The Effect of Splenectomy on Erythropoiesis in the Dog

By Thomas A. Waldmann, Sherman M. Weissman and Nathaniel Berlin

The role of the spleen in the control of the production and destruction of red blood cells has long been a subject of interest. In 1841 Bardeleben published the results of the first experiments directed toward elucidating the role of the spleen in erythropoiesis. He noted a reduction in the red count, an increase in the white count and hyperplasia of the marrow following splenectomy. In 1932, Krumhhaar was able to list 75 publications on 15 species, reporting the hematologic response following removal of the normal spleen. In 55 of the studies, a significant but temporary reduction in hemoglobin and red count following splenectomy was reported, while in 20 studies no anemia was observed. The mean life span of the red cell following splenectomy has been reported as normal or shortened due to random destruction of red cells.

These studies have been difficult to evaluate as they were frequently inadequately controlled and did not utilize the technics now available for the quantitation of erythropoiesis.

This report presents data on the effect of splenectomy in the dog on the blood volume, red cell life span and the rate of red cell synthesis.

METHODS

Red cell production and destruction were estimated in 8 adult mongrel dogs during the 6 months preceding and following splenectomy, and in 3 dogs before and after a sham operation. Adult mongrel dogs of both sexes ranging in weight from 13 to 35 Kg. were used. All were inoculated against distemper, rabies and canine hepatitis and were dewormed with butyl chloride. No gastrointestinal parasites were noted at the completion of the study. No evidence of Bartonella infection was detected on periodic postoperative examinations of blood smears nor in blood cultures on blood agar or Huntines special media for Bartonella. The animals were well and maintained approximately constant weight throughout the year of study. The operations were performed under sodium pentobarbital anesthesia utilizing conventional surgical technics. For the sham operation a laparotomy was performed, and approximately 30 Gm. of omental tissue was removed. In addition 50 ml of blood were withdrawn from a peripheral vein. All animals were given prophylactically 1 Gm. of chlorotetracycline orally per day for 3 months following the surgical procedure.

Peripheral blood counts were performed weekly. Microhematocrits were performed according to the method of Strumia. Reticulocytes and platelets were counted by the methods of Brecher et al.

No change in the number of siderocytes stained according to the method of Dacie were observed. The plasma iron concentration was determined by the method of Bothwell and Mallett.

ISOTOPIC METHODS

The isotopic methods have been previously described in detail.

The total red cell volume and apparent red cell half life were estimated with Cr-labeled

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autologous red cells during the control period, and 3, 6, 12 and 25 weeks following splenectomy. Fifteen ml. of heparinized blood from the dog to be studied were incubated with 100 
\( \mu \)c. of Cr\(^{51} \) as sodium chromate for 45 minutes at room temperature. The cells were then washed three times with ACD saline and injected utilizing a calibrated syringe. Blood samples drawn at 15 minutes, 2 hours and twice weekly for 35 days, were counted in a well-type scintillation counter. Cr\(^{51} \) red cell survival curves were plotted on the basis of the counts per milliliter of red blood cells, computed from the hematocrit and whole blood radioactivity. The first part of the Cr\(^{51} \) red cell survival curves was fitted to a straight line on semilogarithmic paper, and the apparent half-times were estimated from the period 2 to

25 days without correction for Cr\(^{51} \) elution.

The red cell life span was estimated following the intravenous administration of 100 
\( \mu \)c. of 2-C\(^{14} \) glycine during the control period and in the period 6 weeks to 6 months following splenectomy. Blood samples were drawn at weekly intervals for 150 days. Extracted hemoglobin was combusted to carbon dioxide, suspended as barium carbonate in a thixotropic gel and counted in a liquid scintillation spectrometer by the method of Nathan et al.\(^{12} \) The mean life span of red cells by the Carbon 14 method was taken as the time between the administration of the isotope and the inflection point on the descending limb of the hemoglobin specific activity curve. Chromium elution rates were calculated according to the formula of Stohlman and Schniederman.\(^{13} \)

