Unidentified Reticuloendothelial Cell Storage Disease

By Theodore I. Malinin

In 1954 Sawitzky, Hyman and Hyman described unidentified cells in the bone marrow and spleen of two young adults with hepatosplenomegaly. Similar cells were previously observed by Moschlin in splenic aspirates and in the bone marrow. From their morphologic appearance these cells were thought to be reticuloendothelial in origin, but they differed from any previously described reticuloendothelial cells, macrophages or mast cells. Two abnormal cell types were noted in the bone marrow smears stained with Wright's stain. One type was a large cell with eccentrically placed nucleus and densely packed blue-staining cytoplasmic granules. The second cell was a large, foamy cell with scattered dark granules. Unlike the first cell, this appeared to be a macrophage of the type not uncommonly noted in the bone marrow. Histochemical studies revealed that the intracytoplasmic granules were PAS positive, regardless of exposure to diastase, and did not stain with toluidine blue. The granules were negative for alkaline phosphatase, did not stain with acetic-carbol-Sudan III, and contained no iron. Smears stained by Feulgen's technic indicated the presence of DNA in the nucleus only. On the basis of these studies the authors concluded that the intracytoplasmic granules were mucopolysaccharides.

Reticuloendothelial cells which exhibit somewhat similar histochemical properties are found in Gaucher's disease. Morphologically, however, Gaucher's cells appear quite different showing a characteristic, uniformly staining, finely granular cytoplasm. Keratin, a glycolipid, has been extracted in large amounts from spleens removed at surgery from patients with Gaucher's disease. This cerebroside in pure form is soluble in chloroform and methanol at room temperature, gives a positive aldehyde reaction with PAS reagent, and is sudanophilic because of its fatty content. On the other hand, material found in Gaucher's cells does not stain with Sudan III and absorbs ultraviolet light at 2800 Å, rather than from 2675 to 2700 Å, as does pure keratin. Kovacs et al. showed that Gaucher cells revealed anilinophilia and a positive PAS reaction, even after treatment in a boiling chloroform-ethanol mixture. However, both of these reactions were abolished by treatment with pyridine. The reaction with pyronin was also positive but was abolished by exposure to HCl. This led them to believe that the cells contained RNA. Franco and Wolman showed that sections of spleen from patients with Gaucher's disease could be rendered sudanophilic.

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by boiling at pH 4.0. Any free fat was removed by previous treatment with ether and acetone. They thought this method to be specific for cerebrosides, particularly for kerasin.

The present investigation was undertaken in order to report a case of reticuloendothelial cell storage disease similar to those reported by Sawitzky et al. and to attempt to establish the chemical nature of this disorder.

**CASE REPORT**

F. S. (U. Va. #333851), a 30 year old gravida VI, para VI, white female was first admitted to the medical service, University of Virginia Hospital on 4-9-60, 1 day post partum because of hepatosplenomegaly of unknown etiology. An enlarged liver and spleen, ankle edema and severe bilateral varicosities had been noted during a prenatal visit on 1-11-60. On April 8, she spontaneously delivered a full term infant without difficulty.

The patient stated that she was in apparently good health until December, 1959, when she developed a “flu” with anorexia, nausea, vomiting and cough productive of mucopurulent, blood-streaked sputum. About three weeks following the onset of symptoms she noted that her urine turned golden yellow. This persisted for approximately seven days. All symptoms subsided in about 4 weeks with the exception of a chronic cough productive of a small amount of mucopurulent sputum. She denied jaundice, light-colored stools, dark urine, and right upper quadrant pain or tenderness.

The physical examination showed a well developed and nourished white female in no distress. The positive findings were: irregular, dark, macular pigmentation over the face, upper chest and shoulders; liver which extended 4 cm. below the right costal arch with the edge reaching the iliac crest laterally; and a spleen palpable 4-6 cm. below the left costal arch.

