Virchows Arch. A Path. Anat. and Histol. 372, 337–342 (1977)

Virchows Archiv A

Red Pulp of the Spleen in Hereditary Elliptocytosis

David Shneidman, Peter Kiessling, John Onstad, and Paul Wolf

Department of Pathology, University of California, San Diego, La Jolla, California 92103, USA

Summary. Electron microscopic study of the spleen of an adult with hereditary elliptocytosis demonstrated features of erythrocyte pooling in the splenic cords with decreased red cells in transit through the basement membrane slits between the sinus littoral cells and decreased erythrocytes in splenic sinuses. Cordal reticulum cells, macrophages, and platelets were prominent. Light microscopy demonstrated relatively empty sinuses, and electron microscopy confirmed that the sinuses contained variable numbers of intact red cells. The morphology of the splenic red pulp in hereditary elliptocytosis was found to simulate that seen in hereditary spherocytosis but to a lesser degree.

Key words: Elliptocytosis – Spleen – Red pulp.

Introduction

Hereditary elliptocytosis is an uncommon autosomal dominant disorder characterized by an alteration in red cell shape in which the red cells are elliptical and have an axial ratio of less than 0.78. In most instances there is no or mild anemia; however, in 10–15% of patients, the condition is characterized by prominent hemolysis, intermittent jaundice, shortened Cr^{51} red cell half-life, bilirubin gallstones, leg ulcers and splenomegaly. Transient splenomegaly, associated with acute infection, causes the onset of hemolysis and thus, overt hemolysis may become manifest for the first time as a result of an intercurrent infection (Cooper, 1972).

The purpose of this paper is to analyze the cause for splenomegaly in hereditary elliptocytosis and correlate the fine structural appearance of the spleen with the light microscopic findings.

Case Report

The patient, a 35 year old Caucasian male, developed a transient viral syndrome associated with splenomegaly with a subsequent fall in hematocrit from 40% to 29%. His peripheral blood smears

