A Microperfusion Study of Phosphate Reabsorption by the Rat Proximal Renal Tubule

EFFECT OF PARATHYROID HORMONE

NORMAN BANK, HAGOP S. AYNEDJIAN, and STEPHEN W. WEINSTEIN

From the Renal Division, Department of Medicine, Montefiore Hospital and Medical Center, and the Albert Einstein College of Medicine, Bronx, New York 10467

ABSTRACT To study the mechanism of phosphate reabsorption by the proximal tubule and the effect of parathyroid hormone (PTH), microperfusion experiments were carried out in rats. Segments of proximal tubule isolated by oil blocks were perfused in vivo with one of three solutions, each containing 152 meq/liter Na^+ and 2 mmol/liter phosphate, but otherwise differing in composition. The pH of solution 1 was 6.05–6.63, indicating that 60–85% of the phosphate was in the form of $H_2PO_4^-$. The pH of solution 2 was 7.56–7.85, and 85–92% of the phosphate was in the form of $HPO_4^=$. Solution 3 contained HCO_3^- and glucose and had a pH of 7.50–7.65. When the proximal tubules were perfused with solution 1, the ^{32}P concentration in the collected perfusate was found to be consistently lower than in the initial perfusion solution. In sharp contrast, when the tubules were perfused with solutions 2 or 3, ^{32}P concentration usually rose above that in the initial solution. Water (and presumably Na^+) reabsorption, as measured with [^{3}H]inulin, was the same with the acid and alkaline solutions. Administration of partially purified PTH clearly prevented the fall in phosphate concentration with the acid solution, but had a less discernible effect on phosphate reabsorption with the two alkaline solutions. Measurements of pH within the perfused segments with antimony microelectrodes demonstrated that PTH enhanced alkalinization of the acid perfusion solution. The findings are consistent with the view that $H_2PO_4^-$ is reabsorbed preferentially over $HPO_4^=$. This can be attributed to either an active transport mechanism for $H_2PO_4^-$ or selective membrane permeability to this anion. PTH appears to either inhibit an active transport process for $H_2PO_4^-$, or to interfere with passive diffusion of phosphate by alkalinizing the tubular lumen.

Received for publication 11 January 1974 and in revised form 17 June 1974.

INTRODUCTION

Recent micropuncture studies have demonstrated several important features of the renal reabsorptive process for inorganic phosphate (1–11). First, the proximal tubule is responsible for most but not all of phosphate reabsorption, the most avid site being the early portion of the proximal convoluted tubule (2, 4). Second, phosphate reabsorption has been found to parallel fluid reabsorption in the proximal tubule under certain experimental conditions (5–11). For example, extracellular fluid volume expansion results in a reduction in proximal sodium, water, and phosphate reabsorption (8, 9, 11). Third, the inhibition of phosphate reabsorption by parathyroid hormone (PTH)[1] may be linked to an inhibitory action of the hormone on proximal fluid reabsorption (5–7), although it is possible to dissociate the two effects (11).

Phosphate is present in plasma and the glomerular filtrate in two different ionic species, $HPO_4^=$ and $H_2PO_4^-$, the ratio between the two forms being dependent upon the pH. The reabsorptive process in the proximal tubule may discriminate between these two forms, and it has been suggested on the basis of clearance studies that $H_2PO_4^-$ is more readily transported than $HPO_4^=$ (12–15). However, no direct evidence for this view has been available. If different mechanisms exist for the reabsorption of $H_2PO_4^-$ and $HPO_4^=$, PTH may act selectively on one of them. To study these problems, experiments were

[1] *Abbreviations used in this paper:* PD, potential difference; PTH, parathyroid hormone; TF/P, tubular fluid-to-plasma.

carried out in which isolated segments of the rat proximal convoluted tubule were microperfused in vivo with one of three solutions, each containing 152 meq/liter Na^+ and 2 mmol/liter phosphate. Solutions 1 and 2 contained no bicarbonate or glucose but differed widely in pH and thus in the ratio $HPO_4^=/H_2PO_4^-$. Solution 3 had an alkaline pH like solution 2, but contained physiological concentrations of bicarbonate and glucose. Each solution contained trace amounts of ^{32}P-labeled inorganic phosphate and [^3H]methoxy inulin.

We found that when the proximal tubule was perfused with the acid solution, the concentration of ^{32}P in the collected perfusate was consistently below that in the initial solution. In sharp contrast, when the tubule was perfused with alkaline solutions 2 or 3, the ^{32}P concentration in the collected perfusate was usually above the level in the initial solution. PTH administration prevented the fall in ^{32}P concentration with the acid perfusion solution and either had no further effect on phosphate reabsorption with the alkaline solutions or caused ^{32}P to rise slightly higher than was observed in the absence of exogenous PTH. PTH was found to enhance alkalinization of the acid perfusion solution, measured in vivo with antimony microelectrodes. The findings indicate that $H_2PO_4^-$ is reabsorbed selectively over $HPO_4^=$. The data are consistent with an active transport mechanism for $H_2PO_4^-$ and a passive diffusion process for $HPO_4^=$. The effect of PTH could be to inhibit the active transport of $H_2PO_4^-$, or to interfere with phosphate reabsorption by alkalinizing the tubular lumen.

METHODS

Microperfusion experiments were carried out in male Sprague-Dawley rats weighing 200–300 g. The animals were maintained on a regular rat pellet diet and tap water. Food but not water was withheld 16 h before surgery. On the morning of the experiment, each rat was anesthetized with i.p. Inactin (Promonta, Hamburg, West Germany) 100 mg/kg body wt, and prepared for micropuncture as previously described (16). This preparation consisted of a tracheostomy and insertion of two PE 50 catheters in a jugular vein, one for infusion of Ringer's lactate solution at a constant rate of 0.05 ml/min, and the other for injection of FD&C green dye, as previously described (17). This dye was injected two or three times during each experiment. Transit time of the dye to the end of the proximal tubule was taken as an index of overall renal function. In all experiments, transit time measured 8–12 s, within the generally accepted normal range. A third PE 50 catheter was inserted in a carotid artery for continuous measurement of blood pressure. The left kidney was isolated surgically and immobilized. Body temperature of the rat was monitored throughout the experiment via a rectal thermistor and telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio), and maintained between 37° and 38°C by a heated animal table. Mineral oil warmed to 38°C flowed freely over the experimental kidney continuously throughout the experiment. A PE 50 catheter was inserted into the urinary bladder for collection of urine from both kidneys.

