Studies on the Effect of Splenectomy on the Total Leukocyte Count in the Albino Rat

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Changes have been noted in the blood following removal of the spleen in normal animals and in human subjects, but the mechanism involved in the production of these changes remains obscure. Both anemia and polycythemia, as well as morphologic and physiologic alterations in the red cells such as target cells, Howell Jolly bodies and decreased osmotic fragility1-4 have been reported following removal of the normal spleen. These changes have been attributed by some investigators to remote effects of the spleen on red cell formation2 and by others to alterations produced by splenectomy in the rate of blood destruction.3 Leukocytosis, which is more prolonged than that following other operations, has been noted after splenectomy in man4,5 and animals.6
Similarly, an increased number of platelets found has been attributed by some to operative trauma,6 by others to a specific effect of the spleen in inhibiting platelet production, and by still others to removal of the normal site of platelet destruction.2,7

The beneficial effect of splenectomy in congenital hemolytic icterus is, at least in part, due to the removal of an organ which destroys large numbers of abnormal red cells.9 The explanation of the results of splenectomy in idiopathic thrombocytopenic purpura and in various leukopenic states is, however, a subject of considerable controversy. Doan and his co-workers10,11 believe that the role of the spleen in these disorders is essentially the same as in congenital hemolytic icterus in that it is a point of sequestration and abnormally rapid destruction of cells. In support of this concept they have described hyperplasia of splenic clasmatoocytes and excessive phagocytosis of blood cells in the spleen in supravital preparations, and report marked differences in the counts of splenic artery and vein blood in these disorders. Others have been unable to confirm these findings.

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EFFECT OF SPLENECTOMY ON LEUKOCYTE COUNT IN ALBINO RAT

Dameshek and his associates, in the case of idiopathic thrombocytopenic purpura, have noted megakaryocytic changes which they attributed to a hormonal effect by the spleen, and have extended this concept of hormonal inhibition to include many leukopenic states as well as thrombocytopenia. In thrombocytopenic purpura, acetone extracts of the removed spleen have been found by many investigators to produce thrombocytopenia in animals, although an approximately equal number of authors have been unable to confirm this. Inconstant leukopenia has also been observed when extracts made of spleens of patients with chronic neutropenia were injected. In our opinion, no conclusive evidence has been offered to support either the hypothesis that the spleen acts primarily as a site of cellular destruction, or that it acts to inhibit cell production or delivery in the marrow.

The purpose of the experiments to be described was to determine the effect of splenectomy as well as of various other operative procedures on the level of circulating leukocytes in the albino rat, and to study the effect on the leukocyte level of subtotal splenectomy, splenectomy in parabiotic rats and splenectomy in rats in which the production of leukocytes was inhibited by pteroylglutamic acid deficiency.

METHODS

Approximately 2000 male rats of the Sprague-Dawley strain, weighing 100-200 grams, were used in this study. They were housed in wire cages and fed Purina Dog Chow and water ad libitum. Rats made deficient in pteroylglutamic acid were housed in individual wire cages and fed, ad libitum, a basal diet of the following composition: Sheffield alcohol extracted casein, 20.0 per cent; sucrose, 61.8 per cent; lard, 11.0 per cent; salt mixture,* 5.0 per cent; succinyl sulfathiazole, 2.0 per cent; and "crude" methyl pteroylglutamic acid antagonist, 0.2 per cent.

Vitamins were mixed with the diet as follows: (mg. per Kg. of diet) thiamin hydrochloride 12.5; riboflavin 6.0; nicotinic acid 60.0; pyridoxine hydrochloride 10.0; calcium pantothenate 25.0; para-aminobenzoic acid 5.0; inositol 10.0; biotin 5.0. In addition, choline chloride was added in the proportion of 2.54 Gm. per kilogram of diet. Fat soluble vitamins were also mixed with the diet as follows: (per Kg. diet) A, 230,000 units; D, 42,500 units; E, 76 mg.; and K, 76 mg. A splenectomized, a control-operated, and an unoperated group were studied on this diet, the operations being carried out after the deficiency had become established. In addition, three similar nondeficient groups were studied. In these the diet was the same except that the antagonist and succinyl sulfathiazole were omitted, and pteroylglutamic acid, 7.5 mg. per kilogram of diet, was added.

