Hereditary Spherocytosis

II. Observations on the Role of the Spleen

By LAWRENCE E. YOUNG, M.D., RICHARD F. PLATZER, M.D., DONALD M. ERVIN, M.D., AND MARY JANE IZZO, M.S.

Splenectomy promptly decelerates the destruction of red cells in practically all cases of hereditary spherocytosis despite the fact that the corpuscles remain abnormal. Consideration of this apparent paradox stimulated the authors to investigate the manner in which the spleen deals with the defective erythrocytes in such patients. The experiments to be reported show that (1) normal red blood cells transfused to persons with hereditary spherocytosis undergo little or no increase in osmotic and mechanical fragility; (2) the spleen in affected persons is capable of sequestering the patient's own spheroidal cells to a greater degree than transfused normal cells; (3) spherocytes trapped in the spleen undergo greater increase in osmotic fragility than do normal cells; and (4) spleens from patients suffering from thrombocytopenic purpura (i.e., non-hemolytic disease) are also capable of trapping perfused spherocytes in preference to normal discoidal red corpuscles.

Observations on the clinical, hematologic and genetic features in 28 cases of hereditary spherocytosis recently studied by the authors are presented in an accompanying report.29

Recent investigations on the circulation of the spleen should be cited as a preface to the observations to be described in this paper. MacKenzie, Whipple and Wintersteiner16, 17 have used Knisely's13,14 method of transillumination in detailed studies of the splenic circulation of various animals. These authors are of the opinion that the arterioles of the spleen terminate in the interstices of the pulp producing an "open" circulation. They believe that the venous sinuses are not intact but have small apertures which permit the entrance of cells from the pulp with some distortion, and within the pulp the circulating cells have been observed to progress at variable rates of speed through tortuous channels before entering the venous sinuses. Whipple18 has suggested that normal discoidal erythrocytes may pass through the pulp and enter the venous sinuses with greater ease than would spherocytes. MacKenzie and associates17 state that "generally speaking, the spleen pulp filters the blood; during the process of filtration, all components of the blood are exposed to whatever influences, direct or indirect, the tissue of the pulp may be capable of exerting upon them." Gibson and co-workers16 have demonstrated that the hematocrit of blood within "small vessels" of dog spleens is approximately twice as high as that found in any other organ.
but the site at which hemoconcentration occurs in the spleen was not investigated by this group.

Bjorkmar's description of the retention within the venous sinuses of (1) spherocytes produced by saponin injection, (2) red cells agglutinated by gelatin and (3) rice grains having a mean diameter of 3.5 μ or more. Normal nonagglutinated, discoidal cells and rice grains of smaller diameter were observed to be filtered from the sinuses into the pulp. Bjorkman's concept of splenic circulation differs from that of MacKenzie and co-workers in that he believes that relatively thick bodies are trapped in the sinuses rather than in the pulp.

The observations to be described in this paper demonstrate that the relatively thick cells from persons with hereditary spherocytosis are readily trapped in the spleen—most likely in the pulp. While sequestered, the red corpuscles are temporarily removed from active circulation and may undergo changes similar to those observed in cells incubated in vitro. These observations confirm the findings of Emerson, Shen, Ham and Castle.

**Methods—I**

**Determination of Osmotic and Mechanical Fragility of Donated and Patient’s Cells in Peripheral Venous Blood**

During the week preceding operation, patients about to be splenectomized were transfused with normal fresh whole blood in acid-citrate-dextrose solution or with red blood cells of type which could be differentiated serologically from those of the recipients by use of the Ashby technic. Transfusions were continued if possible until the number of donated corpuscles in the recipient’s circulation was nearly equal to that of the patient’s own cells. A sample of defibrinated blood was obtained from the antecubital vein of the patient one or two days prior to operation (and at least one or two days after the last of the series of transfusions) and the osmotic and mechanical fragility of the red cells was determined by the methods of Shen, Ham and Fleming, respectively. Further details pertaining to these methods are described in an accompanying paper.

