CHLOROTHIAZIDE administration in normotensive and hypertensive individuals causes saluresis, diuresis, oligemia, increased depressor responses to ganglioplegics, and decreased pressor responses to norepinephrine. These changes in responses to vasoactive drugs are abolished if plasma volume is expanded by salt-free dextran, and we have suggested that they reflect an augmented sympathetic vasomotor tone brought about by oligemia. This suggestion implies that the saluresis evoked by chlorothiazide is important only because it allows the establishment of a negative water balance.

It has been considered that sodium plays a primary role in the development and/or maintenance of hypertension. Since studies of the mechanism of action of oral diuretics suggest a secondary role for sodium and a primary one for water, it becomes important to separate the hemodynamic effects of sodium depletion from those of water depletion. Selective sodium depletion can be achieved by intraperitoneal injection of 5 per cent glucose solution. This fluid will gain sodium and other electrolytes, and its removal, in a few hours, effects sodium depletion without concomitant depletion of body water. Transport of sodium into the peritoneal fluid creates extracellular hypotonicity and, as water moves intracellularly, hypovolemia and hypotension result.

Measurements were made of arterial pressure, plasma-sodium levels, and intravascular volume and estimates of sympathetic vasomotor tone were obtained in normal rats first made hyponatremic, hypovolemic, and hypotensive by intraperitoneal injection of 5 per cent glucose and then given replacements either of 5 per cent albumin (to replace intravascular volume), small volumes of hypertonic sodium-chloride (to replace salt loss), or isotonic sodium-chloride solution (to replace extracellular-fluid loss). These experiments were designed to establish the relative importances of extracellular-sodium and total-blood-volume deficits in this type of hypotension.

Methods

Adult Sprague-Dawley rats weighing between 175 and 414 Gm. were anesthetized with sodium amobarbital given intraperitoneally, 9 mg./100 Gm. A femoral venous polyethylene catheter (PE 10) was used for withdrawal of blood samples and intravenous injections. Arterial pressure was recorded continuously through a femoral arterial polyethylene catheter (PE 50) transduced through a Statham strain gauge (P23D). Two sets of experiments were performed.

Effects on Arterial Pressure, Hematocrit, and Plasma-Sodium Levels of Anesthesia Alone, Hypovolemia Alone, and of Similar Hypovolemia Before and After Various Fluid Replacements

In three rats (group I), arterial pressure was recorded continuously for four to seven hours during sodium amobarbital anesthesia. Twenty-two rats, composing groups II to V, were given 5 per cent glucose solution intraperitoneally, 15 ml./100 Gm. body weight; after two hours, the peritoneal fluid was removed. In three rats (group II), the course of arterial pressure was observed continuously through a femoral arterial polyethylene catheter (PE 50) transduced through a Statham strain gauge (P32D). Two sets of experiments were performed.

Effects on Arterial Pressure, Hematocrit, and Plasma-Sodium Levels of Anesthesia Alone, Hypovolemia Alone, and of Similar Hypovolemia Before and After Various Fluid Replacements

In three rats (group I), arterial pressure was recorded continuously for four to seven hours during sodium amobarbital anesthesia. Twenty-two rats, composing groups II to V, were given 5 per cent glucose solution intraperitoneally, 15 ml./100 Gm. body weight; after two hours, the peritoneal fluid was removed. In three rats (group II), the course of arterial pressure was observed over a four- to five-hour period—control, for two hours after the intraperitoneal injection of 5 per cent glucose solution, and for an additional two hours after removal of peritoneal fluid. The remaining 19 rats were divided into three groups, according to the type of fluid administered intravenously to repair extracellular-fluid and electrolyte deficits produced during a two-hour period by intraperitoneal glucose administration. Fluid replacements were given immediately after peritoneal fluid had been removed. Six rats (group III) were given enough hypertonic sodium chloride to replace the sodium deficit;
in another six (group IV), the intravascular volume was restored with 5 per cent human salt-free serum albumin; while in seven (group V), an attempt was made to replace both the sodium and water extracellular deficits with isotonic sodium-chloride solution. Anesthesia was stable throughout the experiments without additional medications.

