Gaucher's Disease is an inherited disorder of sphingolipid metabolism caused by a deficiency of glucocerebrosidase. The deficiency of this enzyme leads to the accumulation of its substrate, glucocerebroside, in reticuloendothelial cells, imparting to them their characteristic appearance. The presence of Gaucher cells was considered pathognomonic of this hereditary disorder until 1966 at which time Albrecht described a patient with chronic myelocytic leukemia (CML) with typical Gaucher cells in his bone marrow. She then reviewed bone marrow aspirates from 64 patients with CML and found Gaucher cells in ten additional cases.

This report concerns a patient with CML in whom Gaucher cells were seen in the bone marrow and other organs. Biochemical and electron microscopic studies were performed to establish the relationship of these cells to those found in Gaucher's disease. Evidence will be presented showing that these cells are morphologically and biochemically similar to those of the hereditary disorder but arise through a different mechanism.

Case Report

I.B., (MCH #7477) was a 57 year old Negro woman who entered Morrisania City Hospital on August 18, 1967, with the primary complaint of increasing weakness. One and one half years previously a diagnosis of leukemia had been made at another hospital. The patient was treated with Busulfan with good results (no records were available and this information was obtained from her niece). Several months prior to admission she had stopped taking the Busulfan and had become symptomatic.

Diagnoses of diabetes mellitus and hypertension had been made in the past, and at the time of admission the patient was taking chlorpropamide and chlorothiazide. The family history revealed no evidence of Gaucher's disease.

The pertinent findings on physical examination were generalized lymphadenopathy.
and massive hepatosplenomegaly. There were no pingueculae, and the rest of the examination was unremarkable. The hematocrit was 30 per cent, the platelet count 110,000 mm$^3$ and the white count 800,000 mm$^3$. The initial differential revealed 10 per cent polymorphonuclear neutrophils, 16 per cent juveniles, 20 per cent metamyelocytes, 25 per cent myelocytes, 20 per cent promyelocytes, 5 per cent myeloblasts, 2 per cent lymphocytes and 2 per cent eosinophils. Leukocyte alkaline phosphatase* was absent. The serum alkaline phosphatase was 60 King-Armstrong units, the bilirubin 3.8 mg. per cent, and the uric acid 15 mg. per cent. An acid phosphatase performed soon after admission was 20 Babson-Read units (normal up to 5) and a repeat determination 10 days after admission was 10.2. At this time, acid phosphatase by the Bodansky method was 5.6.

A bone marrow aspirate performed on admission was diagnostic of CML. In addition, many large, ovoid cells with small eccentric nuclei were present. Their cytoplasm was pale blue and had the "wrinkled" appearance typical of Gaucher cells (Fig. 1). All subsequent bone marrow examinations showed plentiful numbers of these cells (5-10 Gaucher cells per low power field).

Initially, treatment with Busulfan produced a moderate decrease in the white count and reduction in hepatosplenomegaly. However, after one month there was transformation of her CML into acute myeloblastic leukemia. The patient was then treated with a combination of Vincristine, Methotrexate, 6-Mercaptopurine, Prednisone and Allopurinol. There was a good hematologic response with apparent remission, but she became progressively debilitated and died on December 22, 1967.

At autopsy the liver, spleen and lymph nodes were enlarged and infiltrated by Gaucher cells (Fig. 2). More striking was the bone marrow which contained sheets of these cells (Fig. 3). These cells were Periodic acid-Schiff (PAS) and aniline blue positive. The cause of death was Pneumocystis carinii pneumonia. The only evidence of leukemia was a small infiltrate in the occipital lobe of the brain.

* Method of Kaplow.7
Fig. 2.—Postmortem section of spleen from I.B. with Gaucher cell (arrow) in field of normal splenic tissue. The Gaucher cells are sparsely distributed. No leukemic infiltrate is seen. Mallory’s aniline blue stain. (Original magnification X 1000)

MATERIALS AND METHODS

Bone marrow aspirates for light microscopy were smeared by the particle technic and stained with Wright and Giemsa stains after methanol fixation.

