THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

V. CHANGES IN THE ENDOTHELIAL CELLS OF THE LYMPH NODES AND LIVER IN SPLENECTOMIZED ANIMALS RECEIVING HEMOLYTIC SERUM.*

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PLATE 77.

In the course of our investigation of the relation of the spleen to blood changes and to jaundice, routine histological examinations have been made. These have, for the most part, revealed only the well known lesions—liver necrosis, the degenerations occurring in epithelial organs, exudative lesions of the kidneys, and the general hemorrhages described by various workers who have studied the

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action of hemolytic serum. The liver and lymph nodes, however, presented in a small group of animals changes which appear to be of significance in connection with the general problem of blood destruction in the absence of the spleen. The most striking of these changes, and the one that we shall describe, is the great increase in the phagocytic power of the endothelial cells of the liver and lymph nodes for red blood corpuscles.

It is not our intention to discuss the literature concerning the destruction of red cells. This has been well presented up to 1893 by Gabbi, and up to 1901 by Hunter. The more recent literature has added little either in fact or theory that is new. Out of the mass of contradictory statements there is uniformity of opinion on only two points: (1) that large endothelial cells of the spleen (the red blood corpuscle-carrying cells) have the power to engulf red blood cells; and (2) that the presence (in anemia and malaria) of blood pigment in the cells (Kupfer's cells) of the liver capillaries indicates that these cells play some part in the destruction of red blood cells. On the other hand, it is not generally admitted that the endothelial cells of the lymph nodes likewise have this power. That phagocytosis of red cells, wherever it occurs, leads ultimately to the freeing of hemoglobin which eventually reaches the liver and is transformed into bile pigment is the opinion of all who support the theory that this mechanism plays a part in the destruction of red blood cells. There is, however, no uniformity of opinion as to whether the hemoglobin is set free in the liver from red cells carried there by the phagocytes or whether it is set free by the phagocytes elsewhere and carried to the liver in another way.

It is only with one phase of the subject, the rôle of endothelial cells in engulfing red cells in the absence of the spleen, that we are concerned. Our hypothesis is that in the absence of the spleen the endothelial cells of the lymph nodes and liver compensate for the loss of similar cells of the spleen.

This possibility was first brought to our attention in the routine examination of tissues from splenectomized dogs which had received specific hemolytic immune serum. So striking were some of

the pictures, that we undertook, for the sake of control, the study of the liver and lymph nodes from a number of normal dogs, of normal dogs receiving hemolytic serum, and of dogs which had been splenectomized for various lengths of time, but which had not received hemolytic serum.

Lymph Nodes.—The lymph nodes studied have been, for the most part, the mesenteric, gastrohepatic, prevertebral, and bronchial. In the normal animal these have been examined more particularly for the frequency of mitosis, for the number of endothelial cells in the sinuses, and for the presence of cells containing red blood corpuscles. Careful study of nodes from five normal animals showed that mitotic figures are found only after prolonged search and are usually limited to the follicles. The number of endothelial cells varies, but usually is not great, and they never occur in large masses in the sinuses. These cells, however, not infrequently contain old blood pigment, and occasionally a cell may be seen containing one or two red blood corpuscles.

The lymph nodes of five animals splenectomized for 3, 4 (2), 39, and 84 days and not subjected to the action of hemolytic serum have been studied in the same way. In two animals representing respectively 4 and 84 days, the lymph nodes differed in no way from the normal; in the other animals mitotic figures were abundant in the follicles and the endothelial cells in the sinuses were greatly increased in number. Prolonged search, however, failed to demonstrate mitotic figures in the latter cells, and although they occasionally contained one or two red blood corpuscles, this power of phagocytosis did not appear to be greater than in the non-splenectomized animal. The increase in number of endothelial cells was, however, very striking.

The literature of splenectomy offers little aid in determining the histological changes occurring in the lymph nodes after removal of the spleen. In the literature at our disposal no definite descriptions have been found except those of Warthin,* who found in

sheep and goats an increase in the phagocytic power of the endothelial cells for red blood corpuscles. Gabbi, who worked with the guinea pig, states that a transient increase of the red blood corpuscle-carrying cells may possibly occur in early periods after splenectomy, but that after three to six months they are no more abundant than in the normal animal.

