Influence of Sodium Balance on the Ability of Diuretics to Inhibit Tubular Reabsorption

A Study of Factors that Influence Renal Tubular Sodium Reabsorption in Man

By Laurence E. Earley, M.D., and Joseph A. Martino, M.D.

SUMMARY
Chlorothiazide and ethacrynic acid were given to normal humans and patients with cirrhosis and ascites, with replacement of urinary losses, to obtain information on (1) the influence of different states of sodium balance and excretion on the effectiveness of the drugs in blocking tubular reabsorption and promoting excretion of sodium, and (2) the influence of different states of sodium balance on reabsorption of sodium by different segments of the nephron of man. The results demonstrated that in normal man the natriuretic response to these agents is markedly influenced by the existing state of sodium balance. The differences in response to the drugs related both to differences in the filtered load of sodium and to differences in the fraction of filtered sodium reabsorbed at the diuretic-sensitive distal tubular sites. Sodium loading in the presence of a mineralocorticoid was associated with a thiazide-induced increment in the excretion of sodium which was 10 times greater than that observed during sodium depletion. This effect is suggestive of decreased proximal tubular reabsorption. In patients with cirrhosis and sodium retention the fraction of filtered sodium reabsorbed at both diuretic-sensitive tubular sites was diminished, suggesting increased proximal tubular reabsorption. In addition to demonstrating a striking influence of sodium balance on the natriuretic response to the diuretics, the results suggest that in man sodium diuresis is associated with decreased fractional reabsorption by the proximal tubule and increased fractional and absolute sodium reabsorption by the distal tubule. In patients accumulating edema and ascites, an increase in the fractional reabsorption of sodium appears to occur in the proximal tubule and could account for limited effectiveness of the diuretic agents.

Additional Indexing Words:
Chlorothiazide Cirrhosis Ethacrynic acid

In clinical situations the natriuretic response to diuretic agents is known to differ greatly among patients accumulating edema or ascitic fluids. The response to organomercurial diuretics is diminished in the presence of hypochloremic alkalosis, presumably as a consequence of an interference with some step in the pharmacologic action of the drug at the level of the renal tubule. Diuretic drugs which act exclusively by inhibiting carbonic anhydrase, become less effective in promoting excretion of sodium in the presence of metabolic acidosis. No such specific

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pathways of interference, however, have been identified for thiazide diuretics or ethacrynic acid. In general, decreasing responsiveness to diuretic agents has been attributed to reduced glomerular filtration, increased secretion of aldosterone, and enhanced reabsorption of sodium at tubular sites unaffected by the drug(s).\textsuperscript{3} In view of the apparent distal tubular sites of action of thiazides\textsuperscript{4} and ethacrynic acid,\textsuperscript{5,6} it seems likely that changes in the delivery of sodium to the distal tubule could be a major factor determining the natriuretic effect of these two agents. In the dog and the rat, acute expansion of the extracellular compartment by infusion of saline increases the delivery of filtrate to the distal nephron as a consequence of both increased glomerular filtration and decreased fractional reabsorption by the proximal tubule.\textsuperscript{7,8} Under such conditions of acute volume expansion distal tubular reabsorption of sodium appears to be increased, since the excretion of sodium increases less than does the delivery of sodium from the proximal tubule.\textsuperscript{7,9} Presumably, under conditions of sodium retention, delivery of sodium to the distal nephron would be diminished as a result of increased fractional reabsorption in the proximal tubule and probably decreased glomerular filtration. If such changes in reabsorption and delivery of filtrate to the distal nephron occur also in the human kidney, then the state of sodium balance should markedly influence the response in man to diuretics that act exclusively or predominantly in the distal nephron. Furthermore, to the extent that the major site of action of the diuretic agent is known, such an effect on the response to diuretics should provide presumptive information regarding the influence of sodium balance on the profile of sodium reabsorption by the human nephron.

Accordingly, in the present study the effects of sequential administration of chlorothiazide and ethacrynic acid were studied in normal volunteers during different states of sodium balance and in a group of patients with liver disease and ascites.

**Methods**

Studies were carried out in one female and six male volunteers ranging in age from 28 to 58 years. None had any history, symptoms, or evidence of cardiovascular or renal disease. The subjects were hospitalized on the Clinical Research Center Metabolic Ward of the Thorsdike Memorial Laboratory throughout the period of study. Additional studies were performed in two female and three male patients with nutritional (alcoholic) cirrhosis of the liver and massive ascites with or without edema of the legs. Both the normal volunteers and the patients with liver disease were fully informed of the investigative nature of the studies, and each gave his free and informed consent to participate.