Bone marrow slides of all patients with CML seen at Montefiore Hospital since 1963 were reviewed. No slide was accepted as having Gaucher cells unless all the observers agreed.

The following studies were done on tissues from patient I.B.:

1) For electron microscopy, freshly aspirated bone marrow particles of less than 0.5 mm³ were immediately fixed for one hour in iced 15 per cent glutaraldehyde in 1/15 M phosphate buffer, pH 7.4. The tissue was then washed in buffer and postfixed for one hour in Dalton’s solution. After dehydration in graded alcohols and propylene oxide, the particles were embedded in Epon. Thin sections were cut with a diamond knife on a Porter-Blum microtome, stained with uranyl acetate and examined on either an RCA EMU3F or a Siemens 1A electron microscope.

2) Acid phosphatase preparations for electron microscopic examination were made according to the Goldfischer et al. modification of the Gomori technic. Freshly aspirated marrow particles which had been fixed for one hour in iced 2 per cent glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2, were studied.

3) The assay of glucocerebrosidase-cleaving enzyme was performed on leukocytes as outlined by Kampine et al. with the following modification: Washed leukocytes were homogenized with 20 volumes of cold acetone in a Waring blender. The homogenate was then washed three times in cold acetone and dried in vacuo. The acetone powder were triturated with 10 volumes (w/v) of cold 0.01 M potassium phosphate buffer, pH 6.0, containing 5 mg. of sodium cholate per ml. of solution. The suspensions were kept at 0 C. for 1 hour and then centrifuged for 20 minutes at 25,000 x g. Glucocerebrosidase
Fig. 3.—Post-mortem bone marrow section from I.B. showing sheets of Gaucher cells. Arrow points to typical example. H & E stain. (Original magnification × 1000)

was quantitatively recovered in the supernatant solutions and the amount of enzymatic activity was determined by incubating the extracts in the presence of 14C-labeled glucocerebrosidase. The amount of 14C-glucose liberated was measured in a liquid scintillation counter and the results expressed as nanomoles of substrate hydrolyzed per mg. of protein per hour.

4) The amount of glucocerebroside in spleen tissue was determined in the following manner: An acetone powder of the tissue sample was prepared as indicated above for the leukocyte preparations. Glucocerebroside is not extracted by this procedure since it is not soluble in cold acetone. A 100 mg. portion of the acetone powder was suspended in 3 volumes (w/v) of 0.1 M potassium chloride solution. To this suspension were added 6 ml. of a solution of chloroform-methanol, 2:1 (v/v). The sphingolipids were extracted and partitioned, and nonsphingolipids were hydrolyzed as described previously. Glucocerebroside was isolated from the mixed sphingolipids by preparative thin-layer chromatography. The purified material was eluted from the chromatogram with warm chloroform-methanol, 2:1 (v/v), and the amount of glycolipid was determined by the phenol-sulfuric acid procedure. A portion of the patient's spleen tissue and 2 control spleen samples were analyzed simultaneously. The latter were histologically normal spleens obtained at necropsy of an 88 year old female and at incidental splenectomy performed on a 45 year old female, both of whom had carcinoma.

RESULTS

Review of CML Marrows

Marrow aspirates from 19 patients with CML were reviewed. Gaucher cells were identified in one of these. In addition, the marrow of I.B. and several other marrow specimens contained cells which may represent a transitional phase (Fig. 4) between reticuloendothelial cells and Gaucher cells. These cells were ovoid, pale blue, contained an eccentric nucleus and were the
same size as Gaucher cells. They differed from Gaucher cells in that their cytoplasm was granular and contained scattered thread-like streaks, but lacked the characteristic striations seen in typical Gaucher cells.