The lymph nodes of five normal dogs which had received specific hemolytic immune serum and had died or been chloroformed after periods varying from twenty-four hours to nine days, showed the lesions usually described as common to various cytotoxic sera and especially to lymphotoxic sera. These are edema, increase of polymorphonuclear leucocytes, focal areas of necrosis, abundant mitotic figures in the follicles, and slightly greater frequency of large endothelial cells capable of phagocytosis of red cells.

Of animals that had been splenectomized and had received hemolytic serum as well, twelve were available for histological examination. Of these, three showed no change in the lymph nodes and five showed a well marked increase in the number of endothelial cells in the sinuses, but no increase in power of phagocytosis. In the four remaining animals the sinuses contained a great number of large endothelial cells filled with red blood corpuscles. The analysis of these findings is somewhat difficult, as three factors must be considered: (1) the length of time after splenectomy; (2) the lapse of time between administration of hemolytic serum and the death of the animal; and (3) the degree of red cell destruction caused by the serum.

These factors are brought out in the following table.

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6 Warthin also describes a new formation of hemolymph nodes. This we have not observed in any of the nineteen splenectomized dogs. This, however, is not surprising as the demonstration of hemolymph nodes in the dog is difficult, and, moreover, only three of our animals have survived splenectomy for more than four months.

Gabbi, D., loc. cit.


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<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Period of splenectomy</th>
<th>Period after serum</th>
<th>Effect of serum</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3 dys.</td>
<td>36 hrs.</td>
<td>Hemoglobinuria</td>
<td>Extreme phagocytosis of red cells.</td>
</tr>
<tr>
<td>II</td>
<td>10 mos.</td>
<td>36 hrs.</td>
<td>Severe jaundice</td>
<td>Well marked phagocytosis of red cells.</td>
</tr>
<tr>
<td>III</td>
<td>9½ mos.</td>
<td>18 hrs.</td>
<td>No record</td>
<td>Well marked phagocytosis of red cells.</td>
</tr>
<tr>
<td>IV</td>
<td>7½ mos.</td>
<td>48 hrs.</td>
<td>Hemoglobinuria</td>
<td>Moderate phagocytosis of red cells.</td>
</tr>
<tr>
<td>V</td>
<td>15 dys.</td>
<td>3 dys.</td>
<td>Hemoglobinuria</td>
<td>Negative.</td>
</tr>
<tr>
<td>VI</td>
<td>27 dys.</td>
<td>3 dys.</td>
<td>Hemoglobinuria</td>
<td>Proliferation of endothelial cells.</td>
</tr>
<tr>
<td>VII</td>
<td>33 dys.</td>
<td>4 dys.</td>
<td>Jaundice</td>
<td>Proliferation of endothelial cells.</td>
</tr>
<tr>
<td>VIII</td>
<td>65 dys.</td>
<td>8 dys.</td>
<td>Hemoglobinuria</td>
<td>Negative.</td>
</tr>
<tr>
<td>IX</td>
<td>6 dys.</td>
<td>9 dys.</td>
<td>Hemoglobinuria</td>
<td>Proliferation of endothelial cells.</td>
</tr>
<tr>
<td>X</td>
<td>3 dys.</td>
<td>9 dys.</td>
<td>No hemoglobinuria or jaundice</td>
<td>Negative.</td>
</tr>
<tr>
<td>XI</td>
<td>103 dys.</td>
<td>10 dys.</td>
<td>No hemoglobinuria (spontaneous jaundice)</td>
<td>Proliferation of endothelial cells.</td>
</tr>
<tr>
<td>XII</td>
<td>25 dys.</td>
<td>15 dys.</td>
<td>Jaundice</td>
<td>Proliferation of endothelial cells.</td>
</tr>
</tbody>
</table>

