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Research Article

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A Micropuncture Study of Renal Phosphate Transport in Rats with Chronic Renal Failure and Secondary Hyperparathyroidism

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ABSTRACT Micropuncture studies were carried out in rats to determine changes in tubular transport of phosphate which occur in chronic renal failure and secondary hyperparathyroidism. Rats underwent subtotal nephrectomy (NX) and were fed a low calcium, high phosphorus diet for 3–4 wk. Other groups consisted of normal control animals, normal rats infused with sodium phosphate to raise filtered load of phosphate, subtotal NX rats parathyroidectomized (PTX) on the day of experiment, and normal PTX rats infused with sodium phosphate. It was found that filtered phosphate/nephron is markedly increased in subtotal NX rats due to high single nephron filtration rates, proximal tubular fluid plasma phosphate ratios are >1.0 , and fractional reabsorption of phosphate is decreased in the proximal tubule. More phosphate was present in the final urine than in surface distal convoluted tubules. Acute PTX in subtotal NX rats resulted in a striking increase in proximal phosphate reabsorption, and urinary phosphate became approximately equal to that remaining in surface distal tubules. Phosphate loading in normal rats reduced fractional reabsorption in the proximal tubule, but urinary phosphate was not greater than that at the end of surface distal tubules. Acute PTX in normal phosphate-loaded animals had no significant effect on proximal tubular phosphate reabsorption. These observations suggest that phosphate homeostasis in chronic renal failure is achieved by inhibition of proximal phosphate reabsorption, counteracting a greatly enhanced intrinsic capacity for reabsorption. In addition, the large amount of urinary phosphate is consistent either with secretion by the collecting ducts or with a disproportionately high contribution by deep nephrons. The changes in phosphate transport are mediated by

parathyroid hormone and are completely abolished by acute removal of the hormone.

INTRODUCTION

It is well established that fractional reabsorption of filtered phosphate decreases progressively in the presence of advancing renal disease (1–3), and that this homeostatic process acts to maintain serum phosphorus concentrations within the normal range until the glomerular filtration rate (GFR)¹ falls to $<20\%$ of normal (4). Most evidence indicates that hyperparathyroidism secondary to the renal failure plays a key role in inhibiting tubular reabsorption of phosphate in this condition (2, 3), although some observations suggest that phosphate adaptation can occur in the absence of parathyroid hormone (5, 6).

Aside from the fact that whole kidney fractional reabsorption of phosphate is reduced in renal failure, no information is available concerning the specific sites along the nephron where changes in transport occur, nor the precise nature of these changes. In normal rats and dogs, most phosphate reabsorption takes place in the proximal tubule with little or no reabsorption in more distal segments (7–12). After parathyroidectomy, the distal nephron reabsorbs some phosphate (8, 11, 13–16), indicating that the hormone normally acts to inhibit net reabsorption in distal segments.

The present micropuncture experiments in rats were carried out to examine segmental tubular transport of phosphate under conditions simulating chronic renal

¹Abbreviations used in this paper: EF_{Na} , fractional sodium excretion; $EF_{\text{phosphate}}$, fractional phosphate excretion; GFR, glomerular filtration rate; NX, partial nephrectomy; PTH, parathyroid hormone; PTX, parathyroidectomy; SNGFR, single nephron glomerular filtration rate; TF/P, tubular fluid plasma ratio.

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failure and secondary hyperparathyroidism in man. Various protocols were designed in subtotal nephrectomized (NX) and normal rats to examine the role played by increased filtered phosphate per nephron, plasma phosphate concentration, parathyroid hormone (PTH), and hypertrophy of surviving nephrons in mediating the changes in phosphate excretion of chronic renal failure. We found that fractional reabsorption of phosphate was markedly decreased in the proximal tubule of subtotal NX rats, and urinary phosphate was greater than that remaining in surface distal tubules. A comparable reduction in fractional reabsorption was found in the proximal tubule of normal rats infused with phosphate, but urinary phosphate was not greater than that which remained in surface distal tubules. Acute parathyroidectomy (PTX) in renal-ablated rats resulted in greatly increased reabsorption of phosphate in the proximal tubule, and urinary phosphate became equal to that remaining in surface distal tubules.

METHODS

White male Sprague-Dawley rats were used, initially weighing 250–300 g. Five groups of rats were studied.

Group I. 10 normal rats were maintained on a regular rat pellet diet and tap water sweetened with 5% sucrose. On the day of the experiment, anesthesia was induced with i.p. Inactin, 10 mg/100 g body wt. The trachea was intubated, and a carotid artery and jugular vein were cannulated by methods previously described (17, 18). Blood pressure was monitored continuously via the carotid artery, and arterial blood samples were obtained from the same catheter for measurement of blood gases and various chemicals, described below. The left kidney was exposed through a small lateral abdominal incision and prepared for micropuncture collections as described previously (17, 18). The urinary bladder was externalized through a small suprapubic incision, and PE tubing, flanged at the tip, was inserted and ligated in a manner which minimized bladder dead space. Body temperature was monitored continuously and maintained at 37°–38°C by a heated animal table. Isotonic Ringer's lactate solution containing [*methoxy*-³H]inulin and NaH₂³²PO₄ (New England Nuclear, Boston, Mass.) was infused at 2.3 ml/h throughout the experiment. Arterial blood samples were collected at the beginning and end of the experiment for measurement of calcium, phosphorus, sodium, and potassium.

