# Micropuncture Studies of Phosphate Transport in the Proximal Tubule of the Dog

## THE RELATIONSHIP TO SODIUM REABSORPTION

SUNG-FENG WEN

*From the Nephrology Program and the Department of Medicine, University of Wisconsin Center for Health Sciences, Madison, Wisconsin 53706*

ABSTRACT Micropuncture studies were performed in the dog to examine the relationship between sodium and phosphate transport in the proximal tubule. In hydropenic, thyroparathyroidectomized animals, administration of parathyroid extract, saline, or acetazolamide resulted in a fall in proximal tubule fluid-to-plasma (TF/P) inulin ratio as well as a rise in tubule fluid-to-plasma ultrafilterable (TF/UF) phosphate ratio. A correlation was found between the changes in fractional reabsorption of sodium and phosphate but the phosphate changes were generally greater than those of sodium. Also, a high distal phosphate delivery in the face of low fractional excretion of phosphate in the urine in thyroparathyroidectomized dogs suggests significant phosphate reabsorption in the distal nephron. On the other hand, calcium chloride infusion to saline-loaded, normal dogs to suppress endogenous parathyroid hormone reduced proximal TF/UF phosphate without change in TF/P inulin, while both parameters remained unchanged in saline-loaded, thyroparathyroidectomized dogs after calcium infusion. An increase in proximal TF/UF phosphate associated with unchanged TF/P inulin was also demonstrated by administration of highly purified parathyroid hormone to saline-loaded, thyroparathyroidectomized dogs. It was concluded that although proximal tubule phosphate transport is generally closely related to that of sodium, the two can dissociate under certain experimental conditions, especially under the influence of parathyroid hormone. These observations also indicate that the effect of parathyroid hormone on proximal tubule phosphate transport is not solely dependent upon its effect on sodium transport.

This work was presented in part at the National Meeting of the American Federation for Clinical Research in Atlantic City, New Jersey, April, 1972, and published in abstract form in *Clin. Res.* **20**: 616, 1972.

*Received for publication 14 February 1973 and in revised form 16 July 1973.*

## INTRODUCTION

Renal tubule handling of inorganic phosphate has been studied by clearance methods (1–8) and to a limited extent by micropuncture techniques (9–14). The available information in the literature favors the concept that filtered phosphate is reabsorbed almost exclusively by the proximal tubule and excreted in the urine with little further modification (9). This has led to the use of phosphate clearance as an indicator for proximal tubule transport (15, 16). On the other hand, evidence for distal phosphate reabsorption has been reported (11).

It has also been demonstrated that a correlation exists between phosphate and sodium reabsorption since the maneuvers to inhibit sodium reabsorption, such as extracellular volume expansion or administration of diuretics, lead to phosphaturia (5–8, 17). As previous micropuncture studies have shown that parathyroid hormone, a potent phosphaturic agent, inhibits proximal tubule sodium as well as phosphate reabsorption (12, 13, 18), it has been suggested that proximal tubule phosphate transport may be dependent on reabsorption of sodium in the same segment, and the dissociation in the transport of the two ions occurs only in the distal nephron, where phosphate is presumably poorly reabsorbed (12, 19).

Our present micropuncture studies were undertaken to evaluate the relationship between phosphate and sodium transport in the proximal tubule in thyroparathyroidectomized (TPTX)[1] dogs after various maneuvers such as infusion of parathyroid extract, saline and acetazolamide to inhibit phosphate reabsorption. Also, proximal tubule phosphate transport was studied after maneuvers

[1] *Abbreviations used in this paper:* FE, fractional excretion; FR, fractional reabsorption; GFR, glomerular filtration rate; P, plasma; PAH, para-aminohippurate; PTX, parathyroidectomized; TF, tubule fluid; TPTX, thyroparathyroidectomized; UF, plasma ultrafiltrate.

designed to change the level of parathyroid hormone in saline-loaded animals, such as infusion of calcium chloride and administration of exogenous parathyroid hormone. These experiments were intended to examine whether a dissociation between the proximal tubule transport of the two ions could be demonstrated when sodium reabsorption by this segment was already inhibited. Our results indicate that although proximal tubule phosphate transport generally parallels that of sodium, the two processes can be dissociated under certain experimental conditions, especially under the influence of parathyroid hormone. The data also suggest that a significant fraction of filtered phosphate is reabsorbed in the segment distal to the proximal tubule in the dog nephron in the absence of parathyroid hormone.

## METHODS

Micropuncture studies were performed in 35 mongrel dogs of either sex weighing 12–20 kg. Animals were maintained on and allowed free access to regular dog food, which contained phosphate amounting to 100 mmol/100 g. The average dietary phosphate intake was therefore as high as 100–300 mmol/day. Water was withheld the night before the micropuncture experiments. 1–3 days before the studies, the thyroid and parathyroid glands were exposed and identified by midline neck incision, and these were completely removed. In order to prevent the development of tetany, the animals were then placed on high calcium intake by addition of calcium carbonate to the dog food, and whenever necessary, parenteral calcium gluconate was administered for correction of tetany. The effectiveness of thyroparathyroidectomy was confirmed by the development of hypocalcemia. Recollection micropuncture studies were carried out in six groups of animals.

*Group 1.* In six TPTX dogs, micropuncture experiments were performed during the first phase of hydropenia, followed by the second phase with i.v. administration of parathyroid extract (Parathormone, Eli Lilly & Co., Indianapolis, Ind.). Parathyroid extract was given as a priming dose of 150 U i.v. and this was followed by a sustaining infusion of the same dose per hour.

*Group 2.* Recollection micropuncture studies were performed in six TPTX dogs during hydropenia and after expansion of the extracellular fluid volume to 5% of body weight. The extracellular volume expansion was carried out by i.v. infusion of 0.85% saline at a rate of 24 ml/min until the infused volume reached 5% of body weight, and thereafter saline was infused at an appropriate rate to replace urinary losses.

*Group 3.* Six TPTX dogs were studied in hydropenia and after i.v. administration of 20 mg/kg acetazolamide. A sustaining infusion of acetazolamide at 20 mg/kg/h was also continued throughout the second phase.

*Group 4.* In six normal dogs with intact parathyroid glands, 0.85% saline was infused at 24 ml/min in the first phase to expand the extracellular volume to 5% body weight. In the second phase, isotonic calcium chloride solution was infused at a rate of 40 μeq/kg/min for 30 min, before a sustaining infusion at 10 μeq/kg/min. Another 30–45 min were allowed for equilibration before tubule fluid (TF) collections were resumed.

*Group 5.* Micropuncture studies were performed in five saline-loaded, TPTX dogs following the same protocol as in Group 4.

*Group 6.* In six TPTX dogs, 0.85% saline was infused to 5% body weight in the first phase and highly purified parathyroid hormone (Wilson Laboratories, Chicago, Ill.) 150 U was administered i.v. in the second phase. This was followed by a sustaining infusion at 100 U/h.

In all groups, isotonic saline was also infused as necessary in each phase to replace urine losses.

