Increased Aldosterone Secretion in Response to Blood Loss

By GORDON L. FARRELL, M.D., ROBERT S. ROSNAGLE, A.B. and ELIZABETH W. RAUSCHKOLB, A.B.

A marked increase in the adrenal secretion of the potent electrolyte-active steroid, aldosterone, was observed in the dog in response to loss of blood. The proportionate increase in adrenal output of aldosterone exceeded that of hydrocortisone. The physiologic mechanism by which the increased aldosterone release is mediated remains obscure. The stimulus to increased aldosterone secretion was not a change in the serum electrolytes. Expansion of the volume of the vascular system by the infusion of large amounts of plasma substitutes failed to uniformly prevent the increased aldosterone output.

COMPENSATORY responses to blood loss have been the subject of extensive studies for many years.1 The adrenal cortex plays an important, although as yet ill-defined, role in the resistance of the body to hemorrhage. Indirect indices show that the secretory activity of this gland is increased.1 Recent developments in methodology make it possible to take a direct approach to the characterization and quantitation of the adrenal secretion in response to hemorrhage. In the present study a marked increase in adrenal aldosterone release was found to occur during blood loss. Increased secretion of this potent sodium-retaining steroid may represent an important component of the physiologic response to hemorrhage.

METHODS

Thirty-four male mongrel dogs were assigned to 6 experimental groups with 5 to 7 dogs per group. The basic experimental plan was the same in each instance. The animals were anesthetized with sodium pentobarbital. Femoral arterial blood pressure was recorded by means of a mercury manometer. The adrenal vein was ligated between the gland and the inferior vena cava and the lumbo-adrenal vein cannulated. Heparin was injected intravenously to prevent clotting. Adrenal venous effluent was collected over a 3-hour period; about one third of the calculated blood volume of each dog was removed by this procedure. In order to permit a study of the changes in steroid secretion occurring during the loss of blood, the adrenal venous blood was collected in lots corresponding to 20-minute bleeding periods. Thus 9 lots of blood were obtained, representing the time intervals 0 (beginning of collection) to 20 min., 20 to 40 min., 40 to 60 min., and so forth. The volume of adrenal venous blood and the arterial blood pressure were noted at the end of each 20-minute collection interval. In experimental group C, blood samples were taken for determination of the hematocrit and serum sodium and potassium concentrations.

The adrenal venous blood samples were kept at 0 C. during collection and frozen for storage. Twenty-four to 48 hours later the samples were thawed and extracted with chloroform. The chloroform extracts were taken to dryness under filtered air and stored in vacuum at 3 C. Since the amount of adrenal venous blood in any one such 20-minute sample from a single dog is insufficient for aldosterone isolation, the following procedure was necessary. The extracts obtained from blood collected during the corresponding 20-minute time interval from the 5 to 7 dogs in each experimental group were pooled, that is, the sample from bleeding period 0-20 min. of the first dog was pooled with the corresponding 0-20 min. sample of the second dog, and so forth. Each experiment thus yielded nine large samples, each sample representing the adrenal steroid output of several animals during corresponding time intervals from the beginning of adrenal venous bleeding.

In experiments A, B and C the blood loss was not replaced. In experiments D and E dextran solution (6 per cent in 0.9 per cent NaCl)* was infused at a

* The dextran preparation, Plavolex, was kindly supplied by Mr. James R. McGuinness of the Wyeth Laboratories.
rate equal to twice that of the blood loss. The infusion of dextran solution was begun at a rate of approximately twice the adrenal venous bleeding. The exact volume of adrenal venous blood was determined at the end of the first 20-minute bleeding period and the infusion was then adjusted so that exactly twice that volume was infused during the next 20-minute period. This procedure was followed throughout the experiment. In experiment F, gelatin for intravenous use (5 per cent in 0.9 per cent NaCl)* was infused at a rate twice that of blood loss.

Isolation of hydrocortisone and aldosterone from the adrenal venous blood was done by paper chromatographic methods. Evidence for identity of aldosterone was obtained by mixed chromatogram with authentic aldosterone diacetate, by sulfuric acid chromogen, and by bioassay for sodium retention in adrenalectomized rats. The isolated material was indistinguishable from aldosterone diacetate by these criteria. Quantitation of aldosterone was on the basis of absorption at 240 mμ in methanol (ε = equal to 15,850). Serum sodium and potassium concentrations were determined with a Beckman flame photometer.

**RESULTS**

Aldosterone and hydrocortisone secretion rates are presented in tables 1 and 2. The initial levels of steroid secretion reflect the variation among the different experimental groups. The reason for this variation among different groups of dogs is not immediately evident. However, the determinations were carried out on consecutive samples of adrenal venous blood from the same group. Each group serves as its own control, so that changes in steroid secretory rate in any single group of dogs during the progress of the experiment can be evaluated independently of variation among groups. The steroid secretion rate in the first 20 min. interval of each experiment was taken as control, and each subsequent determination was calculated as percentage of this initial rate. The results of these calculations are presented in figures 1 and 2, together with the average blood pressure and change in blood volume. Indicated changes in blood volume were calculated as ml. blood loss per Kg. body weight. In the experiments in which dextran or gelatin solution was infused, blood volume changes were calculated as the difference between blood loss and infused plasma expander.†

**Steroid Secretion in the Absence of Fluid Replacement.** In experiments A, B and C no fluid replacement was given. The total blood loss calculated as the difference between blood loss and infused plasma expander.†

*The gelatin solution, Plazmoid, was kindly supplied by Dr. Marvin Kuizenga of the Upjohn Company.