Timed tubular fluid sample collections were obtained from surface convolutions by previously described techniques (17, 18). Early and late segments of proximal convoluted tubules were identified and selected for micropuncture by observing the sequential appearance of i.v. pulse-injected FD and C green no. 3 dye (Keystone Aniline & Chemical Co., Chicago, Ill.). Distal convoluted tubules were also identified for micropuncture by the same technique. Immediately after each tubular fluid collection, an arterialized blood sample was collected from the cut end of the tail into a heparinized capillary tube for measurement of plasma ³H and ³²PO₄. The collected tubular fluid samples were transferred into a column of toluene in a constant-bore capillary tube (0.1-mm internal diameter; Corning Glass Works, Science Products Div., Corning, N. Y.), and the length

of the aqueous column was measured under a microscope with an eyepiece micrometer. Portions of plasma and urine were transferred into the same capillary tube for volume measurement. All samples were washed into liquid scintillation counting vials containing 12 ml counting solution: 100 ml Bio-Solv (Beckman Instruments, Inc., Fullerton, Calif.), 42 ml Liquiflor (New England Nuclear, Boston, Mass.) made up to 1 liter with scintillation-grade toluene (Fisher Scientific Co., Pittsburgh, Pa.). Radioactive counts were measured with a Nuclear-Chicago liquid scintillation counter (Unilux II A; Amersham/Searle Corp., Arlington Heights, Ill.). Windows were set to eliminate ³H from the ³²P channel, and spillover of ³²P into the ³H channel was constant at 2.5% of the ³²P counts. This was subtracted from the counts in the ³H channel. Plasma [³H]inulin was corrected for a water content of 94%, determined by a refractometer, and whole plasma ³²PO₄ was corrected for an ultrafilterable value of 96%, determined by an Amicon ultrafiltration system, using a diaflow membrane, type PM 30 (Amicon Corp., Lexington, Mass.). This correction factor is in close agreement with that found by Strickler et al. (7). Moreover, Le Grimellec et al. (19) found that the phosphate concentration in fluid collected from Bowman's capsule of rats agrees closely with that found in ultrafiltrates of whole plasma through Cuprophane membranes (Enka AG., Plant Wuppertal-Bjarmen Wuppertal 2 West Germany). In the text and figures, plasma ³²PO₄ refers to UF ³²PO₄, i.e. ultrafilterable ³²PO₄.

Group II. 12 normal male rats were prepared in exactly the same fashion as described above, except that the i.v. infusion consisted of 300 mmol sodium phosphate (pH 7.4) at 2.3 ml/h instead of Ringer's lactate solution. Arterial blood samples were collected from the carotid artery at 30- to 45-min intervals throughout the experiment for chemical measurement of serum calcium and phosphorus. Radioactive isotopes were added to the i.v. infusion, as in group I experiments, and measured in tubular fluid, plasma, and urine as described above. In these experiments, a separate urine collection was obtained, bracketing in time each distal tubular fluid collection. Ultrafilterable ³²PO₄ was found to be 100% in these experiments.

Group III. 10 rats underwent subtotal NX by excision of the right kidney and ligation of the upper pole and a portion of the dorsal side of the left kidney by a previously described technique (17). This technique minimizes scarring of the renal capsule of the ventral surface of the kidney, which would interfere with subsequent micropuncture collections. 3 days were allowed for recovery from surgery. The rats were then started on a low calcium, normal phosphorus diet, supplemented with vitamins (ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio). Their intake of phosphorus was also supplemented by adding 67.5 mg phosphorus/day, as neutral sodium phosphate, to their drinking solution. They were maintained in individual metabolic cages and given this dietary regimen for 3–4 wk. This regimen in uremic rats has been shown by Kaye (20) to result in marked hypertrophy of the parathyroid glands which contain increased amounts of radioimmunoassayable PTH. 3 days before micropuncture study, the sodium phosphate drinking solution was replaced by 5% sucrose drinking solution to eliminate possible effects of a large exogenous phosphate load on the day of the experiment.

On the day of micropuncture study, the animals were anesthetized with Inactin, 5–7 mg/100 g body wt, and prepared surgically as in the group I and II animals. Timed tubular fluid collections were obtained from ventral surface proximal and distal convolutions of the remnant left kidney for measurement of [³H]inulin and ³²PO₄. As in the group II rats, a separate urine collection was obtained, bracketing in

time each distal tubular fluid collection. Samples of arterial plasma and urine were measured for ^3H and $^{32}\text{PO}_4$ using the same volumetric technique as for tubular fluid samples.

Group IV. Five rats underwent subtotal NX by exactly the same techniques described for group III animals and were maintained on the same diet for 3–4 wk. The only difference was that on the morning of micropuncture study, the two parathyroid glands were cauterized under microscope visualization at least 1 h before starting tubular fluid or urine collections. The glands were enlarged and easily identified. The thyroid gland was left intact. Tubular fluid collections were obtained from proximal and distal convolutions of the remnant left kidney as in the above groups of animals. Blood and urine collections were obtained as in the other groups of animals.

Group V. Six normal rats were prepared in the same fashion as the group II rats, except that the parathyroid glands were cauterized during surgical preparation for micropuncture. The i.v. infusion consisted of 300 mmol neutral sodium phosphate at 2.3 ml/h throughout the experiment, as in the group II animals. Tubular fluid was collected from proximal and distal convoluted tubules as in the other groups of rats for measurement of ^3H inulin and $^{32}\text{PO}_4$. Blood was collected every 30–45 min for measurement of phosphorus and calcium, and timed urine collections were obtained as in the other groups of rats.

In all experiments, sodium and potassium were measured in plasma and urine samples by flame photometry. Total calcium was measured by the method of Meites (21). Serum phosphorus was measured by the method of Gomori (22). Blood pH, pCO_2 , and pO_2 were measured periodically throughout the experiment in a Radiometer blood micro system, model BMS 3 MK2 (The London Co., Cleveland, Ohio). Plasma HCO_3^- was calculated from the pH and pCO_2 measurements by the Henderson-Hasselbalch equation.

Single nephron glomerular filtration rate (SNGFR), whole kidney GFR, fractional water, and $^{32}\text{PO}_4$ reabsorption were calculated by previously described equations (18).