From this analysis it is seen that the proliferation of the endothelial cells did not occur in the animals (V, IX, and X) splenec-tomized for periods of from three to fifteen days, but was evident in five (VI, VII, VIII, XI, and XII) in which the time elapsing since splenectomy was 27 to 103 days. On the other hand, the lymph nodes of these animals did not present evidence of increased phagocytosis of red cells. Whether this was due to the period which had elapsed (three to fifteen days) since injection of serum cannot be determined, but this was probably the case. Certainly it was not due to failure of hemolysis, for at least two of these animals (VI and VIII) presented evidence of extreme blood destruction. That the period of time elapsing may be an important factor is shown by the fact that all animals (four) presenting evidence of extensive phagocytosis of red cells represent periods of eighteen to forty-eight hours after injection of the serum. In the absence of exact knowledge of the length of time necessary for the destruction of red cells by phagocytic endothelial cells, it is useless to offer surmises, but one cannot escape the fact that in this investigation all evidence of active phagocytosis is seen in animals dying within forty-eight hours. It is possible therefore that the destruction of red cells by phagocytosis may be completed within forty-eight hours and this view is supported by the frequency with which pigment is found in the lymph nodes at later periods.
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It is also evident that the time elapsing since splenectomy bears no relation to the occurrence of phagocytosis of red cells, for the most marked example of the latter was seen in a dog dying three days after splenectomy, while moderate and well marked phagocytosis occurred likewise after 7½, 9½, and 10 months.

Liver.—Examination of the stellate endothelial cells (Kupfer's cells) of the liver has been rendered difficult on account of the intense congestion and abundant necroses which occur after the administration of hemolytic immune serum. For this reason we have not always been able to correlate the evidence of phagocytosis in the liver with the lesion described in the lymph nodes during the early (forty-eight hour) period. Definite evidence, however, of phagocytosis has been found in four animals, representing periods of 1, 2, 8, and 9 days after the administration of serum, and representing, respectively, periods of 3 days, 7½ months, 65 days, and 6 days after splenectomy. Also in a fifth animal, twenty-five days after splenectomy, and fifteen days after the administration of the serum, the cells of the capillaries contained small balls of yellow pigment, apparently representing altered hemoglobin.

As controls we have examined the livers of several normal dogs and of nine splenectomized dogs not receiving serum, but without finding evidence of phagocytosis on the part of the cells of the liver capillaries, or of proliferation of these cells. The splenectomies in this series represented periods of from three to eighty-four days, five under ten days and three over twenty days.

Likewise we have examined the livers of nine normal dogs receiving hemolytic serum. In two of these the endothelial cells appeared to be increased somewhat in number, and no undoubted evidence of phagocytosis could be obtained. All other livers examined showed no changes in the cells of the capillaries.

The details of some of these observations follow:

Phagocytosis of Red Blood Cells by Stellate Cells of the Liver.

Dog 32.—Splenectomy was performed under ether anesthesia on July 19, 1911; spontaneous jaundice was noted on March 8, 1912, and specific hemolytic immune serum injected intravenously on this date. Hemolysis resulted. The red cells dropped within three hours from 6,120,000 to 5,200,000 per cubic millimeter, and the hemoglobin, after twenty hours, to 42 per cent. Death occurred after forty-eight hours.
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Histology.—The liver cells are pale, granular, stain poorly, and present here and there small areas of focal necrosis. The capillaries are dilated and contain much granular material and, as seen by the low power, numerous isolated round and oval clumps of red blood corpuscles. By higher power of the microscope these clumps of red cells are found to be, in large part, within endothelial cells (figure 4). Some of the red cells stain well with eosin, others appear as shadows. Other endothelial cells are seen which contain mere fragments of red cells or masses of granular, yellow pigment, or large, yellow hyaline balls of apparently fused, red cells. Attempts to demonstrate similar phagocytic cells in the large vessels of the liver and of other organs failed; they were present, however, in the sinuses of the lymph nodes.

Phagocytosis of Red Cells by Endothelial Cells of the Lymph Nodes.

Dog 34.—Splenectomy was performed under ether anesthesia on March 11, and hemolytic serum administered intravenously on March 14. Death occurred on March 15 after reduction of red cells to 1,060,000 and hemoglobin to 57 per cent. Hemoglobinuria was marked.