The amounts of 5 per cent albumin, hypertonic sodium-chloride, and isotonic sodium-chloride solutions given were based on results of pilot experiments. In rats which received hypertonic sodium-chloride solutions to repair the sodium deficit (group III), the amount of salt necessary was estimated from results of experiments in which measurements were made of volume and sodium concentration of the peritoneal fluid removed two hours after instillation of 5 per cent glucose solution. Volumes recovered ranged between 100 and 120 per cent of the injected fluid and sodium concentrations from 72 to 85 mEq./L. (mean 80 mEq./L). The approximate sodium deficit produced by intraperitoneal glucose injection was calculated to be 1.2 mEq./100 Gm. (0.08 mEq. Na/ml. X 15 ml./100 Gm. = 1.2 mEq./100 Gm.); this was dissolved in about 1 ml. of water and the solution was slowly given intravenously.

In rats given 5 per cent human serum albumin, the amount administered was 16 per cent of the estimated original plasma volume of 5.5 ml./100 Gm. body weight. Previous experiments had shown that in a group of 20 rats, intraperitoneal glucose solution for two hours caused an increase in hematocrit from a mean of 46 ml. per cent to 55 ml. per cent, indicating approximately a 16 per cent decrease in the plasma volume. From this experiment, also, we had estimated that 16 per cent of the original extracellular-fluid volume was lost by this procedure; accordingly, the rats in which extracellular sodium and water deficits were replaced (group V) received a volume of isotonic sodium-chloride solution equivalent to 16 per cent of the original extracellular-fluid volume, taken as 28 ml./100 Gm. body weight (0.16 X 28 ml./100 Gm. = 4.48 ml./100 Gm.).

One-ml. femoral-vein blood samples were taken before and at the end of each experiment; in some cases an additional sample was obtained at two hours, when peritoneal fluid had been removed. Hematocrit was measured by the Wintrobe method and plasma-sodium concentration by internal standard flame photometry.

Vascular Reactivity During Hyponatremic Hypovolemia, Following Deficit Replacements, and After Ganglion Blockade

To study sympathetic vasomotor activity during hyponatremic, hypovolemic, hypotension and following administration of the various replacement solutions, experiments similar to those just described were performed in 18 rats. In addition, estimates of sympathetic vasomotor tone were obtained from responses of arterial pressure to norepinephrine (0.01 μg.) and serotonin creatinine sulfate (3.0 μg.) before, and at 30-minute intervals during, development of hypotension, and following fluid and electrolyte replacements. Vascular responses to these two vasoactive agents have been found to provide information concerning sympathetic activity (see Discussion). These experiments were complemented by an identical series performed in 10 rats following administration of pentolinium (5 mg. in 20 per cent polyvinyl pyrollidone solution, subcutaneously) which was used to produce ganglion blockade.

### Results

#### Effects on Arterial Pressure, Hematocrit, and Plasma-Sodium Concentration of Anesthesia, Hyponatremic Hypovolemia Alone and with Replacements

In the three rats (group I) which were anesthetized only, arterial pressure tended to rise slightly throughout the four to seven hours of observation (fig. 1, table 1). In sharp contrast were the arterial-pressure changes in the three anesthetized rats (group II) observed before, and for four hours after, intraperitoneal administration of glucose solution. During the first hour, arterial pressure fell precipitously, then more gradually, and for the two hours after removal of peritoneal fluid remained constant—mean decrease 30 per cent (fig. 1, table 1). Associated were a 22 per cent decrease in plasma-sodium concentration and a 33 per cent increase in hematocrit.

Following stabilization of hyponatremic, hypovolemic hypotension, repair of the sodium deficit with 1 ml. of hypertonic saline (group III) returned arterial pressure to control levels where it was maintained for about an hour, and thereafter fell, so that two hours after saline injection the mean percentile decrease for the group was 18 per cent (fig. 2, table 1). At the end of the experiment, hematocrit was slightly lower than, and plasma sodium the same as, control values. Albumin given intravenously (group IV) was equally effective in restoring and maintaining arterial
Table 1
Changes in Arterial Pressure, Hematocrit, and Plasma Sodium in Anesthetized Rats and in Rats Made Hyponatremic and Hypovolemic by Intraperitoneal Glucose Injection and Given Replacement Fluids

