The Effects of Splenectomy on the Red Blood Cells of the Dog with Particular Emphasis on the Reticulocyte Response

By Mortimer Lorber

Although numerous papers have been written on the hematologic response to splenectomy, the effect on the reticulocyte count has often been neglected. This report is primarily a study of the reticulocyte level of dogs following removal of the spleen as compared with that occurring subsequent to other types of surgery or phlebotomy. Based on observed differences, inferences have been drawn regarding certain of the mechanisms concerned in erythropoiesis in splenectomized and in nonsplenectomized animals.

Methods

Fifteen male, adult, mongrel dogs were employed. The following procedures were performed on individual animals at 2 to 6 month intervals:

Dog
A  Splenectomy
B  Splenectomy, Phlebotomy
C  Splenectomy, Unilateral Nephrectomy, Phlebotomy
D  Splenectomy, Unilateral Nephrectomy
E  Phlebotomy (splenectomized fifteen months previously)
F,J  Splenic Venous Routing into the Systemic Circulation
G  Splenic Venous Routing into the Systemic Circulation, Phlebotomy
H  Splenic Venous Routing into the Systemic Circ., Phlebotomy, Splenectomy
J  Phlebotomy, Unilateral Nephrectomy
K,M  Phlebotomy
L  Phlebotomy, Unilateral Nephrectomy plus Splenectomy at one operation.
N,O  Unilateral Nephrectomy

The results of splenectomy were observed in 6 dogs; 4 of which (A-I,D) were splenectomized initially and 2 others (H,L) following recovery from other procedures. There were several control groups: (1) in Dogs F-J, the splenic venous blood was rerouted into the systemic circulation, thus affording information as to whether the results of splenectomy were due to the removal of the spleen per se or merely to the removal of the splenic blood from the portal circulation. (2) Four dogs (J-M) were phlebotomized, as was Dog E which had been splenectomized fifteen months previously. (3) Unilateral nephrectomy was performed initially in 2 dogs (N,O), and in one (J) it was done subsequent to recovery from phlebotomy. A total of 11 procedures, including the initial surgery in all but one
EFFECTS OF SPLENECTOMY ON RED BLOOD CELLS

(E), were followed on 7 splenectomized dogs (A-E,H,L) and 15 procedures were performed on 10 control animals (F-O).

The animals were caged and fed a diet of horse meat, dog biscuits, and water. At least 4 sets of hematologic determinations were performed prior to the major procedures. All determinations were performed on blood collected in Wintrobe balanced oxalate tubes, except the platelet counts and smears which were done directly from the needle. Double pipette erythrocyte counts, Wright stained smears for circulating normoblasts and erythrocyte morphology, hemoglobin (Crosby's modification of Drabkin's cyanmethemoglobin method), Wintrobe hematocrit and red blood cell indices, and osmotic fragility determinations were performed. Phase microscope platelet counts were also performed.

Reticulocyte counts using New Methylene Blue, noting the number per 1000-3000 erythrocytes were done. Absolute reticulocyte values were obtained by multiplying the reticulocyte percentage by the erythrocyte count. 150,000±10,000 erythrocytes per hematocrit division were present throughout the entire period of observation in 10 dogs which underwent surgery. In the phlebotomized dogs, red blood cell counts were done but rarely, and a value of 150,000 red cells per hematoцит division was assumed in calculating the absolute reticulocyte values.

All surgery was performed using intravenous Nembutal anesthesia. Splenectomy and unilateral nephrectomy were performed employing routine surgical technics. Routing of the splenic blood into the general circulation was accomplished by an end-to-end anastomosis of the splenic and left renal veins following left nephrectomy. The phlebotomies were performed using Pentothal anesthesia, withdrawing 22%–31% of the estimated blood volume (7% of body weight) from the femoral vein. A smaller phlebotomy of about 10% of the estimated blood volume was done in Dog E.

Iliac crest bone marrow specimens were aspirated at the time of surgery and at various intervals thereafter. Five hundred cell differential counts were done on Wright stained marrow smears.

The data were analyzed statistically by the Cochran and Cox "t" method when the variates are unknown. The limits of significance were such that the likelihood of a significant value being due solely to chance was 5 in 100.