**Normal Sodium Balance**

All seven normal volunteers received a diet containing 50 mEq of sodium daily, and after 3 to 5 days when sodium balance was achieved, the initial study was performed with the subject recumbent and in a fasting state. Between 8 and 9 a.m. the subject ingested approximately 1 L of tap water and an infusion of 5 g of dextrose per 100 ml of water was begun at 5 ml/min. This infusion delivered inulin and p-aminohippurate (PAH) at rates suitable for clearance measurements. Approximately 2 hours after beginning this infusion, control collections were begun and extended for 38 to 129 min. During this time three to seven consecutive collections of urine were taken from an indwelling bladder catheter from most subjects, or by voiding by two of the male subjects. Peripheral venous blood samples were collected at the midpoint of each urinary collection or alternate urinary collections, depending on the duration of the period. Immediately following these control observations, 250 to 500 mg of chlorothiazide were injected intravenously, followed by the constant infusion of chlorothiazide at a rate of 5 mg/min. When urine flow began to increase (within 5 min), an intravenous infusion of 0.9 g\% saline containing 5 mEq/L of potassium chloride was begun at a rate approximately equal to that of urinary flow. Approximately 10 min later when urinary flow was relatively stable, four to seven additional clearance collections were taken. Following this, 50 to 100 mg of ethacrynic acid was injected intravenously and then ethacrynic acid was infused at a rate of 1 mg/min throughout the remainder of the study. Infusion of the replacement solution was increased to rates 2 to 4 ml/min less than the rate of urinary flow. When urinary flow was stable at the increased rate, 5 to 10 additional clearance collections were taken.

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Sodium Loading in the Presence of a Mineralocorticoid

Each of the seven volunteers was given 2 mg of 9 alpha-fluoro-hydrocortisone daily and a diet containing supplemental potassium and 200 mEq of sodium daily. In addition they received an infusion of 1,000 ml of 0.9% saline daily for 2 or 3 days. This technic of sodium loading in the presence of a mineralocorticoid has been shown to permit rapid achievement of sodium balance in the presence of the steroid and therefore permits the study of acute saline diuresis independent of mineralocorticoid suppression. Within 24 hours the subjects' weights had stabilized at an increase of 1.1 to 6.0 kg, and they were excreting the large daily intake of sodium. In other words, they had achieved sodium balance and "escaped" from the sodium-retaining effects of the steroid. Two to four days later the subjects were restudied with diuretic blockade as described above, except that each received 1,000 ml of 0.9% saline intravenously followed by 10 ml/min, prior to beginning the control collections of urine. This resulted in moderate to marked natriuresis during control collections.

Sodium Depletion

Four subjects were permitted to become mildly depleted of sodium by not replacing completely the urinary losses which continued for several hours after completing the acute clearance studies. They were then maintained on a diet containing less than 10 mEq of sodium daily, and 3 to 5 days later the acute clearance study was repeated. Losses of weight at the time of this study ranged from 0.5 to 2.1 kg.

Quiet Standing

The effects of quiet standing on sodium excretion and reabsorption were observed in four subjects in eight different studies. This maneuver is known to result in an acute decrease in sodium excretion in man. After completing collections during recumbency in the presence of both chlorothiazide and ethacrynic acid, the subjects stood in one position by the bed, and clearance collections were continued for an additional 20 to 50 min. In some, but not all, studies the patients returned to the recumbent position, and clearance collections were continued. This maneuver permitted observations of the effects of standing on sodium excretion and residual sodium reabsorption in the presence of both chlorothiazide and ethacrynic acid.

Patients with Pathologic Sodium Retention

Five patients with ascites and edema secondary to cirrhosis of the liver received a daily intake of 25 to 50 mEq of sodium, and each demonstrated positive sodium balance. The acute clearance studies were performed as described above. Two of these patients were restudied 3 and 5 days following the initial study which had resulted in a forced negative sodium balance. The initial study with diuretics resulted in a loss of weight of 2.9 and 11.0 kg in these two patients.

Calculation of Fractional Sodium Reabsorption

Fractional tubular reabsorption of sodium was calculated as:

\[ 1 - \frac{(U_{Na} + U_{K})}{(GFR \times P_{Na})}, \]

where \( U_{Na} \) and \( U_{K} \) are the rates of excretion of sodium and potassium, respectively; GFR is the clearance of inulin; and \( P_{Na} \) is the concentration of sodium in plasma. Tubular fractional sodium reabsorption blocked by chlorothiazide \((T_{Na}^{CTZ})\) was calculated as

\[ \frac{(U_{Na} + U_{K})}{(GFR \times P_{Na})_{CTZ}} - \frac{(U_{Na} + U_{K})}{(GFR \times P_{Na})_{Control}}, \]

where the first part of the expression is fractional sodium excretion during the steady state effect of chlorothiazide, and the part subtracted is the fractional sodium excretion during the pre-diuretic control periods. Tubular sodium reabsorption blocked by ethacrynlic acid \((T_{Na}^{EA})\) was calculated as

\[ \frac{(U_{Na} + U_{K})}{(GFR \times P_{Na})_{EA, CTRZ}} - \frac{(U_{Na} + U_{K})}{(GFR \times P_{Na})_{CTZ}}, \]

where the first part of the expression is fractional sodium excretion in the presence of both ethacrynlic acid and chlorothiazide. Residual fractional reabsorption in the presence of both diuretic agents is equal to \( 1 - \frac{(U_{Na} + U_{K})}{(GFR \times P_{Na})_{EA, CTRZ}} \). Analytical procedures were performed by technics previously described.

