Relationship between clearances of Ca and Na: effect of distal diuretics and PTH

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Costanzo, Linda S., and I. M. Weiner. Relationship between clearances of Ca and Na: effect of distal diuretics and PTH. Am. J. Physiol. 230(1): 67-73. 1976.—One of the objectives of this study was to determine whether or not the absence of parathyroid hormone (PTH) modifies quantitatively the acute action of chlorothiazide (CTZ) to lower the clearance ratio, $C_{Ca}/C_{Na}$. The same group of dogs was studied with standard clearance techniques before and after thyroparathyroidectomy (T-PTX), and after T-PTX during the infusion of PTH. There was no significant difference in the response to CTZ before or after T-PTX, or during the infusion of PTH. The effects of PTH and a maximally effective dose of CTZ were additive. A second objective of this work was to ascertain whether or not two other diuretics which act on the distal tubule, amiloride and triamterene, had actions on $C_{Ca}/C_{Na}$ similar to that of CTZ. Amiloride caused a reduction in $C_{Ca}/C_{Na}$ which, even at maximally effective doses, was much smaller than the effect of CTZ. Maximally effective doses of amiloride and CTZ had additive actions. Triamterene was evaluated at only one dose; it also lowered the ratio $C_{Ca}/C_{Na}$.

Amiloride; triamterene; chlorothiazide; renal distal tubule; dogs

In man and experimental animals there is normally a linear relationship between the fractional excretion of calcium and sodium (28). Thiazide diuretics change the relationship such that the ratio, clearance of calcium/clearance of sodium ($C_{Ca}/C_{Na}$), is diminished. This action is seen on acute (5, 6, 13, 22) as well as during prolonged drug administration (5, 22). Under certain conditions the drugs can produce hypocalcemia, an absolute fall in the excretion of calcium in acute studies. In chronic experiments, hypocalcemia has been observed only when sodium and calcium excretions were initially high (6, 30). In chronic studies, hypocalcemia has been observed after one or more days of drug ingestion (5, 22). There is evidence to suggest that chronic thiazide-induced hypocalcemia requires the presence of parathyroid hormone (PTH) in man (5, 22), but not in rats (19). On the other hand, acute thiazide-induced hypocalcemia has been observed in animals after thyroparathyroidectomy (T-PTX) (6). The present study was designed to ascertain whether or not the presence of PTH influences the magnitude of the acute response to a thiazide. Because the degree of hypocalciuria is in part dependent on the initial rate of calcium and sodium excretions as well as the extent of drug-induced natriuresis (6), the magnitude of the thiazide effect is best evaluated by studying the relationship between the clearances of calcium and sodium in the presence and absence of the drug over wide ranges of electrolyte excretion. In the present study the effects of chlorothiazide were estimated in the same dogs before and after T-PTX. In addition, we tested the effect of parathyroid hormone alone and in combination with chlorothiazide in T-PTX dogs. The results establish that acute thiazide administration reduces $C_{Ca}/C_{Na}$ to quantitatively the same extent in dogs before and after T-PTX. $C_{Ca}/C_{Na}$ was greater in dogs after T-PTX than before, and PTH administration lowered the ratio to the value found in the intact dogs. PTH and chlorothiazide given in combination to T-PTX dogs produced additive depression of $C_{Ca}/C_{Na}$.

Because thiazides change the relationship between calcium and sodium reabsorption in the distal nephron (19), it seemed relevant to test the activity of other diuretics which act on the distal tubule. Amiloride produced hypocalciuria in dogs undergoing osmotic diuresis; a maximally effective dose of the drug also lowered the slope of the line relating calcium and sodium clearances. Chlorothiazide could further lower $C_{Ca}/C_{Na}$ in dogs treated with maximally effective doses of amiloride. Triamterene also decreased $C_{Ca}/C_{Na}$.

METHODS

Sixty-eight clearance experiments were performed on female mongrel dogs anesthetized with sodium pentobarbital, 30 mg/kg. Those animals used for single experiments were maintained on a diet of Purina laboratory chow. Animals used repeatedly received one can of Alpo dog food per day and, in addition, had free access to chow. The canned food served as a vehicle for calcium lactate (25 g/day) and sodium levothyroxine (0.8 mg/day) when these were added to the diet after thyroparathyroidectomy.

