The Experimental Production of Splenomegaly, Anemia and Leukopenia in Albino Rats

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The frequent association of splenomegaly with a decrease in one or more of the formed elements of the blood in such conditions as cirrhosis of the liver (Banti's syndrome),\(^1\) rheumatoid arthritis (Felty's syndrome),\(^2\) visceral leishmaniasis (Kala-Azar),\(^3\) Gaucher's disease,\(^4\) tuberculosis,\(^5\) syphilis, sarcoidosis, malaria and Hodgkin's disease\(^6,\) \(^7\) has long been recognized. In many instances splenectomy has been followed by either temporary or permanent alleviation of the hemopoietic abnormalities even though the underlying disease process has not been altered. To such disorders the term "secondary hypersplenism" has been applied.\(^6,\) \(^7\) The precise manner in which the spleen is related to the hemopoietic abnormalities has been a matter of controversy.\(^6,\) \(^10\)

Because of the many limitations imposed on research in human subjects, studies in experimental animals were begun in this laboratory in 1949 in an effort to elucidate the role of the spleen in hemopoiesis.\(^11\)

In an earlier study we described the well sustained leukocytosis which follows splenectomy in the rat.\(^11\) Partial omentectomy and unilateral nephrectomy produced increases in the leukocyte count of less magnitude and much shorter duration than those which followed splenectomy. When the spleen was removed and as little as 10 per cent of it was transplanted into the abdominal wall, the usual post-splenectomy leukocytosis was not observed, the leukocytosis comparing in magnitude and duration only with that of the control operations. When splenectomy was performed in one partner of parabiotic rats, no rise occurred in the leukocyte count of either animal. If, however, the spleen of the second partner was then removed, there was a rise in the leukocyte count of both animals. When the production of leukocytes in the marrow was suppressed by the administration of a folic acid antagonist, splenectomy was not followed by an increase in the leukocyte count. The results of these experiments were interpreted as supporting, although not conclusively proving, the hypothesis that this organ exerts its influence by controlling the rate of production and/or liberation of leukocytes in the bone marrow.

Since in the above studies removal of the spleen was associated with leukocytosis, it seemed of interest to determine whether splenomegaly in the rat would be associated with leukopenia. If a condition analogous to "secondary hyper-
spleenism” in man could be induced in animals, a means would be available for studying the mechanisms involved. Therefore, experiments were undertaken in an effort to develop a method for producing “secondary hypersplenism.”

Massive splenomegaly has been produced by Hueper in rabbits, dogs and rats by the repeated injection of various nonphysiologic macromolecular polymers such as polyvinyl alcohol, methyl cellulose, acacia, pectin, gelatin and ovalbumin. Apparently, these high molecular polymers are not metabolized in the body and are stored in the tissues, particularly the liver, kidneys and spleen. Although Hueper’s primary interest was in the production of atheromatosis, he recorded various hematologic changes following the administration of these compounds which he designated as the “hematologic macromolecular syndrome.” A transient leukopenia and thrombocytopenia, mild anemia, increased sedimentation of erythrocytes and prolongation of the coagulation time were observed im-

| Group | Condition | No. of Rats | Initial Body Wt. Gm. | Final Body Wt.* Gm. | Ascitic Fluid Gm. | Spleen Wt. Gm. | Spleen Wt. Body Wt. × 100 | Serum Creatinine mg. *%
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<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>274 ±7.5</td>
<td>428 ±9.8</td>
<td>0 ±0.1</td>
<td>1.2</td>
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<td>II</td>
<td>Methyl Cellulose</td>
<td>10</td>
<td>269 ±8.4</td>
<td>396 ±16.9</td>
<td>15 ±0.3</td>
<td>3.8</td>
<td>0.98 ±0.030</td>
<td>0.39 ±0.030</td>
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<tr>
<td>III</td>
<td>Splenectomy</td>
<td>20</td>
<td>276 ±5.5</td>
<td>386 ±8.0</td>
<td>2 ±0.6</td>
<td>0 ±4.9</td>
<td>0 ±6.6</td>
<td>0 ±6.6</td>
</tr>
<tr>
<td>IV</td>
<td>Splenectomy</td>
<td>20</td>
<td>276 ±4.9</td>
<td>422 ±6.6</td>
<td>0 ±4.9</td>
<td>0 ±6.6</td>
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* After withdrawal of the ascitic fluid.
The figures represent mean ± S.E.

