# Micropuncture Study of Diuretic Effects on Sodium and Calcium Reabsorption in the Dog Nephron

B. R. EDWARDS, P. G. BAER, R. A. L. SUTTON, and J. H. DIRKS

*From the Renal and Electrolyte Division, Department of Medicine, Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada*

ABSTRACT A close relationship has been observed between the clearance rates of sodium and calcium under a variety of diuretic conditions. The thiazide diuretics act differently in dissociating the renal tubular reabsorption of sodium and calcium. This phenomenon has been further investigated using recollection micropuncture and clearance techniques in a group of 14 dogs subjected to three consecutive experimental phases: expansion to 3% of body weight (BWt) with Ringer's solution, chlorothiazide infusion at 20 mg/kg/h, and furosemide in a prime of 10 mg/kg and a 10 mg/kg/h infusion. Diuretic losses were balanced with infusion of equal volumes of Ringer's solution throughout the experiment. Chlorothiazide increased the fractional excretion (FE) of sodium almost threefold while $FE_{Ca}$ was not significantly altered. Furosemide increased $FE_{Na}$ and $FE_{Ca}$ to an approximately equal, and more marked, degree. This dissociation of sodium and calcium reabsorption after chlorothiazide was also evident in the superficial distal tubule, where (tubule fluid/plasma sodium) $(TF/P_{Na})$ increased from 0.32 to 0.49 ($P < 0.01$) and TF/(ultrafiltrate)$UF_{Ca}$ was unchanged (0.35–0.31). Furosemide markedly reduced the transtubular concentration gradient for both sodium (0.86) and calcium (0.94). $TF/P_{Inulin}$ decreased progressively from 3.79 to 2.78 to 2.33 in three phases. In the late proximal tubule, chlorothiazide induced a fall of $TF/P_{Inulin}$ from 1.57 to 1.44 ($P < 0.01$), but the ratio $TF/UF_{Ca}:TF/P_{Na}$ was unchanged. Furosemide had no significant proximal effect. It is concluded that acute administration of chlorothiazide reduces sodium reabsorption in the distal nephron, presumably the cortical diluting segment, without affecting calcium reabsorption.

## INTRODUCTION

Examination of renal handling of sodium and calcium by whole kidney clearance techniques reveals that the excretion rates of these ions are proportional during a variety of diuretic conditions. Walser (1, 2) and other investigators (3–5) found that the association between calcium and sodium clearances observed in the hydropenic state is not altered during diuresis induced by saline, mannitol, glucose, or water. Nor is this association diminished by most diuretics, since calcium excretion remains proportional to sodium excretion after administration of ethacrynic acid (6–9), furosemide (7–11), and mercurials (5, 12). However, quantitative differences in the associated degree of calciuresis have been reported. In addition, one group of diuretics, the thiazides, is reported to have a unique effect on renal excretion of sodium and calcium.

Acute administration of thiazides results in rapid onset of diuresis and natriuresis. Urine flow rate and sodium excretion rate are increased three- to fourfold. The reported effect on calcium excretion, however, is variable. Some investigators find an increase in calcium excretion in proportion to, but less than, the increase in sodium excretion (5, 8, 9, 13–15). Others, although observing similar increases in sodium excretion, find that calcium excretion is unchanged (11, 16) or even decreased (17, 18). It has been suggested that this variability may result from differences in protocol for replacement of urinary losses (14, 15, 18), or from the reduction in glomerular filtration rate (GFR) [1] sometimes reported (19, 20).

Chronic thiazide administration produces a characteristic response. After 3 or 4 days, despite continued

[1] *Abbreviations used in this paper:* AA, atomic absorption; BWt, body weight; $C_{H_2O}$, free water clearance; FE, fractional excretion; GFR, glomerular filtration rate; HG, helium glow; P, plasma; PTH, parathyroid hormone; TF, tubular fluid; UF, ultrafiltrate.

drug administration, sodium excretion and urine flow rates return to control levels. Calcium excretion, however, is reduced to about one-half of control level and remains reduced for as long as the drug is administered (13, 21–26).

In this study we have examined the thiazide-induced dissociation of renal tubular handling of sodium and calcium by micropuncture analysis of proximal and distal tubular fluid (TF) during simultaneous whole-kidney clearance measurements. The results clearly reveal that during saline or furosemide diuresis, as in hydropenia, calcium and sodium excretion rates vary proportionately, but during thiazide diuresis, sodium excretion is increased without any coincident increase in calcium excretion, and the clearances of these two ions are clearly dissociated. This disproportionate effect of thiazide was also evident in distal TF where the concentration of sodium was increased without any alteration in calcium ion concentration.

## METHODS

Experiments were performed on 14 mongrel dogs weighing between 11 and 18 kg. The dogs were allowed free access to standard laboratory chow and water until the day of the experiment. Anesthesia was induced by i.v. administration of sodium pentobarbital [30 mg/kg (body weight) BWt] with supplementary doses of sodium pentothal as required. An endotracheal tube was inserted and ventilation maintained with a Harvard respiratory pump (Harvard Apparatus Co., Inc., Millis, Mass.) at an appropriate rate and stroke volume.

