Decreased Resistance to Hemorrhage in Neurohypophysectomized Dogs

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With the technical assistance of Cleveland P. Hickman, Pfc, and John H. Waters, Pfc

The diabetes insipidus dog is more susceptible to hemorrhage than the normal dog as determined by a standardized hemorrhage procedure. When aqueous pitressin is administered in subpressor dosages, this susceptibility to hemorrhage is reversed to or toward normal. Aqueous pitressin does not increase the intact dog's resistance to hemorrhage. The foregoing observations are interpreted as demonstrating that the neurohypophysial pressor principle serves a physiologic role in maintaining blood pressure during hemorrhage.

The total extract of the pituitary gland was demonstrated to possess a potent pharmacodynamic pressor effect by Oliver and Schäfer in 1891. Subsequently, the pressor substance has been demonstrated to be associated with the total extract of the posterior lobe, the pitressin fraction of this extract, and specifically with the extraction of neurohypophysial tissue (pars nervosa and anterior hypothalamus). Du Vigneaud's recent studies support the concept that pitressin (vasopressin) is a discrete molecule which possesses both pressor and antidiuretic activity.

In spite of the foregoing identification of pitressin specifically with neurohypophysial tissue, no definite physiologic vasomotor role has been demonstrated for its pressor activity. No obvious vasomotor deficit has been found to follow neurohypophysial lesions, and no clinical entity has been described in which vascular responses to pitressin are of therapeutic value.

Nevertheless the probability remains that the neurohypophysial pressor principle plays an important physiologic role in animal economy. Krogh has reviewed the early work on this problem. The only suggestion of a therapeutic role in the realm of vasomotor activity has been the recent report by Zweifach that pitressin increased the effectiveness of whole blood transfusion in experimental hemorrhagic shock.

A comprehensive program is in progress in the laboratories of the Department designed to elucidate neurohypophysial functions. Impetus for the program was derived from the realization that functionally the neurohypophysis encompasses, in addition to the pars nervosa, the hypothalamus and/or tissue in its immediate environs. The program has gained momentum with the development of a surgical procedure which is consistent for executing a reasonably selective neurohypophysectomy.

Described below are experiments demonstrating that dogs which exhibit a well-defined diabetes insipidus (neurohypophysectomy) are more sensitive to hemorrhage than are normal dogs. Further, appropriate neurohypophysial substitution therapy (pitressin) re-establishes the resistance to hemorrhage in these preparations.

Experimental Procedures

A. Animals. Adult female dogs, ranging in weight from 9 to 15 Kg., were used; six were unoperated and seven the neurohypophysis was removed. In the operated dogs, hemorrhage experiments were done from two months to two years after the onset of permanent diabetes insipidus.

The animals were housed in a reasonably constant environmental temperature of 24 to 26 C. and maintained on a fixed daily diet consisting of 10 Gm. of Purina Laboratory Chow pellets and 10 Gm. of horse meat per kilogram.

B. Precipitation of Diabetes Insipidus—Neurohypophysectomy. Detailed descriptions of the surgical procedure utilized in precipitating experimental diabetes insipidus are given elsewhere.

In our experience it is necessary to impinge upon the ventral tuberal and ventral anterior hypothalamic, in addition to isolating the pars nervosa com-
ponent of the hypophysis, in order to achieve well-defined functional neurohypophysial deficiencies. Our criteria for having successfully achieved a neurohypophysectomy is the postoperative presence of (1) a well-defined and permanently persisting diabetes insipidus, (2) a marked reduction (50 per cent) in glomerular filtration rate, renal plasma flow and tubular maxima and (3), as described in this report, a decreased resistance to a standardized hemorrhage procedure. That these deficiencies are true neurohypophysial deficits are attested to by the fact that they are all reversed to normal by appropriate administration of neurohypophysial extraction products (replacement therapy). There are reasons, however, for suspecting that even though such preparations exhibit measurable deficiencies they are not totally lacking in the ability to elaborate the active neurohypophysial principles in remnantal amounts; thus, perhaps the neurohypophysectomies were in the near-total rather than the total category, or it may be that these principles are elaborated from non-neurohypophysial tissue in remnantal amounts.

The anatomic extirpation required for executing a functional neurohypophysectomy as delimited above conforms closely with the tissue from which neurohypophysial principles have been extracted. This area also possesses a characteristic vascularity and affinity for acid stains. The hypothalamic component of the area is where secretory neurons are predominantly located.

