Changes in Plasma and Extracellular Fluid Volumes in Patients with Essential Hypertension During Long-Term Treatment with Hydrochlorothiazide

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SUMMARY

In 13 untreated patients with essential hypertension, the plasma volume (T-1824) and the extracellular fluid volume (82Br- distribution space) were determined three times. During treatment with 50 to 100 mg of hydrochlorothiazide daily, determinations of plasma volume and extracellular fluid volume were repeated after treatment for 1 mo (13 patients), 2 mo (13 patients), 4 mo (eight patients), and 6 mo (seven patients). In all cases the average plasma and extracellular fluid volumes were reduced compared with the averages of three control values. The plasma volume was significantly reduced at the 5% level after treatment for 1, 2, and 4 mo.

Corresponding studies from the literature have been reviewed and, on the basis of these studies and our own results, it is concluded that thiazides have a volume-depleting effect which is continued during long-term treatment of patients with essential hypertension.

Additional Indexing Words:
Hemoglobin Thiazide treatment Hematocrit

IN SPITE OF regular clinical application of thiazides for more than 10 years in antihypertensive drug treatment, whether the antihypertensive effect of thiazides can be explained by a reduction in plasma and extracellular fluid volumes needs to be clarified.

It seems well established that, during short-term treatment, the thiazides reduce the fluid volumes of the body,\textsuperscript{1–8} whereas the effect during long-term treatment is only sparsely investigated.\textsuperscript{1, 5, 6, 8–10}

Hence, the object of the present study is to elucidate the antihypertensive effect of the thiazides by investigating the effect of hydrochlorothiazide on plasma and extracellular fluid volumes during long-term treatment of patients with essential hypertension.

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Methods

The group studied comprised 13 patients with essential hypertension. None of them had signs of congestive heart failure and clinically demonstrable edema. They did not suffer from diseases which are known to influence the parameters investigated. Age, height, sex, body weight, retinal vascular changes, heart volume, serum creatinine, and creatinine clearance values are shown in table 1. None of the patients was under treatment, or former antihypertensive therapy had been withdrawn at least 4 weeks before commencement of the study.

The blood pressure (BP) was determined by applying an arm cuff and mercury manometer. Each BP value in table 1 represents the average of three determinations. BP was always determined with the patient in the recumbent position after at least 1 hour’s rest in bed. The mean blood pressure (MBP) was calculated as the diastolic BP plus one third of the blood pressure amplitude.

Before institution of treatment, plasma volume (PV) and extracellular fluid volume (ECV) were determined three times with a 3 to 7-day interval between determinations. The initial examination was carried out in the hospital and the following ones were done in the out-patient clinic under
Table 1

Clinical Data on 13 Patients with Essential Hypertension prior to Antihypertensive Treatment