Dogs were prepared for micropuncture experiments with i.v. pentobarbital anesthesia 30 mg/kg, sustained by injection of small doses as necessary. An endotracheal tube was inserted to maintain adequate respiration by a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.). The jugular, foreleg, and femoral veins were cannulated for infusion of fluid and collection of blood samples. The bladder was exposed by suprapubic incision and both ureters were catheterized for collection of urine. The left kidney was exposed by a flank incision, dissected free of perirenal tissue, and immobilized with a Lucite kidney holder. The main renal artery of the same kidney was punctured with a gauge 27 needle connected to PE 20 polyethylene tubing for injection of lissamine green. A small area of renal capsule (about 1 $cm^2$) was carefully removed to expose the surface tubule structures, which were covered with a continuous drip of mineral oil to prevent evaporation. Under direct visualization with a stereomicroscope, the surface tubules were punctured with oil-filled, glass capillary micropipettes with a Leitz micromanipulator (E. Leitz, Inc., Rockleigh, N. J.). These micropipettes were made by pulling the glass capillary tubing with a micropipette puller (Industrial Science Associates, Inc., Ridgewood, N. Y.) and grinding the tip to about 10 μm in diameter. A long block of castor oil colored with Sudan black was maintained immediately distal to the puncture site to prevent retrograde collection of TF. Gentle suction was applied to initiate the TF collection. The late or the last accessible segments of the proximal tubule were identified by injecting 5% lissamine green 0.1–0.2 ml into the renal artery and timing its appearance at the surface tubules. The late proximal tubule sites were also confirmed by injection into the tubule of a small droplet of colored oil, which either disappeared immediately without reappearing or traveled only a short segment of the surface tubules before disappearing. Recollection micropuncture technique was employed to obtain TF samples at the same puncture site before and after experimental maneuvers. Clearance collection of urine with 15-min periods was carried out throughout the experiments and blood samples were drawn from the femoral vein at the midpoint of each period. Plasma ultrafiltrate (UF) was obtained in two to three plasma samples in each experimental phase by centrifugation of the plasma in collodion bags (20) with optimal pH control. The ultrafilterable fractions of divalent ions in the plasma by this method were 60.1±1.3% (SE) for calcium and 105±2% (SE) for phosphate. A Technicon AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) was used for determination of inulin, paraaminohippurate (PAH) and phosphate in the plasma and urine. Modification of the resorcinol method was applied for inulin (21), ethylenediamine method for PAH (22), and colorimetric method with stannous chloride-hydrazine for phosphate (23). Calcium was measured by atomic absorption spectrophotometry (Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.) and sodium by flame pho-

tometry (Instrumentation Laboratory, Inc., Lexington, Mass.).

Inulin concentration in the TF was determined by modification of ultramicro-fluorometric method of Vurek and Pegram (24) with an AMINCO fluoromicrophotometer (American Instrument Co., Inc., Silver Spring, Md.). Phosphate in the TF was determined by a modification of the microcolorimetric method by Chen, Toribara, and Warner (25). This was carried out by preparation of Reagent C, containing 0.8 N sulfuric acid. 0.25% ammonium molybdate, and 1% ascorbic acid. 5 $\mu$l of Reagent C was placed in a 50 $\mu$l constant-bore glass capillary (Microcap Disposable Micropipettes, Drummond Scientific Corp., Broomall, Pa.) to which 20 nl of TF sample was added. The ends of the Microcap were flame-sealed (with one end tapered to facilitate transfer of the solution), and the solution was mixed by centrifugation in each direction several times and incubated at 37°C for 90 min. The sealed ends of the Microcap were then broken and the reagent-sample mixture was transferred to fill up a 5-$\mu$l glass capillary, made by cutting the same Microcap at 12 mm length. This was placed in an AMINO fiber optic microcolorimeter with both ends of the glass capillary in contact with fiber optic rods, which channeled light longitudinally through the solution-filled capillary and out to a photomultiplier. The optical density of the solution was read at 820 nm against the reagent blank and slight variations in the blank value were minimized by using the same glass capillary for all readings. With this technique, phosphate concentration in 20-nl volumes of 24 human plasma samples was determined after precipitation of plasma protein with 10% trichloroacetic acid. These results ($A$) at the concentration range of 0.54–3.22 mmol/liter were compared with those simultaneously determined by the Auto-Analyzer method with stannous chloride ($B$). The ratio $A/B$ had a mean of 0.99±0.06 SD indicating good correlation between the two methods. A similar study using 18 plasma UF yielded a mean $A/B$ ratio of 0.98±0.07 SD. The presence of lissamine green and inulin in high concentrations, as was encountered in the urine samples of our micropuncture experiments, did not interfere with phosphate determination by the two methods. High calcium levels in the samples up to 15 meq/liter also did not alter phosphate recovery by these methods.

In 20 pairs of recollected and control proximal TF samples in TPTX dogs during hydropenia, mean ratio of the two $TF/P_2$ to $TF/P_1$ was 0.99±0.10 SD, and that of phosphate ratios $TF/UF_2$ to $TF/UF_1$ 1.01±0.10 SD in 23 pairs. During saline diuresis, these values were 1.01±0.09 SD for inulin in 22 pairs and 0.99±0.11 SD for phosphate in 18 pairs of recollected samples.

Plasma values for inulin and sodium, and those in the plasma UF for phosphate, were used for clearance calculations without correction for Donnan distribution. Both micropuncture and clearance data were analyzed statistically by Student's $t$ test (26) for comparison of paired mean values per animal for the two experimental phases.

## RESULTS

*Effect of phosphaturic maneuvers in hydropenic, TPTX dogs.* The relationship between renal transport of phosphate and sodium was studied in 18 hydropenic TPTX dogs before and after enhancement of phosphate excretion by administration of parathyroid extract, isotonic saline, or acetazolamide. The clearance data of the micropunctured kidney in these experiments are summarized in Table I. Mean glomerular filtration rate (GFR) remained unchanged after parathyroid extract and saline but was reduced significantly by 29% after

TABLE I

*Clearance Data in Hydropenic, TPTX Dogs before and after Phosphaturic Maneuvers*

| Exp group | Exp phase | GFR | $UFC_a$ | $UFP_{O_4}$ | $U_{PO_4}V$ | $FE_{PO_4}$ | $U_{Na}V$ | $FE_{Na}$ |
|---|---|---|---|---|---|---|---|---|
| | | *ml/min* | *meq/liter* | *mmol/liter* | *μmol/min* | % | *μeq/min* | % |
| PTE (6) | C | 31.0 | 1.7 | 1.78 | 1.5 | 2.9 | 43 | 0.85 |
| | | ±6.0 | ±0.1 | ±0.13 | ±0.3 | ±0.7 | ±17 | ±0.22 |
| | E | 32.2 | 1.7 | 1.71 | 13.9‡ | 26.4‡ | 56 | 1.11 |
| | | ±6.3 | ±0.1 | ±0.13 | ±3.1 | ±4.4 | ±21 | ±0.25 |
| SAL (6) | C | 34.8 | 1.7 | 2.51 | 4.5 | 4.5 | 26 | 0.55 |
| | | ±4.8 | ±0.2 | ±0.23 | ±1.6 | ±1.2 | ±6 | ±0.12 |
| | E | 32.9 | 1.4* | 2.19* | 11.8‡ | 18.2‡ | 249‡ | 5.72‡ |
| | | ±5.0 | ±0.2 | ±0.18 | ±2.1 | ±3.8 | ±32 | ±1.13 |
| AZE (6) | C | 34.8 | 2.0 | 2.13 | 4.6 | 5.5 | 25 | 0.48 |
| | | ±3.7 | ±0.3 | ±0.10 | ±1.9 | ±2.0 | ±5 | ±0.08 |
| | E | 24.8‡ | 1.9 | 2.32 | 18.1‡ | 31.2‡ | 153‡ | 4.62‡ |
| | | ±4.1 | ±0.3 | ±0.14 | ±3.6 | ±3.6 | ±18 | ±0.52 |

Values are mean±SEM. Parentheses denote number of dogs.