Polyethylene catheters were inserted into both foreleg veins for the administration of Ringer's solution and the appropriate diuretic, and into the left jugular vein for the administration of 5% (wt/vol) inulin solution in 0.9% NaCl. Blood pressure was monitored through a catheter in

the left femoral artery and blood samples were obtained from a catheter in the left femoral vein. Both ureters were cannulated from a suprapubic approach and urine from each kidney collected separately. The left kidney was exposed by a flank incision, carefully freed of perirenal attachments, and mounted in a stable Lucite holder. The left renal artery was cannulated retrogradely with a 27-gauge needle attached to PE-20 tubing for the injection of 4% buffered FD & C green dye. Late proximal and distal tubules were identified by periodic injection of 0.05–0.1 ml of the dye. In our experience, FD & C green dye in the amounts used has no detectable effect on proximal tubular and overall renal sodium and water reabsorption (27).

Each experiment comprised three consecutive phases. In the first phase, the dog was initially expanded to 3% BWt by i.v. infusion of Ringer's solution at 12 ml/min. The composition of the Ringer's solution was: Na 145, K 3.5, Ca 3.0, Cl 131.5, $HCO_3$ 20 meq/liter. Thereafter and throughout the experiment, the rate of infusion of Ringer's solution was adjusted to balance urinary losses as closely as possible. In the second phase, chlorothiazide (Diuril Lyovac, Merck Sharp & Dohme, Inc., Div. of Merck & Co., Inc., West Point, Pa.) in 0.9% NaCl was infused at 0.5 ml/min at a dose of 20 mg/kg/h. In the third phase, the chlorothiazide infusion was discontinued and furosemide (Lasix, Hoechst Pharmaceuticals, Somerville, N. J.) in 0.1 N NaOH was given as a prime of 10 mg/kg, followed by an infusion at 0.5 ml/min of 10 mg/kg/h. In six additional dogs, the results of which are incorporated only in Figs. 6 and 7, the furosemide phase was omitted.

On completion of surgery, inulin was given as a priming dose of 0.4% BWt followed by a sustaining infusion set to yield a constant plasma concentration between 70 and 100 mg/100 ml. At least 45 min were allowed for complete equilibration of inulin in the extracellular compartment. Similarly, 15–30 min were allowed to elapse after the complete administration of the 3% BWt Ringer's load and after the initiation of the chlorothiazide and furosemide infusions.

In phase I, several superficial late proximal and/or distal tubules were identified and punctured. An oil block of 4–5

TABLE I

*Composition of Calcium Standards*

Proximal range

| Urea | 10 mM | |
|------|-------|---|
| Glucose | 100 mg/100 ml | |
| Fructose | 150 mg/100 ml | |
| $MgSO_4$ | 1 mM | +2, 3, 4, 5, 6, 7, 8, 9, 10 meq/liter calcium |
| $KH_2PO_4$ | 4 mM | |
| NaCl | 115 mM | |
| $NaHCO_3$ | 25 mM | |

Distal range

| Urea | 25 mM | |
|------|-------|---|
| Fructose | 400 mg/100 ml | |
| $MgSO_4$ | 0.5 mM | |
| $KH_2PO_4$ | 15 mM | |
| KCl | 10 mM | +0, 0.5, 1.0, 1.5, 2.0 meq/liter calcium |
| $Na_2SO_4$ | 4 mM | |
| $NaHCO_3$ | 10 mM | |
| NaCl | 50 mM | |

TABLE II
Summary of Clearance Data for

| | V | GFR | FE$_{H_2O}$ | U$_{Na}$ | P$_{Na}$ | U$_{Na}$V | FE$_{Na}$ | U$_{Ca}$ |
|---|---|---|---|---|---|---|---|---|
| | ml/ min | ml/ min | % | meq/ liter | meq/ liter | μeq/ min | % | meq/ liter |
| Saline | 1.5 | 33.2 | 5.0 | 141 | 152 | 189 | 3.9 | 2.6 |
| (3% BWt) | ±0.3 | ±1.8 | ±1.2 | ±15 | ±1 | ±32 | ±0.8 | ±0.3 |
| Chlorothiazide | 3.3* | 25.8* | 13.3* | 137 | 153 | 428* | 11.1* | 1.0* |
| | ±0.4 | ±1.5 | ±1.9 | ±11 | ±2 | ±53 | ±1.5 | ±0.1 |
| Furosemide | 9.2* | 26.2* | 35.2* | 139 | 154 | 1300* | 31.9* | 2.8 |
| | ±0.9 | ±1.4 | ±3.0 | ±3 | ±2 | ±146 | ±2.9 | ±0.1 |

V, urine flow; U$_{Na}$, U$_{Ca}$, urinary sodium and calcium concentration, U$_{Na}$V, U$_{Ca}$V, sodium and calcium excretion rate; Pr, plasma protein concentration; BP, mean arterial blood pressure.
* $P < 0.001$, ‡ $P < 0.05$ compared with saline.

tubule diameters in length was maintained distal to the tip of the collecting pipette with a minimum of suction. Any TF samples collected when the oil block had been lost were discarded. In phases II and III, recollections of TF samples were obtained from as many of the previously punctured sites as possible.

