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Erythrophagocytosis by the Sinus Endothelial Cell of the Spleen in Haemolytic Anaemias

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Summary. An ultrastructural study of spleens from patients with a heterogeneous group of haemolytic anaemias was undertaken in order to determine whether sinus endothelial cells have erythrophagocytic ability. In most cases, sinus endothelial cells contained erythrocytes and various stages of intracellular degradation of engulfed erythrocytes were noted. Though the frequency of erythrophagocytosis varied from case to case, phagocytosis of erythrocytes by the endothelial cell was more frequent in cases in which cordal macrophages showed active erythrophagocytosis. These results suggest that the sinus endothelial cells have erythrophagocytic ability in certain pathological states, especially when the demands for the removal of defective erythrocytes are increased. However, the bulk of erythrophagocytosis is carried out by cordal macrophages, and endothelial phagocytosis has a minor significance in the development of haemolytic anaemia. Two possible processes by which erythrocytes come into contact with sinus endothelial cells are suggested.

Key words: Spleen – Sinus endothelial cell – Erythrophagocytosis – Haemolytic anaemias.

Introduction

Splenic sinuses in man are long vascular channels with a unique endothelium and basement membrane (Weiss, 1977). Electron microscopic studies of the spleen in haemoglobin H disease (Wennberg and Weiss, 1968), unstable haemoglobin haemolytic anaemia (Matsumoto et al., 1977), a rhesus monkey infected with plasmodium knowlesi (Schnitzer et al., 1973), and in animals with drug-induced Heinz body haemolytic anaemia (Rifkind, 1965; Lawson et al., 1969; Chen and Weiss, 1973; Klausner et al., 1975; Adachi, 1977) indicate that the

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wall of the splenic sinuses is a critical site in the control of erythrocyte passage through the red pulp. The role of inter-endothelial slits in the wall as a filter of erythrocytes with impaired deformability or with rigid intracellular masses has been emphasized by Chen and Weiss (1973). Following mechanical trapping by the inter-endothelial slit the macrophages in the Billroth cord play a central role in the removal of defective erythrocytes. It is not clear whether the endothelial cell of the splenic sinus is able to participate in this clearance mechanism or not. In this study, we present morphological evidence that sinus endothelial cells of the human spleen have erythrophagocytic ability in response to increased demands for the removal of defective red blood cells.

Materials and Methods

Spleens removed from 22 patients with a heterogeneous group of haemolytic anaemias (10 cases with erythrocyte pyruvate kinase deficiency, 8 cases with hereditary spherocytosis, 2 cases with autoimmune haemolytic anaemia, 1 case with unstable haemoglobin haemolytic anaemia, and 1 with hereditary elliptocytosis) were examined electron microscopically. Small tissue fragments from several different sites were immediately fixed in 4.15% glutaraldehyde in cacodylate buffer at pH 7.4 for 2 h at 4°C. After post-fixation in 1% OsO_4, the specimens were dehydrated in a series of alcohols and embedded in epoxy resin according to the method of Luft (1961). Thin sections cut with an Ivan Sorvall 2B ultramicrotome were stained with uranyl acetate and lead citrate, and viewed and photographed with a Hitachi HS-8 and/or H-300 electron microscope.

For the demonstration of acid phosphatase activity, small pieces of the spleen were fixed in cold 2.1% glutaraldehyde in cacodylate buffer and subsequently incubated in a solution containing lead nitrate and beta-glycerophosphate, and then similarly processed for electron microscopy.

Spleens from 6 patients with idiopathic thrombocytopaenic purpura and 4 patients with gastric cancer were used as controls.

Results

The sinus endothelial cell was characterized by cytoplasm which contained abundant micropinocytotic vesicles, loosely organized cytoplasmic filaments, and basal condensation. These structures were very important in helping to differentiate sinus endothelial cells from the macrophage.

