The Submicroscopic Morphology of Gaucher Cells

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It is the purpose of this paper to describe the submicroscopic morphology of the large cells characteristic of Gaucher’s disease. Electron micrographs of sternal bone marrow biopsy specimens from three patients with Gaucher’s disease are presented. For comparison we have included descriptions of the cells as examined in Wright’s stained preparations with the bright field microscope, and unstained fresh cells examined with the phase contrast microscope and the polarization microscope.

Observations

Wright’s stained cells

Gaucher cells stained with Wright’s stain and examined by bright field appeared as large rounded cells, 50-60 micra in diameter. The cytoplast was faintly purple, while the small, usually eccentric nucleus was blue. Often several rounded nuclei were seen within a single cell. The cytoplast was stringy or fibrillar in appearance, the individual fibrils seeming to cross one another. The cells were readily distinguished from foam cells, whose globular inclusions appeared as empty vacuoles after fixation.

Living cells—phase microscope

A fresh drop of aspirated bone marrow was placed on a clean slide and covered with a cover slip which was ringed with vaseline. It was then viewed with Dark M phase contrast optics (American Optical Company). Phase photomicrographs included in the present paper were taken by the late Dr. Paul Ralph.

Typical Gaucher cells could readily be identified in bone marrow examined in vitro. They appeared as large cells with one to several eccentric nuclei (fig. 1). Considerable bubbling and pseudopod formation was seen at the periphery of the cell. The endoplasm was filled with dense fibrillar or crescent shaped bodies which were moderately refractile and appeared darker than the cytoplasmic contents of neighboring cells. These fibrils were less than 1.2 microns in width and up to several microns in length, and sometimes appeared to be branched. Individual fibrils were often seen to cross one another, the many fibrils comprising a tangled meshwork in the endoplasm. When the cell wall was ruptured by pressure on the cover slip or by allowing the preparation to stand for 24 hours, single fibrils could be visualized (fig. 2). Each fibril seemed to be bounded by a limiting membrane which was too thin to be sharply resolved. At the ends of the fibrils one often saw small tails of this membrane which appeared to be crossed or frayed out, and here could be more clearly resolved (indicated by arrows in figure 2). Occasional small rounded mitochondria were seen between the fibrils.

Polarization microscope

Fresh smears were prepared as for phase microscopy and examined with the Inoué polarization microscope. The irregularities of the ectoplasmic border could be seen even more clearly in these preparations (fig. 7). The fibrillar structures in the endoplasm showed a moderate amount of birefringence when viewed with polarized light (fig. 3). Because of

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This investigation was supported by a research grant (C-1332-C5) from the United States Public Health Service.

Submitted July 3, 1956; accepted for publication Aug. 7, 1956.
Fig. 1.—Living Gaucher cell, Dark M phase contrast (American Optical Company). Two small nuclei (N) can be seen within the tangled meshwork of fibrillar bodies. The fibrils (F) are moderately refractile and are confined to the endoplasm. Occasional small rounded mitochondria (M) are seen between the fibrils. The ectoplasm exhibits extensive bubbling and pseudopod formation.

Fig. 2.—Mass of fibrils from a Gaucher cell which was ruptured by allowing the preparation to stand for 24 hours, Dark M phase. One gets the impression that the single fibrils are bounded by a limiting membrane. At the ends of the fibrils (indicated by arrows) this membrane seems to be frayed out, and is here more clearly resolved. Note that the fibrillar material is denser and more refractile than the cytoplasmic contents of several nearby granulocytes.
FIG. 3.—Living Gaucher cell, polarization microscope. The lower picture was taken after the compensator had been turned 90°. The cell border can be seen on the right, at the edge of an area of thin clear ectoplasm. The tangled fibrils in the endoplasm collectively show a moderate amount of birefringence.

the irregular shape of the fibrils and the fact that, even in a spread cell, many fibrils were piled on top of one another in random orientation, it was not possible to measure the birefringence of individual strands.

*Electron microscope observations on fixed cells*

Immediately after aspiration a portion of the marrow was placed in buffered 0.25 percent osmic acid² with KCl added (1.5 ml. of 1N KCl in 25 ml. fixative). The fixation and
embedding schedule was essentially the same as that used by us for other blood and bone marrow studies. The tissue was sectioned and examined with an RCA-EMU electron microscope with biased gun and 100 micron objective aperture.

