On the Mechanism of Antihypertensive Action of Hydrochlorothiazide in Rats

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Chlorothiazide and congeners, such as hydrochlorothiazide, have been shown to decrease the blood pressure in hypertensive patients\(^1\) and animals.\(^2\) Indeed, all potent diuretics have been suggested to possess some antihypertensive properties either alone or in combination with other agents.\(^3\) Two consequences of the diuretic action of chlorothiazide have been postulated to account for its hypotensive effects: reduction in plasma volume and extracellular fluid volume (ECFV)\(^6\) and reduction in cellular sodium concentration with consequent alteration in the sodium gradient and in cellular function.\(^9\) Although reduction in ECFV following chlorothiazide has been repeatedly demonstrated, continued treatment may result in restoration of ECFV but not of the original blood pressure.\(^6\) Direct evidence for reduction of cellular sodium concentration is lacking.

As has been recently emphasized,\(^11\) the mechanisms whereby blood pressure may be lowered are limited and involve usually either reduction in cardiac output or decrease in vascular resistance to flow. If the hypotensive action of chlorothiazide operated through reduction in extracellular sodium and fluid, it might act by decreasing cardiac output, and this has been reported to occur in hypertensive patients treated with the drug.\(^12\) However, reduction in plasma volume or extracellular fluid volume is not alone sufficient condition for causing reduced blood pressure since a similar reduction in ECFV in normotensive patients does not lower blood pressure. Further reduction of cardiac output does not necessarily reduce the blood pressure in the intact organism.\(^14\) Indeed, chlorothiazide-induced diminution of ECFV may produce hypotension in other more complex ways. Reduction of peripheral resistance might occur by elimination of edema from walls of smaller blood vessels or by reduction of the pressure of surrounding tissues on small blood vessels.\(^6\)\(^9\)

If reduction in cellular sodium concentration is the operative action of chlorothiazide, hypotension might result from some alteration in function of cardiac cells leading to reduced cardiac output or of vascular smooth muscle cells to reduce their tone or responsiveness to humoral excitants.\(^6\)\(^7\) Hence, if chlorothiazide does act on cellular sodium and/or on extracellular sodium, this does not distinguish between the heart and blood vessels as possible sites of its hypotensive effect. However, if chlorothiazide-induced changes in sodium concentration do occur and are restricted either to the heart or to vascular muscle cells, this selectivity of action would provide a clue to the site of the hypotensive effect.

The objects of this investigation were, therefore, (1) to obtain, if possible, direct evidence as to the changes in tissue composition and fluid volumes associated with the hypotensive action of hydrochlorothiazide in rats, and (2) to determine if these were localized in specific tissues in such a way as would be consistent with a mechanism for the hypotensive effect.

Methods

EXPERIMENT NO. 1

Male albino rats of the Wistar strain in matched groups were used throughout. All animals were unilaterally nephrectomized and then had 20-mg desoxycorticosterone acetate (DCA) pellets implanted to produce active hypertension as in a previous experiment.\(^3\) The hypertension was later
assessed by preliminary determinations of femoral arterial pressure. These animals were then divided into four groups. One of these received no hydrochlorothiazide but did receive the vehicle (group 1). The other three groups received hydrochlorothiazide 10 mg./Kg. once a day by mouth for three days and twice on the fourth day. On the fifth day, all animals were nephrectomized and given inulin. 18 hours after food and water had been withdrawn. Two hours later, the animals were anesthetized with ether and prepared for recording femoral arterial pressure and for infusion of saline into the femoral vein. A blood sample was taken, and thereafter the blood pressure was recorded continuously. One hydrochlorothiazide-treated group (group 2) and the untreated hypertensive control group (group 1) were prepared for infusion and left for the interval which would have been required for an infusion, but no infusion was carried out. One of the two remaining groups treated with hydrochlorothiazide was given an infusion of hypotonic (100 mEq./L.) saline at a rate of 5 ml per four minutes until 2 ml per 100 Gm. body weight had been administered (group 3). The other treated group received the same volume of hypertonic saline (200 mEq./L.) at the same rate (group 4). All animals were exsanguinated one minute after stopping the infusion or at the equivalent time in the animals receiving a sham infusion. Exsanguination from the femoral artery was as rapid as possible to minimize shifts in electrolytes secondary to hypotension during this process. Only those animals in which the blood pressure was lowered to zero in less than a minute were taken for analyses. The effect of the period required for exsanguination on tissue electrolytes was further tested by dividing these animals into groups according to whether or not zero pressure was achieved in less than 30 seconds. No significant differences were noted between groups of similarly treated animals subdivided in this way, and the results have been recombined for presentation.

The tissues taken for analysis were psoas muscle, left ventricle, stomach muscle free of mucosa, and aorta free of adventitia. Methods for analysis of tissue water, sodium, potassium, and chloride concentrations have been previously described. For chloride titrations a Collove chloridimeter was used.

Plasma samples from the exsanguinated blood were analyzed after a 1:200 dilution by the same procedures as above. In addition, the inulin concentrations in plasma were determined to permit calculation of the inulin space. Hematocrits were determined after centrifuging for 30 minutes.

