Fine Structure of the Red Pulp of the Spleen in Hereditary Spherocytosis

By Zelma Molnar and Henry Rappaport

The spleens from two children and one adult with hereditary spherocytosis were studied in the electron microscope. Stagnation of the erythrocytes within the splenic cords is attributable to their lack of plasticity as evidenced by the absence of bilobed, tailed, or squeezed forms in transit through the walls of the sinuses. In contrast to the sections studied by conventional light microscopy, the splenic sinuses in hereditary spherocytosis were not "empty," but contained red blood cells, the majority of which had lost their hemoglobin content. Cordal macrophages were increased in all three cases and were abundant in the splenic cords of the adult patient, causing a further impediment to the rapid passage of erythrocytes. Macrophages, and, to a lesser degree, sinus endothelial cells contained the products of hemoglobin breakdown. The macrophages showed active erythrophagocytosis. Sinus endothelial cells rarely contained intact red blood cells, but showed pronounced pinocytotic activity, a probable mechanism of hemoglobin incorporation. Platelets within the endothelial cells of the sinuses were much more frequently seen in the three cases of hereditary spherocytosis than in control spleens. The presence of ferritin in platelets suggests that they too may play a role in clearing the end products of hemolysis from the spleen.

The deformability of red blood cells may be the basic key to the role played by the red pulp of the spleen in certain hemolytic disorders. The plasticity of the red blood corpuscles is put to a particularly grueling test within the microcirculation of the spleen. A multitude of internal and external factors influences the deformability of these cells.¹ ² In hereditary spherocytosis (H.S.) the erythrocytes exhibit a "rigidity" (or lack of plasticity)³ ⁴ making them vulnerable to trapping by the spleen.⁵ In H.S. of long duration a "work hyperplasia" of the macrophages seems to develop, leading to an increased cellularity of the pulp cords.⁶ ⁹ This results in a secondary hypersplenic state in which the increased destruction of red blood cells is no longer solely attributable to the stagnation of the spherocytes, but also to their exposure to increased numbers of macrophages which contributes to the widening of the cords.

The present study was undertaken in order to correlate and reconcile the...
fine structural appearance of the human spleen in H.S. with previously reported histologic features that are not entirely in keeping with the presently accepted biological concepts of the hemolytic disorder. In the description of conventional light microscopic appearance of H.S., emphasis is placed on the highly characteristic structure of the splenic red pulp: mainly, the pronounced widening of the splenic cords; a lack, or scarcity of the nucleated cells in them; “collapse” and/or emptiness of the splenic sinuses, and hyperplasia of the sinus lining cells. We were interested in the problem of correlating the lack of erythrophagocytosis, that could be observed by light microscopy, with the clinical observation, that in spite of the high rate of hemolysis, neither hemoglobinemia nor hemoglobinuria are evident in the disease.10 Finally, we were interested in the ultrastructural details of the mechanism of hemolysis in H.S., in particular, compared with splenic hemolysis in general.

Materials and Methods

The spleens from three patients with hereditary spherocytosis were obtained at operation and form the basis of this study. Pieces measuring 0.5 cu mm were fixed promptly in 3.5% collidine-buffered paraformaldehyde at pH 7.4 and postfixed in 1% osmium tetroxide.11 Dehydration in graded ethanol was followed by embedding in Epon resin. The blocks were sectioned on a Reichert OM U-2 ultramicrotome or on Servall MT-2 ultramicrotomes. Thick sections of plastic-embedded tissues were cut and stained with Mallory’s azure-2 methylene blue stain for selection of the fields in the light microscope. Ultrathin sections were mounted singly or in serial sections on formvar-covered copper grids. They were double-stained with lead citrate and uranyl acetate and were studied in a Philips EM 200 electron microscope. For studies on the presence of ferritin, ultrathin sections with a gray reflection were cut and stained briefly (3 min) with lead citrate alone. Spleens removed because of traumatic injuries from otherwise healthy individuals and uninvolved spleens obtained at staging procedures for malignant lymphoma were used as controls, and processed in an identical manner. The control spleens were from patients of comparable ages. Tissue fragments obtained simultaneously were promptly fixed in 10% formalin and processed for light microscopic studies. Sections were stained with hematoxylin and eosin, the Prussian blue method for iron-containing pigment, and with the periodic acid-Schiff reaction. The clinical data pertaining to the three patients with H.S. are summarized in Table 1. None of the three patients received transfusion prior to splenectomy.