In all experiments the animals received intravenous infusions containing sufficient inulin to produce concentrations in plasma of 40–50 μg/ml. Blood samples from a femoral artery were taken at midpoints of clearance periods through an indwelling Cournand needle. In dogs subjected to multiple experiments, urine was collected...
through indwelling bladder catheters. The bladders were washed out with 10 ml of 5% mannitol prior to the start of a series of clearance periods and at the end of each period. In other dogs, ureters were catheterized via a suprapubic incision.

Effect of CTZ, thyroparathyroidectomy, and PTH. The following two experiments were performed in random order on each of five dogs. Control experiments began with intravenous infusion of 5% mannitol in 0.9% NaCl at 5 ml/min for 40 min; at the end of this time a series of 12 10-min clearance periods were started. Urine flow was varied over a wide range during these periods by increasing the concentration of mannitol in the infusion (to 10%) and varying the rate (5-8.6 ml/min). At the end of the experiments the dogs were given 1 liter of isotonic saline intravenously to prevent mannitol-induced volume contraction. A similar experiment was also performed in each dog in the presence of chlorothiazide. A priming dose of chlorothiazide (2 mg/kg) was given at the same time that the intravenous infusion was started. The initial infusion contained CTZ to deliver 0.25 mg/kg-min. The infusion rate of CTZ was increased to 0.30 mg/kg-min in the last four periods to prevent the drug concentration in plasma from falling as a result of volume expansion.

After successful completion of the two foregoing experiments, each dog was subjected to surgical thyroparathyroidectomy and maintained as described above. In the fed state the concentration of calcium in plasma was usually normal, but on a few occasions signs of tetany developed and the animals were treated with intravenous CaCl₂. The dogs were invariably hypocalcemic during experiments because of the preceding fast. At the end of each experiment, except when PTH was used, the therapeutic saline infusion contained 2.5 mM CaCl₂. Serum thyroxine was monitored; it did not change appreciably from presurgical levels. The control experiment and the experiment with chlorothiazide were repeated. In addition, two similar experiments were conducted in the presence of exogenous PTH (Parathormone, Lilly). A loading dose of 4 U/kg was injected intravenously when the infusion was started and PTH was added to the infusion to deliver 0.12 U/kg-min. The experiments after thyroparathyroidectomy were performed in random order. At least 8 days intervened between successive procedures. The mean weight of the dogs was 22.5 ± 1.7 kg during procedures before T-PTX and 22.7 ± 2.1 kg after T-PTX.

Effect of amiloride on the linear relationship between C$_\text{Ca}$ and C$_\text{Na}$. Five experiments of the same design as the control experiment described above were performed in intact dogs (17.0-30.0 kg), but in the presence of amiloride. A loading dose of 4 mg/kg was given over 30 min. Amiloride was added to the infusion solutions to deliver 0.06 mg/kg-min.

Effect of amiloride on clearances of Ca, Na, and K: a combination with chlorothiazide. Experiments were performed in five intact dogs (15.3-25.0 kg) and three chronically T-PTX dogs (18.5-27.5 kg). After control clearance periods, amiloride was given intravenously in various amounts. Priming doses were 1, 2, or 4 mg/kg and the respective sustaining infusions contained amiloride to provide 0.015, 0.03, and 0.06 mg/kg-min. A detailed protocol of one of these experiments will be given in RESULTS. Additional experiments of similar design were performed in intact dogs (15.8-29.5 kg) to determine the effect of amiloride when given in combination with chlorothiazide. After three control periods one of the drugs was given, its effects evaluated in three clearance periods, and then the second drug was added and the effects of the combination evaluated in three additional clearance periods. The number of dogs and sequence of drugs and doses will be indicated in tables.

Renal arterial infusion of amiloride. Four intact dogs weighing 15.2-29 kg were used; 500 ml of a solution of 5% mannitol in 0.9% NaCl were infused intravenously at 21 ml/min. The infusion was then slowed to 8.6 ml/min for the remainder of the experiment. The left renal artery was exposed via a flank incision. A curved 23-gauge needle was inserted proximal to any bifurcations. The needle was kept open by infusing 0.9% NaCl at 0.68 ml/min. Dogs with multiple renal arteries were not used. Urine was collected separately from each kidney. Forty minutes after slowing the infusion, three 10-min control periods were taken. The arterial infusion solution was changed to one containing amiloride (100 µg/min). After a 5-min wait, three additional collections were taken.