Microperfusion of individual surface proximal convoluted tubules was carried out by techniques previously described in detail (18–20). The delivery system for the perfusion consisted of a 10-μl Hamilton syringe (Hamilton Co., Whittier, Calif.) driven by a Sage pump (Model 255-2, Sage Instruments Div., Orion Research, Inc., Cambridge, Mass.). The pump was set to deliver 12 nl/min. The Hamilton syringe, filled with mineral oil, was connected to a sharply beveled micropipet via thick-walled PE 20 tubing, and the micropipet was mounted on a micromanipulator. The tubing and pipet were filled with one of the three perfusion solutions described below. A second micropipet, filled with Sudan black-stained castor oil, was mounted on a separate micromanipulator and used to inject oil blocks in the tubular lumen, and to collect the perfusion fluid, as described below.

The compositions of the perfusion fluids were as follows: solution 1: Na^+ 150 meq/liter; Cl^- 150 meq/liter; sodium phosphate 2 mmol/liter; pH = 6.05 – 6.63. Solution 2: same as solution 1 except pH = 7.56 – 7.85. Solution 3: Na^+ 150 meq/liter; Cl^- 120 meq/liter; HCO_3^- 30 meq/liter; sodium phosphate 2 mmol/liter; glucose 100 mg/100 ml; the solution was equilibrated with 5% CO_2; pH = 7.50 – 7.65.

In experiments in which phosphate and water absorption were measured, trace amounts of [^{32}P]Na_2HPO_4 or [^{32}P]-NaH_2PO_4 and [^3H]methoxy inulin were added to the perfusion fluid (New England Nuclear, Boston, Mass.). The amounts added were estimated to yield radioactivity at least three to four times above background. No dye was added to the perfusion fluid.

In vivo pH experiments. In nine experiments, intraluminal pH was measured in the perfused proximal segments with antimony microelectrodes. The antimony microelectrodes were prepared according to the method of Vieira and Malnic (21, 22) on the day before the experiment. An antimony microelectrode was mounted on a micromanipulator and connected to a model 2E 25 Radiometer pH meter (London Co., Cleveland, Ohio). The reference calomel electrode was inserted into the abdominal cavity of the animal, its tip covered with Ringer's-soaked cotton. An electrical bridge was constructed between the calomel electrode and the kidney. This consisted of a cotton wick that extended from the calomel electrode to a cotton pad in the bottom of the kidney dish upon which the kidney rested. All cotton was soaked in Ringer's solution. Each antimony microelectrode was standardized in vitro at the beginning and end of the experiment with three different phosphate buffers of known pH (determined by glass electrode). The standardization procedure was carried out by placing the buffer solutions in small plastic wells on the surface of the kidney, and introducing the tip of the antimony electrode into each solution. This was done to eliminate any electrical potential differences (PD) between the two electrodes, due to junction potentials or intervening membranes, except for the PD that may have been present across the proximal tubular epithelium (23). In addition to the three standard buffers, the pH of the perfusion solution was measured on the surface of the kidney. In preliminary experiments it was found that large concentration differences of phosphate affect the response of the antimony electrode. It was essential, therefore, to standardize each electrode with buffers containing the same concentration of phosphate as that used in the perfusion solutions. Accordingly, 2-mM phosphate in 150 meq/liter NaCl was used as the standardizing solution. No significant effect on the electrodes was found

upon reducing the phosphate concentration from 2 to 1 mM, a reduction that could occur from phosphate reabsorption by the proximal tubule. When the standardization procedure had been completed, a voltage vs. pH plot was made, the slope determined, and the pH of the perfusion solution determined from the plot. An antimony microelectrode was accepted for use in an experiment only if the pH value of the perfusion solution agreed within ±0.15 U with glass electrode determinations.

Intraluminal pH of the perfusion solutions were determined as follows: A segment of a proximal tubule was isolated between two long oil blocks and perfused with either solution 1 or 2 at a pump setting calibrated to deliver 12 nl/min. The details of the perfusion technique correspond to method 3 described in a previous publication (20). This method allows identification of the tubular segments being perfused without the presence of a dye in the perfusion fluid. After the perfusion had been maintained for 2–3 min, an antimony electrode was inserted into the lumen in a perfused convolution as distant from the perfusion pipet as possible. The microelectrode was positioned in the center of the tubular lumen. Millivolt readings were accepted only if they remained stable throughout the entire time of observation, which ranged from 30 s to 3 min. The antimony electrode was then withdrawn and reinserted in the lumen as close to the perfusion pipet as possible, and a second voltage reading made. Two or three tubules were perfused in each rat. In six normal control rats, perfusion solutions 1 and 2 were used in each animal. In three additional rats, a purified preparation of parathyroid hormone (546 U/mg) (Wilson Laboratories, Chicago, Ill.) was administered i.v. at a rate of 16 U/h for 2 h. Surface proximal tubules were then microperfused with solution 1, to which 0.1 U/ml of PTH had been added, and pH was measured as in the six control rats. At the end of the pH measurements, each tubule used was injected with yellow latex (General Biological Supply House, Inc., Chicago, Ill.) and the length of the tubule between the two points of pH measurement was determined by microdissection.