### Table 1.—Rates of Secretion of Aldosterone in Dogs During Bleeding With and Without Plasma Volume Replacement

<table>
<thead>
<tr>
<th>Time in min. from beginning of bleeding</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No replacement of blood volume</td>
</tr>
<tr>
<td></td>
<td>A (6)*</td>
</tr>
<tr>
<td>0-20</td>
<td>8.8†</td>
</tr>
<tr>
<td>20-40</td>
<td>10.6</td>
</tr>
<tr>
<td>40-60</td>
<td>22.2</td>
</tr>
<tr>
<td>60-80</td>
<td>26.7</td>
</tr>
<tr>
<td>80-100</td>
<td>26.2</td>
</tr>
<tr>
<td>100-120</td>
<td>15.2</td>
</tr>
<tr>
<td>120-140</td>
<td>11.9</td>
</tr>
<tr>
<td>140-160</td>
<td>8.9</td>
</tr>
<tr>
<td>160-180</td>
<td>22.5</td>
</tr>
</tbody>
</table>

* Number of dogs in each experimental group.
† Values are expressed in μg. steroid/Kg. body weight/hr.

### Table 2.—Rates of Secretion of Hydrocortisone in Dogs During Bleeding With and Without Plasma Volume Replacement

<table>
<thead>
<tr>
<th>Time in min. from beginning of bleeding</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>No replacement of blood volume</td>
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<tr>
<td></td>
<td>A (7)*</td>
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<tr>
<td>0-20</td>
<td>17.5</td>
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<tr>
<td>20-40</td>
<td>16.6</td>
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<tr>
<td>40-60</td>
<td>25.2</td>
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<td>60-80</td>
<td>37.3</td>
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<td>28.3</td>
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<td>100-120</td>
<td>17.4</td>
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<td>120-140</td>
<td>20.6</td>
</tr>
<tr>
<td>140-160</td>
<td>13.3</td>
</tr>
<tr>
<td>160-180</td>
<td>12.6</td>
</tr>
</tbody>
</table>

* Number of dogs in each experimental group.
† Values are expressed in μg. steroid/Kg. body weight/hr.
† The actual increase was probably somewhat less than calculated because of leakage of the plasma expanders from the vascular system. However, the work of Davis and co-workers indicates that such leakage is probably not a serious factor under the conditions of these experiments.
over the 3-hour period was, on the average, about 30 ml./Kg. The rates of bleeding were on the average 3.1 ml./min., which, over the 3-hour period of bleeding, would have been expected to induce irreversible shock as a later consequence in a fair percentage of the animals.4

Mean arterial pressure (fig. 1) fell progressively during the experiment to an average of approximately 80 mm. Hg. During the blood
loss a marked increase in aldosterone secretion occurred which amounted to a threefold increase in experiments A and C, and somewhat less than twofold increase in experiment B.

It will be noted that the increase in aldosterone secretion was not maintained steadily in each experiment. The reasons for this variability in adrenal output are not clear. Possibilities include: (1) the discharge and temporary exhaustion of preformed steroid or steroid precursor in the adrenal gland, (2) the operation of a homeostatic system in which the initial burst of steroid release induced suppression of a central exciting mechanism which in turn leads to a temporary suppression of adrenal output of steroid, or (3) failure of metabolic processes in the gland consequent to changes in oxygen tension of the blood or other sequellae of the procedure. There appeared to be no consistent correlation with adrenal blood flow.

The adrenal output of hydrocortisone was also increased as a consequence of the bleeding. This is seen particularly in experiments A and C; the small increase seen during the time interval 100-120 min. in experiment B may not be significant. It is of considerable interest that the relative increase in aldosterone secretion rate exceeded that of hydrocortisone in each instance. The maximum increases in hydrocortisone were 213 per cent, 111 per cent and 320 per cent in the same experiments, respectively, while that of aldosterone was 303 per cent. The pattern of response of hydrocortisone secretion was variable. There appeared to be no significant difference in hydrocortisone secretion as compared to experiments A, B and C in which the blood loss had not been replaced. In these volume replacement experiments the disparity of response patterns of aldosterone and hydrocortisone was again observed. In both experiments F and A a marked increase in aldosterone secretion was observed during the first 2 hours.

Steroid Secretion When Fluid Replacement Was Given. In experiments D and E the blood loss was replaced with twice the volume of dextran solution. Arterial blood pressure was maintained above 125 mm. Hg throughout experiment D and during the first 2 hours of experiment E. Aldosterone secretion in experiment D remained near the initial level until the third hour of the experiment when a modest increase occurred. In experiment E aldosterone secretion again seemed to be suppressed early in the experiment but rose in the third hour. In experiment F in which gelatin was infused, a marked increase in aldosterone secretion was observed during the first 2 hours.