EXPERIMENT NO. 2

The second experiment was carried out in the same way as the first with the following exceptions. The animals with DCA pellets were given 1 per cent saline to drink for five days (days 5 to 10), while DCA pellets were present in order to intensify the hypertensive effect of DCA. Pellets were removed on day 34 at the onset of treatment with hydrochlorothiazide. Hydrochlorothiazide (10 mg./Kg.) was given daily for seven days and twice on the eighth day. The basic arrangement of groups was similar, and corresponding group numbers are therefore maintained. Two additional groups were prepared, one a control group (contr.) which had unilateral nephrectomy but received no DCA and the other (group 5) a further group of animals treated with hydrochlorothiazide (HCT). As in the first experiment, group 1 contained hypertensive animals untreated with HCT, while groups 2, 3, and 4 contained HCT-treated animals, either not infused, infused with hypotonic saline, or infused with hypertonic saline, respectively. The extra HCT-treated group (group 5) was infused with 0.5 M dextrose solution 2 ml./100 Gm. body weight.

The concentration of the hypotonic infusion fluid used (group 3) was reduced to 50 mEq./L., and the hypertonic infused fluid (group 4) was increased to 250 mEq./L, in order to alter the sodium gradient more drastically. The animals were anesthetized with a mixture of phenobarbital I. F. and pheno-barbital S. C.

The same tissues were analyzed, but the results for psoas and stomach muscle will not be reported in full since psoas muscle cellular water and electrolytes did not differ significantly in the various groups and since the changes in stomach electrolytes resembled those found in the first series of animals. The data in experiment 1 are presented in terms of fat-free dry weight (FFDW), fat-free wet weight (FFWW), and mEq./L. cell water; in experiment 2, in terms of dry weight (DW) and wet weight (WW). The standard error of the mean has been used to indicate variation in data.

Results

HYDROCHLOROTHIAZIDE EFFECTS (GROUP 1 VERSUS GROUP 2)

Blood Pressure (Table 1)

The systolic and the diastolic blood pressures of the animals treated with hydrochlorothiazide were initially significantly less than the corresponding pressures in the untreated animals in the first experiment (systolic -22.4 mm. Hg, P < 0.05; diastolic -22.5 mm. Hg, P < 0.01). The means of the diastolic pressures of the three groups of treated animals were very close, and each was significantly
different from the value for untreated animals. There was greater variation in systolic pressures in the groups of treated animals so that, although each mean was less than that of the untreated control group, no one of these alone was significantly less.

Interpretation of blood pressure measurements was complicated in the second experiment (table 1) by the presence of a moderate hypertension in the control groups (161.6/114.1). The use of barbiturate anesthetics may have contributed to the hypertension by virtue of their parasympathetic blocking actions. The higher concentration of sodium in the aorta and ventricles of these animals (see later) suggest also that this hypertension may have been of renal origin. The blood pressure was, however, significantly elevated above this base by DCA to 199.1/145.4 and reduced by hydrochlorothiazide. The mean reduction achieved in 38 animals was very close to that in the first experiment (-22.6 mm. Hg systolic and -22.8 mm. Hg diastolic), and the $P$ values in both instances were < 0.01. There were some animals in each group with pressures in the same range as in the untreated hypertensive group, and it may be speculated that in these instances the elevated pressure was not a result only of the exposure to DCA and saline. In any case, as a consequence, the

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TABLE 2

Values from Analysis of Blood—Postinfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Na mEq./L. plasma</th>
<th>Cl mEq./L. plasma</th>
<th>K mEq./L. plasma</th>
<th>Plasma H2O Gm./Kg.</th>
<th>Inulin space ml/100 Gm.</th>
<th>Hematocrit percent cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>146.1</td>
<td>100.6</td>
<td>2.73</td>
<td>923.9</td>
<td>22.07</td>
<td>51.4</td>
</tr>
<tr>
<td>DCA</td>
<td>0.8*</td>
<td>0.9</td>
<td>0.21</td>
<td>1.4</td>
<td>0.50</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>146.6</td>
<td>96.4</td>
<td>2.46</td>
<td>923.8</td>
<td>21.65</td>
<td>52.7</td>
</tr>
<tr>
<td>DCA and HCT</td>
<td>0.9</td>
<td>0.8</td>
<td>0.10</td>
<td>1.0</td>
<td>0.64</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>146.5</td>
<td>99.3</td>
<td>2.41</td>
<td>933.5</td>
<td>25.16</td>
<td>47.5</td>
</tr>
<tr>
<td>DCA and HCT</td>
<td>1.0</td>
<td>0.9</td>
<td>1.13</td>
<td>1.3</td>
<td>0.55</td>
<td>1.0</td>
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<tr>
<td>+ 100 mM NaCl</td>
<td>4</td>
<td>149.0</td>
<td>104.0</td>
<td>2.38</td>
<td>933.2</td>
<td>24.84</td>
</tr>
<tr>
<td>+ 200 mM NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Contr.</td>
<td>146.5</td>
<td>114.9</td>
<td>3.11</td>
<td>927.9</td>
<td>22.88</td>
<td>48.4</td>
</tr>
<tr>
<td>1</td>
<td>152.2</td>
<td>117.7</td>
<td>3.09</td>
<td>927.2</td>
<td>21.14</td>
<td>49.1</td>
</tr>
<tr>
<td>DCA</td>
<td>2.5</td>
<td>1.7</td>
<td>0.33</td>
<td>2.2</td>
<td>0.49</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>146.3</td>
<td>111.6</td>
<td>2.57</td>
<td>988.4</td>
<td>22.06</td>
<td>46.6</td>
</tr>
<tr>
<td>DCA and HCT</td>
<td>1.8</td>
<td>1.2</td>
<td>0.14</td>
<td>1.2</td>
<td>0.66</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>142.5</td>
<td>110.1</td>
<td>3.11</td>
<td>954.2</td>
<td>25.61</td>
<td>44.9</td>
</tr>
<tr>
<td>DCA and HCT</td>
<td>1.2</td>
<td>0.7</td>
<td>0.11</td>
<td>1.0</td>
<td>0.36</td>
<td>0.6</td>
</tr>
<tr>
<td>+ 50 mM NaCl</td>
<td>4</td>
<td>148.3</td>
<td>120.0</td>
<td>2.46</td>
<td>966.3</td>
<td>23.99</td>
</tr>
<tr>
<td>+ 250 mM NaCl</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>120.8</td>
<td>97.4</td>
<td>2.71</td>
<td>959.0</td>
<td>24.21</td>
<td>37.7</td>
</tr>
<tr>
<td>DCA and HCT</td>
<td>1.9</td>
<td>0.85</td>
<td>0.61</td>
<td>1.1</td>
<td>0.87</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*Standard error of the mean.