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr), sex</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Blood pressure (mm Hg)</th>
<th>Heart volume (ml/m²)</th>
<th>F.H.</th>
<th>Creatinine clearance per m² BSA X 1.73</th>
<th>Creatinine (mg/100ml)</th>
<th>Control values</th>
<th>Plasma volume* (ml)</th>
<th>Extracellular fluid volume* (ml)</th>
<th>Treatment with hydrochlorothiazide (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.M.</td>
<td>57 M</td>
<td>172</td>
<td>81.2</td>
<td>190/125</td>
<td>590</td>
<td>II</td>
<td>1.5</td>
<td>69</td>
<td>3722</td>
<td>20,509</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>K.O.</td>
<td>49 M</td>
<td>181</td>
<td>85.7</td>
<td>230/155</td>
<td>522</td>
<td>II</td>
<td>1.1</td>
<td>87</td>
<td>3361</td>
<td>18,415</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>H.O.H.</td>
<td>62 M</td>
<td>169</td>
<td>66.8</td>
<td>190/110</td>
<td>508</td>
<td>II</td>
<td>1.1</td>
<td>93</td>
<td>4047</td>
<td>18,568</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>P.A.L.</td>
<td>48 M</td>
<td>182</td>
<td>90.0</td>
<td>210/120</td>
<td>551</td>
<td>III</td>
<td>1.1</td>
<td>85</td>
<td>3959</td>
<td>20,045</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>E.M.</td>
<td>42 M</td>
<td>171</td>
<td>64.9</td>
<td>170/115</td>
<td>357</td>
<td>II</td>
<td>0.9</td>
<td>87</td>
<td>2989</td>
<td>14,674</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>O.R.C.</td>
<td>55 M</td>
<td>173</td>
<td>66.3</td>
<td>230/135</td>
<td>508</td>
<td>III</td>
<td>1.6</td>
<td>59</td>
<td>3120</td>
<td>15,674</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>O.T.O.</td>
<td>37 M</td>
<td>182</td>
<td>78.7</td>
<td>170/120</td>
<td>406</td>
<td>I</td>
<td>1.4</td>
<td>68</td>
<td>3738</td>
<td>19,214</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>S.E.L.</td>
<td>47 M</td>
<td>168</td>
<td>68.9</td>
<td>205/130</td>
<td>392</td>
<td>III</td>
<td>1.1</td>
<td>104</td>
<td>3192</td>
<td>16,229</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>E.P.</td>
<td>44 F</td>
<td>169</td>
<td>68.3</td>
<td>175/120</td>
<td>314</td>
<td>III</td>
<td>1.1</td>
<td>87</td>
<td>2720</td>
<td>14,357</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>E.B.</td>
<td>42 F</td>
<td>159</td>
<td>53.4</td>
<td>200/130</td>
<td>268</td>
<td>II</td>
<td>1.0</td>
<td>70</td>
<td>2096</td>
<td>11,641</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>I.H.</td>
<td>45 F</td>
<td>153</td>
<td>59.6</td>
<td>185/120</td>
<td>419</td>
<td>II</td>
<td>0.7</td>
<td>86</td>
<td>2537</td>
<td>11,991</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>I.J.</td>
<td>61 F</td>
<td>163</td>
<td>72.5</td>
<td>195/105</td>
<td>520</td>
<td>II</td>
<td>0.8</td>
<td>—</td>
<td>3217</td>
<td>16,167</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>A.J.</td>
<td>63 F</td>
<td>137</td>
<td>66.9</td>
<td>215/120</td>
<td>530</td>
<td>III</td>
<td>1.3</td>
<td>52</td>
<td>2825</td>
<td>14,041</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

Mean: 50.1, Range: 37-63
Height: 153-182, Weight: 53.4-90.0, Blood pressure: 170-230/105-155, Heart volume: 268-590, F.H.: 0.7-1.6

*The individual values of plasma and extracellular fluid volume are the average of three determinations.

Abbreviation: F.H. = Eye background changes (Keith-Wagner).
identical conditions. Before each examination the patient rested in bed for at least 1 hour and had fasted for at least 9 hours. The averages of these three determinations were used as control levels for PV and ECV. These average values appear in table 1.

The patients were then given from 50 to 100 mg of hydrochlorothiazide daily (mean, 86 mg). The intensity of treatment was not changed during the experimental period, and no KCl was given.

The determinations of PV and ECV were repeated after 1, 2, 4, and 6 mo of treatment. Five patients had to be excluded from the study after 2 mo of treatment: two of the five did not cooperate, one had a coronary occlusion, one emigrated, and one received supplementary antihypertensive therapy. After 4 mo of treatment, another patient was excluded because of lack of cooperation.

PV was determined with the Evans blue dye method (T-1824). The amount administered was determined gravimetrically. Blood samples were taken at 0, 15, 30, 45, and 60 min after injection from an indwelling needle in a cubital vein. The needle was rinsed continuously with isotonic glucose, using a volume of fluid corresponding to the number of milliliters of blood (70 ml) taken.