Subsequent to operation the animals were in good health and exhibited no gross clinical symptoms other than a well-defined diabetes insipidus. Preoperative and postoperative blood pressure determinations revealed no significant blood pressure changes as a result of the operation.

C. Hemorrhage Procedure. Dogs were not fed for at least 24 hours prior to the procedure. The femoral artery was exposed under local anesthesia and cannulated. Blood was drawn and blood pressure measured through this cannula. Pressures were measured using a Sanborn electromanometer and Visocardette.

Blood was withdrawn at the rate of 3 to 5 cc. per kilogram per 10 minutes by withdrawing 25 to 30 cc. of blood every five minutes. Blood pressure was recorded immediately prior to each blood withdrawal in order that transient falls would not confuse results. Thus, at least four minutes elapsed following blood withdrawal before pressures were taken. When mean blood pressure had fallen to approximately 40 mm. Hg, blood was reinfused. This procedure was repeated in each animal three to six times. An interval of at least two weeks elapsed between each hemorrhage. Hematocrit determinations and eosinophil counts were obtained prior to and during most experiments.

If the artery was exposed as far distally as palpable the same artery could be utilized at least three times. Gangrene or disability following this routine was never encountered in a total of over 20 dogs.

RESULTS

A. Resistance to Hemorrhage in Normal Dogs. Removal of 35 to 50 cc. of blood per kilogram was required to lower mean blood pressure below 70 mm. Hg* in normal dogs. The data for this group of experiments are presented in table 1. The observed values approximate those reported by other workers. In any given dog, however, the range was quite narrow during each of several repeated hemorrhage procedures performed at two-week intervals. Also, although not apparent from the tabulated data, the characteristics of blood pressure fall were consistent in individual dogs; that is, in some animals blood pressure fell precipitously, in others, gradually. Changes in pulse rate were variable but pulse rate tended to increase progressively during the bleeding procedure.

B. Hemorrhage in Diabetes Insipidus Dogs. Withdrawal of only 20 to 35 cc.† of blood per kilogram lowered mean blood pressure below 70 mm. Hg in diabetes insipidus dogs. The 16 experiments in which these results were obtained are tabulated in table 2. The changes in pulse rate and pulse contour, and the characteristics of blood pressure fall were essentially the same as observed in the normal dog. Note that two of these dogs had been bled preoperatively and had fallen well within the normal range at that time.

C. Aqueous Pitressin Therapy During Hemorrhage. In four experiments on normal dogs aqueous pitressin was given intra-arterially at 5 or 10 minute intervals during the hemorrhage (table 1). Pitressin was not started until at least 100 cc. of blood had been withdrawn. This management did not alter the response to hemorrhage which the dogs had manifested without pitressin therapy. In six experiments,

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* Seventy mm. mean is used throughout the tabulation of data for convenience. Actually blood pressure was lowered to 40 to 50 mm., and similar results and conclusions are obtained if 60 mm. is used as the arbitrary value.

† In one instance 40 cc. per kilogram were required. Repeated values in this dog were below 35. (Dog # 203 —Table 2.)
<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Wt. (Kg.)</th>
<th>No Therapy, cc./Kg.</th>
<th>On Pitressin Throughout, cc./Kg.</th>
<th>Terminal Pitressin Rx*, cc./Kg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/20 u</td>
<td>1/10 u</td>
<td>1/5 u</td>
</tr>
<tr>
<td>100</td>
<td>17</td>
<td>25.0 (1)</td>
<td>28.8 (2)</td>
<td>23.2 (3)</td>
</tr>
<tr>
<td>203</td>
<td>10</td>
<td>40.0 (1)</td>
<td>34.3 (2)</td>
<td>30.0 (3)</td>
</tr>
<tr>
<td>204</td>
<td>17.3</td>
<td>20.8 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>245</td>
<td>16.5</td>
<td>22.1 (1)</td>
<td>22.0 (2)</td>
<td></td>
</tr>
<tr>
<td>217</td>
<td>11.3</td>
<td>21.9 (1)</td>
<td>26.8 (2)</td>
<td>28.5 (4)</td>
</tr>
<tr>
<td>256‡</td>
<td>14</td>
<td>32.2 (1)</td>
<td>32.2 (2)</td>
<td></td>
</tr>
<tr>
<td>286‡</td>
<td>12.2</td>
<td>28.7 (1)</td>
<td>33.3 (3)</td>
<td></td>
</tr>
</tbody>
</table>