The plasma and red cell iron turnovers were measured in 8 dogs during the control period and at 6 to 12 weeks post-splenectomy. Five to 10 ml. of autologous plasma were incubated with 10 \( \mu \)c. of Fe\(^{59} \) as ferrous citrate for 30 minutes at room temperature. Plasma samples were obtained for radioassay at 15 minute intervals during the first hour, and at approximately 2, 3 and 4 hours. Whole blood samples were drawn on day 1, 3, 7, 11 and biweekly thereafter and counted for Fe\(^{59} \) until a plateau in red cell radioactivity had been reached. Samples containing both radioiron and radiochromium were differentiated in a single channel gamma-ray spectrometer. The plasma radioiron disappearance curves were fitted to a single exponential. The plasma and red cell iron turnovers were calculated according to the single dynamic pool model of Huff et al.\(^{14} \)

**RESULTS**

**Platelets.**—The mean platelet count for the 8 splenectomized dogs increased from the preoperative level of 320,000/cu.mm. to a maximal level by the third week of 740,000/cu.mm. (fig. 1). The mean platelet count persisted at least 75 per cent over control values for the 16 weeks of observation.

No significant change in the platelet count was noted in the 3 dogs given sham operations and 5 additional dogs undergoing other major surgical procedures.

**White blood count.**—The mean of 12 determinations per dog prior to splenectomy was 15.2 \( \pm \) 1.3 \( \times \) 10\(^3 \)/cu.mm., while the mean of 11 determinations per dog, 1 to 15 weeks post-splenectomy was 16.6 \( \pm \) 1.4 \( \times \) 10\(^3 \)/cu.mm.

**Hematocrit.**—The mean hematocrit during the control period was 48 \( \pm \) 3.

The mean hematocrit fell gradually to 78 per cent of that of the control period by 6 to 8 weeks post-splenectomy. The mean hematocrit remained at least 15 per cent below the control values throughout the 6 months post- splenectomy. Following the sham operation, the mean hematocrit of the 3 dogs fell transiently, but returned to control values by the third to fourth week (fig. 2).

**Blood volume.**—The total circulating red cell volume decreased 31 per cent from the mean control value of 38 \( \pm \) 3 ml./Kg. to 26 \( \pm \) 2 ml./Kg. by the twelfth week following splenectomy persisting at this level for at least the remaining 3 months of the study (table 1). There was a concomitant persistent
Fig. 1. (at top).—The effect of splenectomy on the platelet count. The mean platelet count of 8 dogs given sham operations, shown with the solid line, and the mean platelet count of 8 splenectomized dogs, shown with the dashed line, are plotted as the per cent of the control value. The control value is taken as the mean of 10 preoperative determinations for each dog. The vertical bars represent one standard deviation.

Fig. 2 (at bottom).—The effect of splenectomy on the venous hematocrit. The mean hematocrit of 3 dogs given sham operations, shown with the solid line, and the mean hematocrit of 8 splenectomized dogs, shown with the dashed line, are plotted as the per cent of the control value. The control value is taken as the mean of 10 preoperative determinations for each dog. The vertical bars represent one standard deviation.

16 to 18 per cent reduction in the plasma volume from 47 ± 3 to 38 ± 2 ml./Kg. by the twelfth week post-splenectomy. Following splenectomy the extrapolation of Fe$^{59}$ disappearance curves gave values for the plasma volume 7 per cent higher than those obtained with Cr$^{51}$-labeled cells, while in the normal dog
there was no significant difference in the estimates of the plasma volume by these two methods.

No significant difference in the total circulating red cell volume or plasma volume was observed in the dogs given sham operations between the determinations made during the control period and those performed 4 weeks following surgery.

Red cell life span.—There was no significant alteration from control values in the apparent red cell life span studied by the autologous Cr\(^{51}\)-labeled cell technic 4 weeks post-sham-operation, and in 13 determinations performed at 3, 6, 12 or 25 weeks post-splenectomy. The mean red cell life span determined with Cr\(^{51}\)-labeled glycine was 96 ± 4 days following splenectomy, compared to 103 ± 4 days during the control period (table 2).