Erythrophagocytosis by sinus endothelial cells was observed in most cases with haemolytic anaemia, but not in control spleens. Although the frequency of endothelial phagocytosis varied from case to case, the bulk of erythrophagocytosis was carried out by cordal macrophages and endothelial phagocytosis was a minor aspect of erythrocyte destruction in the spleen (Fig. 1). It was apparent, however, that phagocytosis of erythrocytes by sinus endothelial cells was more easily demonstrated in the spleens in which cordal macrophages showed active erythrophagocytosis (Fig. 2). As to the relation between morphological findings and clinical data, erythrophagocytosis by cordal macrophages was frequently demonstrated in cases either with long-standing haemolytic anaemias, such as hereditary spherocytosis and unstable haemoglobin haemolytic anaemia or with rapidly progressive haemolysis due to immune mechanism, and splenectomy was markedly effective in these cases. As we described previously (Matsumoto

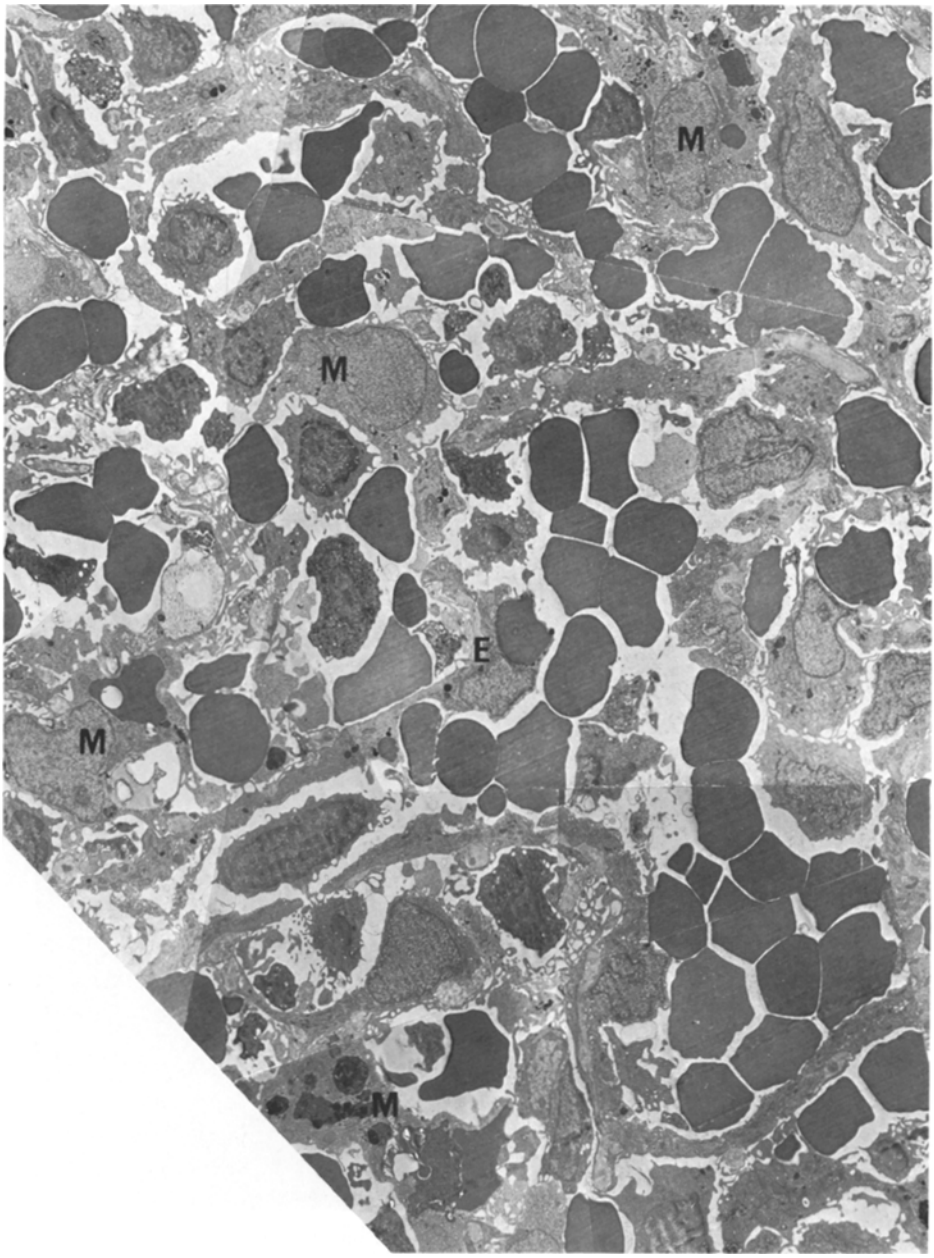

Fig. 1. A low-power electron micrograph shows an overview of the erythrophagocytosis within the spleen. Cordal macrophages (*M*) reveal active erythrophagocytosis. A sinus endothelial cell (*E*) contains an erythrocyte on the luminal aspect of the cytoplasm. Hereditary spherocytosis. ×2,100

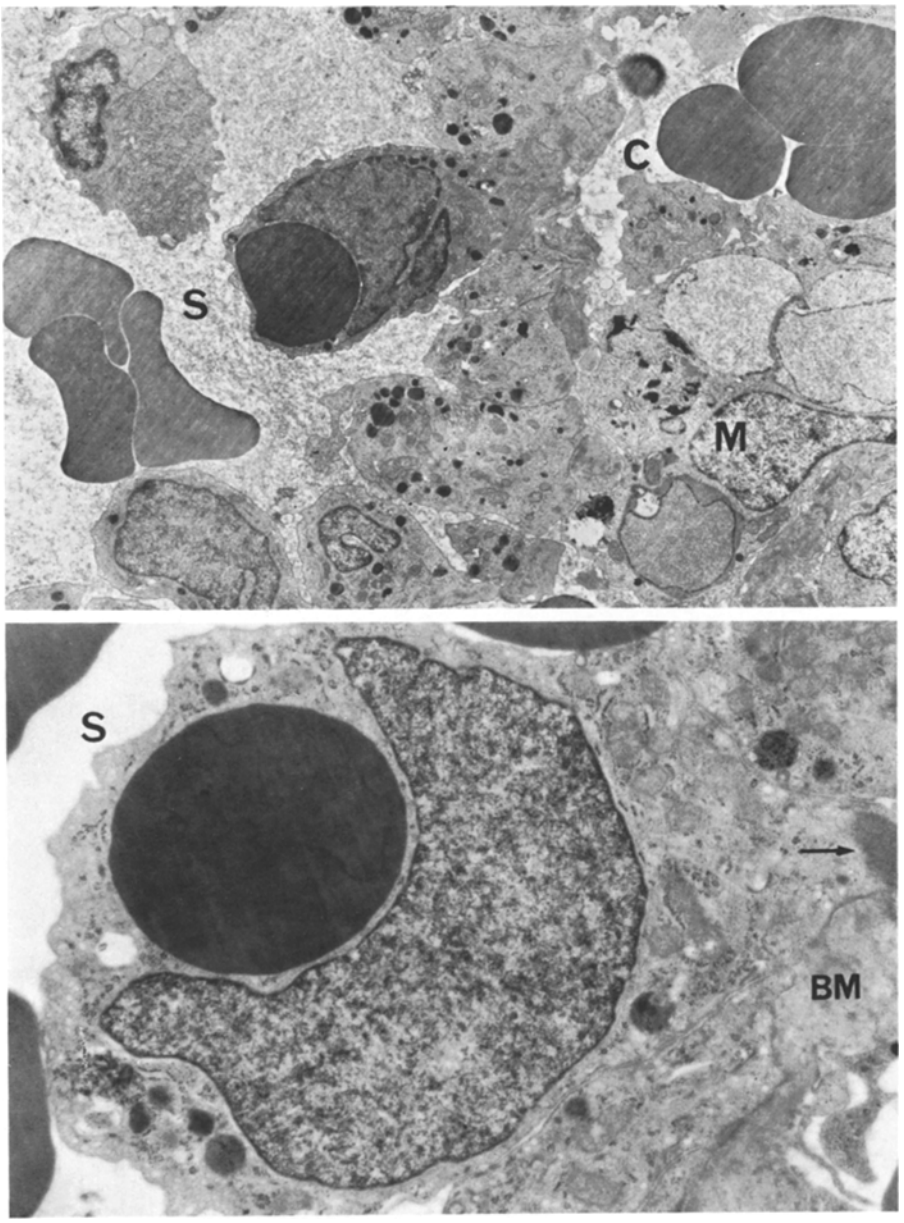

Fig. 2. A sinus endothelial cell containing an erythrocyte on the luminal aspect protrudes into the sinus umen (*S*). A macrophage (*M*) in the cord contains several erythrophagocytic vacuoles. *C*, Billroth cord. Autoimmune haemolytic anaemia. × 5,000