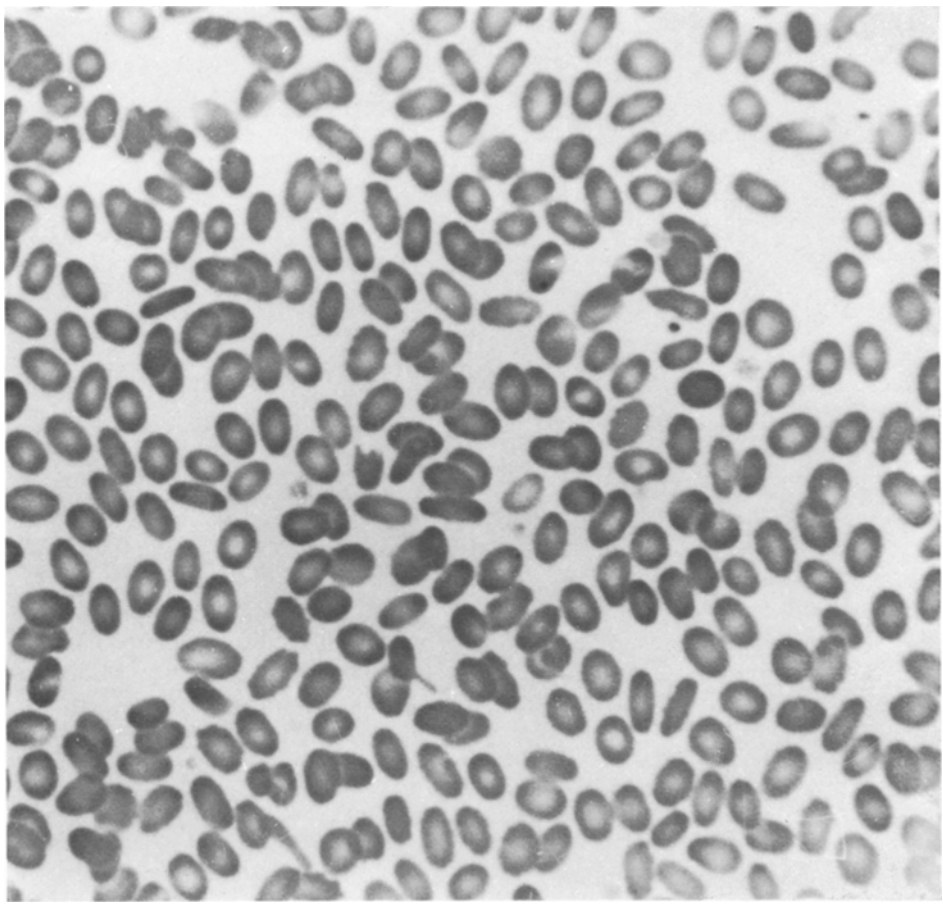

Fig. 1. Patient's peripheral blood smear from admission for elective splenectomy demonstrating that the majority of the red blood cells are elliptical. ×400. Wright-Giemsa stain

showed moderate elliptocytosis. The reticulocyte count rose to 3.5%. He was consequently diagnosed as having hereditary elliptocytosis with recent acute hemolysis. At the termination of the viral episode, his hematocrit stabilized at 40% with a reticulocyte count of 2.8%, and he became asymptomatic. He had an elective splenectomy three years later; there were no abnormal physical findings except a palpable spleen 5 cm below his left costal margin. Peripheral blood smears showed (Fig. 1) that a majority of his red cells were elliptical. Laboratory values included a hematocrit of 41%, hemoglobin of 14.9 gm/dl, WBC 9400/cu mm with 81 polymorphonuclear neutrophils, 1 band neutrophil, 15 lymphocytes, 3 monocytes. The platelet count was 240,000/cu mm.

Methods and Materials

Sections of spleen for light microscopy were fixed in formalin and stained with hematoxylin and eosin, periodic acid Schiff and Giemsa stains. Splenic tissue for electron microscopy was cut into 1 mm cubes, fixed in 3% glutaraldehyde with 0.15 phosphate buffer, and then postfixed in 1% osmium tetroxide. The tissue was cut with an ultramicrotome and stained with uranyl acetate and lead citrate and then examined with a Siemen's model 101 electron microscope.