Effect of triamterene on clearances of Ca, Na, and K. Experiments were performed in five intact dogs (18.8-22.0 kg); 500 ml of a solution containing 0.25% inulin and 5% mannitol in 0.9% NaCl were delivered intravenously at 21 ml/min and the infusion was slowed to 8.6 ml/min for the remainder of the experiment. Forty minutes after slowing the infusion, three 10-min control periods were taken. In a separate vein a loading dose of triamterene, 5 mg/kg, in 10 ml of methylcellulose was injected, and an infusion of triamterene (0.25 mg/kg-min) dissolved in methylcellulose was started at 0.5 ml/min. After a 5-min wait, three additional clearance periods were taken. In five additional dogs (15.4-23.0 kg) control experiments were performed in which methylcellulose without triamterene was infused during the second set of three periods.

Analytical methods. Inulin (18), calcium (27), chlorothiazide (3), and amiloride (2) were determined according to published methods. Sodium and potassium were determined by flame photometry. Ultrafiltrates of plasma were prepared in an atmosphere of 95% O₂, 5% CO₂ at 37°C according to Toribara et al. (26).

Published methods for triamterene are known to be nonspecific (17). Consequently we developed the following procedure. To 1 ml of sample or dilution, 0.5 ml 1 N NaOH and 30 ml of chloroform containing 1.5% (v/v) isomyl alcohol were added. The mixture was shaken for 30 min; 20 ml of the chloroform phase was shaken with 5 ml of 0.1 N HCl for 15 min. The fluorescence of a portion of the aqueous phase was determined in a spectrofluorometer, excitation at 355 nm, emission at 440 nm. Recoveries from plasma and urine ranged from 85 to 90%. The specificity of the method was established with paper chromatography using the solvent system, isopropyl alcohol:water:25% aqueous NH₃ (w/v): 140:50:10. Urine from animals treated with triam-
terene contained materials which gave multiple fluorescent spots. However, the CHCl₃ extract gave only one fluorescent material which had the same mobility as authentic triamterene.

Data are shown as a means ± standard error. Linear regressions were calculated by the method of least squares. Student's t test (paired or unpaired as appropriate) was used for statistical evaluation. The interaction of the parathyroid states and the magnitudes of chlorothiazide effects were tested in two stages. The data on C_CAL/C_S (Table 1) were converted to logarithms and the effects of CTZ in T-PTX dogs with and without PTH were compared, as were the effects of PTH in the presence and absence of CTZ. Since no statistically significant difference was detected, these data were pooled to give the best estimate of the CTZ effect in T-PTX dogs and compared to the data from the dogs in the intact state.

Clearances and clearance ratios in figures and tables are usually calculated in terms of total plasma calcium. Sufficient data are provided to convert these ratios to fractional excretions.

RESULTS

Effect of CTZ, thyroparathyroidectomy, and PTH on the relationship between calcium and sodium clearances. Figure 1 displays the regression lines obtained from six experiments performed in each of five dogs. The top panel (intact) confirms the normal linear relationship between the clearances of calcium and sodium ions and the effect of CTZ thereon. The middle panel (T-PTX) shows the control relationship in these dogs after T-PTX and in the presence of CTZ. The lower panel (T-PTX + PTH) displays these relationships in T-PTX dogs in the presence of exogenous PTH. In each case CTZ lowered calcium excretion at any given level of sodium excretion, changing the slopes significantly (P < 0.001).

Statistical analysis of the mean data on C_CAL/C_S from the experiments after thyroparathyroidectomy (Table 1) revealed that the effect of simultaneous CTZ and PTH infusion was not different from the sum of the effects when the two agents were infused separately, i.e., the effect of CTZ in T-PTX dogs was the same whether or not PTH was given. There was no significant difference in the effect of CTZ on C_CAL/C_S before and after T-PTX.

In the absence of CTZ there was a significant change in C_CAL/C_S attributable to thyroparathyroidectomy (P < 0.01). In T-PTX dogs, PTH administration lowered the ratios of clearances significantly both in the presence and absence of CTZ (P < 0.01). This statistical analysis confirms the results of comparison of the regression lines (Fig. 1).

