Splenic Erythroclasia: An Electron Microscopic Study of Hemoglobin H Disease

By EMMA Wennberg and Leon Weiss

The spleen selectively removes damaged and aged erythrocytes from the circulation. It is anatomically adapted for this function by: (a) circulation of the erythrocyte from arterial termination to sinus through a tortuous cordal reticular meshwork beset by macrophages, and (b) passage of erythrocytes through slits between the lining cells of the vascular sinuses to reach the sinal lumen and thence the venous circulation. The spleen may remove erythrocytes from the circulation by causing stasis, thus allowing mildly damaged or sphered cells to be phagocytized in toto, by mechanical fragmentation of red cells and by "pitting," i.e. the removal of rigid precipitates from the red cells.

The spleen’s role in erythroclasia is heightened in hemoglobin H disease: fragmented, tear-drop- and bizarre-shaped red cells are numerous in the circulation of patients with spleens, but diminished in number after splenectomy; conversely, after splenectomy, there is a great increase in inclusion-bearing cells in the peripheral circulation.

We studied the spleen of a patient with hemoglobin H disease to attempt to elucidate a morphologic basis for these hematologic findings.

Pertinent Clinical Findings

The patient was a 29 year old white female of non-Mediterranean origin. She had a history of anemia since the age of six, with hemoglobin levels ranging from nine to 12 grams. Despite the fact that the anemia was iron-refractory, she was treated with iron-containing preparations intermittently from this time until hemoglobin H was demonstrated at the Johns Hopkins Hospital both by the formation of characteristic inclusions on incubation with brilliant cresyl blue and by its electrophoretic behavior. Sixteen percent of the total hemoglobin was hemoglobin H. (A sister of the patient was also found to have a hemoglobin H fraction in her blood.) Pre-operatively, she was found to have splenomegaly (duration undetermined) and her blood smear was described as being markedly hypochronic with anisocytosis and poikilocytosis. Many burr cells, target cells and numerous fragmented cells were present. The reticulocyte count was five percent. Splenectomy was performed because of thrombocytopenia and portal hypertension. (At autopsy, the liver was markedly fibrosed and contained heavy iron deposits.) We were not able to study the post-splenectomy blood because the patient died of post-operative complications.

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Fig. 1.—This light micrograph of a section of plastic embedded spleen demonstrates the great dilatation of the sinuses which are empty of blood except for a layer of erythrocytes which appear attached to the sinus lining cells. Toluidin blue x 250.

MATERIALS AND METHODS

The spleen was obtained at operation and 1 mm.² pieces were fixed in two percent glutaraldehyde in cacodylate buffer at pH 7.4 for two hours, rinsed in buffer, post-fixed in one percent OsO₄ similarly buffered for one hour, dehydrated in graded alcohols and embedded in Araldite.⁵ The blocks were sectioned in a Model MT 2 Servall Porter-Blum Microtome. The sections were mounted on bare copper grids, stained with lead citrate and uranyl acetate, and studied in a Siemens Elmiskop I. Thicker sections of plastic embedded materials were stained with one percent toluidine blue in borate buffer at pH 8.0 and studied by light microscopy.⁵ Larger portions of the spleen were fixed in formaldehyde, embedded in paraffin, and stained for the presence of iron deposits by the method of Comori.¹⁰

OBSERVATIONS

The spleen was grossly enlarged and congested; on cutting, much blood was lost from the cut surface.

Light Microscopy

The sinuses were enormously distended but almost empty of blood except for a layer of red cells on the inner surface of the vascular sinuses. Each red blood cell had a thin process which attached it to the sinus wall, thus giving it a tear-drop shape (Figs. 1 and 2). The cords were not congested, but had an excessive number of large macrophages containing broken-down erythrocytes. The sinus lining cells were heavily loaded with iron-containing pigment (Fig. 3).
Fig. 2.—The sinuses (S) are widely dilated. The erythrocytes in the lumen are tear-drop in shape. They are held in position by a thin process detained between the sinus lining cells. A large macrophage (M) lies in a cord. Light micrograph, Toluidin blue × 400.

Fig. 3.—This paraffin embedded section was stained for the presence of iron. The sinus walls which contain pigment appear black; the cords, which are pigment free, are light. The iron deposits are in the sinus lining cells, not in the basement membrane. × 500.