Immediately following a single injection. Longer continued administration of the compounds was accompanied by a moderately severe anemia and thrombocytopenia, but no striking alteration in the leukocyte count was observed. Noting the similarity between the lesions produced by macromolecular storage diseases in animals and the lesions of Gaucher's disease and glycogen storage disease, Hueper suggested that the anemia observed in his animals might be the result of inhibition of the bone marrow secondary to the marked proliferation of the reticulum cells observed in the large spleens of these animals.

In the present paper we have reported investigations with methyl cellulose in rats which show that long continued administration results in splenomegaly with anemia, leukopenia and, in some instances, thrombocytopenia, and that the hematologic abnormalities are greatly modified by the absence of the spleen.

**METHODS**

Sixty male albino rats of the Sprague-Dawley strain were used in this study. They were housed in individual wire cages and fed Purina dog chow ad libitum. The animals were divided into four groups as shown in table 1. Splenectomy was performed on the animals in Groups III and IV by a technic previously described. Two days after splenectomy a
leukocyte count and hemoglobin determination were made on each animal, in all four
groups, from freely flowing tail vein blood.

Methyl cellulose of a viscosity grade of 400 centipoises was obtained as a dry powder,
and dissolved by slowly adding 2.5 Gm. to 100 ml. of distilled water at a temperature of
approximately 80 C. with constant stirring. The mixture was then allowed to cool and a
clear viscous solution formed which was refrigerated until used.

Two ml. of the methyl cellulose solution were injected intraperitoneally twice weekly
through an 18 gauge needle into each animal in Groups II and III. After 15 weeks, leukocyte
and hemoglobin determinations were made from tail vein blood. All animals were then
anesthetized with pentobarbital and weighed. The abdominal cavity was opened, any
ascitic fluid present was drained and the animals reweighed. For the determination of red
cell indexes, reticulocytes and platelet counts, approximately 8 ml. of blood were withdrawn
from the aorta and 5 ml. placed in bottles containing balanced oxalate as an anticoagulant.
Serum for creatinine and bilirubin determinations was obtained from the remainder
of the blood. Portions of spleen, bone marrow, liver and kidney were taken for histologic study.
All hematologic determinations were performed according to technics described elsewhere, the dry method for enumerating reticulocytes being used. Platelets were determined by the
Rees-Ecker method.

RESULTS

No immediate effects of the injections were noted in the animals, although acute hematologic abnormalities were not looked for. After the injections had been continued for six to eight weeks, the animals in Group II began to appear listless, pallor could be noted in their eyes, and enlargement of the abdomen was observed. In some of the animals the enlarged spleen could be readily felt in the abdomen. These signs became more pronounced as the injections were continued. By the fifteenth week 2 animals had died. At autopsy massive splenomegaly was present and, therefore, the experiment was terminated as previously outlined.

Upon opening the abdomen of the animals in Groups II and III, varying amounts of straw colored, slightly opaque ascitic fluid were observed. The greatest quantity was found in the animals of Group II, where up to 60 ml. were obtained from a single rat. The greatest amount found in the abdomen of the animals in Group III was 12 ml.; in both groups some animals had no fluid and fluid was not found in the abdomen of any animal not receiving methyl cellulose. The average amount of fluid for each group is shown in table 1.

All animals in Group II were found to have massively enlarged spleens as compared with the animals in Group I. The spleen weight of Group II animals ranged from 2.63 to 5.24 Gm. and averaged approximately 1 per cent of the total body weight. The spleens from the control animals ranged from 0.84 to 1.52 Gm., with an average of approximately 0.3 per cent of the total body weight. Frequently, whitish-gray areas were seen on the surface of the spleen. Figure 1 is a photograph of four spleens from animals of comparable weight from each of these two groups.

Microscopic examination of the spleens of the animals in Group II revealed that approximately one-third of the red pulp was taken up by round or ovoid clusters of macrophages ranging from about 100 to 250 \( \mu \) in diameter. Many of the clusters contained a few lymphocytes and reticulum cells. An occasional cluster was composed almost entirely of reticulum cells, fibroblasts and lymphocytes and resembled a granuloma. Frequently, the clusters were surrounded by
a rim of lymphocytes which made the cluster stand out distinctly. There was also diffuse hyperplasia of the reticulum of the red pulp. The Malpighian corpuscles were relatively decreased in number and did not contain clusters of storage cells. Storage of hemosiderin was absent, in contrast to the control spleens.

The conspicuous features of the bone marrows of the animals given methyl cellulose (Groups II and III) were increased cellularity and a moderate number of storage cell clusters and granulomas. The marrow cavities were depleted of fat cells. The hemopoietic elements were increased in number. The marrows of the splenectomized control group (Group IV) were more cellular than those of the non-splenectomized control group (Group I), but the difference was not striking.