There was a tendency for GFR to be lower in experiments with CTZ (Table 2), but the difference reached significance only in the last pair of protocols (P < 0.05). Walser (29) has shown that a change in GFR does not of itself alter C_CAL/C_S. The value for P_Ca was lower in the hypoparathyroid state than in the intact state (P < 0.01), and this deficiency was eliminated during the infusion of PTH. The fraction of P_Ca ultrafilterable varied slightly among the different experiments. P_NO was essentially constant. P_CTZ was not significantly different in the dogs before and after T-PTX or in the presence of exogenous PTH. The differences in C_CTZ/GFR were minor.

Effect of amiloride on Ca Na relationship. The effect of amiloride on the relationship between calcium and sodium clearances was tested in a series of five intact dogs. These animals were normal with respect to GFR and plasma electrolytes (not shown). The results are displayed in Fig. 2. Also shown in Fig. 2 is the regression line for the relationship of calcium and sodium clearances in dogs not treated with diuretics. This line was constructed from data in the present study (Fig. 1, top panel) and from a previous study in which the same protocol was used (6). The slope of the line for the experiment in which amiloride was used is different from that of the control line (P < 0.001). When control data from the present study alone were used, P < 0.01. The foregoing experiments with amiloride were per-
TABLE 1. Values for $C_{Ca}/C_{Na}$ and $C_{UFCa}/C_{Na}$ in five dogs before and after T-PTX under various experimental conditions

<table>
<thead>
<tr>
<th>Dog</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Means + SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{Ca}/C_{Na}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>0.70</td>
<td>1.08</td>
<td>1.04</td>
<td>1.08</td>
<td>0.64</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>+ CTZ</td>
<td>0.21</td>
<td>0.28</td>
<td>0.20</td>
<td>0.29</td>
<td>0.38</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>+ T-PTX</td>
<td>1.00</td>
<td>1.04</td>
<td>1.06</td>
<td>1.04</td>
<td>0.96</td>
<td>1.10 ± 0.08</td>
</tr>
<tr>
<td>+ CTZ</td>
<td>0.36</td>
<td>0.26</td>
<td>0.27</td>
<td>0.37</td>
<td>0.50</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>$C_{UFCa}/C_{Na}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>1.33</td>
<td>1.75</td>
<td>1.40</td>
<td>1.22</td>
<td>0.87</td>
<td>1.33 ± 0.14</td>
</tr>
<tr>
<td>+ CTZ</td>
<td>0.33</td>
<td>0.59</td>
<td>0.37</td>
<td>0.53</td>
<td>0.47</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>+ T-PTX</td>
<td>2.00</td>
<td>2.50</td>
<td>1.46</td>
<td>2.03</td>
<td>1.41</td>
<td>1.84 ± 0.17</td>
</tr>
<tr>
<td>+ CTZ</td>
<td>0.67</td>
<td>0.77</td>
<td>0.54</td>
<td>0.43</td>
<td>0.55</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
<td>+ T-PTX + PTH</td>
<td>1.19</td>
<td>1.11</td>
<td>1.16</td>
<td>0.80</td>
<td>0.88</td>
<td>1.08 ± 0.12</td>
</tr>
<tr>
<td>+ CTZ</td>
<td>0.32</td>
<td>0.44</td>
<td>0.34</td>
<td>0.42</td>
<td>0.41</td>
<td>0.39 ± 0.02</td>
</tr>
</tbody>
</table>

formed after a loading dose of the drug (4 mg/kg) and during the infusion of 0.06 mg/kg-min. Plasma amiloride varied from 1.70 to 3.25 µg/ml among the experiments and, as a result of the sustaining infusion, remained essentially constant within each experiment. In these experiments the clearance of amiloride was 3 times the GFR (range 2.79–3.35).

