Observations on the Foam Cell in Thalassemia

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Whipple and Bradford were the first to describe the presence of large, pale mononuclear cells having frothy or granular cytoplasm and small vesicular nucleus in the spleen and the bone marrow of two cases of Cooley's anemia. They termed these cells "foam cells." It was subsequently noted that these cells stained but faintly with sudan stains. It is recognized at the present time that these cells may be present in the spleen and the bone marrow in thalassemia; but the literature does not provide any information regarding the origin or the significance of these cells. In the course of our study of the spleen in a series of 30 cases of thalassemia syndrome comprising cases of homozygous thalassemia and hemoglobin E-thalassemia, the foam cells were seen in large numbers in 4 cases only in hematoxylin-eosin-stained preparations. With the periodic acid–Schiff (PAS) method, the cytoplasm of the foam cells was stained deep red and these cells were thus very prominently displayed. Detailed investigations were then undertaken to determine the cytochemical make-up of the foam cell and to study the frequency of occurrence of these cells in the thalassemic cases. The results obtained are presented and discussed in this communication.

Material and Methods

The 30 patients whose spleens were examined, consisted of homozygous thalassemia, 6 cases, and hemoglobin E thalassemia, 24 cases. The age of the patients varied from 6/12 to 30 years. Sixteen were males and 14 females. All were anemic with the haemoglobin level varying from 2.0 to 9.5 g% 100 ml of blood. The weight of the spleen varied from 110 to 1285 gm.

The material examined consisted of the following: (a) paraffin sections of formol-saline-fixed specimens of spleen obtained by splenectomy in 29 cases and at autopsy in one case; (b) formol-saline-fixed frozen sections of an accessory spleen removed surgically from one of the above series of cases; (c) paraffin sections of spleen from different splenomegalic conditions, viz., tropical splenomegaly including cases of postmalarial splenomegaly, post-hepatitic splenomegaly and splenomegaly associated with Laennec's cirrhosis, hereditary spherocytosis, acquired hemolytic anemia, idiopathic thrombocytopenic purpura, aplastic anemia, myeloid leukemia, erythroleukemia, Niemann-Pick's disease, congestive splenomegaly of unknown origin and kala-azar.

The sections from all the cases were stained (1) with hematoxylin and eosin, and (2) by the PAS method. The sections from the spleens showing fairly large number of foam cells were subjected to the following histochemical methods.

For proteins (paraffin sections).—(a) Danielli's coupled tetrazonium reaction for simple protein containing tryptophane, tyrosine or histidine; (b) Millon reagent for tyrosine containing protein; (c) Feulgen's nuclear reaction; (d) Pyronin-methyl green stain with controls hydrolyzed in N HCl at 60 C. for 10 minutes, or subjected to the action of ribonuclease (Kunitz), 1 mg. per milliliter of distilled water, at 37 C. for 1 hour.
For lipids (frozen sections).—(a) Sudan black B in 70 per cent alcohol with controls subjected to extraction in pyridine at 60 C. for 20 to 22 hours; (b) Fettrot in 70 per cent alcohol; (c) Danielli's reaction4 for free and acetal aldehydes which are usually found in relation with lipids; (d) Performic acid-Schiff reaction (Pearse).5

For carbohydrates (paraffin and frozen sections).—(a) Periodic acid—Schiff reaction with controls subjected (1) to ptyalin digestion for 1 hour at 37 C., (2) acid hydrolysis for 10 minutes at 60 C., (3) extraction in pyridine at 60 C. for 20 to 22 hours, (4) hyalase (testicular hyaluronidase) digestion for 3 hours at 37 C. (b) Staining with toluidine blue (purified according to the method of Robinson and Bacsich); the sections were stained till the collagen in the splenic trabeculae showed gamma metachromasia, mounted in water and examined immediately; the controls were subjected to (1) hyalase digestion, (2) ribonuclease action and (3) hot pyridine; (c) staining with alcian blue.5

OBSERVATIONS

Morphology of the foam cell.—The foam cells were polygonal, rounded or oval in shape measuring (in formol-fixed paraffin sections stained with hematoxylin and eosin) on an average 18.5 x 13.1 μ, the range being, length 9.6–35.2 and breadth 8.0–24.0 μ. The nucleus was vesicular, rather small in size and was excentrically placed. As described by Whipple et al.,1 the cytoplasm appeared pale, foamy or frothy or granular (fig. 1).

