CONCISE REPORT

Intrasplenic Microcirculation in Rats With Acute Hemolytic Anemia

By L. T. Chen

Intrasplenic microcirculation in rats with acute hemolytic anemia has been studied by means of the microsphere method. Alteration in the intrasplenic microcirculation was observed in the anemic rats. The open circulation was reduced from 98% in the normal rats to 67% in the anemic rats, while the closed circulation was increased from 2% in the normal rats to 33% in the anemic ones. The reduction in the open circulation in the anemic rats appears to be attributed to the congestion in the splenic cords as a result of the retardation of the passage of damaged red blood cells. Restoration of the open circulation in rats on the first day of full recovery from anemia seems to be associated with a decrease in the congestion in the splenic cords.

THE FUNCTION of the spleen is closely related to the nature of its intrasplenic microcirculation. However, the regulation of intrasplenic microcirculation is not well understood. The intrasplenic microcirculation could be altered in the spleens with various pathologic conditions. This is suggested by the mixing kinetic studies of $^{31}$Cr-labeled red blood cells, which show that the time required for complete mixing of labeled red blood cells in the spleen is much longer in the pathologic spleens than in the normal ones. The mechanisms for such alterations are poorly understood because of lack of methodology for investigating the intrasplenic microcirculation. Recently, a method for measuring the relative proportion of blood traveling the open circulation and closed circulation in the spleen has been reported. Such a method can be used to study the nature of the intrasplenic microcirculation in the pathologic spleens.

The purpose of this study was (1) to investigate if there were any changes in the proportion of blood taking the open and closed circulation in the spleen of rats with acute hemolytic anemia, and (2) to determine the extent of such changes and its relationship to the splenomegaly.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 250–300 g were purchased from Charles River, Wilmington, Mass.

Induction of Acute Hemolytic Anemia

Acute hemolytic anemia was induced by a single intraperitoneal injection of phenylhydrazine hydrochloride (Baker Chemical Co., N.J.) at a dose of 10 mg/100 g body weight. Phenylhydrazine hydrochloride was prepared as a 0.2% solution in saline. Phenylhydrazine-treated rats served as controls. Hematocrits, reticulocyte counts, and spleen weights of phenylhydrazine-treated rats are shown in Table 1. The hematocrit dropped from 45% to 25% 1 day after phenylhydrazine injection and reached its lowest level (23%) on day 3. It began to rise on day 4 and returned to the normal value on day 7. Day 7 is considered the first full day of recovery from acute hemolytic anemia. The spleen weight was increased gradually following the phenylhydrazine treatment, doubled on day 3, and reached its highest level on day 7.

RESULTS

A single i.p. injection of phenylhydrazine (10 mg/100 g body weight) in rats resulted in an acute hemolytic anemia. The hematocrits, reticulocyte counts, and spleen weights of phenylhydrazine-treated rats are shown in Table 1. The hematocrit dropped from 45% to 25% 1 day after phenylhydrazine injection and reached its lowest level (23%) on day 3. It began to rise on day 4 and returned to the normal value on day 7. Day 7 is considered the first full day of recovery from acute hemolytic anemia. The spleen weight was increased gradually following the phenylhydrazine treatment, doubled on day 3, and reached its highest level on day 7.

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In the normal rats, about 98% of the blood entering the spleen traveled the open circulation and the other 2% traveled the closed circulation (Table 2). However, in the anemic rats with an enlarged spleen (day 3 following phenylhydrazine treatment), the open circulation was reduced to 67% while the closed circulation increased to 33%. On day 7, rats recovered from acute hemolytic anemia still had an enlarged spleen. At this time the open circulation and the closed circulation had returned to their near normal level.

Morphological studies reveal that in the spleen of normal rats, erythropoiesis was not apparent and a few red blood cells were scattered in the cords of the red pulp (Fig. 1). In contrast to the normal rats, there were numerous red blood cells localized in the cords and concentrated around the sinuses in the spleens of anemic rats. Extensive erythropoiesis in the red pulp became apparent (Fig. 2). Many red blood cells contained Heinz bodies and were seen in the slits of the sinus wall (Fig. 3). In rats that had recovered from acute hemolytic anemia, erythropoiesis in the spleen had subsided. The number and distribution of red blood cells in the spleen were similar to those of normal rats (Fig. 4).

**DISCUSSION**

A new method employing a suitable size of rigid microspheres for investigating the nature of intrasplenic microcirculation has been recently reported. This method demonstrates that both the open circulation and closed circulation exist in the spleen and that over 90% of the blood takes the open route of circulation in the normal unanesthetized rabbit. By applying the microsphere method to the study of the splenic microcirculation in rats, the present report shows that there is an alteration in the intrasplenic microcirculation in rats with acute hemolytic anemia. In the normal anesthetized rats, about 98% of the blood travels the open route of circulation and 2% takes the closed route. In contrast to the normal rats, only 67% of the blood takes the open route and the rest travels the closed route in the anemic rats. The alteration in the intrasplenic microcirculation in the anemic rats is restored to the near normal state shortly following the recovery of rats from anemia.