The unhemolyzed cells remaining in the bottom of each tube employed in the measurements of fragility were resuspended in 0.85 per cent solution of sodium chloride. The density of these suspensions was adjusted visually to match that of a similar tube containing 1 part of normal whole blood in 200 parts of saline. Heavier suspensions were prepared in some instances in order to provide larger numbers of unagglutinated cells following differential agglutination with appropriate antisera. The usual procedure was to transfer 0.2 ml. of each cell suspension to each of 4 small test tubes (8 x 60 mm.). Dried anti-M or anti-A serum (Lederle) was then added to 2 of the tubes and the proportions of donated and recipient’s cells were determined by the previously described method used in this laboratory.

The per cent hemolysis of the donated cells in each tube used in the fragility tests was computed by the formula:

\[
\text{Per cent hemolysis of donated cells} = \left[ A - \frac{(B \times C)}{A} \right] \times 100
\]

in which \(A\) = average per cent donated cells in 3 or more tubes showing no hemolysis; \(B\) = per cent of donated cells remaining in given tube after hemolysis has occurred; \(C\) = fraction of all cells unhemolyzed in given tube.

Per cent hemolysis of the recipient’s red cells in each tube was calculated in the same manner.
Determinations of Osmotic and Mechanical Fragility of Donated and Patient’s Cells in Spleenic Blood

All spleens were removed under nitrous-oxide and ether anesthesia.* In each case the vasa brevia were separated and the spleen was freed from surrounding tissues, after which the entire splenic pedicle was clamped no longer than from 3 to 5 minutes before the organ was delivered from the abdomen. Care was taken to prevent engorgement of the spleen which would occur if the splenic vein were ligated prior to ligation of the splenic artery.

As soon as the spleen had been removed from the patient, the hemostats were released from the splenic vein and the freely flowing blood was collected and a portion used for measurements of erythrocyte fragility. A large part of the spleen was then minced in a meat grinder and the ground tissue suspended in compatible normal serum or in the patient’s serum. Large tissue particles were removed by filtering the suspension through coarse gauze, after which the erythrocytes were washed 3 times in serum and were separated as completely as possible from the white blood corpuscles and splenic cells. Erythrocytes thus obtained from the minced spleen were suspended in sufficient compatible normal serum or in the patient’s serum to give a hematocrit of 35 per cent. Fragility of the donated cells and the patient’s cells in the splenic minced blood was then determined by the same methods used for venous blood.

Description of Cases

The procedures described were carried out in 3 patients with hereditary spherocytosis and in a 15 year old girl with pancytopenia and multiple congenital abnormalities (Fanconi syndrome). None of these patients was in crisis at the time the studies were made. The principal features of these cases† are listed in table 1. The following additional information is also pertinent:

Case 1 (J. X., Case 14 of report 1). One liter of whole blood was transfused into this patient on the fifth day and another liter on the fourth day before splenectomy. Each transfusion was followed by a mild febrile reaction.

Case 2 (L. E., Case 26 of report 1). One-half liter of whole blood was given on the eighth day and a second transfusion of 450 ml. on the seventh day before splenectomy. A mild febrile reaction followed the first transfusion.

Case 3 (S. D., Case 3 of report 1). Transfusions of 250 ml. of whole blood were given without reaction on the third, fourth and fifth days before splenectomy. Since the patient’s red blood cell count was 4.4 M. before transfusion, 100 ml. of blood were removed from the patient prior to each of the 3 transfusions.

Case of Fanconi syndrome (C. O.). This patient, who weighed only 30 Kg., was subjected to splenectomy chiefly with the hope that recurrent thrombocytopenic purpura might be arrested. She was given 500 ml. of packed group 0 red blood cells on the day preceding operation and the transfusion was followed by a febrile reaction but there was no hemoglobinemia or hyperbilirubinemia.

* The authors are indebted to Drs. J. J. Morton, H. E. Pearse, W. J. M. Scott, T. B. Jones and E. B. Mahoney for their cooperation in performing splenectomy in cases referred to in both Part I and Part II of this paper.

