The Pathogenesis of Gaucher's disease has not yet been clarified. Recently this problem has been investigated by studying the submicroscopic morphology of Gaucher cells in the bone marrow, spleen and liver. These studies have demonstrated that the cerebroside or the cerebroside-protein complex (Uzman's lipoprotein) is aggregated in tubular elements which appear as dense rings in cross section, the outside diameter measuring about 400 A. A variable amount of tubules, together with a pale matrix, is contained in cytoplasmic vacuoles, called Gaucher bodies. These very characteristic bodies, because of their high content of acid phosphatase activity and other enzymes, could be considered lysosomal in nature.

Moreover, Lee et al. have found that isolated cerebroside has the same tubular morphology as observed in the Gaucher cells. Nevertheless, the precise mechanism involved in the accumulation of intracellular cerebroside has not yet been completely elucidated. De Marsh and Kautz support the hypothesis that the Gaucher cells incorporate a lipoprotein precursor of extracellular origin by pinocytosis rather than by endogenous synthesis. On the contrary, Ross et al. and Fisher and Reidbord have found some morphologic features of the Gaucher bodies which suggest that the mitochondria participate in the formation of these structures. Lee et al., studying iron metabolism in Gaucher's disease, support the concept that destroyed phagocytized red cells are the source of cerebroside in the Gaucher cells. Finally, support for a lack of glucocerebroside-cleaving enzyme comes from studies by Brady et al. using glucose-14C cerebroside as substrate. The results of Fraccaro et al. with in vitro culture of spleen cells from a case of Gaucher's disease are in keeping with the hypothesis of cerebroside accumulation by phagocytic activity.

The present paper is concerned with the fine structure of the Gaucher cell in the spleen and liver. In particular, we have investigated the possible correlation between erythrophagocytic activity and the morphogenesis of Gaucher bodies.

Case Report

L.E., a 5 year old girl, was admitted to the Pediatric Clinic because of splenomegaly, frequent hemorrhagic manifestations and anemia. The first symptoms appeared between two and three years of age. The family history was not contributory.
Fig. 1.—Gaucher cells in a spleen follicle observed in a thick section with the light microscope. Gaucher cells surround the follicular arteriole and show an abundant cytoplasm filled with elongated vacuoles. × 510.

The patient was physically underdeveloped (height 90 cm., weight 13 Kg.) but mentally normal with an I.Q. of 107. The spleen was grossly enlarged to the level of the iliac crest. The liver was also enlarged 3 cm. below the costal margin. No changes were detected in the skin, bones and central nervous system. The hemoglobin was 5.5 Gm. per cent; hematocrit 20 per cent; white blood cells 1,800 per cu. mm.; platelet count was 40,000 per cu. mm.; the serum acid phosphatase level was 148 I.U.

Bone marrow examination revealed numerous Gaucher cells.

The patient underwent splenectomy. The spleen weighed 780 Gm. and complete absence of glucocerebrosidase activity was detected.11

MATERIALS AND METHODS

Small fragments obtained from both spleen and liver were fixed in 2 per cent glutaraldehyde in phosphate buffer pH 7.4 at 4 C. After a 2 hour fixation, the tissue was postfixed in 2 per cent osmium tetroxide in the same buffer, dehydrated through graded alcohols, passed through two changes of propylene oxide and embedded in Epon 812.12 Thick section (0.5 μ) were cut with a Porter-Blum microtome and subsequently stained with Giemsa.
MORPHOGENESIS OF GAUCHER CELLS

Fig. 2.—Gaucher cells in the liver observed in a thick section with the light microscope. Gaucher cells (G) are located among the laminae of the hepatocytes. Note the presence of two dark hepatocytes (arrows). X 1,500.

The sections were then examined under the light microscope for the presence of Gaucher cells. Thin sections were then cut from the same blocks, using an LKB microtome, with a glass knife. Sections were placed on bare grids (200 mesh) and stained with uranyl acetate followed by lead citrate. All preparations were observed in a Hitachi HU 11 A electron microscope at 75 kv.

Results

Gaucher cells, stained with Giemsa and examined by light microscope, appear as large, round cells, 48-80 μ in diameter. The cytoplasm is filled with many vacuoles and the nucleus is usually eccentric with an evident nucleolus. These cells are found both in the follicles and red pulp of the spleen (Fig. 1). In the liver, numerous large Gaucher cells are interspersed among the hepatocytes and have the same morphologic characteristics described above. Modified "dark" hepatocytes are also seen in these thick sections (Fig. 2).

With the electron microscope, Gaucher cells appear as a type of reticuloendothelial cell, frequently in contact with plasma cells or small lymphocytes in the spleen (Fig. 3) or as a modified Kupffer cell in the liver (Fig. 4).
Fig. 3.—Electron micrograph of a Gaucher cell in the spleen. Numerous large vacuoles (called Gaucher bodies) filled with small tubules are visible in the cytoplasm. Note multiple contact between a plasma cell (Pc) and the Gaucher cell (G). Upper right a lymphocyte (L). × 7,500.