Phosphorus and water reabsorption measurements. In 16 normal rats, reabsorption of phosphorus and water was measured in isolated segments of proximal convoluted tubules during perfusion with solutions 1, 2, or 3. The microperfusion system was the same as described in the preceding section. However, in this case, the perfusate was collected quantitatively, as described in method 3 of reference 20. In these experiments, trace amounts of [^{32}P]Na_2HPO_4 or [^{32}P]NaH_2PO_4 and [^{3}H]methoxy inulin were added to the perfusion solutions. The collections of the perfusate were carefully timed with a stopwatch. Each collected perfusate and samples of the initial perfusion solution were transferred to a 0.1-mm ID constant-bore glass capillary (Corning Glass Works, Science Products Div., Corning, N. Y.) for measurement of volume (24), and were then washed into liquid scintillation vials for radioactive counting in a Nuclear-Chicago counter, Unilux II A (Nuclear-Chicago Corp., Des Plaines, Ill.). Separation of the two isotopes was almost complete. Disintegrations per minute were calculated for each isotope, with ^{133}Ba as an external standard, by the channels ratio method. Counting was carried out for approximately 16 h immediately after each experiment. The decay of ^{32}P for this period of time was negligible and therefore ignored in the statistical analysis of the ^{32}P concentrations.

In nine additional rats, PTH (546 U/mg) was administered i.v. at a rate of 16 U/h and 0.1 U/ml was added to the perfusion solutions. Phosphorus and water absorption was measured in proximal tubular segments perfused with solution 1, 2, or 3, as described above.

The following calculations were carried out for each tubular perfusion: (*a*) perfusion rate = collected vol/min × $C/I_{^3H}$, where $C/I_{^3H}$ is the ratio of concentrations of [^{3}H]-methoxy inulin in the collected perfusate (C) over that in the initial perfusion fluid (I); (*b*) percent water absorbed = $(1 - I/C_{^3H}) \times 100$; and (*c*) collected/injected phosphate concentration = $C/I_{^{32}P}$.

The use of ^{32}P as an indicator of chemical phosphate absorption was evaluated in preliminary experiments. C/I phosphate ratios were measured in the same samples by ^{32}P and the chemical method of Chen, Toribara, and Warner (25), modified by Maesaka, Levitt, and Abramson (26). In two rats, proximal tubules were perfused with solution 1. The C/I phosphate ratios measured by the two methods agreed with one another by ±8% and variations in the ratios among different samples paralleled each other closely. In addition to this evidence, Murayama, Morel, and Le-Grimellec (3) compared chemical phosphate absorption, as measured by electron probe analysis, with ^{32}P absorption in microperfusion experiments in hydropenic rats. They found that with a phosphate concentration of 2 mmol in the perfusion fluid, as used in the present experiments, there was no statistical difference between the radioactive and electron probe measurements in the collected perfusate, and that the specific activity of the isotope remained constant over the length of tubule perfused (up to 3 mm). Thus, under hydropenic conditions, transport of phosphate in the proximal tubule seems to be essentially a unidirectional process.

In most experiments, the tubules that had been perfused were injected with yellow latex and were microdissected for determination of the length of the perfused segment.

At the end of each experiment, arterial blood was collected from the abdominal aorta for measurement of pH, total CO_2, Na^+, and K^+ by methods previously described (27). Timed urine collections, obtained from the urinary bladder, were analyzed for the same constituents. Inorganic phosphorus in plasma and urine was measured by the method of Gomori (28).

RESULTS

Tables I and II show the plasma and urinary electrolyte data for the control and PTH-infused rats. As can be seen in Table I, the only significant difference in plasma values between the two groups was the phosphorus, which was lower in the PTH-infused rats ($P < 0.001$). Urinary excretion data (Table II) reveal significantly higher values for flow rate, sodium, bicarbonate, and phosphorus excretion in the animals infused with PTH.

In vivo pH measurements. The observations on changes of the pH of perfusion solutions 1 and 2 during perfusion of the proximal tubule are shown in Fig. 1. The length of the perfused segment, determined by microdissection, is shown on the abscissa. The circles represent measurements in control rats and the triangles measurements in rats given exogenous PTH. The ionic form of phosphate shown in the enclosure indicates the predominant species present in the initial perfusion solution. The lines connect values measured at two points

TABLE I

Plasma Electrolyte Data in Control and PTH-Infused Rats

	pH	HCO_3^-	pCO_2	Na^+	K^+	P
		meq/liter	*mm Hg*	*meq/liter*	*meq/liter*	*mg/100 ml*
Control (22)						
Mean	7.45	23.0	34.2	145.7	4.5	7.7
±SE	0.01	0.8	1.6	1.3	0.1	0.4
PTH (12)						
Mean	7.43	23.7	37.7	143.3	4.5	5.7
±SE	0.03	0.5	2.9	2.1	0.1	0.2
P	NS	NS	NS	NS	NS	<0.001

NS = $P > 0.05$. The numbers in parentheses are the number of animals.

in the same tubule. As can be seen, the pH of the more alkaline solution, represented by open circles, fell by 0.10–0.30 U. In contrast, the pH of the more acid solution rose by 0.15–0.45 U in the control rats (closed circles). In the rats given exogenous PTH, the pH of the acid perfusion solution also rose by 0.20–0.50 U, but this occurred within shorter lengths of tubule, so that the rate of rise appeared to be greater than in the control rats.

Phosphate and water absorption measurements. The microperfusion rates for the control and PTH animals, measured with [^{3}H]inulin (Eq. *a*), were 11.6 nl/min ± 0.4 SE(control) and 12.9 nl/min ±0.4 (PTH), respectively. Although these values are significantly different from one another ($P < 0.05$), it seems unlikely that this fortuitous small difference effected the patterns of phosphate and water absorption.