Results

Response to Chlorothiazide

In seven normal volunteers in sodium balance on a daily intake of 50 mEq of sodium, \( U_{Na} \) averaged \( 114 \pm 57 \) (standard deviation) \( \mu \)Eq/min prior to administration of diuretics. The filtered load of sodium averaged \( 16.4 \pm 3.5 \) mEq/min. During the steady state effect of chlorothiazide \( U_{Na} \) increased an average of \( 690 \pm 249 \) \( \mu \)Eq/min, and the

\*Urinary potassium represents predominantly secretion of potassium in "exchange" for reabsorbed sodium at a tubular site distal to the proximal tubule and distal to the sites of action of both chlorothiazide and ethacrynic acid. Therefore, excreted potassium should represent an amount of sodium at least equal to that reabsorbed distal to the sites of diuretic action.
Table 1
Summary of Effects of Sequential Administration of Chlorothiazide and Ethacrynic Acid to Normal Volunteers during Different States of Sodium Balance and to Patients with Cirrhosis and Ascites

<table>
<thead>
<tr>
<th></th>
<th>GFR</th>
<th>C\textsubscript{PAR}</th>
<th>FF</th>
<th>F\textsubscript{Na}</th>
<th>U\textsubscript{Na\textsubscript{K}}V</th>
<th>U\textsubscript{Na}V</th>
<th>(1 - \left(\frac{U_{Na+K}V}{F_{Na}}\right))</th>
<th>(\Delta U_{Na}V)</th>
<th>(\Delta U_{Na+K}V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>119</td>
<td>576</td>
<td>0.20</td>
<td>16.4</td>
<td>114</td>
<td>96</td>
<td>98.7</td>
<td>(± 26)</td>
<td>(± 10)</td>
</tr>
<tr>
<td>CTZ (n = 7)</td>
<td>99</td>
<td>532</td>
<td>0.18</td>
<td>13.7</td>
<td>803</td>
<td>116</td>
<td>93.2</td>
<td>(± 21)</td>
<td>(± 20)</td>
</tr>
<tr>
<td>CTZ + EA (n = 7)</td>
<td>106</td>
<td>600</td>
<td>0.18</td>
<td>14.6</td>
<td>4245</td>
<td>291</td>
<td>68.7</td>
<td>(± 16)</td>
<td>(± 11)</td>
</tr>
<tr>
<td>Sodium loading and 9-(\alpha)-fluorohydrocortisone (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>156</td>
<td>635</td>
<td>0.25</td>
<td>21.6</td>
<td>883</td>
<td>180</td>
<td>95.3</td>
<td>(± 25)</td>
<td>(± 24)</td>
</tr>
<tr>
<td>CTZ (n = 7)</td>
<td>137</td>
<td>561</td>
<td>0.25</td>
<td>19.2</td>
<td>2437</td>
<td>285</td>
<td>86.0</td>
<td>(± 18)</td>
<td>(± 17)</td>
</tr>
<tr>
<td>CTZ + EA (n = 7)</td>
<td>146</td>
<td>644</td>
<td>0.23</td>
<td>20.7</td>
<td>7058</td>
<td>422</td>
<td>63.6</td>
<td>(± 19)</td>
<td>(± 18)</td>
</tr>
<tr>
<td>Sodium depleted (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>112</td>
<td>692</td>
<td>0.17</td>
<td>15.0</td>
<td>4</td>
<td>138</td>
<td>99.1</td>
<td>(± 14)</td>
<td>(± 13)</td>
</tr>
<tr>
<td>CTZ (n = 4)</td>
<td>96</td>
<td>555</td>
<td>0.17</td>
<td>12.9</td>
<td>158</td>
<td>345</td>
<td>95.9</td>
<td>(± 15)</td>
<td>(± 14)</td>
</tr>
<tr>
<td>CTZ + EA (n = 4)</td>
<td>110</td>
<td>594</td>
<td>0.19</td>
<td>14.9</td>
<td>4104</td>
<td>520</td>
<td>69.0</td>
<td>(± 12)</td>
<td>(± 11)</td>
</tr>
<tr>
<td>Patients with cirrhosis and ascites (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>79</td>
<td>413</td>
<td>0.20</td>
<td>10.5</td>
<td>18</td>
<td>37</td>
<td>99.3</td>
<td>(± 14)</td>
<td>(± 13)</td>
</tr>
<tr>
<td>CTZ (n = 7)</td>
<td>69</td>
<td>380</td>
<td>0.19</td>
<td>9.1</td>
<td>299</td>
<td>118</td>
<td>95.5</td>
<td>(± 16)</td>
<td>(± 15)</td>
</tr>
<tr>
<td>CTZ + EA (n = 7)</td>
<td>68</td>
<td>471</td>
<td>0.15</td>
<td>9.1</td>
<td>1995</td>
<td>211</td>
<td>75.7</td>
<td>(± 10)</td>
<td>(± 9)</td>
</tr>
</tbody>
</table>

*Values are the means and standard deviations of the means of multiple clearance periods from individual experiments. n = the number of experiments.

