Relationship of Appearance of Abnormal Plasma Hemin Pigment to Development of Irreversible Hemorrhagic Shock in Dogs

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Irreversible hemorrhagic shock in dogs was associated with a progressive rise in plasma hemoglobin levels and the appearance of an abnormal hemin pigment in the plasma. Both findings were related to mucosal necrosis of the bowel occurring as a result of prolonged hemorrhagic shock. In dogs perfused through the superior mesenteric artery during hemorrhagic shock, the bowel remained normal, irreversibility was prevented and the abnormal plasma findings did not occur.

In recent studies we showed that irreversibility of hemorrhagic shock could be prevented in almost all normal or Eck-fistula dogs by cross-perfusing the bowel via the superior mesenteric artery with donor arterial blood. The perfusion apparently prevented critical bowel ischemia with resultant mucosal necrosis. Irreversibility was not prevented by similar perfusion of an equal amount of donor arterial blood into the inferior vena cava or the lower abdominal aorta during shock. Furthermore, irreversibility could not be consistently prevented in other groups of dogs by perfusing the liver either via the celiac axis or via the portal system, or by perfusing the brain via a carotid artery.

A constant finding in all dogs dying of irreversible hemorrhagic shock, whether perfused or not, was increasing opacity of the plasma in the period following retransfusion. Just before death the plasma in some dogs was almost black. Plasma hemoglobin levels increased markedly during this period and this apparently accounted for the color change. Moreover, the same abnormal hemin pigment found by Nemir et al. in “black” peritoneal fluid and plasma of dogs dying of strangulated obstruction of the small bowel was found to be present in the plasma of dogs dying of irreversible hemorrhagic shock. This pigment could be identified by its characteristic spectral absorption curve. The analysis of these findings forms the basis for this report.

Methods

Mongrel dogs sedated with morphine sulfate (2 mg./Kg. subcutaneously) were bled according to our modification of the Lamson-Fine technic. When the dogs had reached a sustained mean arterial pressure of 35 mm. Hg, varying types of cross-perfusion were initiated with arterial blood from an unanesthetized donor dog and a double Sigmamotor pump. Following a 4½ to 5 hour period in shock, the perfusions were stopped and all shed blood was returned to the shocked dog.

Prior to the experiments, and within ½ hour after retransfusion, plasma hemoglobin levels were measured in the dog with shock by the method of Crosby and Furth. Three and 7 hours following retransfusion plasma hemoglobins from the previous dog with shock were again measured. A final plasma hemoglobin determination was made at 24 hours after the transfusion if the dog was still alive. At the same time plasma hemoglobin was again measured and serum bilirubin determinations were made by the Ducci-Watson modification of the Malloy-Evelyn technic. At similar intervals, blood samples were also taken for determinations of the plasma spectral absorption curve according to the method of Nemir et al. Following centrifugation, samples of plasma were treated with NaCN, and of plasma
IRREVERSIBLE HEMORRHAGIC SHOCK

TABLE 1.—Plasma Hemoglobin in Hemorrhagic Shock*

<table>
<thead>
<tr>
<th>Type of perfusion</th>
<th>Number of dogs</th>
<th>Number of survivors</th>
<th>Hours to death after retransfusion</th>
<th>No. dogs studied</th>
<th>Plasma hemoglobin in mg %</th>
<th>Post-retransfusion (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pre-hemorrhage</td>
<td>3</td>
</tr>
<tr>
<td>I. Superior Mesenteric</td>
<td>30</td>
<td>27</td>
<td>—</td>
<td>15</td>
<td>Normal†‡</td>
<td>17.5</td>
</tr>
<tr>
<td>II. Superior Mesenteric Eck fistula</td>
<td>10</td>
<td>9</td>
<td>—</td>
<td>2</td>
<td>Normal†‡</td>
<td>20.5</td>
</tr>
<tr>
<td>III. Inferior Vena Caval</td>
<td>10</td>
<td>2</td>
<td>5.86 = 3.1</td>
<td>1</td>
<td>Normal†‡</td>
<td>17.3</td>
</tr>
<tr>
<td>IV. Inferior Vena Caval Eck fistula</td>
<td>3</td>
<td>0</td>
<td>17.5 = 6.7</td>
<td>3</td>
<td>Normal†‡</td>
<td>20.8</td>
</tr>
<tr>
<td>V. Aortic</td>
<td>10</td>
<td>2</td>
<td>7.03 = 6.1</td>
<td>5</td>
<td>Normal†‡</td>
<td>23.2</td>
</tr>
<tr>
<td>VI. Celiac</td>
<td>10</td>
<td>4</td>
<td>12.5 = 6.8</td>
<td>5</td>
<td>Normal†‡</td>
<td>28.5</td>
</tr>
<tr>
<td>VII. Portal</td>
<td>10</td>
<td>3</td>
<td>9.64 = 4.5</td>
<td>8</td>
<td>Normal†‡</td>
<td>23.2</td>
</tr>
<tr>
<td>VIII. Carotid</td>
<td>10</td>
<td>2</td>
<td>26.2 = 4.0</td>
<td>6</td>
<td>Normal†‡</td>
<td>28.5</td>
</tr>
<tr>
<td>IX. None</td>
<td>15</td>
<td>1</td>
<td>6.39 = 3.9</td>
<td>7</td>
<td>Normal†‡</td>
<td>41.3</td>
</tr>
<tr>
<td>X. None Eck fistula</td>
<td>8</td>
<td>0</td>
<td>18.5 = 9.1</td>
<td>6</td>
<td>Normal†‡</td>
<td>18.7</td>
</tr>
</tbody>
</table>

* Mean values with standard deviations.
† Survived 72 hours or more.
‡ In some instances the prehemorrhagic plasma specimen was noted to be colorless indicating a normal plasma hemoglobin and the quantitative determination was therefore omitted.