Fig. 3. A recently phagocytized erythrocyte is seen on the luminal aspect of the sinus endothelial cell, which is characterized by the dense filaments (*arrow*) in the basal cytoplasm. *BM*, basement membrane; *S*, sinus. Hereditary spherocytosis. × 11,700

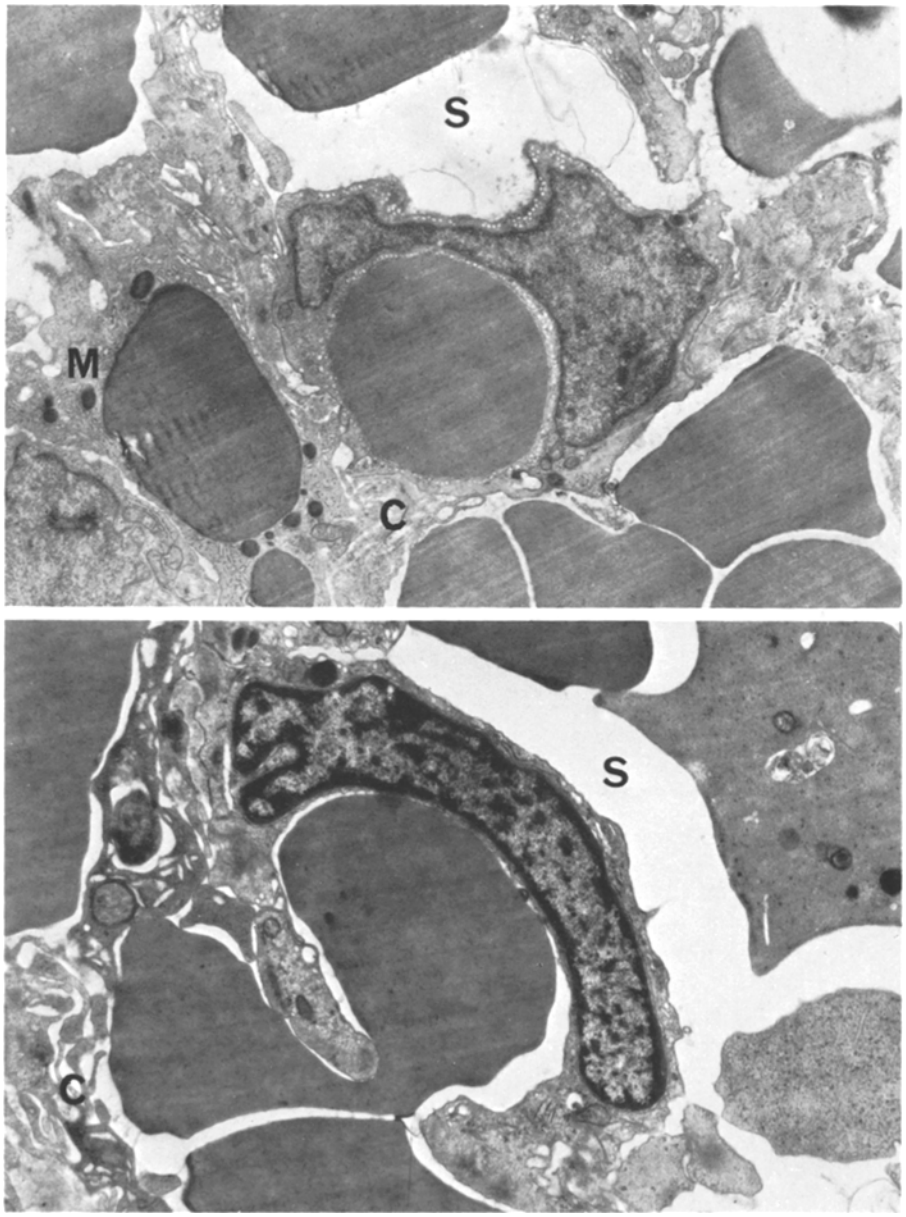

Fig. 4. An engulfed erythrocyte is seen on the basal side of the sinus endothelial cell, characterized by numerous micropinocytotic vesicles. A macrophage (*M*) in the cord also contains an erythrocyte. *S*, sinus; *C*, Billroth cord. Hereditary spherocytosis. ×6,200