In marrow prepared for electron microscopy the typical Gaucher cells were easily identified by their large size and characteristic morphology (fig. 4). They lacked the specific granules, polymorphic nucleus and other morphologic features of megakaryocytes, which are the only other cells of large size with which they might have been confused. Each Gaucher cell contained one to several small rounded nuclei, usually located in an eccentric position. Each nucleus showed patches of dense chromatin, often in a peripheral ring along the nuclear membrane, and one to several dense nucleoli. The cytoplasm contained numerous “fibrils” (described in more detail below), elongate mitochondria 1\(\mu\) to 1\(\frac{1}{2}\) micron long, and occasional dense walled vacuoles approximately 1\(\frac{1}{2}\) micron in diameter. One could also identify endoplasmic reticulum, clusters of Golgi membranes with their associated vesicles, numerous other small vesicles not associated with the Golgi membranes but of similar dimensions (about 600 \(\AA\)), and occasional other very dense inclusion bodies. The small non-Golgi vesicles were seen singly or clustered in rosettes throughout the cytoplasm, and were

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**Fig. 4.**—Osmic acid fixed cell, electron micrograph. The eccentric nucleus (N) shows part of a nucleolus (Nu), and a dense peripheral ring of chromatin. Mitochondria (M), vacuoles (V) and Gaucher fibrils (F) are seen in the cytoplasm.
Fig. 5.—Portion of the cytoplasm of a Gaucher cell, electron micrograph. The elongate mitochondria (M) display typical cristae. Golgi membranes with associated vesicles are seen at G. Small amounts of endoplasmic reticulum (er) are seen at several points in the cytoplasm. Many small vesicles are seen singly or in clusters (several are indicated at mv). The numerous fibrils (F) within the cytoplasm are seen as broad bands of medium dense material, and are sometimes branched.

Fig. 6.—Higher power view of an area from the cell shown in fig. 5, showing several fibrils. The limiting membrane (lm) appears dense, and single at this level of resolution. Within the fibrils is an apparently homogeneous matrix, in which are seen longitudinally arranged tubular subunits. These appear as dense rings in cross-section (xs) and as double filaments in favorable longitudinal sections (ls). They measure approximately 130 Å in total diameter. While these tubular elements are in general oriented in the long axis of the fibril, we detect no particular orientation or spacing with respect to one another.
especially numerous near the cell membrane. A more detailed description of the area near the cell membrane is given below.

The "fibrils" of the Gaucher cell appeared in electron micrographs as broad bands of finely granular, medium dense material, irregular in outline and bounded by a limiting membrane (fig. 5). They showed no particular orientation within the endoplasm. Single
"fibrils" varied in width from a fraction of a micron to several microns, and were often seen to be branched. In sections they were often many microns long. Sometimes the entire endoplasm was filled with a network of these fibrils. At low magnifications one received an impression of oriented subunits within the fibrils. At higher power (fig. 6) the individual fibrils revealed a single, dense, apparently continuous limiting membrane. This enclosed

Figs. 9, 10, 11.—Three electron micrographs showing the junctions between pairs of Gaucher cells. The cell border is extended into ridge like projections which, when sectioned across the base, appear as pairs of membranes parallel to the cell membrane. These are seen to be continuous with the cell membrane (arrows). Numerous microvesicles are seen between and along the inner surfaces of cell membranes (mv).
a lighter ground substance in which were embedded dense-walled tubular structures oriented along the long axis of the fibril. These tubular elements appeared as dense rings in cross section and as double filaments where longitudinally sectioned. They measured approximately 130 Å in outside diameter.