The data on phosphate and water absorption in isolated perfused proximal tubular segments are shown in Figs. 2–5. Fig. 2 compares the $C/I_{^{32}P}$ to C/I_{in} data for the tubular perfusions in 16 control animals. It is apparent that when the tubule was perfused with the more acidic solution, the concentration of phosphate in the collected perfusate fell significantly below the concentration in the initial perfusion fluid. In some instances, the $C/I_{^{32}P}$ ratio was as low as 0.60 when C/I_{in} was only 1.25–1.50, suggesting rapid absorption of phosphate in relatively short segments of tubule. In only one instance was a $C/I_{^{32}P}$ ratio higher than 1.0 observed. There was generally an inverse relation between $C/I_{^{32}P}$ and C/I_{in}. In sharp contrast, when the tubules were perfused with either of the two alkaline solutions, the concentrations of phosphate in the collected perfusates were generally higher, and in most instances were above the concentration in the initial solution. Up to a $C/I_{^3H}$ ratio of 2.0, there was no apparent difference in the $C/I_{^{32}P}$ ratios for solution 2 and 3; i.e., the rise in phosphate concentration was the same whether bicarbonate and glucose were present in the perfusion solution or not. Beyond that point, there are too few points for a valid comparison.

In Fig. 3, fractional water absorption is plotted against length of perfused proximal segment for the control experiments. There was no apparent difference for solutions 1 and 2, and an aggragate linear regression line has been calculated ($y = 16.6x - 3.7$). Water

TABLE II

Urinary Electrolyte Data in Control and PTH-Infused Rats

	V	pH	$U_{HCO_3}V$	$U_{Na}V$	U_KV	U_PV
	μl/min/kg		*μmol/min/kg*	*μeq/min/kg*	*μeq/min/kg*	*μg/min/kg*
Control (22)*						
Mean	39.6 (37)‡	6.42	0.2	2.1	3.5	7.3
±SE	4.6	0.07	0.06	0.4	0.3	1.0
PTH (12)*						
Mean	124.4 (31)‡	6.59	0.9	9.0	5.8	15.9
±SE	22.8	0.16	0.2	2.4	1.6	2.7
P	<0.001	NS	<0.01	<0.01	NS	<0.01

* Number of animals.

‡ Number of samples for all determinations within the group of animals.

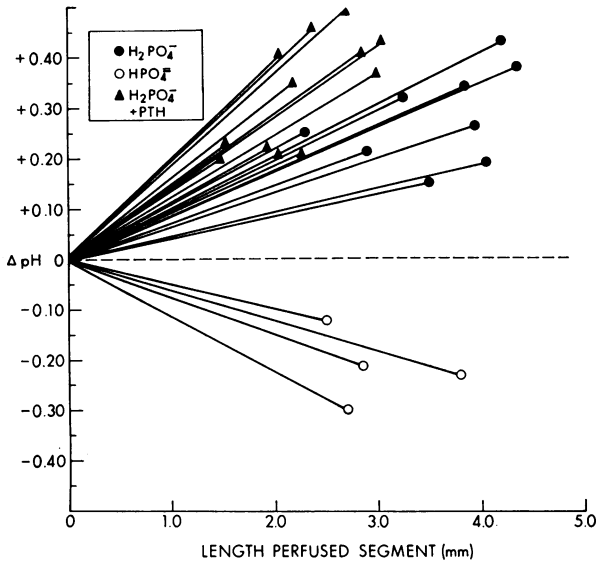

FIGURE 1 In vivo changes in pH of perfusion solutions. The circles represent measurements in normal control rats, and the triangles measurements in PTH-infused rats. The ionic form of phosphate shown in the key is the predominant form in the initial perfusion solution.

absorption with solution 3 had the same slope but the intercept was higher ($y = 15.5x + 4.7$). As can be seen, the lengths of tubule perfused with the three different solutions were comparable.

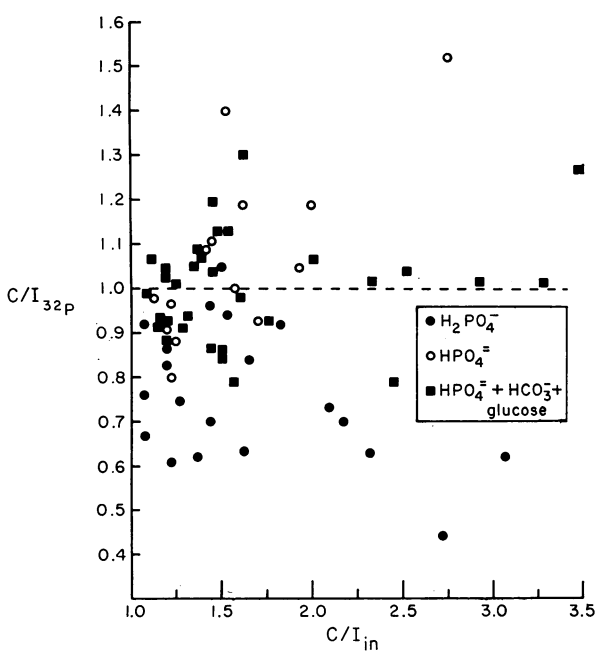

FIGURE 2 ^{32}P and [^3H]inulin concentrations in collected (C) and injected (I) perfusates measured in control rats. The ionic form of phosphate shown in the key indicates the predominant form in the initial perfusion solution.

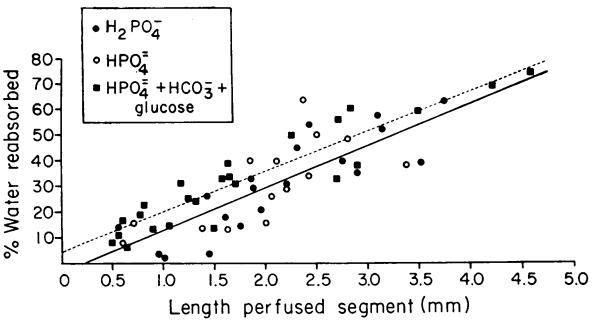

FIGURE 3 Fractional water absorption in perfused proximal tubular segments of control rats. The data are from the same experiments shown in Fig. 2. The solid line is the linear regression line for solutions 1 and 2, and the interrupted line is for solution 3. See text for equations.