RESULTS

In Table I are shown the data on blood acid-base, GFR, and fractional sodium excretion (EF_{Na}) at the beginning (I) and end (F) of the micropuncture experiments. All pH values were within the normal range, and there were no significant differences from the normal group I animals. Plasma pCO_2 fell during the course of the experiment in four of the five groups, presumably as a result of decreasing levels of anesthesia and hyperventilation, and blood pH rose slightly. However, the values at the end of the experiment were not significantly different among the five groups. Plasma HCO_3^- was within the normal range and remained relatively constant throughout the experiment except for a moderate fall in group IV rats (NX-PTX). Metabolic acidosis had not developed spontaneously in any of the subtotal NX rats. Whole kidney GFR was reduced by approximately 90% in the subtotal NX rats, as compared with the normal rats. EF_{Na} was significantly higher in all experimental groups, as compared with the group I control rats. There was no significant difference in EF_{Na} between the NX and NX-PTX groups (III and IV) ($P > 0.30$) or between the two phosphate-loaded groups (II and V) ($P > 0.35$).

In Table II are presented the serum calcium, phosphorus, sodium, and potassium data at the beginning (I) and end (F) of the micropuncture experiments for the five groups of rats. In all groups, there was a tendency for serum calcium to fall during the course of the experiment, the most marked fall occurring in the two PTX groups (IV and V). There were no significant differences in initial serum calcium concentrations among the five groups. The initial serum phosphorus concentrations were comparable in all five groups, and changed significantly during the course of the experiments only in group II and V rats infused with isotonic sodium phosphate. Serum phosphorus was normal in the group III (NX) rats. Serum sodium and potassium concentrations were within the normal range in all five groups of animals.

Mean blood pressure (BP) averaged between 110 and 140 mm Hg in all five groups. In the group III (NX) rats, mean BP at the beginning of the experiments was $139 \text{ mm Hg} \pm 4 \text{ SEM}$, a value significantly higher than the normal group I rats ($123 \text{ mm Hg} \pm 3 \text{ SEM}$). In the two acute PTX groups (IV and V), mean BP fell 14 and 8 mm Hg, respectively, during the course of the experiments. However, a comparable fall in BP occurred in the intact group II rats infused with sodium phosphate (12 mm Hg). There were no statistical differences in final BP among any of the five groups, although the average value in the NX rats was 10 mm Hg higher than in the NX-PTX rats.

The average values for SNGFR for each group are presented in Table III. SNGFR was calculated from distal tubular collections as well as proximal tubular collections. This served as a useful check on the technical adequacy of individual tubular fluid collections, because errors caused by back flow of tubular fluid or plugged pipettes are readily detected. Samples that yielded grossly different SNGFR values from the majority of collections in each animal were discarded. SNGFR measured from proximal tubular collections were comparable in groups I, II, and V, but was more than twofold higher in group III (NX) and IV (NX-PTX) rats. SNGFR measured from distal tubular collections was slightly higher than values measured from proximal collections in four of the five groups, but the differences were statistically significant only in group II ($2P < 0.005$). Also shown in Table III are the mean tubular fluid plasma inulin ($\text{TF}/\text{P}_{\text{in}}$) values obtained from the last surface convolution of proximal convoluted tubules. The mean values in the NX and NX-PTX groups were significantly lower than in the normal group I rats.

In Fig. 1 are shown $\text{TF}/\text{P}_{^{32}\text{PO}_4}$ ratios for the proximal and distal tubules, plotted against the corresponding $\text{TF}/\text{P}_{\text{in}}$ ratio measured in the same sample, for groups I–III. In the group I normal rats, $\text{TF}/\text{P}_{^{32}\text{PO}_4}$ in the proximal tubule was < 1.0 in all but five instances. The linear regression line for the proximal tubule

TABLE I
Blood Acid-Base and GFR Values in Five Groups of Rats

	pH		pCO ₂		HCO ₃ ⁻		GFR	EF _{Na} ⁺
	I	F	I	F	I	F		
			mm/Hg		meq/liter		ml/min/kg	%
Group I (10)*	7.37‡	7.45	43.2	31.2	24.0	20.7	9.36	0.06
	0.02	0.06	2.7	4.9	1.0	0.8	0.64	0.02
Group II (12)	7.40	7.44	37.9	34.0	22.8	22.2	8.87	0.38§
	0.01	0.02	0.8	2.9	0.9	1.5	0.56	0.05
Group III (10)	7.38	7.37	40.7	40.3	22.9	21.9	0.84§	0.66§
	0.02	0.03	2.9	4.4	1.7	1.0	0.13	0.14
Group IV (5)	7.39	7.41	41.8	33.6	24.9	19.2	0.98§	0.70§
	0.02	0.06	1.7	6.5	0.9	1.2	0.16	0.21
Group V (6)	7.40	7.49	39.5	30.5	24.1	22.6	9.83	0.36§
	0.02	0.03	2.4	2.8	1.7	1.2	2.16	0.06

* Numbers in parentheses are the number of animals. I, initial sample; F, final sample.

‡ Data presented as mean ± SEM.

§ 2 P < 0.01 compared with group I control animals.

($y = -0.06x + 0.77$) was not statistically significant ($r = -0.17$). In the distal tubule, TF/P_{32PO₄} was >1.0, but the calculated regression line was again not statistically significant ($y = 0.06x + 0.04$; $r = 0.34$). These observations are in agreement with previous micro-puncture studies of TF/P_{PO₄} ratios, measured by chemical methods, in normal hydropenic rats (7, 8, 12). In the group II rats, loaded with sodium phosphate, proximal tubular TF/P_{32PO₄} was above 1.0 in all but one collection and tended to rise as TF/P_{In} rose. The linear

regression line is $y = 0.29x + 0.8$ (2P < 0.01). In the distal tubule TF/P_{32PO₄} continued to rise with higher TF/P_{In} ratios, reaching values much higher than in the nonloaded normal rats. The linear regression line for the distal tubule is $y = 0.37x + 1.64$ (2P < 0.01). The same pattern of TF/P_{PO₄} ratios in the proximal distal tubules, measured chemically, has been described previously in normal phosphate-loaded rats (7, 12). In the group III rats (NX), the proximal and distal tubular TF/P_{32PO₄} ratios overlapped completely with the