In a control series of experiments designed to test the validity of the micropuncture technique, three-phase recollection distal micropuncture studies were performed in five dogs expanded to 3% BWt with Ringer's solution but without subsequent diuretic administration. In all other respects, these experiments were identical to those previously described. In 15 distal tubules from five dogs, mean values ±SEM for the three phases were: TF/P$_{inulin}$, 3.31±0.10 to 3.28±0.11 to 3.37±0.11; TF/P$_{Na}$, 0.34±0.01 to 0.33±0.01 to 0.34±0.01; and TF/UF$_{Ca}$, 0.34±0.01 to 0.32±0.02 to 0.35±0.02. None of these changes were statistically significant.

In each phase, urine collections of 15 min duration were made during the time in which micropuncture samples were being obtained. A blood sample was obtained at the mid-point of each clearance period and the hematocrit and plasma protein concentration determined. Ultrafiltrates (UF) were obtained from at least two plasma samples in each phase. Amicon Centriflo ultrafiltration cones were employed (Amicon Corp., Lexington, Mass.). 2–3 ml of freshly prepared plasma was placed under mineral oil in the cone and the UF collected under oil during centrifugation at approximately 800$g$ for 30 min at room temperature. Under these conditions, we have found good agreement for UF$_{Ca}$ and % UF$_{Ca}$ with this rapid method as compared with more complex systems (28) involving the control of P$_{CO_2}$ and temperature of the plasma sample. Respective values±SEM for the Amicon- and P$_{CO_2}$/temperature-controlled methods were: for UF$_{Ca}$, 3.25±0.06 and 3.22±0.06 meq/liter, and for % UF$_{Ca}$, 65.5±1.1 and 65.0±1.3% ($n = 11$). An analysis of the Amicon method is described by Raman (29). UF$_{Ca}$ for additional plasma samples in each phase were determined by interpolation.

Plasma and urine samples were analyzed for inulin by the anthrone method of Führ, Kaczmarczyk, and Krüttgen (30), for sodium and potassium by flame photometry
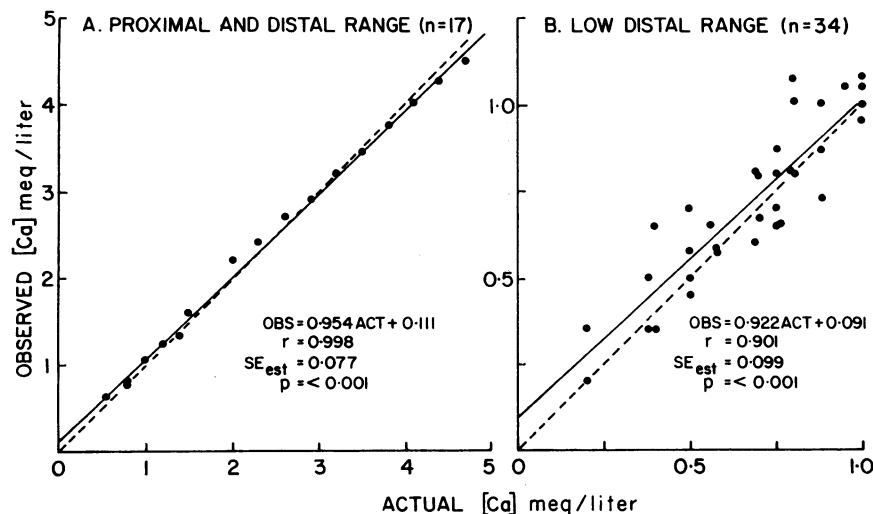


FIGURE 1  Calcium unknowns in artificial TF analyzed by HG photometer. Dashed line represents line of identity. OBS, observed values; ACT, actual values.

| Pca | UFca | UcaV | FEca | FEca/FENa | FEk | Hematocrit | Pr | BP |
|---|---|---|---|---|---|---|---|---|
| meq/ liter | meq/ liter | μeq/ min | % | | % | % | g/ 100 ml | mm Hg |
| 4.9 | 3.1 | 3.4 | 3.5 | 0.93 | 35.7 | 39 | 4.8 | 127 |
| ±0.1 | ±0.1 | ±0.6 | ±0.8 | ±0.08 | ±3.2 | ±1 | ±0.2 | ±4 |
| 4.9 | 3.0 | 3.5 | 4.9 | 0.39* | 67.4* | 40 | 4.8 | 130 |
| ±0.1 | ±0.1 | ±0.7 | ±1.2 | ±0.05 | ±4.4 | ±1 | ±0.2 | ±4 |
| 4.9 | 3.0‡ | 26.3* | 34.6* | 1.06 | 105.2* | 42* | 5.0 | 130 |
| ±0.1 | ±0.1 | ±3.2 | ±4.2 | ±0.06 | ±4.6 | ±1 | ±0.2 | ±5 |