The level of amiloride used in the preceding experiments was chosen as greater than that necessary to produce the maximal effect on the basis of experiments of the type illustrated in Table 3. Amiloride was given at two dosages after control periods to an intact dog. At each level of amiloride, the concentration of the drug in the plasma was stable for the three periods. Neither plasma electrolytes (not shown) nor GFR was altered at either level of the drug. In this experiment, none of the changes in electrolyte excretion were greater at the higher than at the lower dosage. Amiloride increased both urine volume and $C_{Na}/GFR$ to a small extent, while $C_{Ca}/GFR$ decreased by about 84% and $C_{Ca}/C_{Na}$ decreased by 51%. $C_{Ca}/C_{Na}$ decreased substantially (62%). This experiment and the results of four others in intact dogs are summarized in Table 4 (group I). The results were generally similar to those in the illustrative experiment. There was no difference in the magnitude of the depression in $C_{Ca}/C_{Na}$ caused by the three doses of amiloride.

T-PTX dogs also show a hypocalciuric response to amiloride (Table 4, group II). The difference in response to the two doses of amiloride was not significant, nor were these responses different from those seen in intact dogs.

The results of experiments where amiloride was given in combination with chlorothiazide are also summarized in Table 4. When amiloride was given first (group III), subsequent administration of chlorothiazide further increased $C_{Na}/GFR$ and further decreased $C_{Ca}/C_{Na}$ ($P < 0.001$). Chlorothiazide partially reversed the hypocalciuria produced by amiloride. When chlorothiazide was administered first (group IV), it produced a substantial increment in $C_{Na}/GFR$ and a smaller increment in $C_{Ca}/GFR$. The net effect of these changes was a reduction in $C_{Ca}/C_{Na}$. The basis for this kind of response to chlorothiazide has been discussed elsewhere (6). Against the background of the chlorothiazide effect, amiloride produced a further increment in $C_{Na}/GFR$, but produced a sharp reduction in $C_{Ca}/GFR$ to approximately the control level. As a consequence, the ratio $C_{Ca}/C_{Na}$ fell to a level lower than that observed in the presence of chlorothiazide alone ($P < 0.025$). In these experiments the GFR was about 20% below control during the periods when both drugs were infused simultaneously. As already noted, changes in GFR per se do not change the sodium-calcium relationship (29). Amiloride reversed the chlorothiazide-induced hypercalciuria.

Renal arterial infusion of amiloride. Amiloride (100 µg/min) was infused into the left renal arteries of four dogs (Fig. 3). Mean urinary drug excretions were 105 ± 11 and 3.5 ± 1.6 µg/min for the left and right kidneys, respectively. There was essentially no effect of the drug on GFR or urinary volume from either kidney. $C_{Na}/GFR$ increased slightly on the experimental side while falling on the control side. However, $C_{Ca}/GFR$ and $C_{Ca}/C_{Na}$ fell; in each instance the decline on the left side was greater than on the right. As a result, $C_{Ca}/C_{Na}$ fell to a considerably greater extent on the left than on the right side. The results are consistent with a direct renal effect of amiloride to decrease calcium (and potassium) clearance relative to sodium clearance. In preliminary experiments with a higher rate of amiloride infusion, 400 µg/min, enough drug escaped excretion by the infused kidney to produce essentially equivalent bilateral effects.

Effect of triamterene on the Ca-Na relationship.
Triamterene at the dose employed increased $C_{\text{ca}}/GFR$, depressed $C_{\text{Na}}/GFR$, and had no consistent effect on $C_{\text{Ca}}/GFR$. However, the ratio $C_{\text{Ca}}/C_{\text{Na}}$ decreased significantly ($P < 0.05$). Because of the insolubility of triamterene in conventional vehicles, methylcellulose was employed for intravenous infusion of the drug. In order to ascertain whether or not the unobserved changes in electrolyte excretion were influenced by the presence of the solvent, five experiments were performed with the solvent alone. Infusion of methylcellulose produced small changes in the clearance ratios of the electrolytes, the net effect of which was an increase in $C_{\text{ca}}/C_{\text{Na}}$, a change opposite to that observed when triamterene was added to the infusion. Thus it is clear that triamterene has an effect on $C_{\text{Ca}}/C_{\text{Na}}$ which is qualitatively similar to that of amiloride and CTZ.

Table 5 also includes information on the excretion of triamterene. The clearance of the drug was less than GFR, but since a large fraction of it was bound to plasma proteins, the results indicate net secretion.

