Restitution of blood volume after hemorrhage: role of the adrenal cortex

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PIRKLE, J. CARL, JR., AND DONALD S. GANN. Restitution of blood volume after hemorrhage: role of the adrenal cortex. Am. J. Physiol. 230(6): 1683-1687. 1976.—Splenectomized and adrenalectomized-splenectomized dogs, anesthetized with pentobarbital, were bled so that the role of cortisol in restitution of blood volume could be examined. Intact dogs and adrenalectomized dogs infused with cortisol at a high rate (17 μg/min) showed restoration of blood volume and plasma protein at 24 h, preceded by an early increase in plasma osmolality. Adrenalectomized dogs infused with cortisol at basal rates (2 μg/min) showed no increase in plasma osmolality and no restoration of blood volume or plasma protein at 24 h unless extracellular fluid volume was expanded by exogenous fluid. It is concluded that hemorrhage leads to an increase in extracellular fluid expansion which reduces capillary pressure. Because large doses of cortisol have been shown to shift intracellular fluid to the interstitium (5, 30, 31), we postulated (25) that the increased secretion of cortisol observed after hemorrhage (12) induces restoration of protein through a fluid shift from cells to interstitium. On the basis of preliminary experiments, Marks (23) has proposed that increased circulating levels of cortisol after hemorrhage were necessary for full restitution of blood volume. The present experiments were performed to investigate this possibility and to begin to define the underlying mechanisms.

METHODS

Experimental protocol. Thirty-two adult mongrel dogs weighing 15-25 kg were splenectomized, and 22 of these were also adrenalectomized, at least 2 wk prior to experiment. The adrenalectomized dogs were maintained on 5 mg cortisol acetate and 2 mg deoxycorticosterone (DOC) daily. Twenty-four hours prior to experiment, cortisol acetate was withheld and the adrenalectomized dogs were infused (via a Sigma pump through a jugular vein catheter) with 2 μg/min of cortisol. This corresponded to the resting morning secretion rates measured in intact dogs (12). On the morning of the experiment, after 18 h without food or water, the dogs were anesthetized with 30 mg/kg of sodium pentobarbital; maintenance doses of 3 mg/kg were given every 15 min during the experiment. In addition to the jugular vein catheter, catheters were placed in a femoral artery and cephalic vein.

After a resting period of 2-3 h, the dogs were given 3,000 U of sodium heparin derived from beef lung. Plasma volume was determined and control blood samples were obtained. The dogs were then bled through the arterial cannula at a rate of 7.5 ml/kg over a period of 3 min. The experiments involving adrenalectomized dogs were divided into three groups: groups I and III (12 and 2 experiments, respectively) continued to receive 2

interstitial fluid expansion; extracellular osmolality; lymphatic return; splenectomized dogs

EXPERIMENTS IN MAN and in splenectomized dogs indicate that restitution of blood volume after hemorrhage involves both a rapid first stage and a slower second stage. The first stage requires 15 min to 2 h and involves transcapillary absorption of interstitial fluid (5, 30, 31). This early influx of fluid follows precapillary vasoconstriction (17) which reduces capillary pressure. Because the entering fluid is free of colloidal protein, however, plasma oncotic pressure is reduced (7). Thus, transcapillary exchange appears to reach a pseudosteady state before blood volume is fully restored (5, 30, 31).

The second stage of restitution of blood volume is much slower, requiring ½2-2 days for completion (1, 30, 31). It appears to be secondary to restoration of oncotic pressure (1, 7, 30), which results mainly from a shift of preformed interstitial protein to the vascular system (1). Increased hepatic synthesis is believed to have a minor role in plasma protein restoration during this time, but may serve to replace interstitial protein during the next 2-3 days (1, 22, 30). Several hypotheses have been advanced to explain this shift of interstitial protein. Recently, we proposed a mathematical model which was used to evaluate these hypotheses (25). The most plausible explanation of the protein recovery was a shift of intracellular fluid to the interstitium. Such a shift would increase interstitial pressure, thus accelerating lymphatic movement of interstitial protein to the vascular system. This lymphatic movement could occur via lymphovenuous shunts (4, 35), which would explain the lack of increase in left thoracic duct flow after hemorrhage (7, 18, 38). Since large doses of cortisol have been shown to shift intracellular fluid to the interstitium of both intact and adrenalectomized dogs (34), we concluded that hemorrhage leads to an increase in extracellular osmolality mediated in part by increased cortisol concentrations. As a consequence, there is a shift of intracellular fluid to the interstitium. This results in a reequilibration of extracellular fluid toward the plasma, thus completing the restitution of blood volume. The osmotically active agents mobilized by cortisol do not appear to be glucose, sodium, or potassium.
μg/min of cortisol; group II (8 experiments) received 17 μg/min cortisol after hemorrhage; group III (7 experiments) received in addition 7.5 ml/kg of 5% dextrose in isotonic saline infused over a period of 1 h. Infusions of cortisol were maintained for 24 h, with a total volume of 25 ml. The infusion rate of 17 μg/min of cortisol for the group II experiments corresponds to the secretion rate of extracellular fluid necessary for full restitution of blood volume as determined by a computer simulation (25). All adrenalectomized dogs were given 5 mg DOC intramuscularly upon hemorrhage so that increased secretion of aldosterone would be mimicked. More than one experiment was performed on some adrenalectomized dogs; in this case, a recovery period of at least 2 wk was allowed between experiments. Blood samples were obtained every 5 min for 30 min, then every 15 min for 2 h; then at 2, 3, 5, 12, 18, and 24 h. Pentobarbital was discontinued at 2 h, and the dogs were allowed to recover. Food and water were withheld while the dogs were monitored over the next 22 h.