(Model 143, Instrumentation Laboratory, Inc., Lexington, Mass.), and for calcium by atomic absorption (Model 303, Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.). Inulin in TF samples was analyzed by the fluorometric method of Vurek and Pegram (31), and sodium and calcium in TF and UF samples by the helium glow photometer (Montreal Polycrafters, Montreal, Que.). The analytical errors of the microinulin and microsodium determinations have been previously reported (32). Modifications of the helium glow (HG) to read calcium included a calcium filter (4227 Å) and the use of a higher wire heat. Calcium standards have been made up in artificial TF for both the proximal and distal tubular range (slightly modified after Marcus and Jamison [33] [Table I]). A series of 17 calcium standards, analyzed as unknowns, yielded excellent agreement between observed and actual calcium concentrations (Fig. 1). A second series of 34 calcium standards in the lower distal range (0–1.0 meq/liter) produced a similar, though less well defined, relationship: observed = 0.922, actual + 0.091±0.099, $r = 0.90$, $P < 0.001$. 111 plasma UF's from hydropenic dogs analyzed for calcium by atomic absorption (AA) and the HG gave a mean HG/AA ratio of 1.01±0.01 SEM, which is not significantly different from 1.00 ($P < 0.2$). Addition of $CaCl_2$ to plasma UF's sufficient to raise the calcium concentration approximately 1.5 and 2.5 times yielded recoveries of 101.0±1.5 ($n = 20$) and 100.9±1.5% ($n = 18$), respectively.

Standard formulae have been employed in the calculation of nonreabsorbed fractions of filtered load. Statistical analysis of the data incorporated the paired Student $t$ test. Unless otherwise specified, the levels of significance cited in the text for the results of phases II and III are always in comparison with the corresponding data for phase I.

## RESULTS

### Clearance data

For each dog, all clearance periods in each phase of the experiment have been averaged and the mean values treated as individual data in the calculation of overall group means. Table II summarizes the clearance data for the three experimental phases. The results from all 14 dogs have been incorporated in this table, since the protocol was identical and overall clearance effects

were similar, irrespective of whether proximal or distal tubules or both were sampled. Data for the left (micropuncture) kidney are presented. The results for the right kidney are in no respect significantly different from the left.

Notable changes include a decrease of GFR after chlorothiazide with no further change in the furosemide phase. Chlorothiazide induced a significant diuresis, natriuresis, and kaliuresis. In contrast to the increase of absolute and fractional excretion (FE) of sodium, that of calcium remained essentially unchanged. The increase of FEca from 3.5 to 4.9% was not statistically significant. Ultrafilterable calcium and plasma sodium and calcium concentrations remained stable. Mean arterial blood pressure was also unchanged and no significant alteration of hematocrit or plasma protein concentration occurred, testifying to the relative stability of overall fluid balance. The characteristic response to chlorothiazide can be depicted by the ratio FEca/FENa which fell from 0.93 to 0.39 ($P < 0.001$).

When furosemide was substituted for chlorothiazide in the final phase, an even more pronounced diuresis resulted. Urine flow rate increased sixfold. Absolute and fractional excretion of sodium was markedly increased and net secretion of potassium was evident in most animals. There was a massive increase in absolute and fractional excretion of calcium such that the ratio FEca/FENa returned to, and slightly surpassed, the initial value. Again, mean arterial blood pressure and plasma sodium and calcium concentrations remained relatively unchanged. A slight decrease of UFca occurred, possibly related to the imperfect maintenance of fluid balance as reflected by the small increase of hematocrit and plasma protein concentration. While in a few dogs there was a tendency for GFR to return towards the initial level, this was not reflected in the overall group mean.

| | Proximal tubule | | | | Distal tubule | | | |
|---|---|---|---|---|---|---|---|---|
| | $TF/P_{In}$ | $TF/P_{Na}$ | $TF/UF_{Ca}$ | $TF/UF_{Ca:Na}$§ | $TF/P_{In}$ | $TF/P_{Na}$ | $TF/UF_{Ca}$ | $TF/UF_{Ca:Na}$§ |
| Saline (3% BWt) | 1.57±0.06 | 0.96±0.03 | 1.11±0.04 | 1.15±0.04 | 3.79±0.25 | 0.32±0.04 | 0.35±0.04 | 1.14±0.07 |
| Chlorothiazide | 1.44±0.06‡ | 0.95±0.02 | 1.06±0.04 | 1.13±0.04 | 2.78±0.17‡ | 0.49±0.04‡ | 0.31±0.05 | 0.63±0.10* |
| Furosemide | 1.65±0.08¶ | 0.94±0.01 | 1.08±0.01 | 1.16±0.03 | 2.33±0.17*¶ | 0.86±0.03*‖ | 0.94±0.05*‖ | 1.10±0.05‖ |
| No. of dogs | 12 | 9 | 9 | 9 | 10 | 10 | 10 | 10 |

* $P < 0.001$, ‡ $P < 0.01$ compared with saline; § $TF/UF_{Ca:Na} = TF/UF_{Ca}:TF/P_{Na}$, ‖ $P < 0.001$, ¶ $P < 0.05$ compared with chlorothiazide.