A minor component of random red cell destruction up to 0.2 per cent per day was evident in the Cr\(^{51}\) curves of two of the dogs, both before and after splenectomy. No significant difference was observed in the fraction of cells destroyed by random mechanisms in the post-splenectomy period compared to the control period. The rate of Cr\(^{51}\) elution from the red cells was comparable in the pre- and post-splenectomy period, 1.8 per cent/ per day compared to 1.6 per cent/ per day.

RBC synthesis.—The mean plasma radioiron disappearance T\(_{1/2}\) determined before splenectomy was 74 minutes compared to 72 minutes after splenectomy (table 3).

Following splenectomy, the mean plasma iron turnover was 0.47 mg./Kg./per day, 32 per cent below the control value of 0.69 mg./Kg./per day. The red cell iron turnover was 0.36 mg./Kg./per day, 37 per cent below the control value of 0.57 mg./Kg./per day, suggesting a comparable reduction in red cell synthesis.

### Table 1.—The Effect of Splenectomy on the Total Red Cell and Plasma Volume

<table>
<thead>
<tr>
<th>Period</th>
<th>Sham Operations (3 dogs)</th>
<th>Splenectomy (8 dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 4 weeks P.O.*</td>
<td>Control 3 weeks P.O.</td>
</tr>
<tr>
<td>C(^{51}) RBC Vol.</td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td>ml. Kg.</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>Percentage change</td>
<td>—</td>
<td>+5</td>
</tr>
<tr>
<td>Plasma Vol.</td>
<td>—</td>
<td>+4</td>
</tr>
</tbody>
</table>

P.O. = postoperative.

### Table 2.—The Effect of Splenectomy on the Red Cell Life Span

<table>
<thead>
<tr>
<th></th>
<th>Cr(^{51}) T(_{1/2}) (days)</th>
<th>C(^{51}) mean red cell life span (days)</th>
<th>Cr(^{51}) elution (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24 ± 3</td>
<td>103 ± 4</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Post-sham-operation</td>
<td>24</td>
<td>96 ± 4</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Post-splenectomy</td>
<td>25 ± 2</td>
<td>96 ± 4</td>
<td>1.6 ± 0.3</td>
</tr>
</tbody>
</table>
Table 3.—The Effect of Splenectomy on Erythrocyte Iron Turnover

<table>
<thead>
<tr>
<th>Pre-splenectomy</th>
<th>Post-splenectomy</th>
<th>Percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron (μg.%)</td>
<td>105 ± 20</td>
<td>87 ± 17</td>
</tr>
<tr>
<td>Plasma volume (ml./Kg.)</td>
<td>47 ± 3</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Plasma Fe²⁺ disappearance (T½; min.)</td>
<td>74 ± 10</td>
<td>72 ± 13</td>
</tr>
<tr>
<td>Per cent incorporation of Fe²⁺ in RBC</td>
<td>82 ± 4</td>
<td>77 ± 7</td>
</tr>
<tr>
<td>Plasma iron turnover (mg./Kg./day)</td>
<td>0.69 ± 0.08</td>
<td>0.47 ± 0.11</td>
</tr>
<tr>
<td>RBC iron turnover (mg./Kg./day)</td>
<td>0.57 ± 0.06</td>
<td>0.36 ± 0.09</td>
</tr>
</tbody>
</table>

The rate of red cell synthesis may also be estimated during the steady state from the mean red cell life span and the total red cell volume. The data show a 25 per cent decrease in red cell synthesis from 0.37 ml./Kg./per day to 0.28 ml./Kg./per day between the control period and the period from 6 weeks to 6 months following splenectomy.

Reticulocyte life span (table 4).—The mean reticulocyte percentage was calculated for each dog from 10 preparations prior to surgery, and 10 preparations obtained 6 to 26 weeks post-splenectomy. Following splenectomy, the mean reticulocyte percentage was 0.93 per cent compared to 0.39 per cent during the control period.