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Experimental conditions</th>
<th>Replacement</th>
<th>Per cent change MBP*</th>
<th>Hct* (ml/100 ml)</th>
<th>PNa* (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>...</td>
<td>...</td>
<td>+15</td>
<td>43</td>
<td>148</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>Intraperitoneal glucose</td>
<td>...</td>
<td>+14</td>
<td>57</td>
<td>116</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Intraperitoneal glucose</td>
<td>Hypertonic NaCl</td>
<td>+33</td>
<td>51</td>
<td>145</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>Intraperitoneal glucose</td>
<td>5 per cent albumin</td>
<td>+34</td>
<td>45</td>
<td>147</td>
</tr>
<tr>
<td>V</td>
<td>7</td>
<td>Intraperitoneal glucose</td>
<td>Isotonic NaCl</td>
<td>+9.7</td>
<td>47</td>
<td>146</td>
</tr>
</tbody>
</table>

*Abbreviations: MBP = mean arterial pressure; Hct = hematocrit; PNa = plasma-sodium concentration.

Values in parentheses indicate range of values.

<table>
<thead>
<tr>
<th>Figure 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes in arterial pressure during sodium amobarbital anesthesia alone, and following intraperitoneal glucose injection.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 2</th>
</tr>
</thead>
</table>
| Changes in arterial pressure of anesthetized rats given 5 per cent glucose solution intraperitoneally and also in lowering the hematocrit pressure and also in lowering the hematocrit which at the end of four hours, was slightly less than control values. Severe hyponatremia was evidenced by a mean plasma-sodium value of 112 mEq/L. Following administration of isotonic sodium chloride solution (group V), arterial pressure never returned to control levels and was significantly lower than in groups III and IV. Plasma-sodium concentration was only partially restored; hematocrit was not different than control values. Vascular Reactivity During Hyponatremic Hypovolemia and Following Replacements. Arterial pressure responses to norepinephrine and serotonin changed in a consistent pattern during the development of hypotension and following the various replacements. During the first two hours, as oligemia progressed, the pressor response to norepinephrine and serotonin declined. After injection of norepinephrine, arterial pressure was higher than control values. Following administration of isotonic sodium chloride solution (group V), arterial pressure was significantly lower than in the control rat. Plasma-sodium concentration was only partially restored; hematocrit was not different than control values. **
norepinephrine became greatly diminished and the depressor phase of the serotonin response was prolonged. Following electrolyte and/or fluid replacements, pressor responses to norepinephrine returned to control values and the duration of the serotonin depressor response was shortened. The constancy of the inverse relationship between the magnitude of the norepinephrine response and the duration of the serotonin depressor phase is shown in figure 3. The level of plasma sodium did not seem to play a role in responses of arterial pressure to these two vasoactive agents; the responses were similar following replacement with hypertonic sodium-chloride, 5 per cent albumin, or isotonic sodium-chloride solutions. A representative experiment is shown in figure 4.

The constancy of the changed responses to norepinephrine and serotonin suggested augmented sympathetic vasomotor activity during oligemia that decreased with subsequent expansion of the intravascular volume. For the study of the participation of sympathetic activity in these responses, experiments were repeated after pentolinium had been given to achieve some degree of ganglion blockade. After pentolinium, control arterial pressure was considerably lower than that observed in other experiments (table 2). Following intraperitoneal glucose injection, blood pressure fell still more and pulse pressure became very narrow. With each replacement fluid, arterial pressure was restored above control levels and the magnitude of the percentile change was greater than previously observed. Pressor responses to norepinephrine were enhanced by pentolinium administration, but as oligemia progressed, they diminished as previously (fig. 5, table 2). Repair solutions restored responsiveness to control values independent of plasma-sodium concentration. In contrast with changes in norepinephrine responses, the serotonin depressor phase was minimal after pentolinium treatment and remained unchanged throughout development of oligemia and its repair with the various replacement solutions (fig. 5).