### DISCUSSION

The present results confirm that in the intact dog chlorothiazide changes the relationships between calcium and sodium excretion such that for any level of sodium excretion, calcium excretion is diminished (6). In these experiments the concentrations of CTZ in plasma and urine were in excess of those shown to produce maximal effects (6). The results show definitively that acute thiazide administration changes the calcium-sodium relationship to essentially the same extent in normal and chronically thyroparathyroidectomized dogs. Hypoparathyroid humans show a similar response to acute thiazide administration (unpublished observations). The findings provide no information as to why hypoparathyroid humans do not show sustained hypocalciuria with chronic thiazide administration. However, it is quite clear that the initial dissociating effect of thiazide is not impaired in chronic hypoparathyroidism in dogs and humans, and therefore does not require the presence of PTH.

The ratio of clearances, $C_{\text{Ca}}/C_{\text{Na}}$, was higher after thyroparathyroidectomy than before, probably a result of the lack of parathyroid hormone. PTH administration lowered the ratio to values found in intact dogs. This effect produced by PTH at the dose employed was qualitatively similar to that of chlorothiazide, but the thiazide effect was of much greater magnitude. In the presence of maximally effective levels of chlorothiazide in T-PTX dogs, PTH further lowered the slope of the line relating the two clearances, the effects of the two agents being additive. Thus it appears that the two substances have mechanisms or sites of action which are not overlapping. Both thiazides and PTH are believed to exert their effect on calcium and sodium transport such that for any level of sodium excretion, calcium excretion is diminished (6).

The mechanism by which thiazides acutely elicit hypocalciuria and decrease calcium relative to sodium excretion is not known. It is clear that the effect does not require volume depletion or PTH (6). Walser (28) has proposed that thiazides enhance calcium reabsorption secondary to decreased sodium reabsorption in the distal tubule. He suggested that the thiazide-induced decrease in sodium transport might result in diminished transepithelial potential difference (PD) in the distal tubule. Such a reduction in intratubular negativity might be expected to favor reabsorption of calcium ions. There is recent evidence, however, that CTZ does not cause a reduction in transepithelial potential difference (7).

### TABLE 3. Effect of amiloride on clearance ratios of Na, Ca, and K in an intact dog (25.0 kg)

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Vol, ml/min</th>
<th>GFR, ml/min</th>
<th>$C_{\text{Na}}/GFR$</th>
<th>$C_{\text{Ca}}/GFR$</th>
<th>$C_{\text{Ca}}/C_{\text{Na}}$</th>
<th>$C_{\text{Ca}}/C_{\text{Na}}$</th>
<th>P&lt;sub&gt;amin&lt;/sub&gt;, mg/ml</th>
<th>P&lt;sub&gt;max&lt;/sub&gt;, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Slow infusion to 8.6 ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75-85</td>
<td>5.0</td>
<td>84.2</td>
<td>0.028</td>
<td>0.023</td>
<td>0.83</td>
<td>0.134</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>85-95</td>
<td>5.2</td>
<td>76.7</td>
<td>0.023</td>
<td>0.027</td>
<td>0.82</td>
<td>0.179</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>95-105</td>
<td>5.4</td>
<td>68.4</td>
<td>0.028</td>
<td>0.031</td>
<td>0.81</td>
<td>0.227</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean</td>
<td>5.2</td>
<td>76.1</td>
<td>0.023</td>
<td>0.027</td>
<td>0.82</td>
<td>0.177</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The mean fractions of calcium in plasma which was ultrafilterable were 70 ± 6, 67 ± 4, 69 ± 6, and 71 ± 5 for the four groups, respectively; these values did not change during the experiments.

### TABLE 4. Summary of clearance experiments using various doses of amiloride alone and in combination with chlorothiazide