Abbreviations: AZE, acetazolamide; C, control phase; E, experimental phase; PTE, parathyroid extract; SAL, saline; $U_{Na}V$, sodium excretion; $U_{PO_4}V$, phosphate excretion.

* $P < 0.05$.

‡ $P < 0.01$.

acetazolamide. Mean UF phosphate was slightly reduced only after saline infusion. Mean UF calcium was low in all three groups, indicating effective parathyroidectomy. Prominent phosphaturia occurred in all groups with mean fractional excretion (FE) of phosphate increasing from 2.9 to 26.4% after parathyroid extract, from 4.5 to 18.2% after saline, and from 5.5 to 31.2% after acetazolamide. On the other hand, significant natriuresis was observed only after saline and acetazolamide with increases in mean FE sodium from 0.55 to 5.72% and from 0.48 to 4.62%, respectively. After parathyroid extract administration, mean FE sodium was unchanged at 0.85 and 1.11% ($P > 0.1$), indicating a dissociation of phosphaturic effect of parathyroid extract from that on sodium transport.

Proximal tubule micropuncture data before and after phosphaturic maneuvers in TPTX dogs for each experiment are shown in Table II. In groups of six animals each, mean proximal TF/UF phosphate increased from 0.69 in hydropenia to 0.92 after parathyroid extract, from 0.75 to 0.85 after saline, and from 0.78 to 0.97 after acetazolamide. The individual TF/UF phosphate values for all three groups of experiments are plotted against the corresponding TF/P inulin in Fig. 1. During hydropenia, all but two points are scattered below unity with the overall mean at 0.74, and appear to be unchanged at different levels of fluid reabsorption in the late proximal tubule. Mean proximal TF/P inulin in the three groups during hydropenia at 1.70, 1.67, and 1.71 were reduced significantly to 1.47 after parathyroid extract, 1.28 after saline, and 1.42 after acetazolamide (Table II). Fractional reabsorption(FR) of phosphate in the proximal tubule was reduced by 21–22% of the filtered load by the three maneuvers, while that of water

TABLE II

*Proximal Tubule Micropuncture Data in Hydropenic, TPTX Dogs before and after Phosphaturic Maneuvers*

| Dog No. | $n$ | $TF/UF_{PO_4}$ | | $TF/P_{Inulin}$ | | $FR_{PO_4}$ | | $FR_{H_2O}$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | C | E | C | E | C | E | C | E |
| | | | | | | % | | % | |
| Parathyroid extract administration | | | | | | | | | |
| 12 | 5 | 0.70 | 0.97 | 2.14 | 1.49 | 67 | 35 | 53 | 33 |
| 14 | 2 | 0.58 | 0.75 | 1.31 | 1.21 | 56 | 38 | 24 | 17 |
| 15 | 5 | 0.53 | 0.74 | 1.46 | 1.37 | 64 | 46 | 32 | 27 |
| 16 | 7 | 0.96 | 1.16 | 1.87 | 1.72 | 49 | 36 | 47 | 42 |
| 18 | 6 | 0.77 | 0.93 | 1.48 | 1.32 | 48 | 30 | 32 | 24 |
| 56 | 4 | 0.62 | 0.98 | 1.91 | 1.73 | 68 | 43 | 48 | 42 |
| Mean | | 0.69 | 0.92‡ | 1.70 | 1.47* | 59 | 38‡ | 39 | 31* |
| ±SE | | 0.06 | 0.06 | 0.13 | 0.04 | 4 | 2 | 5 | 4 |
| Saline infusion | | | | | | | | | |
| 20 | 5 | 0.70 | 0.75 | 1.69 | 1.13 | 59 | 34 | 41 | 12 |
| 21 | 6 | 0.65 | 0.75 | 1.61 | 1.26 | 60 | 40 | 38 | 21 |
| 22 | 7 | 0.86 | 0.85 | 1.55 | 1.32 | 45 | 36 | 35 | 24 |
| 23 | 6 | 0.82 | 1.05 | 1.56 | 1.18 | 47 | 11 | 36 | 15 |
| 24 | 4 | 0.69 | 0.85 | 1.69 | 1.57 | 59 | 48 | 41 | 36 |
| 25 | 3 | 0.79 | 0.85 | 1.90 | 1.20 | 58 | 29 | 47 | 17 |
| Mean | | 0.75 | 0.85* | 1.67 | 1.28‡ | 55 | 33‡ | 40 | 21‡ |
| ±SE | | 0.03 | 0.04 | 0.05 | 0.06 | 5 | 5 | 2 | 3 |
| Acetazolamide administration | | | | | | | | | |
| 26 | 5 | 0.85 | 1.08 | 1.63 | 1.50 | 48 | 28 | 39 | 33 |
| 27 | 6 | 0.79 | 0.89 | 1.60 | 1.19 | 51 | 25 | 37 | 16 |
| 28 | 3 | 0.69 | 0.98 | 1.91 | 1.50 | 64 | 35 | 48 | 33 |
| 29 | 7 | 0.83 | 1.01 | 1.62 | 1.67 | 49 | 40 | 38 | 40 |
| 30 | 4 | 0.65 | 0.92 | 1.91 | 1.29 | 66 | 29 | 48 | 22 |
| 53 | 4 | 0.87 | 0.93 | 1.61 | 1.36 | 47 | 32 | 38 | 26 |
| Mean | | 0.78 | 0.97‡ | 1.71 | 1.42* | 54 | 32‡ | 41 | 28* |
| ±SE | | 0.04 | 0.03 | 0.06 | 0.07 | 3 | 2 | 2 | 4 |

Abbreviations: C, control phase; E, experimental phase.

* $P < 0.05$.

‡ $P < 0.01$.

was reduced by 8% after parathyroid extract, 19% after saline, and 13% after acetazolamide, respectively.

In Fig. 2, mean fractions of filtered phosphate reabsorbed at the late proximal tubule before and after the phosphaturic maneuvers are compared with the corresponding values at the level of final urine. During hydropenia, mean FR phosphate of 55–60% at the late proximal tubule in the three groups are markedly contrasted with the much higher values at the final urine at 95–97%, suggesting a significant further reabsorption of phosphate distal to the proximal tubule puncture site. After the three phosphaturic maneuvers, FR phosphate in the proximal tubule was reduced similarly by 20–25% of filtered load in all three groups and these effects were reflected in the similar reduction in FR phosphate at the urine level in parathyroid extract and acetazolamide groups. However, the phosphaturic effect of saline infusion was of considerably smaller magnitude than its effect in the proximal tubule (reduction in FR of 14% vs. 22%).

*Experiments in saline-loaded dogs.* Since the foregoing experiments demonstrated a correlation between changes in phosphate and sodium transport in the proximal tubule, additional studies were performed in saline-loaded dogs to examine whether changes in proximal tubule phosphate transport could occur under the conditions in which proximal tubule sodium reabsorption was already inhibited. In six normal dogs, after saline infusion to 5% body weight, recollection micropuncture experiments were carried out before and after infusion of calcium chloride to suppress endogenous parathyroid hormone. The results were compared with those of five saline-loaded, TPTX dogs studied under the same experimental protocol. The clearance data of these experiments are summarized in Table III. Mean GFR was reduced by 18–22% when mean UF calcium was increased by calcium chloride infusion from 2.7 to 4.7 meq/liter in the normal and from 1.9 to 4.2 meq/liter in the TPTX animals. Mean plasma phosphate increased prominently in the two groups, but as a result of reduction in ultrafilterable fraction of phosphate from 104–108% to 81–84% after calcium infusion, the increases in mean UF phosphate were slightly less prominent, from 1.50 to 2.01 mmol/liter and from 1.76 to 2.19 mmol/liter, respectively. The ultrafilterable fraction of phosphate remained stable during the entire phases of micropuncture. Mean FE phosphate in the normal dogs remained unchanged at 27.8 and 28.2%, while in the TPTX dogs, mean FE phosphate increased from 7.6 to 21.1%. Mean FE sodium also increased after calcium infusion in both groups of animals.

