The Reservoir Function of the Spleen and Its Relation to Postsplenectomy Anemia in the Dog

By NICOLAS ARIAS ELENES, ROGER A. EWALD AND WILLIAM H. CROSBY

Following removal of the spleen from normal dogs anemia develops and becomes maximal 3 to 12 weeks after the splenectomy. Thereafter the red cell mass returns to normal in approximately six months. During the anemia the plasma iron turnover is reduced, reflecting diminished hemoglobin production, and the red cell life span is normal. The anemia, apparently, is due to reduction of erythropoiesis. The mechanism of this change in marrow activity and its relation to the absence of the spleen are not known.

When a normal dog is awake and excited or under the influence of epinephrine the spleen is tightly contracted and contains little blood. On the other hand, when the dog is resting, asleep or under the influence of barbiturates, his spleen becomes greatly enlarged and filled with red cells. As much as 30 per cent of the total red cell mass may be sequestered in the spleen and temporarily excluded from active circulation. The venous hematocrit is considerably reduced so that the animal's blood presents the picture of anemia. Contraction of the spleen quickly corrects the picture. The homeostatic system which controls erythropoiesis, and thereby the size of the red cell mass, evidently does not respond to the temporary anemia: it seems to recognize as "normal" the small volume of cells which circulates during sleep. If there were a stimulus to erythropoiesis during sleep when the animal was aroused and his spleen contracted the red cell mass would be larger than "normal." It has been suggested that this may be the basis of the anemia which gradually develops post-splenectomy. The splenic pool of red cells is no longer available to augment the normal mass during waking hours, and as a consequence the circulating red cell mass becomes gradually reduced to that size which is recognized as normal by the animal's homeostatic system.

This report describes the manner in which this hypothesis was challenged. The results indicate that the reservoir function of the spleen is not involved in postsplenectomy anemia of the dog.

Material and Methods

Eight healthy mongrel dogs weighing 11 to 18 Kg. and with initial hematocrits of 40 or above were selected. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. Examination of blood smears revealed no evidence of Hemobartonella at any time during the experiment.

In five of the dogs, a sterile heavy nylon net was snugly sutured around the spleen (fig. 1). Under pentobarbital anesthesia (30 mg./Kg.) a midline laparotomy was performed, the spleen identified and carefully lifted from the peritoneal cavity. All vessels

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Fig. 1.—Contracted dog spleen completely enshrouded in a tightly sutured nylon net. Note that portions of the net were passed between the vessels of the pedicle to avoid compromising the splenic blood supply.

of the pedicle were identified and isolated. Strips cut in one portion of a rectangular sheet of net (cold sterilized with zepharin) were passed between the vessels of the pedicle and sutured together. At this time an intravenous injection of epinephrine (1 ml. 1:1000) was given. After the spleen had completely contracted, the remaining portion of the net was tightly wrapped about the spleen and the free edges sutured together with 4-0 silk. No sutures were placed in the splenic capsule or tissue of the pedicle. Excess net was carefully trimmed. The entire spleen was thus encased within a single layer of nylon net without compromise of the splenic blood supply.

The remaining three animals† underwent a sham operation which consisted of laparotomy, handling of the spleen, removal of 30 Gm. of omental tissue and of 30 ml. of blood.

In both groups, before the initial surgery and throughout the experiment the following tests were performed repeatedly: microhematocrits by the method of Strumia,5 platelet and reticulocyte counts by the methods of Brecher6,7 and leukocyte counts by the standard procedure. Red cell mass determinations were carried out by the Cr51 method as described by Sterling.8 In these studies 15 ml. of whole blood was mixed with 10 μc. of Cr51 in ACD solution* for 45 minutes at room temperature. The tagging reaction was stopped by the addition of 100 mg. of ascorbic acid. The Cr51 activity of the injected sample was 6 to 8 μc.

Samples were taken at 20 and 30 minutes after injection; in the splenectomized dogs the first sample was taken at 10 minutes because of decreased mixing times. Radioactivity of the blood was measured in a Packard Auto Gamma Crystal Scintillation counter.

The t test for small samples of paired observations9 was employed in the statistical analysis.

All dogs were splenectomized two months after the initial surgery.

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*100 per cent nylon net, Jacknit Mills, 108 W. 39th Street, New York, New York.
†Only two sham animals completed the experiment. The third dog (No. 48X) escaped from the kennels on day 40.
RESERVOIR FUNCTION OF THE SPLEEN

RESULTS

Four of the five dogs with spleens enshrouded in the nylon nets did not develop anemia during the 9 weeks after their surgery. Neither did the sham-operated dogs (table 1). To ascertain if the nylon nets effectively prevented splenic expansion during sleep the following study was conducted during the fifth week: duplicate hematocrit determinations were made with the dogs awake, while anesthetized with intravenous sodium pentobarbital (Nembutal, 30 mg./Kg.), and after intravenous injection of 1 ml. epinephrine (1:1000). Significant changes in the awake versus asleep hematocrit values of the sham group (p < .05) compared to the net group (p > .05) indicated that the nets successfully inhibited the reservoir function of the spleen (table 2). A similar study performed after splenectomy revealed no significant hematocrit changes in either group.

The increased hematocrit values following epinephrine injection (table 2) were attributed to complete splenic contraction and emptying of the splanchic bed. As a group, there were no significant (p > .05) changes in the awake versus epinephrine hematocrit values.

On the sixty-fourth day splenectomy was performed in both groups of dogs. During the operation the effectiveness of the nets to inhibit splenic changes was again challenged. The absence of significant changes in splenic size following pentobarbital and epinephrine was visually confirmed. The spleens were not removed until after the epinephrine had time to be effective. The netted spleens, which had not changed size, weighed considerably less than spleens removed from normal dogs under nembutal anesthesia with no epinephrine (table 3).