† Dr. John S. Lawrence gave generous assistance in early phases of this study and enabled the authors to conduct studies on Case 1. Cases 2 and 3 and the patient with Fanconi syndrome were investigated through the kindness of Dr. S. W. Clausen.
# HEREDITARY SPHEROCYTOSIS. II.

## RESULTS—I

The proportions of donated and patient's cells in splenic mince blood and in venous blood, taken in each case at the time the splenic pedicle was clamped, are given in table 2. The relatively large proportion of patient's cells in the splenic blood, as compared with peripheral venous blood, indicated that the spheroidal cells were selectively retained in the spleens of the 3 patients with hereditary spherocytosis. In the patient with the Fanconi syndrome, on the other hand, the difference in the proportions of patient's and donated cells in the samples of peripheral and splenic blood was not significant.

### TABLE 1—Principal Features in 3 Cases of Hereditary Spherocytosis and Case of Fanconi Syndrome Transfused Prior to Splenectomy (Part I) and in Spherocytic Donor (Part II)

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Red cell count before transfusion</th>
<th>Highest pre-op reticulocyte count</th>
<th>Highest pre-op serum bilirubin</th>
<th>Spherocytosis in patient</th>
<th>Spherocytosis in relatives</th>
<th>Coombs antigen-antibulin test on red cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>48</td>
<td>M</td>
<td>3.4</td>
<td>5.0</td>
<td>1.3</td>
<td>slight</td>
<td>slight</td>
<td>neg.</td>
</tr>
<tr>
<td>No. 2</td>
<td>7</td>
<td>M</td>
<td>3.7</td>
<td>2.1</td>
<td>1.5</td>
<td>moderate</td>
<td>none*</td>
<td>neg.</td>
</tr>
<tr>
<td>No. 3</td>
<td>4</td>
<td>F</td>
<td>4.4</td>
<td>6.5</td>
<td>5.9</td>
<td>moderate</td>
<td>marked</td>
<td>neg.</td>
</tr>
<tr>
<td>Fanconi syndrome</td>
<td>15</td>
<td>F</td>
<td>2.4</td>
<td>2.9</td>
<td>—</td>
<td>none</td>
<td>not examined</td>
<td>neg.</td>
</tr>
<tr>
<td>Donor of spherocytes part II</td>
<td>27</td>
<td>M</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>moderate</td>
<td>reported</td>
<td>neg.</td>
</tr>
</tbody>
</table>

### TABLE 2—Response to Splenectomy

<table>
<thead>
<tr>
<th>Case</th>
<th>Erythrocyte fragility</th>
<th>Patients' blood types</th>
<th>Donors' blood types</th>
<th>Response to Splenectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>fresh blood</td>
<td>incubated blood</td>
<td>osmotic mechanical</td>
<td>osmotic mechanical</td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>slightly increased</td>
<td>markedly increased</td>
<td>OMNRh</td>
<td>ONRh−</td>
</tr>
<tr>
<td>No. 2</td>
<td>increased</td>
<td>markedly increased</td>
<td>OMNRh−</td>
<td>ONRh−</td>
</tr>
<tr>
<td>No. 3</td>
<td>increased</td>
<td>markedly increased</td>
<td>AMNRh+</td>
<td>ANRh+</td>
</tr>
<tr>
<td>Fanconi syndrome</td>
<td>slightly decreased</td>
<td>normal</td>
<td>AMNRh+</td>
<td>OMNRh+</td>
</tr>
<tr>
<td>Donor of spherocytes part II</td>
<td>increased</td>
<td>markedly increased</td>
<td>OMNRh+</td>
<td>—</td>
</tr>
</tbody>
</table>

* Patient's father could not be reached for examination.
† Red cell counts and serum bilirubin concentration of the spherocyte donor were normal at the time of donations.
‡ The donor of the spherocytes had been splenectomized with excellent response about 5 years prior to these experiments.
The gross and microscopic findings in the spleen in all 3 spherocytic cases were similar. The splenic tissue was engorged with red cells most of which were thought to be within the pulp while the venous sinusoids appeared relatively empty and collapsed. Red cells were not numerous in the spleen removed from the patient exhibiting the Faneconi syndrome.