RESULTS

Platelets. In all the splenectomized dogs, a significant thrombocytosis of 6 to 22 weeks duration occurred, indicating the removal of all splenic tissue which was eventually confirmed at autopsy or laparotomy.

Circulating Normoblasts. In all but one of six dogs, circulating normoblasts were absent prior to splenectomy. In the former animal, only one was noted in four preoperative differential counts. After removal of the spleen, four of the dogs had intermittent normoblastemia during regeneration. Two of these were not followed in the maintenance phase. In the remaining two (table 1), circulating normoblasts persisted for almost five months in one and in the other for over six months, after which observations were no longer made. By contrast, in one unilaterally nephrectomized animal and in one with a surgically altered splenic circulation, an occasional normoblast was seen only during the phase of regeneration and not thereafter. None were noted in the remaining controls or in Dog E 15 months following splenectomy.

Hematocrit, Hemoglobin and Erythrocyte Count. Similar maximal postoperative hematocrit depressions (table 2) occurred following splenectomy and following unilateral nephrectomy. The former dogs regenerated within two to three weeks and the latter required three to four weeks for recovery. The dogs whose splenic venous circulation were rerouted experienced a
greater postoperative hematocrit depression and regenerated in two to five weeks, except for one animal which developed mange and required 12 weeks. The regeneration of erythrocyte and hemoglobin values, in general, paralleled the hematocrit.

Erythrocyte Morphology. Splenectomy resulted in the appearance of lepto-
cytes (target cells) which usually were large and polychromatophilic when first noted on the second day following surgery. They became more numerous and most of them decreased in size and lost their polychromatophilia by the seventh postoperative day. The maximum number was reached in two to four months. Thereafter, although persisting in each animal at about a constant level for the entire period of observation, including Dog E 22 months after splenectomy, they varied in number from about 1 to 30 per cent in different animals.

All splenectomized dogs developed an increased osmotic resistance (table 1) characterized by a slight decrease of the maximal fragility value and a greater decrease of the minimal value. Howell-Jolly bodies, however, were rare and found in only two of the splenectomized dogs. There were no Howell-Jolly bodies, leptoocytes or appreciable changes in osmotic fragility in the control dogs. The erythrocyte indices were essentially unchanged in all the animals. No Bartonellae were observed within the erythrocytes of any animal.

Reticulocytes. As reticulocytes are usually considered to be evidence of active erythropoiesis, each postprocedure reticulocyte response was divided into two phases: that phase occurring during recovery from blood loss and termed the phase of regeneration, and that occurring subsequent to the return of the hematocrit to that range which existed prior to the procedure and termed the maintenance phase. As the absolute reticulocyte values differed greatly between individual animals, the ratios of the mean values during the regeneration or maintenance phases to the mean value in the preprocedure phase (table 2) were usually better means of expressing the effect of the

<table>
<thead>
<tr>
<th>Dog:</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoblasts:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>Post</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,202*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>130*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>140</td>
<td></td>
</tr>
</tbody>
</table>

Indices (MCV,MCH,MCHC):

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td>64, 22, 34</td>
<td>64, 22, 33</td>
</tr>
<tr>
<td>MCH</td>
<td>66, 22, 33</td>
<td>66, 24, 33</td>
</tr>
<tr>
<td>MCHC</td>
<td>66, 24, 36</td>
<td>66, 24, 35</td>
</tr>
</tbody>
</table>

Osmotic Frag:

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>% NaCl</td>
<td>.52-.36</td>
<td>.44-.28</td>
</tr>
</tbody>
</table>

No. Days Observed

| | 226 | 197 | 190 |

*Statistically significant.
TABLE 2.—Reticulocyte and Hematocrit Data

The figures for maximum postprocedure hematocrit depression (column 4) are on the basis of 100 as the preprocedure mean value.

