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Research Article

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Tracer Microinjection Study of Renal Tubular Phosphate Reabsorption in the Rat

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ABSTRACT To determine the sites of tubular phosphate reabsorption in the nephron, microinjection studies were undertaken, utilizing isotonic electrolyte solutions, containing either 1.4 or 8.0 mM phosphate and radioactive PO_4-^{33}P and inulin-^3H, in rats made mildly diuretic by infusion of mannitol. The injected sites were localized by the technique of latex dissection.

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The data indicate that: (*a*) no phosphate reabsorption occurs in the distal convoluted tubule or in the collecting duct, (*b*) phosphate efflux per unit tubular length is greater in the first one-third of the proximal tubule than in the remaining two-thirds, and (*c*) in the last two-thirds of the proximal tubule, absolute phosphate reabsorption is relatively small and might be limited by factors other than the amount or concentration of injected phosphate.

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INTRODUCTION

Previous investigations have provided conflicting conclusions concerning the site and nature of tubular phosphate reabsorption. It has been suggested that phosphate is reabsorbed mostly if not totally in the proximal tubule (1) whereas others have concluded that reabsorption may occur to some extent in the distal tubule as well (2). A micropuncture study by Amiel, Kuntziger, and Richet (2) additionally suggested that the bulk of proximal tubular phosphate reabsorption occurred early in the tubule. The present study was carried out in order to help resolve these controversies, utilizing the tracer microinjection technique in the proximal and distal tubules with solutions containing two phosphate concentrations, 1.4 mM (physiological) and 8.0 mM (supraphysiological), to vary the injected loads of phosphate.

The microinjection method has been used by other groups to study reabsorption of cations and other anions (3–9) but has not been previously applied to the study of phosphate reabsorption. When latex dissections of microinjected tubules accompany the determination of urinary recovery of the injected ions, precise and accurate localization of tubular reabsorptive processes may be obtained. Other investigators who have not used latex dissections, have localized injection sites by measuring lissamine green transit times or tubular fluid to plasma inulin ratios (2, 8). These two latter parameters have been shown to vary as functions of glomerular filtration rate and rate of sodium reabsorption, and are, therefore, less accurate indicators of tubular location than the direct measurement obtained with latex dissections (10).

METHODS

Experiments were performed on 38 female Wistar rats (150–250 g) anesthetized with Inactin, 100 mg/kg, intraperitoneally. PE 50 polyethylene catheters were placed in the left ureter, bladder, and left jugular vein. The left kidney was exposed through a flank incision and the rat was

placed on a heated table to maintain the rectal temperature between 37° and 38°C. The experimental kidney was covered with warm mineral oil (37°C), supported in a metal cup, and illuminated via a fiber optic light source.

10% mannitol in 0.9% saline was started at a rate of 0.1 ml/min, and after administration of 1% body weight, the rate of infusion was decreased to 0.05 ml/min for the remainder of the experiment. Urine was replaced with 0.45% saline at a rate equivalent to urine flow less 0.05 ml/min.

After an equilibration period of at least 30 min, when urine flow rate had stabilized, the microinjections were performed. The urine flow of the experimental kidney was always equal to or greater than 75% of that of the control kidney. Mean urine flows for all injection periods were 53.7 ±27.7 μl/min (mean±SD) and 59.2±33.7 μl/min, for the left and right kidneys, respectively.

Stock solutions containing 145–153 mEq/liter Na, 4 mEq/liter K, 10 mEq/liter HCO_3^-, 139 mEq/liter Cl^-, and either 1.4 or 8.0 mM phosphate were prepared, to 0.5 ml of which was added 250 μCi of inulin-^3H [1] and 25 μCi of $PO_4^{-33}P$.[1] The calculated phosphate concentration of the final injected solution was verified by electron probe microanalysis, utilizing the method of Lechene (11).

Eight droplets (7.3 nl) of labeled stock solutions were placed under oil in a siliconized Petri dish and collected into oil-filled micropipettes (tip diameter, 6–8 μ). Contents of two or three pipettes were counted directly to determine the ^3H and ^{33}P injected. The reproducibility of the volume of the droplets was ±0.5%.

Microinjection sites were selected with the aid of 5% lissamine green (0.05 ml, intravenously). The microdroplets were injected with care taken not to precede or follow the intratubular injection with any mineral oil. Mean±SD of the duration of injections was 35.9±10.1 sec.