Gaucher cells have an abundant cytoplasm with a rather complex cell border which extends into small pseudopodia or ridge-like projections similar to irregular microvilli. These frequently enclose erythrocyte fragments or small tubules (Fig. 5). The cytoplasm contains numerous oval or crescent-shaped
Fig. 4.—Electron micrograph of two Gaucher cells in the liver. Gaucher cells appear as modified Kupffer cells by accumulation in the cytoplasm of the cerebroside tubular material. Note the evident space of Disse encircling the Gaucher cells (arrows). $\times 6,000$.

bodies of variable size, generally surrounded by a single smooth membrane and with internal tubular structures (Fig. 6). These tubules appear as rings in cross section which are moderately osmiophilic and measure approximately 400–600 Å in diameter (Fig. 7). Tubules are set in a pale matrix in the largest bodies and in a rather dense substance in medium and small bodies which appear numerous in some Gaucher cells (Figs. 8, 9, 10 and 11). Further-
Fig. 5.—Phagocytic activity of the Gaucher cells in the spleen. On the cell border there are irregular microvilli which enclose red cells fragments and small tubules (arrows). In the cytoplasmic bodies tubules are embedded in a matrix of a different density. (Above) $\times 10,000$. (Below) $\times 14,400$. Inset $\times 30,000$. 
Fig. 6.—Cytoplasmic border of adjacent Gaucher cells in the spleen. Many ridge-like projections line one of the cell borders. Close to the plasma membrane are evident some pinocytic vesicles (arrows). In the cytoplasm many large Gaucher bodies (LGB) containing tubular elements. × 14,400.

more, there are a variable number of dense erythrophagosomes in which tubular structures are sometimes detected (Fig. 12).

However, in the larger cytoplasmic Gaucher bodies, the limiting membrane is frequently interrupted and the tubular elements are spilled into the extracellular space (Fig. 13).

In summary, there is a variable number of cytoplasmic bodies: the largest are the most typical and are characterized by the presence of tubular structures; the smallest, frequently seen at the periphery of the cell (Fig. 11), contain tubules in a dense matrix and sometimes appear as irregular cytoplasmic striations. Among these structures, dense bodies interpreted as erythrophagosomes are observed.

The cytoplasm contains both rough and smooth endoplasmic reticulum; clusters of free ribosomes; and a fair number of pinocytic vesicles (Fig. 6). The mitochondria surrounding the limiting membrane of the cytoplasmic
bodies are usually swollen and show rarefaction of the cristae and breakdown of the external double membrane (Fig. 12 and 14). The rough endoplasmic reticulum appears as tubular structures rich in ribonucleoprotein particles or as dilated and isolated vesicles (Fig. 14). A small Golgi apparatus is sometimes evident. The nuclei are often eccentrically located exhibiting a nuclear envelope with an evident perinuclear space and large pores. There is a distinct nucleolus and chromatin is frequently condensed in aggregates disposed around the periphery of the nucleus (Fig. 13).

**DISCUSSION**

The correlation of electron microscopic features of Gaucher cells with those observed by light microscope in thick sections, clearly indicates that the cytoplasmic vacuoles correspond to the large Gaucher bodies noted with the electron microscope. Furthermore, with the electron microscope it is possible to detect other cytoplasmic bodies which appear in longitudinal section as
Fig. 8.—Ultrastructure of Gaucher bodies. Note the different density of the amorphous material surrounding the tubules. × 38,100.

particular cytoplasmic striations because of the presence of a dense intertubular material. The Gaucher cells which contain a great number of these striations (Fig. 11) correspond histologically to Gaucher cell type II with a granular or amorphous cytoplasm as described by Földish.13

In agreement with Jordan2 and Neimann et al.,14 we have seen in Gaucher cells some erythrophagosomes (Fig. 12); on the other hand we have noted a rather intense phagocytic and pinocytic activity of Gaucher cells with accumulation of tubular structures in the smallest cytoplasmic bodies (Figs. 5, 6 and 10). These observations suggest the extracellular origin of tubular cerebroside material by means of direct pinocytosis of small lipoproteic precursors taken up from the extracellular space or from partial digestion of the red cell stroma in the erythrophagosomes.

Lipid storage begins with partial digestion of erythrocytes by lysosomal enzymes; afterwards, there is also an active secondary incorporation of the cerebroside material which is continuously liberated from lysed Gaucher cells. We think that this last mechanism is much more evident in the late clinical stages of Gaucher's disease when a great quantity of cerebroside is present in the spleen and in other organs (Fig. 15).
Fig. 9.—Characteristic aspect of the cytoplasm at the periphery of two Gaucher bodies. Note the presence of intracytoplasmic tubular aggregates. × 38,100.