## Micropuncture data

The results have been calculated on the basis of mean values per phase of experiment per dog. Individual TF/P data are also presented. Mean values±SEM of individual data are indicated in the figures.

*Proximal tubule.* 30 paired tubule fluid samples were obtained in 12 experiments and analyzed for inulin. Because of technical difficulties, sodium was not analyzed in three pairs, and calcium in five. Table III summarizes the mean values of $TF/P_{Inulin}$ ratios together with the $TF/P_{Na}$ and $TF/UF_{Ca}$ data. The only significant change is a decrease of $TF/P_{Inulin}$ from 1.57 (Ringer's) to 1.44 during chlorothiazide infusion ($P < 0.01$). The mean values for the nonreabsorbed fraction (fractional rejection) of filtered water, sodium, and calcium are shown in Table IV. The relationship between proximal calcium and sodium reabsorption ($TF/UF_{Ca}:TF/P_{Na}$) was unaltered by any of the experimental maneuvers (Fig. 5).

*Distal tubule.* 25 paired TF samples were obtained in 10 experiments and analyzed for inulin. Two of these pairs were not analyzed for electrolytes, although sodium and calcium data were obtained for two additional pairs, in which insufficient volume was available for inulin determination. Tables III and IV delineate the main findings. Individual micropuncture data are depicted in Fig. 2 for inulin, and electrolyte data are shown in Fig. 3 (chlorothiazide) and Fig. 4 (furose-

mide). $TF/P_{Inulin}$ showed a progressive fall from 3.79 to 2.78 after chlorothiazide ($P < 0.01$), and to 2.33 after furosemide ($P < 0.001$). Furosemide, the more potent diuretic, markedly reduced the transtubular gradient for both sodium (0.86) and calcium (0.94). While chlorothiazide induced an increased $TF/P_{Na}$ ratio (0.32 to 0.49) ($P < 0.01$), $TF/UF_{Ca}$ showed a slight, though nonsignificant, decrease (0.35 to 0.31). Consequently, fractional rejection of sodium at the distal puncture site increased 10%, whereas that of calcium was essentially unchanged (Table IV). The ratio $TF/UF_{Ca}:TF/P_{Na}$ illustrates this distal dissociation of calcium from sodium reabsorption with chlorothiazide (Fig. 5).

From Table IV, it can be seen that fractional reabsorption of filtered water, sodium, and calcium in the proximal tubule is reduced approximately 4–6% after chlorothiazide. This trend is reversed by the administration of furosemide. With chlorothiazide, fractional reabsorption of filtered water at the distal tubule puncture site had decreased 9%, and sodium 10%, but calcium only 2%. Hence, between the late proximal and the distal tubule, fractional reabsorption of filtered water decreased 3%, sodium 5%, whereas that of calcium increased 2%. The changes in fractional reabsorption in the different nephron segments induced by furosemide were much more marked. No distinct effects were evident at the late proximal site. Between the late proximal and the distal tubule, fractional reabsorption of

| | Proximal tubule | | | Distal tubule | | |
|---|---|---|---|---|---|---|
| | $P/TF_{In}$ | $TF/P_{Na:In}$ | $TF/P_{Ca:In}$ | $P/TF_{In}$ | $TF/P_{Na:In}$ | $TF/P_{Ca:In}$ |
| | % | % | % | % | % | % |
| Saline (3% BWt) | 65.2±2.5 | 65.6±2.1 | 70.4±1.5 | 28.0±2.1 | 9.4±1.7 | 10.4±2.1 |
| Chlorothiazide | 71.0±2.6‡ | 71.0±1.8§ | 74.8±3.2 | 37.3±2.3‡ | 19.5±2.2* | 12.7±2.4 |
| Furosemide | 62.6±3.0 | 60.8±3.9 | 69.2±3.5 | 45.2±2.8* | 41.6±3.3* | 47.4±3.7* |
| No. of dogs | 12 | 9 | 9 | 10 | 9 | 9 |

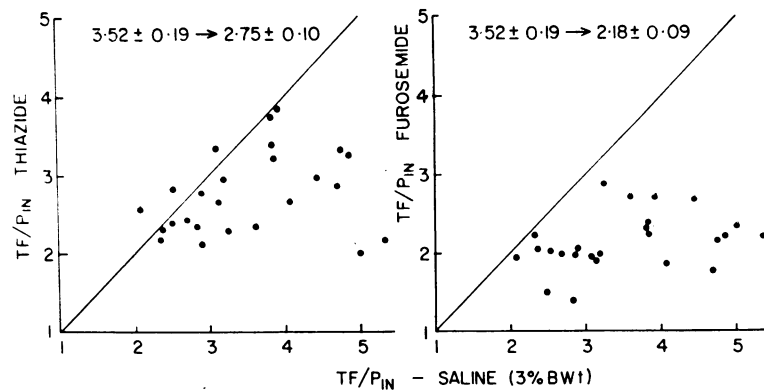* $P < 0.001$, ‡ $P < 0.01$, § $P < 0.02$ compared with saline.