To localize injection sites, the kidney containing the injected tubules filled with latex was digested in 6 N HCl for 24 hr. The dissected cast was projected onto the ground glass screen of a Nikon projection head (Nikon, Inc., Garden City, N. Y.), from which a tracing was made and tubular length measured with a metric map measure.

Urine was collected from the left kidney into 10 ml of Scintisol. One 1-min and two 15-sec collections were made before each injection for determination of background. After the injection, 20 15-sec collections, followed by 5 1-min collections were obtained from the experimental kidney. Two 5-min collections were made from the right kidney.

Four clearance periods in two rats prepared identically with those used for microinjection were obtained in order to assess the glomerular filtration rate. The mean left (experimental) and right (control) kidney glomerular filtration rates for all clearance periods were 0.90 ml/min and 1.04 ml/min, respectively. Left and right kidney urine flows were 41.6 and 43.1 μl/min, respectively, comparable with those obtained for the microinjected animals.

^{33}P and ^3H were determined with a model 3320 Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.) Corrections were made for background activity. Quench corrections for counting efficiency and correction for overlap of ^{33}P activity in the ^3H window were done by the channels ratio method. In order to obtain stability of our counting system, we found that the addition of 1 ml of distilled water allowed us to reproducibly count both ^{33}P and H^3 over a period of 2 months without shift in the pulse height analysis, quench curve equation, or recovery. The per cent recovery of ^{33}P (per cent $^{33}P'$) was calculated with the formula: Per cent $^{33}P'$ = [($^{33}P/^3H$)urine ÷ ($^{33}P/^3H$)injectate] × 100.

The inulin and phosphate excretion pattern was identical in both proximal and distal tubules. Time of recovery for 50% of the injected inulin was 2.70±1.36 min (mean±SD) and 1.63±0.64 min for proximal and distal injections respectively. There was no detectable delayed recovery of the ^{33}P for both proximal and distal tubular injections. There was no measureable ^{33}P or ^3H excreted by the right kidney unless inulin-^3H recovery from the left kidney was less than 90% of the injected amount. Only injections with ^3H recovery greater than 93% were considered.

All samples were counted to a minimum of 3× background, the samples containing 95% of the excreted radioactivity being counted to a minimum of 5000 counts. The Student's t test was employed for evaluation of statistical significance and the regression equations were determined by the method of least squares.

RESULTS

Proximal tubular injections. Inulin recoveries for all injections at both phosphate concentrations used were always greater than 93% (mean±SD = 95.6±1.7% ^3H recovery). Figs. 1 and 2 demonstrate that there is no apparent relationship between inulin-per cent ^3H recovery and per cent proximal tubular length (PTL).[2] The relationship between per cent ^{33}P recovery and per cent PTL for 17 microinjections of 1.4 mM phosphate solution is shown in Fig. 1. There is a significant correlation between per cent ^{33}P recovery and per cent PTL. The regression equation is $y = 1.28\ x + 2.34$, $r = 0.608$, $P < 0.01$). This correlation is still significant ($r = 0.5$, $P < 0.05$) despite the elimination of the two lowest points in Fig. 1. This relationship indicates almost complete recovery (> 90%) of phosphate injected beyond 70% PTL.

Fig. 2 shows the corresponding relationships for the 50 microinjections of 8.0 mM phosphate (a concentration chosen to simulate large phosphate delivery). There is a smooth curvilinear relationship between per cent ^{33}P recovery and per cent PTL. This curve, which exhibits a rapid rate of rise early in the proximal tubule, shows a sharp decrease in slope beyond 30% PTL. The curve was arbitrarily divided at that point into two segments which were analyzed separately. Regression analysis for both populations (0–30% PTL and 31–70% PTL) indicates significant correlations in both cases. For 0–30% PTL, $y = 1.23\ x \pm 45.01$, $r = 0.587$, $P < 0.001$. For 31–70% PTL, $y = 0.33\ x + 72.32$, $r = 0.478$, $P < 0.02$. The difference between the slopes of these two regression lines is statistically significant ($P < 0.01$).

Since the volume, phosphate concentration, and per cent radioactive phosphate recovery were known for all

[1] New England Nuclear Corp., Boston, Mass.