Fig. 5. A large portion of an erythrocyte is surrounded by the cytoplasmic processes of the endothelial cell. Basal parts of the endothelial cell contain filamentous, electron-dense materials. *S*, sinus; *C*, Billroth cord. Unstable haemoglobin haemolytic anaemia. ×9,000

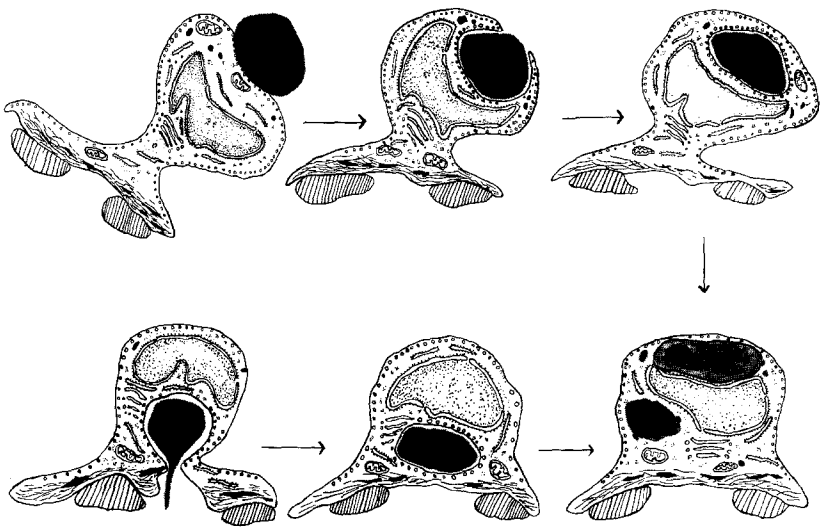

Fig. 6. Schematic representation of two possible methods of erythrophagocytosis by the sinus endothelial cell in the spleen

et al., 1972), phagocytosis of reticulocytes by cordal macrophages was a characteristic feature of erythrocyte pyruvate kinase deficiency. Endothelial phagocytosis of reticulocytes or mature erythrocytes was rarely observed, and splenectomy was partially beneficial in these patients.

In most instances, erythrocytes were phagocytized at the luminal side of the cell surface (Fig. 3). In some cases, sinus endothelial cells containing erythrocytes protruded into the sinus lumen suggesting their active erythrophagocytic ability (Fig. 2). These findings indicated that the erythrocytes were phagocytized during stasis in the sinus lumen. Occasionally, sinus endothelial cells ingested erythrocytes on the basal side (Fig. 4). Initially, erythrocytes passing through the pores of the basement membrane came into direct contact with sinus endothelial cells on the basal side (Fig. 5). Ingestion of erythrocytes from the basal aspect of the endothelial cell was observed in hereditary spherocytosis and unstable haemoglobin haemolytic anaemia, in which inter-endothelial slits were obliterated by poorly deformable spherocytes or Heinz body-containing erythrocytes respectively. These findings suggest two possible methods by which erythrocytes come into contact with sinus endothelial cells (Fig. 6). Most frequently contact occurs on the luminal side, uncommonly it takes place at the basal side, especially when inter-endothelial slits are obliterated by poorly deformable erythrocytes.

In the early phase of erythrophagocytosis, engulfed erythrocytes were surrounded by cytoplasmic membranes of sinus endothelial cells but were still intact. Usually, the erythrocytes were engulfed whole. As intracellular degradation progressed, the matrix of phagocytized erythrocytes was degraded and condensed into coarsely granular or mottled material with increased electron density. Acid phosphatase activity was not shown in non-phagocytizing endothe-

lial cells, but weak enzyme activity was demonstrated at the periphery of the erythrophagocytic vacuoles.