Laboratory Findings: Hct. 42; WBC 7,100; Diff. J 1, B 8, Sg 49, L (large) 18, L (small) 15, M 6, E 3; Sed. rate 19 mm/hr.; BSP 4% retention hr.; Cephalin floc. 3+, repeated neg.; VandenBergh direct 0.15 mg., indirect 0.30 mg.; Total protein 6.37 gm., Albumin 52%, A globulin 5%, A1 globulin 11%, B2 globulin 14%, G globulin 18%, LDH 128 units; SGOT 18 units; SGPT 9 units; urinary Bence-Jones protein 0; OT 1:333 neg., Fungus skin tests neg. The urinalysis, electrolytes, cholesterol, blood glucose, serum alkaline phosphatase, urea, and the stool benzidine were within normal limits. Acid phosphatase was 1.75 mg. % on one occasion, but two subsequent determinations were negative. The roentgenograms of the chest were within normal limits.

Examination of the bone marrow smear stained with Wright’s stain showed it to be hypercellular, erythronormoblastic with an increase in young forms of the myeloid series. Three per cent of nucleated bone marrow cells were large cells with dark blue cytoplasmic granules and cells with foamy cytoplasm.

Because of severe varicosities, patient underwent a bilateral tubal ligation, at which time biopsies consisting of a wedge of liver and a mesenteric lymph node were obtained. At operation the liver was large, but was of a usual appearance and consistency. The patient was last seen on 8-10-60. She was asymptomatic and apparently well.

**METHODS OF STUDY**

**General Morphology**

Two abnormal cell types were noted in the bone marrow smears stained with Wright’s stain. The predominant cell was a large reticuloendothelial-like cell with a small eccentrically placed nucleus. The cytoplasm contained numerous dark sea blue, densely packed round and oval granules (fig. 1). The second type showed a foamy cytoplasm, scattered dark blue granules, and an eccentric nucleus with a prominent nucleolus (fig. 2). The latter cells had an appearance
Fig. 1.—Reticuloendothelial-like cell from the bone marrow ("young" form). The cytoplasmic granules are dark blue. Wright's stain. × 1200.

Fig. 2.—Bone marrow cell with a foamy cytoplasm and a few residual dark blue granules ("old" form). Wright's stain. × 1200.
of a macrophage. Remnants of granulocytes were seen in several of them. An “intermediate” stage was also present. It was represented by cells whose cytoplasm contained vacuoles as well as dark blue granules.

The liver showed normal architecture. Several parenchymal cells were vacuolized. Abnormal cells similar to those seen in the bone marrow were found in the sinusoids and in the periportal connective tissue. The cytoplasmic granules were not visible in hematoxylin and eosin preparations but stained dark blue with Giemsa and Wright’s stains (fig. 3).

The lymph node appeared normal. Scattered reticuloendothelial cells closely resembling those observed in the bone marrow and the liver were present, primarily around the medullary strands. The cytoplasmic granules did not stain with hematoxylin and eosin but stained with Giemsa and Wright’s stains (fig. 4).

Histochemistry

The bone marrow smears were air-dried before staining. No fixative was used. The tissues were fixed in Carnoy’s fluid for 4 hours, transferred to abso-
Fig. 4.—Essentially normal lymph node showing abnormal cells (arrows) in the stroma of the medulla. Giemsa $\times 160$.

Lute ethanol for 4 hours, then to 3 changes of dioxan, 1 hour in each. After immersion into 3 changes of paraffin for a total of 4 hours the specimens were embedded in fresh paraffin. Sections 6 $\mu$ thick were cut. Sections to be used for protein stains were placed on slides without adhesive, and incubated at 60° C. overnight. Sections to be used for other stains were placed on slides smeared with Myer's albumin. A small piece of liver was fixed in formalin. It was used for frozen sections. The following stains were used and are summarized in table 1.

1. Periodic acid Schiff's reaction (PAS).—The cytoplasmic granules stained dark red. The staining was more intense at the peripheries of the granules than in their centers (fig. 5). The nuclei did not stain. Cells exposed to diastase before staining showed no change in the staining capacity of the granules. This indicated that the PAS-positive material was not glycogen.