Results

Light Microscopic Studies

Studies in the light microscope revealed the classical pattern of hereditary spherocytosis12-13 in all three patients (Fig. 1A). The splenic cords contained numerous erythrocytes, while the splenic sinuses appeared relatively empty. A hyperplasia of the lining cells was evident; it was pronounced in the spleen of the adult patient, less conspicuous in the children. Hemosiderin pigment was present in the particulate form within macrophages and in a more diffuse form in sinus endothelial cells. It is of interest that the amount of iron pigment was focally conspicuous within the endothelial cells of the dilated pulp veins immediately before they enter the trabecular veins (Fig. 1B).

Erythrophagocytosis was not demonstrable in the cordal compartment, presumably because it may have been obscured by the marked congestion.13 It could be demonstrated within the sinuses, particularly after ultrastructural studies called our attention to its presence. It was of considerable interest
## Table 1.—Clinical Data in Three Patients with Hereditary Spherocytosis (H.S.)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Significant Symptoms and/or Signs</th>
<th>Significant Laboratory Data Prior to Splenectomy</th>
<th>Blood Film</th>
<th>Osmotic Fragility</th>
<th>Weight of Spleen</th>
<th>Pertinent Observations in Family Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.E.P.</td>
<td>23</td>
<td>F</td>
<td>Anemia since age 14 (treated with iron) became severe during pregnancy at age 22</td>
<td>Hgb: 11.0 g/100 ml Reticulocytes: 3–20%</td>
<td>Spherocytosis evident</td>
<td>Increased (initial 0.55%, complete 0.35%)*</td>
<td>350 g</td>
<td>Gall bladder disease in six close blood relatives</td>
</tr>
<tr>
<td>J.A.A.</td>
<td>4</td>
<td>M</td>
<td>Jaundice diagnosed as “congenital hemolytic”</td>
<td>Hgb: 7.8 g/100 ml Reticulocytes: 17.0%</td>
<td>Spherocytes</td>
<td>Increased (initial 0.55%, complete 0.42%)*</td>
<td>85 g</td>
<td>Father: anemia with spherocytosis; mother and both grandmothers had anemia</td>
</tr>
<tr>
<td>T.B.</td>
<td>6</td>
<td>M</td>
<td>Enlarged spleen discovered on admission for upper respiratory infection</td>
<td>Hgb: 13.2 g/100 ml Reticulocytes: 5.7%</td>
<td>Many spherocytes</td>
<td>Increased (initial 0.55%, complete 0.35%)*</td>
<td>165 g</td>
<td>Father and two uncles had splenectomy in infancy because of spherocytic anemia, grandfather also had hereditary spherocytosis</td>
</tr>
</tbody>
</table>

*Normal value: initial, 0.46–40% saline; complete, 0.36–30% saline.
†After 24-hr incubation:

<table>
<thead>
<tr>
<th>Patient</th>
<th>R.E.P.</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85% saline</td>
<td>46.6%</td>
<td>1.5%</td>
</tr>
<tr>
<td>0.65% saline</td>
<td>100%</td>
<td>25.0%</td>
</tr>
<tr>
<td>0.45% saline</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
that intact red blood cells and hemosiderin pigment were evident within the same macrophages (Fig. 1C). We also noticed, following study of the electron micrographs, that the sinuses were not empty but only appeared to be so because most of the red blood cells within them were laked and thus had the characteristic appearance of so-called “ghosts” (Fig. 1D).