Discussion

It is well recognized that macrophages in the Billroth cords phagocytize defective erythrocytes and play a major role in the development of some haemolytic anaemias. This function of the spleen is known as "culling" (Crosby, 1958). Inter-endothelial slits of the sinus wall are known to constitute a major rout by which the spleen selectively processes erythrocytes (Chen and Weiss, 1973). Thus, the slits in the sinus wall act as a simple filter for erythrocytes with impaired deformability and the site where the culling function of the spleen is performed.

Whether or not the sinus endothelial cells have a phagocytic ability is still a matter of debate. Ingestion of iron granules (Moore et al., 1961) and colloidal carbon (Burke and Simon, 1970), and the presence of haemosiderin granules in various kinds of haemolytic diseases (Willand and Smith, 1956; Rappaport and Crosby, 1957) all suggest that sinus endothelial cells may have phagocytic ability under certain circumstances. From the results of histochemical studies, Snodgrass (1968) reported that sinus endothelial cells of the rabbit's spleen had strong nonspecific esterase activity, although acid phosphatase activity was very slight. He concluded that sinus endothelial cells were inactive in terms of phagocytosis when compared with macrophages in the cords. Burke and Simon (1970), on the other hand, have demonstrated endothelial phagocytosis of colloidal carbon in the rabbit and have proposed the development of phagocytic ability in sinus endothelial cells after a functional overload. Some evidence of erythrophagocytosis by the sinus endothelial cell has been described in long-standing haemolytic diseases such as hereditary spherocytosis (Molnar and Rappaport, 1972; Matsumoto et al., 1973), or in autoimmune haemolytic anaemia with massive destruction of blood cells (Matsumoto et al., 1978).

In this study, erythrophagocytosis by sinus endothelial cells was observed in the spleens from patients with either chronic haemolytic anaemias, such as hereditary spherocytosis and unstable haemoglobin haemolytic anaemia, or with rapidly progressive haemolysis due to immune mechanism. In both cases, cordal macrophages revealed frequent erythrophagocytosis, and removal of the spleen was markedly beneficial. In contrast, splenectomy resulted in only partial improvement of the patient's condition in erythrocyte pyruvate kinase deficiency. In the spleens from the patients with erythrocyte pyruvate kinase deficiency, morphological evidence of red cell destruction in the spleen was not prominent compared with the former group of haemolytic anaemias, and endothelial phagocytosis was rarely encountered. Thus it should be emphasised that the bulk of erythrophagocytosis in the spleen is carried out by cordal macrophages. Sinus endothelial cells are relatively inactive compared with cordal macrophages, and the phagocytic ability of endothelial cells seems to be a reserve function in response to increased demands for the removal of defective erythrocytes. Although erythrophagocytosis by sinus endothelial cells plays a minor role in the development

of haemolytic anaemia it may represent morphological evidence of a functional overload in the spleen.

Two methods by which erythrocytes come into contact with endothelial cells have been suggested as the initial events in erythrophagocytosis by the sinus endothelial cell (Fig. 6). Most frequently contact occurs on the luminal side of the endothelial cell. Some of the defective erythrocytes traversing the sinus wall and remaining in the lumen may be vulnerable to endothelial phagocytosis. How endothelial cells recognize defective erythrocytes remains unknown. The second process, though this seems to be very rare, occurs when erythrocytes, which have passed through the basement membrane fenestrations, encounter sinus endothelial cells from the basal side. Such erythrocytes may have much difficulty in passing through the sinus wall and may be detained there and finally engulfed either by cordal macrophages or by sinus endothelial cells. Entry from the basal side may be more likely when the inter-endothelial slits are blocked or occupied for a long time by erythrocytes with reduced deformability or by intracellular rigid masses. In fact, erythrophagocytosis from the basal aspect of the endothelial cell has been recognized in hereditary spherocytosis and unstable haemoglobin haemolytic anaemia. Further studies, including examinations of serial sections, are necessary to prove that endothelial phagocytosis occurs on luminal and basal sides of the endothelial cell.

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