Differences between individual groups and the untreated groups were usually not significant. Elimination of data from animals with severe hypertension persisting after hydrochlorothiazide (diastolic pressures greater than 140 mm. Hg) did not alter any of the conclusions drawn from electrolyte data, and they have been included.

**Extracellular Fluid Spaces and Plasma Composition**

There was no statistically reliable decrease in the inulin spaces accompanying the hypotensive effects of hydrochlorothiazide in either experiment. The electrolyte concentrations and other data derived from analyses of plasma are summarized in table 2. In both experiments, hydrochlorothiazide treatment (group 2) caused slight hypokalemia and alkalosis as judged by the decrease in plasma chloride concentration compared to untreated hypertensive animals in group 1. The plasma potassium concentrations in both experiments were lower in all groups than the normal range of values (4 to 4.5 mEq./L.) in our laboratory. Treatment with DCA and/or hydrochlorothiazide may contribute to this difference but cannot account for the low potassium in the control group in experiment 2. In animals which had not received hypertonic saline in the first experiment, the plasma chlorides were also decreased (normal 105 to 110 mEq./L.), while in the second experiment they were slightly elevated. The diets and sodium intakes in these two experiments were different, perhaps accounting for some of the differences.

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### TABLE 3

**Effect of Hydrochlorothiazide on Tissue Composition**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Experiment 1*</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1 (DCA)</td>
<td>Group 2 (DCA + HCT)</td>
<td>Group 1 (DCA + saline)</td>
<td>Group 2 (DCA + saline HCT)</td>
</tr>
<tr>
<td><strong>Psoas muscle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mEq./Kg. DW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>130.7 ± 6.1</td>
<td>137.4 ± 2.9</td>
<td>127.1 ± 10.4</td>
<td>121.0 ± 9.6</td>
</tr>
<tr>
<td>Cl</td>
<td>56.8 ± 0.9</td>
<td>52.3 ± 1.1</td>
<td>50.2 ± 1.9</td>
<td>48.1 ± 1.3</td>
</tr>
<tr>
<td>K</td>
<td>430.0 ± 8.6</td>
<td>416.2 ± 10.0</td>
<td>426.1 ± 12.5</td>
<td>430.2 ± 20.6</td>
</tr>
<tr>
<td>ml./Kg. WW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>772.4 ± 1.1</td>
<td>770.2 ± 0.8</td>
<td>760.5 ± 1.1</td>
<td>762.8 ± 1.3</td>
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<tr>
<td>Cl space</td>
<td>116.7 ± 1.6</td>
<td>112.6 ± 2.1</td>
<td>93.4 ± 4.4</td>
<td>93.3 ± 2.7</td>
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<tr>
<td>mEq./L. cell water</td>
<td>18.6 ± 2.1</td>
<td>22.2 ± 0.9</td>
<td>24.1 ± 3.3</td>
<td>22.4 ± 3.7</td>
</tr>
<tr>
<td>K</td>
<td>151.0 ± 3.2</td>
<td>144.2 ± 3.5</td>
<td>122.0 ± 12.3</td>
<td>122.7 ± 7.1</td>
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<td><strong>Ventricle muscle</strong></td>
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<td></td>
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<tr>
<td>mEq./Kg. DW</td>
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<td></td>
</tr>
<tr>
<td>Na</td>
<td>160.1 ± 5.4</td>
<td>168.8 ± 4.15</td>
<td>211.0 ± 10.9</td>
<td>185.3 ± 7.2</td>
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<tr>
<td>Cl</td>
<td>87.7 ± 1.25</td>
<td>84.0 ± 1.15</td>
<td>95.5 ± 4.5</td>
<td>99.0 ± 3.7</td>
</tr>
<tr>
<td>K</td>
<td>301.0 ± 8.6</td>
<td>312.8 ± 4.7</td>
<td>310.8 ± 12.0</td>
<td>323.5 ± 9.6</td>
</tr>
<tr>
<td>ml./Kg. WW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>765.8 ± 1.0</td>
<td>766.5 ± 1.3</td>
<td>769.2 ± 1.8</td>
<td>771.2 ± 1.7</td>
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<tr>
<td>Cl space</td>
<td>185.8 ± 3.1</td>
<td>183.2 ± 3.2</td>
<td>170.0 ± 8.7</td>
<td>184.8 ± 6.9</td>
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<tr>
<td>mEq./L. cell water</td>
<td>16.5 ± 2.1</td>
<td>20.6 ± 1.2</td>
<td>37.4 ± 3.2</td>
<td>25.5 ± 3.3</td>
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<tr>
<td>K</td>
<td>118.6 ± 2.6</td>
<td>123.8 ± 2.5</td>
<td>128.8 ± 4.6</td>
<td>129.5 ± 4.0</td>
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<tr>
<td><strong>Aorta</strong></td>
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<tr>
<td>mEq./Kg. DW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>293.1 ± 18.1</td>
<td>290.3 ± 19.7</td>
<td>334.4 ± 16.0</td>
<td>337.2 ± 21.2</td>
</tr>
<tr>
<td>Cl</td>
<td>220.7 ± 2.8</td>
<td>204.4 ± 3.3</td>
<td>221.3 ± 6.9</td>
<td>225.2 ± 9.0</td>
</tr>
<tr>
<td>K</td>
<td>117.1 ± 4.7</td>
<td>114.8 ± 5.4</td>
<td>134.7 ± 4.0</td>
<td>137.0 ± 12.1</td>
</tr>
<tr>
<td>ml./Kg. WW</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>870 ± 5</td>
<td>679 ± 6</td>
<td>645 ± 6</td>
<td>668 ± 5</td>
</tr>
<tr>
<td>Cl space</td>
<td>596 ± 10</td>
<td>601 ± 9</td>
<td>607 ± 19</td>
<td>610 ± 24</td>
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<tr>
<td><strong>Stomach muscle</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>mEq./Kg. DW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>312.4 ± 5.9</td>
<td>313.9 ± 16.1</td>
<td>279.2 ± 8.5</td>
<td>279.3 ± 11.0</td>
</tr>
<tr>
<td>Cl</td>
<td>221.1 ± 4.9</td>
<td>313.8 ± 8.7</td>
<td>298.6 ± 5.5</td>
<td>279.1 ± 6.0</td>
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<tr>
<td>K</td>
<td>365.9 ± 4.9</td>
<td>344.8 ± 6.6</td>
<td>308.6 ± 7.2</td>
<td>329.8 ± 8.9</td>
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<td>ml./Kg. WW</td>
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<tr>
<td>H₂O</td>
<td>802.4 ± 2.0</td>
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<td>783.4 ± 0.8</td>
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<tr>
<td>Cl space</td>
<td>566.6 ± 8.7</td>
<td>588.2 ± 11.9</td>
<td>496.2 ± 12.5</td>
<td>491.1 ± 8.2</td>
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</table>