The pattern of response of hydrocortisone secretion in these experiments was variable. There appeared to be no consistent correlation with adrenal blood flow. Other evidence has been accumulated in this laboratory which supports this concept.11

Electrolyte Changes During the Bleeding. The levels of serum sodium and potassium were determined during experiment C to establish whether a fall in serum sodium or rise in serum potassium occurred which stimulated the observed increase in aldosterone release according to the concept of regulation of aldosterone secretion by the adrenal cortex.
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dosterone secretion via blood electrolytes, or (2) whether the increased aldosterone secretion might itself bring about changes in serum electrolytes. Serum sodium and potassium and the hematocrit were determined on each dog at each time interval (fig. 3).

The electrolyte changes (table 3) provide no evidence that the increase in aldosterone secretion was associated with or was preceded by a fall in serum sodium or increase in serum potassium. On the average, a slight increase in serum sodium levels occurred due, in large part, to a marked rise in serum sodium occurring in 2 of the animals. In these animals (dogs 1 and 2) the serum sodium rose throughout the experiment to values of 176.1 and 183.5 mEq./L. The remaining 4 animals in this group failed to show a marked change in serum sodium, although the final level in animal no. 4 was considerably higher than the preceding values.

The markedly elevated serum sodium in dogs 1 and 2 is of special interest. In dog 1 the increase in serum sodium concentration was 25.2 mEq./L., and in dog 2 it was 37.9 mEq./L. It seems evident that this elevation in serum sodium concentration could have occurred as the result of addition of sodium to the extracellular fluid compartment without an iso-osmotic equivalent of water, or of the loss of water without a corresponding loss in sodium. Only a fraction of the increase can be accounted for by total renal tubular sodium reabsorption during the excretion of a normal volume of urine. The elevation of serum sodium may have been the result of a selective outflow of sodium from the intracellular compartment or from bone, perhaps as a result of increased blood levels of aldosterone. On the other hand, the passage of water from the interstitial compartment into cells, as suggested by Ashworth and Kregel, who also noted an elevation of serum sodium following acute blood loss, could also account for the enhanced serum sodium concentrations reported here. However, this is difficult to envision in the absence of change in hematocrit since the vascular compartment would be expected to share equally in the water redistribution with a consequent hemococoncentration. No significant change in the hematocrit was found in our bleeding experiments.

Progressive hemodilution was observed in the experiments in which blood loss was replaced by plasma expander. This indicated that a considerable fraction of the plasma substitute remained in the vascular system. The hematocrit was 17.1 on the average at the end of the experiments as compared to an initial average of 42. This observation is in agreement with that of Davis and co-workers, who found that approximately three fourths of dextran solution administered to hypovolemic dogs remained in the vascular system at the end of 4 hours. The hemodilution did not appear to interfere with steroid secretion. In experiments D and E, for example, the highest steroid secretion rates were seen at the end of the experiment at a time when hemodilution was the greatest.

The Nature of the Stimulus to Increased Aldosterone Secretion. The two physiologic parameters, change in serum electrolytes and total fluid volume, which have been suggested as regulators of aldosterone secretion do not appear to explain the increased aldosterone release found in these experiments. No change in the ratio of serum sodium to potassium was seen at a time when aldosterone secretion was increased threefold. Although replacement of

![Fig. 3. Hematocrit, serum sodium, serum potassium and aldosterone secretion rates during progressive blood loss (experiment C).](http://circres.ahajournals.org/doi/fig/10.1161/01.RES.36.3.610)
blood volume with an excess of plasma substitute appeared to prevent the increase of aldosterone secretion in one experiment, it merely delayed the response in a second and failed entirely to prevent the response in a third.

The actual mechanism whereby blood loss leads to enhanced aldosterone secretion remains obscure. Rauschkolb and Farrell have accumulated evidence that the secretion of aldosterone may be regulated by a diencephalic center. It will be of considerable interest to determine if reduction in blood pressure or blood flow through this region of the brain constitutes the effective stimulus to the increased aldosterone secretion during bleeding.

**SUMMARY**

Acute loss of blood induces a marked increase in adrenal aldosterone secretion accompanied by a less consistent increase in hydrocortisone secretion. The response is only partially prevented or delayed by infusion of plasma substitute. The response cannot be correlated with changes in serum electrolytes or total blood volume. The suggestion is made that alterations in cerebral hemodynamics may represent the stimulus to increased adrenal output of aldosterone.

**SUMMARIO IN INTERLINGUA**

Acute perdita de sanguine induce un marcate augmento del secretion de aldosterona adrenal accompaniate per un minus uniforme augmento del secretion de hydrocortisone. Le responsa es prevenibile solmente in parte per le infusion de substituto de plasma. Ilo non se correlaciona con alterationes del electrolytos serial o del total volumine de sanguine. Es sti-

**REFERENCES**

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