All blood counts were taken directly from freely flowing tail vein blood obtained by cutting off one or two distal vertebrae. Total leukocyte counts were done with ordinary white cell diluting pipet, and differential counts were made using cover slip preparations and Wright's stain. Hemoglobin determinations were performed with the Evelyn photoelectric colorimeter. Since it has been reported that leukocyte counts of heart and tail blood differ in the rat,† total leukocyte counts and hemoglobin determinations on blood from these two sites were compared in ninety-day postoperative splenectomized, partially

* The salt mixture was of the following composition (expressed in per cent): NaCl 13.5; MgCO₃, 8.5; KH₂PO₄, 5.7; CaH₂PO₄, 46.3; KCl 6.7; KI 0.227; CaCO₃, 16.0; Fe₃(PO₄)₂, 32.1; CuSO₄, 0.22; MnCl₂, 0.019; ZnO, 0.016; and CoCO₃, 0.016.
omentectomized, unilaterally nephrectomized and unoperated groups. The animals were lightly anesthetized with ether when blood was drawn for both counts. The results are presented in table 1. Although the counts were generally slightly lower in heart blood, no significant difference could be demonstrated in any group, or when the counts of all groups were combined. These results are in agreement with those of Nichols and Miller.17

All operative procedures were done using 1 percent sodium pentobarbital as an anesthetic, in a dose of 40 mg. per kilogram, given intraperitoneally. Splenectomy and unilateral nephrectomy were carried out as described by Ingle and Griffith.21 Partial omentectomy was performed making a longitudinal incision in the left side of the abdomen which extended about an inch below the costal margin. A portion of the greater omentum was delivered through the wound and was tied off a few millimeters from its gastric attachment and excised. Partial splenectomy was performed in a manner similar to total splenectomy except that the spleen was divided across its middle and, in one group one-half, and in another group, three-fourths of the spleen was removed, leaving the remainder unsutured and with its blood supply intact. No difficulty was encountered with excessive hemorrhage from the cut surface of the spleen. Splenic transplants were carried out by first removing the entire spleen, and then cutting a small piece, amounting to approximately 5 to 10 percent of the total weight of the spleen, from one tip. This fragment was then placed in a pocket made in the abdominal wall subcutaneous tissue adjacent to the incision. In another group of rats the fragment was placed intraperitoneally in the left upper quadrant near the original spleen site. The leukocyte counts of these animals were followed for thirty to thirty-five days, after which they were reexplored. In most cases the spleen fragments were easily recognizable, and were removed, fixed in 10 percent formalin, and sectioned for histologic examination. Parabiosis was carried out according to the technic of Bunster and Meyer22 except that metal skin clips instead of sutures were used to close the skin incisions. Both the scapulae and iliac crests were sutured together, and no communication was made between the peritoneal cavities. Two weeks after they were joined, counts were made for a base line period of one week. Splenectomy, or, in the controls, omentectomy, was performed in the right hand partner, and the leukocyte level observed for seventeen to twenty days. At the end of this time the same operation was performed in the left hand partner, and counts made for another seventeen to twenty days. Only approximately 30 percent of the pairs joined have been included in the study. The remainder were omitted because of the death of one partner, or because they became separated before the completion of the experimental period.

No evidence of Bartonella muris infection has been encountered in the strain of rats used, although at the time of this writing over 2000 splenectomies have been performed.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Tail blood Hb. Gm./100 ml.</th>
<th>Tail blood WBC X1000 per mm.</th>
<th>Heart blood Hb. Gm./100 ml.</th>
<th>Heart blood WBC X1000 per mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenectomized</td>
<td>11</td>
<td>14.4 ± 0.5*</td>
<td>17.3 ± 1.2</td>
<td>13.6 ± 0.3</td>
<td>15.7 ± 1.6</td>
</tr>
<tr>
<td>Omentectomized</td>
<td>9</td>
<td>14.0 ± 0.8</td>
<td>17.0 ± 1.5</td>
<td>12.1 ± 0.5</td>
<td>13.4 ± 1.3</td>
</tr>
<tr>
<td>Nephrectomized</td>
<td>10</td>
<td>18.6 ± 0.2</td>
<td>11.4 ± 0.7</td>
<td>17.2 ± 0.4</td>
<td>11.0 ± 0.9</td>
</tr>
<tr>
<td>Unoperated</td>
<td>12</td>
<td>16.0 ± 0.3</td>
<td>14.2 ± 1.2</td>
<td>16.3 ± 0.3</td>
<td>12.6 ± 1.2</td>
</tr>
<tr>
<td>Total, all groups</td>
<td>42</td>
<td>15.8 ± 0.3</td>
<td>14.9 ± 0.7</td>
<td>14.9 ± 0.4</td>
<td>13.1 ± 0.7</td>
</tr>
</tbody>
</table>