FIGURE 2 Distal $TF/P_{inulin}$ ratios: effects of chlorothiazide (left) and furosemide (right). Means±SEM for 25 tubules in 10 dogs are shown.
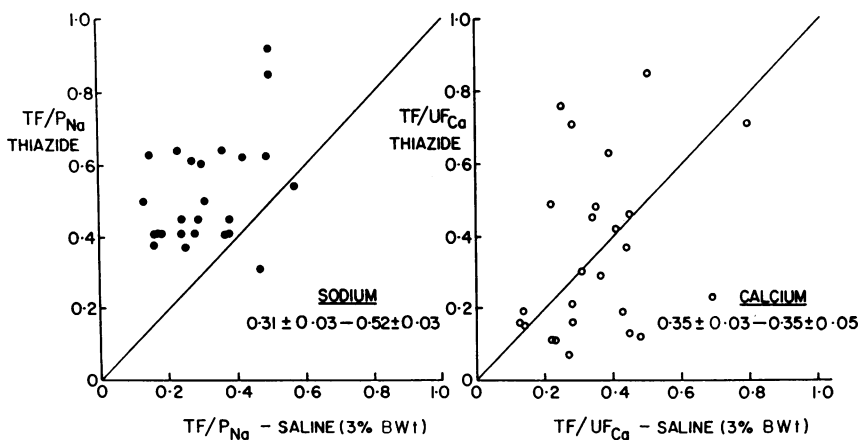


FIGURE 3 Distal $TF/P_{Na}$ and $TF/UF_{Ca}$ ratios: effects of chlorothiazide. Means±SEM for 25 tubules in 10 dogs are shown.
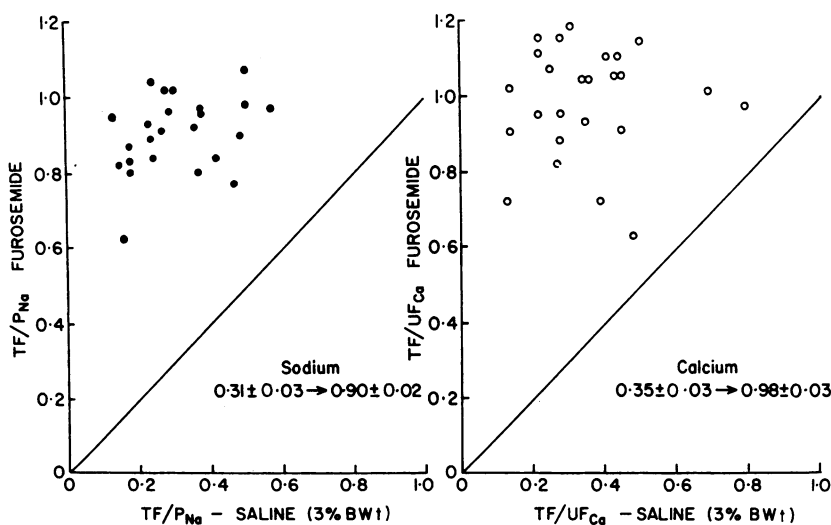


FIGURE 4 Distal $TF/P_{Na}$ and $TF/UF_{Ca}$ ratios: effects of furosemide. Means±SEM for 25 tubules in 10 dogs are shown.
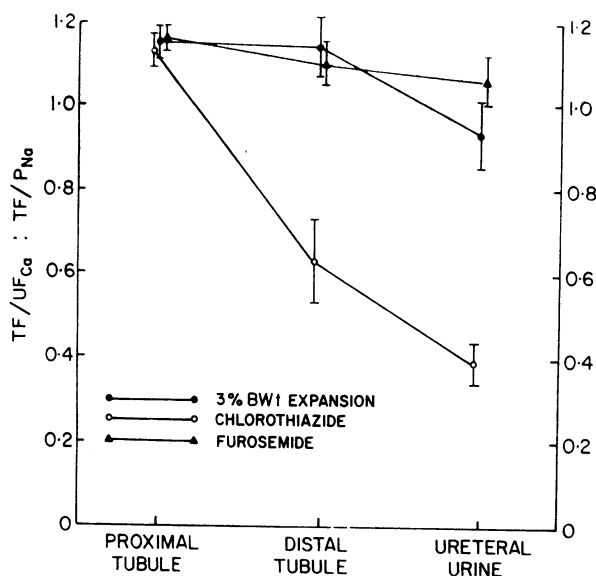
FIGURE 5 Ratio of nonreabsorbed fraction of filtered calcium to sodium in late proximal tubule, distal tubule, and final urine. Each point represents mean±SEM of experimental means.

filtered water, sodium, and calcium decreased 20, 37, and 38%, respectively.