Fig. 4 shows the $C/I_{^{32}P}$ and inulin ratios for nine animals given exogenous purified PTH. The clearest difference between these data and those shown for the control animals in Fig. 2 is that the $C/I_{^{32}P}$ ratios did not fall to low levels during perfusion with the acid solution. Thus, all $C/I_{^{32}P}$ ratios were above 0.80 with the acid solution, and in 8 of the 16 samples were above 1.0. In the case of perfusions with solution 2, $C/I_{^{32}P}$ ratios generally rose above 1.0, as in the control rats, and no clear-cut effect of PTH was observed. With perfu-

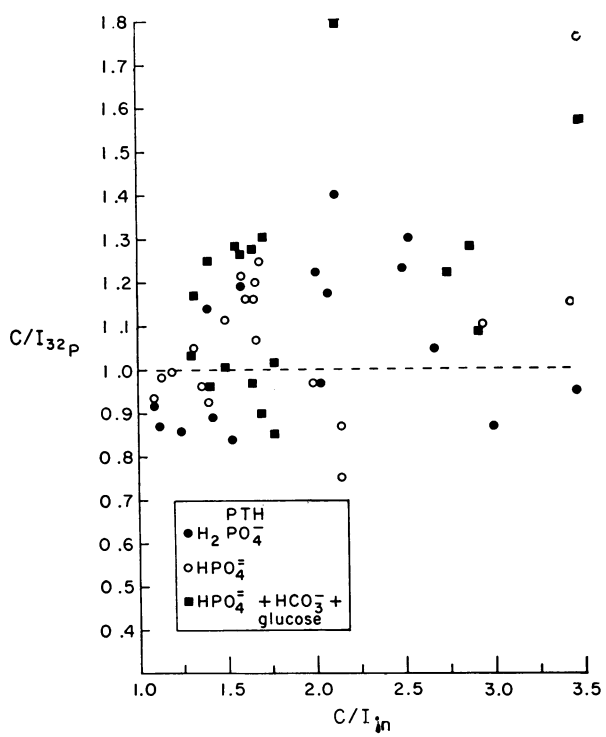

FIGURE 4 ^{32}P and [^3H]inulin concentrations in collected (C) and injected (I) perfusates measured in PTH-infused rats.

sion solution 3, however, $C/I_{^{32}P}$ appeared to be higher than in the control rats, as 8 of the 17 samples had ratios over 1.2 whereas only 3 out of 31 ratios above 1.2 were observed in the control animals.

Fig. 5 shows the data on fractional water absorption vs. length of tubular segment for the rats infused with PTH. It is apparent that there was no difference in water absorption when the tubules were perfused with solutions 1 or 2. The aggregate linear regression line for these two perfusion solutions is $y = 17.3x + 6.3$. Fractional water absorption at 3.0-mm length was 58%, a value higher than that observed in the absence of exogenous PTH (46%). With perfusion solution 3, water absorption was significantly reduced, when compared with perfusion solutions 1 and 2. The linear regression line for these data is $y = 19.3x - 9.6$. As in the case of the control experiments, comparable lengths of tubule were perfused with the three different solutions.

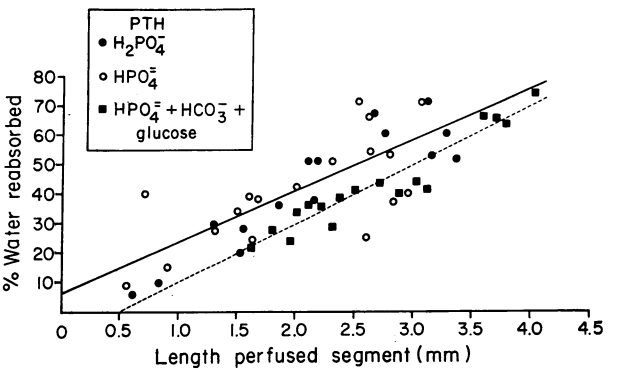

FIGURE 5 Fractional water absorption in perfused proximal tubular segments of PTH-infused rats. The data are from the same experiments shown in Fig. 4. The solid line is the linear regression line for solutions 1 and 2, and the interrupted line is for solution 3. See text for equations.

DISCUSSION

The results of the present microperfusion experiments demonstrate that the two major forms of phosphate normally present in the glomerular filtrate, $HPO_4^=$ and $H_2PO_4^-$, are not reabsorbed equally in the proximal tubule. When a relatively acid solution containing predominantly $H_2PO_4^-$ was used to perfuse the proximal tubule, the ^{32}P concentration in the collected perfusate was found to be consistently lower than in the initial solutions, whereas with alkaline solutions, ^{32}P in the collected perfusate was often higher than in the initial solution. These observations could not be accounted for by any difference in the rate of water absorption or by differences in the length of perfused segments (Fig. 3). The findings are consistent with the view that $H_2PO_4^-$ is reabsorbed preferentially over $HPO_4^=$.

The mechanism that might be responsible for a selective transport of $H_2PO_4^-$ is uncertain, but two possibilities can be considered. First, the data are consistent with an active transport process for $H_2PO_4^-$. The estimated concentration of total chemical phosphate in the tubular lumen fell to as low as about as 3.0 mg/100 ml, whereas the serum phosphate concentration averaged 7.7 mg/100 ml. Thus, reabsorption occurred against a significant chemical gradient. The electrical potential across the proximal tubular epithelium has recently been found to vary according to the composition of the tubular fluid (23, 29). Kokko has found that when rabbit proximal tubules are perfused in vitro with a saline solution containing no glucose or amino acids and only small amounts of bicarbonate, the lumen is electrically positive relative to the peritubular fluid (23). Perfusion solution 1 in the present study resembles such a solution, and therefore it might be expected that the PD was positive during perfusion with this solution. If this were the case, then $H_2PO_4^-$ would have been reabsorbed against an electrical as well as a chemical gradient. This would be consistent with the view that $H_2PO_4^-$ is reabsorbed by an active transport process.