TABLE II
Serum Calcium, Phosphorus, Sodium and Potassium Concentrations in Five Groups of Rats

	Total calcium		Phosphorus		Na ⁺		K ⁺	
	I	F	I	F	I	F	I	F
	mg/100 ml		mg/100 ml		meq/liter		meq/liter	
Group I (10)	9.1	8.8	6.7	7.6	146.9	147.8	4.1	4.6
	0.1	0.2	0.3	0.7	1.7	4.1	0.4	0.7
Group II (12)	9.0	8.6	6.7	18.4*	148.7	156.4	4.6	4.3
	0.2	0.3	0.3	1.1	2.3	2.0	0.3	0.3
Group III (10)	8.8	8.2	6.4	7.3	145.9	143.1	4.2	4.4
	0.4	0.6	0.8	0.8	2.0	1.1	0.2	0.3
Group IV (5)	9.0	7.1*	5.9	7.6	145.9	142.0	4.5	4.6
	0.3	0.3	0.4	0.5	2.4	0.9	0.1	0.2
Group V (6)	8.5	6.1*	6.4	17.2*	147.1	149.9	4.3	3.7
	0.3	0.5	0.4	2.0	2.2	2.4	0.2	0.2

I, initial sample; F, final sample.

* 2 P < 0.01 compared to group I control animals.

TABLE III
SNGFR and End-Proximal TF/P Inulin Ratios

	Proximal SNGFR	Distal SNGFR	End-proximal TF/P _{In}
	nl/min	nl/min	
Group I	37.9 (24)* 2.1	43.0 (18) 3.3	3.10 (11) 0.26
Group II	36.2 (32) 1.4	48.0 (19) 4.4	2.88 (19) 0.20
Group III	91.9‡ (14) 7.0	92.8‡ (14) 12.3	2.46‡ (17) 0.11
Group IV	84.0‡ (21) 6.0	95.4‡ (22) 12.0	2.40‡ (8) 0.19
Group V	45.4 (26) 4.6	45.7 (16) 2.6	2.74 (10) 0.12

* Numbers in parentheses are the number of observations.
‡ $P < 0.05$ compared with group I control animals.

group II values. The linear regression line for the proximal tubule is $y = 0.28x + 0.76$ ($2P < 0.01$) and for the distal tubule is $y = 0.38x + 1.24$ ($2P < 0.01$). Thus, the pattern of TF/P_{32PO₄} ratios in the proximal and distal tubules was closely comparable in normal phosphate-loaded rats and subtotal NX rats, even though serum phosphorus was much different in the two groups.

The linear regression lines for these two groups of rats were not significantly different from one another. The pattern differed strikingly from that in nonloaded normal control rats.

In Fig. 2 is plotted the percent reabsorption of ultra-filtered ³²PO₄ against percent fluid reabsorption on the abscissa, for groups I and II rats. In the group I rats, phosphate was reabsorbed progressively along the length of the proximal tubule. The linear regression line has the equation $y = 0.78x - 0.39$ ($r = 0.76$; $2P < 0.001$). Thus, at the end of the accessible portion of the proximal tubule, approximately 90% of the filtered phosphate had been reabsorbed. These observations are in agreement with previous micropuncture studies of phosphate reabsorption by the proximal tubule of normal rats (7-12, 23). In the distal tubule, no definite reabsorption of phosphate was apparent, although the small amount delivered to this segment under normal conditions makes detection of reabsorption difficult. The observations in the distal tubule are in agreement with previous studies (7, 8, 12, 23). In the group II phosphate-loaded rats, a much smaller percentage of filtered phosphate was reabsorbed by the proximal tubule. The linear regression line for the proximal tubule is $y = 0.63x + 4.3$ ($r = 0.61$; $2P < 0.001$). At the end of the accessible proximal tubule, approximately 45-50% of filtered phosphate had been reabsorbed. The data for the distal tubule

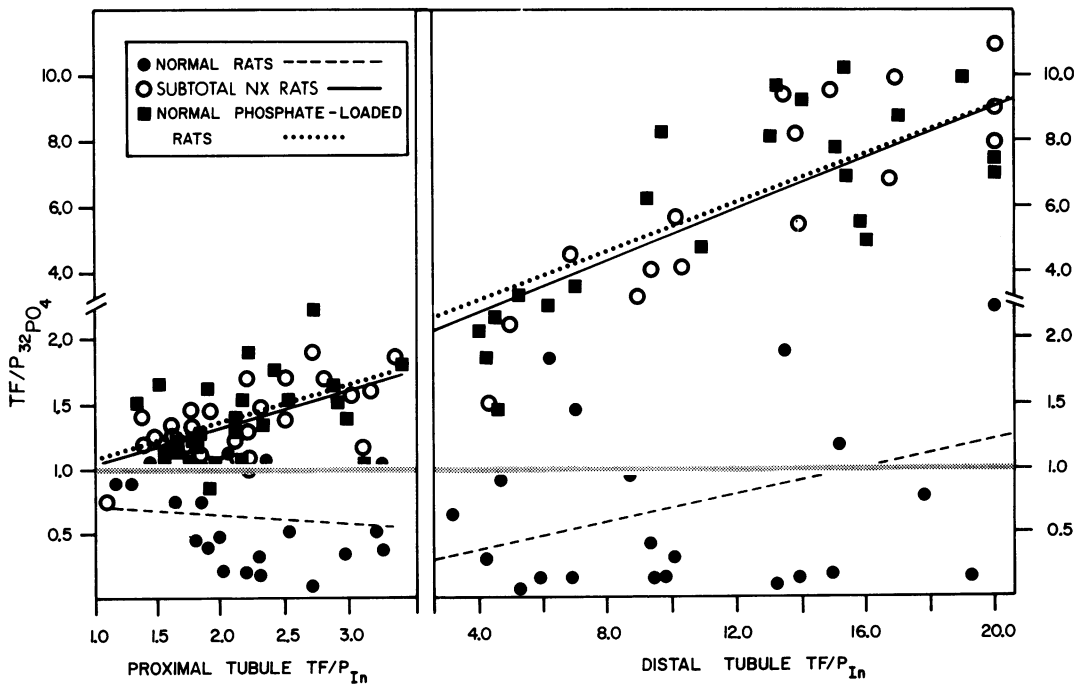


FIGURE 1 Tubular fluid-plasma ³²PO₄ ratios (TF/P_{32PO₄}) related to corresponding tubular fluid/plasma inulin ratios (TF/P_{In}) in three groups of rats. The lines are linear regression lines. See text for equations.

