Splenic Macrophages in Thrombocytopenia

By B. G. Firkin, R. Wright, S. Miller and E. Stokes

SALTZSTEIN,1 AND LANDING ET AL.2 in 1961 described the occurrence of lipid laden histiocytes in spleens examined in patients with idiopathic thrombocytopenic purpura following splenectomy. These workers suggested that these changes were due to an accumulation of lipids following the ingestion of platelets by the splenic reticuloendothelial system. Similar interpretations have been made by Hill et al., (1963).3 In this study splenic tissue has been obtained following splenectomy in patients with a number of disorders and examined under the electron microscope. In the majority of patients with idiopathic thrombocytopenic purpura, but not in other instances, platelets have been seen within splenic histiocytes in various stages of digestion. The end result is the production of myelin figures and lipid droplets. These findings support the previous contention that the splenic lipidosis seen in idiopathic thrombocytopenic purpura is a direct result of platelet phagocytosis.

METHODS

Spleens were obtained at operation from patients with the following hematological disorders: idiopathic thrombocytopenic purpura (I. T. P.) (9), aplastic anemia (1), myeloid metaplasia (1), and congenital spherocytosis (3). With the exception of the latter group, all patients on whom splenectomy was performed were thrombocytopenic and purpuric, and the major aim of the operation was to correct this disability. Eight of the nine patients with I. T. P. had platelet survival times estimated by the 51Cr technic using isologous platelets.4 In five patients the platelet life span was less than six hours, in one twelve hours, and in the remaining two, three days compared to the normal survival time of eight to ten days. Immediately postoperatively the spleen was sectioned longitudinally and an area selected for biopsy some 5 cm., in from the capsule. The surface of this area was first rinsed with 6.5 per cent glutaraldehyde in cacodylate buffer and a small block a ½ cm.3 was removed with a razor blade. This block was sliced into fine slivers of tissue and fixed in 6.5 per cent glutaraldehyde, post fixed in 1 per cent osmium tetroxide in Palades buffer and processed in the usual manner for electron microscopy. Some four or five specimens were obtained in a similar fashion from each spleen.

RESULTS AND DISCUSSION

Figure 1 shows a splenic macrophage in a patient with I. T. P. In this cell, platelets can be seen in various stages of intracellular digestion. A platelet is contained within a membrane and its organelles appear completely intact (Fig. 1 and Fig. 2). The initial change is some loss in the definition of the platelets' organelles and this is frequently accompanied by some darkening of the cell's cytoplasm, which usually occurs in the cell's periphery and is often localized to one point. These stages progress until it would be impossible to recognize the cell with certainty (lowermost arrow, Fig. 1) if it were not
Fig. 1.—Arrows indicate three platelets in varying stages of dissolution in splenic histiocytes. The uppermost thrombocyte has not undergone any significant change whereas the one at 10 o'clock is markedly altered with loss of organelle definition and change in cytoplasmic density. The lowermost cell is barely recognisable as a platelet showing considerable breakdown and disintegration with progressive darkening of the cytoplasm, vacuolisation and rounded areas which are densely osmophilic. In the remaining cytoplasm of the histiocyte, rounded areas of variable diameter with large osmophilic inclusions are seen. It is thought that these represent lipid deposits following further digestive breakdown of the platelet similar to the one indicated by the lowermost arrow.

For the observation of the progression of events. As the digestion of the platelet proceeds the external membrane becomes less defined and the ingested cell presents a variegated appearance, containing densely osmophilic areas which vary in size. Occasionally vacuoles are prominent (Fig. 3). Further alterations occur until the ingested cell lacks definition and consists entirely of rounded densely osmophilic masses which are believed to be lipid in nature (Fig. 1). The latter structures may undergo a further change giving rise to inclusions which resemble myelin figures (Fig. 4) without passing through the stages pictured in Figure 3.

These ultrastructural findings substantiate Saltzstein's hypothesis that the increased phospholipid he observed in histiocytes was due to ingested platelets since this study has observed all phases of platelet degradation in splenic histiocytes, down to myelin figures and dense osmophilic inclusions. The myelin-like structures have been extremely prominent in some of the spleens examined (Fig. 5). Although the changes observed in the platelets' ultrastructure during their digestion by the macrophages differ in some respects from those observed during this cell's autolysis on storage, there were some sim-
Fig. 2.—Platelets in a splenic macrophage viewed at a higher magnification. Each cell is surrounded by a double membrane (see text) and the characteristic platelet granules are present in the two cells on the right.

Fig. 3.—Further stages of platelet digestion in a splenic macrophage. Note the myelin like figure developing in the top right corner. The lower lip of this inclusion is very similar to the cytoplasm of the partially digested platelet immediately below. Numerous other inclusions representative of differing stages of digestion may be seen.
Fig. 4.—Arrows indicate three platelets contained in a tongue-like extension of a splenic histiocyte. To the left and above is a lymphocyte and a plasma cell. To the right and above another histiocyte containing a number of myelin like figures. The uppermost and lowermost arrowed platelets show the intra-thrombocytic appearance of myelin figures in the phagocytosed platelets.

Similarities in the intracellular disruption and organelle changes seen on platelet incubation at 37 C. for 24 hours and those seen in the splenic histiocytes (Fig. 3 and 4). Stored platelets rarely exhibited myelin figure formation and then only after one to two weeks at 4 C. These findings would suggest that
the platelet is destroyed within the phagocytic cell by a combination of the histiocytes' own enzyme systems as well as the catalytic enzymes of the platelet itself.

These changes must be extremely frequent in idiopathic thrombocytopenic purpura since they were observed in eight of the nine spleens so far examined. This is further emphasised by the fact that our biopsy technic was purely random, and that platelet digestion has not been observed in the spleens obtained from the five patients with other hematologic disorders. The changes were similar in all patients although myelin figures were more prominent in some and lipid droplets in others. The one patient in whom these changes were not observed had a platelet life span of three days. This may be of some significance although one patient with a similar three day life span had identical findings to those observed in patients with platelet life spans of six hours. Somewhat similar morphologic observations have been noted by Casley-Smith et al. (1967) in an investigation of changes in artificial thrombi in pulmonary arteries of rabbits. These authors support Chandler and Hand's (1961) suggestion that some of the lipid accumulations in blood vessels may be due to phagocytosed platelets.

**Summary**

Electron microscopic examination of splenic tissue obtained from patients with a variety of hematologic disorders has shown ultrastructural evidence for platelet breakdown within splenic histiocytes in seven out of eight patients

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Fig. 5.—Portion of splenic histiocyte with numerous myelin figures from a patient with I.T.P.
with idiopathic thrombocytopenic purpura (I. T. P.). The morphologic characteristics of this intracellular digestion have been described.

**SUMMARIO IN INTERLINGUA**

Le examine a microscopia electronic de tissu splenic obtenite ab patientes con un varietate de disorderes hematologic ha revelate evidentia ultrastructural pro un decomposition plaquetal intra histiocytos splenic in septe de octo casos de idiopathic purpura thrombocytopenic. Le characteristicas morphologic de iste digestion intracellular es discutite.

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**REFERENCES**