*Fat-free dry weight in experiment 1.

**Tissue Composition (Table 3)**

In psoas muscle, a decreased chloride concentration per Kg. fat-free dry weight (FFDW) in experiment 1 was the only significant change (P < 0.05) accompanying the hypotensive effect of hydrochlorothiazide. This change was not accompanied by a significant decrease in the chloride space (in ml./Kg. fat-free wet weight (FFDW) of this tissue) owing to the slightly lower plasma chloride concentration in the treated animals. In ventricular muscle, the only noteworthy change was in cellular sodium concentration in experiment 2, which was decreased in the hydro-
chlorothiazide-treated animals ($P < 0.05$). In experiment 2, but not in experiment 1, the sodium concentration per Kg. in aortae of treated animals was less, and the water concentration per Kg. was significantly more, leading to a significant decrease in sodium concentration per liter of aorta water. The hypotensive effect of hydrochlorothiazide was not accompanied by significant changes or even consistent trends in the composition of the stomach muscles of these rats (table 4). Neither the Na, K, Cl, or H$_2$O concentrations nor the chloride spaces of these tissues were significantly altered. These inconsistent data, along with the lack of significant change in the inulin space, support the conclusion that in this experiment the hypotensive effect of hydrochlorothiazide was accompanied neither by shrinkage of the extracellular fluid volume nor by any consistent alteration in cellular sodium concentration. Alterations in resistance of the small blood vessels secondary to electrolyte shifts could not be ruled out by the present data, but a generalized shift of electrolytes did not occur.

The sodium concentrations of aorta and ventricle in both groups in experiment 2 were higher than in the comparable tissues in experiment 1. This was not a systematic error since the sodium concentrations were lower in psoas and stomach muscles in experiment 2.

**EFFECTS OF EXPANSION OF EXTRACELLULAR FLUID VOLUME**

**Blood Pressure (Table 1)**

During the saline or sham infusions, the blood pressure fell slightly and comparably in all groups in the first experiment. Therefore, in these experiments, acute re-expansion of the extracellular fluid volume in two of these groups did not restore the blood pressures to the values in the untreated control group of hypertensive animals. In particular, the mean diastolic pressures in each of the hydrochlorothiazide-treated groups were significantly less than those of the untreated controls after the infusions.

Since hypotonic infusions have been found to reverse the hypotensive effects of hydrochlorothiazide in other experiments in rats,$^4$ the experiment was repeated using more extreme variation in tonicity of the infused solutions.

Infusion of hypotonic saline (50 mM/L.) elevated blood pressure (11.2/10.1) in group 3 in contrast to the slight fall (-2.5/-0.5) in group 2 which received a sham infusion. Infusion of hypertonic saline (250 mM/L.) increased systolic pressure (+18.5) but had little effect on diastolic pressure (+2.2) in group 4. The animals receiving hypertonic dextrose solution (group 5) had decreased pressures (-4.7/-12.0). At the conclusion of the infusions, the untreated hypertensive animals (group 1) and the treated animals perfused with hypotonic saline (group 3) had very similar pressures while all other groups had lower pressures, diastolic pressure being significantly lower in all but group 2 (sham infusion). Systolic pressures were significantly lower in groups 2 and 5 (dextrose infusion). Acute re-expansion of extracellular and plasma volume was obviously not sufficient to reverse the hypotensive effects of hydrochlorothiazide in agreement with recent studies of patients,$^{10}$ animals,$^8$ and the results in experiment 1.