**Electron Microscopy**

Erythrocytes lying in the sinuses were markedly distorted and varied in hemoglobin content as indicated by differences in density (Figs. 4, 5 and 12). It is evident that most cells remaining in the lumen of the sinuses were in
Fig. 4.—Two sinus lumens (S) are seen at right upper and lower corners; in the upper sinus lumen, the erythrocytes show wide variations in density from light grey to black. The basement membrane (BM) contains an abnormal material of mixed electron lucent patches with dense portions similar to that seen in the lower sinus lumen (arrow). A portion of a large macrophage (M) is seen on the left. It contains one large fragment of an erythrocyte and many inclusions with further degraded erythrocytes. × 2000.

Fig. 5.—A large sinus (above) contains many bizarre-shaped erythrocytes, some of which have long, thin processes towards the sinus wall. The cord below contains portions of macrophage cytoplasm and some membrane-like structures. × 2000.
Fig. 6.—An erythrocyte is seen straddling two sinus lining cells. The major portions of it are now in the sinus lumen. × 5000.
PLENOC ERYTHROCLASIA

The process of entering it from the cord and thin portions of many of these erythrocytes were observed remaining in the cord or between sinus lining cells (Figs. 4, 8, 9 and 13). Several cells containing large, dense bodies were observed both in the sinuses and the cords (Figs. 14 and 16). Erythroblasts were seen very rarely (Fig. 14).

A striking and common phenomenon was the bisection of erythrocytes in relationship to the sinus lining cells. It appears that an erythrocyte may simultaneously enter the sinus via two sides of the lining cells with the result that the red cell is seen to be straddled over the lining cell (Figs. 6, 7, 8, and 9). Thence, the red cell may break into two (perhaps sphered) fragments which lie free in the sinus, and a very thin connecting portion which may be retained in the cordal compartment and phagocytized (Figs. 13 and 14).

Another manifestation of fragmentation of erythrocytes at the same site in the spleen was the apparent retention in the cord of a small portion of the red cell, generally dense and spherical, the major portion having passed into the sinus lumen (Figs. 10, 11, and 12). This is suggestive of the pitting of intracellular rigid bodies as postulated by Crosby7 and described by Koyama et al.8

The basement membrane of the sinuses was moderately increased in thickness. It contained abnormal material composed of a mixture of electron lucent patches and very dense membrane-like structures (Figs. 4, 7, 12, and 13), the latter resembling material in the sinus lumen (Fig. 4).

The cords were striking only in their content of large numbers of macrophages which contained erythrocytic breakdown products. We saw two types of phagocytized material in early stages of breakdown. One consisted of the thin “tails” of erythrocytes which resulted from the splitting or drawing out of these cells at the sinus wall (see above, and Figs. 13 and 14); the second consisted of large portions of or entire erythrocytes (Figs. 4, 12, and 15) some of which contained dense precipitates (Fig. 15). Thus, this is a spleen which is very active in the fragmentation and phagocytosis of abnormal erythrocytes.

We have over the years examined a number of human spleens including normal ones and one from a patient with portal hypertension. In these and in animal spleens examined, we never noted changes such as we have described above.

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Fig. 7.—An erythrocyte being bisected by a sinus lining cell which has been sectioned through its nucleus. Note the abnormal substance present in the basement membrane (BM) and the thin fragments of erythrocyte in the cordal compartment (arrow). × 4000.

Fig. 8.—A sinus (above) contains the two major fragments of an erythrocyte which appears in process of bisection and portions of other erythrocytes detained similarly in passage from cord to sinus. The cord (below and left) contains several erythrocytes and a dense granulocyte. × 2000.

Fig. 9.—Immediately below the sinus lining cell (SL) at which an erythrocyte is bisected, there is a portion of macrophage cytoplasm (M) enveloping a long process of another erythrocyte (arrow). × 4000.
Fig. 10.—The major portion of an erythrocyte (E) has passed from cord to sinus leaving a smaller, spherical portion in the cord. × 9000.

Fig. 11.—A portion of an erythrocyte (star) is being pitted during its passage from cord to sinus. The sinus lining cells are heavily loaded with pigment granules which are both dispersed in the cytoplasm and collected into aggregates (arrow). × 8000.
Fig. 12.—The sinus at left has many bizarre-shaped red blood cells, one of which (arrow) has a globular portion of cytoplasm detained in the cord. A large macrophage at right contains several large portions of erythrocytes in its cytoplasm (stars). Collagen fibrils at right are part of the wall of a nearby arteriole. × 4000.
Fig. 13.—A large macrophage filling most of this micrograph contains in its cytoplasm the broken off thin “tails” of erythrocytes (arrows) and many degradation vacuoles. Some free thin portions of erythrocytes are seen between and just below the sinus lining cells at the upper right corner. Patches of basement membrane are between the sinus lining and the macrophage. × 12000.