The nets had provoked little or no reaction in the abdomen (figure 2) and examination of histological sections of the spleens revealed only moderate thickening and fibrosis of the splenic capsule with occasional foci of mononuclear cells. In both groups of animals sections of splenic parenchyma were essentially unremarkable.

Post-splenectomy all dogs developed a reduction of the red cell mass (table 1). The onset of the anemia and the period of recovery varied. Blood cultures and smears from the splenectomized animals were negative for Hemobartonella.

Mild leukocytosis and reticulocytosis were evident in all the dogs after splenectomy. The increase in reticulocytes was maximal just prior to the onset of recovery from anemia. Thrombocytosis was not a consistent finding and there was a wide variation in platelet counts from animal to animal, in the same animal from day to day, and between the two groups.

*Ten ml. A-C-D Solution Modified (Squibb).
†One dog (No. X44) developed infection in the surgical wound and became anemic. At the time of splenectomy the nylon-encased spleen was heavily adherent especially to the liver and colon. Attempts to remove the spleen were unsuccessful and the animal was killed.
Table 1.—Results of the Red Cell Mass Determinations (ml./Kg. body weight)

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Group</th>
<th>Baseline</th>
<th>Days</th>
<th>0</th>
<th>21</th>
<th>60</th>
<th>64</th>
<th>84</th>
<th>134</th>
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<tbody>
<tr>
<td>Y39</td>
<td>Sham</td>
<td>34.0</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4X7</td>
<td>Sham</td>
<td>32.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1W4</td>
<td>Net</td>
<td>28.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24X</td>
<td>Net</td>
<td>32.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X63</td>
<td>Net</td>
<td>30.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>97X†</td>
<td>Net</td>
<td>33.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>33.9</td>
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<td>37.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Performed on day 100.
†Expired postsplenectomy during hematocrit change study (nembutal anesthesia).
‡This animal developed a postoperative peritonitis and showed a decreased red cell mass on days 21 and 69.

Table 2.—Peripheral Hematocrit Changes Following Injections of Nembutal and Epinephrine

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Group</th>
<th>Awake</th>
<th>Asleep</th>
<th>Epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y39</td>
<td>Sham</td>
<td>43.5</td>
<td>38.0</td>
<td>44.5</td>
</tr>
<tr>
<td>4X7</td>
<td>Sham</td>
<td>41.5</td>
<td>35.5</td>
<td>49.0</td>
</tr>
<tr>
<td>45X</td>
<td>Sham</td>
<td>43.5</td>
<td>32.8</td>
<td>41.0</td>
</tr>
<tr>
<td>1W4</td>
<td>Net</td>
<td>39.5</td>
<td>37.0</td>
<td>43.5</td>
</tr>
<tr>
<td>24X</td>
<td>Net</td>
<td>45.0</td>
<td>42.0</td>
<td>44.0</td>
</tr>
<tr>
<td>97X</td>
<td>Net</td>
<td>38.5</td>
<td>37.5</td>
<td>38.5</td>
</tr>
<tr>
<td>X44</td>
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<td>40.5</td>
<td>39.3</td>
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<tr>
<td>X63</td>
<td>Net</td>
<td>39.8</td>
<td>38.0</td>
<td>42.5</td>
</tr>
</tbody>
</table>

*Mean values.

Table 3.—Weights of Netted and Normal Spleens Removed during Nembutal Anesthesia

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Body Weight (Kg.)</th>
<th>Spleen (Gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Net</td>
<td>11.0</td>
<td>61.0</td>
</tr>
<tr>
<td>2 Net</td>
<td>10.0</td>
<td>33.7</td>
</tr>
<tr>
<td>3 Normal</td>
<td>11.4</td>
<td>200.0</td>
</tr>
<tr>
<td>4 Normal</td>
<td>10.5</td>
<td>197.0</td>
</tr>
</tbody>
</table>

The spleens were initially weighed with pedicle clamps in place to prevent loss of pooled blood. The above figures represent gross splenic weights minus the weights of the surgical clamps, the pedicle, and the omental adhesions.

**DISCUSSION**

The results of this study have demonstrated that the reservoir function of the spleen is not involved in postsplenectomy anemia of the dog. Dogs with intact spleens which were prevented from functioning as blood reservoirs did not develop anemia; however, following removal of these spleens, typical postsplenectomy anemia was observed. The canine spleen is not an erythropoietic organ so its removal does not diminish the volume of erythropoietic tissue. However, the spleen may be a source of erythropoietin, or similar substances, in the dog, and its removal could produce a temporary aregenerative anemia which is not corrected until other sources of erythropoietin increase sufficiently to compensate for loss of the spleen.
The results of this experiment leave unresolved the problem of the homeostatic control of the red cell mass in animals with spleens possessing a large reservoir function. In such animals, what is the "normal" circulating red cell mass?

**Summary**

Nylon nets sutured around intact dog spleens mechanically prevented them from functioning as red cell reservoirs. Failure of the animals to develop anemia indicates that loss of the reservoir function of the spleen is not involved in the postsplenectomy anemia of the dog.

**Summario in Interlingua**

Retes de nylon que esseva suturate circum intacte splenes canin preveniva los mechanicamente de funcionar como reservoirs de erythrocytos. Le facto que le animales non disveloppava anemia indica que le perdita del function del splen como reservoir non es interessate in le anemia post splenectomia in le can.

**ACKNOWLEDGMENT**

The technical assistance of M/Sgt. Allen A. Young is gratefully acknowledged.

**REFERENCES**

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