The reduction in the proportion of the blood traveling the open circulation in the spleen of anemic rats could be partly due to congestion in the open route. This is strongly suggested by the presence of a large number of red blood cells containing Heinz bodies in the cordal meshwork and around the splenic sinuses of the anemic rats. Red blood cells, which contain Heinz bodies, are known to be less pliant than the normal ones and consequently are delayed or retarded in their passage through the cordal meshwork and the slits of the sinus wall. As a result, the splenic congestion ensues. Increased erythropoiesis in the cords may also contribute to the congestion in the cords. Because of the cordal congestion, a part of the blood traveling the open circulation may be diverted to the closed circulation. This may explain an increase in the closed circulation in the anemic rats.

In rats that have recently recovered from an acute hemolytic anemia, and whose spleen weight is 2.5 times the normal one, the splenic congestion appears to be greatly reduced. This is suggested by a large decrease in the number of normal red blood cells and developing red blood cells in the cords. As a result of

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**Table 1. Hematocrit, Reticulocyte Count, and Spleen Weight of Rats Following a Single i.p. Injection of Phenylhydrazine (10 mg/100 g Body Weight)**

<table>
<thead>
<tr>
<th>Time After Injection (Day)</th>
<th>Hematocrit (%)</th>
<th>Reticulocyte Count (%)</th>
<th>Spleen Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control†</td>
<td>Ph‡</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>6</td>
<td>47.6 ± 0.6</td>
<td>2.0 ± 0.2</td>
<td>0.79 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>46.3 ± 4.0</td>
<td>3.1 ± 0.7</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>43.8 ± 1.5</td>
<td>1.8 ± 0.3</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>45.0 ± 1.3</td>
<td>2.0 ± 0.6</td>
<td>0.86 ± 0.02</td>
</tr>
<tr>
<td>7</td>
<td>45.8 ± 1.2</td>
<td>2.5 ± 0.9</td>
<td>0.85 ± 0.04</td>
</tr>
<tr>
<td>14</td>
<td>44.9 ± 1.8</td>
<td>1.4 ± 0.8</td>
<td>0.89 ± 0.05</td>
</tr>
</tbody>
</table>

*Mean values ± 1 SD calculated from a group of 5-8 rats.
†Saline-treated rats.
‡Ph, phenylhydrazine-treated rats.
§The difference statistically significant ($p < 0.01$) when compared to the control group.

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**Table 2. Splenic Microcirculation During Acute Anemia: Relative Distribution of Microspheres (3-4 μm) in the Spleen of Rats With Acute Anemia**

<table>
<thead>
<tr>
<th>Time After Phenylhydrazine Injection (Day)</th>
<th>Number of Microspheres in Anemias</th>
<th>Percentage* of Microspheres in Microspheres Counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0†</td>
<td>6</td>
<td>98.1 ± 0.4 1.9.0 ± 0.4 6.165</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>67.0 ± 3.6 33.0 ± 3.6 5.039</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>93.6 ± 0.9 6.4 ± 0.9 5.700</td>
</tr>
</tbody>
</table>

*Mean ± 1 standard deviation.
†Rats injected with saline served as controls.
Fig. 1. Red pulp of the spleen of a normal rat. A few erythrocytes were scattered in the cord. A microsphere in the cord is indicated by an arrow. S, sinus (x 500).

Fig. 2. Red pulp of the spleen of an anemic rat (day 3 after phenylhydrazine injection). Numerous erythrocytes were localized in the cord and concentrated around the sinus (S). Erythropoiesis (E) became apparent (x 500).

Fig. 3. Red pulp of the spleen of an anemic rat (day 3 after phenylhydrazine injection). Slits of the sinus wall were occupied by erythrocytes (arrow) that contained many Heinz bodies. C, cord; S, sinus (x 8000).

Fig. 4. Red pulp of a rat that had recovered from acute hemolytic anemia (day 7 after phenylhydrazine injection). The number and distribution of erythrocytes in the cord were similar to those of the normal rat (x 500).
the reduced congestion in the cords, the open circulation would be restored to the near normal level, in spite of splenomegaly.

It has been reported that the total blood flow to the spleen is altered in rats with acute hemolytic anemia. Such an alteration is paralleled to the changes in the intrasplenic microcirculation observed in the present study. That is, the total blood flow to the spleen is reduced in the anemic rats and restored to the near normal level in rats recovering from anemia. The reduction in the total splenic blood flow in the anemic rats may be attributed to the congestion in the splenic cords. This is also supported by the observation that the total splenic blood flow in the normal rats can be reduced by two-thirds after obstruction of the open circulation by application of rigid spherocytes.

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REFERENCES

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