Incidence.—These cells occurred in islands or small collections in the red pulp, the number of cells varying considerably in different cases. The foam cells could be demonstrated by the PAS method in 27 of the 30 cases studied. It could not be found in one case of homozygous thalassemia and two cases of hemoglobin E-thalassemia. In one of the latter cases it could, however, be demonstrated in the histologic section of the bone marrow.

Histochemical Observations

The cytoplasm.—PROTEINS. Simple protein was demonstrated by Millon's reagent and by the coupled tetrazonium reaction in the cytoplasm which
appeared to contain pale granular or globular areas which showed gamma metachromasia on counterstaining with toluidine blue though the color of the coupled tetrazonium was changed to dirty black by this procedure. With pyronin-methyl green stain, the cytoplasm was stained lightly with pyronin. On hydrolysis (N HCl or ribonuclease action), which completely removed the cytoplasmic pyroninophilia of other cells, there was only partial loss of pyroninophilia in the foam cells which showed granular pale red material in the cytoplasm. The above reactions showed that the cytoplasm contained protein and ribonucleic acid (RNA), the latter showing rather weak reaction, besides other material that was not removed by ribonuclease or acid hydrolysis and was stained metachromatically by toluidine blue. There was no desoxyribonucleic acid (DNA) in the cytoplasm.

LIPIDS. The cytoplasm was lightly stained with Fettrot and Sudan black B in frozen sections. After hot pyridine extraction, the sudanophilia appeared to be slightly less. Danielli's reaction for free and acetal aldehydes showed a faint pink coloration and the performic acid-Schiff reaction did not show a convincing reaction. The cytoplasm was thus found to contain small amounts of neutral lipids and possibly protein-bound lipids and phospholipids which were not removed by pyridine extraction of formol-fixed frozen sections. It was doubtful if any lipids with free unsaturated bonds were present.

CARBOHYDRATES. With the PAS method, the cytoplasm showed an intense reaction, and it appeared to be packed with coarse granules of PAS-positive material in both frozen and paraffin sections (fig. 2). Ptyalin digestion did not affect the PAS reaction. After acid hydrolysis, the cytoplasm still continued to be equally PAS-positive. The reaction was unaffected in both frozen and paraffin sections subjected to hot pyridine extraction at 60 C. for 20 to 22 hours. After hyalase digestion, the PAS reaction was slightly less intense than in the controls.

Fig. 2.—Section from the same spleen as shown in figure 1 stained by PAS method. The foam cells are prominently shown.
On staining with toluidine blue, the cytoplasm showed gamma metachromasia which was practically unaffected by hyalase digestion, was only slightly reduced by ribonuclease and unaffected by hot pyridine extraction.

The cytoplasm was stained bright blue with alcian blue. It could be concluded that the PAS-positive material was obviously not glycogen. From its metachromatic reaction with toluidine blue and bright blue staining with alcian blue, it appeared to be an acidic mucopolysaccharide. This mucopolysaccharide was mainly of the chondroitin sulfuric acid type (CSA), but there was possibly a very small fraction of hyaluronic acid associated with CSA, slight loss of intensity of PAS reaction after hyalase indicating this possibility. Slight loss of metachromasia after ribonuclease digestion indicates that the metachromatic coloration was only to a small extent due to RNA. In view of the fact that only weakly staining reactions could be obtained for lipids and gamma metachromasia was present, it seems unlikely that phospholipids had any contributory role in the causation of intense PAS-positive reaction.

The nucleus.—The nucleus was found to contain simple protein and RNA in the nuclear membrane and the nucleoplasm. The chromatin showed a strong reaction for protein and the presence of DNA and RNA. No lipids or carbohydrates could be seen in the nucleus.

Examination of Sections of the Spleen in Conditions Other than Thalassemia for the Presence of Foam Cells

Histologic sections of the spleen from the 14 conditions associated with splenomegaly and listed under Material and Methods were stained with hematoxylin and eosin and by the PAS method. Foam cells, similar in morphology and PAS staining reaction to those described in thalassemia, were not found in any of the nonthalassemic conditions so far studied by us.