The livers of the animals given methyl cellulose (Groups II and III) showed a scattering of storage cell clusters and granulomas. These were smaller than those seen in the spleen and were present in both the center and the periphery of the lobules. The liver cells adjacent to the granulomas showed a moderate degree of pressure atrophy. Otherwise, the parenchymal cells and sinusoidal lining cells were not remarkable. No transitions from parenchymal or endothelial cells to storage cells were seen. Storage cell clusters and granulomas were somewhat more frequent in the splenectomized animals (Groups III) than in the non-splenectomized animals (Group II) but the difference was not impressive.

The kidneys of the rats which had received methyl cellulose showed pronounced changes. Most of the glomeruli contained numerous storage cells. The capillary bed was totally or subtotally obliterated. A rare glomerulus showed only one or two storage cells. The involved glomeruli were enlarged and appeared as grape-like clusters of storage cells with an occasional patent capillary coursing between the clusters of storage cells. The epithelium of many tubules showed moderate to severe degenerative changes. The cortical stroma contained occasional bands or clusters of storage cells. There was also a moderate
Fig. 2.—Showing the histopathologic alterations.

1.—Spleen from rat given methyl cellulose (Group II). Storage cell cluster at left, surrounded by irregular rim of lymphocytes. Small “granuloma” at right, in periphery of Malpighian body. X 301

2.—Splenic capsule of rat given methyl cellulose (Group II). Note thickening of capsule, bands of macrophages, and surface adhesions. Also note storage cell clusters in red pulp below. X 109

3.—Bone marrow of control rat (Group I). X 251

4.—Bone marrow of rat given methyl cellulose (Group II). Note increased cellularity and absence of fat cells. X 251
irregular infiltration of the stroma with inflammatory cells. No conspicuous differences between the kidneys of the animals in Group II and Group III were observed.

The histopathologic alterations are illustrated in figure 2.

The hematologic data are given in table 2. Prior to the administration of methyl cellulose the splenectomized rats showed significantly greater leukocyte counts than the control rats, but did not show significant differences in hemoglobin or body weight. Following fifteen weeks of methyl cellulose administration a marked decrease in the leukocyte count of the animals in Group II was present, whereas no significant change was seen in the leukocyte counts of any of the other groups. Thus, although animals in Group III received the same amount

<table>
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<th>Group</th>
<th>Condition</th>
<th>Initial Values</th>
<th>Final Values</th>
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<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>19.6 ± 1.47</td>
<td>17.6 ± 0.25</td>
</tr>
<tr>
<td>II</td>
<td>Methyl Cellulose</td>
<td>22.7 ± 1.69</td>
<td>17.8 ± 0.11</td>
</tr>
<tr>
<td>III</td>
<td>Splenectomy</td>
<td>35.4 ± 2.04</td>
<td>16.9 ± 0.11</td>
</tr>
<tr>
<td>IV</td>
<td>Splenectomy</td>
<td>35.7 ± 2.33</td>
<td>17.1 ± 0.30</td>
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</table>

The figures represent the mean ± S.E.

The initial values in Groups III and IV were obtained two days after splenectomy. P.M.N., polymorphonuclear cells including eosinophils and basophils; M.N.C., mononuclear cells including lymphocytes and monocytes; M.C.V., mean corpuscular volume; M.C.H.C., mean corpuscular hemoglobin concentration.

of methyl cellulose, no leukopenia was observed in any of the animals; the only other known difference between these two groups was the absence of the spleen in Group III rats. From the differential counts it may be observed that the leukopenia in Group II was due almost entirely to a reduction in the mononuclear cells. In individual animals, there was no definite correlation between spleen size and leukocyte count.

(5)—"Granuloma" in bone marrow of rat given methyl cellulose (Group II). Note storage cells in periphery of granuloma. × 511

(6)—Liver of rat given methyl cellulose (Group II). At left, storage cell cluster in periportal space. Small granuloma at right and at bottom. × 251

(7)—Liver of rat given methyl cellulose (Group II). Clusters of clear storage cells surrounding nodule of lymphoid cells in periportal space. × 511

(8)—Kidney of rat given methyl cellulose (Group II). Glomerulus in center subtotally replaced by storage cells, with capillary crossing its center. At right and above, glomerulus with isolated storage cells in capillary tufts. × 143

(9)—Kidney of rat given methyl cellulose (Group II). Cluster of interstitial storage cells in center. Note degeneration of tubular epithelium. × 715
A significant decrease in hemoglobin concentration was observed in the animals in Group II. A decrease in erythrocyte count and in volume of packed red cells of similar degree was present, so that the red cell indexes were not significantly altered from the normal (Group I). In the splenectomized animals receiving methyl cellulose (Group III) a decrease in hemoglobin also occurred although it was much less marked than that in the nonsplenectomized animals. Red cell indexes in these rats were also not significantly different from those of the control animals. In addition to the anemia, striking reticulocytosis developed in all animals in Group II, whereas no significant change in reticulocytes was observed in the other groups. Serum bilirubin determinations were made in all animals but none could be detected.