* Standard error = \[ \frac{\sum (X - \bar{X})^2}{\sqrt{n(n - 1)}} \]
RESULTS

A. The Effect of Splenectomy on the Leukocyte Count as Compared with Control Operations

The mean value of the leukocyte counts of 1200 rats eight days after splenectomy along with its standard error may be seen in table 2. The means and standard errors of an unoperated group, a partially omentectomized group, and an unilaterally nephrectomized group are also shown, along with the comparison between each of these groups and the totally splenectomized group. The difference is highly significant in each case. The distribution of these counts about their means may be seen in figure 1.

A number of animals were observed for longer periods of time after the operations. The course of the mean leukocyte level in each of the four groups is plotted in figure 2. It may be seen that the splenectomized group maintained a greater degree of leukocytosis than the unoperated and control operated groups for more than two months, gradually approaching the level of the other groups after this time. The means and standard errors of these groups, thirty days postoperatively, are shown and compared in table 2. The difference between the leukocyte level of the splenectomized group and each of the three operated groups at this period is still highly significant.

In figure 3 are shown the mean total leukocyte count, and means of absolute values of polymorphonuclear and mononuclear cells of a group of 10 splenectomized rats with their nonoperated controls. This demonstrates that the rise which occurs is shared by both types of leukocytes.

B. The Effect of Partial Splenectomy

In table 2 are shown the means and standard errors of the leukocyte counts of two groups of rats in which 50 per cent and 75 per cent, respectively, of the
FIG. 1.—Distribution of total leukocyte counts of four groups of rats one week post-operatively.

FIG. 2.—Course of the leukocyte counts of splenectomized, omentectomized, nephrectomized and unoperated rats. Each line represents mean of a group of 12 animals.
spleen was removed. Comparison with the totally splenectomized group indicates that there is a significant difference in the leukocyte counts both at one week and one month postoperatively.

When more than 75 per cent of the spleen was removed, it was frequently difficult to find recognizable splenic tissue after thirty days because of scar formation at the operative site. For this reason, and in order to determine if it was necessary for the spleen to remain in situ, transplants were made of small pieces of spleen intraperitoneally, and into the abdominal wall. After two weeks,
there were intraperitoneal implants tended to have somewhat higher counts than
the group with subcutaneous implants during the first three postoperative weeks,
at no time was there a significant difference between these two groups; consequen-
tly their counts have been combined. The mean of the counts of both groups
with its standard error may be seen in table 2 where comparison is made with
the totally splenectomized group. The difference is significant at both the one
week and the one month period.

C. The Effect of Splenectomy in Parabiotic Rats

The course of the mean leukocyte levels of each member of parabiotics is
shown in figure 5. The right hand partner is designated "A," and is represented
by a solid line; the left hand partner is designated "B," and is represented by
a dotted line. Twelve pairs are averaged in the splenectomized group and ten
pairs in the omentectomized group. It will be noted that following the first op-
eration no rise occurred in the white count of either partner. Following the sec-
ond operation, a significant rise occurred in the leukocyte level of both partners
in the splenectomized group which resembled the rise seen when single rats were
splenectomized.

D. The Effect of Splenectomy in Rats Made Leukopenic by
Pteroylglutamic Acid Deficiency

Forty-five rats were made deficient, and, after definite leukopenia had de-
developed, were divided into three groups of 15 rats each. One group was splen-
Fig. 5.—Course of the leukocyte counts of parabiotic partners. Solid lines represent first operated partners (A); broken lines represent second operated partners. (B) splenectomized group—12 pairs; omentectomized group—9 pairs.