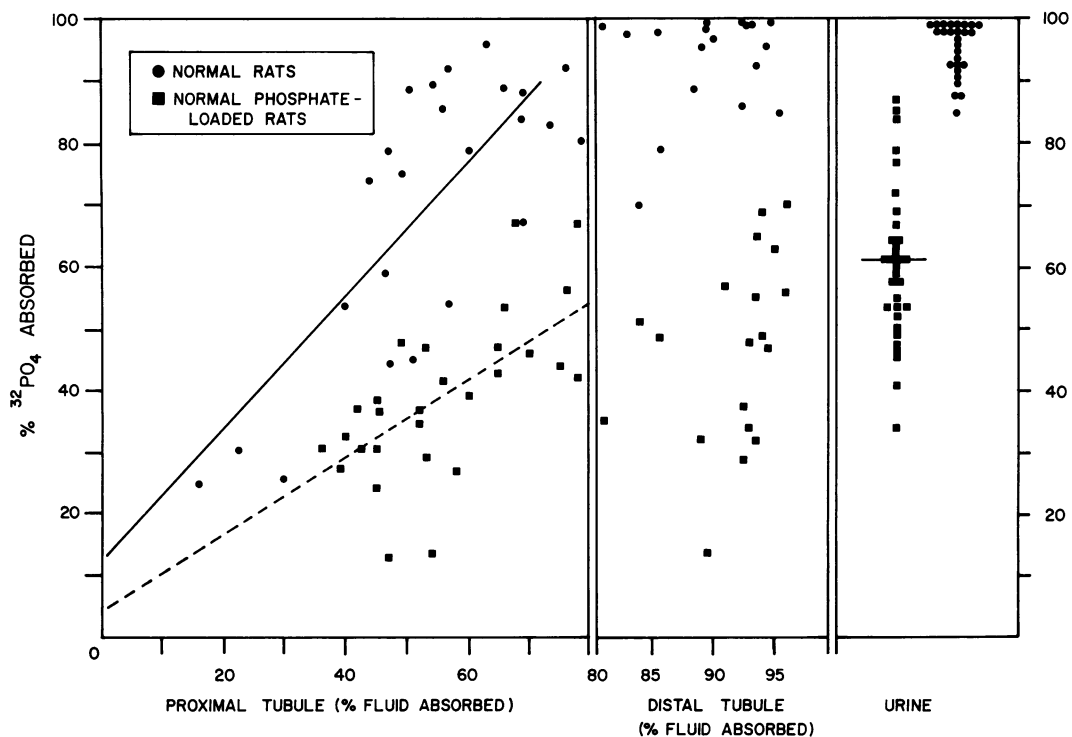


FIGURE 2 Percent $^{32}\text{PO}_4$ absorbed related to corresponding percent fluid absorbed in normal rats and normal phosphate-loaded rats. See text for linear regression equations.

showed considerable scatter, and no clear pattern of phosphate reabsorption or secretion was apparent. A similar wide scatter is evident in the distal tubular data of previous micropuncture studies of phosphate-loaded normal rats (7, 12). The reason for this is not clear. When the end-proximal results are compared to the final urine, shown at the right of Fig. 2, it appears that some additional phosphate was reabsorbed beyond the accessible proximal tubule. The mean fractional reabsorption for the whole kidney in the group II rats was 61%.

In Fig. 3 are presented the data on percent phosphate reabsorbed for the NX and the NX-PTX rats (groups III and IV). It is clear that in the NX rats with secondary hyperparathyroidism, approximately 45–50% of filtered phosphate was reabsorbed in the proximal tubule, the pattern of reabsorption being quite similar to that in the normal phosphate-loaded animals (Fig. 2). The linear regression line for the proximal tubule is $y = 0.65x + 2.0$ ($r = 0.68$; $2P < 0.001$). No phosphate reabsorption or secretion was discernible along the length of the distal convoluted tubule. Percent phosphate reabsorption for the whole kidney, shown at the right of Fig. 3, was lower than observed in the surface distal convolutions (mean = 37%). In sharp contrast to these results, the NX-PTX rats showed much greater phosphate reabsorption in the proximal tubule, reaching values of approximately

97% by the end of the accessible proximal convolutions. Less than 5% of filtered phosphate remained in the distal lumen in these animals, and $\text{EF}_{^{32}\text{PO}_4}$, shown at the right, ranged from 8 to <1% (mean = 2.32%). Thus, acute PTX in subtotal NX rats had a profound effect on phosphate reabsorption in the proximal convoluted tubule and on phosphate excreted in the final urine.

In Fig. 4 are plotted the data on percent phosphate reabsorbed for the group V rats (normal phosphate-loaded plus PTX). The data from the group II (normal phosphate-loaded rats with intact parathyroid glands) have been included for comparison. It can be seen that acute PTX had no significant effect on percent phosphate reabsorption in the proximal tubule during phosphate loading. The linear regression line for the PTX group is $y = 0.78x - 0.39$ ($r = 0.76$; $2P < 0.001$). This line is not significantly different from that for the intact phosphate-loaded rats. The distal tubular values showed a wide scatter in the PTX rats, as in the intact rats. No pattern of phosphate reabsorption or secretion was apparent, although the mean percent reabsorption in the distal tubule of the PTX rats was significantly higher than in the intact rats (62 vs. 47%; $P < 0.0025$). Similarly, percent phosphate reabsorption for the whole kidney was significantly higher for the phosphate-loaded PTX rats than for the intact phosphate-loaded rats (79 vs. 61%; $P < 0.001$).