Even when corrected for the 31 per cent reduction in total red cell volume, the total number of circulating reticulocytes was increased 67 per cent. The intravascular reticulocyte maturation time determined from the product of the mean reticulocyte percentage, and the red cell life span was increased from 0.40 days to 0.90 days.

DISCUSSION

The present data indicate that splenectomy in the dog causes a significant reduction in the circulating red cell volume with decreased erythrocyte production, but a normal erythrocyte life span.

There is a marked variation in the reported hematologic changes following splenectomy. The majority of the previous studies including the extensive work of Pierce, Krumbhaar, Musser and co-workers15–18 have reported a significant reduction in hematocrit and red count following splenectomy. Other workers, however, have noted no change when compared to sham operated controls.19–21 Some of these discrepancies may have been due to species variation in the size of the spleen. Another significant cause of confusion is that the degree of reduction in the circulating red cell volume following

Table 4.—The Effect of Splenectomy on the Reticulocyte Life Span

<table>
<thead>
<tr>
<th>Reticulocyte %</th>
<th>Mean C¹⁴ red cell life span (days)</th>
<th>Reticulocyte life span (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-splenectomy</td>
<td>0.39</td>
<td>103</td>
</tr>
<tr>
<td>6 weeks to 6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>post-splenectomy</td>
<td>0.93</td>
<td>96</td>
</tr>
<tr>
<td>Percentage change</td>
<td>+138</td>
<td>-7</td>
</tr>
</tbody>
</table>
splenectomy is obscured when evaluated from peripheral indices, such as the hematocrit, by the concomitant reduction in the plasma volume.22–24

The data of Reeve, Sear, Gregersen and co-workers22–24 on the effect of splenectomy in the dog on the discrepancy between the blood volume determined with labeled red cells and labeled plasma proteins provided sufficient data to calculate the changes in the red cell and plasma volume per kilogram of body weight following splenectomy. Their data revealed a 31 per cent lower circulating red cell volume of 28 ml./Kg. in 19 splenectomized dogs compared to 40 ml./Kg. in 10 normal controls. The plasma volume per Kilogram of body weight was 13 per cent lower in 10 splenectomized dogs than in 10 normal controls. These findings are very similar to those of the present report.

The normal red cell life span following splenectomy is in agreement with findings of Miescher4 utilizing Cr31-labeled red cells in a single study in man and Singer and Weiss.3 who utilized the Hawkins-Whipple bile fistula technic in the dog. Drabkin,5 however, found in the dog a significant component of random red cell destruction following splenectomy as studied with glycine 2-Cl6.

Many hypotheses have been proposed to explain the post-splenectomy anemia. It is quite improbable that the anemia is a result of postoperative blood loss as the reduction in total red cell volume is gradual in onset, maximal by 6 to 12 weeks and of at least 5 months duration. A comparable anemia was not seen in sham operated controls.

Bartonella infection causing increased red cell destruction and anemia has been noted in the rat, dog and cat following splenectomy.25 Much of the early work has been criticized in that Bartonella infection was not excluded as a possible cause of the post splenectomy anemia. No evidence for Bartonellosis was noted on careful examination of the animals in the present study. The absence of any significant component of random red cell destruction excludes Bartonella infection as the cause of anemia.

A significant consideration is that splenectomy in the dog may remove an important reservoir of red cells. Barcroft26–27 Cruickshank,29 Izquierdo and Cannon30 and Hahn et al.,31 have estimated that from 5 to 20 per cent of the blood volume may be sequestered in the spleen of the normal dog especially during barbiturate anesthesia. Barcroft28 has also shown that the spleen during life may be 2 to 5 times the size of the spleen at autopsy of the exsanguinated dog. In the present study the average weight of the spleens removed at operation under pentobarbital anesthesia was 63 Gm. while the mean decrease in total red cell volume was 300 ml. and the mean drop in blood volume was 500 ml. Thus although the exact significance of the loss of the splenic red cell reservoir cannot be evaluated, it does not appear to be an adequate explanation for the observed reduction in the red cell and plasma volume.