The osmotic fragility of donated and patient's erythrocytes in venous and splenic mince blood is shown graphically in figure 1. Tonicities at which more than 90 per cent hemolysis occurred are not plotted in the graphs of this paper because their determination is considered relatively inaccurate by the method employed. The marked increase in fragility of the patients' spheroidal cells, which had been trapped in the spleen, is in contrast to the relatively small and irregular shifts in fragility of donated cells. Irregularities encountered in determining fragility of donated cells in splenic mince blood are attributable in part to technical difficulties in measuring hemolysis of the small proportion of donated corpuscles in the splenic cell population. The osmotic fragility of the patients' cells in peripheral venous blood was only slightly greater than that of the normal donated corpuscles in Cases 1 and 3, while in Case 2 the curve for patient's cells showed a "tail," such as is often seen in this disease prior to splenectomy. The apparently greater than normal fragility of the donated cells in peripheral blood of Case 1 may have been the result of difficulties in maintaining the pH of the blood specimens at 7.4 in this experiment.

Figure 2 (Left) shows a smear prepared from the capillary blood of Patient 1 (J. N.) at the time of splenectomy and figure 2 (Right) shows a smear of splenic mince blood from the same patient. Only 2 or 3 spheroidal cells are seen in the former while many spherocytes are evident in the latter. In view of the fragility curves in figure 1, it is reasonable to assume that most, if not all, of the spherocytes in the splenic smear are the patient's corpuscles. The patient's red cells comprised 61 per cent of those in the peripheral blood and 84 per cent of the red cells in the splenic blood.

The mechanical fragility of the normal donated cells in the samples of venous blood drawn after transfusion, but before splenectomy, was not increased in any of the 3 cases. The mechanical fragility of the patients' cells was increased to approximately the same extent observed prior to transfusion. The differential mechanical fragility of donated and patients' cells in splenic mince blood could not be measured to our satisfaction.

The osmotic fragility curves for donated and recipient's cells in the splenic

---

**Table 2—Proportions of Patient's and Donated Erythrocytes in Peripheral Venous Blood and in Splenic Mince Blood of Patients Transfused Prior to Splenectomy**

<table>
<thead>
<tr>
<th>Case</th>
<th>Per Cent Patient's Cells In</th>
<th>Per Cent Donated Cells In</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peripheral Venous Blood</td>
<td>Splenic Mince Blood</td>
</tr>
<tr>
<td>No. 1. (J. N.)</td>
<td>61</td>
<td>84</td>
</tr>
<tr>
<td>No. 2. (L. E.)</td>
<td>43</td>
<td>80</td>
</tr>
<tr>
<td>No. 3. (S. D.)</td>
<td>49</td>
<td>90</td>
</tr>
<tr>
<td>Faneconi Syndrome (C. O.)</td>
<td>53</td>
<td>55</td>
</tr>
</tbody>
</table>
NORMAL DONATED CELLS
IN PT. FRESH VENOUS BLOOD
NORMAL DONATED CELLS
IN PT. SPLENIC MINCE BLOOD
PT CELLS IN OWN FRESH VENOUS BLOOD
PT CELLS IN OWN SPLENIC MINCE BLOOD

CASE I.

CASE 2

CASE 3

Fig. 1.—Osmotic fragility curves of donated and patients' erythrocytes in peripheral venous blood and in splenic mince blood of 3 patients with hereditary spherocytosis. The lower portion of the curve for donated cells in mince blood of Case 2 is omitted because of irregularities encountered in measuring partial hemolysis of the small numbers of cells present.

range for normal fresh venous blood in this laboratory. These results, together with the figures given in table 1, show clearly that in this control experiment the nonspheroidal cells of the recipient were not selectively trapped in the spleen and that the relatively small numbers of cells found in the spleen after operation
had not undergone increase in fragility. Despite the fact that all of the donated cells in this case had been transfused during the day immediately preceding operation, the proportion of donated corpuscles in the splenic blood was nearly the

Fig. 2.—(Left) Smear from capillary blood of Case 1 at the time of splenectomy. (Right) Smear of washed splenic mince blood from Case 1.