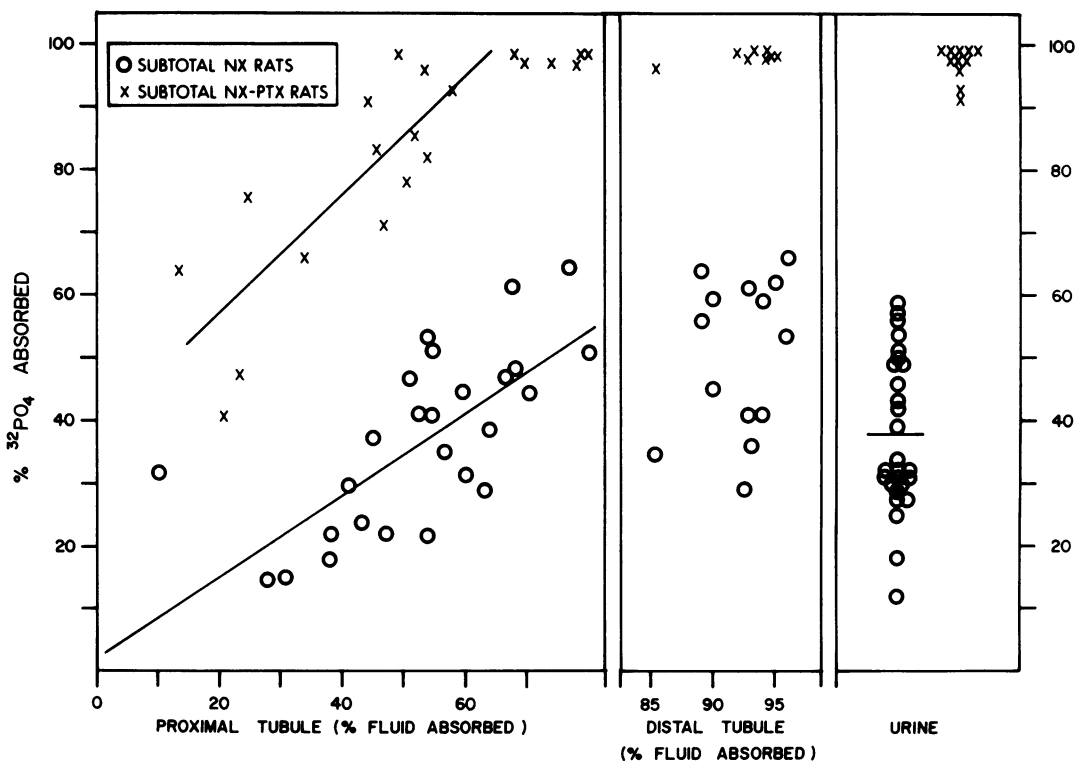


FIGURE 3 Percent $^{32}\text{PO}_4$ absorbed related to corresponding percent fluid absorbed in subtotal NX rats and subtotal NX-PTX rats. See text for linear regression equations.

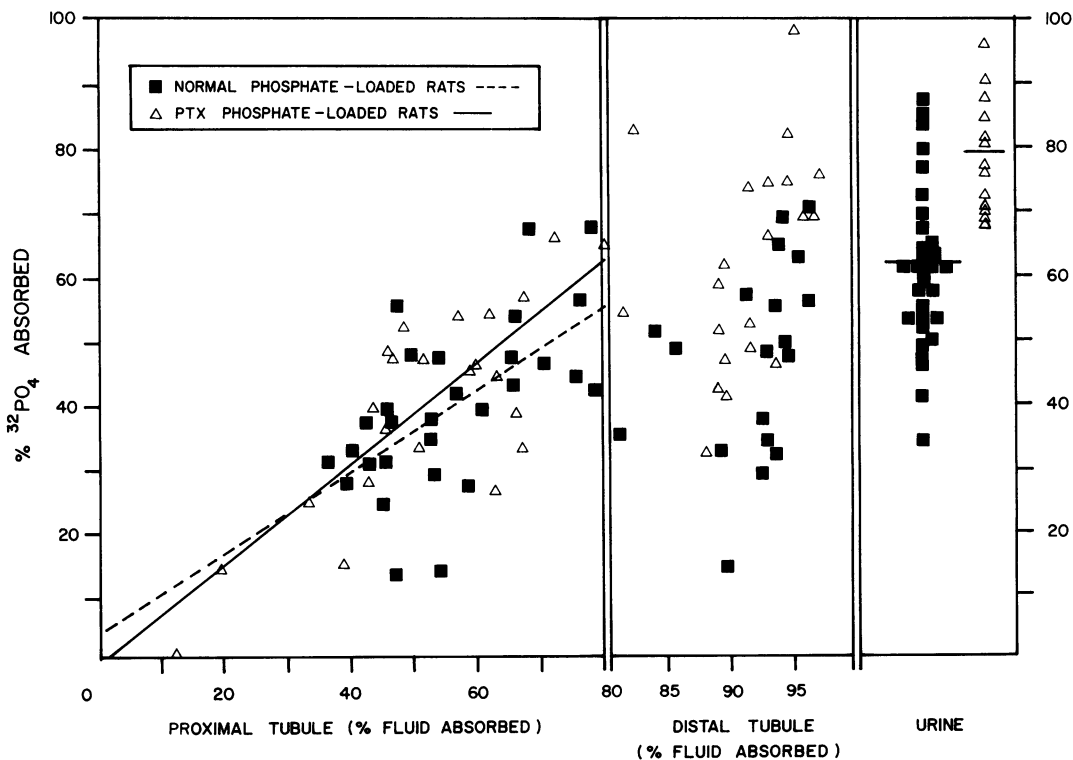


FIGURE 4 Percent $^{32}\text{PO}_4$ absorbed related to corresponding percent fluid absorbed in PTX phosphate-loaded rats. Data for normal phosphate-loaded rats from Fig. 2 are included to allow comparison. See text for linear regression equations.