An alternative hypothesis to account for the observations is that the proximal tubular epithelium is highly permeable to $H_2PO_4^-$, but not to $HPO_4^=$. Under these conditions, the main determining factor for the movement of phosphate might be the transepithelial concentration gradient of $H_2PO_4^-$. Although the concentration of total phosphate in the initial perfusion solution was close to that in rat blood, the concentration of $H_2PO_4^-$ in the acid solution was undoubtedly much higher than $H_2PO_4^-$ in the more alkaline peritubular blood. If the proximal tubule were highly permeable to this univalent anion, passive diffusion down a steep concentration gradient would be expected to occur. Furthermore, with continuing H^+ secretion and conversion of $HPO_4^=$ to $H_2PO_4^-$, the concentration gradient for $H_2PO_4^-$ would be maintained even though the total phosphate concentration in the tubular lumen fell below the total phosphate concentration in the surrounding capillaries. Net reabsorption would stop only when the concentrations of $H_2PO_4^-$ on the two sides of the tubular epithelium were equal. Such a mechanism, which is analogous to the non-ionic diffusion process for ammonia excretion, would not require an active transport step. This hypothesis seems as plausible as the argument for active transport of $H_2PO_4^-$, but the two cannot be distinguished on the basis of the present experiments.

The mechanism of reabsorption of $HPO_4^=$ is less certain. During perfusion with solution 2, which also contained no glucose, amino acids, or bicarbonate, the PD would again be expected to be positive. It is possible that the electrical attraction of a positive PD for the divalent anion was strong enough to retard reabsorption

and thus mask an active transport mechanism. However, this seems unlikely, since perfusion with solution 3 resulted in a comparable rise in ^{32}P concentration (Fig. 2). Solution 3 contained 30 mmol/liter bicarbonate and 100 mg/100 ml glucose and should, according to the observations of Kokko (23), have resulted in a negative PD. Thus, a strong driving force should have existed under these conditions for transfer of phosphate out of the lumen. Since the reabsorptive pattern was not appreciably affected (Fig. 2), we assume that the PD does not play a major determining role in the reabsorption of $HPO_4^=$ or $H_2PO_4^-$. The observations indicate that reabsorption of $HPO_4^=$ lagged behind reabsorption of water when the perfusion solutions were alkaline. These data are consistent with the view that $HPO_4^=$ is either reabsorbed by passive diffusion down a chemical concentration gradient or by conversion to $H_2PO_4^-$ by H^+ secretion into the tubular lumen.

It should be noted that the differences in ^{32}P absorption found with the acid and alkaline solutions (1 and 2) probably represent minimum differences in the tubular handling of $H_2PO_4^-$ and $HPO_4^=$, since the pH of the two solutions tended to become more alike during perfusion of the tubules (Fig. 1). The precise mechanisms responsible for the pH changes shown in Fig. 1 are not clear. It seems possible, however, that the rise in pH of the more acid solution was due in part to an influx of HCO_3^- from the epithelial cells or peritubular blood, since in a previous microperfusion study we found that the proximal tubule of the rat has a finite permeability to HCO_3^- (18). Another possible explanation for the rise in pH of the more acidic solution is the preferential reabsorption of $H_2PO_4^-$, leaving $HPO_4^=$ in the lumen. The observations in the rats given exogenous PTH, however, suggest that this was not the primary reason for the rise in pH, since in these experiments $H_2PO_4^-$ absorption was inhibited and the rise in pH was even greater than in the control rats. The findings are thus more consistent with the view that HCO_3^- influx occurred in the control rats, and that this influx was somehow enhanced by the administration of PTH. The possible effect of enhanced HCO_3^- influx on phosphate reabsorption will be discussed below. Whatever the correct explanation, the fact that the pH values of the perfusion solutions changed toward one another would have had the effect of minimizing the observed differences in reabsorption of $H_2PO_4^-$ and $HPO_4^=$.

Free-flow micropuncture studies of phosphate reabsorption by the rat and dog proximal tubule have shown that under normal hydropenic conditions, tubular fluid-to-plasma (TF/P) phosphate ratios are below 1.0 and most of the phosphate in the glomerular filtrate is reabsorbed by the early segments of the proximal tubule (1–7, 10, 11). It should be pointed out that the $C/I_{^{32}P}$ data shown in Figs. 2 and 4 are not comparable to TF/P phosphate ratios found in free-flow micropuncture experiments. The concentrations of phosphate in the injected and collected perfusates of the present control experiments (Fig. 2) were probably below the plasma phosphate concentration or only slightly above it in some instances. Therefore, a rise in $C/I_{^{32}P}$ with increasing C/I_{in} ratios does not necessarily imply that luminal phosphate concentration would rise similarly under free-flow conditions, since the concentration of phosphate in the perfusion solutions (6.4 mg/100 ml) was somewhat lower than that normally present in the glomerular filtrate (7.7 mg/100 ml). It seems reasonable that the rise in $C/I_{^{32}P}$ ratios observed with the alkaline solutions occurred because $HPO_4^=$ is relatively impermeant, and the intraluminal concentration must be at least as high as in the peritubular blood before outward diffusion occurs. The microperfusion data thus demonstrate the relative rates of phosphate and water reabsorption for solutions of different chemical composition and cannot be directly extrapolated to free-flow conditions.