[2] *Abbreviations used in this paper:* PTL, proximal tubular length; $(TF/UF)_{PO_4}$, tubular fluid/ultrafiltrate phosphate concentration ratio.

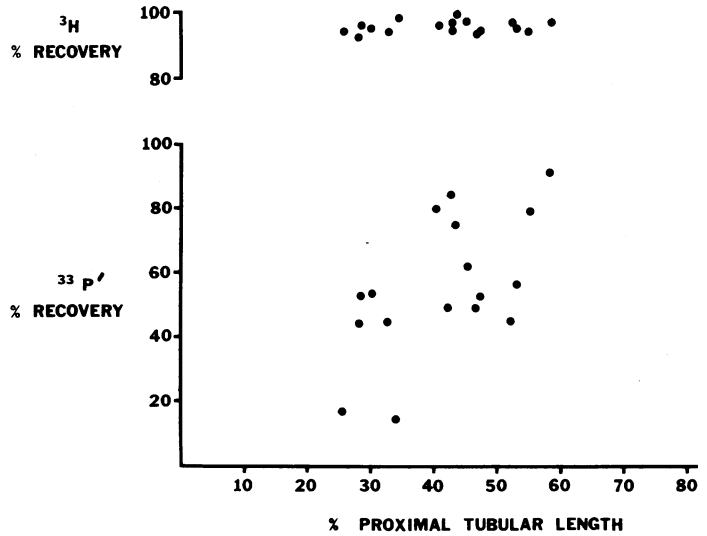

FIGURE 1 Relationship between proximal tubular length and inulin-^{3}H and $^{33}P'$ recoveries for 1.4 mM phosphate injections. Per cent $^{33}P'$ recovery is per cent ^{33}P recovery corrected for ^{3}H recovery. See text.

injections, the absolute reabsorption of injected phosphate could be calculated. Fig. 3 shows the relationship between the absolute reabsorption of phosphate and per cent PTL. By inspection, there does not seem to be a difference in the amount of phosphate reabsorbed from both concentrations injected beyond 25% PTL. This obtains despite nearly a sixfold difference between the two concentrations and, consequently, between the amounts of phosphate injected. We should point out, however, that although we could find no statistical difference in ab-

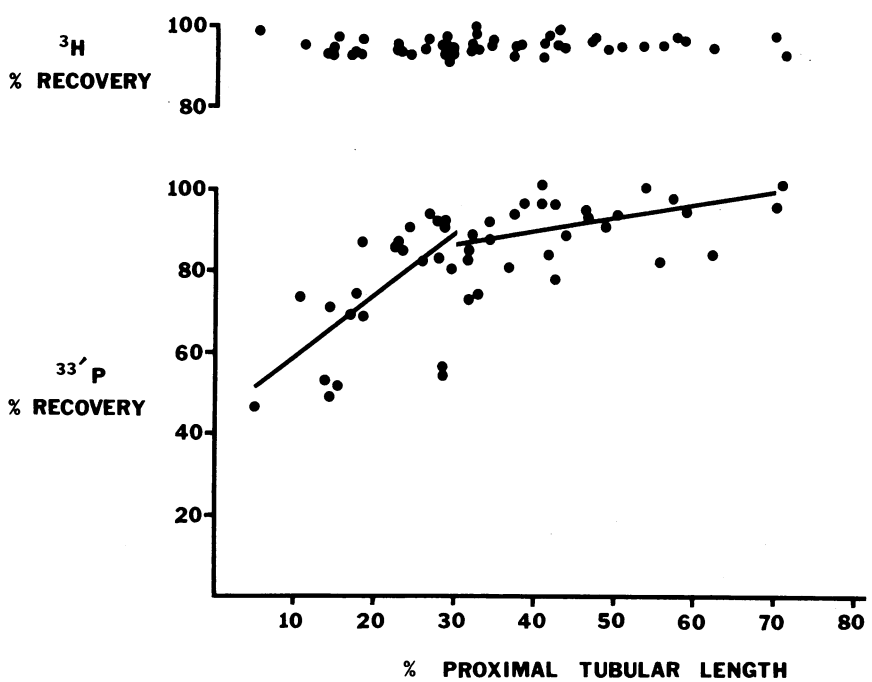

FIGURE 2 Relationship between proximal tubular length and inulin-^{3}H and $^{33}P'$ recoveries for 8.0 mM phosphate injections. Per cent $^{33}P'$ recovery is per cent ^{33}P recovery corrected for ^{3}H recovery. See text.