In Fig. 5 is plotted a comparison of percent phosphate remaining in the distal tubular urine with that in the final urine ($EF_{^{32}PO_4}$) for all five groups. Because no reabsorption or secretion could be detected along the length of the distal convoluted tubule in any of the five groups, the mean value of all distal collections was used in each group. Similarly, the mean

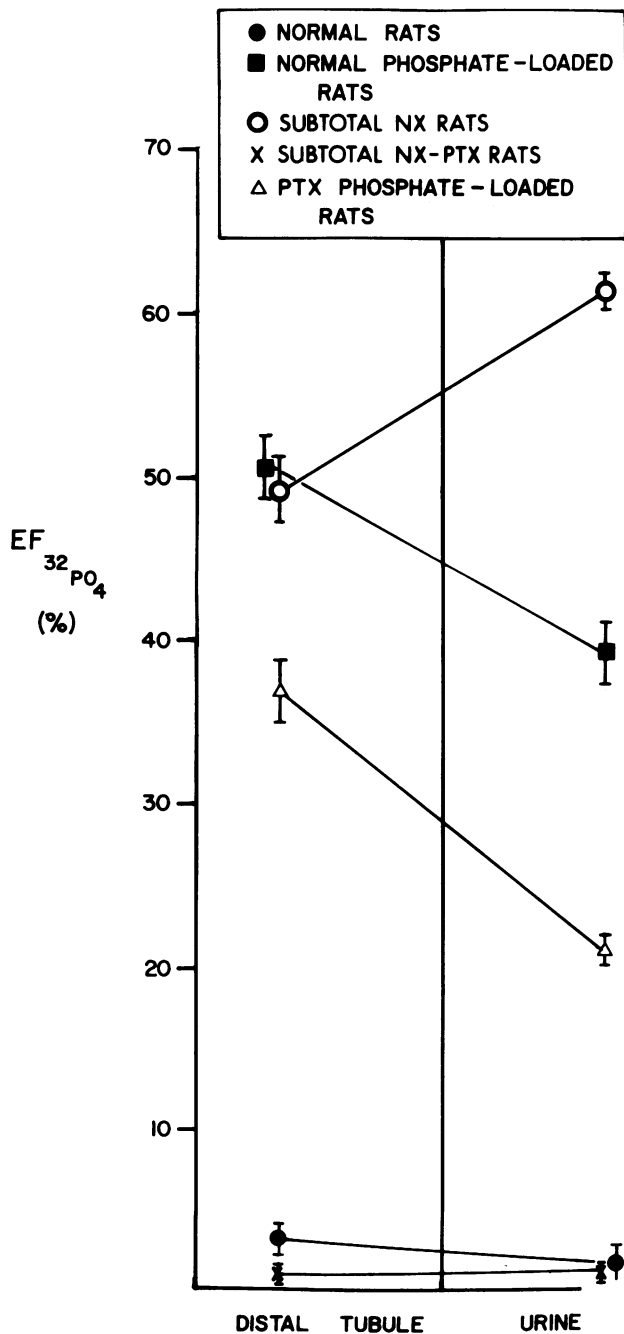


FIGURE 5 Percent of filtered $^{32}PO_4$ remaining in surface distal convoluted tubules and final urine in five groups of rats. Vertical lines = 1 SEM.

$EF_{^{32}PO_4}$ in the final urine of each group was used in this plot. As can be seen, there was less $^{32}PO_4$ in the final urine than in surface distal convoluted tubules in the normal group I rats, although the difference was only 4%. In the intact phosphate-loaded rats (group II), the difference between the surface distal tubules and final urine was larger, amounting to 12% of the large filtered load. In the PTX phosphate-loaded rats (group V), the difference between the surface distal tubules and the final urine was 15.6% of the filtered load. In contrast to these results, in the subtotal NX rats with secondary hyperparathyroidism, there was an average of 14.5% more phosphate in the final urine than in the surface distal convoluted tubules ($P < 0.01$). Acute PTX in NX rats reduced $EF_{^{32}PO_4}$ to very low levels, with only 2% remaining in the surface distal tubules and final urine.

To analyze the data for the distal tubules and final urine more closely, the individual values of paired distal tubular fluid and urine samples, collected simultaneously, are presented in Table IV for groups II and III. In the group II animals, in all but two paired collections, there was either less or the same amount of $^{32}PO_4$ in the final urine as in surface distal convoluted tubules. In the subtotal NX rats, in all but one paired collection, there was more $^{32}PO_4$ in the final urine than in surface distal convoluted tubules.

DISCUSSION

The results of the present study show that fractional reabsorption of filtered phosphate is markedly reduced in the proximal convoluted tubule of subtotal NX rats with chronic renal failure and secondary hyperpara-

TABLE IV
Percent $^{32}PO_4$ Remaining in Distal Tubules and Final Urine during Paired Collections for Group II and III Rats

Group II (intact phosphate-loaded)		Group III (subtotal NX)	
Distal tubule	Urine	Distal tubule	Urine
%	%	%	%
65.1	59.1	40.6	68.8
65.7	47.9	38.8	68.8
43.1	38.9	37.8	53.4
48.7	37.5	35.4	50.1
52.9	30.4	63.9	71.4
34.7	20.3	58.9	69.2
29.7	41.2	55.1	58.0
50.7	50.5	48.8	68.4
44.9	52.7	33.9	45.7
51.4	52.7	40.0	49.1
36.7	22.8	58.8	88.1
52.1	37.8	46.3	81.9
		70.4	66.4

thyroidism. In addition, only a small amount seemed to be reabsorbed between the last proximal convolutions and early distal tubule, or along the distal tubule itself, but wide scatter of the distal tubular data makes any conclusion about these segments uncertain. In every NX animal with hyperparathyroidism, more phosphate was observed in the final urine than was present in the distal tubules on the surface of the kidney (Table IV). This is consistent either with secretion of phosphate by the collecting ducts or with a disproportionately large contribution to urinary phosphate by deep nephrons (24). The present observations cannot distinguish between these two possibilities.