Since the cells in these electron microscope preparations were fixed immediately upon removal from the marrow cavity, they had no chance to spread as did the cells prepared for in vitro light microscope studies. The ectoplasm was therefore thinly disposed around the endoplasm (figs. 4 and 8). As in light microscope studies, the ectoplasm was free of Gaucher fibrils, and usually also free of mitochondria and other organelles. The cell surface presented a complex picture. Sometimes on a free surface (fig. 8), and often when the cell was crowded against another Gaucher cell or other cell (figs. 9, 10, 11), the cell border was extended into numerous long projections and ridges. These projections were longer and less regular than the microvilli seen on the free surfaces of many absorptive cells. Sometimes several rows of membranes were arranged parallel to the border of the cell. These were seen to be continuous with the cell membrane, and apparently represented sections across the bases of ridge-like projections from the cell surface. Occasional rounded cross sections of finger-like projections were also encountered. Some of these projections were swollen at the tip in a rounded bleb. Small vesicular structures (approximately 600 Å in diameter) were often seen within the ectoplasmic projections, in the blebs, and lined up between and along the inner surfaces of the membranes which bordered the cell. These vesicles were similar in size to other small vesicles seen throughout the endoplasm, and also to the Golgi vesicles. A few large vacuoles lay near the cell border. The vacuole wall was similar in appearance to the cell membrane.

**DISCUSSION**

In an attempt to interpret these new morphologic findings, the literature concerning various aspects of Gaucher's disease is summarized below. The biochemical and histochemical findings are largely from the work of Uzman. Blood chemistry and organ analyses on Gaucher subjects have been done by several groups of workers. Physical findings on the three patients included in the present study, as well as on a number of other patients with Gaucher's disease seen by the senior author, agree well with the classical clinical picture of the disease. Diagnosis was confirmed in all cases by the presence of Gaucher cells in the stained bone marrow smear.

**Biochemical and histochemical**

The glycolipid, kerasin, has been extracted in large amounts from spleens removed at surgery from patients with Gaucher's disease. This cerebroside is composed of sphingosine, lignoceric acid and a hexose—galactose, or sometimes glucose. Pure kerasin is soluble at room temperature in 1:1 chloroform: methanol, absorbs in the ultraviolet at 2675–2700 Å, gives a positive aldehyde reaction with periodic acid Schiff reagent, and might be expected to be sudanophilic and osmophilic, because of its fatty acid component. Since the kerasin in dehydrated tissue sections from Gaucher patients is removed only after harsh extraction procedures (boiling organic solvents), sectioned Gaucher cells absorb at 2800 Å (the typical peak for many proteins) but not at the lower range, and for several other reasons, Uzman concluded that the lipid fraction is probably firmly bound to a larger chemical entity in the Gaucher cell. This author found that the extracted lipoprotein contained 62 per cent kerasin and 38 per cent protein. The kerasin was envisioned to be imbedded in a mesh of peptide coils, and firmly bound to the protein moiety. Uzman suggested that the protein component may be some nor-
normal protein component of the cell. Morphological evidence related to this hypothesis will be discussed below.

When blood chemistry studies have been done on Gaucher’s patients, it has been found that blood levels of fats, cholesterol and lecithin were within normal limits. Total cholesterol and ester cholesterol levels, as well as cerebrosides, were elevated in the spleen. Keratin could not be detected in the blood or urine.\(^6\)\(^7\)

**Clinical**

Gaucher’s disease has often been viewed as a generalized disturbance of lipid metabolism resulting in abnormal lipid storage.\(^5\) Several facts contradict this. The substance within Gaucher cells is neither sudanophilic nor osmophilic. Lipemia is not associated with the disease. Generalized lipid metabolism does not appear to be disturbed, nor is the particular glycolipid, keratin, found in the serum in either normal or Gaucher’s subjects. In addition the sites of lipid or lipoprotein accumulation are rather limited. In particular the spleen and bone marrow are preferentially involved. Patients with Gaucher’s disease are detected clinically only after the disease is far enough advanced to result in complaints related to splenomegaly, marrow destruction, anemia, thrombopenia or bone lesions. Histories indicate that this is probably an hereditary condition—i.e., one of the so-called “inborn errors of metabolism.”

Although the disease is intracellular in manifestation, the fact that Gaucher cells are found in several sites suggests that a whole system, for instance the reticulo-endothelial system, is involved.\(^8\) It seems unlikely that the spleen and marrow are “robbing” other organs of a necessary metabolite. Rather one suspects either an overproduction of keratin by these organs, or a “trapping” of keratin (or its immediate precursors) as it circulates in trace amounts. The latter interpretation finds some support in the morphological findings presented in the present electron microscope study.