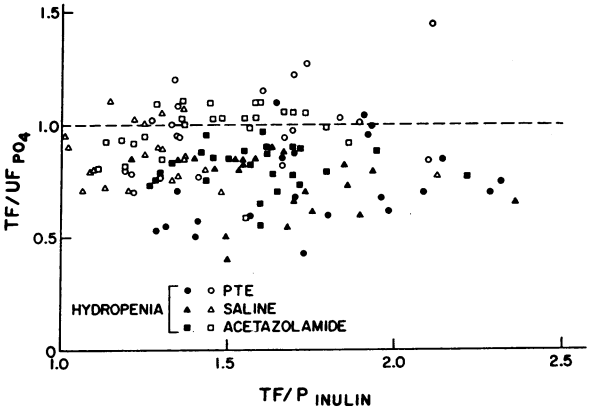


FIGURE 1 Proximal TF/UF phosphate ratios plotted against the corresponding TF/P inulin ratios before and after three phosphaturic maneuvers in hydropenic, TPTX dogs. Each point represents the values for an individual TF sample. PTE, parathyroid extract.

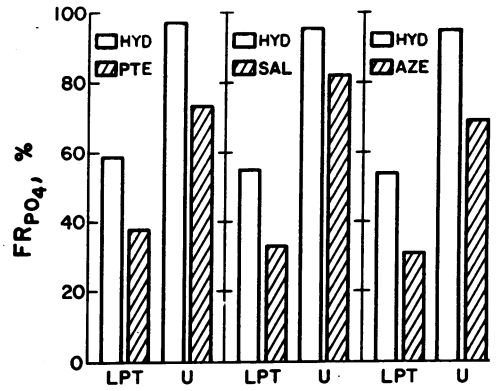


FIGURE 2 Mean FR phosphate at the late proximal tubule (LPT), and final urine (U) before and after phosphaturic maneuvers in hydropenic, TPTX dogs. AZE, acetazolamide; HYD, hydropenia; PTE, parathyroid extract; SAL, saline.

Proximal tubule micropuncture data are summarized in Table IV. Mean proximal TF/UF phosphate was reduced from 0.89 to 0.66 after calcium infusion in the normal group but mean TF/P inulin did not change significantly ($P > 0.1$). This increase in proximal tubule FR phosphate without corresponding change in sodium was mainly due to suppression of endogenous parathyroid hormone, since both proximal TF/UF phosphate and TF/P inulin remained unchanged in TPTX dogs under similar experimental conditions. The individual proximal TF/UF phosphate values before and after calcium infusion are plotted in Fig. 3, which illustrates the difference in response to calcium infusion in the two groups.

Fig. 4 compares the mean FR phosphate at the late proximal tubule and at the level of final urine before and after calcium infusion. In the saline-loaded normal dogs, mean FR phosphate at the late proximal tubule was increased by 18% of filtered load after calcium infusion, but remained unchanged at the final urine. In contrast,

TABLE III

*Summary of Clearance Data in Saline-Loaded Dogs*

| Dogs | Exp. phase | GFR | $C_{PAH}$ | $UFC_a$ | $P_{PO_4}$ | $UFP_{O_4}$ | $U_{PO_4}V$ | $FE_{PO_4}$ | $U_{Na}V$ | $FE_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *ml/min* | *ml/min* | *meq/liter* | *mmol/liter* | *mmol/liter* | *μmol/min* | *%* | *μeq/min* | *%* |
| Normal (6) | Saline | 45.1 | 125.0 | 2.7 | 1.39 | 1.50 | 18.2 | 27.8 | 240 | 3.84 |
| | | ±4.0 | ±12.3 | ±0.08 | ±0.17 | ±0.18 | ±4.4 | ±5.3 | ±11 | ±0.78 |
| | $CaCl_2$ | 34.6‡ | 74.5‡ | 4.7‡ | 2.39* | 2.01* | 18.9 | 28.2 | 270 | 5.59* |
| | | ±4.1 | ±7.7 | ±0.3 | ±0.22 | ±0.20 | ±3.3 | ±4.2 | ±40 | ±0.87 |
| TPTX (5) | Saline | 33.8 | 71.0 | 1.9 | 1.69 | 1.76 | 4.8 | 7.6 | 129 | 2.37 |
| | | ±3.9 | ±11.3 | ±0.2 | ±0.32 | ±0.34 | ±1.6 | ±0.2 | ±23 | ±1.11 |
| | $CaCl_2$ | 27.5* | 45.3* | 4.2‡ | 2.72* | 2.19* | 12.9‡ | 21.1‡ | 211 | 5.19* |
| | | ±2.7 | ±6.4 | ±0.2 | ±0.23 | ±0.19 | ±2.3 | ±0.2 | ±49 | ±1.24 |
| TPTX (6) | Saline | 41.3 | 96.9 | 1.9 | 1.43 | 1.50 | 7.6 | 10.2 | 289 | 4.26 |
| | | ±5.0 | ±10.2 | ±0.2 | ±0.20 | ±0.20 | ±4.3 | ±4.1 | ±72 | ±0.70 |
| | PTH | 42.2 | 98.4 | 1.9 | 1.33 | 1.39 | 20.3‡ | 36.4‡ | 339 | 4.86 |
| | | ±5.5 | ±17.2 | ±0.1 | ±0.17 | ±0.19 | ±4.5 | ±5.5 | ±79 | ±0.86 |

Values are mean±SEM. Parentheses denote number of dogs.
$C_{PAH}$, PAH clearance; PTH, parathyroid hormone; other abbreviations as in Table I.
* $P < 0.05$.
‡ $P < 0.01$.

in the saline-loaded, TPTX dogs, mean FR phosphate at the urine level was reduced by 14% of filtered load while that at the late proximal tubule remained unchanged after calcium infusion.

In order to further demonstrate the dissociation between phosphate and sodium transport in the proximal tubule, highly purified parathyroid hormone was administered to six saline-loaded, TPTX dogs, and the results are also summarized in Tables III and IV. Mean FE phosphate increased from 10.2 to 36.4% while mean GFR and mean FE sodium remained unchanged. There was a significant increase in mean proximal TF/UF phosphate from 0.81 to 1.01 without change in corresponding TF/P inulin.