A number of workers have presented evidence to suggest that the normal spleen produces one or more hormones that control the production, maturation and release of red cells from the marrow. An increase in peripheral hematocrit and red cell count has been observed following the subcutaneous or oral
administration of saline or protein free extracts to dogs and rabbits.\textsuperscript{15,18,32-34,58,57} Patton et al.\textsuperscript{38} and Jeney,\textsuperscript{29-40} however, did not find such an erythropoietic effect from the administration of splenic extracts. Although these data suggest that the spleen may secrete a hematopoietic hormone, it is clear that the spleen is not the sole controlling organ for erythropoiesis as the splenectomized animal responds to anoxia by the production of erythropoietine\textsuperscript{41} and the development of polycythemia.\textsuperscript{5-21}

The alterations in the peripheral reticulocyte percentage, red cell morphology and bone marrow examination provide evidence to suggest that the spleen controls the maturation of red cells and their release from the marrow. The reticulocyte percentage following splenectomy has been reported as reduced\textsuperscript{42} unchanged\textsuperscript{20,21} or increased.\textsuperscript{18,19,43-45} The present data show a significant increase in the reticulocyte percentage following splenectomy despite a reduction in the rate of production of red cells. This was the result of a 125 per cent increase in the intravascular life span of the reticulocyte. This apparent increase in the reticulocyte life span may be due to release of reticulocytes from the marrow at an earlier stage of development, slower maturation of the reticulocyte once released into the peripheral circulation, or the loss of an organ that normally sequesters reticulocytes and "pits" the red cell of intracellular inclusions.

Jandl\textsuperscript{46} and Berendes\textsuperscript{47} have reported that the reticulocyte percentage of the splenic pulp is greater than that of the peripheral blood suggesting splenic sequestration of reticulocytes. The data of Motulsky et al.\textsuperscript{48} are not in agreement with these findings. In one case they demonstrated the exclusion of reticulocytes from the splenic pulp.

Crosby\textsuperscript{49} has shown that the normal spleen removes solid particles from the red cell such as siderotic granules without damaging the red cell. It has also been suggested that the spleen "pits" red cells of other inclusions, Howell-Jolly bodies, Heinz bodies and Bartonella organisms.\textsuperscript{50} It is possible that the normal spleen "pits" the reticulum from young red cells.

Another explanation for the observed reticulocytosis is a slower rate of maturation of circulating reticulocytes. Jacobsen and Plum have shown that reticulocytes mature more rapidly in vitro when incubated in normal plasma than in the plasma of the splenectomized animal whose reticuloendothelial system was blocked with trypan blue.\textsuperscript{51,52} They suggest that this is due to a reduction in the circulating erythrocyte-ripening factor normally synthesized in the reticuloendothelial system. A comparable reduction in the rate of maturation of red cell precursors might also explain the bone marrow hyperplasia noted by some workers following splenectomy.\textsuperscript{2,18,53-55}

Lorber\textsuperscript{19} has presented evidence to suggest that reticulocytes are released at an earlier stage of development in the splenectomized than in the control animal. An increase in the number of more immature reticulocytes classified on the basis of the amount of reticulum was noted in the splenectomized animal. The occasional presence of circulating normoblasts following splenectomy has also been interpreted as indicating that the spleen controls the release of red cells from the marrow.\textsuperscript{19,43,54,57} Thus although it is clear that a
rетiculoctosis with increased reticulocyte life span is present post-splenectomy more work is required before it can be determined which of the various possible explanations for this phenomenon is correct.

Conclusions

1. The erythrokinetics of 8 dogs were studied during a control period and following splenectomy and compared to 3 dogs given sham operations.

2. A significant elevation of the platelet count and a minimal elevation of the WBC were noted in the 15 weeks following splenectomy. No such changes were noted in the sham operated controls.