Fig. 14.—A macrophage (M) at right is in contact with several erythrocyte “tails” (star) curled up underneath the sinus lining. An erythrocyte is in passage between two lining cells which are heavily loaded with iron pigment. A bizarre erythrocyte at the upper left corner contains an ill-defined dense area (arrow). An erythroblast (Eb) is in the sinus lumen. × 12000.
Fig. 15.—A macrophage which contains two large portions of erythrocyte. The upper erythrocyte fragment has a dense inclusion in its center. × 12000.

DISCUSSION

Slater et al. have discussed at length and demonstrated by light microscopy how the spleen may be responsible for the pitting of precipitated α chains in cases of β thalassemia. In the case we have described, there appear to be two patterns of splenic action upon the abnormal erythrocytes. The first is the removal of intra-cellular precipitates (in this case, consisting of oxidized β chains). The second, and more unusual pattern of action, consists of the
Fig. 16.—A group of erythrocytes lying free in a sinus demonstrate two dense inclusions (arrow) and marked variation in shape. × 10000.

splitting of the erythrocytes into two or more fragments by their passage from cord to sinus. Nathan and Gunn describe the morphology of erythrocytes in thalassemic patients as consisting of a mixed population of large, pale cells and small, dense cells with intermediate forms, tear-drop cells and small fusiform fragments. The spleen we have studied illustrates how a large, pale cell
may be split into two small, dense cells leaving behind a small fragment to be phagocytized by splenic macrophages.

Several factors may contribute to the phenomenon of erythrocyte fragmentation. In the case under study, the splenic sinus walls may have been so stiffened by iron pigment deposits as to have allowed the cells to pass through them only with much difficulty. If this were an important factor, however, the cords would have been very congested, and they were not. The principal abnormal factors then must lie in the erythrocytes. The red blood cell, by virtue of the abnormal hemoglobin it contains, may be abnormally rigid; that is, it may easily and continuously be deformed, but will not regain its original shape once the deforming force is removed. This explains the bizarre-shaped cells commonly seen in the peripheral circulations of the patient. Slater et al. have suggested that the hemoglobin in β thalassemic cells may be partially gelated to account for this observation.

In order for them to be able to span around sinus lining cells and reach the circulation simultaneously through two openings, there has to be both a slow oozing of the cell (due to its gelated contents) and a high stroma to content ratio. The two resulting fragments will be sphered, thereby being less deformable than biconcave cells and thus readily subject to destruction by further fragmentation or lysis.

**Summary**

This is an electron microscopic study of the spleen in a case of hemoglobin H disease. The patient showed evidence in the peripheral blood of marked erythrocyte fragmentation.

The spleen acted upon the abnormal erythrocytes in two major ways:

1. The erythrocytes entered the sinus lumen simultaneously via two intercellular slits on opposite sides of a lining cell and then split into two sphered fragments (which lay free in the sinus lumen) and the long, thin connecting fragment (which was retained in the cordal compartment and phagocytized).
2. Small, dense, spherical portions of erythrocytes were retained in the cord while the major portion of the cell was released into the sinus lumen. This is suggestive of pitting of rigid intracellular precipitates.

The erythrocytes emerged from their passage through the cords and maintained bizarre forms in the sinus lumen. It is possible that the contents of these cells may be partially gelated to account for this. This gelation is thought to largely underlie their behavior in their circulation through the spleen. Evidence of increased erythrocytic breakdown was found in the numerous large macrophages present in the cords.

**SUMMARIO IN INTERLINGUA**

Es reportate un studio electrono-microscopic del splen de un patiente con morbo de hemoglobina H. Le sanguine del patiente monstrava in specimens peripheric evidentia de un marcate fragmentation erythrocytic.

Le splen ageva super le erythrocytos anormal in duo major manieras:
1. Le erythrocytos entrava in le lumine del sinus simultaneemente via duo fissuras intercellular a lateres opposite de un cellula de revestimento e postea se divideva in duo frag-ments spheroide (que jaceva liberemente intra le lumine del sinus) e un longe tenue fragmento de connexion (que esseva retenite in le compartimento cordal e phagocytisate).

2. Micre e dense portiones spheric de erythrocytos eseva retinite in le corda durante que le portion major del cellula eseva liberate ad in le lumine del sinus.

Le erythrocytos emergeva ab lor passage a transverso le cordas e manteneva formas bizarre in le lumine del sinus. Il es possibile que le contento de iste cellulas es partialmente gelate. (Isto explicarea le phenomeno.) Un tal gelation es reguardate como responsable pro le comportamento del cellulas durante lor passage a transverso le splen. Evidentia pro un augmentate destruction de erythrocytos eseva trovate in le facto que numerose grande macrophagos eseva presente in le cordas.

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REFERENCES

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