If Dog F, which developed mange, be omitted, the mean time required for complete regeneration of the hematocrit to occur (column 7) in the dogs with the splenic venous shunt into the systemic circulation is reduced from 40 to 25 days.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenectomy</td>
<td>A</td>
<td>25,315</td>
<td>88</td>
<td>79,891*</td>
<td>3.2*</td>
<td>15</td>
<td>63,827*</td>
<td>2.5*</td>
<td></td>
</tr>
<tr>
<td>Splenectomy</td>
<td>B</td>
<td>5,265</td>
<td>79</td>
<td>101,618*</td>
<td>19.3*</td>
<td>22</td>
<td>34,191*</td>
<td>6.5*</td>
<td></td>
</tr>
<tr>
<td>Splenectomy</td>
<td>C</td>
<td>13,665</td>
<td>88</td>
<td>108,129*</td>
<td>7.9*</td>
<td>18</td>
<td>76,944*</td>
<td>5.6*</td>
<td></td>
</tr>
<tr>
<td>Splenectomy</td>
<td>D</td>
<td>20,906</td>
<td>87</td>
<td>51,725</td>
<td>2.5</td>
<td>17</td>
<td>79,330*</td>
<td>3.8*</td>
<td></td>
</tr>
<tr>
<td>Splen. of Shunt</td>
<td>H</td>
<td>20,400</td>
<td>77</td>
<td>90,190*</td>
<td>4.4*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Splen. + Nephr.</td>
<td>L</td>
<td>2,650</td>
<td>71</td>
<td>96,706*</td>
<td>36.5*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>14,700</td>
<td>82</td>
<td>88,043</td>
<td>12.3</td>
<td>18</td>
<td>63,498</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Phlebotomy of dogs</td>
<td>B</td>
<td>30,185</td>
<td>73</td>
<td>93,398*</td>
<td>3.1*</td>
<td>15</td>
<td>47,893*</td>
<td>1.6*</td>
<td></td>
</tr>
<tr>
<td>Splenectomized dogs</td>
<td>C</td>
<td>66,487</td>
<td>77</td>
<td>122,460*</td>
<td>1.8*</td>
<td>16</td>
<td>89,060</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td>E</td>
<td>55,625</td>
<td>99</td>
<td>74,070*</td>
<td>1.4*</td>
<td>10</td>
<td>68,960</td>
<td>1.9*</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>50,099</td>
<td>81</td>
<td>96,609</td>
<td>2.1</td>
<td>14</td>
<td>68,686</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Unilateral Nephrectomy of dogs</td>
<td>D</td>
<td>42,060</td>
<td>74</td>
<td>112,737*</td>
<td>2.7*</td>
<td>19</td>
<td>108,037*</td>
<td>2.6*</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>62,075</td>
<td>81</td>
<td>99,720</td>
<td>1.9</td>
<td>18</td>
<td>93,517</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Splenic Venous Shunt into the systemic circulation</td>
<td>F</td>
<td>20,152</td>
<td>71</td>
<td>17,068</td>
<td>0.9</td>
<td>86</td>
<td>23,895</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Systemic Circulation</td>
<td>I</td>
<td>61,966</td>
<td>71</td>
<td>45,960</td>
<td>1.9</td>
<td>23</td>
<td>27,355</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>25,321</td>
<td>73</td>
<td>59,146</td>
<td>2.3</td>
<td>40</td>
<td>26,807</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Phlebotomy of Shunted Dogs</td>
<td>G</td>
<td>21,120</td>
<td>87</td>
<td>38,960*</td>
<td>1.8*</td>
<td>12</td>
<td>19,096</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>18,941</td>
<td>81</td>
<td>39,223</td>
<td>2.1</td>
<td>16</td>
<td>20,348</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Phlebotomy of Normal Dogs</td>
<td>J</td>
<td>16,950</td>
<td>86</td>
<td>18,387</td>
<td>1.1</td>
<td>13</td>
<td>11,677</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Normal Dogs</td>
<td>K</td>
<td>7,987</td>
<td>83</td>
<td>14,055</td>
<td>1.8</td>
<td>10</td>
<td>11,391</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>18,941</td>
<td>81</td>
<td>39,223</td>
<td>2.1</td>
<td>16</td>
<td>20,348</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Unilateral Nephrectomy of Normal Dogs</td>
<td>N</td>
<td>23,445</td>
<td>85</td>
<td>38,411</td>
<td>1.6</td>
<td>28</td>
<td>36,566</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Normal Dogs</td>
<td>O</td>
<td>9,391</td>
<td>79</td>
<td>26,364</td>
<td>2.8*</td>
<td>21</td>
<td>21,118</td>
<td>2.8*</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>18,475</td>
<td>84</td>
<td>28,921</td>
<td>2.4</td>
<td>23</td>
<td>22,083</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant.

various procedures on the reticulocyte count than were changes in the absolute numbers of reticulocytes.