Fig. 3.—Osmotic fragility curves for donated and patient’s red cells in splenic mince blood of patient with Fanconi syndrome.

same as in venous blood. In the spheroctytic cases, on the other hand, the proportion of donated cells in the splenic blood was relatively small even though these cells had been transfused from four to eight days prior to splenectomy.

Figure 4 shows the osmotic fragility curves of red cells in splenic mince blood,
spleenic vein (pedicle) blood and in peripheral venous blood of a fourth patient, (P. D.), who was subjected to splenectomy without having been transfused. This patient, like his 2 brothers, exhibited mild icterus, spherocytosis, reticulocytosis and splenomegaly but no anemia. The intermediate position of the curve for blood from the splenic vein or pedicle was also observed in the transfused cases of hereditary spherocytosis. In our experience, and in that of Dacie and others, the “tail” of the osmotic fragility curve has largely disappeared after splenectomy in such patients. This finding, together with the larger tail in the curve for blood from the splenic vein, suggests that the cells most susceptible to lysis in hypotonic saline may be those that have passed through the spleen after having been imprisoned for a time in the splenic “incubator.” Curves for

![Graph showing osmotic fragility curves](https://example.com/graph.png)

**Fig. 4.—** Osmotic fragility curves obtained with erythrocytes in samples of blood from minced spleen, splenic vein and antecubital vein of P. D., nontransfused patient with hereditary spherocytosis (Case 9 of report I9).

cells taken directly from the splenic pulp (mince blood) reveal greatly increased fragility of a large proportion of the corpuscles, and are similar to curves obtained when peripheral blood from patients with hereditary spherocytosis (either pre- or post-splenectomy) is incubated in vitro at 37 C. for 24 hours.29

Figure 5 shows a lateral plot of the osmotic fragility of the red cells in the peripheral venous blood of patient F. C. (Case 17 of report I) before, during and after splenectomy, which was performed under nitrous-oxide and ether anesthesia. This patient, like P. D., was not anemic and was not transfused. Fragility was maximal in the sample drawn one hour after clamping of the splenic pedicle, possibly because many fragile cells were forced into the circulation from the spleen when the organ was mobilized just prior to clamping the pedicle. The spleen in this case weighed 730 Gm. and the patient weighed 55 Kg.
There was a sharp decrease in fragility between the first and third postoperative days followed by a more gradual decrease during the ensuing weeks.

The results thus far described are essentially in agreement with those obtained by Emerson, Shen, Ham and Castle, whose methods were employed with minor deviations in Part I of this study. These experiments demonstrated that the spleen in patients with hereditary spherocytosis is capable of selective sequestration of spheroidal cells. It then remained to be determined whether the selective filtration was due to a peculiarity of the spleen in this disease or to the effect which almost any human spleen might have upon relatively thick corpuscles. The observations described in Part II were made in order to throw light on this question.

**Fig. 5.—Lateral plot of osmotic fragility of red corpuscles in peripheral venous blood of patient, F. C., showing fluctuations in fragility related to splenectomy.**

**Method—II**

The second method of study involved perfusion of spleens obtained at operation from patients suffering with idiopathic thrombocytopenic purpura. Such spleens cannot be considered normal but nevertheless they were removed from patients whose red cells were not being rapidly destroyed. Blood used to perfuse the spleens was a mixture of group O erythrocytes from a splenectomized individual with hereditary spherocytosis (Case 13 of report 19) and normal group A or group B blood which was compatible with the blood of the thrombocytopenic patient and could be differentiated serologically from the spheroidal cells. The group O cells from heparinized blood were washed 3 times with normal heparinized A or B plasma and suspended in the same plasma before being mixed with the normal A or B heparinized whole blood. The mixtures of spherocytic and normal blood were prepared about 18 to 20 hours prior to perfusion, and were stored at 4° C. until 30 to 60 minutes before the start of perfusion, at which time they were placed in an incubator at 37° C.