To determine the effect of an increased rate of infusion of cortisol on the plasma osmolality (see below) of adrenalectomized dogs without hemorrhage, another series of experiments was carried out. Eight adrenalectomized dogs (group IV) were not hemorrhaged, but the infusion rate of cortisol was increased from 2 to 17 μg/min. Blood samples were taken just before and 3, 5, 10, 15, 20, 30, and 45 min after the increase in rate of infusion.

Measurement of blood volume and arterial pressure. The control plasma volume (CPV) was measured by dilution techniques (6) using T-1824 (Evans blue dye) or 141I-labeled albumin. Postinjection samples were taken at 10, 15, 20, 25, and 30 min and the concentrations of dye or isotope were extrapolated to zero time on a semilog plot. Large-vessel hematocrits (LVH) were obtained at 10, 15, 20, 30, and 45 min after the increase in rate of infusion.

Results

Restoration of blood volume. Restoration of blood volume after hemorrhage is shown in Table 1 as percent of the shed volume; that is, % BVR = 100 (BV-(CBV-HEM))/HEM, where % BVR is the percent of shed blood volume that is restored. In intact dogs, significant partial restoration of blood volume occurred by 30 min after hemorrhage (P < 0.001). By 2 h, restoration of blood volume averaged 47 ± 9% (mean ± SE) complete, and at 24 h, restoration had climbed to 124 ± 8%. Partial restoration of blood volume was also observed in all groups of adrenalectomized dogs for the first 2-6 h after hemorrhage with no significant difference from the intact group. By 12 h, the blood volume in dogs adrenalectomized and infused with cortisol at the basal rate declined, and restoration was insignificant (12 ± 10%) by 24 h. In contrast, in dogs adrenalectomized and infused with cortisol at the increased rate, restoration was significantly lower at 24 h (80% vs. 124%; P < 0.001) than that of the intact dogs, but was significantly higher (P < 0.001) than that of the dogs infused at the basal rate. In dogs adrenalectomized and infused with cortisol at

<table>
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<th>Type of Experiment</th>
<th>Minutes</th>
<th>Hours</th>
<th>Time After Hemorrhage</th>
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<tbody>
<tr>
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<td>1</td>
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<tr>
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<td>±10</td>
<td>±9</td>
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<td>45</td>
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<td>±14</td>
<td>±9</td>
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<td>38</td>
<td>39</td>
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<tr>
<td>n = 8</td>
<td>±5</td>
<td>±10</td>
<td>±6</td>
</tr>
<tr>
<td>Group III</td>
<td>11</td>
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<td>27</td>
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<tr>
<td>n = 7</td>
<td>±7</td>
<td>±11</td>
<td>±8</td>
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Values are means ± SE of percents of shed volumes. Intact: splenectomized; group I: adrenalectomized-splenectomized, 17 μg/min cortisol posthemorrhage; group II: adrenalectomized-splenectomized, 2 μg/min cortisol posthemorrhage; group III: adrenalectomized-splenectomized, 2 mg/min cortisol posthemorrhage infused with 7.5 ml/kg 5% dextrose in 0.9% NaCl.
the basal rate and with 7.5 ml/kg infusion of 5% dextrose in isotonic saline, restoration was significantly lower (98% vs. 124%; *P < 0.01) than that of the intact dogs by 24 h, but was significantly higher than that of adrenalectomized dogs infused with cortisol at the increased rate (98% vs. 80%; *P < 0.05).