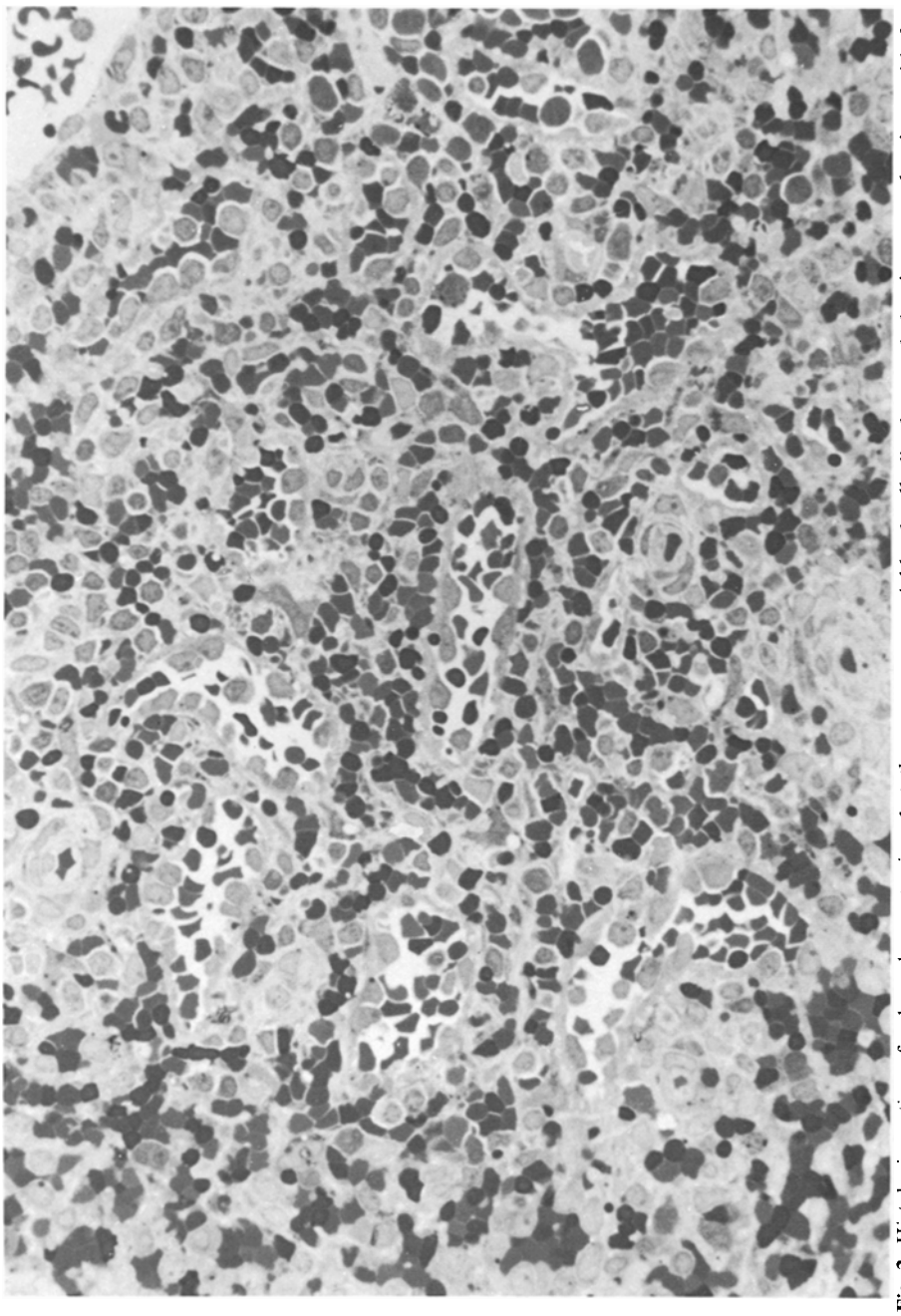

Fig. 2. Histologic section of spleen demonstrating that there are many red blood cells lined up and abutting upon the sinus, with few in passage. ×160. Epon embedded, Methylene blue stain