Abbreviations: GFR = glomerular filtration rate (clearance of inulin); C\textsubscript{PAR} = clearance of p-aminohippurate; FF = filtration fraction (GFR/C\textsubscript{PAR}); F\textsubscript{Na} = filtered load of sodium (GFR \times plasma sodium concentration); U\textsubscript{Na\textsubscript{K}}V = rate of sodium excretion; U\textsubscript{Na}V = rate of potassium excretion; 1 - \(\frac{U_{Na+K}V}{F_{Na}}\) = fraction of filtered sodium reabsorbed.

Control = collections prior to administration of diuretics; CTZ = collections during administration of chlorothiazide alone; CTZ + EA = collections during administration of chlorothiazide and ethacrynic acid; \(\Delta U_{Na}V\) = change in the rate of excretion of sodium produced by the diuretic; \(\Delta(U_{Na+K}V)/F_{Na}\) = fraction of the filtered load of sodium blocked from reabsorption by the diuretic.
Table 2

Effects of Sequential Administration of Chlorothiazide and Ethacrynic Acid on Tubular Sodium Reabsorption in a Normal Volunteer on a Daily Intake of 50 mEq of Sodium

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>GFR (ml/min)</th>
<th>C_PAH (ml/min)</th>
<th>F_Na (mEq/min)</th>
<th>U_NaV (μEq/min)</th>
<th>U_KV (μEq/min)</th>
<th>U/P_{Osm}</th>
<th>1 - (U_{Na,KV} \times 100)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-180 to -120</td>
<td>1,000 ml tap water PO; began intravenous infusion of inulin and PAH in 5 g % dextrose in water at 5 ml/min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 6</td>
<td>130</td>
<td>592</td>
<td>18.1</td>
<td>82</td>
<td>126</td>
<td>0.5</td>
<td>99</td>
<td>116/70</td>
</tr>
<tr>
<td>6 - 21</td>
<td>118</td>
<td>502</td>
<td>16.4</td>
<td>71</td>
<td>107</td>
<td>0.5</td>
<td>99</td>
<td>120/72</td>
</tr>
<tr>
<td>21 - 31</td>
<td>128</td>
<td>530</td>
<td>17.9</td>
<td>82</td>
<td>103</td>
<td>0.9</td>
<td>99</td>
<td>124/64</td>
</tr>
<tr>
<td>31 - 66</td>
<td>159</td>
<td>653</td>
<td>22.1</td>
<td>104</td>
<td>104</td>
<td>1.3</td>
<td>99</td>
<td>130/70</td>
</tr>
<tr>
<td>Chlorothiazide, 500 mg intravenously and then 5 mg/min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>75</td>
<td>Began intravenous infusion of replacement solution at 5 ml/min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 - 92</td>
<td>109</td>
<td>558</td>
<td>15.3</td>
<td>910</td>
<td>250</td>
<td>1.5</td>
<td>93</td>
<td>125/60</td>
</tr>
<tr>
<td>92 - 107</td>
<td>114</td>
<td>582</td>
<td>16.0</td>
<td>993</td>
<td>216</td>
<td>1.6</td>
<td>92</td>
<td>136/70</td>
</tr>
<tr>
<td>97 - 112</td>
<td>120</td>
<td>582</td>
<td>16.5</td>
<td>949</td>
<td>195</td>
<td>1.6</td>
<td>93</td>
<td>112/68</td>
</tr>
<tr>
<td>107 - 117</td>
<td>101</td>
<td>482</td>
<td>13.9</td>
<td>779</td>
<td>151</td>
<td>1.6</td>
<td>93</td>
<td>130/70</td>
</tr>
<tr>
<td>112 - 117</td>
<td>112</td>
<td>525</td>
<td>15.5</td>
<td>847</td>
<td>156</td>
<td>1.6</td>
<td>94</td>
<td>108/70</td>
</tr>
<tr>
<td>Ethacrynic acid 50 mg intravenously and then 1.0 mg/min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>Increased rate of infusion of replacement solution to 24 ml/min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>135 - 145</td>
<td>113</td>
<td>659</td>
<td>15.5</td>
<td>4633</td>
<td>421</td>
<td>1.0</td>
<td>66</td>
<td>125/80</td>
</tr>
<tr>
<td>145 - 150</td>
<td>96</td>
<td>495</td>
<td>13.1</td>
<td>3850</td>
<td>370</td>
<td>1.0</td>
<td>68</td>
<td>122/80</td>
</tr>
<tr>
<td>150 - 155</td>
<td>105</td>
<td>622</td>
<td>14.4</td>
<td>4435</td>
<td>352</td>
<td>1.0</td>
<td>67</td>
<td>124/74</td>
</tr>
<tr>
<td>155 - 161</td>
<td>105</td>
<td>559</td>
<td>14.4</td>
<td>4567</td>
<td>348</td>
<td>1.0</td>
<td>66</td>
<td>110/72</td>
</tr>
<tr>
<td>162</td>
<td>Subject assumed standing position for remainder of study.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>161 - 167</td>
<td>95</td>
<td>457</td>
<td>13.2</td>
<td>2936</td>
<td>256</td>
<td>1.0</td>
<td>76</td>
<td>94/72</td>
</tr>
<tr>
<td>167 - 177</td>
<td>79</td>
<td>358</td>
<td>11.9</td>
<td>1975</td>
<td>231</td>
<td>1.0</td>
<td>81</td>
<td>104/70</td>
</tr>
<tr>
<td>177 - 181</td>
<td>109</td>
<td>453</td>
<td>15.0</td>
<td>2135</td>
<td>280</td>
<td>1.0</td>
<td>84</td>
<td>98/74</td>
</tr>
<tr>
<td>181 - 186</td>
<td>99</td>
<td>407</td>
<td>13.7</td>
<td>1757</td>
<td>245</td>
<td>1.0</td>
<td>85</td>
<td>98/70</td>
</tr>
</tbody>
</table>