**Restoration of total plasma protein.** Restoration of total plasma protein (plasma volume times the plasma protein concentration) is shown in Table 2 as percent of the shed amount (lost through hemorrhage and through blood sampling). Total plasma protein was restored in intact (141 ± 11%; *P < 0.001) and in adrenalectomized dogs receiving increased cortisol (92 ± 18%; *P < 0.001), as well as in adrenalectomized dogs receiving only basal cortisol and exogenous fluid (190 ± 50%; *P < 0.001). Restoration of protein was significantly greater in intact dogs than in adrenalectomized dogs infused with cortisol at the increased rate (*P < 0.01). In adrenalectomized dogs receiving only basal cortisol after hemorrhage, however, restoration of protein occurred only transiently and was zero by 24 h (0 ± 36%; *P > 0.5). Restoration of protein was significantly greater for all other groups (*P < 0.001 in each case).

**Plasma cortisol.** Plasma concentrations of cortisol are shown in Table 3. Posthemorrhage concentrations of cortisol rose both in intact dogs and in dogs infused with cortisol at the increased rate (*P < 0.01), but remained near resting levels for those adrenalectomized dogs infused with cortisol at the basal rate. The elevation was still significant at 2 h (*P < 0.001) in intact dogs and in adrenalectomized dogs infused at 17 μg/min (*P < 0.01), but was not significant by 6 h (*P > 0.5) for intact rats; *P > 0.05 for adrenalectomized rats). The fall in plasma concentration of cortisol in the adrenalectomized dogs despite continued infusion implies an unexplained change in distribution or in metabolism of the hormone.

**Plasma osmolality, sodium, potassium, and glucose.** Plasma osmolality in intact dogs was elevated during the first 2–6 h after hemorrhage, beginning at 5 min after hemorrhage (time of first sample). The plasma osmolality of adrenalectomized dogs infused with cortisol at the increased rate also was elevated at 5 min, and this elevation persisted for the first 1.5 h after hemorrhage. During this period, the elevation was not significantly different from the elevation observed in intact dogs. In contrast, in adrenalectomized dogs infused with cortisol at the basal rate, osmolality decreased between 10 and 30 min (*P < 0.01), with return to levels not significantly different from control by 45 min (*P > 0.1). No significant change in plasma osmolality was observed in the adrenalectomized dogs whose cortisol infusion rate was increased from 2 to 17 μg/min without hemorrhage (Table 4). Plasma concentrations of sodium and potassium did not differ significantly from control at any time in any group. Variations in plasma glucose levels (Table 5) appeared too small to contribute significantly to the changes in plasma osmolality (1 mosmol/kg H2O = 18 mg/100 ml glucose) in any of the experiments.

**Mean arterial pressure.** Mean arterial pressure was well maintained in all experiments during the first 2 h after hemorrhage. Thus infusion of cortisol at 2 μg/min appeared adequate to maintain normal vasomotor tone in adrenalectomized-splenectomized dogs.

**Discussion**

The data on restitution of blood volume after moderate hemorrhage in splenectomized dogs appear to be in agreement with those of Chien (6) and with those of Skillmann et al. (31). After a 7.5 ml/kg hemorrhage, restoration reaches a plateau within 15 min to 2 h and is only partially complete. This partial restoration is accompanied by a dilution of plasma protein, indicating that the fluid entering the vascular system at this time is low in colloidal protein. The further expansion of plasma volume over the next 22 h is associated with an increase in total plasma protein to slightly above control.
level, restoring oncotic pressure. Adrenalectomized-splenectomized dogs infused with cortisol at the basal rate also demonstrate partial restoration of blood volume within 2 h after hemorrhage, so that a rise in plasma concentration of cortisol does not appear necessary for the full expression of this phase.

However, only those bled dogs either with increased rates of infusion or secretion of cortisol or with infusion of extra fluids exhibited the second phase of restitution of blood volume and of plasma protein by 24 h. Adrenalectomized-splenectomized dogs infused with cortisol at the basal rate had significantly less restoration of blood volume at 24 h than did either intact dogs (P < 0.001) or adrenalectomized-splenectomized dogs infused with cortisol at the increased rate (P < 0.001; Table 1). Furthermore, restoration of plasma protein did not occur in adrenalectomized dogs infused at the basal rate, as it did in others (Table 2). The results confirm the concept (7) that the second phase of restitution of blood volume is mediated through restoration of plasma protein. Since an increased rate of infusion of cortisol is required for restoration both of plasma volume and of plasma protein, the role of cortisol in mediating these effects cannot be simply permissive, as suggested previously by Ingle et al. (19).