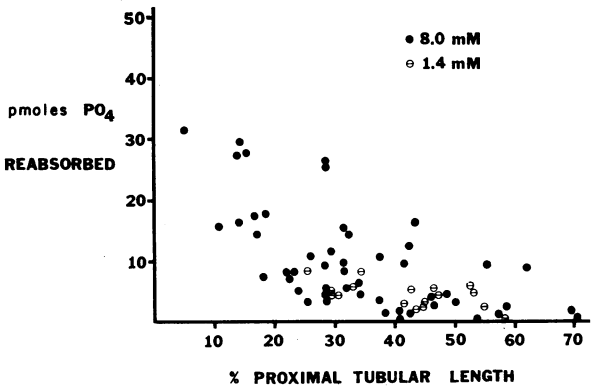

FIGURE 3 Relationship between proximal tubular length and absolute reabsorption of injected phosphate for both 1.4 and 8.0 mM phosphate injections.

solute phosphate reabsorption between the high and low phosphate injections, there is inherent in these calculations a high degree of potential error because the calculations of reabsorption depend on subtraction of a large number of counts from the total injected. Thus although the data suggest no difference in absolute reabsorption between the two injections, they do not absolutely support this conclusion.

Distal tubular injections. Inulin recoveries for microinjections of both phosphate concentrations were always greater than 93% (mean±SD = 99.0±2.3 per cent ^{3}H-recovery), and there was no relationship between inulin-^{3}H recovery and per cent distal tubular length (Fig. 4). The results of eight distal tubular microinjections of 1.4 mM phosphate and nine injections of 8.0 mM

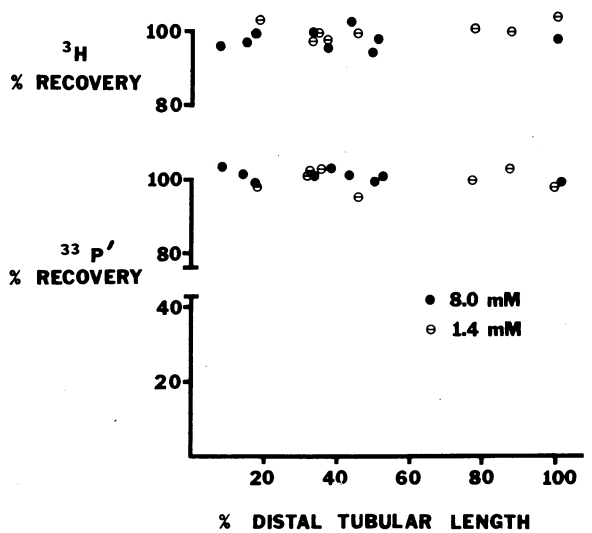

FIGURE 4 Relationship between distal tubular length and inulin-^{3}H and ^{33}P′ recoveries for both 1.4 and 8.0 mM phosphate injections. Per cent ^{33}P′ recovery is per cent P^{33} recovery corrected for ^{3}H recovery. See text.

phosphate show that ^{33}P recovery was complete along the entire length of the distal tubule for all injections.

DISCUSSION

The results of injections of both concentrations into distal tubules indicate that virtually no phosphate efflux occurs distal to the macula densa. Since the intratubular administration of 8.0 mM phosphate corresponds to a tubular fluid/ultrafiltrate phosphate concentration ratio $(TF/UF)_{PO_4}$ of 4, an extremely low permeability to phosphate for the distal tubule is demonstrated. This supports the earlier suggestions of Strickler, Thompson, Klose, and Giebisch (1) that phosphate reabsorption is largely a proximal tubular phenomenon. Since we can see no evidence of any phosphate efflux distally, we may conclude that no measureable net phosphate reabsorption occurs in the distal tubule or collecting ducts of the superficial tubules of the rat. Consequently, phosphate reabsorption detected by proximal tubular microinjections may be ascribed to efflux in the portion of the proximal tubule beyond the site of injection and in the loop of Henle.