It has been reported in iron deficiency anemia7 and pernicious anemia8 that a correlation exists between the erythrocyte level and the subsequent
reticulocytosis in response to treatment. It was, therefore, fortunate that despite differences in individual animals, the lowest postoperative mean hematocrit values of all but one of the groups were similar, being 81-84 per cent of the preprocedure mean. The sole exception was the group of dogs whose splenic venous blood had been rerouted. In this group, due to greater hemorrhage during the creation of the venous anastomoses, the maximal mean hematocrit depression was 73 per cent of the preoperative value. One of the main factors believed to govern reticulocytosis was thus kept constant in all but one of the groups. However, despite the greater blood loss experienced by those four dogs whose splenic venous flow was rerouted, their mean reticulocyte value during the regenerative phase was but 2.3 times greater than preoperatively. This was of the same order of magnitude as occurred subsequently when two of them were phlebotomized, and as also occurred in three dogs which had undergone unilateral nephrectomy. Less of a reticulocyte elevation (1.3 times) occurred during regeneration in the four normal dogs which were phlebotomized as their initial procedure.

Of the 13 procedures other than splenectomy performed on control dogs F-O (fig. 1, table 2), only six procedures on five dogs (G,H,I,J,O) were followed by significant reticulocytosis during the phase of regeneration. One dog (M) even experienced a significant decrease of reticulocytes at that time. In only two animals (G,O) did the reticulocytosis persist, although to a lesser degree, during the maintenance phase. Whereas, in two others (L,M) a significant decrease was noted during that phase. Considering all the control procedures, the reticulocyte levels during regeneration ranged from 0.6 to 4.1 times the preprocedure level, with a mean of 2.0. During the maintenance phase they were 0.5 to 2.7 times the preprocedure level, the mean being 1.2.

In marked contrast, the splenectomized animals developed a reticulocytosis during the phase of regeneration of 2.5 to 36.5 times the preprocedure mean, the mean for all animals being 12.3. In addition, during the maintenance phase the reticulocyte levels were elevated from 2.5 to 6.5 times, the mean being 4.6 times the preoperative value. Thus, the splenectomized animals exhibited a reticulocytosis during both phases which was usually greater than that seen in the controls.

That the observed reticulocytosis was not a function of hematocrit depression was evidenced by Dog L whose hematocrit the day after splenectomy rose from 53.5 per cent to 60.0 per cent due to dehydration, and whose reticulocyte count, nevertheless, increased 13 fold, from 7,000 to 90,000. Dog C had no change in his 41.0 per cent hematocrit on the first postsplenectomy day, despite which his reticulocytes also increased more than 12 times from 13,000 to 172,000.

It was noted that in three out of four splenectomized dogs, a further significant elevation of the reticulocyte level could be induced by phlebotomy or unilateral nephrectomy performed two or more months following splenectomy. The mean reticulocyte elevation amounted to about a twofold increase during the phase of regeneration and somewhat less than that during the maintenance phase.
EFFECTS OF SPLENECTOMY ON RED BLOOD CELLS

phase. This was a higher incidence of animals with reticulocytosis than was noted following similar secondary procedures in eight nonsplenectomized controls, four of which had an elevation of reticulocyte level during the regeneration phase, which persisted during the maintenance phase in but one.

Considering the initial and secondary procedures together, the mean reticulocyte values of all the procedures performed in the splenectomized group were 92,502 during regeneration and 71,878 during maintenance. Whereas, in all the control procedures they were 38,033 during the former phase and 20,301 in the latter. In addition, there was scarcely any overlap of the absolute values, further emphasizing the difference in reticulocyte response between the splenectomized and nonsplenectomized animals.