RESULTS

Table 1 summarizes the results obtained in the various hemorrhagic shock experiments. The analysis of plasma hemoglobin was not started until midway through this study.

Almost all dogs in groups I and II survived (table 1). Plasma hemoglobin reached a peak in these dogs just after retransfusion and then dropped to a normal level by the following day. In all other groups, plasma hemoglobin continued to increase with the passage of time following retransfusion and reached a peak just before death. Nonperfused dogs in groups IX and X also showed increasing plasma hemoglobin levels following retransfusion, indicating that the hemolysis was not entirely due to damage to red blood cells by the Sigmamotor pump used in the perfusion experiments. Dogs in groups VIII and X had the longest survival of any of the
FIG. 1 Top. Normal plasma spectrum 8 hours following retransfusion in a dog perfused via the superior mesenteric artery.

FIG. 2 Bottom. Abnormal plasma spectrum 8 hours following retransfusion and just prior to death in a dog perfused via the inferior vena cava.

dogs which went into irreversible shock, and with the exception of dogs in group IX, correspondingly had the highest plasma hemoglobin concentration prior to death. Non-perfused normal dogs (group IX) had the highest plasma hemoglobin levels prior to death and at autopsy had the most marked congestion and necrosis in the small and large bowel.

In a few experiments blood samples were taken for plasma hemoglobin determination from the shocked dog and from the blood reservoir prior to retransfusion. In all such cases the values were less than those observed after retransfusion.

**Bilirubin.** Serum bilirubin determinations were not found to be abnormal in any of the dogs in any of the groups, whether in irreversible shock or not, indicating that the plasma color change was wholly due to hemoglobin and its products, or to some unknown colored substance.

**Abnormal Hemin Pigment.** Figure 1 is a normal spectral absorption curve of plasma taken from a bowel-perfused dog 8 hours following retransfusion. Figure 2 is an abnormal spectral absorption curve of plasma taken from a dog dying of irreversible hemorrhagic shock just prior to death.

The spectral characteristics of the plasma sample treated with Na$_2$S$_2$O$_4$ are similar to those of the abnormal hemin pigment found by Xemir et al. in the peritoneal fluid and plasma of dogs following intestinal strangulation. In almost all cases, an abnormal spectral absorption curve was found in the plasma samples obtained just prior to death of dogs in irreversible shock. Samples taken earlier than this showed a transition from a normal to an abnormal spectral absorption curve.

**Clinical Course.** Dogs dying of irreversible hemorrhagic shock followed a typical course following retransfusion. There was a temporary return of blood pressure to normal but the dogs remained lethargic. Coincident with the onset of bloody diarrhea, the blood pressure again declined to shock levels and death usually occurred in 8 hours or less following retransfusion. Bloody diarrhea, the cardinal clinical sign of irreversibility, continued unabated until death.

**Autopsy.** At autopsy, the principal lesions present were severe congestion and extensive mucosal necrosis both in the small and large bowel. The lumen of the bowel was filled with black bloody fluid. The liver usually was congested. The peritoneal fluid was normal in color and amount.

**Effect of Bowel Perfusion via Superior Mesenteric Artery During Hemorrhagic Shock.** This procedure prevented irreversible shock following retransfusion. The dogs so treated became increasingly alert and did not develop bloody diarrhea. The day following the experiment, they were able to eat and drink. When sacrificed at varying periods following an experiment, these dogs had congested livers but their bowels appeared to be normal or slightly congested.

**Source of Increased Plasma Hemoglobin.** The source of the increasing amounts of plasma hemoglobin in dogs in irreversible hemorrhagic shock apparently lies in the damaged
bowel. During the prolonged hypotension the bowel mucosa apparently suffers critical ischemia leading to necrosis. With retransfusion, bloodflow to the bowel is temporarily increased above normal levels. The injured and necrotic mucosa becomes congested and some blood leaks out into the lumen of the bowel where it is hemolyzed by digestive enzymes and bacteria. Much of this blood leaves the bowel as bloody diarrhea, but some is also reabsorbed through the damaged mucosa. This is what causes the progressive increase in plasma hemoglobin concentration.

The hemin pigment with the abnormal spectral absorption curve has been shown by Nemir and Drabkin to be produced by the action of trypsin on erythrocytes. Although the presence in the blood stream of this abnormal pigment is associated with a moribund condition of the dog in both irreversible hemorrhagic shock and strangulation obstruction, there is as yet no evidence that the pigment is toxic in itself. However, it is very likely that along with the absorption of this pigment, other more toxic products are also being absorbed from the necrotic bowel.

**Summary**

Irreversible hemorrhagic shock in dogs was associated with a progressive rise in plasma hemoglobin levels and the appearance of an abnormal hemin pigment in the plasma prior to death. Both findings were apparently related to the development of mucosal congestion and necrosis of the bowel which occur during prolonged hemorrhagic shock. In dogs perfused through the superior mesenteric artery during hemorrhagic shock, the bowel remained normal, irreversibility was prevented and the abnormal plasma findings did not occur.

**Acknowledgment**

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**REFERENCES**

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