Abbreviations: U/P_{Osm} = ratio of urine to plasma of total solute concentration. For other abbreviations see table 1.
increment in total cation excretion averaged 5.5 ± 1.4% of the filtered load of sodium. When the same subjects were studied during volume expansion in the presence of 9-α-fluorohydrocortisone, control rates of sodium excretion averaged 883 ± 405 μEq/min, in association with a higher filtered load of sodium which averaged 21.6 ± 3.9 mEq/min. During chlorothiazide a larger increase in UNaV occurred and averaged 1,549 ± 607 μEq/min. The increment in total cation excretion averaged 9.3 ± 2.9% of the filtered load of sodium which was significantly greater than that observed on the low sodium diet (P = 0.01). Thus, both the absolute and fractional inhibition of sodium reabsorption by chlorothiazide was increased when the subjects were in a state of sodium loading with high control rates of sodium excretion. When four of the subjects were studied in a state of moderate sodium depletion, UNaV averaged 4 ± 4 μEq/min in association with a filtered load of sodium which averaged 15.0 ± 1.3 mEq/min. Under these conditions the natriuretic effect of chlorothiazide was strikingly decreased. This decrease in the natriuretic effect of chlorothiazide in the sodium-depleted individuals was associated with a higher rate of potassium excretion than was observed under any other conditions of the study (table 1), and, therefore, the reduction in sodium excretion during chlorothiazide was proportionately greater than the reduction in fractional cation excretion (figs. 1 and 2). The increase in UNaV averaged only 155 ± 83 μEq/min, and the increment in cation excretion averaged 3.2 ± 1.2% of the filtered load of sodium. Both of these changes were significantly less than those observed in the absence of sodium depletion (P < 0.01 for both changes).

In seven studies on five patients with cirrhosis and ascites, control UNaV averaged 18 ± 15 μEq/min in association with a filtered load of sodium averaging 10.5 ± 2.3 mEq/min. Chlorothiazide resulted in an increase in UNaV averaging 281 ± 151 μEq/min and an increment in cation excretion which averaged 3.8 ± 1.3% of the filtered load of sodium. Both of these changes were significantly less (P < 0.01 for both) than those observed in the normal individuals on the control sodium diet. However, the increases in UNaV and fractional excretion of cation were not significantly different from the increases observed in the normal individuals when studied during sodium depletion.

Details of a single study in a normal volunteer are given in table 2, and data on the

![Figure 1](http://circ.ahajournals.org/)

**Figure 1**

Increase in the excretion of sodium due to chlorothiazide in normal individuals under different conditions of sodium balance and in patients with cirrhosis and ascites. Values are the means of multiple collections during a steady-state effect of the diuretic, and lines connect the same individual under the different experimental conditions.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2**

Effects of chlorothiazide on fractional cation excretion in normal individuals under different conditions of sodium balance and in patients with cirrhosis and ascites. Values are the means of multiple collections during a steady-state effect of the diuretic and lines connect the same individual under the different experimental conditions.

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Effects of chlorothiazide from all studies are summarized in table 1 and figures 1 and 2.