The correlation of distal tubular $TF/UF_{Ca}$ and $TF/P_{Na}$ during 3% BWt Ringer's expansion and chlorothiazide administration is illustrated in Figs. 6 and 7. Data from six additional dogs, not subsequently infused with furosemide, are included. During saline diuresis (Fig. 6) the slope of the regression line was not sig-
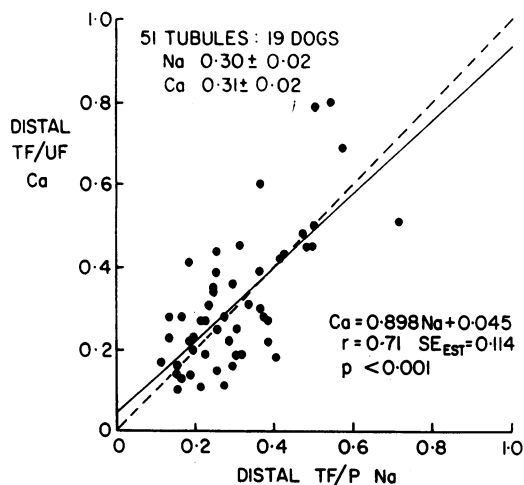


FIGURE 6 Relationship of $TF/UF_{Ca}$ to $TF/P_{Na}$ in the distal tubule of dog expanded to 3% of its BWt with isotonic Ringer's solution. Means±SEM for 51 tubules in 19 dogs are shown. Dashed line represents line of identity; solid line represents calculated regression line.
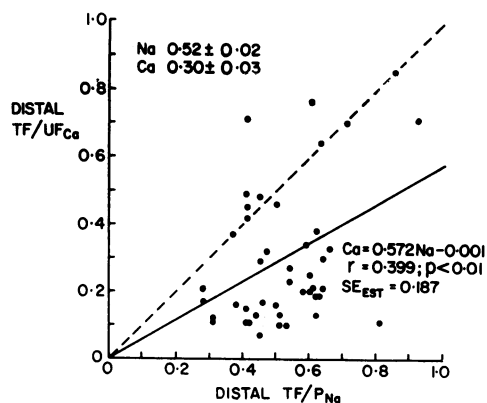


FIGURE 7 Relationship of $TF/UF_{Ca}$ to $TF/P_{Na}$ in the distal tubule of dog after chlorothiazide infusion. Means ±SEM for 44 tubules in 17 dogs are shown. Dashed line represents line of identity; solid line represents calculated regression line.

nificantly different from the line of identity. The correlation coefficient of 0.71 was highly significant at $P < 0.001$. Fig. 7 illustrates the reduced correlation of calcium and sodium concentration ratios in the distal tubule after chlorothiazide. The slope and correlation coefficient were reduced to 0.57 and 0.4, respectively.

## DISCUSSION

In our verification of the HG technique for calcium, there was a very close and highly significant correlation between observed and actual calcium concentrations of the standards analyzed as unknowns. Although there was relatively more inherent variability at very low calcium concentrations, there remained a good overall correlation. In addition, a good agreement between calcium concentrations of 111 plasma UF analyzed both on the HG and by AA was observed. If care is taken to avoid dust contamination of samples, we feel that the HG photometer provide an accurate and reproducible means of picomole analysis of calcium.

The three-phase control experiments indicate the normal stability of this preparation and that the changes observed in the experimental group represent phenomena unrelated to the experimental technique per se. We have demonstrated the validity of our micropuncture and calcium technique in the hydropenic dog (34).[2] The diuretic drugs were given against a background of mild volume expansion in order to enhance fluid delivery to the distal nephron to facilitate distal collection. This maneuver does not alter the proportionality of sodium and calcium reabsorption (1, 2). Saline loading also precluded the occurrence of volume depletion, a

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[2] Edwards, B. R., R. A. L. Sutton, and J. H. Dirks. 1973. Effect of calcium infusion on renal tubular reabsorption in the dog. Manuscript in preparation.

factor considered important in the mechanism of action of thiazides (35).

Previous reports have revealed the proportionate nature of sodium and calcium clearance rates under a variety of diuretic conditions (1–12). In the present studies we have confirmed that the ratio of calcium to sodium clearance rates is close to unity during 3% BWt Ringer's expansion and after the acute administration of furosemide. The calcium:sodium proportionality can also be demonstrated in the superficial distal tubule. In other studies (34) [2] we reported a close correlation between distal TF/UF$_{Ca}$ and TF/P$_{Na}$ in the hydropenic dog. The relationship Ca = 0.875 Na + 0.017 ±0.111 (SE of estimate) yielded a correlation coefficient of 0.75 ($P < 0.001$). In the present study, the relationship between distal sodium and calcium concentration ratios during saline diuresis was almost identical to that observed during hydropenia. In addition, furosemide appeared to inhibit calcium and sodium reabsorption in the distal nephron to an equal degree.

In view of the normally close correlation between distal sodium and calcium reabsorption, the effects of chlorothiazide are particularly striking. A dissociation of calcium from sodium reabsorption in the distal nephron segments is clearly evident. However, the correlation coefficient (Fig. 7), although reduced to 0.4, was still significant at $P < 0.01$. 6 of the 44 points illustrated in Fig. 7 have a TF/UF$_{Ca}$ ratio of more than twice the population mean. These TF samples were obtained from dogs in which the more typical response was also observed and therefore cannot be attributed to an overall unresponsiveness to chlorothiazide. They could represent early distal tubule samples where the responsiveness to chlorothiazide is perhaps less pronounced than in later distal areas. Alternatively, dust contamination during analysis could account for the rise in calcium but not sodium concentration. If these six samples are temporarily eliminated from the calculation, the regression equation becomes Ca = − 0.0009 Na + 0.232±0.119, $r = 0$. All the evidence indicates that acute administration of chlorothiazide selectively inhibits sodium reabsorption somewhere in the distal nephron, while having no significant effect on calcium reabsorption. Three questions remain to be answered: what is the precise site of action of thiazide drugs? what is their mechanism of action? and what is the nature of the pump(s) affected?