Bone Marrow. Careful study of smears suggested that splenectomy did not cause any significant alteration in cellularity of the marrow. There was a slight nonspecific postoperative megakaryocytosis of several months duration, which also has been noted following laparotomy and unilateral nephrectomy. No marked change in the myeloid-erythroid ratio was ever observed despite the increased numbers of reticulocytes evident in the peripheral blood.

DISCUSSION

Platelets. The significant thrombocytosis which in all of the splenectomized dogs persisted up to five months postoperatively is a well-known finding and has been observed both in experimental animals and in people whose spleens have been removed following trauma or for a variety of hematologic disorders. In this latter group, its duration has been reported to be a matter of years. However, the former cases are more truly comparable to the experimental animals in that they are both otherwise free of disease. It is, therefore, of interest that Ek and Bayner, although observing reticulocytosis in 11 out of 18 people, were unable to note unusually high platelet levels several years after the removal of traumatized healthy spleens. The present data are in accord with that observation that reticulocytosis tends to persist longer than thrombocytosis following splenectomy in otherwise normal animals and man.

Circulating Normoblasts. Although it has been stated that normoblastemia is rare in dogs except immediately following splenectomy, the present findings indicate that it is more prevalent both immediately after splenectomy and thereafter. The intermittent normoblastemia noted in several of the splenectomized animals months after complete recovery from postoperative blood loss has also been reported in man, but has not been observed several years following removal of the spleen.

Hematocrit, Hemoglobin, and Erythrocyte Count. Three or four decades ago, it was claimed that in contrast to other surgical procedures of similar magnitude, splenectomy resulted in a greater decrease of red blood cell values which persisted for several months to almost one year, due to a disturbance of unspecified mechanisms concerned in maintaining the normal blood picture. No mention was made of the possible presence of Barto-
Fig. 1.—Reticulocyte data, postprocedure minimum hematocrit (HCT↓), based on 100 as the preprocedure value, and preprocedure mean hematocrit (HCTm) values. The columns encompass the range of the reticulocyte values and the enclosed dot indicates the mean value. The reticulocyte values are illustrated by three columns representing the preprocedure, regeneration and maintenance levels. However, Dogs L and H in the upper left corner of the figure were not followed during the latter phase and, therefore, each has only the first two columns of the usual three.
EFFECTS OF SPLENECTOMY ON RED BLOOD CELLS

In those reports. In contrast, the findings in this paper and others\textsuperscript{13,17,24} have failed to reveal any more marked anemia than was attributable to blood loss during splenectomy. Neither the degree nor duration of the anemia appeared to be greater in the splenectomized than in the control dogs and, other than in one splenectomized and one control dog, there was no lag of hemoglobin regeneration behind the red blood cells and hematocrit as had also been claimed.\textsuperscript{21} In addition, there was no subsequent significant elevation of those values, thereby also differing from previous reports.\textsuperscript{8,10}

\textbf{Erythrocyte Morphology.} Although it has been stated\textsuperscript{9} that no changes in the appearance of erythrocytes occur following splenectomy, it is now generally agreed that such changes are among the most striking results of removal of the spleen.\textsuperscript{10,17} The main developments are the formation of leptocytes (target cells), siderocytes which contain nonhemoglobin iron and about which nothing further shall be said, and the occurrence of Howell-Jolly bodies. Although in man,\textsuperscript{14} the latter are said to be a constant result of splenectomy, they were rather scarce and, unlike leptocytes, were not found in all the dogs described in this paper.