2. Feulgen's reaction.—The nuclear chromatin stained dark red violet. The cytoplasmic granules did not stain, indicating an absence of DNA in the granules (fig. 6).
### Table 1.—Histochemical Analysis of Cytoplasmic Granules

<table>
<thead>
<tr>
<th>Stain</th>
<th>Result</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>Unstained</td>
<td>Colorless</td>
<td></td>
</tr>
<tr>
<td>H &amp; E</td>
<td>Does not stain</td>
<td>Stain evenly</td>
</tr>
<tr>
<td>Wright's</td>
<td>Dark Blue</td>
<td></td>
</tr>
<tr>
<td>Giemsa</td>
<td>Dark Blue</td>
<td>Stain evenly</td>
</tr>
<tr>
<td>Feulgen</td>
<td>Does not stain</td>
<td></td>
</tr>
<tr>
<td>PAS</td>
<td>Dark Red</td>
<td>The granule stains more intensely at the periphery.</td>
</tr>
<tr>
<td>PAS after treatment with diastase</td>
<td>Dark Red</td>
<td>Stain evenly</td>
</tr>
<tr>
<td>Toluidine Blue</td>
<td>Dark Blue</td>
<td></td>
</tr>
<tr>
<td>Mallory Aniline Blue</td>
<td>Does not stain</td>
<td></td>
</tr>
<tr>
<td>Smith-Dietrich</td>
<td>Does not stain</td>
<td></td>
</tr>
<tr>
<td>Nile Blue Sulphate</td>
<td>Dark Blue</td>
<td>Stain evenly</td>
</tr>
<tr>
<td>Sudan III</td>
<td>Dark Blue</td>
<td>Stain evenly</td>
</tr>
<tr>
<td>Sudan IV</td>
<td>Does not stain</td>
<td></td>
</tr>
<tr>
<td>Sudan Black</td>
<td>Faint staining</td>
<td></td>
</tr>
<tr>
<td>After boiling at pH 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan III</td>
<td>Orange-Red</td>
<td>Stain evenly</td>
</tr>
<tr>
<td>Sudan IV</td>
<td>Orange-Red</td>
<td>Stain evenly</td>
</tr>
<tr>
<td>OTA</td>
<td>Dark Brown</td>
<td>The granule stains more intensely at the periphery.</td>
</tr>
<tr>
<td>FOTA</td>
<td>Dark Brown</td>
<td>The intracytoplasmic reticulum in foamy cells stains dark brown.</td>
</tr>
<tr>
<td>Methyl Green Pyronin</td>
<td>Bright Red</td>
<td></td>
</tr>
<tr>
<td>Methyl Green Pyronin after treatment with ribonuclease</td>
<td>Bright Red</td>
<td></td>
</tr>
</tbody>
</table>

3. **Toluidine blue.**—The intracytoplasmic granules stained intensely deep blue, without metachromasia. The absent metachromasia probably meant bound or masked electronegative surface changes or the absence of them. No specific groups can be inferred from this reaction.8

4. **Mallory Aniline Blue.**—The cytoplasm did not stain at all, a finding which differs from that in Gaucher's disease.

5. **Smith-Dietrich** stain for phospholipids.9—No phospholipid was demonstrated in the granules or the endoplasmic reticular network.

6. **Fat Stains.**—Both the granules and the nuclear chromatin stained dark blue with Nile blue sulfate. Lack of red color indicated the absence of triglycerides.10 Frozen sections, smears, and permanent sections stained with Sudan III and Sudan IV gave a negative reaction. Sections and smears treated with Sudan black showed only a faint coloration in the granules. Following treatment with boiling acetate buffer at pH 4.0 for 60 seconds, the cytoplasmic granules stained evenly dark red-orange with Sudan III and IV (fig. 7). The sudanophilia was present not only in the smears and frozen sections but also in the paraffin sections. Treatment with ethanol, xylene, and acetone before boiling did not
abolish the reaction. These findings indicated that the granules contained bound fat or fatty acid complexes and that these complexes could be broken up by harsh treatment, thus rendering them sudanophilic.

8. Oxidized tannin azo (OTA) technic.7—The intracytoplasmic granules and the nuclear membrane stained dark yellowish brown, indicating an abundance of tannophilic protein in these cells. The staining was more intense in the periphery of the granule than in the center (fig. 8). In the vacuolated cells the intracytoplasmic reticulum stained dark brown, also showing its protein nature.

9. Feulgen-oxidized tannin azo (FOTA) method.11,14—The results of staining by the FOTA technic were similar to those in the OTA method alone, except that the nuclear DNA stained dark red (fig. 9). Again, this reaction showed that the granules contained abundant protein and the hydrolysis with HCl did not interfere with the ability of this protein to bind tannic acid.