* In these experiments pitressin was given at 5 or 10 minute intervals in the amount indicated when mean blood pressure had fallen below 70 mm. Hg. Blood pressure was then maintained above this value until the amount of blood shown had been withdrawn.
† Represents order in which hemorrhage procedures were performed.
‡ Preoperative values of 37.3 (256) and 45 cc./Kg. (286) checked on two occasions.
TABLE 1.—Hemorrhage in Normal Dogs with and without Pitressin Therapy

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Wt. (Kg.)</th>
<th>Wt. (Kg.)</th>
<th>Blood Removal Required to Lower Mean Blood Pressure Below 70 mm. Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No Therapy, cc./Kg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/20 u</td>
</tr>
<tr>
<td>250</td>
<td>15.8</td>
<td>42.2</td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)*</td>
<td>(2)</td>
</tr>
<tr>
<td>262</td>
<td>12.2</td>
<td>45.0</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td>270</td>
<td>11.1</td>
<td>40.5</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>(2)</td>
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<td>275</td>
<td>10.1</td>
<td>34.6</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td>316</td>
<td>9.9</td>
<td>35.4</td>
<td>.005 u/Kg. X 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>348</td>
<td>11.0</td>
<td>47.7</td>
<td>.009 u/Kg. X 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
</tbody>
</table>

* Values parenthesized represent order in which hemorrhage experiments on any given dog were performed.
† Values represent the number of times the given pitressin dose was injected during the course of the hemorrhage experiment.

Aqueous pitressin (.05 to .20 u) was not given until mean blood pressure had fallen below 70 mm. Hg. Following this procedure, in no instance did pressure return to values above 70 mm.

In experiments on dogs with diabetes insipidus, pitressin was given at 5 or 10 minute intervals throughout the bleeding procedure in the same manner as in control animals (table 2). This procedure significantly altered hemorrhage response. In order to lower mean blood pressure below 70 mm. Hg, it was necessary to withdraw 5 to 15 cc. of blood per kilogram more from the dogs given pitressin than from dogs without benefit of pitressin.

In five additional experiments pitressin administration (.05 to .20 u) was delayed until mean blood pressure had fallen below 70 mm. Hg. The increase in blood pressure under these conditions was striking as compared with the absence of this response in the normal dogs. Blood pressure rise occurred despite continued hemorrhage and was sustained for variable intervals of continued bleeding (table 2, "Terminal Pitressin"). Pulse changes following pitressin therapy were inconsistent.

D. Noradrenaline Therapy. Infusion of noradrenaline (2 to 6 μg per 10 minutes) throughout the hemorrhage procedure in the dogs with diabetes insipidus caused no noteworthy change in the animals' response to hemorrhage (table 3). When noradrenaline was given in varying doses (2 to 20 μg) after mean blood pressure had fallen below 70 mm. Hg, pressure was increased only slightly and transiently. This was in marked contrast to the effects of pitressin in the hemorrhaged dog with diabetes insipidus.

E. Other Possible Factors that Might Increase Sensitivity to Hemorrhage in the Diabetes Insipidus Dog. Studies in this Laboratory and elsewhere have revealed no alterations in blood volume, mannitol space, plasma sodium or chloride in the dog with diabetes insipidus.
In three dogs, pitressin in oil was given several days prior to the hemorrhage procedure in sufficient dosage to restore fluid exchange to normal (5 units pitressin tannate intramuscularly). In two of these dogs pressure fell as it had in the untreated state; in the third, pressure was maintained slightly longer.

Urine output during hemorrhage of the dogs with diabetes insipidus was not excessive. Urine flow fell rapidly and total urine output during the entire procedure averaged 25 cc. of dilute urine.

Hematocrit readings were made during most experiments. No significant differences were noted between normal and experimental animals during hemorrhage.

Careful observations were made for evidence of adeno-hypophysial dysfunctions in the dogs with diabetes insipidus. No clinical abnormalities were noted. Insulin tolerance studies were within or near the normal range, there was no change in coat texture, and no significant changes were found in the adrenal or other endocrine glands at autopsy. Findings on this type of preparation are presented in detail elsewhere.

