clin. Invest.* **58:** 152–163.
15. Glorieux, F. H., C. L. Morin, R. Travers, E. E. Delvin, and R. Poirier. 1976. Intestinal phosphate transport in familial hypophosphatemic rickets. *Pediatr. Res.* **10:** 691–969.
16. Roof, B. S., C. F. Piel, and G. S. Gordon. 1972. Nature of the defect responsible for the familial vitamin D-resistant rickets (VDRR) based on radioimmunoassay for parathyroid hormone (PTH). *Trans. Ass. Amer. Physicians.* **85:** 172–180.
17. Glorieux, F., and C. R. Scriver. 1972. Loss of a parathyroid hormone-sensitive component of phosphate transport in x-linked hypophosphatemia. *Science (Wash. D. C.).* **175:** 997–999.
18. Dent, C. E. 1952. Rickets and osteomalacia from renal tubular defects. *J. Bone Jt. Surg. Br. Vol.* **34B:** 266–274.
19. Eicher, E. M., J. L. Southard, C. R. Scriver, and F. H. Glorieux. 1976. Hypophosphatemia: mouse model for human familial hypophosphatemic (vitamin D-resistant) rickets. *Proc. Natl. Acad. Sci. U. S. A.* **73:** 4667–4671.
20. Staum, B. B., R. J. Hamburger, and M. Goldberg. 1972. Tracer microinjection study of renal tubular phosphate reabsorption in the rat. *J. Clin. Invest.* **51:** 2271–2276.
21. Agus, Z. S., L. B. Gardner, L. H. Beck, and M. Goldberg. 1973. Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium, and phosphate. *Am. J. Physiol.* **224:** 1143–1148.
22. LeGrimellec, C., N. Roinel, and R. Morel. 1974. Simultaneous Mg, Ca, P, K, and Cl analysis in the rat tubular fluid IV. During acute phosphate plasma loading. *Pfluegers. Arch. Eur. J. Physiol.* **346:** 189–204.
23. Brownlee, K. A. 1965. Statistical theory and methodology in science and engineering. John Wiley & Sons, Inc. New York.
24. Chanard, J., R. Black, M. Purkerson, J. Lewis, S. Klahr, and E. Slatopolsky. 1977. The effects of colchicine and vinblastine on parathyroid hormone secretion in the rat. *Endocrinology.* **101:** 1792–1800.
25. Hruska, K. A., R. Kopelman, W. E. Rutherford, S. Klahr, and E. Slatopolsky. 1975. Metabolism of immunoreactive parathyroid hormone in the dog. *J. Clin. Invest.* **56:** 39–48.
26. Rodriguez, H. J., J. Walls, J. Yates, and S. Klahr. 1974. Effects of acetazolamide on the urinary excretion of cyclic AMP and on the activity of renal adenyl cyclase. *J. Clin. Invest.* **53:** 122–130.
27. Steiner, A. L., D. M. Kipnis, R. Utiger, and C. Parker. 1969. Radioimmunoassay for the measurement of adenosine-3′,5′-cyclic phosphate. *Proc. Natl. Acad. Sci. U. S. A.* **64:** 367.
28. Kotani, M., A. Yamashita, M. Miyamoto, K. Seiki, K. Tasaki, T. Thimizu, S. Terauchi, and I. Horii. 1968. Composition of serum and lymph of rats with aminonucleoside-induced nephrosis. *Jpn. Circ. J.* **32:** 995–1001.
29. Harris, C. A., P. G. Baer, E. Chirito, and J. H. Dirks. 1974.

Composition of mammaliam glomerular filtrate. *Am. J. Physiol.* **227:** 972–976.
30. Le Grimellec, C., P. Poujeol, and C. de Rouffignac. 1975. ^{3}H-inulin and electrolyte concentrations in Bowman's capsule in rat kidney. Comparison with artificial ultrafiltration. *Pfluegers. Arch. Eur. J. Physiol.* **354:** 117–131.
31. Giasson, S. D., M. G. Brunette, G. Danan, N. Vigneault, and S. Carriere. 1977. Micropuncture study of renal phosphorus transport in hypophosphatemic vitamin D resistant rickets mice. *Pfluegers. Arch. Eur. J. Physiol.* **371:** 33–38.
32. Brunette, M., D. Chabardes, M. Imbert, M. Montegut, A. Clique, and F. Morel. 1977. Hormone sensitive adenylate cyclase activity along the nephron of genetic hypophosphatemic mice. *In* Abstracts of the Annual Meeting of the American Society of Nephrology, Washington, D. C. 2.
33. Dennis, V. W., E. Bello-Reuss, and R. R. Robinson. 1977. Response of phosphate transport to parathyroid hormone in segments of rabbit nephron. *Am. J. Physiol.* **233**(Suppl. 1): F29–F38.
34. Colindres, R. E., E. Pastoriza-Munoz, W. E. Lassiter, and C. Lechene. 1976. Effect of extracellular volume expansion of phosphate reabsorption along the nephron in thyroparathyroidectomized rats. *In* Proceedings of the 9th Annual Meeting of the American Society of Nephrology, Washington, D. C. 2.
35. Beck, L. H., and M. Goldberg. 1973. Effects of acetazolamide and parathyroidectomy on renal transport of sodium, calcium and phosphate. *Am. J. Physiol.* **224:** 1136–1142.
36. Knox, G. G., and C. Lechene. 1975. Distal site of action of parathyroid hormone on phosphate reabsorption. *Am. J. Physiol.* **229:** 1556–1560.
37. Amiel, C., H. Kuntziger, and G. Richet. 1970. Micropuncture study of handling of phosphate by proximal and distal nephron in normal and parathyroidectomized rats. Evidence for distal reabsorption. *Pfluegers. Arch. Eur. J. Physiol.* **317:** 93–109.
38. Pastoriza-Munoz, E., C. Lechene, W. E. Lassiter, and R. E. Colindres. 1975. Effects of purified parathyroid hormone on phosphate reabsorption along the nephron in acutely thyroparathyroidectomized rats. *Physiologist.* **19:** 325.
39. Tenenhouse, H. S., C. R. Scriver, R. R. McInnes, and F. H. Glorieux. 1978. 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:** 236–244.
40. DeFronzo, R. A., M. Goldberg, and Z. S. Agus. 1976. The effects of glucose and insulin on renal electrolyte transport. *J. Clin. Invest.* **58:** 83–90.
41. DeFronzo, R. A., M. Goldberg, and Z. S. Agus. 1975. Effects of phlorizin and insulin on tubular phosphate reabsorption: evidence for two transport pathways. *In* Proceedings of the 8th Annual Meeting of the American Society of Nephrology. 76.
42. Dennis, V. W., and P. C. Brazy. 1977. Influence of glucose transport on phosphate absorption by isolated proximal tubules. *Clin. Res.* **25:** 429A. (Abstr.)
43. Baron, D. T., T. J. Hahn, and L. V. Avioli. 1977. Glucose induced decrease in maximal tubular reabsorption of phosphate in vitamin D resistant rickets. *Clin. Res.* **25:** 560A. (Abstr.)
44. O'Doherty, J. A., H. F. DeLuca, and E. M. Eicher. 1976. Intestinal calcium and phosphate transport in genetic hypophosphatemic mice. *Biochem. Biophys. Res. Commun.* **71:** 617–621.