The theoretical extinction in plasma \( (D_o) \) at time \( t = 0 \) was found by extrapolation from a semi logarithmic graph of the plasma extinctions at \( t = 15, 30, 45, \) and 60 min. Fresh plasma was used, pipetted off after centrifugation for 20 min at 800 \( \times \) g. The determinations were made at 620 m\( \mu \) in a spectrophotometer (Spectronic 20). By applying \( D_o \) and a standard curve, the concentration of T-1824 \( (C_o) \) was calculated in mg per ml of plasma. PV was calculated as:

\[
PV = \frac{m}{C_o} \text{ml.}
\]

The hematocrit value \( (\text{hct}) \) was determined in an Ecco-Quick microcentrifuge at 17,000 \( \times \) g. All values were determined as duplicate determinations. No correction was made for “trapped” plasma.\(^{11}\)

ECV was determined as the distribution space of \( ^8\text{Br}^- \) according to previous studies.\(^{12}\) About 30 \( \mu \)c \( ^8\text{Br}^- \) in a sterile solution were injected.\(^*\) The volume administered was determined gravimetrically and, on the basis of a standard solution, the volume of radioactivity administered \( (A) \) was calculated in counts per second. Following a period of equilibration of 4 hours, blood samples were taken without stasis and, during the same period, collection of urine was made. The radioactivity in the samples was determined under identical conditions in a well-type counter (Selectronic). The net volume of \( ^8\text{Br}^- \) administered was corrected for loss to erythrocytes \( (T_E) \) and urine \( (T_U) \). \( T_E \) was calculated on the basis of the hct and the radioactivity in plasma and whole blood. In calculating the distribution space of \( ^8\text{Br}^- \) correction was made for plasma protein and the Gibbs-Donnan effect.

The plasma protein correction factor \( (p) \) was determined as\(^{13}\):

\[
p = \frac{100 - 0.73 \times pp}{100}
\]

where 0.73 is the volume of liquid displaced by 1 g of protein in 100 ml of plasma and pp is the protein concentration in g/100 ml. To compensate for the Gibbs-Donnan effect, a stable correction factor, \( r = 1.02,^{13} \) was applied.

ECV was calculated as the distribution space of \( ^8\text{Br}^- \) employing the following equation\(^{13}\):

\[
ECV = \frac{P \left[ \frac{A - (T_E + T_U)}{C_{pl}} - PV \right] + PV}{r}
\]

where \( C_{pl} \) is the radioactivity concentration in counts/sec/ml of plasma.

Changes in the parameters investigated were calculated as differences between the averages of the three control levels and values determined after 1, 2, 4, and 6 mo of treatment with hydrochlorothiazide.

For testing of the zero-hypothesis, Student’s \( t \)-test was applied.

Results

Changes in PV after 1, 2, 4, and 6 mo of treatment with hydrochlorothiazide are shown in figure 1. In all the cases a reduction was observed in the average values of PV. The reduction was significant after 1, 2, and 4 mo, whereas after 6 mo, at which time only seven patients were included in the study, it was not significant at the 5% level. In the eight patients who were followed up for 4 to 6 mo, the average PV reductions, after 1, 2, 4, and 6 mo, were: 159 ml (sd, 165), 160 ml (sd, 155), 195 ml (sd, 204), and 128 ml (sd, 154), respectively. Also in these cases the reduction was significant at the 5% level after 1, 2, and 4 mo but not after 6 mo.

Changes in ECV after 1, 2, 4, and 6 mo of treatment with hydrochlorothiazide are shown in figure 2. In all the cases an average

\*Radiochemical Centre, Amersham, England.
The effect of hydrochlorothiazide on plasma volume after 1, 2, 4, and 6 mo of treatment. The horizontal lines of the columns represent the average values and the whole column indicates 2 standard deviations.

reduction in ECV was demonstrated, but it was significant only after 1 and 2 mo of treatment. In one patient ECV presented an increase of 2,809 ml after 4 mo of treatment. A corresponding increase in body weight was not observed, and at this stage PV was still reduced. The cause of this considerable deviation is not known but, presumably, it can be explained as a methodological or analytical error. If the average reduction in ECV after 4 mo is calculated without including this value, an average reduction of 551 ml is found, which is of the same order of magnitude as the ECV reductions after 1 and 6 mo of treatment.