Ultrastructural Study

Red Cell Distribution Between Splenic Sinuses and Cords: In contrast to the classic histopathologic descriptions of the disease, in all three cases of H.S. examined, the sinuses as well as the cords contained red blood cells (Fig. 2); there were focal differences in the same spleen, ranging from tightly packed cords and sinuses to areas where RBC were scanty. Such local differences have been observed by Gripwall14 in the light microscope. The RBC within the cords and sinuses in the spleen with H.S. showed a spectrum of electron density indicating great variations in the hemoglobin content as reported by Wennberg and Weiss.15 The variations in electron density were particularly conspicuous within the cords. In them, the red blood cells showed either normal electron density or varying degrees of hemoglobin loss which was often extreme. In the sinuses, red blood cells with greatly decreased electron density predominated, indicating an abundance of so-called laked red blood cells or red cell ghosts (Fig. 3). Red blood cells in the cords had a plump appearance and often straddled narrow passages between the collagen-reticulin fibers of the cordal compartments as if unable to squeeze through (Fig. 4). The H.S. spleens examined lacked cells with long tails, compressed, or bilobed forms of erythrocytes in transit through the sinus wall. This ultrastructural evidence of deformability was observed in all of our control spleens (Fig. 5). Siderocytes with iron granules, and red blood cells containing vesicles or small vacuoles with electron-dense particles were seen only occasionally in the spleens in H.S. Pitting of such components, or fragmentation of the RBC was not observed. In one of our spleens with H.S. (R.E.P.) occasional red blood cells had angulated shapes and a higher electron density than the surrounding laked red blood cells or ghosts (Fig. 3). Similar red blood cells were also seen occasionally in several other spleens with or without known hemoglobin abnormality. "Triangulated" red blood cells were reported earlier by Dacie et al.16 in hemolytic anemia, as occurring under conditions of partial hemolysis.

Cordal Cellularity: Cordal Macrophages: A conspicuous finding in the three H.S. spleens was an increase in macrophages (Fig. 4). As was reported earlier

Fig. 1.—(A) Representative area of the red pulp of the spleen from a patient with hereditary spherocytosis. The cords are conspicuously widened, and the sinuses appear relatively empty. × 380. (B) Spleen of patient with hereditary spherocytosis. Iron pigment is abundant in endothelial cells of a pulp vein immediately before its junction with the trabecular vein. × 380. (C) Sinus of spleen of patient with hereditary spherocytosis contains several macrophages. Various phases of erythrophagocytosis are indicated, including seemingly intact, recently ingested RBC and hemosiderin pigment (arrow). × 1200. (D) Sinus of spleen from patient with hereditary spherocytosis containing laked red blood cells, some of which appear to be within a macrophage (center of sinus). Most of the erythrocytes in the surrounding cord retain their hemoglobin content. × 1200.
by Wennberg and Weiss,\textsuperscript{15} there was evidence of many more macrophages than was suspected from the study in the light microscope. Macrophages were found in the compartments of the widened cordal spaces among the red blood cells and were seen in various phases of transit into the sinuses (Figs. 2, 4). The number of mononuclear cells devoid of phagosomes was also increased, and like the

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Spleen of patient with H.S. Erythrocytes of varying electron density are seen in the cord (C) (lower left) and sinus (S) (right). A large area of the sinus is occupied by a macrophage (Ma). The pseudopod of another macrophage (arrow) extends between two endothelial cells (E). \times 8400.}
\end{figure}
Fig. 3.—Spleen of a patient with H.S. Laked red blood cells and an angular erythrocyte are seen within the splenic sinus (S). The nucleus of the endothelial cell (E) is deeply indented. Numerous pinocytotic vesicles, some of which contain a highly electron-dense material (arrows), are seen near the cell surfaces. The electron-dense basal material (arrowheads) is present in segments of the cell which extend between the interrupted basement membrane (bm). \( \times \) 13,800.

macrophages, they appeared in close contact with erythrocytes, often partially enclosing them (Fig. 6). The number of macrophages and nonphagocytosing monocytes varied in different areas of the same spleen and in the three H.S. spleens examined: they were moderately increased in the spleen of the youngest patient (J.A.A.), in comparison with control spleens. They were more numerous in the slightly older patient (T.B.) and abundant in the spleen of the oldest patient (R.E.P.).