In removing the spleen an attempt was made to clamp the splenic artery and vein simultaneously not more than a few minutes before the spleen was actually delivered from the body. Once the organ was excised, it was immediately brought to the laboratory and one
or two of the largest splenic arteries were cannulated within 10 to 20 minutes. Before perfusion was started, a small portion of the spleen was sliced off one end of the organ and used for preparation of fixed tissue sections to be compared with sections made after perfusion. Perfusion was accomplished by the use of an automatic pipeting machine* adjusted to deliver approximately 20 ml. per minute (usually 10 strokes of 2 ml. each). Red cells were washed from the spleen by perfusing the organ with warm normal saline for about 10 minutes or until the washings were grossly clear. The mixture of spheroidal and normal cells was then added to the vessel in which the spleen was placed so that the intake tube of the pipeting machine drew up the blood and pumped it through the spleen. The perfused mixture emerged from the splenic veins, and from the pole of the spleen which had been sliced open for preparation of tissue sections, and ran into the vessel from which it was again drawn up by the intake tube. The temperature was maintained at 37 C. throughout the experiment by use of a water bath. The blood was mixed gently every 2 or 3 minutes and at appropriate intervals samples of approximately 0.5 ml. were removed with a Pasteur pipet for measurements of hematocrit and for estimating the proportions of the 2 types of cells by differential agglutination.

Perfusion was in each case continued for 2 or 3 hours during which time the volume of the mixture diminished due to evaporation and to loss of cells to the spleen (table 3). As soon as perfusion was discontinued, fixed tissue sections were prepared from the central portion of the spleen, which was believed to have been most thoroughly perfused. Most of the central portion was then ground and the red cells from the minced spleen were washed and suspended in compatible normal serum by use of the same technic employed in part I.

**Table 3—Weight of Spleens and Volume of Perfusion Mixtures Before and After Perfusion**

<table>
<thead>
<tr>
<th>Perfusion Number</th>
<th>Weight of Spleen Before Perfusion</th>
<th>Volume of Perfusion Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gm.</td>
<td>ml.</td>
</tr>
<tr>
<td>1</td>
<td>238</td>
<td>130</td>
</tr>
<tr>
<td>2</td>
<td>225</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>120</td>
</tr>
<tr>
<td>control</td>
<td>98</td>
<td>60</td>
</tr>
</tbody>
</table>

* Decrease in volume of perfusion mixture was due to (1) loss of blood to the spleen, (2) evaporation and (3) removal of samples.

Results—II

Perfusion was carried out with spleens removed from 4 patients with idiopathic thrombocytopenic purpura.† Three of the spleens were perfused with a mixture of group O spheroidal cells and normal A or B cells, while the fourth spleen was perfused as a control with a mixture of two normal blood specimens. The spherocytic blood was obtained in each instance from the same splenectomized individual with hereditary spherocytosis (Case 13 of report I). The erythrocytes of the spherocytic group O donor measured 6.7 μ in diameter and 2.7 μ in thickness at the time of the first perfusion which was five years after the donor's spleen had been removed. Other features of the donor's case have been included in table 1. Additional information concerning the perfusion studies is provided in table 3.

* Manufactured by the Baltimore Biological Laboratory, Baltimore, Md.