3. After splenectomy there was a 15 to 22 per cent reduction in the peripheral hematocrit, a 31 per cent reduction in the total circulating red cell volume and a 16 to 18 per cent reduction in the plasma volume, maximal by 6 to 12 weeks and persisting at least the 3 remaining months of the study.

4. There was no significant alteration from control values in the apparent red cell life span estimated with Cr\textsuperscript{51} 3, 6, 12 and 25 weeks following splenectomy nor in the mean red cell life span and fraction of red cells destroyed by random mechanisms estimated with C\textsuperscript{14}-labeled glycine in the period 6 weeks to 6 months post-splenectomy.

5. The plasma iron turnover and red cell iron turnover performed in the period 6 weeks to 6 months post-splenectomy were 32 to 36 per cent below control determinations.

6. The rate of red cell synthesis estimated from the C\textsuperscript{14} glycine life span and the red cell volume was reduced 25 per cent following splenectomy from 0.37 to 0.28 cc/kg/day.

7. During the period from 6 weeks to 6 months post-splenectomy there was a 138 per cent increase in the reticulocyte percentage compared to control values accomplished by a 125 per cent increase in the intravascular reticulocyte maturation time.

8. The decreased production of red cells and resultant anemia following splenectomy could be due either to (a) the removal of a splenic red cell "reservoir" or (b) the removal of an organ (spleen) that normally produces a humoral factor controlling the production of red blood cells.

Summario in Interlingua

1. Le erythrocinetica de 8 canes esseva studiate durante un periodo de controlo e post splenectomia. Le resultatos esseva comparate con illos obtenite in simile studios in tres canes subjicite a operationes fictitie.

2. Un elevation significative del numeracion plachettal e un elevation minimal del numeracion leucocytic esseva notate durante le 15 septimanas post le splenectomia. Nulle tal alterationes esseva notate in le animales de controlo subjicite a operationes fictitie.

3. Post splenectomia il occurreva un reduction de 15 a 22 pro cento in le hematocrite peripheric, un reduction de 31 pro cento in le total volumine de erythrocytos circulante, e un reduction de 16 a 18 pro cento in le volumine de plasma. Iste alterationes esseva maximal post inter 6 e 12 septimanas a partir del operation e persisteva durante al minus le tres remanente menses del studio.
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4. In comparison con le valores de controlo, il non havéa un alteration de character significative in le apparente duration del vita del erythrocytos, estimate 3, 6, 12, e 25 septimeyas post le splenectomia per medio de Cr-114, e etiam non in le superviventia medie del erythrocytos o del proportion del erythrocytos destriute per mechanismi de hasardo, estimate durante le periodo ab 6 septimeyas usque a 6 menses post le splenectomia per medio de glycina marcate con Cr-114.

5. Le transition de ferro in le plasma e le transition de ferro in le erythrocytos, mesurate durante le intervallo ab 6 septimeyas usque a 6 menses post le splenectomia, esseva inter 32 e 36 pro cento infra le determinatione de controlo.

6. Le synthese de erythrocytos, estimate super le base del superviventia (mesurate con glycina a Cr-114) e le volumine de erythrocytos, esseva reducette per 25 pro cento post le splenectomia. Illo havéa amontate a 0,37 cm³/kg/die, e post le splenectomia illo amontava a 0,28 cm³/kg/die.

7. Durante le intervallo de tempore ab 6 septimeyas usque a 6 menses post le splenectomia, il havéa un augmento de 138 pro cento in le proportione de reticuloeytos in comparison con le valores de controlo. Isto esseva accompaniate de un augmento de 125 pro cento in le tempore de maturation intra-vascular del reticuloeytos.

8. Le reducette production de erythrocytos e le resultante anemia post le splenectomia poteva esser le effecto (a) del elimination de un reservoir splenic de erythrocytos o (b) del elimination de un organo, i.e. del spleen, que produce normalmente un factor humoral que ha le function de regular le production de erythrocytos.

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