Response to Ethacrynic Acid

The steady-state effects of adding ethacrynic acid to the administration of chlorothiazide are shown in tables 1 and 2 and figures 3 and 4. In the normal individuals on a daily intake of 50 mEq of sodium, ethacrynic acid produced an increment in $U_{\text{Na}}V$ averaging 3.442 ± 0.695 µEq/min and an increment in cation excretion which averaged 24.4 ± 2.0% of the filtered load of sodium. When the subjects were studied during sodium loading, the increment in $U_{\text{Na}}V$ averaged 4.594 ± 0.954 µEq/min which represented an increase above that observed in the presence of the 50mEq daily intake in five of the seven subjects (fig. 3). However, during sodium loading the increment in cation excretion averaged 22.4 ± 4.4% which was not significantly different from that observed on the low sodium diet (table 1 and fig. 4). In the four subjects studied during sodium depletion the increments in $U_{\text{Na}}V$ and fractional cation excretion also were not significantly different from those of the group as a whole when studied in the absence of sodium depletion (table 1 and fig. 4).

In the patients with cirrhosis and ascites ethacrynic acid produced an increment in $U_{\text{Na}}V$ averaging 1.696 ± 0.504 µEq/min which was significantly less ($P < 0.01$) than that observed in the normal individuals during either state of sodium balance. The increment in cation excretion in the patients averaged 19.8 ± 3.9% of the filtered load of sodium and was significantly less than that observed in the normal individuals on the 50-mEq sodium diet and during sodium depletion ($P < 0.02$ and $< 0.01$, respectively). Thus, in the patients with cirrhosis the natriuretic response to ethacrynic acid was distinctly less than that in the normal individuals during either state of sodium balance, and this diminished response related to both a smaller filtered load of sodium and a diminished fractional inhibition of sodium reabsorption (table 1).

Residual Tubular Reabsorption of Sodium

In the presence of both chlorothiazide and ethacrynic acid with replacement of urinary losses, residual tubular reabsorption of sodium averaged 68.7 ± 3.7% of the filtered load of sodium. When the same subjects were studied during sodium loading in the presence of 9α-fluorohydrocortisone, residual tubular reabsorption was significantly decreased ($P < 0.01$) and averaged 63.6 ± 1.3% of the filtered load of sodium (table 1 and fig. 5). However, GFR and absolute tubular reabsorption were increased during sodium loading. In the four subjects studied during sodium depletion residual tubular reabsorption averaged 69.0 ±
Residual fractional tubular reabsorption of sodium in the presence of chlorothiazide and ethacrynic acid administration in normal individuals in different states of sodium balance and in patients with cirrhosis and ascites. Values are the means of multiple collection periods during a steady-state effect of the two diuretic agents and replacement of urinary losses. Lines connect the same individual under different experimental conditions.

1.7% of the filtered load of sodium which was not different from the value (68.7 ± 3.7%) in the presence of the 50-mEq intake of sodium. In the patients with cirrhosis and ascites residual tubular reabsorption averaged 75.7 ± 4.4% of the filtered load of sodium (table 1 and fig. 5) which was significantly greater (P < 0.01) than that observed in the normal individuals during either state of sodium balance. Thus, in the presence of both diuretic agents the fraction of filtered sodium reabsorbed was depressed in the normals during sodium loading and increased in the patients with cirrhosis.

In eight studies in four individuals, measurements during the combined administration of chlorothiazide and ethacrynic acid were made in both the recumbent and standing positions. Results of one of these studies are given in detail in table 2, and all eight studies are summarized in figure 6. Residual tubular reabsorption increased from an average of 66.5 ± 4.4% of the filtered load of sodium during recumbency to an average of 76.4 ± 5.8% during standing (P < 0.01). This change was associated with a fall in glomerular filtration rate from an average of 126 ± 28 to 101 ± 24 ml/min. In several of the subjects residual fractional reabsorption of sodium was as high or higher during standing as that observed in the patients with cirrhosis or in the same individuals during sodium depletion, even though GFR was lower in the latter two situations (compare figure 6 and table 1). Thus, although the higher residual fractional reabsorption during standing related to a fall in GFR, the absolute value for residual reabsorption did not relate to the absolute value of GFR when comparison is made among the groups of studies.

In none of the present studies did differences in the effects of the individual diuretic agents or differences in residual fractional reabsorption relate in a predictable manner to differences in filtration fraction, calculated as GFR/CpAH (table 1; fig. 6).