Other Hematogenous Cells: Polymorphonuclear leukocytes appeared to be numerically increased in the splenic cords of two of the patients (R.E.P. and T.B.). This, however, was also observed in some of the controls. No increase in lymphocytes or plasma cells was evident. No red cell precursors were observed.

Cellularity of Sinuses: The Endothelial Lining Cells: Electron microscopic study of the sinus lining cells in both H.S. and control spleens revealed no differences in the basic components of these cells from those previously
Fig. 4.—Splenic cord in H.S. is occupied by macrophages (Ma) in various stages of erythrophagocytosis. Erythrocytes with rather plump shapes appear to straddle narrow passages (arrows) within the cord, partially enclosed by macrophages. × 10,750.

described in human spleens; the cells had abundant cytoplasmic filaments, particularly in the basal portion of the cells surrounding the electron-dense basal material (Figs. 2, 3, 5, 6, and 7). Nuclei of the lining cells often had deep indentations, and the surface of the cells had abundant pinocytotic and coated vesicles.
Fig. 5.—Control spleen, removed because of gun-shot wound. Plasticity of normal erythrocytes is indicated by formation of squeezed, tailed (A) and bilobed forms (B) during passage through the sinus wall. The nucleus of the endothelial cell (E) is indented. The electron-dense basal material (arrow) is prominent in areas of the endothelial cells which extend to the interrupted basement membrane (bm). The sinus lumen(s) occupies the upper part of both pictures. × 13,800.

As in the classic histologic description of the H.S. spleens, the light microscopic pathology indicated “prominent littoral cells” in the spleen of patient R.E.P.; at the ultrastructural level, a numerical increase of the sinus lining
cells was not clearly demonstrable in cross-sections of the sinuses. Macrophages in transit into the lumen, however, were frequently conspicuous. Although their nuclei were often surrounded by only a narrow rim of cytoplasm, these macrophages were readily separable from the endothelial lining cells (Figs. 2, 6), a separation that was not observable by light microscopy.

The number of macrophages, and other monocytes, was conspicuously increased in the splenic sinuses of one of our patients (R.E.P.) (Figs. 2, 4, 6), as was found by Wennberg and Weiss. In our studies, we observed regional
Fig. 7.—Spleen of patient with H.S. Longitudinal section through a row of sinus endothelial cells. Note the filamentous, electron-dense basal material (arrows), and abundant, longitudinally oriented cytoplasmic filaments. Numerous pinocytotic and coated vesicles are seen at the surface of the cells. Some have electron-dense contents (arrowheads). × 51,250.

Differences in the numbers of macrophages. These variations were much more pronounced in the sinuses than in the cords, ranging from sinuses that were tightly packed with macrophages (Fig. 2), to areas in the same spleen where sinuses appeared to be filled solely with ghosts of erythrocytes (Fig. 3). The number of macrophages and monocytes in the sinuses of the other two H.S. spleens examined appeared to be somewhat increased compared with the control spleens.

Erythrophagocytosis: Various phases of this activity were observed within cordal macrophages, including recently ingested, almost intact RBC, and the breakdown products of erythrocytes, often within the same macrophage (Figs. 4, 8).

Erythrocytes within sinus endothelial cells were occasionally observed in the three cases of H.S. but never in the control spleens. It is of interest that some of these endothelial cells contained platelets in addition (Fig. 9). Nuclei of such endothelial cells were highly indented. Platelets, exhibiting various degrees of degranulation, were often conspicuous in the sinus lining cells of the three H.S. spleens (Fig. 10), but only occasionally observed in the endothelial cells of our control spleens.