TABLE IV

*Summary of Proximal Tubule Micropuncture Data in Saline-Loaded Dogs*

| Dogs | *n* | Exp. phase | $TF/UFP_{O_4}$ | $TF/P_{In}$ | $FR_{PO_4}$ | $FR_{H_2O}$ |
|---|---|---|---|---|---|---|
| | | | | | % | % |
| Normal (6) | 38 | Saline | 0.89 | 1.51 | 39 | 31 |
| | | | ±0.09 | ±0.12 | ±9 | ±6 |
| | | $CaCl_2$ | 0.66* | 1.59 | 57* | 35 |
| | | | ±0.07 | ±0.13 | ±6 | ±5 |
| TPTX (5) | 31 | Saline | 0.80 | 1.43 | 44 | 29 |
| | | | ±0.03 | ±0.07 | ±4 | ±4 |
| | | $CaCl_2$ | 0.82 | 1.49 | 45 | 32 |
| | | | ±0.05 | ±0.09 | ±7 | ±4 |
| TPTX (6) | 26 | Saline | 0.81 | 1.52 | 46 | 33 |
| | | | ±0.07 | ±0.09 | ±6 | ±4 |
| | | PTH | 1.01* | 1.54 | 32* | 33 |
| | | | ±0.13 | ±0.11 | ±10 | ±5 |

Values are mean ±SEM. Abbreviations as in Tables IV and V.
* $P < 0.05$.

*Comparison of phosphate and sodium transport in the proximal tubule.* Correlation between phosphate and sodium transport in the proximal tubule was evaluated by comparing changes in FR in this segment calculated from our micropuncture data. Mean increments in FR of the two ions for each experiment are plotted against each other in Fig. 5. The diagonal line represents one-to-one relationship for the two ions. In hydropenic, TPTX animals, indicated by closed symbols, there was a significant correlation ($r = 0.69$) between the transport changes of the two ions when their FR was reduced by administration of parathyroid extract, saline, or acetazolamide. However, all but three points are above the diagonal line, indicating greater effect of these maneuvers on FR phosphate than FR sodium. On the other hand, dissociation between phosphate and sodium reabsorption was demonstrated in the saline-loaded animals, indicated by open symbols, with increased FR phosphate without sodium changes in normal dogs after calcium infusion, and reduced phosphate transport with unchanged sodium reabsorption in TPTX dogs after parathyroid hormone.

## DISCUSSION

Available micropuncture studies in the literature (9–14) have shown that phosphate is reabsorbed by the proximal tubule in greater proportion than the corresponding

plasma (or plasma UF) level with mean proximal TF/P (or TF/UF) phosphate at about 0.6–0.7. This is in contrast to proximal tubule handling of major cations such as sodium (27, 28), potassium (27, 28), calcium (10, 29), and magnesium (30), which all have a proximal TF/P (or TF/UF) ratio of unity under various physiological and experimental conditions. Constancy of TF/P (or TF/UF) ratio implies that proximal tubule reabsorption of these cations takes place in parallel fashion and hence a good correlation between the transport of sodium and other cations should obtain. Indeed, FE calcium (31–33) and FE magnesium (32, 33) have been shown to be linearly related to that of sodium. A similar linear correlation between FE sodium and FE phosphate has also been demonstrated in normal (6) and TPTX (6, 17) animals. In our micropuncture studies, after administration of parathyroid extract, saline, or acetazolamide, the reduction in FR phosphate was generally proportional to but characteristically greater than that of sodium. This was born out by reduction in proximal TF/P inulin and increase in TF/UF phosphate with presumably unchanged TF/P sodium (28) after the three maneuvers. The mechanism by which correlation between sodium and phosphate transport occurs in the proximal tubule is not clear. Assuming an active sodium reabsorption as the major driving force for proximal tubule transport, reduction in sodium reabsorption may be followed by a similar reduction in the reabsorption of other ions, through diminished bulk flow across proximal tubule epithelium probably via intercellular channels (34). Under this condition, the proximal TF/P (or TF/UF) ratio of cations such as potassium (28), calcium (29), and magnesium (30) remains unchanged at unity whereas that of phosphate invariably increases. This increase in proximal TF/P (or TF/UF) phosphate after inhibition of sodium reabsorption, as was

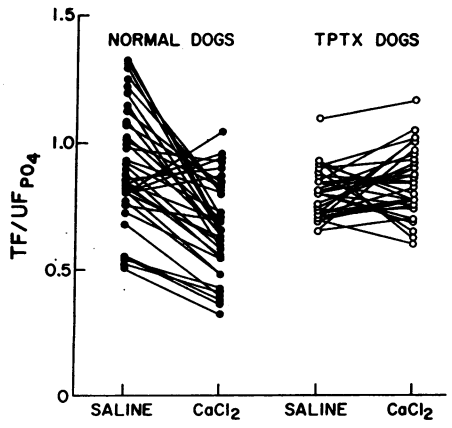


FIGURE 3 Proximal TF/UF phosphate ratios before and after calcium chloride infusion in saline-loaded dogs. The values for individual TF samples are plotted.

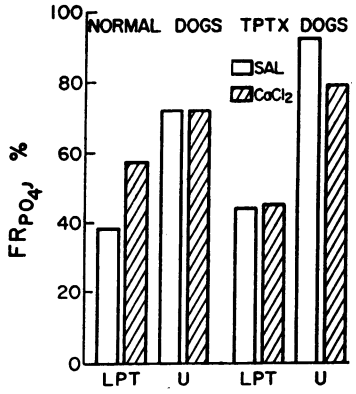


FIGURE 4 Mean FR phosphate at the late proximal tubule (LPT), and final urine (U) before and after calcium chloride infusion in saline-loaded dogs. SAL, saline.

demonstrated in the present studies, is not unexpected since phosphate in the proximal tubule reabsorbate is nearly always higher than that in the plasma (or plasma UF) and thus a reduction in bulk reabsorption should lead to increased TF/P (or TF/UF) phosphate. Although the alternative possibility of a direct coupling for sodium and phosphate transport in the proximal tubule cannot be excluded, such a fixed relationship has been questioned by Kuntziger, Amiel, and Gaudebout (35).

Our mean proximal TF/UF phosphate of 0.74 in hydropenic, TPTX dogs is comparable to the values reported in the intact dogs (12, 19) and rats (9, 10, 13) and much higher than those reported in parathyroidectomized (PTX) rats (13, 14) and dogs (36). Although the reason for the difference is not readily apparent, high dietary intake of phosphate in our dogs may be responsible for the higher proximal TF/UF phosphate in our studies, since reducing intestinal absorption of phosphate by administration of aluminum-hydroxide gel resulted in a lower mean proximal TF/UF phosphate of 0.45.[3] Furthermore, Van Stone and Hano (37) showed that urinary excretion of phosphate increased similarly on increased dietary phosphate in both intact and PTX rats, indicating the importance of dietary phosphate intake on renal phosphate transport. Although the longer duration of TPTX in our animals, as compared to acutely PTX state studied by others, may also be responsible for the higher proximal TF/UF phosphate, our micropuncture data in acutely TPTX dogs (38), as well as those by Amiel, Kuntziger, and Richet (11) in acutely PTX rats, demonstrated a similarly high proximal TF/UF phosphate. Whatever the reason for the relatively high proximal TF/UF phosphate, calculation of the fraction of filtered phosphate remaining at the end of proximal tubule in our studies, assuming end proximal

[3] Unpublished observation.