† The spleen used in perfusion experiment 2 was removed by Dr. Bradford Simmons at the Clifton Springs Sanitarium and Clinic. Dr. Samuel Munford kindly provided facilities at the Clinic which made this experiment possible.
The results of the perfusion experiments are shown graphically in figure 6. It is evident that in each instance the proportion of spheroidal cells became less and the proportion of normal cells became greater during perfusion. Selective trapping of the group O spherocytes within the spleen was confirmed at the end of each experiment by demonstrating that these cells were present in the samples of splenic mince blood in significantly greater proportions than in the perfused mixtures. These findings are more remarkable in view of the fact that the thrombocytopenic donors of the spleens belonged to the same major blood groups (B,
A and A respectively) as the donors of the normal cells used in the perfusion mixtures. Any cells from the thrombocytopenic blood that had not been washed from the spleen during perfusion with saline would therefore have contributed to the proportion of normal cells found in the splenic mince samples after perfusion. Despite some evaporation during perfusion, a decrease in hematocrit was to be expected because of the loss of red cells to the spleen, and because of hemolysis of a small portion of the cells during the procedure.

In the control experiment the proportions of normal ON and normal OMN cells in the perfused mixture did not change significantly and the two types of normal cells were found in the splenic mince blood in essentially the same proportion as in the perfused mixture.

Sections taken from the tips of the spleens prior to perfusion contained relatively few red blood cells while those prepared after perfusion with spherocytes showed considerable packing of erythrocytes in the pulp, as had been observed in spleens removed from patients with hereditary spherocytosis.

Spleens from normal human beings or from persons having no blood dyscrasia could not be obtained for perfusion during the period of this study. It is, nevertheless, significant that spleens from patients having no hemolytic disease proved capable of selective trapping of spheroidal cells.

**Discussion**

The observations of Emerson, Shen, Ham and Castle as well as those described in this paper and in an earlier report from this laboratory make it clear that cells from patients with hereditary spherocytosis are more readily trapped in the spleen than are normal red corpuscles. The perfusion experiments show, moreover, that spleens from patients with idiopathic thrombocytopenic purpura may be capable of selective imprisonment of spheroidal cells in essentially the same manner as spleens from patients with spherocytosis. It seems likely that spleens from normal individuals would also selectively retain spheroidal cells. This supposition is supported by the fact that red corpuscles from patients with hereditary spherocytosis survive only a short time after transfusion to normal recipients. Since splenectomy greatly decelerates red cell destruction in patients with spherocytosis, it is not surprising that spheroidal cells survive for much longer periods when transfused into splenectomized recipients.

Dacie has summarized existing reports on the microscopic findings in the spleen in cases of hereditary spherocytosis, and he concludes that engorgement of the organ with red cells is the most striking abnormality. He perfused excised human spleens with easily recognizable fowl’s cells and his results indicate that the spleen in hereditary spherocytosis has capillary-to-sinus pathways as direct as in normal individuals. He found it difficult, however, to wash spleens from patients with spherocytosis free of blood by perfusion with saline. He concluded that in this disease the splenic pulp “may be a backwater outside the main current of the blood stream.”

Whipple has suggested that spherocytes may be trapped in the pulp because their greater thickness does not permit them to pass as readily as normal discoidal cells through slit-like openings into the venous sinusoids. This hypothesis seems reasonable, but it has not yet been proved that the trapping phenomenon
is dependent solely upon the greater thickness of spheroidal cells. With regard to the site of sequestration, we are of the opinion that the cells become lodged in the pulp rather than in the venous sinususes. Examination of fixed sections of spleens removed from 15 patients with hereditary spherocytosis in the Strong Memorial Hospital revealed the presence of large numbers of red cells that appeared to be for the most part in the pulp, but in many areas it was impossible to determine the exact site in which the cells were trapped. The appearance of the sections was essentially the same in cases that had been transfused and in those that had not. Erythrophagocytosis was not a prominent finding in these sections, nor has it been in the experience of most observers.

Although the part which the spleen plays in disposing of normal erythrocytes may not be of great importance, it is well established that splenectomy decelerates red cell destruction in patients with hereditary spherocytosis. Erythrocytes from these individuals undergo greater increase in osmotic and mechanical fragility than do normal cells when incubated in vitro at body temperature, and it is therefore not surprising that similar differences in fragility are observed in cells that have stagnated in the backwater of the spleen. Dacie has observed, moreover, that cells from patients with hereditary spherocytosis undergo lysis more rapidly than normal cells when incubated in vitro and that such hemolysis is independent of complement and is not inhibited by normal plasma. It has been suggested that lysolecithin may be a factor in producing hemolysis of stagnating red cells, but the exact role of lysolecithin in in vivo hemolysis has not yet been established.