The lowest platelet counts were observed in Group II, whereas the platelet counts of splenectomized animals were significantly higher than in the nonsplenectomized groups. The platelet counts of the animals in Group II ranged from 190,000 to 842,000 but, with the exception of the one animal with a count of 842,000, all the rats in this group had platelet counts below 500,000. The platelet counts of the control (Group I) animals ranged from 500,000 to 630,000. The difference between the means of these two groups did not prove to be significant although, with the exception of the one animal, there was no overlapping of counts in the two groups.

Differential counts were performed on marrow smears from three animals in each group but no significant differences were observed.

**Discussion**

The resemblance of the disorder produced in the rats by the injection of methyl cellulose to the clinical syndrome in man to which the term “secondary hypersplenism” has been applied is rather striking. This syndrome, both in rats and in man, is characterized by splenomegaly, hyperplasia of the bone marrow, normocytic, normochromic anemia and leukopenia. In man the disorder is associated frequently, although not invariably, with thrombocytopenia. In the rats a mild thrombocytopenia was observed in 9 out of 10 animals. In man the hematologic alterations are alleviated by splenectomy. In the rat, splenectomy prevented the development of anemia, leukopenia and thrombocytopenia.

The experiments presented herein, other than demonstrating a relationship between the presence or absence of the spleen and the reduction in the cellular elements in the blood, offer no explanation for the pathogenesis of these changes. That infiltration of the bone marrow by methyl cellulose-containing macrophages is not the explanation is suggested by the following observations: (1) infiltration of the marrow by such macrophages was only moderate; (2) pancytopenia failed to develop in splenectomized animals even though an equal degree of infiltration of the marrow was present; and (3) the bone marrows of the animals given methyl cellulose were characterized by hyperplasia of the blood-forming tissue.

The finding of normocytic, normochromic anemia with reticulocytosis suggests that hemolysis may play a role in the pathogenesis of the anemia. The failure to observe bilirubinemia in the animals does not mitigate strongly against the anemia being hemolytic because of the apparent ease with which rats clear
large amounts of bilirubin from the plasma. Since the kidneys were heavily laden with methyl cellulose-containing cells, the possibility exists that the blood changes were due to impaired renal function. This seems unlikely since the blood changes were not present in the splenectomized rats and nitrogen retention, as evidenced by serum creatinine levels, was not present.

The etiology of the ascites in the animals given injections of methyl cellulose is not obvious. Fluid retention in the abdomen may have resulted from the increased intraperitoneal colloid osmotic pressure produced by the presence of the methyl cellulose, or from increased portal venous pressure, perhaps with low serum protein levels resulting as a consequence of impaired hepatic function. It is regrettable that liver function was not studied in these animals. Although the serum creatinine level was not increased, it is possible that diminished renal function with fluid retention could be an explanation.

With this simple procedure for developing a syndrome in rats similar to "secondary hypersplenism" in man, it should be possible to carry out definitive studies concerning the mechanism whereby splenomegaly influences the circulating levels of the formed elements of the blood.

**Summary**

The intraperitoneal administration of methyl cellulose into rats over a period of fifteen weeks resulted in the development of a syndrome characterized by massive splenomegaly, hyperplasia of the bone marrow elements, normocytic, normochromic anemia, reticulocytosis, leukopenia, a mild thrombocytopenia in 9 of the 10 animals, ascites, and infiltration of the spleen, liver and kidneys with "storage-cell" macrophages. The administration of methyl cellulose to rats previously splenectomized produced similar histologic lesions but failed to produce the hematologic abnormalities.

It is suggested that the syndrome produced by methyl cellulose may represent an experimental form of the process to which, in man, the term "secondary hypersplenism" has been applied.

**REFERENCES**

EXPERIMENTAL PRODUCTION OF SPLENOMEGALY IN RATS

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The Experimental Production of Splenomegaly, Anemia and Leukopenia in Albino Rats

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