**Extracellular Fluid Spaces and Plasma Composition (Table 2)**

Infusion of slightly hypotonic saline (group 3) in the first experiment caused no significant differences in ion concentration, but the more dilute solution used (50 instead of 100 mM/L.) in the second experiment produced a significant dilution of sodium although not of potassium and chloride in plasma. Potassium concentration was in fact elevated in this instance. The animals receiving hypertonic saline infusions (group 4) had increased sodium and chloride concentrations in plasma with more pronounced changes in the second experiment using a more concentrated solution. Hypertonic dextrose solution infused into group 5 animals (experiment 2) markedly diluted plasma sodium and chloride concentrations. All animals which received infusion had decreased concentrations of plasma solids, and the inulin spaces in these animals were elevated.
That the plasma volume as well as the extracellular space was indeed expanded in the two groups infused with hypo- or hypertonic saline can be inferred from several calculations. The inulin spaces in these groups infused with saline in experiment 1 were 3.5 and 3.2 ml. greater per 100 Gm. body weight than in the hydrochlorothiazide-treated group that received only a sham infusion. Indeed, they were 3.1 and 2.8 ml. per 100 Gm. body weight greater than in the group not treated with hydrochlorothiazide.

In experiment 2, the corresponding values were 0.55 and 1.93 per 100 Gm. body weight in the saline-infused groups and 2.15 ml./100 Gm. body weight in the group infused with dextrose solution. All these values, however, are subject to the criticism that the infusion period was too short to allow the inulin to equilibrate, and the fact that the increases in the inulin spaces were sometimes greater than the volume of the infused fluid (2 ml./100 Gm.) suggests that this was probably so.

However, the decrease in the proportion in plasma solids permits calculation of the increase in plasma volume (table 2). The assumption was made that no plasma solids were lost or gained (neglecting the tiny amount of solids infused). The results calculated in terms of a 275-Gm. rat in experiment 1 and in terms of a 250-Gm. rat in experiment 2 are presented in table 4.

Similar calculations were made using the decrease in hematocrit, but they led to large values for hypertonic solutions (e.g., 112 per cent of the 0.5 M dextrose solution retained in plasma). The assumption that red cell volume was constant was used and may have been invalid if shrinkage of red cells occurred in hypertonic solutions. The dilution of plasma chloride and sodium after dextrose infusion requires percentages of 15.8 and 14.1, respectively, which correspond approximately to the values from decrease in solids (table 4). In any case, there is no doubt that a substantial increase in plasma volume occurred. Therefore, if reduction of plasma volume had been the primary factor in the hypotensive

<table>
<thead>
<tr>
<th>Calculation based on</th>
<th>Per cent increase in plasma volume by</th>
<th>Per cent of infusion retained in plasma</th>
<th>Calcium 1</th>
<th>250 Gm. rat</th>
<th>Plasma solids</th>
<th>Calcium 2</th>
<th>250 Gm. rat</th>
<th>Plasma solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>100 mM NaCl</td>
<td>200 mM NaCl</td>
<td>29</td>
<td>11.6</td>
<td>12.4</td>
<td>29</td>
<td>10.2</td>
<td>6.8</td>
</tr>
<tr>
<td>275 Gm. rat</td>
<td>50 mM NaCl</td>
<td>50 mM NaCl</td>
<td>59</td>
<td>6.8</td>
<td>10.2</td>
<td>26</td>
<td>15.0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Circulation Research, Volume XI, December 1968
TABLE 5

Effect of Various Infusions on Composition of Left Ventricles of HCT-Treated Hypertensive Animals

<table>
<thead>
<tr>
<th>Group</th>
<th>mEq. per Kg. fat-free dry weight</th>
<th>mEq. per liter cell water</th>
<th>ml./Kg. fat-free wet weight</th>
<th>mEq. per liter water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
<td>Cl</td>
<td>K</td>
<td>Na</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No HCT</td>
<td>160.1</td>
<td>87.7</td>
<td>301.0</td>
</tr>
<tr>
<td>2</td>
<td>HCT</td>
<td>5.4*</td>
<td>125</td>
<td>8.6</td>
</tr>
<tr>
<td>3</td>
<td>HCT + 50 mM NaCl</td>
<td>109.8</td>
<td>84.0</td>
<td>312.8</td>
</tr>
<tr>
<td>4</td>
<td>HCT + 100 mM NaCl</td>
<td>4.15</td>
<td>115</td>
<td>4.6</td>
</tr>
<tr>
<td>5</td>
<td>HCT + 0.5 M dextrose</td>
<td>118.5</td>
<td>89.3</td>
<td>306.0</td>
</tr>
<tr>
<td></td>
<td>Contr.</td>
<td>4.4</td>
<td>2.1</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No HCT</td>
<td>211.0</td>
<td>95.5</td>
<td>310.8</td>
</tr>
<tr>
<td>2</td>
<td>HCT</td>
<td>10.9</td>
<td>4.5</td>
<td>12.0</td>
</tr>
<tr>
<td>3</td>
<td>HCT + 50 mM NaCl</td>
<td>185.3</td>
<td>99.0</td>
<td>323.5</td>
</tr>
<tr>
<td>4</td>
<td>HCT + 250 mM NaCl</td>
<td>7.2</td>
<td>3.7</td>
<td>9.6</td>
</tr>
<tr>
<td>5</td>
<td>HCT + 0.5 M dextrose</td>
<td>108.0</td>
<td>94.9</td>
<td>288.3</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>8.1</td>
<td>2.9</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Contr.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Standard error of the mean.
†Values in terms of wet weight and dry weight in experiment 2.
‡Control values included for ease of comparison.

effect of hydrochlorothiazide, the saline and dextrose infusions should have restored the blood pressure. Further doubt regarding such a mechanism of action is also implicit in the fact that no statistically reliable decrease of extracellular volume (inulin space) was measured in hydrochlorothiazide-treated animals with decreased blood pressure (group 2) compared to untreated hypertensive animals.