MBP was in all cases reduced significantly ($P < 0.005$) during treatment with hydrochlorothiazide. The average reduction in MBP was 13 mm Hg in 13 patients, 21 mm in 13, 19 in eight, and 26 mm Hg in seven, after 1, 2, 4, and 6 mo of treatment respectively. A definite correlation between reduction in PV and the fall in MBP could be demonstrated only after 2 mo of treatment ($r = 0.62$, $P < 0.05$).

Both hemoglobin (hgb) and hct values showed an average increase during treatment. The average increase in hgb was 0.54, 0.68, 0.55 and 0.59 g/100 ml after 1, 2, 4, and 6 mo of thiazide treatment, respectively, and the average increase in hct was 1.1, 1.15, 0.5, and 1.0% in the corresponding periods of time. The increase in hgb was significant following 1 and 2 mo of treatment, whereas the average increase in hct was significant only after 2 mo.

The body weight was reduced an average 1.2 kg during the period of treatment. Since many of the patients in connection with the establishment of the diagnosis of hypertension had voluntarily reduced their weight, no great importance can be attached to the weight reduction. There was no correlation between the reduction in ECV and the loss in weight.

**Discussion**

Since the introduction of the thiazides in antihypertensive therapy, the mechanism of their antihypertensive action has been much disputed. A number of studies on the initial effect of thiazides (less than 15 days of treatment) on the PV showed that PV is reduced by about 300 ml, on an average.$^{1-8}$ Therefore, the antihypertensive effect was explained as a depletion of fluid volume, resulting in reduced cardiac output and BP.$^{2,4,14}$ This theory was supported by several studies showing that the effect on the BP could be reversed by infusion of dextran, either in isotonic glucose or in physiologic saline.$^{2,3,7,8,10,15}$

This initial fall in PV, however, could not be found again in all the cases after more than 1 mo of treatment and, correspondingly, no definite reduction in cardiac output was found.$^{1,14}$

These findings gave rise to the presumption that, apart from the effect causing a depletion in volume which might be of importance for the initial fall in BP, the thiazide might also have another mechanism of action, reducing the peripheral resistance.

The number of studies available relating to the effect of thiazides during long-term treatment is very limited. Table 2 presents studies regarding the effect on PV of thiazides.
Changes in Fluid Volume in patients

The effect of hydrochlorothiazide on extracellular fluid volume after 1, 2, 4, and 6 mo of treatment. The horizontal lines of the columns represent the average values and the whole column indicates 2 standard deviations.

in patients suffering from essential hypertension. In all cases, PV was reduced, but in several groups of patients the reduction was not significant.\textsuperscript{1, 5, 9} In some of the investigations the patients received other antihypertensive therapy during the experimental period with drugs which are known to influence the fluid volume of the body. In some of the studies, earlier instituted therapy was maintained\textsuperscript{5, 8, 10} whereas in others\textsuperscript{5} the administration of additional therapy was reported only during the experimental period. In a few studies, the patients were given a fixed amount of salt in the diet\textsuperscript{6, 8} but in most of the cases the intake of salt was not restricted.\textsuperscript{1, 5, 9, 10}

The definition of the hypertension varied greatly in the studies reported and, most often, the patient's age was not considered. In some studies the patients had slight hypertension, although the level of the BP was not stated\textsuperscript{5, 6, 8, 9}, in other studies the patients exhibited labile BP with the diastolic BP ranging below 100 mm Hg.\textsuperscript{1, 9}

In a few cases the patients had previously undergone sympathectomy,\textsuperscript{1, 5} which is known to change the sensitivity to thiazides.

Various methods for the determination of PV have been applied (\textsuperscript{131}I-albumin, T-1824, Congo-red), but even when the same test substance was applied, the methodology varied. Furthermore, the requirements as to fasting and rest in bed varied considerably.

In the two reports published by Conway and Lauwers\textsuperscript{1, 9} in 1960 it appears that several of the patients are included in both studies, and consequently, these studies can only be considered as representing one group of patients.