Because of their acid phosphatase and esterase activity, together with the morphologic evidence of a single bounding membrane, Gaucher bodies could be considered lysosomes in which the tubules represent residual material. The demonstration of a deficiency of glucocerebrosidase-cleaving enzyme activity in splenic tissue supports this morphologic data. Moreover, the mitochondrial alterations observed seem to be non-specific modifications related to the rupture of the limiting membrane of the large Gaucher bodies (Fig. 14). Finally, in the spleen, we have observed plasma cells surrounding Gaucher cells showing sometimes plasma membrane contact (Fig. 3). This observation, together with the finding of gamma-globulins in Gaucher bodies, might suggest a possible immunologic mechanism in the pathogenesis of Gaucher’s disease. Further studies are needed to clarify this critical point.

The synthesis in situ of cerebroside by reticuloendothelial cells and the possibility of a re-absorption of tubular material in some Gaucher bodies must be also discussed. Since we have seen very active pinocytosis and phagocytosis [numerous microvilli on cell border engulfing amorphous material, tubules and erythrocyte debris (Figs. 5 and 6)], we feel that the hypothesis of a real synthesis in the Gaucher cell cannot be supported while the extracellular origin of this material with possible aggregation in macromolecular
Fig. 10.—Intracytoplasmic structures of a Gaucher cell in the spleen. Filamentous structures and small Gaucher bodies (SGB) with a dense matrix are evident. This cell corresponds to type II Gaucher cell as described by Födisch. ×14,400. Inset ×30,000.
cytoplasmic complex seems more realistic. Moreover, it must be considered that in other pathologic conditions in which there is an increased cerebroside load produced by cellular lysis, typical Gaucher cells can be detected. In regard to the tubular re-absorption in the Gaucher bodies, we feel that morphologic evidence supports the leakage of these structures from lysed Gaucher cells (Fig. 13) instead of being destroyed in situ. This spilled material present in the extracellular space either as tubules or as soluble precursors can be re-accumulated by other reticuloendothelial cells (Fig. 15).

Fig. 11.—Ultrastructure of the small Gaucher bodies. These bodies are generally found at the periphery of the cell and contain tubules surrounded by a dense substance. × 48,900.

Summary

The ultrastructure of Gaucher cells in the spleen and in the liver from a case of juvenile type of Gaucher's disease is described. Different types of cytoplasmic bodies are observed in Gaucher cells. These are surrounded by a single smooth membrane and contain tubular or ring-like structures that in the smaller bodies are set in a rather dense substance. Among these structures some erythrophagosomes have been detected. The limiting membrane of larger Gaucher bodies is frequently interrupted and the tubules appear in the extracellular spaces.

These observations support the hypothesis of the extracellular origin of tubular cerebroside material with a continuous production of cerebroside from digested erythrocytes associated with pinocytosis of the cerebroside subsequently liberated from the lysed Gaucher cells.
Fig. 12.—Erythrophagosomes in Gaucher cells in the spleen. Erythrophagosomes showing dense homogeneous material and tubular like structures (arrows). × 15,000.
Fig. 13.—Lysis of a Gaucher cell in the spleen. The cytoplasm of this cell is partially disrupted and tubules spill into extracellular space (arrows). Note the regressive changes of the cytoplasmic organelles. × 6,000. Inset × 12,000.

SUMMARIO IN INTERLINGUA

Le ultrastructura de cellulas Gaucher in le splen e in le hepate ab un patiente con morbo de Gaucher de typo juvenil es describite. Differente typos de corpores cytoplasmatic esseva observate in le cellulas Gaucher. Istos es circumsed per un sol membrana lisie, e illos contine structuras tubular o de configuration anular le quales, in le caso del corpores plus micre, as disponite in un satis dense substantia. Inter iste structuras un certe numero de erythrophagosomas esseva detegite. Le membrana limitatori del corpores Gaucher plus grande es frequentemente interrumpite, e le tubulos appare in le spatio extracellular.

Iste observationes supporja le hypothese del origine extracellular de material cerebrosidic tubular con un production continue de cerebrosida ab erythrocytos digerite in association con pinocytosis del cerebrosida subsequentemente liberate ab le lysate cellulas Gaucher.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Giuseppe Tridente for helpful advice concerning the
Fig. 14.—Mitochondrial damage in a Gaucher cell. (Higher magnification of Figure 13). Mitochondria are swollen and show a breakdown of the double membrane in connection with LGB (arrow). × 11,000.

performance of this study. The technical assistance of Mr. Gilberto Miotti-Scapin is gratefully acknowledged.

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Fig. 15.—Schematic representation of Gaucher cell morphogenesis. Red cell fragments are phagocytized and gradually transformed into cytoplasmic bodies containing tubules. The broken arrow indicates the possibility of a secondary pinocytosis of Gaucher cell. This second mechanism is more evident when Gaucher's dis-ery is clinically manifested.


The Morphogenesis of Gaucher Cells Investigated by Electron Microscopy

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