Histology.—A mesenteric lymph node shows hemorrhage, edema, and extensive infiltration with polymorphonuclear leucocytes. The sinuses, both peripheral and central, are closely packed with large, pale, endothelial cells, nearly all of which contain red blood cells, a single high power field showing thirty to forty phagocytic cells (figures 1 and 2). The number of engulfed red cells varies but is usually large, ten to twenty not infrequently being found in a single cell. In many endothelial cells, on the other hand, the red cells have fused to form large, round, or oval hyaline masses still staining deeply with eosin. Between the phagocytic cells is much granular, eosin-staining material suggesting disintegrated red cells, mingled with serum, through which run irregular threads of fibrin. Here and there in the follicles are small areas of necrosis. Moderate leucocytic infiltration is seen throughout the section. Phagocytic cells cannot be demonstrated in the blood-vessels or in a tangle of lymphatic vessels present at one side of the node.

Other lymph nodes (gastrohepatic, prevertebral, and bronchial) present the same lesions.

The liver of this animal showed widespread necrosis but in the non-necrotic areas phagocytic endothelial cells are found in the capillaries (figure 3).

SUMMARY.

In a large proportion of dogs that have been splenectomized for periods of two weeks or more, one finds a great increase in the number of endothelial cells of the lymph nodes. In most splenectomized dogs that succumb to an injection of hemolytic immune serum within forty-eight hours, the sinuses of the lymph nodes contain large numbers of endothelial cells, phagocytic for red cells. This is not seen in normal dogs receiving hemolytic serum. Like-
wise a similar power of phagocytosis is seen frequently in the stel-
late cells of the capillaries of the liver. Both in the lymph nodes
and the liver these cells appear to be formed in situ; we find no
evidence that they have been transported to these organs.

Such findings suggest the development of a compensatory func-
tion on the part of the lymph nodes and possibly of the liver. Nor-
mally the spleen contains cells which have the power to engulf and
presumably to destroy the red blood corpuscles. In certain patho-
logical conditions this function is frequently greatly augmented and
may sometimes be shared by the lymph nodes, for example, in ty-
phoid fever, as was first clearly shown by Mallory.\textsuperscript{9} Our observa-
tions suggest that in the absence of the spleen, this function of
forming red blood corpuscle-phagocytizing cells, normally a minor
activity of the lymph nodes, becomes highly developed in the latter
organs, and that these cells, and the stellate cells of the liver, thus
assume, in part at least, the function of destroying red blood cor-
puscles by phagocytosis.

In view of the somewhat limited material at our disposal, we
offer this, not as definitely conclusive, but as evidence which, in con-
nection with the work of others, is highly suggestive of the pos-
sibility of the lymph nodes assuming some of the function of the
spleen.

Whether this activity of the endothelial cells of the lymph nodes
and the liver has any bearing on the anemia that follows splenec-
tomy and on the occurrence of spontaneous jaundice in the late
periods after splenectomy, is not yet clear.

\textsuperscript{9} Mallory, F. B., A Histological Study of Typhoid Fever, \textit{Jour. Exp. Med.},
1898, iii, 611.
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EXPLANATION OF PLATE 77.

The drawings were made with the camera lucida and with a Spencer microscope, objective, 4 mm., ocular, 8.

Figures 1, 2, and 3 represent lesions in a dog that was splenectomized on March 11, 1912, received hemolytic serum on March 14, and died on March 15. The actual lapse of time was about thirty-six hours.

Fig. 1. Peripheral sinus of a mesenteric lymph node containing large numbers of endothelial cells filled with red blood corpuscles and occasionally also polymorphonuclear leucocytes.

Fig. 2. Similar cells in a central sinus of the same lymph node.

Fig. 3. A section of liver with two Kupfer cells containing red blood corpuscles.

Fig. 4. Similar to figure 3, but from a dog that was splenectomized on July 19, 1911, received hemolytic serum on March 8, 1912, and died after forty-eight hours.