\textbf{Reticulocytes.} Diverse reports on the reticulocyte level in various species following splenectomy have been published. The response has been reported to be either diminished,\textsuperscript{23} unchanged,\textsuperscript{9,24} slightly increased,\textsuperscript{9} or a definite reticulocytosis.\textsuperscript{11,16,17} The present findings, in agreement with the latter observations, suggested the question as to whether the observed reticulocytosis necessarily implied alterations in the normal rate of hemoglobin formation or destruction. It has previously been shown that following splenectomy the life span of the dog erythrocyte was unchanged\textsuperscript{25} and the fecal urobilinogen excretion was decreased.\textsuperscript{13} Drabkin, in his studies of C\textsuperscript{14} glycine-labeled hemin and bile pigments, was unable to demonstrate a definite hemolytic process in splenectomized dogs.\textsuperscript{13} The reticulocytosis, therefore, was evidently not a response to increased hemolysis. If it were entirely a manifestation of increased erythropoiesis, more rapid regeneration from blood loss and greater alterations in the myeloid-erythroid ratio of the marrow might have been expected in splenectomized animals than in those with intact spleens, yet recovery occurred at a normal rate, as has been the experience of others.\textsuperscript{23,26} That the reticulocytosis during the phase of regeneration, however, did represent to a great extent the addition of new erythrocytes to the circulation was evidenced by the rise of red cell values from their postoperative minimum. By contrast, in the succeeding maintenance phase, despite a definite reticulocytosis elevation, there was no further rise in erythrocyte, hemoglobin or hematocrit values, suggesting that the reticulocyte increase at that time was no longer primarily a manifestation of increased erythrocyte manufacture. Of pertinent interest is the statement by Minot and Castle\textsuperscript{27} that the presence of increased numbers of reticulocytes per se does not always mean increased formation or delivery of red blood cells by normal physiologic processes. They noted reticulocytosis without resultant elevation of hemoglobin values in myelophthisic involvement of the bone marrow, as have others following the use of caffeine, arsenic or cobalt compounds,\textsuperscript{28} or following the administration of yeast in pernicious
Unfortunately, studies to rule out concomitant hemolytic processes were not reported in those papers.

Another possible cause of the apparent reticulocyte increase could have been decreased "ripening" (maturation) of the erythrocytes, as has been reported by Jacobsen and Plum. This would have increased the life span of circulating reticulocytes beyond the one to two days usually agreed on and could have accounted for their apparent increase in numbers unaccompanied by elevations of red cell values. It is assumed here that the reticulocytosis did not represent an increased rate of maturation of normoblasts. The reticulocytoses could either develop into adult erythrocytes or undergo possible dyspoiesis or destruction. The latter two possibilities, although perhaps theoretically operative in some autoimmune states, are, normally, probably not of great importance in either intact or splenectomized animals in which an important site of blood destruction has been removed.

Having considered occurrence of reticulocytosis without evidence of further increases of erythrocyte levels, let another apparent paradox now be mentioned; i.e., the regeneration from blood loss without much or any of a reticulocyte increase as has been noted in nonsplenectomized animals and man. It has been stated that all red blood cells are released from the bone marrow as reticulocytes. If that were true, then during regeneration an appreciable reticulocytosis should have occurred in the nonsplenectomized dogs. As that was usually not the case, unless the reticulocytes have an unusually short life span, so that a small reticulocyte count in reality represents more cells than it appears to, most red cells released during regeneration must be mature erythrocytes. This belief has been expressed by Minot et al. in discussing the therapy of pernicious anemia, and by Jacobsen. It has also been implied by who noted that in recovery from acetylphenylhydrazine anemia, the reticulocyte counts of dogs returned to normal long before the erythrocyte count, hemoglobin and hematocrit values did.

The concept that the spleen regulates hematopoiesis through hormonal means, first propounded by Isaac, has subsequently been adhered to by others, some of whom believe that the spleen also controls the emission of cells from the bone marrow. The presence of normoblastemia, even though the blood loss following splenectomy has long since been repaired, has been presented as evidence for a change in the usual release mechanism subsequent to removal of the spleen. The elevated reticulocyte level observed following splenectomy is likewise compatible with this belief.