In addition, an adrenal factor other than cortisol appears to be involved in the full expression of the second phase of restitution of blood volume. There was significantly less restoration of blood volume (80 vs. 124%; P < 0.001) and of plasma protein (92 vs. 141%; P < 0.01) in adrenalectomized-splenectomized dogs infused with cortisol at the increased rate than in intact dogs (Table 1), even though concentrations of cortisol in the former were greater than or equal to those in the intact dogs (Table 3). Catecholamines secreted by the adrenal may be involved in the additional restitution of volume observed in the intact dog, but the vasoconstrictive property of these substances could not account for more restitution of plasma protein. Further, although more intense precapillary vasoconstriction could lead to additional transcapillary absorption of interstitial fluid by reducing capillary pressure, the resulting decrease in interstitial hydrostatic pressure would reduce the rate of lymphatic movement of interstitial protein to the vascular system.

To determine the magnitude of the fluid shift from cells to interstitium necessary to effect full restitution of blood volume by 24 h following a 7.5 ml/kg hemorrhage, a simulation was performed with the previously developed mathematical model (25). This simulation predicted that a net expansion of 7.5 ml/kg in extracellular fluid volume (after dehydration and urinary losses) will lead to full restitution of blood volume. The shift of fluid to the interstitium from cells increases interstitial hydrostatic pressure (14) because of the low compliance of interstitial space (15). The calculations indicate an increase up to 2 mmHg in interstitial hydrostatic pressure with a consequent fourfold increase in lymphatic return (16). As colloidal protein is shifted from the interstitium to the vascular system, plasma oncotic pressure is restored, resulting in redistribution of extracellular water toward the plasma. An additional simulation was then carried out for splenectomized-adrenalectomized dogs infused with cortisol at the basal rate and with 7.5 ml/kg of 5% dextrose in saline for 1 h after hemorrhage. The results of the simulation predicted full restitution of blood volume by 24 h and, as shown in Fig. 1, agreed closely with experimental results. Accordingly it seems plausible that without administration of exogenous fluid, the second phase of restitution of blood volume and of plasma protein depends upon a shift of fluid from cells to interstitium, mediated by increased extracellular osmolality.

Osmolality increased after hemorrhage only if the plasma concentration of cortisol rose, suggesting strongly that the increased osmolality is mediated at least in part by increases in cortisol which follow hemorrhage (11, 12). However, an increased infusion rate of cortisol in the absence of hemorrhage did not lead to an increase in plasma osmolality in adrenalectomized dogs. This suggests that at least one additional factor, activated by hemorrhage, must be involved in mediation of the increase in osmolality. Such factors may be neural or hormonal or both, but have not yet been identified. Since osmolality increased within 5 min, the factors involved must begin to act very rapidly.

The nature of the increase in plasma osmolality is not clear. Glucose contributes significantly to the plasma hyperosmolality observed in hemorrhagic shock (2, 3, 20, 29), but appears to play a minor role in the case of the rapid, moderate hemorrhages reported here. No

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**TABLE 5. Change in plasma glucose in response to hemorrhage**

<table>
<thead>
<tr>
<th>Type of Experiment</th>
<th>Minutes</th>
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<tr>
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<td>&lt;0.03</td>
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<td>Group I</td>
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<td>5.1</td>
</tr>
<tr>
<td>Group II</td>
<td>2.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Group III</td>
<td>0.6</td>
<td>3.0</td>
</tr>
<tr>
<td>n = 8</td>
<td>2.7</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; mg/dl/mL. Groups identified as in Table 1. Significance level of changes in glucose: * P < 0.001; ** P < 0.01; *** P < 0.05; all others not significant (P > 0.05).

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**FIG. 1. Restitution of blood volume for adrenalectomized-splenectomized dogs infused with cortisol at basal rate and with 7.5 ml/kg of 5% dextrose in saline.**
significant changes were observed for sodium or potassium during the period of osmotic change. Other osmotically active agents may include free fatty acids and amino acids, which have been reported to increase after injury (29). Studies are in progress to characterize the increased solute. In the present experiments, plasma osmolality increased about 7 mosmol/kg H₂O within 5 min. Moderate stress in rats also has been reported to induce a similar increase in osmolality in 1.5 min which was not explained by changes in glucose (9).

Some investigators have failed to observe a shift of fluid from cells to interstitium in response to hemorrhage. However, the experiments either involved apparent prehemorrhage stress with high plasma concentration of cortisol (24) or hemorrhagic shock (8, 28). Other investigators observed a shift of fluid to the interstitium after sublethal hemorrhage (21, 33, 36).

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