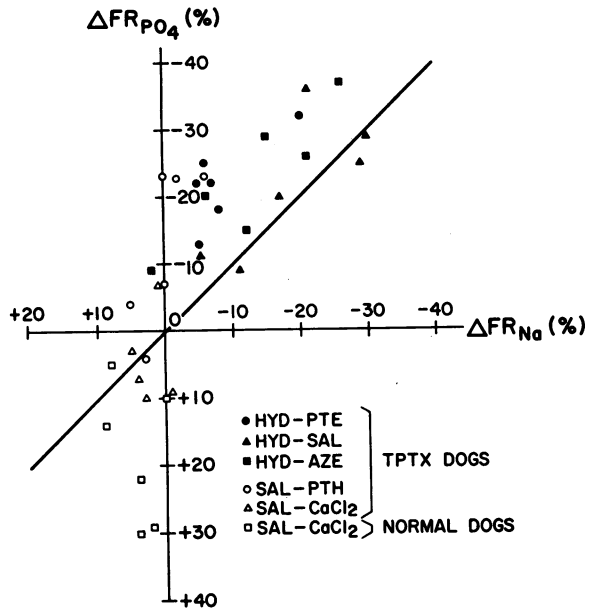


FIGURE 5 Mean increments in FR phosphate ($\Delta FR_{PO_4}$) in the proximal tubule as related to the corresponding sodium increments ($\Delta FR_{Na}$) in all six groups of animals. Each point represents mean values for each experiment. Correlation coefficient ($r$) for the first three groups (closed symbols) is 0.69 ($P < 0.01$). No apparent correlation is seen in saline-loaded animals (open symbols). AZE, acetazolamide; HYD, hydropenia; PTE, parathyroid extract; PTH, parathyroid hormone; SAL, saline.

TF/P inulin as 2.5, revealed that approximately 30% of filtered phosphate was delivered to the distal nephron during hydropenia. Since the corresponding FR phosphate in the final urine was about 5%, as much as 25% of filtered phosphate could be reabsorbed by the distal nephron in TPTX dogs. It may be argued that phosphate transport in the superficial nephrons as obtained by micropuncture studies may not represent that of all nephrons (39, 40) and greater FR phosphate by the deep nephrons may be responsible for the difference in the remaining fractions between the superficial end proximal tubule and the final urine. However, functional heterogeneity in the dog kidney is probably relatively small (41), as almost 100% of the nephrons possess long loops of Henle (42) with the ratio of superficial to deep nephron GFR at about 0.8 (43). Since cortical nephrons functionally comprise the majority of total nephrons, even a complete reabsorption of filtered phosphate by the deep nephron proximal tubules cannot account for 25% difference in the remaining fractions observed in our studies. Although the 25% discrepancy may also represent further reabsorption by the pars recta of the proximal tubule, reabsorption in this segment is thought to be of much smaller magnitude than that in the pars convoluta (44). Also the generous assumption of 2.5 as the end proximal TF/P inulin in the dog used in our calculation of remaining phosphate fraction should greatly minimize the possibility of additional proximal tubule reabsorption over our estimated value. Therefore, it is more reasonable to assume that further phosphate reabsorption occurred distal to the proximal tubule in our TPTX dogs. A similar conclusion was reached by Amiel et al. (11) in the rat, and additional evidence in our studies (*vide infra*) also supports the existence of distal phosphate reabsorption. However, distal tubule micropuncture study of phosphate reabsorption is necessary to further confirm these observations. Our finding of possible distal phosphate reabsorption in TPTX dogs is at variance with microinjection (45, 46) and microperfusion (47) studies in intact rats in which no significant distal reabsorption was demonstrated. The reason for this difference is unknown but it is possible that demonstration of distal phosphate reabsorption requires the absence of endogenous parathyroid hormone. It may also represent a species difference in renal handling of phosphate. The generally higher plasma phosphate level in the rat (9, 11, 13) than that in the dog (6, 48) under physiological conditions also attests to the existence of difference in phosphate metabolism between the two species.

The effect of parathyroid extract on proximal tubule sodium and phosphate reabsorption in TPTX dogs in our studies was similar to that observed in the intact animals reported in the literature (12, 18). In our experiments, the reduction in FR phosphate by the proximal tubule by 21% of filtered load was reflected in 24% increase in FE phosphate in the urine, indicating that the major site of action of parathyroid extract on renal phosphate transport must reside in the proximal tubule. Similar changes in both proximal tubule FR phosphate and urinary FE phosphate by 22% and 26%, respectively, were observed after administration of acetazolamide. In contrast, after saline infusion, although reduction in proximal tubule FR phosphate by 22% was comparable to parathyroid extract and acetazolamide groups, the increase in FE phosphate in the urine was only 14%. This blunting of the phosphaturic effect by saline infusion has also been reported in TPTX dogs by Massry, Coburn, and Kleeman (6), Slatopolsky, Gradowska, Caglar, and Rutherford (49), and in PTX rats by Frick (5). Hebert, Rouse, Eknoyan, Martinez-Maldonado, and Suki (50) showed that the diminished phosphaturic effect of saline infusion in acutely TPTX dogs was related to low serum phosphate relative to an increased threshold or tubule maximum, or decreased splay. In our studies, the observed reduction in phosphaturic effect appears not to be solely related to low plasma phosphate value since mean UF phosphate in this group was relatively high at 2.2–2.5 mmol/liter.

Since fractional delivery of phosphate to the distal nephron was similar in the first three groups after phosphaturic maneuvers, the blunted phosphaturic response observed after saline infusion must have occurred as a result of an increase in distal FR phosphate relative to its delivery. A similar conclusion was reached by others (14, 51) in the rat after volume expansion. Conversely, administration of parathyroid extract and acetazolamide, in addition to its predominently proximal tubule effect, appears to prevent such a compensatory increase in FR phosphate in the distal nephron, resulting in unabated phosphaturia. These observations lend further support to the existence of distal phosphate reabsorption in the dog nephron.

The effect of acetazolamide or other carbonic anhydrase inhibitors on proximal tubule sodium transport has been studied by micropuncture technique in the past but the results were equivocal in the dog (52) and contradictory in the rat (53–55). In our micropuncture study in TPTX dogs, proximal tubule FR sodium was reduced by 13% of the filtered load after acetazolamide. Since this occurred despite 29% fall in GFR, absolute sodium reabsorption by the proximal tubule must be greatly reduced. Our findings of reduced proximal tubule phosphate as well as sodium reabsorption after acetazolamide in TPTX dogs were also supported by the similar observation by Beck and Goldberg (36). It is not known whether the effect of acetazolamide on proximal tubule phosphate transport is secondary to its sodium effect or a direct result of carbonic anhydrase inhibition.