Hemosiderosis: Electron-dense material consistent with iron pigment was abundant in the macrophages of the cords and sinuses. It was also evident in some sinus endothelial cells but absent from others in the same field. In the macrophages the pigment was present in membrane-limited bodies
Fig. 8.—Splenic cord in H.S. containing three macrophages (Ma) which show various phases of erythrophagocytosis, including recently ingested erythrocytes (Rc) and products of hemoglobin breakdown in lysosomes with a heterogeneous content (arrows). × 8400.
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Fig. 9.—Sinus endothelial cell in spleen of patient with H.S. containing an erythrocyte and a platelet (arrow). Another endothelial cell of the same sinus also contains a platelet (arrowheads). The sinus (S) is filled with laked RBC. Consecutive serial sections of this and other similar areas have shown that the erythrocyte and platelets are within the endothelial cell cytoplasm. × 8400.

(lysosomes) presumably as part of the heterogeneous breakdown product of hemoglobin (Fig. 8). In contrast, most of the pigment in the sinus endothelial cells was in lysosomes with a more homogeneous content (siderosomes) (Fig. 11) often associated with small lipid droplets. Siderosomes or lysosome-like particles were rarely observed in control spleens, except in one out of two spleens injured by gun shot, in which the sinus endothelial cells had many siderosomes. The observations in the sinus endothelial cells suggest the uptake of products of intravascular hemolysis from the sinus lumen via the pinocytotic or the coated vesicles that were numerous in these cells (Fig. 7).

Part of the iron pigment in macrophages and in sinus endothelial cells was in the form of ferritin. Most platelets in the cords and in sinuses also contained ferritin, and platelets that contained abundant ferritin had fewer granules (Figs. 12A and B). Ferritin was rarely seen in platelets of the control spleens.

DISCUSSION

Ultrastructural studies of the spleen in three cases of hereditary spherocytosis are not in keeping with prevalent descriptions based on conventional light microscopy. The sinuses were not empty but were occupied by laked red blood cells or ghosts. The pronounced increase of cordal macrophages, previ-
Fig. 10.—Sinus endothelial cell of the spleen in H.S. containing three structures interpreted as platelets, showing various degrees of degranulation. A fourth structure of similar size, which consists mainly of lipoprotein membrane whorls, presumably represents the end-state of platelet degradation (arrowheads). Basal parts of the endothelial cells contain the electron-dense material (arrows) in areas extending between the interrupted basement membrane (bm). × 22,750.

Ously reported by Wennberg and Weiss,¹⁵ and not evident in light microscopic sections, was confirmed. The sinus endothelium contained electron-dense material consistent with hemosiderin, probably derived from hemoglobin that was taken up by pinocytotic activity. Erythrophagocytosis by cordal macrophages was a prominent feature. Additional findings included that the platelets contained ferritin, an observation consistently scanty or absent in our control spleens. Platelets were observed frequently within the splenic sinus endothelium in H.S., but only occasionally in controls.

A conspicuous finding in all three H.S. spleens was the lack of deformability of the RBC, manifested by the absence of compressed, hour-glass-shaped, bilobed, or long tailed red blood cells in transit through the walls of the sinuses. These features were seen in our control spleens and have been described in other hemolytic anemias.¹⁸

One of the problems requiring elucidation was the observation that, in spite of the high rate of hemolysis, neither hemoglobinemia nor hemoglobinuria is evident in this disease. Our electron microscopic observations seem to indicate that much of the degradation of the red blood cells and hemoglobin occur
Fig. 11.—Sinus endothelial cell of spleen in H.S. containing membrane limited bodies with an electron dense content (siderosomes) (arrowheads). The interrupted basement membrane (bm) and the basal, electron-dense material of the endothelial cell are seen on the right (arrow). × 22,500. Higher magnification of one of the siderosomes (insert) shows ferritin particles (circles). × 77,360.