Fig. 6.—Course of the leukocyte counts of splenectomized and unoperated folic acid deficient rats (each line represents the mean of 15 rats) and of splenectomized and unoperated nondeficient rats (each line represents the mean of 5 rats).
ectomized, one group partially omentectomized, and the third group was left unoperated. Three similar nondeficient groups of 5 rats each were studied at the same time. The mean white cell counts of the splenectomized and unoperated groups are shown in figure 6. The omentectomized groups have been omitted for the sake of clarity; their curves were not significantly different from the unoperated groups.

It may be seen that in the pteroylglutamic acid deficient rats, splenectomy did not increase the leukocyte count, nor did it change the downward course as the deficiency progressed. After thirty-five days, many of the animals had died, and the remainder were treated with 20 mg. of pteroylglutamic acid intraperitoneally. At this time there were 8 splenectomized and 10 unoperated animals. A rapid increase in leukocytes occurred, the white cell count in the splenectomized group rising to somewhat higher levels, although the difference was not significant.

**DISCUSSION**

These experiments demonstrate that, in the rat, leukocytosis due to an increase in both neutrophils and lymphocytes occurs following splenectomy. Since this leukocytosis is more marked and more prolonged than that which follows other operative procedures, it seems evident that the spleen of normal rats plays a role in the control of the level of circulating leukocytes.

If the spleen acts by destroying cells, then the rate of destruction might be expected to be a function of the total mass of splenic tissue. Removal of part of the spleen should result in an increase in the leukocyte count, and the more spleen tissue that is removed, the greater should be the rise. On the other hand, if the spleen acts through a hormonal mechanism, partial splenectomy might have no effect, although admittedly this would not necessarily be true. Analogy might be drawn between the thyroid, in which removal of all but a small portion results in lowering of the basal metabolic rate, and the ovary, in which a small bit of remaining ovarian tissue is sufficient to maintain complete ovarian function.

Since as small an amount as 9.7 per cent of the spleen is capable of maintaining the leukocyte count at the same level as 100 per cent of the spleen, thirty days postoperatively, a hormonal effect of the spleen on the leukocytes would seem more likely than an effect through destruction of the circulating leukocytes. At eight days postoperatively, there was considerable variation in the counts of all groups and, although at this time there is a linear relationship between the amount of spleen present and the degree of leukocytosis, this correlation may not be valid since the numbers of animals were relatively small and the day to day variations in the leukocyte level at this period after operation were rather great.

The results in parabiotic animals also suggest that the spleen of one partner liberates a substance which suppresses the postsplenectomy leukocytosis in the other partner. It has been demonstrated, however, that there is blood exchange between parabiotic partners which, in matings of animals from highly inbred strains, occurs at the rate of approximately 1 per cent of the blood volume per hour. The number of cells found in one animal which had been produced in
his partner would, therefore, be a function of the height of the count in the producer, the rate of transfer and the rate of destruction in the recipient. Van Dyke has found that, in animals in which one partner is made non-leukocyte producing, the level of leukocytes is approximately one-third that of the producing partner. Presumably, since our rats were less highly inbred than his, the rate of transfer was less. If it is assumed that splenectomy in one partner results in decreased destruction of his own leukocytes, then his own count should rise, and, if his partner's spleen destroyed cells at the same rate, his partner's count should also rise slightly. Even if the remaining spleen destroyed cells at an increased rate, the count in the splenectomized partner should remain somewhat higher. Such was not the case in our animals, as splenectomy in one partner resulted in no perceptible rise in the count of either animal. On the other hand, if the spleen of one animal liberates a substance which crosses freely to his partner to influence the count, then the animals would be in a position roughly analogous to a single animal with 50 per cent of his spleen removed, and in this situation, as demonstrated in the 50 per cent splenectomized group, no rise occurs. Because of the relative crudeness of the methods, it cannot be dogmatically stated that this experiment is conclusive, although it does lend support to the hormonal concept of splenic function. We have noted that parabiotics show much less variation in their leukocyte counts than do single rats, both between partners and in the same partner at different times.