Discussion

From the morphology of the ‘foam cell’ as seen in the tissue sections, it is apparent that these cells belong to the reticuloendothelial system and are histiocytes. The histochemical investigations carried out so far indicate that the main chemical constituent in this PAS-positive material that is responsible for the foamy appearance is an acidic mucopolysaccharide of the chondroitin sulfuric acid type. Other substances are also present in the cell in minute or relatively small amounts, viz., lipids, hyaluronic acid, RNA, protein, etc.

Study of the incidence of the foam cells in thalassemia and other conditions indicates that the foam cells are demonstrable in varying numbers in almost all cases of thalassemia but not in any of the other conditions studied by us.

We have next to consider the possible source of this acidic mucopolysaccharide in the foam cell in thalassemia.

Two possibilities suggest themselves. This mucopolysaccharide may be derived from extrinsic sources and stored by the histiocytes in their cytoplasm. For example, in Whipple’s disease foamy cells containing PAS-positive mucopolysaccharide occur in the submucosa of the small intestines, mesenteric lymph nodes, portal tracts, subendothelial tissue and elsewhere, but not in
the spleen and the bone marrow. It has been claimed that this mucopolysaccharide is derived from the intestinal mucosa and stored in the histiocytes, which thus appear as foamy cells. Alternatively, the polysaccharide may be produced in the cell itself as a result of an inherent disorder in cell physiology. Von Gierke's disease shows such an intrinsic defect in the hepatic cell physiology, viz., deficiency of the enzymes required for the conversion of glycogen (PAS-positive) into glucose, particularly of glucose-6-phosphatase, which leads to accumulation of glycogen in the hepatic cells.

Astaldi et al.\textsuperscript{10} recorded the presence of a PAS-positive substance in the erythroid cells in thalassemia. This finding was confirmed by Chatterjea et al.,\textsuperscript{11} who found PAS-positive material in the late erythroblasts and normoblasts in this disease. Astaldi et al.\textsuperscript{10} regarded this substance as neutral mucopolysaccharide because it did not show any metachromasia with toluidine blue and did not stain by the Hale method. It may be mentioned that the Hale technic is not regarded as a procedure suitable for critical histochimistry.\textsuperscript{5} We have stained smears of bone marrow from two recent cases of thalassemia with purified toluidine blue; the normoblasts showed the presence of granules stained reddish in color (gamma metachromasia), thus indicating that the substance in these granules (which were PAS-positive) was an acidic mucopolysaccharide. It appears probable that the mucopolysaccharide in the erythroid cells and that described by us in the foam cells are identical or closely related. It has been recently shown that immature erythroid cells are destroyed in the bone marrow in thalassemia major.\textsuperscript{12} The mucopolysaccharide liberated by the lysis of the abnormal erythroid cells in thalassemia may be taken up by the histiocytes. This PAS-positive substance accumulates in their cytoplasm, thus giving rise to their foamy appearance.

Astaldi et al. regard the presence of the mucopolysaccharide as an inherent abnormality in the erythron in Cooley's anemia. It is possible that this anomaly representing a specific intracellular defect of carbohydrate synthesis in the developing blood cells\textsuperscript{13} is shared by some of the cells of the reticuloendothelial system, viz., the histiocytes. But the nature of abnormality in the physiology of the erythron in Cooley's anemia is yet unknown, and in the absence of precise information on this subject further speculation does not appear to be justified.

**Summary**

Histologic and histochemical study of the foam cell in the spleen in thalassemia has shown that the foamy appearance is due to the accumulation of an acidic mucopolysaccharide of the chondroitin sulfuric acid type in the cytoplasm of this histiocytic cell. This polysaccharide shows intense red coloration with the periodic acid Schiff reaction, which is thus of great value in demonstrating the foam cell in tissue sections. The possible sources of this mucopolysaccharide have been discussed.

**Summario in Interlingua**

Studies histologic e histochemic del cellula spumose in le splen in casos de thalassemia ha demonstrate que le apparentia spumose del cellula es debite
al accumulation de un mucopolysaccharido acidic del typo acido chondroitin-sulfuric in le cytoplasma de iste cellula histiocytic. Iste polysaccharido monstra un intense coloration rubie in le reaction de Schiff a acido periodic, lo que es de grande valor in demonstrar le presentia del cellula spumose in sectiones de tissu. Le possibile origine de iste mucopolysaccharido es discutite.

REFERENCES
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