Fig. 12.—Platelets in spleen of patients in H.S. (A) Small segment of a platelet within cord of spleen showing specific granules (arrows) and some ferritin particles (circles). × 51,250. (B) Higher magnification of a portion of a partially degranulated platelet within sinus of spleen contains numerous ferritin micelles (circles). The low gray reflection, stained briefly with lead acetate only. × 200,000.
intracellularly, and that if any hemoglobin escapes this intracellular process, it probably is incorporated within the sinus endothelium by way of pinocytosis. Whether or not the platelets contribute further to the clearing of hemoglobin from the vascular compartment of the splenic red pulp is not clear. That this may be the case, however, is suggested by the fact that ferritin is found within platelets. Platelets have previously been reported to be capable of phagocytic activity when exposed in vitro to ferritin. The fate of the platelets containing ferritin may be twofold; they may be phagocytosed by macrophages or they may carry the ferritin to the bone marrow. The latter is suggested by the presence of ferritin-containing platelets in the sinuses of the spleen seen in the present study and by the observation of survival of platelets after phagocytosis by Vegge et al. The occasional observation of red blood cells within the sinus lining cells is in keeping with the general concept that although the endothelial cells are not incapable of phagocytosis they exhibit a much lower phagocytic activity than macrophages. It is of interest that whenever this occurred it seemed to be accompanied by platelets within the same cells. The possibility that the lysosomes of platelets play a role in digestion of the red blood cells should be considered. Earlier histochemical studies by Dorfman have indicated the lack of lysosomal enzymes within the endothelial lining cells of the sinuses compared to macrophages. Those findings agree well with our morphological studies, indicating the absence of lysosomes from sinus lining cells of control spleens. We speculate that the frequent presence of intracellular platelets in the three H.S. cases examined may partially account for the clearing of the lining cells of products of red cell breakdown by utilizing the platelet lysosomal enzymes. The interaction between platelets and endothelial cells is well known from the studies of Johnson and her co-workers and Marchesi, who reported platelets within endothelial cells in acute inflammation. In our studies on human spleens we have occasionally seen platelets within the sinus endothelial lining cells of control spleens. It appears, however, that their intracellular presence is much more frequent in hemolytic disease. We have observed it in both hereditary spherocytosis and in sickle cell disease. It is well known from previous studies that the leakage of adenosine-diphosphate (ADP) causes adheriveness of platelets. Leakage of ADP from RBC may explain the increased number of platelets adhering to and entering into the sinus endothelial cells when splenic hemolysis is increased. The numerous nuclear indentations in lining cells that contained platelets, and the abundant (contractile?) cytoplasmic filaments suggest that one of the results of the interaction of platelets with endothelial cells is the contraction of the latter. Such contraction of the longitudinally oriented lining cells may have an effect upon the splenic intermediate or sinus circulation, or both, by facilitating the passage of cells. This may explain the release of spherocytes from the splenic pool under the effect of adrenalin as reported by Prankerd.

Our findings in three cases of H.S. lead us to believe that the mechanism of hemolysis in hereditary spherocytosis is as follows: the inherent rigidity of the erythrocyte membrane in H.S. results in the stasis of the red blood cells within the cords, where the adverse conditions, together with the inherent abnormality of the red blood cell membrane, results in leakage of red blood...
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cell contents. Phagocytosis of damaged erythrocytes in toto is primarily carried out by cordal macrophages, while endothelial cells, and perhaps platelets, contribute to the uptake of products of intravascular hemolysis. We wish to emphasize the possibly dual role that the platelets appear to play in H.S. and perhaps in other hemolytic anemias, namely an increased endothelium-platelet interaction\(^{22,23}\) and the contribution of platelets to the clearing of products of hemolysis from the splenic circulation.

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