<table>
<thead>
<tr>
<th>Group (Condition)</th>
<th>Drug</th>
<th>GFR</th>
<th>$C_{\text{Na}}/GFR$</th>
<th>$C_{\text{Ca}}/GFR$</th>
<th>$C_{\text{Ca}}/C_{\text{Na}}$</th>
<th>$C_{\text{Ca}}/C_{\text{Na}}$</th>
<th>P&lt;sub&gt;amin&lt;/sub&gt;, mg/ml</th>
<th>P&lt;sub&gt;max&lt;/sub&gt;, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Intact)</td>
<td>Amil.</td>
<td>1 mg/kg</td>
<td>2</td>
<td>+7 ± 3</td>
<td>+38 ± 8</td>
<td>-21 ± 5</td>
<td>-47 ± 5</td>
<td>-82 ± 2</td>
</tr>
<tr>
<td>II (TPTX)</td>
<td>Amil.</td>
<td>1 mg/kg</td>
<td>4</td>
<td>+9 ± 3</td>
<td>+52 ± 5</td>
<td>-16 ± 12</td>
<td>-50 ± 6</td>
<td>-82 ± 7</td>
</tr>
<tr>
<td>III (Intact)</td>
<td>Amil.</td>
<td>3 mg/kg</td>
<td>3</td>
<td>-2 ± 10</td>
<td>-36 ± 5</td>
<td>-52 ± 6</td>
<td>-72 ± 4</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>IV (Intact)</td>
<td>Amil.</td>
<td>5 mg/kg</td>
<td>5</td>
<td>+9 ± 3</td>
<td>+38 ± 7</td>
<td>-25 ± 10</td>
<td>-41 ± 8</td>
<td>-53 ± 9</td>
</tr>
<tr>
<td>CTZ</td>
<td>CTZ.</td>
<td>10 mg/kg</td>
<td>9</td>
<td>-2 ± 5</td>
<td>-127 ± 28</td>
<td>-4 ± 21</td>
<td>-59 ± 8</td>
<td>-42 ± 12</td>
</tr>
</tbody>
</table>

Values are percent change from control, mean ± SE. Amil = amiloride. For groups I and II, the loading dose of amiloride was 4 mg/kg and the sustaining rate was 0.2 mg/kg/min. For groups III and IV, the loading dose of amiloride was 4 mg/kg and the sustaining rate was 0.2 mg/kg/min. For groups I, II, III, and IV, the control values for GFR (in ml/min) were 60 ± 2, 70 ± 3, 81 ± 5, and 59 ± 5, respectively; for $C_{\text{na}}/GFR$, 0.95 ± 0.01, 0.96 ± 0.02, 0.97 ± 0.03, and 0.98 ± 0.01, respectively; for $C_{\text{Ca}}/GFR$, 0.04 ± 0.01, 0.06 ± 0.02, 0.04 ± 0.01, and 0.05 ± 0.01, respectively; for $C_{\text{Ca}}/C_{\text{Na}}$, 0.85 ± 0.00, 0.90 ± 0.09, 0.64 ± 0.09, and 0.01 ± 0.05, respectively; for $C_{\text{Ca}}/C_{\text{Na}}$, 0.30 ± 0.32 ± 0.11, 0.35 ± 0.06, 0.27 ± 0.05, respectively; N refers to the number of experiments at the indicated dose(s). The fraction of calcium in plasma which was ultrafilterable during control period was 70 ± 6, 67 ± 4, 69 ± 6, and 71 ± 5 for the four groups, respectively; these values did not change during the experiments.
The foregoing observation seems to eliminate decreased distal tubular potential as a mechanism for the CTZ effect on calcium excretion, but it does not eliminate the possibility that other drugs influence calcium excretion through a change in transepithelial potential. Amiloride which has an action on Cc/GFR qualitatively similar to that of CTZ has been reported to diminish distal tubular potential (11). This latter observation was made before the profile of potential differences along the distal tubule (33) was appreciated, and thus the magnitude of PD change is uncertain. However, there is no reason to doubt that PD is diminished by amiloride. Recently, amiloride has shown to decrease the transepithelial PD in isolated perfused distal convoluted tubules (16) and cortical collecting tubules (16, 25). The latter observations not only strengthen the idea that the calcium-retaining actions of CTZ and amiloride are mediated by mechanisms which are either fundamentally different or differ in the extent of the tubules involved. The magnitude of the effect of chlorothiazide on Cc/GFR is much greater than that of a maximally effective dose of amiloride. Moreover, each of the drugs could produce a further depression of Cc/GFR when administered in the presence of a maximally effective dose of the other. There are, however, some superficial similarities between the actions of CTZ and amiloride. Neither drug requires the presence of PTH for its effect on Cc/GFR and each of the drugs can produce a largely unilateral effect on this ratio when it is infused into one renal artery (ref. 6, Fig. 3).