Tissue Composition

One of the limitations of these experiments is the possibility that changes in electrolyte concentration which are physiologically decisive may not be detectable against the background of biological and experimental variation. The fact that changes in tissue composition as a result of saline infusions were detected gives some reassurance that such changes had a reasonable chance of detection. Complete data for ventricles are given in table 5 and for aortae in table 6, and those for psoas muscle and for stomach muscle are mentioned in the text where pertinent.

After infusion of hypertonic saline (group 4 vs. group 2), consistent changes were noted in ventricular and psoas muscles in experiment 1. In psoas muscle, the chloride concentra-
### TABLE 6

<table>
<thead>
<tr>
<th>Group</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na (mEq./per mg. fat-free dry weight)</td>
<td>Cl (ml./Kg. fat-free wet weight)</td>
</tr>
<tr>
<td>No HCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>293.1</td>
<td>290.0</td>
</tr>
<tr>
<td>2</td>
<td>290.3</td>
<td>296.9</td>
</tr>
<tr>
<td>HCT</td>
<td>281.3</td>
<td>286.9</td>
</tr>
<tr>
<td>HCT +</td>
<td>7.9</td>
<td>7.5</td>
</tr>
<tr>
<td>300 mM NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>305.8</td>
<td>306.9</td>
</tr>
<tr>
<td>HCT +</td>
<td>18.5</td>
<td>18.5</td>
</tr>
<tr>
<td>200 mM NaCl</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>383.4</td>
<td>383.4</td>
</tr>
<tr>
<td>No HCT</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>2</td>
<td>357.2</td>
<td>357.2</td>
</tr>
<tr>
<td>HCT</td>
<td>21.2</td>
<td>21.2</td>
</tr>
<tr>
<td>3</td>
<td>356.7</td>
<td>356.7</td>
</tr>
<tr>
<td>HCT +</td>
<td>17.4</td>
<td>17.4</td>
</tr>
<tr>
<td>50 mM NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>380.8</td>
<td>380.8</td>
</tr>
<tr>
<td>HCT +</td>
<td>13.3</td>
<td>13.3</td>
</tr>
<tr>
<td>250 mM NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>365.3</td>
<td>365.3</td>
</tr>
<tr>
<td>HCT +</td>
<td>16.4</td>
<td>16.4</td>
</tr>
<tr>
<td>0.5 M dextrose</td>
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<td></td>
</tr>
<tr>
<td>Contr.</td>
<td>356.7</td>
<td>356.7</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Standard error of the mean.

1Values in terms of wet weight and dry weight in experiment 2.

1Control values included for ease of comparison.

The chloride concentration was increased significantly (from 52.3 to 63 mEq./Kg. FFDW), and the chloride space was increased significantly (from 112.6 to 124.3 ml./Kg. FFWM). Similar significant changes occurred in ventricular muscle, mean chloride concentration increasing from 84 to 98.4 mEq./Kg. FFDW and chloride space from 183 to 199 ml./Kg. FFWM. In addition, the sodium concentrations were increased (psoas 137 to 154 and ventricle 170 to 179 mEq./Kg. FFDW) when calculated in Gm. of dry weight but not when calculated in terms of cellular water. Similar changes were noted in experiment 2. All these changes were consistent with an increase in extracellular fluid at the expense of cellular fluid. The effects of infusion of hypertonic saline on the aorta (table 6) and stomach muscle were surprisingly different. The chloride concentrations were increased, but not significantly so except in stomach muscle in experiment 2 (from 279 to 323 mEq./Kg. DW, P < 0.01), and the chloride spaces were either significantly decreased, i.e., aorta from 601 to 567, stomach muscle from 585 to 454 ml./Kg. FFWM (experiment 1), or not significantly altered (experiment 2). There is evidence of a less freely diffusible fraction of chloride in...
these tissues so that this difference in behavior in the first experiment cannot be definitely attributed to a paradoxical shrinkage of the extracellular space. In any case, such findings reinforce previous evidence that changes in smooth muscle cannot be reliably deduced from ion movements observed in extracellular fluid in vivo.

The changes following infusion of hypotonic saline were different (group 3 vs. group 2). The chloride concentration was increased significantly in experiment 1 in psoas (52 to 61 mEq./Kg. FFDW) and ventricular muscle (84 to 89 mEq./Kg. FFDW) and unaltered in experiment 2, but the chloride space was not reliably changed in any tissue in either experiment. In all tissues except aortae, the increase in tissue water concentrations (e.g., in experiment 1, psoas from 770 to 780; ventricle from 766.5 to 772; stomach muscle from 800 to 806; aorta 678 to 680 ml./Kg. WW) following hypotonic saline was significant. The potassium concentration of psoas and ventricular muscles was decreased in both experiments, but these changes were usually not significant. In experiment 1, although not in experiment 2, a significant increase in tissue sodium concentration of psoas muscle occurred when calculations were made in terms of dry weight going from 137 to 153 mEq./Kg. FFDW, but fiber sodium was not changed significantly in either experiment. All these changes were consistent with the movement of a portion of the injected fluid into cells which, combined with retention of fluid (vide supra) in the cardiovascular system, resulted in insignificant changes in interstitial fluid concentration.