Nevertheless, the findings help to provide an explanation for several observations concerning phosphate reabsorption under free-flow conditions. First, the reason for the avid reabsorption of phosphate by early segments of the proximal tubule has not been clear (2, 4). Theoretically, it could be due to an inherent property of the early convolutions with regard to phosphate transport, or might be secondary to other events occurring in this portion of the nephron. Since the segments chosen for microperfusion in the present study were randomly selected, and therefore probably are representative of most of the accessible proximal tubule, our findings suggest that all portions of the proximal tubule on the surface of the kidney are capable of avid reabsorption of phosphate. In the case of the acid perfusion solution, as much as 70% of the phosphate was reabsorbed over a 3.5-mm length of tubule. Thus, it seems likely that the findings in free-flow micropuncture studies do not reflect a special property of the early proximal tubule, but rather other events occurring in this portion of the nephron. It has been found that the pH of tubular fluid falls from 7.4 in the glomerular filtrate to approximately 6.7 in the early segments of the proximal tubule and remains relatively constant at that level along the remainder of the proximal tubule (21). This early fall in pH would result in a change of the $HPO_4^=/H_2PO_4^-$ ratio from approximately 4/1 in the glomerular filtrate to less than 1/1 at a pH of 6.7. Conversion of $HPO_4^=$ to $H_2PO_4^-$ and either active transport or passive diffusion of $H_2PO_4^-$ could readily account for the avid reabsorption of phosphate by early proximal segments.

Second, the present findings provide at least a partial explanation for observations in dog and man that alkalinization of the urine by bicarbonate administration results in depressed phosphate reabsorption by the renal tubules (13–15, 30). Since bicarbonate administration prevents the normal fall in proximal tubular pH (21), the filtered phosphate would tend to remain predominantly in the $HPO_4^=$ form, thereby reducing the availability of $H_2PO_4^-$ for active transport or passive diffusion. Whether this is the entire explanation or not is unclear, as extracellular fluid volume expansion was probably produced by $NaHCO_3$ infusion in the human and dog experiments. Volume expansion per se has subsequently been shown to reduce proximal phosphate reabsorption (8, 9, 31, 32). Furthermore, administration of acetazolamide, which tends to lower the *in situ* pH in the proximal tubule (21), also results in inhibition of phosphate reabsorption (10, 11, 30). This latter effect has recently been attributed to stimulation of renal adenyl cyclase by acetazolamide (33), a mechanism of action thought to be similar to that of PTH (5). Thus, while the ratio of $HPO_4^=/H_2PO_4^-$ in the tubular lumen may be an important factor in determining phosphate reabsorption, other mechanisms undoubtedly also play a role.

Our observations on the effect of PTH are compatible with the view that this hormone inhibits the transfer of $H_2PO_4^-$ out of the lumen, but has little or no direct effect on $HPO_4^=$. Thus, in the experiments shown in Fig. 4, PTH prevented the fall in $C/I_{^{32}P}$ when the tubule was perfused with the acid solution, and in most instances the ratio was over 1.0. In the case of perfusion solution 2, made up predominantly of $HPO_4^=$ and only small amounts of $H_2PO_4^-$, PTH had no clear-cut effect on phosphate absorption. The main effect thus appeared to be to block absorption of $H_2PO_4^-$. The mechanism might be a specific inhibition of an active transport process. Alternatively, the reduction in $H_2PO_4^-$ absorption with PTH could be accounted for by alkalinization of the tubular fluid (Fig. 1). If this hormone either inhibits H^+ secretion or increases the permeability of the tubule to HCO_3^-, the expected rise in intraluminal pH would result in a greater proportion of the phosphate being in the poorly permeable $HPO_4^=$ form. That PTH has a significant effect on urinary bicarbonate excretion is supported by the present findings (Table II) and by similar observations in man (34) and dog (35). Inhibition of H^+ secretion might also account for what appeared to be impaired $HPO_4^=$ reabsorption during perfusion with solution 3. Since solution 3 contained HCO_3^-, inhibition of H^+ secretion by PTH might have caused intraluminal pH to rise further as water was absorbed, and thus result in even more of the phosphate being in the $HPO_4^=$ form.

In previous micropuncture studies in the dog (5, 6, 11) and rat (7), it was found that administration of PTH produces a small but significant reduction in fluid reabsorption in the proximal tubule, as measured by free-flow TF/P inulin ratios. In the present study, a significantly higher urine flow rate and sodium excretion was observed in the rats receiving PTH, but no reduction in proximal fluid reabsorption was detectable when the tubules were perfused with solutions 1 or 2 (Fig. 5). In fact, fluid reabsorption was actually somewhat higher in these rats than in the controls. This might have been due to the higher urine flow rates in the rats given PTH and consequent volume contraction. In the case of perfusion with solution 3, fluid absorption was significantly reduced in the PTH-infused rats. Presumably, they had undergone a degree of volume contraction comparable to the other PTH-treated rats. The reason for the different response to PTH is uncertain. Solution 3 had a higher osmolality than solutions 1 or 2, because of the presence of glucose. This may have countered an osmotic force favoring reabsorption, and thus allowed an inhibitory effect of PTH on sodium transport to become manifest. Alternatively, if a major action of PTH is to inhibit H^+ secretion, the presence of HCO_3^- in solution 3 may account for the reduced fluid absorption observed in these experiments. Clearly, further studies are needed to determine the effect of PTH on proximal tubular pH and fluid absorption under various physiological conditions.

ACKNOWLEDGMENTS

The authors are grateful for the excellent technical assistance of Mrs. Cathy Cesario and Mr. Saul Cohen.

This research was supported by grants from the U. S. Public Health Service (5 R01-HL 14720) and the New York Heart Association.

REFERENCES

1. Strickler, J. C., D. D. Thompson, R. M. Klose, and G. Giebisch. 1964. Micropuncture study of inorganic phosphate excretion in the rat. *J. Clin. Invest.* **43**: 1596–1607.
2. Amiel, C., H. Kuntziger, and G. Richet. 1970. Micropuncture study of handling of phosphate by proximal and distal nephron in normal and parathyroidectomized rat. Evidence for distal reabsorption. *Pflügers Arch. Eur. J. Physiol.* **317**: 93–109.
3. Murayama, Y., F. Morel, and C. LeGrimellec. 1972. Phosphate, calcium and magnesium transfers in proximal tubules and loops of Henle, as measured by single nephron microperfusion experiments in the rat. *Pflügers Arch. Eur. J. Physiol.* **333**: 1–16.
4. 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.