To determine whether splenectomy resulted not only in an increased number of reticulocytes, but in the release of more immature ones as well, differential reticulocyte counts classifying the cells on the basis that the amount of reticulum was inversely proportional to the maturity of the cells were done on four of the splenectomized dogs, with two of the normal phlebotomized animals and two of the unilaterally nephrectomized dogs as controls (table 3). The results suggested that in all four splenectomized dogs during the phase of regeneration, but not during maintenance, about twice as many of the youngest forms were present as before splenectomy. Of the controls, only one
dog (K) developed a similar shift; the other three had but slight increases, the largest being half again as many as preoperatively (O). That a shift to the left is not an inherent manifestation of the release of large numbers of reticulocytes was indicated by the differential reticulocyte counts in Dog I, whose splenic blood was diverted into the systemic circulation, at times when his reticulocyte counts were 281,000 (3.1 per cent) and 409,000 (9.1 per cent), respectively. It should be emphasized that the reproducibility of repeated differential reticulocyte counts was not as good as that of the differential white blood cell count, perhaps because only 25 reticulocytes per slide were counted because of the necessity at times of having to peruse as many as 25,000 erythrocytes before finding 25 reticulocytes. Nevertheless, the data did suggest a release of more immature reticulocytes following splenectomy than were usually seen during recovery from blood loss in the nonsplenectomized animals. If this be true, it would tend to support the belief that removal of the spleen affects the bone marrow, resulting in earlier release of erythroid cells into the circulation. It might, thus, partly explain the delayed reticulocyte maturation that was noted following splenectomy,39-40 since the younger the reticulocytes are on entering the circulation, the longer the time before they disappear by completing their maturation.

Many of the findings that were noted following splenectomy may be interpreted as either being due to the increased release of reticulocytes and normoblasts from the bone marrow because of the absence of normal splenic inhibition or to the removal of an organ in which they complete their maturation into adult erythrocytes. If the latter were the case, then one would normally expect to find circulating normoblasts in the peripheral blood, albeit perhaps in small numbers, on their way from the bone marrow to the spleen to com-

| Table 3.—Differential Reticulocyte Counts in Four Splenectomized and Four Control Dogs (Based on a Series of Counts of 25 Reticulocytes) |
|---|---|---|---|---|---|---|
| Dog A | Presplectonemy | Most | 2.8 | 17.6 | 4.6 | Prephlebotomy |
| Regeneration | 6.7 | 15.8 | 2.5 | Regeneration | 3.0 | 19.7 | 2.3 |
| Maintenance | 4.3 | 16.8 | 3.9 | Maintenance | 5.8 | 14.5 | 4.7 |
| Dog B | Presplectonemy | Most | 3.3 | 14.4 | 7.3 | Prephlebotomy |
| Regeneration | 9.3 | 14.8 | 0.9 | Regeneration | 7.8 | 15.6 | 1.6 |
| Maintenance | 4.6 | 17.2 | 3.2 | Maintenance | 7.2 | 15.5 | 2.3 |
| Dog C | Presplectonemy | Most | 3.6 | 18.4 | 3.0 | Prephlebotomy |
| Regeneration | 7.1 | 15.7 | 2.0 | Regeneration | 6.0 | 15.3 | 2.7 |
| Maintenance | 4.9 | 18.7 | 1.4 | Maintenance | 3.0 | 18.0 | 4.0 |
| Dog D | Presplectonemy | Most | 2.3 | 17.7 | 5.0 | Prephlebotomy |
| Regeneration | 5.3 | 17.2 | 2.5 | Regeneration | 2.4 | 19.4 | 3.2 |
| Maintenance | 2.6 | 18.4 | 4.0 | Maintenance | 1.5 | 19.0 | 4.5 |
| MEAN (splenectomy) | Presplectonemy | Most | 3.0 | 17.0 | 5.0 | Preprocedure |
| Regeneration | 7.1 | 15.9 | 2.0 | Regeneration | 4.8 | 17.4 | 2.7 |
| Maintenance | 4.1 | 17.8 | 3.1 | Maintenance | 4.4 | 16.8 | 3.9 |
plete their development. The fact that in nonsplenectomized animals circulating normoblasts are absent or very rare suggests that that is not the case. In addition, two of the dogs had no depression of hematocrit on the first post-splenectomy day, despite which, within 18 hours of the surgery, they had a 12 and 13 fold increase in their circulating reticulocytes. It is somewhat difficult for the author to conceive that this increase merely represented a passive backlog or reticulocytes, whose normal life span is but one or two days, that continued to circulate because their usual site of maturation was removed. It rather suggests that a change in the release mechanism from the bone marrow had occurred, permitting the release of not only more but younger red cell precursors into the circulation.