In the second experiment, hypertonic dextrose (0.5 M) was infused as well as hypotonic and hypertonic saline. The results from this group (group 5) were compared to the appropriate control (group 2). Tissue composition was little altered by dextrose infusion. Chloride concentration was significantly decreased in aortae (225 to 203 in mEq./Kg. DW) but not in other tissues. As a consequence of the decreased plasma chloride concentrations, the chloride space was significantly increased in all tissues but aorta (psoas 93 to 114; ventricle 185 to 215; stomach muscle 491 to 558; aorta 610 to 649 ml./Kg. WW). These results are compatible with expectations from the infusion of a hypertonic solution containing no salts, for tissues other than aorta, while the changes in aorta emphasize once again the uniqueness of smooth muscle shifts in water and electrolyte.

Comparison between the electrolyte compositions of tissues from animals infused with hypo- and hypertonic saline revealed the expected results. For example, the sum of sodium and potassium per Kg. dry weight and per L. of fiber water was significantly greater in ventricles from animals perfused with the hypertonic saline in both experiments (145 vs. 136 mEq./L. in experiment 1; 161 vs. 130 in experiment 2). Similar differences also occurred in composition of psoas muscles. However, significant differences in the total cation concentrations of stomach muscle and aorta were not obtained in either experiment between groups infused with hypo- and hypertonic saline, although the mean values differed in the expected ways.

**Discussion**

The results of these experiments on rats with DCA hypertension indicate that hypotensive effects of hydrochlorothiazide may occur without producing detectable changes in extracellular fluid volume and without producing important or consistent changes in the composition of heart muscle, psoas muscle, stomach muscle, or aorta. Furthermore, expansion of the extracellular fluid volumes to values greater than in the untreated group did not reverse the hypotensive effects of hydrochlorothiazide. Therefore, these results support the conclusion of Friedman et al. that decrease in the volume of extracellular fluid is not a necessary link in the hypotensive effect of hydrochlorothiazide in rats with active DCA hypertension. Studies of hypertensive patients in which blood pressure changes were correlated with changes in ECFV may not be in contradiction to these.
results since the changes in ECFV were produced either by addition of salt to the diet so that alterations in renal function and aldosterone secretion and secondary changes of various sorts may have affected the issue—or, when infused, may have resulted from increased cardiac output, as was possibly the case in group 4, experiment 2, in which infusion of hypertonic saline produced an increase of systolic but not of diastolic pressures. Examination of the data also indicates that changes in the water concentration in plasma and tissues did not accompany the hypotensive action of hydrochlorothiazide in contradiction to the suggestion of some authors. Therefore, these results strongly suggest that the hypotensive action of hydrochlorothiazide should be dissociable from its effect on extracellular and cellular fluid volume in patients with essential hypertension.

The experiments do not provide any evidence supporting the contention that the effect of hydrochlorothiazide is mediated by an increase in the ratio \([\text{Na}]_o/\text{[Na]}_i\). Friedman et al. suggest that hydrochlorothiazide lowers blood pressure in hypertensive rats by depleting intracellular sodium and increasing the ratio \([\text{Na}]_o/\text{[Na]}_i\) and that infusion with hypotonic saline restores blood pressure whereas infusion with hypertonic saline does not, because in the first instance the ratio is decreased (effect of hydrochlorothiazide antagonized), while in the second instance it may be further increased. Although our experiments show that if the ratio is initially decreased by hypertonic saline, the blood pressure may rise slightly, while with a more profound initial decrease in this ratio by hypertonic dextrose, the blood pressure tends to fall. Hence, the ratio \([\text{Na}]_o/\text{[Na]}_i\) is not the major determinant of blood pressure. The fact that alterations in \([\text{Na]}_o\) cause secondary alterations in \([\text{Na]}_i\), so that the ratio in some tissues such as the ventricles ultimately rises (see table 7), cannot mitigate this conclusion since the same general blood pressure effects were obtained from the beginning of the infusion to the time the animals were killed.

Table 7

<table>
<thead>
<tr>
<th>Group</th>
<th>(\text{Ventricle Experiment 1} )</th>
<th>(\text{Ventricle Experiment 2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[\text{Na}]_o/[\text{Na]}_i</td>
<td>[\text{Na}]_o/[\text{Na]}_i</td>
</tr>
<tr>
<td>2</td>
<td>8.9</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>7.1</td>
<td>5.7</td>
</tr>
<tr>
<td>4</td>
<td>8.0</td>
<td>7.1</td>
</tr>
<tr>
<td>5</td>
<td>7.6</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Insofar as the ventricle muscle is concerned, the results are likewise not consistent with this hypothesis, since the ratio was less in all animals treated with hydrochlorothiazide in the first experiment, including those infused with saline and in which the hypotensive effect of hydrochlorothiazide persisted (table 7). In the second experiment, the sodium ratio was increased in group 2 HCT-treated rats, but was highest in group 3 which were as hypertensive as group 1 at the time the tissues were removed. The ratio was less than that in group 1 in groups 4 and 5. The differences between the two experiments may have resulted from the same cause (renal disease or barbiturate anesthesia) which initiated “spontaneous” hypertension and induced higher sodium concentrations in aortae and ventricles. The presence of this pre-existing hypertension did not prevent the hypotensive effect of hydrochlorothiazide, but it may have altered the shifts in electrolytes induced by this agent. An elevated cellular sodium concentration may have been more readily influenced by the saluretic action of HCT. However, the important fact is that the hypotensive effect of HCT occurred irrespective of the direction of change in cellular sodium (qualitatively identical although quantitatively less striking results were derived from examination of the data for psoas muscle).