Our present concept of the role of the spleen in hereditary spherocytosis may be summarized as follows: spheroidal cells are readily trapped in the splenic pulp because their abnormal thickness does not permit them to escape easily through slit-like openings into the venous sinusoids. A normal spleen, however, is probably capable of trapping spheroidal cells as readily as the spleen in a patient with hereditary spherocytosis. Cells stagnating in the pulp are removed from the protective factors present in actively circulating blood and osmotically active substances accumulate within the cell (Ham and Castle). Some of the cells with greatly increased thickness manage to escape from the spleen and these cells probably account for the “tails” of osmotic fragility curves obtained with blood from the splenic vein and with peripheral venous blood from patients with intact spleens. Such cells are thought to be relatively susceptible to destruction by the wear and tear of circulation. Many of the cells remaining in the spleen undergo lysis, with the result that evidence of phagocytosis of whole red corpuscles in the spleens of such patients is not striking.

The exact nature of the abnormality of the erythrocyte in this disease and of the changes that occur in these cells when sequestered, or when incubated in vitro, is not clear. The pathogenesis of crises in individuals with hereditary spherocytosis also remains a mystery. It is difficult to explain all of the features of the crisis, especially the arrested maturation of erythroid cells in the marrow, purely on the basis of excessive sequestration of thick red cells in the spleen. Other forms of splenic activity postulated by Owren, Dameshek and Bloom and by Doan and Wright are in need of further investigation with particular reference to the crisis.
SUMMARY

1. Three patients with hereditary spherocytosis and 1 patient with the Fanconi syndrome (pancytopenia and multiple congenital abnormalities) were transfused prior to splenectomy with normal erythrocytes of types which could be differentiated serologically from those of the recipients. The proportions of donated and patient’s cells in peripheral blood and in blood washed from the minced spleens were determined by differential agglutination, and the osmotic and mechanical fragilities of the two types of cells in peripheral and splenic blood were measured by differential agglutination of the corpuscles remaining in each test tube after partial hemolysis had occurred.

2. In each case of hereditary spherocytosis the proportion of recipient’s cells was much higher in splenic than in peripheral blood, indicating selective retention of the thicker corpuscles within the spleen. Osmotic fragility of the patient’s red cells was much greater in samples of splenic minced blood than in peripheral venous samples, while the fragility of the donated red corpuscles was normal or nearly normal in both splenic and peripheral blood.

In the patient exhibiting the Fanconi syndrome, on the other hand, neither the patient’s red cells nor donated red cells were retained to any extent in the spleen and the fragility of neither type of cell was altered.

3. Spleens removed surgically from 3 patients with idiopathic thrombocytopenic purpura were perfused with mixtures of normal A or B cells and group O cells drawn from a splenectomized individual with hereditary spherocytosis. During perfusion the spheroidal cells were selectively removed from the mixtures and at the end of each experiment red cells of this type predominated in the blood samples washed from the minced spleens. A fourth excised spleen was perfused with a mixture of two types of normal cells, neither of which was retained to any extent by the spleen during perfusion. The perfusion experiments show that spleens from patients with nonhemolytic disease are also capable of selective trapping of spheroidal cells.

4. The experiments described indicate that the spleen acts as a filter and trap and as an “incubator” in accelerating destruction of red corpuscles in patients with hereditary spherocytosis.

REFERENCES


9 — — — AND —: Unpublished observations.


26 Whipple, A. O.: Recent studies in the circulation of the portal bed and of the spleen in relation to splenomegaly. Tr. & Stud., Coll. of Physicians, Philadelphia 8: 203, 1941.


Hereditary Spherocytosis: II. Observations on the Role of the Spleen

LAWRENCE E. YOUNG, RICHARD F. PLATZER, DONALD M. ERVIN and MARY JANE IZZO