Pretreatment with cortisone of the dog with diabetes insipidus (25 mg. daily for seven days) did not alter the increased sensitivity to hemorrhage (table 3). If increased susceptibility to hemorrhage were a result of diminished corticotropin production by the anterior pituitary, cortisone therapy should be effective, as has been demonstrated in adrenalectomized dogs.

Eosinophil counts were performed prior to, during and following most of the hemorrhage experiments. Both normal dogs and dogs with diabetes insipidus had comparable falls ranging from 50 to 100 per cent. Maximum eosinopenia in two hypophysectomized dogs (ordinary, pars anterior and posterior lobes were removed) following hemorrhage was 12 per cent and 40 per cent.

**DISCUSSION**

The demonstrated increased sensitivity to a standardized bleeding procedure in the dog with diabetes insipidus and the reversal of this susceptibility by the administration of pitressin is compatible with the following interpretations. First, the increase in susceptibility to hemorrhage is due to a deficiency of the neurohypophysial pressor principle which normally enters the blood stream in appropriate amounts when a decrease in circulating blood volume is threatened. Second, when pitressin is appropriately administered it is efficacious in replacing the deficiency present in the neurohypophysectomized animal. Third, the presence of an excessive dosage of the pressor fraction does not increase resistance to hemorrhage beyond that which exists in the normal animal. Thus, in all probability there is present in the circulating blood of the normal animal an overabundance of the pressor principle at the time blood pressure falls. In other words, the fall in blood pressure occurs when the circulating pressor substance is no longer able to compensate for the progressive decrease in blood volume. The pitressin dosage used in these experiments approximated the quantities of pitressin O'Connor has estimated can be released during maximum dehydration.

Other possible interpretations of the data have been considered. Altered fluid or electrolyte levels might affect hemorrhage response. However, hematocrit, blood volume, extracellular fluid volume (mannitol space) and plasma electrolytes were within the normal range in the type of neurohypophysectomized dogs observed. The hematocrit readings during hemorrhage were similar in normal dogs and dogs with diabetes insipidus. Furthermore, pitressin in oil therapy, which restores fluid exchange to normal, had no influence on response to hemorrhage in two of three dogs in which this treatment was used. The amount of circulating pitressin provided in this manner (oil
depot) is not sufficient to delay hemorrhagic hypotension.

The possibility existed that the action of pitressin was not specific, its effects being similar to any pharmacodynamic pressor substance. To test this possibility, noradrenaline was infused throughout the hemorrhage procedure, and was found to have no influence on the increased susceptibility to hemorrhage in the dogs with diabetes insipidus. Further, when the mean blood pressure of untreated, hemorrhaged, normal dogs fell below 70 mm., injected pitressin had little or no effect on blood pressure. However, in four of five similarly treated dogs with diabetes insipidus, there was significant blood pressure elevation after comparable pitressin doses and no beneficial response to noradrenaline in these circumstances.

The question arises as to the possibility of associated anterior hypophysial damage being responsible for the increased susceptibility to hemorrhage in this type of preparation. It has been repeatedly verified that the type of animal utilized exhibits no obvious adenohypophysial dysfunction. There is no marked sensitivity to insulin; eosinophil responses to surgery and hemorrhage remain within the normal range; the coat does not change; sex functions are retained in instances and pancreatectomy precipitates a full-blown diabetes mellitus. Further, cortisone therapy did not influence hemorrhage susceptibility in these preparations (table 3), whereas it is known that this treatment increases the resistance to hemorrhage in the adrenalectomized dog.16 It is conceivable and probable, however, that slight adenohypophysial dysfunction exists in an occasional preparation.20 The inability to return hemorrhage response completely to normal in two of the six dogs with diabetes insipidus may be related to such a factor although it is also possible that this was a matter of inappropriate pitressin dosage. Finally, the therapeutic effect of aqueous pitressin in the dog with diabetes insipidus is inexplicable if one assumes the deficit to be solely adenohypophysial.

**Summary and Conclusions**

The dog with diabetes insipidus is more susceptible to hemorrhage than the normal dog as determined by a standardized hemorrhage procedure.

When aqueous pitressin is administered in subpressor dosages, this susceptibility to hemorrhage is reversed to or toward normal.

Aqueous pitressin does not increase the intact dog's resistance to hemorrhage.

The foregoing observations are interpreted as demonstrating that the neurohypophysial pressor principle serves a physiologic role in maintaining blood pressure during hemorrhage.

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