In the initial studies concerning the effect of thiazides on the body fluid volumes, the patients and methods varied to such an extent that the results obtained appeared contradictory and difficult to interpret. However, all the studies tended to find that the PV was reduced to varying extents. In more recent studies the requirements in respect to patients, methods, and performance of the examinations have been much more strict. In Hansen's report\textsuperscript{6} a definite reduction in PV was found for a period of up to 3 mo after institution of

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Plasma volume changes (ml)</th>
<th>% change</th>
<th>P value</th>
<th>Duration of treatment (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson and Freis\textsuperscript{8}, 1959</td>
<td>11</td>
<td>-80</td>
<td>-</td>
<td>0.05 &lt; P &lt; 0.1</td>
</tr>
<tr>
<td>Conway and Lauwers\textsuperscript{1}, 1960</td>
<td>11</td>
<td>-155</td>
<td>-</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lauwers and Conway\textsuperscript{8}, 1960</td>
<td>10</td>
<td>-90</td>
<td>-3.3</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Gifford et al\textsuperscript{8}, 1961</td>
<td>11</td>
<td>-51</td>
<td>-2.0</td>
<td>&gt;0.4</td>
</tr>
<tr>
<td>Winer\textsuperscript{9}, 1961</td>
<td>16</td>
<td>-39</td>
<td>-1.3</td>
<td>0.4 - 0.5</td>
</tr>
<tr>
<td>Hansen\textsuperscript{6}, 1968</td>
<td>18</td>
<td>-151</td>
<td>-6.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hansen\textsuperscript{6}, 1968</td>
<td>11</td>
<td>-238</td>
<td>-7.5</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>
thiazide therapy. This finding has been confirmed by our own results, PV being significantly reduced after 1, 2, and 4 mo of treatment.

If the body is exposed to continued influence of thiazide, the initial rather considerable PV reduction will most likely result in a counteracting mechanism in the body. Thereby, within a few weeks, a new level of plasma volume will be obtained, representing a balance between the thiazide effect and the volume-control and counteracting-control mechanisms of the body. This was confirmed by Wilson and Freis who, after discontinuance of long-term thiazide treatment in hypertensive patients, found an average increase in PV of 241 ml (so, 100, $P < 0.01$), which was 120 ml higher than the plasma volume value found before institution of treatment.

The varying results obtained in individual patients by repeated PV determinations must be ascribed to changes in one of the numerous, partly unknown, factors which controls the dynamic equilibrium of PV. Consequently in combination with the limited groups of patients examined in the various studies, it is difficult to obtain indisputable results, since only one single deviating value will cause the average reduction in PV to become insignificant. Therefore, attention should be drawn to the fact that all studies concerning the long-term effect of thiazide on PV revealed an average reduction in PV and that the reduction was significant in several cases.

Several other facts support the theory that the thiazides exert a continued volume-depleting effect following long-term treatment. Hence, hemoglobin and hematocrit values were found to be slightly increased during long-term treatment. Our study revealed an average increase in hemoglobin of 0.5 g/100 ml. This increase is of the same order of magnitude as the increase in hemoglobin demonstrated by a retrospective study of hypertensive patients who had been treated with thiazide for several years. In that study the hemoglobin in 42 patients who had been treated with thiazide for an average of 3 years showed an average increase of 0.55 g/100 ml.

Recently Tarazi and co-workers showed a constant and significant decrease in PV during long-term treatment with thiazide. Discontinuance of treatment gave rise to a "rebound phenomenon" during the first week, during which PV rose to values higher than those obtained without treatment. At the same time significant changes in plasma renin activity were demonstrated, and these variations in peripheral renin activity were reversed related to the changes in PV.

Hence, it appears justifiable to suppose that the volume depletion which is produced initially by thiazide treatment, is maintained during long-term treatment, although at a lower degree. Most likely this feature is of importance with regard to the continued reducing effect of thiazides on BP, but whether this can explain a maintenance of the blood pressure reduction, or whether other mechanisms of action also play a role, cannot be decided on the basis of the present findings.

References

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