Since the parallelism between the changes in sodium and phosphate reabsorption in the proximal tubule after the three phosphaturic maneuvers suggests some linkage in the transport of the two ions, attempts were made to determine whether the two can be dissociated by changing the endogenous or exogenous level of parathyroid hormone without changing proximal tubule sodium transport. This was carried out by infusion of calcium chloride in saline-loaded normal dogs or administration of highly purified parathyroid hormone to saline-loaded TPTX dogs. Saline-loaded animals were used because it has been shown that proximal tubule FR sodium in the dog, once suppressed by saline infusion, tends to remain unaffected by further inhibitory maneuvers such as graded volume expansion (56, 57). Indeed, neither calcium infusion nor parathyroid hormone administration, both of which have been shown to reduce proximal tubule sodium reabsorption (12, 18, 58), significantly altered proximal TF/P inulin in the saline-loaded animals in the present studies. Increased FE sodium observed in the final urine after calcium infusion, therefore, was a result of reduced sodium reabsorption in the distal nephron. This is consistent with the observation by Suki, Eknoyan, Rector, and Seldin (59) that both free water clearance and free water reabsorption were reduced in hypercalcemia, suggesting a reduction in sodium transport in the loop of Henle. Calcium infusion in the saline-loaded normal dogs resulted in no significant changes in FE phosphate. Several factors may be involved in this group of animals to influence renal phosphate handling. First, calcium infusion was associated with a fall in GFR, probably as a result of reduction in renal plasma flow, and this may reduce urinary phosphate excretion. Second, there was a rise in UF phosphate as well as total plasma phosphate despite a reduction in its ultrafilterable fraction, which occurred as a result of hypercalcemia (60). Increase in UF phosphate should lead to enhanced phosphate excretion. Although the mechanism for the rise in plasma phopshate after calcium infusion is not known, it was apparently derived from nonrenal origin since urinary phosphate excretion was not diminished (61). Third, acute calcium infusion suppresses parathyroid hormone (62) and stimulates calcitonin (63), both hormones known to reduce proximal tubule phosphate reabsorption (12–14, 64). Since calcium infusion in the intact animals enhanced phosphate reabsorption in the proximal tubule in our present studies, the effect of parathyroid hormone must predominate over that of calcitonin. Finally, calcium infusion may directly reduce phosphate reabsorption (65) since phosphate excretion and FE phosphate increased in the TPTX dogs in our studies. Whatever factors may predominate after calcium infusion in intact animals, unchanged FE phosphate in the face of enhanced proximal tubule phosphate reabsorption strongly suggests an inhibitory effect of calcium infusion on distal phosphate reabsorption offsetting its proximal tubule effect. This was further supported by the observation in the saline-loaded TPTX dogs, in which FE phosphate increased when fractional proximal tubule phosphate transport remained unchanged after calcium infusion (Fig. 4).

In our studies, a dissociation in sodium and phosphate transport in the proximal tubule was demonstrated when endogenous parathyroid hormone was suppressed by calcium infusion in saline-loaded normal dogs and when exogenous parathyroid hormone was administered to saline-loaded TPTX dogs. These observations indicate existence of an independent transport system for phosphate in the proximal tubule, especially under the influence of parathyroid hormone. The demonstration of an independent transport system for the two ions is in contrast to the earlier findings in hydropenic animals during phosphaturic maneuvers and those reported in the literature (12, 18, 19), in which parallel changes in the transport of the two ions were generally found. The concept of an independent phosphate transport system is helpful in understanding the role of renal excretion in

the maintenance of phosphate homeostasis. It is known that when phosphate load to the body is increased through dietary intake or i.v. administration, urinary phosphate excretion is enhanced not only by an increase in the filtered load but by a reduction in tubule FR either through its direct effect on the tubule or, secondarily, through stimulation of parathyroid hormone (1, 4, 37). It appears that this effective homeostatic regulation of renal phosphate transport is, at least in part, dependent on the metabolic influences of phosphate rather than solely related to changes in renal sodium transport. However, the relative importance of sodium-independent and -dependent transport systems for phosphate in determining phosphate excretion in the final urine remains to be elucidated.

## ACKNOWLEDGMENTS

The author wishes to thank Frank Emspak, Kathleen Zweifel, and Patricia Simmons for their expert technical assistance. He is also indebted to Drs. John Mangos and Thomas Steele for allowing the use of their analytical equipments in the present studies, and Drs. Richard Rieselbach and Frank Gutmann for carefully reviewing the manuscript.

This investigation was supported by Research Grant 1-P01-AM 15512-01 from the National Institutes of Health, Public Health Service.

## REFERENCES

1. Pitts, R. F., and R. S. Alexander. 1944. The renal reabsorptive mechanism for inorganic phosphate in normal and acidotic dogs. *Am. J. Physiol.* **142**: 648.
2. Thompson, D. D., and H. H. Hiatt. 1957. Renal reabsorption of phosphate in normal human subjects and in patients with parathyroid disease. *J. Clin. Invest.* **36**: 550.
3. Foulks, J. G., and F. A. Perry. 1959. Renal excretion of phosphate following parathyroidectomy in the dog. *Am. J. Physiol.* **196**: 554.
4. Hellman, D., H. R. Baird, and F. C. Bartter. 1964. Relationship of maximal tubular phosphate reabsorption to dietary phosphate in the dog. *Am. J. Physiol.* **207**: 97.
5. Frick, A. 1969. Mechanism of inorganic phosphate diuresis secondary to saline infusions in the rat. *Pflügers Arch. Eur. J. Physiol.* **313**: 106.
6. Massry, S. G., J. W. Coburn, and C. R. Kleeman. 1969. The influence of extracellular volume expansion on renal phosphate reabsorption in the dog. *J. Clin. Invest.* **48**: 1237.
7. Suki, W. N., M. Martinez-Maldonado, D. Rouse, and A. Terry. 1969. Effect of expansion of extracellular fluid volume on renal phosphate handling. *J. Clin. Invest.* **48**: 1888.
8. Steele, T. H. 1970. Increased urinary phosphate excretion following volume expansion in normal man. *Metab. (Clin. Exp.)*. **19**: 129.
9. 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.
10. Morel, F., N. Roinel, and C. LeGrimmellec. 1969. Electron probe analysis of tubular fluid composition. *Nephron.* **6**: 350.
11. 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. *Pflüger Arch. Eur. J. Physiol.* **317**: 93.
12. 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.
13. Gekle, D. 1971. Der Einfluss von Parathormon auf die Nierenfunktion. I. Tierexperimentelle Untersuchungen. *Pflügers Arch. Eur. J. Physiol.* **323**: 96.
14. Frick, A. 1972. Proximal tubular reabsorption of inorganic phosphate during saline infusion in the rat. *Am. J. Physiol.* **223**: 1034.
15. Rastegar, A., Z. Agus, T. B. Conner, and M. Goldberg. 1972. Renal handling of calcium and phosphate during mineralo-corticoid "escape" in man. *Kidney Int.* **2**: 279.
16. Martinez-Maldonado, M., G. Eknoyan, and W. N. Suki. 1971. Natriuretic effects of vasopressin and cyclic AMP: possible site of action in the nephron. *Am. J. Physiol.* **220**: 2013.
17. Eknoyan, G., W. N. Suki, and M. Martinez-Maldonado. 1970. Effect of diuretics on urinary excretion of phosphate, calcium and magnesium in thyroparathyroidectomized dogs. *J. Lab. Clin. Med.* **76**: 257.
18. 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 KG, Berlin. 380.
19. Puschett, J. B., Z. S. Agus, D. Senesky, and M. Goldberg. 1972. Effects of saline loading and aortic obstruction on proximal phosphate transport. *Am. J. Physiol.* **223**: 851.
20. Osmun, J. R. 1967. Total and ultrafilterable calcium and magnesium in human plasma: improved method and normal values. *Am. J. Med. Technol.* **33**: 448.
21. Roe, J. E., J. H. Epstein, and N. P. Goldstein. 1949. A photometric method for the determination of inulin in plasma and urine. *J. Biol. Chem.* **178**: 839.
22. Harvey, R. B., and A. J. Brothers. 1962. Renal extraction of para-aminohippurate and creatinine measured by continuous in vivo sampling of arterial and renal vein blood. *Ann. N. Y. Acad. Sci.* **102**: 46.
23. Kraml, M. 1966. A semi-automated determination of phospholipids. *Clin. Chim. Acta.* **13**: 442.
24. Vurek, G. G., and S. E. Pegram. 1966. Fluorometric method for the determination of nanogram quantities of inulin. *Anal. Biochem.* **16**: 409.
25. Chen, P. S., Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28**: 1756.
26. Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, New York. 67.
27. Seely, J. F., and J. H. Dirks. 1969. Micropuncture study of hypertonic mannitol diuresis in the proximal and distal tubule of the dog kidney. *J. Clin. Invest.* **48**: 2330.
28. Dirks, J. H., and J. F. Seely. 1970. Effect of saline infusions and furosemide on the dog distal nephron. *Am. J. Physiol.* **219**: 114.
29. Duarte, C. G., and J. F. Watson. 1967. Calcium re-