**Bone Marrow.** Although in 1914, Pearce and Pepper noted no macroscopic increase of red marrow in dogs after splenectomy, subsequent reports of bone marrow hyperplasia have appeared. The present data, however, afford no particular evidence of bone marrow changes subsequent to splenectomy, and are thus more in agreement with the recent observation of erythroid hyperplasia in but 2 of 12 patients, a number of years after removal of the spleen.

**Summary and Conclusions**

In splenectomized dogs the following observations on the erythroid elements were made:

1. There was no unusual postoperative depression of erythrocyte, hemoglobin or hematocrit values, and regeneration occurred at the same rate as in the control dogs.

2. A significant increase in the number of circulating reticulocytes was noted, which persisted for a longer duration than the associated thrombocytosis. There is a suggestion that the reticulocytes circulating during regeneration were more immature than those of the control animals.

3. Varying numbers of target cells accompanied by increased osmotic resistance were present in all the splenectomized dogs. Most also developed normoblastemia, particularly during active regeneration, which persisted thereafter in some of the animals. Howell-Jolly bodies were rare.

4. No significant bone marrow changes were noted.

It is suggested that:

1. In nonsplenectomized animals, most erythrocytes released from the bone marrow in response to blood loss are mature red cells rather than reticulocytes.

2. Postsplenectomy reticulocytosis is not dependent on a fall in hematocrit and is not a reflection of hemolysis or even of increased erythropoiesis, except, perhaps, in part during regeneration from the blood loss incurred at surgery.

3. Postsplenectomy reticulocytosis and normoblastemia are unaccompanied by alterations in the myeloid-erythroid ratio of the bone marrow and are probably largely due to a diminution of the normal inhibition of erythroid release from the marrow, permitting their earlier entry into the circulation.

4. The aforementioned changes are due specifically to the removal of the spleen itself, rather than merely to the removal of splenic blood from the portal circulation.
In canes splenectomisate le sequente observationes esseva facite con respecto al elementos erythroide:

1. Occurreva nulle inusual depression post-operatori in le valores del erythrocytos, del hemoglobina, o del hematocrite, e le regeneration se faceva con le mesme intensitate como in le canes de controlo.

2. Esseva notate un augmento significative in le numero del reticulocytos circulante. Iste augmento persisteva plus longemente que le associate thrombocytosis. Certe observationes pare indicar que le reticulocytos circulante durante le regeneration esseva plus immatur que illos in le animales de controlo.

3. Varie numeros de cellulas a oculo de ave, accompaniante de augmentate resistentia osmotic, esseva presente in omne le canes subjicite a splenectomy. In le majoritate del casos il habeva etiam un disvelopamento de normoblastemia, specialmente durante le regeneration active. Isto persisteva subsequentemente in certes del animales. Corpores de Howell-Jolly esseva rar.

4. Esseva notate nulle alterationes significative del medulla ossee.

Le sequente conclusiones es proponite:

1. In animales non-splenectomisate, le majoritate del erythrocytos liberate per le medulla ossee in responsa a un perdita de sanguine es cellulas matur plus tosto que reticulocytos.

2. Reticulocytosis post-splenectomic non depende de un abassamento del valores del hematocrite e non es un reflexion de hemolyse o mesmo de un accelerate erythropoiese, excepte—forsan—in parte durante le regeneration post perditas de sanguine causate per le intervention chirurgic.

3. Reticulocytose post-splenectomic e normoblastemia non es accompaniante de alterationes del proportion myeloido-erythroide in le medulla ossee e resulta probablemente in grande mesura ab le diminution del inhibition normal del liberation de erythrocytos ab le medulla con le efecto de un entrata plus precoce de illos a in le circulation.

4. Le mentionate alterationes es effectuate specificamente per le ablation del splen per se e non simplemente per le elimination de sanguine splenic ab le circulation portal.

REFERENCES


EFFECTS OF SPLENECTOMY ON RED BLOOD CELLS

45. Schonsboe, J. S.: Two cases of splenic control of the cell emission from bone marrow. Acta med. Scandinav. 103:123, 1940.
The Effects of Splenectomy on the Red Blood Cells of the Dog with Particular Emphasis on the Reticulocyte Response

MORTIMER LORBER