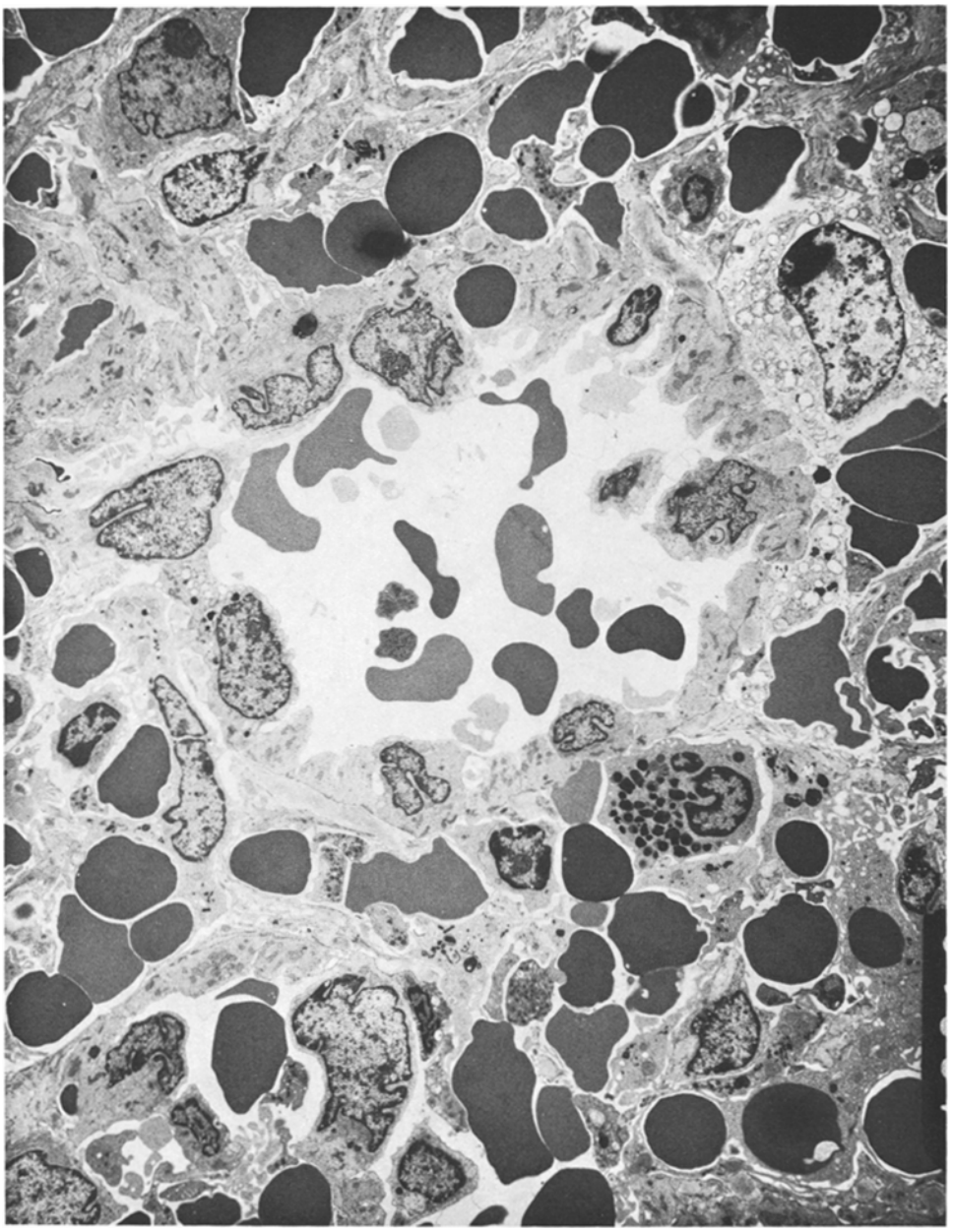

Fig. 3. Electron microscopic section of spleen demonstrating that splenic cords are congested with erythrocytes and many platelets are present. A marked decrease of red cells in passage from the cord to the sinus is evident. ×3000. Uranyl acetate stain

Gross Findings

The surgical specimen consisted of an intact spleen measuring $17 \times 12 \times 6$ cm and weighing 410 g. In addition, there was a small accessory spleen measuring $2.5 \times 1.5 \times 1.5$ cm which weighed 2.5 g. On section both were composed of moderately firm, beefy red parenchyma with indistinct Malpighian bodies.

Light Microscopic Findings

Multiple sections of spleen showed a characteristic architecture of the red pulp consisting of widening and congestion of the splenic cords with somewhat empty splenic sinuses. Surrounding many sinuses, a layer of red cells seemed to abut upon the sinus basement membrane (Fig. 2).

Ultrastructural Findings

In contrast to the light microscopic findings, both splenic cords and sinuses contained red cells with variable crowding. Some of the splenic cords and sinuses were tightly packed, while others were less full of erythrocytes. The red cells showed a variation in electron density, a finding indicative of variable hemoglobin content (Vennberg, 1969). Many red cell abutted upon the endothelial slits of the splenic cordal endothelial cells, but there was a decrease of red cells with long tails or with compressed or bilobed shapes in transit through the endothelial slits that line the endothelial wall (Fig. 3). These "in transit" red cells are inevitably seen in good number in normal spleens by electron microscopy (Burke, 1970; Molnar, 1972).

In addition, sections of the spleen demonstrated a prominence in the number of cord reticulum cells and macrophages which were in close contact with the red cells. The sinus lining cells were not increased in number or altered in morphology. Erythrophagocytosis was evident in the cords with myelin figure breakdown products of ingested red cells in the phagocytic histiocytes (Simon, 1970). A large number of platelets were also present.