To examine the underlying mechanisms involved in the changes in transport, we investigated the possible roles played by increased filtered loads of phosphate per nephron, plasma phosphorus levels, PTH, and hypertrophy of the remaining nephrons. With regard to increased filtered loads of phosphate, it is clear from the elevated SNGFR and normal serum phosphorus levels in the group III rats that load of filtered phosphate per nephron was much higher in the NX rats than in normal rats. Infusion of neutral sodium phosphate into normal rats raised serum phosphorus without reducing SNGFR, and therefore increased the filtered load per nephron markedly. Calculation of load of filtered phosphorus per nephron yielded values of 5.71 ± 0.27 ng/min for group II, and 6.34 ± 0.57 ng/min for group III rats. Under these conditions of elevated filtered phosphate, fractional reabsorption of phosphate in the proximal tubule fell to almost identical values in these two groups (Figs. 2 and 3). TF/P_{32PO_4} ratios in the proximal tubule were consistently above 1.0 in both the NX rats and normal phosphate-loaded rats (Fig. 1), and rose progressively in the distal tubule in a manner indistinguishable from one another. Therefore, it seemed possible from these observations that the pattern of reabsorption of phosphate seen in the proximal and distal convoluted tubules of the NX rats could be accounted for by the high filtered load of phosphate per nephron. It should be pointed out that several important physiologic differences existed between the group II and III animals. First, SNGFR was much higher in the subtotal NX rats. Second, plasma phosphorus was normal in group III, but markedly elevated in the phosphate-loaded group II rats. Third, whole kidney GFR was 90% lower in group III than in group II, and presumably an "azotemic" state was present in group III. Fourth, proximal fractional water reabsorption was lower in group III than in group II (Table III). In spite of these various differences, proximal and distal tubular reabsorption of phosphate in superficial nephrons was indistinguishable between these two groups. It seems reasonable to assume, therefore,

that none of these factors in themselves played a critical role in determining the pattern of proximal and distal phosphate reabsorption, except the filtered load of phosphate.

However, it is clear from the findings in the NX-PTX rats (group IV) and the normal PTX phosphate-loaded rats (group V) that the filtered load of phosphate does not account entirely for the observations in the proximal tubule of the group III NX rats. Thus, after acute PTX in NX rats, proximal phosphate reabsorption increased markedly, even though the calculated filtered load per nephron was approximately the same as in the group III NX rats (6.10 ± 0.47 ng/min). In contrast, in the group V rats, with a calculated single nephron filtered load of phosphate of 6.19 ± 0.35 ng/min, acute PTX caused little or no increase in proximal phosphate reabsorption (Fig. 4). These observations suggest, therefore, that the hypertrophied proximal tubule has a greatly increased intrinsic capacity for phosphate reabsorption which is uncovered by acute removal of PTH. PTH apparently has a much greater effect on the hypertrophied proximal tubule than on the normal tubule to inhibit net phosphate reabsorption. It is also apparent from the observations that the large increase in proximal phosphate reabsorption in NX-PTX rats was not accompanied by any significant change in proximal sodium or fluid reabsorption (Table III). Thus, under these experimental conditions, proximal sodium and phosphate reabsorption were disassociated. Net phosphate reabsorption in the proximal tubule of NX rats seems to be determined by at least three factors: a high filtered load of phosphate; a greatly enhanced intrinsic capacity for phosphate transport; and an increased inhibitory action of PTH.

In addition to the decrease in fractional reabsorption of phosphate in the proximal tubule, the percent of $^{32}PO_4$ in the final urine of the NX rats with hyperparathyroidism was almost always greater than remained in the lumen of the distal tubules on the surface of the kidney (Fig. 5, Table IV). In the normal phosphate-loaded rats (group II), there was usually less phosphate in the urine than in the surface distal tubules. This difference between the two groups is all the more striking in view of the fact that plasma phosphate was much higher in the normal phosphate-loaded rats than in the NX rats. Such high plasma levels might be expected to promote secretion of phosphate (12, 24-27). Boudry et al. (12) and Knox et al. (24) found greater amounts of phosphate in the urine than in surface distal tubules of volume-expanded phosphate-loaded normal rats. Although we did not observe this in our normal phosphate-loaded rats, perhaps because they were not volume-expanded, the phenomenon was quite consistent in the group III NX rats.

Two possible explanations can be considered for the high urinary phosphate in the group III NX rats. One is that deeper nephrons contributed a disproportionately large amount of phosphate to the final urine, either because of a higher SNGFR than the superficial nephrons or to a greater inhibition of reabsorption. Knox et al. (24) compared phosphate in the tubular lumen of the ascending limb of Henle's loop of deep nephrons with that in surface distal convoluted tubules and final urine. They found that under the experimental conditions of volume expansion, phosphate loading, and PTH administration, deep nephrons contribute more phosphate to the collecting ducts than superficial nephrons. They suggested that finding more phosphate in the urine than in superficial distal tubules could be due to a disproportionately large contribution by deep nephrons. A second possibility is that addition of phosphate occurred along the collecting ducts of the hyperparathyroid NX rats. The presence of a renal secretory mechanism for phosphate has been a controversial subject for many years, but has been reported in a few clearance studies in phosphate-loaded normal dogs (25, 26) and man (27). Phosphate secretion has also been reported in human subjects with X-linked hypophosphatemia (28). Our observations are consistent with secretion by the collecting ducts under conditions of reduced renal mass and secondary hyperparathyroidism. However, the data cannot distinguish between collecting duct secretion and a disproportionately large contribution to the final urine by deep nephrons. It should be pointed out that acute PTX in NX rats completely abolished the difference between surface distal tubular phosphate and urinary phosphate (Fig. 3). This implies that PTX either equalized the contribution of superficial and deep nephrons to the final urine or that the hormone is necessary for secretion by the collecting ducts. Further studies are needed to clarify this point. In any case, PTH appears to play a central role in phosphate homeostasis in subtotal NX rats.

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