absorption in proximal tubule of the dog nephron. *Am. J. Physiol.* **212**: 1355.
30. Brunette, M., S. F. Wen, R. L. Evanson, and J. H. Dirks. 1969. Micropuncture study of magnesium reabsorption in the proximal tubule of the dog. *Am. J. Physiol.* **216**: 1510.
31. Walser, M. 1961. Calcium clearance as a function of sodium clearance in the dog. *Am. J. Physiol.* **200**: 1099.
32. Massry, S. G., J. W. Coburn, L. W. Chapman, and C. R. Kleeman. 1967. The effect of NaCl infusion on urinary $Ca^{++}$ and $Mg^{++}$ during reduction in their filtered loads. *Am. J. Physiol.* **213**: 1218.
33. Gonda, A., N. Wong, J. F. Seely, and J. H. Dirks. 1969. The role of hemodynamic factors on urinary calcium and magnesium excretion. *Can. J. Physiol. Pharmacol.* **47**: 619.
34. Giebisch, G. 1969. Functional organization of proximal and distal tubular electrolyte transport. *Nephron.* **6**: 260.
35. Kuntziger, H., C. Amiel, and C. Gaudebout. 1972. Phosphate handling by the rat nephron during saline diuresis. *Kidney Int.* **2**: 318.
36. 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.
37. Van Stone, J. C., and J. E. Hano. 1972. Phosphate excretion in the parathyroidectomized rat receiving parathyroid hormone. *Metab. (Clin. Exp.).* **21**: 849.
38. Wen, S. F. 1973. Effect of volume expansion on renal phosphate transport in unilateral remnant kidney of the dog. *Clin. Res.* **21**: 713.
39. Horster, M., and K. Thurau. 1968. Micropuncture studies on the filtration rate of single superficial and juxtamedullary glomeruli in the rat kidney. *Pflügers Arch. Gesamte Physiol. Menschen Tiere.* **301**: 162.
40. De Rouffignac, C., and J. P. Bonvalet. 1970. Étude chez le rat des variations du debit individuel de filtration glomerulaire des nephrons superficiels et profonds en function de l'apport sode. *Pflügers Arch. Eur. J. Physiol.* **317**: 141.
41. Liebau, G., D. Z. Levine, and K. Thurau. 1968. Micropuncture studies on the dog kidney. I. The response of the proximal tubule to changes in systemic blood pressure within and below the autoregulatory range. *Pflügers Arch. gesamte Physiol. Menschen Tiere.* **304**: 57.
42. Schmidt-Nielsen, B., and R. O'Dell. 1961. Structure and concentrating mechanism in the mammalian kidney. *Am. J. Physiol.* **200**: 1119.
43. Carriere, S., P. Boulet, A. Mathieu, and M. Brunette. 1972. Isotonic saline loading and intrarenal distribution of glomerular filtration in dogs. *Kidney Int.* **2**: 191.
44. Burg, M. B., and J. Orloff. 1968. Control of fluid absorption in the renal proximal tubule. *J. Clin. Invest.* **47**: 2016.
45. Gagnan-Brunette, M., L. Taleb, and S. Carriere. 1971. Effect of parathyroid hormone upon phosphate reabsorption along the nephron. Abstracts of the Annual Meeting of the American Society of Nephrology. **5**: 24.
46. 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.
47. Murayama, Y., F. Morel, and C. Le Grimellec. 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.
48. Laflamma, G. H., and J. Jowsey. 1972. Bone and soft tissue changes with oral phosphate supplements. *J. Clin. Invest.* **51**: 2834.
49. Slatopolsky, E., L. Gradowska, S. Caglar, and E. Rutherford. 1971. The influence of parathyroid hormone on the phosphaturia of extracellular fluid volume expansion in the dog. Abstracts of the Annual Meeting of the American Society of Nephrology. **5**: 74.
50. Hebert, C. S., D. Rouse, G. Eknoyan, M. Martinez-Maldonado, and W. N. Suki. 1972. Decreased phosphate reabsorption by volume expansion in the dog. *Kidney Int.* **2**: 247.
51. Maesaka, J. K., M. F. Levitt, and R. G. Abramson. 1971. Micropuncture study of phosphate transport during hydropenia and progressive extracellular volume expansion in rat. Abstracts of the Annual Meeting of the American Society of Nephrology. **5**: 48.
52. Dirks, J. H., W. J. Cirksena, and R. W. Berliner. 1966. Micropuncture study of the effect of various diuretics on sodium reabsorption by the proximal tubule of the dog. *J. Clin. Invest.* **45**: 1875.
53. Weinstein, S. W. 1968. Micropuncture studies of the effects of acetazolamide on nephron function in the rat. *Am. J. Physiol.* **214**: 222.
54. Malnic, G., M. Mello Aires, and F. Lacaz Vieira. 1970. Chloride excretion in nephrons of rat kidney during alterations of acid-base equilibrium. *Am. J. Physiol.* **218**: 20.
55. Kunau, R. T., Jr. 1972. The influence of the carbonic anhydrase inhibitor, benzolamide (CL-11,366), on the reabsorption of chloride, sodium, and bicarbonate in the proximal tubule of the rat. *J. Clin. Invest.* **51**: 294.
56. Davis, B. B., M. J. Walters, and H. V. Murdaugh, Jr. 1969. Renal response to graded saline challenge. *Am. J. Physiol.* **217**: 1604.
57. Wen, S. F., N. L. M. Wong, R. L. Evanson, E. A. Lockhart, and J. H. Dirks. 1973. Micropuncture studies of sodium transport in the remnant kidney of the dog. The effect of graded volume expansion. *J. Clin. Invest.* **52**: 386.
58. DiBona, G. F. 1971. Effect of hypercalcemia on renal tubular sodium handling in the rat. *Am. J. Physiol.* **220**: 49.
59. Suki, W. N., G. Eknoyan, F. C. Rector, Jr., and D. W. Seldin. 1969. The renal diluting and concentrating mechanism in hypercalcemia. *Nephron.* **6**: 50.
60. Hopkins, T., J. E. Howard, and H. Eisenberg. 1952. Ultrafiltration studies on calcium and phosphorus in human serum. *Bull. Johns Hopkins Hosp.* **91**: 1.
61. Bernstein, D., C. R. Kleeman, R. Rockney, J. T. Dowling, and M. H. Maxwell. 1962. Studies on the renal clearance of phosphate and the role of the parathyroid glands in its regulation. *J. Clin. Endocrinol. Metab.* **22**: 641.
62. Aurbach, G. D., and J. T. Potts, Jr. 1967. Parathyroid hormone. *Am. J. Med.* **42**: 1.
63. Copp, D. H. 1967. Hormonal control of hypercalcemia. *Am. J. Med.* **43**: 648.
64. Gekle, D., and K. Kossmann. 1968. Der Einfluss von Thyreocalcitonin auf die Phosphätreabsorption der Niere (Mikropunktions-untersuchungen). *Monatsschr. Kinderheilkd.* **116**: 308.
65. Eisenberg, E. 1965. Effects of serum calcium level and parathyroid extracts on phosphate and calcium excretion in hypothyroid patients. *J. Clin. Invest.* **44**: 942.