Discussion

It is generally accepted that the red pulp of the spleen is an important site for the destruction of aged and abnormal red cells. The red pulp consists of cords and sinuses. Exactly how the red cells get into cords is still a matter of controversy and is debated by the schools of the closed and open circulation. Once in, the erythrocytes lie in intimate contact with a mixed population of reticulum cells and macrophages. The mechanism of how red cells exit the cords has been established: all must pass through the border slits of the sinus endothelial cells, temporarily enlarging the potential space between them. These endothelial slits are limited to an 0.5 microns maximum by intraendothelial microfilaments and this passageway controls the rate of RBC escape from the cords to the sinuses (Chen, 1973). Normal red cells are pliant and plastic enough to slip easily through perhaps because of their contractile proteins which regulate their shape. However, the membranes of red cells of hereditary elliptocytosis are more rigid and have difficulty in traversing this passageway. Red cells line up focally around the sinus basement membrane, and there are decreased numbers seen in transit: a case where "many are called but few are chosen." Thus, RBCs sequester in the cords, macrophages proliferate, and splenomegaly results from the increased RBCs and subsequent reaction to the stagnant degenerating erythrocytes.

It is important to compare the fine structural changes with those seen in the spleen in hereditary spherocytosis, a similar but more severe intrinsic hemolytic anemia, which is practically always associated with splenomegaly. In hereditary spherocytosis, the splenic cords are intensely congested with hyperplasia of cordal macrophages and prominent erythrophagocytosis. The basic splenic pathophysiology in both these diseases is the same: the inability of the red cell to pass from the cord into the sinus. Molnar and Rappaport reported an increase in cordal platelets, attracted there, they surmised, by the leakage of ATP from the defective spherocyte RBC membrane (Molnar, 1972). The number of platelets seen in the splenic cords in hereditary elliptocytosis may be due to a similar cause, as these erythrocytes also have a more permeable membrane associated with a more rapid than normal decline in cellular ATP and 2,3 DPG, and with a 40–50% increase in ouabain-inhibited sodium efflux (Cooper, 1972). Phosphorylation of the red cell contractile protein, spectrin, is necessary to permit a conformational change in the RBC membrane protein, which regulates the erythrocyte shape. It has been established that there is decreased phosphorylation of spectrin in RBC membrane in hereditary spherocytosis (Greenquist, 1974). The changes of hereditary elliptocytosis are less marked than those of hereditary spherocytosis – the cordal congestion is less intense and erythrophagocytosis is not as prominent, perhaps because the elliptocytes are not as rigid. However, the changes seen in these two diseases are largely differences in degree, and the morphology seen in the red pulp in hereditary elliptocytosis is in the spectrum between normal and the more severe pathologic changes of hereditary spherocytosis.

References

Burke, J.S., Simon, G.T.: Electron microscopy of the spleen. I. Anatomy and microcirulation. Amer. J. Path. **58**, 127 (1970)

Chen, L.P., Weiss, L.: The role of the sinus wall in the passage of erythrocytes through the spleen. Blood **41**, 529 (1973)

Cooper, R.A., Jandl, J.H.: Hereditary elliptocytosis. Textbook of hematology, edit. by William Williams, p. 387. New York: McGraw-Hill 1972

Greenquist, A.C., Shohet, S.B.: Defective protein phosphorylation in membranes of hereditary spherocytosis erythrocytes. Fed. Europ. Biochem. Soc. Lett. **48**, 133 (1974)

Molnar, Z., Rappaport, H.: Fine structure of the red pulp in hereditary spherocytosis. Blood **39**, 1 (1972)

Simon, G.T., Burke, J.S.: Electron microscopy of the spleen. III. Erythroleukophagocytosis. Amer. J. Path. **58**, 451 (1970)

Vennbert, E., Weiss, L.: The structure of the spleen and hemolysis. Ann. Rev. Med. **20**, 29 (1969)

Received September 30, 1976