5. Agus, Z. S., J. B. Puschett, D. Senesky, and M. Goldberg. 1971. Mode of action of parathyroid hormone and cyclic adenosine 3',5'-monophosphate on renal tubular phosphate reabsorption in the dog. *J. Clin. Invest.* **50**: 617–626.
6. 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.
7. Gekle, D., D. Rostock, and G. Kurth. 1969. A micropuncture investigation of the effect of parathormone on proximal tubular fluid reabsorption in the rat kidney. *In* Progress in Nephrology. G. Peters and F. Roch-Ramel, editors. Springer-Verlag New York, Inc. 380–384.
8. Frick, A. 1972. Proximal tubular reabsorption of inorganic phosphate during saline infusion in the rat. *Am. J. Physiol.* **223**: 1034–1040.
9. Kuntziger, H., C. Amiel, and C. Gaudebout. 1972. Phosphate handling by the rat nephron during saline diuresis. *Kidney Int.* **2**: 318–323.
10. 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.
11. Wen, S.-F. 1974. Micropuncture studies of phosphate transport in the proximal tubule of the dog. *J. Clin. Invest.* **53**: 143–153.
12. Carrasquer, G., and W. A. Brodsky. 1961. Elimination of transient secretion of phosphate by alkalinization of plasma in dogs. *Am. J. Physiol.* **201**: 499–504.
13. Mostellar, M. E., and E. P. Tuttle, Jr. 1964. Effects of alkalosis and plasma concentration and urinary excretion of inorganic phosphate in man. *J. Clin. Invest.* **43**: 138–149.
14. Fulop, M., and P. Brazeau. 1968. The phosphaturic effect of sodium bicarbonate and acetazolamide in dogs. *J. Clin. Invest.* **47**: 983–991.
15. Puschett, J. F., and M. Goldberg. 1969. The relationship between the renal handling of phosphate and bicarbonate in man. *J. Lab. Clin. Mad.* **73**: 956–969.
16. Bank, N. 1962. Relationship between electrical and hydrogen ion gradients across the rat proximal tubule. *Am. J. Physiol.* **203**: 577–582.
17. Bank, N., and H. S. Aynedjian. 1973. A micropuncture study of potassium excretion by the remnant kidney. *J. Clin. Invest.* **52**: 1480–1490.
18. Bank, N., and H. S. Aynedjian. 1967. A microperfusion study of bicarbonate accumulation in the proximal tubule of the rat kidney. *J. Clin. Invest.* **46**: 95–102.
19. Bank, N., W. E. Yarger, and H. S. Aynedjian. 1971. A microperfusion study of sucrose movement across the rat proximal tubule during renal vein constriction. *J. Clin. Invest.* **50**: 294–302.
20. Bank, N., and H. S. Aynedjian. 1972. Techniques of microperfusion of renal tubules and capillaries. *Yale J. Biol. Med.* **45**: 312–317.
21. Vieira, F. L., and G. Malnic. 1968. Hydrogen ion secretion by rat renal cortical tubules as studied by an antimony microelectrode. *Am. J. Physiol.* **214**: 710–718.
22. Malnic, G., and F. L. Vieira. 1972. The antimony microelectrode in kidney micropuncture. *Yale J. Biol. Med.* **45**: 356–367.
23. Kokko, J. P. 1973. Proximal tubule potential difference. Dependence on glucose, HCO_2, and amino acid. *J. Clin. Invest.* **52**: 1362–1367.
24. Windhager, E. E., and G. Giebisch. 1961. Micropuncture study of renal tubular transfer of sodium chloride in the rat. *Am. J. Physiol.* **200**: 581–590.
25. Chen, P. S., Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28**: 1756–1758.
26. Maesaka, J. K., M. F. Levitt, and R. G. Abramson. 1973. Effect of saline infusion on phosphate transport in intact and thyroparathyroidectomized rats. *Am. J. Physiol.* **225**: 1421–1429.
27. Yarger, W. E., H. S. Aynedjian, and N. Bank. 1972. A micropuncture study of postobstructive diuresis in the rat. *J. Clin. Invest.* **51**: 625–637.
28. Gomori, G. 1953. Inorganic phosphate. *Stand. Methods Clin. Chem.* **1**: 84–87.
29. Barratt, L. J., F. C. Rector, Jr., J. P. Kokko, and D. W. Seldin. 1974. Factors governing the transepithelial potential difference across the proximal tubule of the rat kidney. *J. Clin. Invest.* **53**: 454–464.
30. Malvin, R. L., and W. D. Lotspeich. 1956. Relation between tubular transport of inorganic phosphate and bicarbonate in the dog. *Am. J. Physiol.* **187**: 51–56.
31. Frick, A. 1968. Reabsorption of inorganic phosphate in the rat kidney. I. Saturation of transport mechanism. II. Suppression of fractional phosphate reabsorption due to expansion of extracellular fluid volume. *Pflügers Arch. Eur. J. Physiol.* **304**: 351–364.
32. Frick, A. 1969. Mechanism of inorganic phosphate diuresis secondary to saline infusions in the rat. Excretion of sodium, inorganic phosphate and calcium in normal and in parathyroidectomized rats. *Pflügers Arch. Eur. J. Physiol.* **313**: 106–122.
33. 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.
34. Nordin, B. E. C. 1960. The effect of intravenous parathyroid extract on urinary pH, bicarbonate, and electrolyte excretion. *Clin. Sci. (Oxf.).* **19**: 311.
35. Hellman, D. E., W. Y. W. Au, and F. C. Bartter. 1965. Evidence for a direct effect of parathyroid hormone on urinary acidification. *Am. J. Physiol.* **209**: 643–650.