Injections of physiological concentrations (1.4 mM) of phosphate along the length of the proximal tubule indicate that approximately 90% of the injected ^{33}P is recovered from injections early in the last third of the proximal tubule (Fig. 1). Extrapolation of this relationship between ^{33}P recovery and proximal tubular length indicates that if phosphate efflux were to continue at the same rate per unit tubular length, then phosphate efflux would be essentially completed by the time the tubular fluid reached the last quarter of the proximal tubule. This indicates, therefore, that little phosphate reabsorption occurs in the last 25% of the proximal tubule and in the loop of Henle.

In Fig. 3 is illustrated the relationship between ^{33}P recovery and proximal tubular length for the 8.0 mM phosphate injections. The points appear to lie on a smooth curve of progressively decreasing slope. The slopes of the two arbitrarily selected regression lines are a function of the efflux per unit tubular length. The difference in the slopes of these two lines indicates a quantitative difference in the manner in which the injected, and presumably, the filtered phosphate is handled in these two segments of the proximal tubule. The conclusion to be drawn from these data is that the rate of efflux, and probably, the rate of net reabsorption is grossly higher in the first third than in the middle third of the proximal tubule.

If the rate of ^{33}P efflux in different areas of the tubule can be considered to be indicative of the rate of net reabsorption of phosphate, then the data presented here can provide some useful information concerning the role of different areas of the tubule in normal phos-

phate reabsorption. Efflux of ^{33}P injected into the last quarter of the proximal tubule appears to be less than 10%. Assuming a $(TF/UF)_{PO_4} \cong 0.6$ and a tubular fluid/plasma inulin ratio $\cong 2.5$–3.0 in the last accessible convolutions of the superficial proximal tubule, the amount of filtered phosphate remaining in the tubule at this point should not exceed 30%. If total efflux from this point on were to be 10%, the reabsorption of phosphate which would occur in the final quarter of the proximal tubule and in the loop of Henle would be less than 3% of the filtered load. Therefore, at most, a small fraction of the total phosphate reabsorption occurs in the last 25% of the proximal tubule and the loop of Henle.

Analysis of absolute total efflux of injected phosphate suggests that phosphate reabsorption may be independent of concentration or delivery over the last three-quarters of the proximal tubule (Fig. 3). The amount reabsorbed appears to be a function of tubular length but is independent of sixfold differences in injected phosphate concentrations or injected load of phosphate. It is possible that phosphate reabsorption in this area of the tubule is coupled to Na reabsorption in a molar ratio specific for any given state of sodium reabsorption. This may explain the observations of others that phosphate reabsorption is T_m-limited (1) and variable for different states of sodium reabsorption (12).

With regard to the mode of proximal tubular phosphate reabsorption, our data reveal that the largest fraction of phosphate efflux occurs early in the tubule where the efflux per unit tubular length ("permeability") is highest. Later in the tubule, as the phosphate "permeability" falls, phosphate reabsorption continues in close relationship to sodium reabsorption. These observations are compatible with free flow micropuncture data on proximal tubular phosphate reabsorption obtained from the dog in this laboratory (13), and from the rat (2) suggesting that the differences in ^{33}P efflux between the early and late proximal tubule are indicative of the pattern of net phosphate reabsorption in the proximal tubule.

Some investigators have suggested that a capacity for distal phosphate reabsorption exists and is "stimulated" by saline loading (2, 14). Our data do not support this concept of increased distal phosphate reabsorption secondary to the increase in distal delivery of phosphate which would occur during saline loading, since increase in delivery produced in our experiments by increased injectate phosphate concentration was not associated with an increase in reabsorption.

It appears, therefore, that the ability of the tubule to reabsorb phosphate decreases markedly as fluid travels down the tubule. It is clear (*a*) that fractional efflux of phosphate decreases with increasing distance along the proximal tubule, (*b*) the ability of the last 25% of the proximal tubule and the loop of Henle to reabsorb phosphate is small, and (*c*) the distal tubule and collecting duct can reabsorb essentially no phosphate. Additionally, it is suggested that absolute phosphate reabsorption in the last 75% of the proximal tubule is independent of concentration and delivery of phosphate.

Addendum. A preliminary report utilizing the tracer microinjection technique to study phosphate reabsorption in the rat nephron has recently been presented (15). Although there were some quantitative differences between the data in this report and our data, the authors concluded (similar to us) that the bulk of phosphate reabsorption occurs in the proximal tubule and that there is little or no phosphate efflux in the distal-convoluted tubule and collecting duct.

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