Despite the considerable volume of work published on the site of action of thiazides, a good deal of controversy still exists. Early stop-flow studies claimed to reveal a decreased fluid reabsorption in the proximal tubule after chlorothiazide (36, 37). However, interpretation of proximal events by stop-flow analysis may be hazardous (38). Dirks, Cirksena, and Berliner (39)

showed a slight increase of proximal fractional water reabsorption in the dog after hydrochlorothiazide. This was attributed to acute salt depletion, since diuretic losses were not replaced. It was also concluded that furosemide had no proximal effect, provided fluid balance was maintained. Furosemide has also been reported as having no proximal effect in the rhesus monkey (40), although a more specific response has been demonstrated in the rat proximal tubule (41). Indirect evidence for a proximal effect of thiazides comes from the work of Eknoyan, Suki, and Martinez-Maldonado (42) who interpreted an increase of fractional excretion of phosphate in thyroparathyroidectomized dogs as evidence of a proximal action. In a preliminary study, Burke, Marshall, Clapp, and Robinson (43) suggested that the vasoconstricting action of chlorothiazide masked a major proximal effect of the drug. Elimination of vasoconstriction by simultaneous acetylcholine infusion produced a significant fall of proximal TF/P$_{inulin}$ ratio. Whether or not chlorothiazide exerts a major influence in the proximal tubule, sodium and calcium reabsorption are affected equally and therefore do not detract from the differential response observed in more distal parts of the nephron.

The action of thiazides in more distal nephron segments has been more clearly elucidated, although much of the evidence is indirect. Several investigators have reported that thiazides reduce free water clearance (C$_{H_2O}$) and have no effect on free water reabsorption (44–46). The usual interpretation has been that thiazides inhibit sodium reabsorption in the diluting segment in the ascending limb of Henle's loop. On the other hand, Heinemann, Demartini, and Laragh (19) failed to detect any change in C$_{H_2O}$ after chlorothiazide. Two micropuncture studies in the rat have shown an increased TF/P$_{Na}$ ratio in the distal tubule with hydrochlorothiazide (47, 48). No data for calcium were reported.

Distal free-flow micropuncture (49–51) has shown almost parallel variations in calcium and sodium concentration. Together with the large body of evidence indicating a close parallelism in clearances of these two cations, these data have been interpreted by Walser (1, 2) to indicate a degree of coupling between sodium and calcium transport at one or more sites along the nephron. However, the existence of a common pump mechanism has not been clearly established. Indeed, the dissociation of calcium and sodium excretion during thiazide treatment has weighed against the common pump theory, at least in the distal nephron. Seldin, Eknoyan, Suki, and Rector (52) proposed that thiazides inhibited sodium reabsorption in the late ascending limb of Henle's loop or the early distal convoluted

tubule at a site where the amount of calcium reabsorbed was assumed to be trivial. Antoniou, Eisner, Slotkoff, and Lilienfield (11) also concluded that chlorothiazide acted in the distal nephron to reduce sodium reabsorption at a site where calcium was unaffected.

This postulate has been adopted to explain the long-term effects of thiazides, namely the return of sodium excretion to control levels after a transient increase, and a prolonged reduction in calcium excretion. The role of fluid volume depletion in this phenomenon has been explored by Suki, Eknoyan, and Martinez-Maldonado (35). An alternate mechanism for the chronic action of thiazides is that their effect may be mediated by parathyroid hormone (PTH). Several studies indicate that normal circulating PTH levels may be a prerequisite for the hypocalciuric effect of thiazides (25, 26).

In the present studies, where chlorothiazide was administered acutely, diuretic losses were replaced with equivalent amounts of Ringer's solution. Overall fluid balance, as judged by hematocrit and plasma protein concentration, remained stable. There was no evidence of increased tubular calcium reabsorption after chlorothiazide administration. These observations suggest that chlorothiazide, when given acutely, reduces net tubular sodium reabsorption without affecting calcium reabsorption, and that this effect presumably occurs in the cortical diluting segment and distal tubule. The data also indicate an additional effect of chlorothiazide beyond the distal tubule puncture site. Meng reached a similar conclusion in his study with the rat (48). Whether the drug is acting in the late distal tubule, or collecting duct, or both, remains unanswered. In addition, the contribution of deeper nephrons to the final urine, and their response to chlorothiazide, is unknown. An equally important question is whether calcium and sodium are transported independently across the tubular epithelium in the distal nephron with chlorothiazide influencing solely the sodium transport, or whether there is at least some degree of linkage in the transport of these cations, at least in that locus where furosemide is effective.

## ACKNOWLEDGMENTS

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