10. Methyl green pyronin.16—The intracytoplasmic granules stained bright
Fig. 6.—Bone marrow cell. The eccentric nucleus is Feulgen-positive. Feulgen reaction. × 1200.

red. Prolonged treatment with ribonuclease did not inhibit the reaction, indicating that apparently no RNA was present in the granules.

Discussion

An unusual cell with cytoplasmic granules was found in the bone marrow, liver and a lymph node of a young white adult female with incidentally discovered hepatosplenomegaly. Morphologically, the cell seemed to be reticuloendothelial in origin. Its presence in three different organs far removed from each other suggested a wide distribution of this cell throughout the reticuloendothelial system. Two varieties of the cell were observed. One containing densely packed cytoplasmic granules was thought to be a “young” form, while the other cell with foamy cytoplasm was regarded as an “old” form.

Presence of tannophilic protein and sudanophilic material in the cytoplasmic granules indicated they were composed of an insoluble unknown chemical complex which contained lipid and protein. The tannophilic protein seemed
Fig. 7.—Bone marrow cell after boiling at pH 4.0. The cytoplasmic granules stain positive for lipid. Sudan III. × 1200.

Fig. 8.—Bone marrow cell. The tannophilic protein (black) is found primarily at the periphery of the granules. OTA. × 1200.
to be abundant near the periphery of the granule while the lipid material was seen chiefly in the center of the granule. Unfortunately, the amount of tissue available was not sufficient for further biochemical analysis of the lipid. The fact that sudanophilic material was uncovered by treatment with boiling acetate buffer did not seem to indicate that it was specifically kerasin, as thought by Kovacs et al.6 The intracytoplasmic reticulum of “older” foamy cells stained only for protein and no lipid.

The clinical manifestations shown by the patient and the morphology of abnormal cells were in many respects similar to those described by Sawitzky et al.,1 but from the data available it was impossible to state whether the cells in the present case were identical to those described by these authors. The similarity rested in the presence of nonglycogenic PAS-positive material in the granules, and the difference in the negative toluidine blue reaction in Sawitzky’s cases.

The cells were strikingly different from those in Gaucher’s disease in their nuclear and cytoplasmic appearances. Likewise, the described cells failed to stain with Mallory stain, while Gaucher’s cells attain characteristically dark blue color with this technic.14 Niemann-Pick’s disease can be disregarded because of striking histochemical and morphologic dissimilarities.

Certain similarities between these cells and tissue mast cells were evident. However, they were morphologically and histochemically different from mast cells since their granules had a sea blue color, varied in size and shape, were not metachromatic and developed sudanophilia after acid hydrolysis.
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It was believed that the foamy type of cell represented degenerated forms which had lost their granules. The presence of the "intermediate" stage seemed to support this viewpoint. The fact that granulocytes were occasionally present in the cytoplasm of the foamy cells suggested a phagocytic ability. It was impossible to determine whether the lipid- and protein-containing granules were the phagocytized material or the product of cell's metabolism.

SUMMARY

1. An abnormal cell of probably reticuloendothelial origin with prominent cytoplasmic granules was observed in the bone marrow, liver and a lymph node of an adult white female with incidentally discovered, asymptomatic hepatosplenomegaly.
2. Histochemical studies revealed the granules to consist of a chemical complex which contained bound lipid and protein.
3. The cells differed from those of Gaucher's disease, Niemann-Pick's disease and other storage diseases. The significance of these cells remains unknown.

SUMMARIO IN INTERLINGUA

1. Un cellula anormal, probablemente de origine reticuloendothelial, con prominente granulos cytoplasmic esseva observate in le medulla ossee, le hepate, e un nodo lymphatic de un adulte feminina de racia blanc con hepatosplenomegalia asymptomatic de discoperta incidental.
2. Studios histochimic revelava que le granulos consisteva de un complexo chimic a contento de ligate lipido e proteina.
3. Lecellulas differeva de illos de morbo de Gaucher, morbo de Niemann-Pick, e altere morbos de magasinage. Le significacion de iste cellulas remane incognoscite.

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REFERENCES


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