When rats are made deficient in pteroylglutamic acid, the circulating leukocytes decrease progressively as a result of a decreased rate of production of white cells. If the hypothesis that the spleen is primarily a white cell destroying organ is true, then in such animals splenectomy should result in a decrease in the rate of development of the leukopenia since the cells which are being produced should survive longer. On the other hand, if the spleen suppresses the synthesis or release of leukocytes from the marrow, then, in the presence of the much more powerful inhibiting factor of pteroylglutamic acid deficiency, little or no decrease in the rate of development of leukopenia should be noted after splenectomy. In the experiments reported here, no change in the rate of development of leukopenia was found. This would suggest that the correct theory is the latter. Two criticisms might be raised of this experiment, however, which prevent it from being conclusive. First, the stress of the operation, by increasing pteroylglutamic acid requirements, might accelerate the deficiency and mask any rise which might occur. However, if this were true, the control operated group should have shown a more rapid fall, which was not the case. Secondly, splenic function might be considerably impaired by the deficiency so that splenectomy simply removed an already inactive organ.

Although none of these experiments can be said to be conclusive, they support the concept of a hormonal regulation of the leukocyte level by the spleen, rather than the concept of the spleen as an organ of destruction only. These experiments throw no light on the nature or mechanism of this hormonal influence.

Since the venous drainage of the spleen is into the portal vein, it has been suggested that the liver may play a role in the mechanism by which the spleen influ-
ences the number of blood cells. Leukopenia has been described in experimental carbon tetrachloride poisoning, and Singer has reported that the injection of certain organ extracts into rats whose liver function was impaired as a result of the administration of carbon tetrachloride produces thrombocytopenia, whereas animals with normal liver function failed to develop thrombocytopenia. In this laboratory studies in 60 rats of the effects of acute and chronic liver damage due to carbon tetrachloride poisoning have not revealed leukopenia, nor have any differences between the response of splenectomized and intact rats been noted. Attempts to produce acute liver insufficiency by partial heptectomy in approximately 15 rats have also failed to demonstrate any difference in the leukocyte count of animals with and without spleens.

Bock and Frenzel and Jombres have reported a transient reduction of all blood cellular elements following ligation of the splenic vein with resulting congestion of the spleen, or after by-passing of the liver by splenic venous blood. We have repeated their operative procedure, as well as a number of variations of it, in 20 rabbits, 50 rats, and 3 dogs, and have found no change in the numbers of blood cells.

Attempts to produce leukopenia in intact rats, and to reduce the leukocytosis of splenectomized rats by the injection of various types of extracts of rat, beef and human spleen have thus far failed to demonstrate any leukocyte reducing effect which was specific for the spleen. Extracts of human spleen removed from patients with idiopathic thrombocytopenic purpura, acquired hemolytic anemia, and pancytopenia relieved by splenectomy have been injected, but no effects have been noted on the leukocyte count, either after single injections or following prolonged administration.

**Summary**

1. Following splenectomy in the albino rat, the total leukocyte count increased approximately 100 per cent in seven days, and remained significantly elevated for seventy to ninety days, after which time the leukocytes returned to normal levels. This increase in circulating leukocytes was due to an increase in both neutrophils and mononuclear cells. Partial omentectomy and unilateral nephrectomy produced increases of less magnitude and much shorter duration than those which followed splenectomy.

2. Removal of as much as 75 per cent of the spleen resulted in a leukocyte increase resembling in magnitude and duration that of control operations.

3. When small portions (less than 10 per cent) of the spleen were transplanted to other sites, the leukocyte response also resembled that which followed the control operations.

4. When splenectomy was performed in one partner of parabiotic rats, no rise occurred in the leukocyte count of either animal. When the spleen of the second partner was then removed, a rise in the leukocyte count of both animals occurred.

5. When rats were made leukopenic by pteroylglutamic acid deficiency, no rise in the leukocytes in the peripheral blood occurred following splenectomy.

6. It is concluded that in the rat the spleen exerts an influence on the level of
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circulating leukocytes, and that the results of these experiments support but do not conclusively prove the hypothesis that this organ exerts this influence by controlling the rate of production and/or liberation of leukocytes in the bone marrow. These studies do not exclude the possibility that under certain circumstances the spleen may destroy white cells.

REFERENCES


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