Our studies with triamterene are less complete than with the other two drugs. We are limited in part by the insolubility of the drug in conventional vehicles and by the toxicity of the vehicle employed. (Preliminary experiments of longer duration than those reported or with higher rates of infusion revealed falling glomerular filtration rates and markedly diminished values of Cc/GFR. These phenomena were observed even when triamterene itself was omitted from the infusions.) The information available established that triamterene changes Cc/GFR in the same direction as CTZ and amiloride. The magnitude of the effect observed was of the same order as that produced by amiloride and smaller than that produced by CTZ. However, we did not establish that this effect of triamterene was maximal.

The thiazides, amiloride, and triamterene have all been studied in amphibian membranes. Each of the drugs has been reported to diminish short-circuit current, transepithelial potential difference, and electrical conductance (4, 8, 10, 15, 23, 24). It is not known at the present time which, if any, of these drug-induced changes are relevant to electrical properties or calcium transport in the mammalian distal tubule.

In summary, all four of the substances studied herein, CTZ, amiloride, triamterene, and PTH are natriuretic (1-3, 20) and all four produce a fall in Cc/GFR. Three of the substances have been shown to produce absolute hypocalciuria (6, 32; Tables 3 and 4) and it seems probable that the fourth, triamterene, may under certain experimental conditions do the same. Two of the substances, PTH and CTZ, produce kaliuresis (3, 14) and two, amiloride and triamterene, produce potassium retention (2, 20). At the present time there is no unifying hypothesis which would relate the various effects of the drugs on the excretions of these electrolytes. It may be as Meng (21) suggested that a single agent may influence the pattern of electrolyte excretion by more than one mechanism.

**TABLE 5. Summary of clearance experiments with triamterene or triamterene vehicle**

<table>
<thead>
<tr>
<th></th>
<th>GFR, ml/min</th>
<th>Cc, GFR</th>
<th>Cc, GFR</th>
<th>Cc, GFR</th>
<th>Cc, GFR</th>
<th>Ptrans, mumol</th>
<th>Ctrans, GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.1</td>
<td>0.046</td>
<td>0.033</td>
<td>0.72</td>
<td>0.22</td>
<td>0.001</td>
<td>0.044</td>
</tr>
<tr>
<td>Triam</td>
<td>&lt;5.5</td>
<td>&lt;0.007</td>
<td>&lt;0.005</td>
<td>&lt;0.02</td>
<td>&lt;0.04</td>
<td>&lt;0.001</td>
<td>&lt;0.048</td>
</tr>
<tr>
<td>Control</td>
<td>6.0</td>
<td>0.010</td>
<td>0.006</td>
<td>0.05</td>
<td>0.012</td>
<td>0.002</td>
<td>0.084</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>58.1</td>
<td>0.053</td>
<td>0.037</td>
<td>0.73</td>
<td>0.284</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>0.7</td>
<td>0.038</td>
<td>0.037</td>
<td>0.97</td>
<td>0.346</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Triam = triamterene. Statistical comparison of the change in Cc/GFR between the two groups yielded P < 0.001. The fractional ultrafiltrability of triamterene in plasma was 0.35 ± 0.02. For the first group of experiments the fractional ultrafiltrability of calcium was 74 ± 3% in control periods and 75 ± 2% during triamterene administration. For the second group, the values were 76 ± 2% for control and 78 ± 1% during solvent infusion.
In all of the experiments reported in this paper we monitored the concentrations of the various drugs in plasma and urine. Previous experience established that the effects of CTZ on $C_{\text{ctz}}/C_{\text{ctz}}$ were determined in part by the concentration of the drug in urine (tubular fluid) (6). In no instance in the present study could we attribute variations in response to variations in the concentration of any of the drugs in either plasma or urine. The renal clearance of CTZ in normal animals has been extensively studied and our results are similar to those in previous reports. We previously reported a subtle defect in CTZ excretion in dogs 48 h after thyparathyroidectomy (6). In the present study there was only a 10% difference in the ratio $C_{\text{ctz}}/\text{GFR}$ between intact dogs and the same dogs 1–8 wk after T-PTX. It seems possible that the larger deficit previously reported (35%) was, in part, a nonspecific complication of the postoperative period. In humans with chronic hypoparathyroidism we were unable to identify a defect in CTZ excretion (unpublished observations). Our results demonstrating tubular secretion of both triamterene and amiloride confirm those in the literature (20, 31).

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