Discussion

These experiments were designed to compare and contrast the effects of intravascular and interstitial sodium and water deficits on arterial pressure and sympathetic vasomotor tone. The technique of intraperitoneal glucose injection was used because it allows a reduction in body sodium without a concomitant reduction in body water. Following glucose injection, extracellular electrolytes move into the peritoneal fluid, extracellular hypotonicity...
develops, water moves into cells, and the resulting hypotensive hypovolemia is accompanied by hypotension. This hypotension could be due either to a decreased intravascular volume such as occurs in hemorrhagic shock, or, in some way, to sodium deficit, or to both changes. Our results show that in hypotensive, hypovolemic rats, hypotension is relieved equally as well by salt-free albumin solution as by hypertonic sodium-chloride solution and is, therefore, more dependent on a decreased intravascular volume than on extracellular sodium deficit. Following albumin administration, severe hypotension persisted; but after hypertonic sodium chloride, normal plasma-sodium levels were obtained. Both solutions seemed to replace the intravascular volume equally well, as hematocrit levels fell slightly below control values; it may be, also, that hematocrit was lowered by transfer of water out of red cells. Isotonic sodium-chloride solution did not relieve the hypotension so effectively. Either an insufficient amount was given or the tonicity of the solution was not great enough to draw water out of cells and repair the extracellular deficits. In the latter regard, Winkler, Danowski, and Elkinton found that isotonic sodium-chloride solution did not restore hemodynamic functions in hypotensive, hypovolemic dogs as well as did albumin and hypertonic sodium-chloride solutions.

Since hemorrhagic oligemia enhances sympathetic vasomotor outflow, it seemed important to obtain estimates of sympathetic activity during hypotensive hypovolemia and following replacements. The height of the norepinephrine pressor response and the duration of the serotonin depressor response were taken as these estimates. The pressor response to norepinephrine is depressed during hemorrhage and chlorothiazide-induced oligemia — both situations in which there is either direct or indirect evidence of enhanced sympathetic vasomotor tone; it becomes augmented when sympathetic outflow is diminished either by surgical sympathectomy or by ganglion blockade. Page, McCubbin, and Green have shown clearly that norepinephrine responses are determined, in large measure, by sympathetic vasomotor outflow. Buffer nerve section, which greatly increases outflow, either reduces norepinephrine pressor effect or does not affect it, while greatly enhancing angiotensin responsiveness. If, however, three buffer nerves are sectioned and one carotid sinus perfused at systemic arterial-pressure levels, sympathetic outflow is normal, but buffering capacity is absent; in this situation, norepinephrine as well as angiotensin response is greatly augmented. Norepinephrine infusion, which partly mimics neurogenic hypertension, diminishes responses to superimposed injections of norepinephrine, but does not affect angiotensin response. These experiments show that if buffer nerves are intact, norepinephrine responses are valid estimates of sympathetic vasomotor outflow.

**Table 2**

<table>
<thead>
<tr>
<th>Repair fluid</th>
<th>No. of rats</th>
<th>Control</th>
<th>5% glucose IP*</th>
<th>Fluid replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertonic saline</td>
<td>4</td>
<td>MBP*—mm. Hg</td>
<td>82</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NE resp.—mm. Hg</td>
<td>50</td>
<td>38</td>
</tr>
<tr>
<td>5% albumin</td>
<td>3</td>
<td>MBP—mm. Hg</td>
<td>77</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NE resp.—mm. Hg</td>
<td>47</td>
<td>33</td>
</tr>
<tr>
<td>Isotonic saline</td>
<td>3</td>
<td>MBP—mm. Hg</td>
<td>78</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NE resp.—mm. Hg</td>
<td>55</td>
<td>40</td>
</tr>
</tbody>
</table>

*Abbreviations: IP = intraperitoneal; MBP = mean arterial pressure; NE resp. = pressor response to norepinephrine.
As concerns serotonin responsiveness, Page and McCubbin\textsuperscript{7} have demonstrated that it, also, is dependent on the degree of sympathetic tone; when tone is high, the depressor phase becomes prominent and prolonged, but when tone is low, it is diminished or absent. In these experiments, as oligemia progressed, pressor responsiveness to norepinephrine lessened and the depressor phase of the serotonin response became prolonged (fig. 4), indicating an increased sympathetic vasomotor tone. Following fluid and/or electrolyte replacement, these vascular responses returned to control values. Thus, vasomotor tone seemed unaffected by changes in extracellular sodium concentrations, but dependent upon changes in intravascular volume, being increased by oligemia and decreased by the various replacements.