Electron Microscopy

The ultrastructural appearance of the Gaucher cells (Fig. 5) was identical to those reported in the hereditary disorder. The cytoplasm was filled with numerous spindle-shaped membrane-bound inclusion bodies. These bodies contained variable numbers of relatively uniform tubules measuring 200 to 750 A in diameter, oriented in roughly parallel bundles. In addition to phagocytosis of erythrocytes by Gaucher cells, a finding previously described, phagocytosis of nucleated cells was seen (Fig. 6). The phagocytosed nucleated cells had lobulated nuclei and large nucleoli but only rarely contained specific granules. These cells most probably represent immature white cells; since at the time of the study, the marrow specimen consisted almost entirely of myeloblasts and Gaucher cells. Acid phosphatase reaction product was present in the Gaucher’s cells (Fig. 7). Control specimens prepared in the same manner but omitting substrate were negative.

Glucocerebrosidase Activity

The mean of two determinations of glucocerebrosidase-cleaving enzyme in extracts of acetone powders of the patient’s white cells was 19.5 nanomoles
of substrate cleaved per mg. of protein per hour. This figure is markedly higher than the normal value obtained in the same laboratory (mean = 6.6).

**Glucocerebrosidase Content of Spleen Tissue**

The glucocerebrosidase content of the patient's spleen was 131 nanomoles per 100 mg. of acetone powder. The amount of glucocerebrosidase in the two control specimens was 70.9 and 76.7 nanomoles respectively. Spleens from patients with Gaucher's disease contain ten to forty times the normal amount of glucocerebrosidase, depending upon the degree of infiltration by Gaucher cells.
COMMENTS

The electron microscopic appearance of the cells and the elevated glucocerebrosidase level in the spleen confirm the identity of the cells seen in our patient with those of hereditary Gaucher’s disease. This represents, to the best of our knowledge, the first time that the presence of Gaucher cells in CML has been reported in the English language literature. The association was originally recognized by Albrecht, but no biochemical or electron microscopic
Fig. 7.—Acid phosphatase preparation of bone marrow. Reaction product (arrows) and erythrophagocytosis (RBC) are demonstrated. (Original magnification × 14,280)

studies were done on her patients. Our findings confirm Albrecht’s conclusion that the cells seen are Gaucher’s cells.

The frequency of this association is not known at present. Albrecht found Gaucher cells in ten of her series of sixty-four patients with CML, and we were able to find one additional case in nineteen CML patients reviewed. Thus, it appears that the occurrence of Gaucher cells in CML is not rare. Elevation of serum acid phosphatase level may serve as a clue to their presence. Acid phosphatase determinations should be made prior to anti-
leukemic therapy, since the elevated levels in our patient returned to normal after therapy.

The pathogenesis of hereditary Gaucher’s disease has been clarified in recent years. Trams and Brady demonstrated that the synthesis of glucocerebroside, the sphingolipid which accumulates in Gaucher’s cells, is normal in Gaucher’s disease. Further investigation revealed that both splenic tissue and leukocytes from patients with Gaucher’s disease are deficient in the enzyme glucocerebrosidase. These findings are consistent with the hypothesis that the accumulation of glucocerebroside in Gaucher’s disease results not from overproduction, but from lack of destruction, of this sphingolipid, secondary to deficiency of glucocerebrosidase. However, the glucocerebrosidase activity in our patient’s leukocytes was elevated, as has been previously described in leukemic granulocytes. This rules out the possibility that she had the hereditary form of Gaucher’s disease and suggests an entirely different pathogenesis for this acquired form of Gaucher’s disease.