The results were, in experiment 1, not inconsistent with those of Freed and St. George who found an increase in the ratio of myocardial sodium to potassium accompanying the hypotensive effect of a low potas-
TABLE 8

Changes in Aorta Sodium Relative to Extracellular Sodium and Blood Pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>[Na]$_t$ mEq./L. H$_2$O</th>
<th>[Na]$_t$/[Na]$_i$</th>
<th>Diastolic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>132</td>
<td>1.11</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>132</td>
<td>1.11</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>130</td>
<td>1.13</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>140</td>
<td>1.06</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>177</td>
<td>0.73</td>
<td>110</td>
</tr>
</tbody>
</table>

* [Na]$_t$ is calculated by dividing tissue sodium per Kg. by the volume of water per Kg.

sium diet and a reversion of this ratio along with restoration of the blood pressure in animals treated with cortisone and prednisone. There is no evidence that thiazide diuretics have extrarenal effects so the increase in the ratio Na$_t$/K was probably the result of the renal actions of this compound promoting potassium loss. In the second experiment, with a higher cellular sodium concentration in the ventricle, HCT evoked a decrease in the ratio of cellular sodium to cellular potassium. Therefore, change in this ratio in ventricle was not critical for the hypotensive effects of HCT.

Cellular concentrations of sodium and potassium cannot be calculated from the chloride space in aorta and stomach muscle because of the high value for the chloride space and the evidence that sodium and chloride are distributed in several fractions in these tissues. However, the overall changes in electrolyte composition of aorta (expressed in mEq./L. tissue water) were not readily compatible with a decrease in intracellular sodium as the determinant of blood pressure (table 8). Moreover, alterations in the ratio [Na]$_t$/[Na]$_i$, insofar as they reflect changes in the sodium gradient, are not consistent with the view that this is the determinant of blood pressure (compare the changes in ratios from that in group 1 to those in groups 2 and 4 to the changes in blood pressure for these groups).

Previous studies from this laboratory and elsewhere have emphasized that contractility (response to a supramaximal concentration of a drug) or reactivity (response to a submaximal concentration of a drug) of aorta strips is not uniquely determined by the sodium gradient, but may be related to the potassium gradient or to the interactions of calcium and magnesium with the contractile machinery. Since reactivity could be altered by changes (1) in excitability, (2) ability of the contractile proteins to respond, or (3) in the link between excitation and contraction, each of which may respond differently to altered ionic gradients, it is not surprising that somewhat contradictory results have been obtained. A further feature complicating interpretations is that contractility, as defined above, would be determined chiefly by changes in (2) and (3), and ionic gradients might have different effects from those obtained when reactivity was studied.

Recently, evidence has been presented that hypertonic fluids decrease vascular resistance while hypotonic solutions increase it. The mechanism of the effects is obscure, but sodium concentration was irrelevant. While the conditions of these experiments were not comparable to those cited, it is noteworthy that hypertonic dextrose did reduce blood pressure, and hypotonic saline did tend to elevate it in experiment 2. Hypertonic saline decreased systolic but not diastolic pressure—a result attributed to increased cardiac output from hypervolemia. It is evident that an increase in cardiac output and a decrease in peripheral resistance may have occurred to varying degrees with both hypertonic dextrose and saline. However, the concentrations of sodium and chloride in plasma were scarcely altered by hypo- and hypertonic saline.
The data obtained in this study do not eliminate vascular muscle as the site of decreased peripheral resistance as a mechanism of the hypotensive action of hydrochlorothiazide in rats since, among other reasons, peripheral resistance was not measured and the changes in composition (not only Na, K, and Cl, but also Ca) of arteriolar muscle were not examined. They do suggest the possibility that an action on cardiac muscle may be involved sometimes since changes were observed in its composition accompanying the hypotensive actions of hydrochlorothiazide.

Conclusions that the thiazides do not act in rats by depleting extracellular fluid volume or producing oligemia or by depleting cellular water or sodium are in accord with recent studies carried out in hypertensive patients.6, 8, 9

Summary

Hydrochlorothiazide significantly diminishes blood pressure in rats with DCA hypertension. Infusion with hypertonic saline or dextrose failed to reverse the blood pressure effects of hydrochlorothiazide. Infusion of hypotonic saline failed to restore blood pressure to the levels of hypertension in untreated controls in one experiment but tended to do so in another. There was no significant diminution of the inulin space accompanying the hypotensive action of hydrochlorothiazide, nor any relation between the expansion of the extracellular and cardiovascular volumes by various infusions and their effects on blood pressures of thiazide-treated rats. Plasma or extracellular fluid volume alteration was, therefore, not the primary action of hydrochlorothiazide in these rats. Analyses of the composition of plasma, aorta, stomach muscle, psoas muscle, or left ventricle did not support the theory that hydrochlorothiazide acts by decreasing the concentration of intracellular sodium or increasing the ratio of extracellular to intracellular sodium. Changes in composition of the left ventricle suggested that alterations in its function may have influenced some of the blood pressure changes noted. It is suggested that attention should be paid to the action of hydrochlorothiazide on other constituents in vascular and cardiac muscle.

Acknowledgment

The author wishes to express his gratitude to Dr. S. M. Friedman for providing the hypertensive rats for this study and determining their inulin spaces and for much helpful discussion and criticism of the results and of this manuscript. It should be mentioned that some of the interpretations are not accepted by Dr. Friedman, so that this expression of appreciation should not be construed as implying his concurrence with the conclusions.

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On the Mechanism of Antihypertensive Action of Hydrochlorothiazide in Rats
E. E. Daniel

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