In order to investigate further the role of sympathetic activity in these changed responses, similar experiments were performed following administration of pentolinium. In the presence of ganglion blockade, serotonin depressor response was slight, or completely absent, during control observations and was unaffected by oligemia and the various replacements. In contrast, the pressor effect of norepinephrine, which was enhanced by pentolinium, was again somewhat lessened by hypovolemia and restored by repair of intravascular volume without regard to the plasma-sodium concentration achieved by the repair solution (fig. 5, table 2). The data suggest that pressor responsiveness to norepinephrine is determined not only by sympathetic vasomotor outflow, but also by total blood volume; it seems unrelated to extracellular-intracellular sodium gradient.

Friedman, Jamieson, and Friedman\textsuperscript{11} have suggested that vascular smooth-muscle tone and responsiveness to vasoconstrictor agents are increased when the extracellular-intracellular sodium gradient ($\text{Na}_0\text{Na}_i$) is low and decreased when it is high. Our results do not lend support to this suggestion. Although no attempt was made to estimate intracellular sodium quantitatively, during hyponatremic hypovolemia, $\text{Na}_0\text{Na}_i$ must have been very low; yet, responsiveness to norepinephrine was greatly diminished, but was returned to control values by repair of the intravascular volume, regardless of the sodium gradient that resulted. On the one hand, when 5 per cent albumin solution was used as replacement, hyponatremia persisted and $\text{Na}_0\text{Na}_i$ should have been very low; on the other hand, when hypertonic sodium-chloride solution was used, plasma-sodium concentration was normal and $\text{Na}_0\text{Na}_i$ should have been restored to control values. If extracellular-intracellular sodium gradient is an important determinant of vascular smooth-muscle responsiveness, the conditions of our experiments must not have been appropriate for demonstrating this.

Glucose solution given intraperitoneally causes changes in electrolytes, other than sodium, that might affect arterial pressure and vasomotor tone. This experiment was designed only to study effects of extracellular sodium and water changes; no attempt was made to

\textsuperscript{7}Page and McCubbin, 1960

\textsuperscript{11}Friedman, Jamieson, and Friedman, 1960
prevent other electrolyte deficits nor to correct them once they had developed. Since arterial pressure and vasomotor tone were dependent on intravascular volume, deficiencies of electrolytes other than sodium are assumed to be as unimportant as a deficiency of sodium was found to be.

Summary

In rats made hyponatremic, hypovolemic, and hypotensive by intraperitoneal injections of 5 per cent glucose solution, arterial pressure was returned to control values by correction of the intravascular-volume deficit with either 5 per cent salt-free albumin or small volumes of hypertonic sodium-chloride solution. In the former, severe hyponatremia persisted; in the latter, it was corrected. Isotonic sodium-chloride solution, given in amounts considered sufficient to repair the extracellular-fluid and electrolyte deficits, was not so effective either in controlling hypotension or in expanding the intravascular volume. Using the height of the norepinephrine pressor response and the duration of the serotonin depressor phase as estimates of sympathetic activity, sympathetic vasomotor tone was found to be enhanced during hyponatremic, hypovolemic hypotension and depressed by administration of 5 per cent albumin, hypertonic and isotonic sodium-chloride solutions. These changes in estimates of sympathetic activity seemed dependent on changes in intravascular volume and not in extracellular sodium concentrations. Experiments performed following pentolinium-induced ganglion blockade showed that the depressor phase of the serotonin response was dependent on sympathetic vasomotor outflow, but that the height of norepinephrine pressor response was dependent not only on sympathetic activity, but also on the intravascular volume. In this study of selective sodium deficit, intravascular volume was found to be an important determinant of arterial pressure and sympathetic vasomotor tone.

References

Relationships Among Intravascular Volume, Total Body Sodium, Arterial Pressure, and Vasomotor Tone
HIDEO TAKAGI, HARRIET P. DUSTAN and IRVINE H. PAGE

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