The source of glucocerebroside which accumulates in Gaucher’s disease has never been clearly defined. It has been proposed that this material arises from the degradation of erythrocyte sphingolipid. This hypothesis was examined by calculating erythrocyte sphingolipid turnover utilizing known figures for red cell lipid and glycolipid content and red cell mass. Our calculations indicate that 5 to 10 mg. of sphingolipid are normally released daily from senescent erythrocytes. Using data for granulocyte kinetics and granulocyte lipid and glycolipid content to calculate granulocyte sphingolipid turnover, we estimate that 350-400 mg. of sphingolipid are derived daily from leukocytes. This figure is forty to eighty times greater than the amount derived from erythrocytes. Hence, it appears that the granulocyte, not the erythrocyte, is normally the major source of sphingolipid. The production of sphingolipid from granulocytes must be greatly augmented in CML since the total body granulocyte pool and the granulocyte turnover rate (GTR) are markedly increased in this disorder. For example, a patient with CML and a white blood count of 400,000 mm³ was shown by Athens et al. to have a GTR of ten times normal. It is likely that our patient, with a white count of 800,000 mm³, had an even greater turnover of granulocytes and, consequently, of sphingolipid. Increased sphingolipid turnover has been shown experimentally to elevate tissue levels of glucocerebrosidase. The increased level of this enzyme seen in our patient probably represents an example of enzyme induction secondary to substrate overload.

We postulate, then, that the acquired form of Gaucher’s disease seen in CML is due to an increased GTR which results in overproduction of glucocerebroside, which in turn induces increased glucocerebrosidase activity. However, the increased enzyme activity is insufficient to metabolize the substrate excess, and glucocerebroside accumulates and is stored in RE cells. This situation is in direct contrast with hereditary Gaucher’s disease in which tissue levels of glucocerebrosidase are markedly reduced and sphingolipid accumulation results from decreased destruction, not from overproduction, of the substrate glucocerebroside.
SUMMARY

A patient with CML is presented in whom Gaucher’s cells were seen in the bone marrow and other tissues. Biochemical and electron microscopic studies established the similarity of these cells to those found in Gaucher’s disease. Evidence is presented which indicates that the granulocyte is normally the major source of sphingolipid and that the Gaucher’s cells seen in CML arise from excessive granulocyte turnover.

SUMMARIO IN INTERLINGUA

Es presentate le caso de un patiente con chronic leucemia myeloblastic in qui cellulas de Gaucher esseva observate in le medulla ossee e in altere tissus. Studios biochimic e electron-microscopic establiva le similitude de iste cellulas con illos trovate in morbo de Gaucher. Es presentate evidentia indicante que le granulocyto es normalmente le fonte major de sphingolipido e que le cellulas de Gaucher vidite in chronic leucemia myeloblastic ha lor origine in un excessive metabolismo de granulocytos.

APPENDIX

The following calculations were performed to determine the amount of glycolipid derived daily from (1) red cell and (2) white cell breakdown:

(1) Red cell (RBC) lipid = \(0.45 \times 10^{12}\) Gm./cell

Red cell glycolipid = 5 to 10% of total lipid

= \(23 \times 45 \times 10^{12}\) mg./cell

Total red cells for 70 Kg. man = \(24.5 \times 10^{12}\) cells

Total RBC glycolipid = \((23 \times 45 \times 10^{12}\) mg./cell\) \times \(24.5 \times 10^{12}\) cells

= 563 to 1126 mg.

Daily RBC glycolipid turnover = \(\frac{1}{120}\) \times 563 to 1126 mg.

= 5 to 10 mg.

(2) Granulocyte lipid = \(21.5 \times 10^{12}\) Gm./cell

Granulocyte glycolipid = 16% of total lipid = \(3.4 \times 10^{12}\) Gm./cell

Granulocyte turnover rate for 70 Kg. man = \(1.14 \times 10^{11}\) cells/day

Granulocyte glycolipid turnover = \((1.14 \times 10^{11}\) cells/day\) \times \(3.4 \times 10^{-12}\) Gm./cell

= 387 mg./day

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ADDENDUM

Since this manuscript was submitted for publication, Smith et al. have sent a letter to the editors of Lancet (Lancet 2:780, 1968) describing another patient with CML and Gaucher cells.

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