**Discussion**

The present results demonstrate in normal man that the absolute natriuretic response to chlorothiazide and ethacrynic acid is markedly influenced by the existing state of sodium balance. The increment in sodium excretion effected by chlorothiazide in the presence of salt loading and saline diuresis was more than twice that observed when the individuals were in balance on a limited intake of sodium and was 10 times as great as that observed when...
the individuals were depleted of sodium. In sodium-depleted normal individuals, however, the limited natriuretic effect of chlorothiazide was associated with a large increase in the excretion of potassium, suggesting that the excretion of sodium was limited by enhanced reabsorption at the potassium secretory site distal to the site of action of chlorothiazide. It is not likely that the smaller effect of the drug on fractional cation excretion in the presence of sodium restriction and sodium depletion was due to a greater tubular effect of aldosterone, since the studies during salt loading were performed in the presence of an exogenous mineralocorticoid. Although the possibility cannot be excluded that the state of sodium balance may in some unknown way condition the pharmacologic effect of chlorothiazide at its tubular site of action, this explanation does not seem likely.

These differences in the natriuretic effect of chlorothiazide can be explained by an effect of sodium balance to change the amount of sodium reabsorbed at the drug-sensitive site. In both man and the dog, the major site at which thiazides interfere with sodium reabsorption appears to be in the distal nephron beyond the medullary portion of the loop of Henle and functionally proximal to the site of potassium secretion. Micropuncture studies have demonstrated that thiazides may not alter net proximal tubular reabsorption and the diuretic decreases sodium reabsorption at a site proximal to the first accessible portion of the distal convoluted tubule. Taken together, the evidence favors the view that the major tubular site of action of thiazide diuretics is the cortical portion of the loop of Henle.

The differences in the absolute natriuretic effect of chlorothiazide observed in the present study related both to the filtered load of sodium and to changes in the fraction of filtered sodium excreted in the presence of the drug. The results are consistent with the view that in man saline diuresis in the presence of a mineralocorticoid is associated with increased absolute and fractional reabsorption of sodium by the chlorothiazide-sensitive segment of the distal tubule, presumably the cortical portion of the ascending limb of Henle's loop. In the patients with cirrhosis and in the normal individuals during sodium depletion, absolute and fractional reabsorption at this tubular site was diminished. These changes related in a direct manner to differences in glomerular filtration rate and inversely to the fractional reabsorption of sodium by the remainder of the tubule that was not affected by the thiazide.

The addition of ethacrynic acid in the presence of chlorothiazide permitted a more complete estimate of distal tubular reabsorption. The major site of action of ethacrynic acid appears to be in the medullary portion of the loop of Henle where the drug may virtually abolish sodium reabsorption. The inhibitory effects of ethacrynic acid and chlorothiazide on sodium reabsorption and urinary dilution have been shown to be additive in the dog. In the present study when ethacrynic acid was administered to normal individuals in sodium balance, there was an increment in sodium excretion which averaged 24% of the filtered load of sodium. This is in close agreement with the effect of this drug when given alone to man and reinforces the conclusion that in the present study the two agents were exerting separate and additive effects. The influence of sodium balance on fractional sodium reabsorption at the ethacrynic acid-sensitive site was proportionally less than that observed at the chlorothiazide-sensitive site. The absolute effect of ethacrynic acid on sodium excretion was increased in salt-loaded individuals above that observed in those on the low sodium diet. This greater influence was related primarily to a greater filtered load of sodium, and the fraction of filtered sodium excreted was not statistically different under these conditions. Also, sodium depletion had little effect on the extent to which ethacrynic acid inhibited fractional sodium reabsorption. In the patients with cirrhosis the absolute effect of ethacrynic acid to inhibit sodium reabsorption was distinctly less than its effect in either of the studies in normal individuals, and this lesser response related to both a decreased filtered
load of sodium and diminished fractional inhibition of sodium reabsorption. These observations suggest that in the salt-loaded human, reabsorption of sodium by the medullary loop of Henle is primarily increased as a result of increased delivery of filtrate to this site. In the patients with cirrhosis reabsorption of sodium at the ethacrynic acid-sensitive site was less than in normals and was due to both a decreased filtered load of sodium and decreased fractional reabsorption at this drug-sensitive site.

Residual tubular reabsorption in the presence of both chlorothiazide and ethacrynic acid may provide an indirect index of proximal tubular reabsorption. Although micropuncture evidence is conflicting as to whether ethacrynic acid acts to inhibit proximal tubular reabsorption, a proximal tubular effect has been apparent only when glomerular filtration rate is maintained at a high value. Thus, the major natriuretic effect of ethacrynic acid appears to be due to extensive inhibition of sodium reabsorption in the medullary loop of Henle. The evidence that these two diuretic agents together extensively interfere with distal tubular sodium reabsorption is convincing, and apparently any inhibition of reabsorption in the proximal tubule produced by these drugs is readily overcome by other factors which alter proximal reabsorption. Previous studies in the dog utilizing a similar technic of diuretic-induced distal tubular blockade yielded data on apparent proximal tubular reabsorption which were previously, or have since been, supported by micropuncture studies. Thus, the ability of increased arterial pressure and saline infusion to decrease residual tubular reabsorption during distal tubular blockade, and the ability of aortic constriction and hyperoncotic albumin to increase residual reabsorption have been shown by micropuncture to be proximal tubular events. Therefore, even if these diuretic agents have some inhibiting effect on reabsorption in the proximal tubule, this segment still responds to other maneuvers which alter reabsorption.

It is suggested then that residual sodium reabsorption in the presence of chlorothiazide and ethacrynic acid in man represents predominantly proximal tubular reabsorption. Accordingly, the present study indicates that fractional reabsorption by the proximal tubule of man is depressed during saline diuresis and that this depression is not due to decreased mineralocorticoid effect. Coupled with increased glomerular filtration this depressed proximal fractional reabsorption results in a large absolute increase in delivery of sodium beyond the proximal tubule and increased reabsorption of sodium by the distal tubule (predominantly the loop of Henle). Such an effect of saline infusion to increase delivery of filtrate beyond the proximal tubule and to augment the reabsorption of sodium distally has been demonstrated by micropuncture technics in the dog and rat. In normal individuals sodium depletion was not associated with increased residual tubular reabsorption (in the presence of both drugs), even though a smaller fraction of the filtered sodium was excreted in response to chlorothiazide. This limited natriuretic effect of chlorothiazide in sodium-depleted normal individuals was due to a combination of reduced glomerular filtration, a somewhat greater fractional reabsorption at the more proximal ethacrynic acid-sensitive site and enhanced sodium reabsorption at tubular sites distal to the site affected by chlorothiazide (as evidenced by a greater excretion of potassium). In the patients accumulating ascitic fluid residual fractional sodium reabsorption in the presence of the two drugs was significantly greater than in the normals. This observation is consistent with the view that fractional reabsorption by the proximal tubule was increased in the patients. Coupled with a diminished filtered load of sodium such enhanced fractional reabsorption by the proximal tubule would limit delivery to, and reabsorption of, sodium by the distal nephron, and would adequately explain the diminished effectiveness of the drugs in blocking sodium reabsorption in the patients with cirrhosis and ascites.
These conclusions are entirely consistent with micropuncture data in the rat and dog which indicate that acute saline diuresis is associated with decreased fractional reabsorption by the proximal tubule and increased fractional and absolute reabsorption by the distal nephron. These conclusions are consistent also with the known effects in man of sodium restriction or depletion to limit water diuresis and of sodium loading which enhances water diuresis. These effects of sodium balance on the excretion of dilute urine indicate also that sodium loading increases the delivery of filtrate to the distal nephron where increased reabsorption of sodium increases the volume of dilute urine excreted during water diuresis.

In the present study residual fractional reabsorption increased acutely when the individuals assumed the upright posture. This observation suggests that the increased overall reabsorption and decreased excretion of sodium associated with assuming the upright posture is, in part at least, a result of increased fractional reabsorption by the proximal tubule. Glomerular filtration rate was also decreased in the standing position, so that the excretion of sodium was diminished as a result of both a decreased filtered load and increased fractional reabsorption of sodium. Whether a cause and effect relationship exists between the fall in filtration and the rise in fractional reabsorption, or whether these are simply associated events is unknown. Although an increase in fractional reabsorption and a fall in glomerular filtration were associated events during standing, when the various groups of studies are compared, there was no relationship between the absolute rate of filtration and the residual sodium reabsorption in the presence of the two diuretic agents. In the presence of salt loading, standing resulted in an increased fractional reabsorption at a time when filtration rate was greater than that observed in the absence of salt loading and lower fractional reabsorption. There was no evidence that the different states of sodium balance were accompanied by absolute changes in tubular reabsorption in a direction opposite to the changes in sodium excretion. Due to the associated changes in glomerular filtration, the absolute rate of sodium reabsorption was increased during sodium loading and was decreased during sodium depletion and in the patients with ascites (when the latter are compared with the normal individuals).

The present data demonstrate an influence of sodium balance on the quantitative effect of distally acting diuretic agents, and these changes in response to the diuretic agents provide indirect evidence in man for an influence of sodium balance on the relationship between sodium reabsorption in the proximal and distal segments of the nephron. The relatively small natriuretic response of the patients with ascites to the diuretics resembled the response of the normal individuals when depleted of sodium, and was qualitatively opposite to the response of the normals during saline loading. This limited response of the patients with ascites was related to both a diminution in the filtered load of sodium and decreased fractional inhibition of tubular sodium reabsorption. Since the major site of action of these agents is in the distal tubule, the most likely explanation for these findings is that in the patient accumulating ascitic fluid the excretion of sodium is limited by both a reduced filtered load and enhanced fractional reabsorption in the proximal tubule. Conversely, in normal man salt loading increases excretion of sodium as a result of both increased filtered load and decreased fractional reabsorption by the proximal tubule.

References
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Influence of Sodium Balance on the Ability of Diuretics to Inhibit Tubular Reabsorption: A Study of Factors that Influence Renal Tubular Sodium Reabsorption in Man

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