**Morphologic**

Gaucher cells present some rather interesting morphological features which may be correlated with the clinical and biochemical findings in Gaucher’s disease. In the phase microscope the large fibrils of Gaucher cells are moderately refractile. They are not as refractile as other bodies composed entirely or predominately of lipid. In fixed cells, either for Wright’s staining or for electron microscopy, the fibrils are not extracted by the alcohol used in dehydration, as is the substance within foam cells. In electron micrographs of osmic acid fixed cells, the fibrils are not highly osmophilic as would probably be the case with many lipid bodies. The submicroscopic tubular elements within the fibrils are moderately electron dense. This could indicate either native density or osmophilia, due for instance to either protein or lipid concentrated at these sites. The moderate amount of birefringence of the fibrils when the cells are viewed with the polarization microscope could be explained by oriented lipid or protein, or it could be a scattering effect.

The size of these submicroscopic units (approximately 130 Å in diameter) is of the order of large molecules. They show no definite length, nor can one detect periodicity such as is seen in many long molecules known to be polymers of shorter repeating units (e.g., collagen, muscle myofilaments).\(^7\) Uzman calculated the molecular weight of the lipoprotein molecule to be about 320,000.\(^6\) The
dimensions of other molecules in this molecular weight range are of the same order of magnitude as the submicroscopic elements in Gaucher fibrils (e.g., fibrin, hemoglobin). It is suggested here that the submicroscopic tubular component seen within Gaucher fibrils may well represent the macromolecular lipid or lipoprotein unit characteristic of the Gaucher cell.

Uzman suggested that the keratin in Gaucher cells was probably bound to some normal protein component of the cell. Other cells rich in structural protein, especially ribonucleoprotein, display large amounts of endoplasmic reticulum, or ergastoplasm, arranged in double membranes. These are often highly oriented and sometimes fill the entire cytoplasm (e.g., in the pancreas, plasma cells). This finding has been correlated with the high degree of cytoplasmic basophilia of these cells. Palade suggested that the membrane system associated with cytoplasmic basophilia (ergastoplasm) is one form of a more general membrane system to which the nuclear membrane, cell membrane, vacuolar and vesicular membranes, and Golgi membranes also belong. Wright’s stained Gaucher cells do not exhibit a high degree of cytoplasmic basophilia. In electron micrographs one sees occasional endoplasmic reticulum, as in many other marrow cells, but this does not reach the proportions of that seen in plasma cells or pancreatic acinar cells. The limiting membranes of the fibrils do not have the appearance of ergastoplasm. They are not studded with the fine particulate component (particles of Palade) usually seen on ergastoplasmic membranes. If the glycolipid keratin is bound to some architectural protein in the Gaucher cell, it does not appear that this protein is the ergastoplasm. From morphological findings the fibrils, which apparently represent the lipoprotein complex, are an abnormal constituent of marrow cells. No cells of similar size and appearance are seen in normal marrow, and no such fibrils are seen in any of the cells of normal blood or marrow, or in fixed or free reticulo-endothelial cells, in our experience.

The surface membrane of the Gaucher cell also presents some interesting features. The pseudopodia and ridge-like projections of the surface membrane have been described. They do not resemble the microvilli seen on many absorptive cells (e.g., chick chorio-allantoic membrane, mesothelium, gall bladder, intestinal epithelium, placenta). However, the increased surface area seen in spread cells in vitro, and in electron micrographs of sectioned cells would suggest that the Gaucher cell is active at the surface. The appearance of tiny vesicles (approximately 600 Å in diameter) near the surface membrane is reminiscent of similar structures seen in endothelial cells (vesicles of Palade), lung endothelium and epithelium, gall bladder epithelium, and kidney endothelium. In these cases the vesicles are concentrated along surface membranes of the cells, and often appear to have pinched off from invaginations of the cell surface (caveoli intracellularis). It has been postulated that such vesicles may represent a mechanism of active transport of substances across cell membranes.

In Gaucher’s disease one of several situations may prevail: (a) the Gaucher cells may manufacture the large lipoprotein molecules in situ from smaller precursor metabolites; or (b) the lipoprotein may be synthesized elsewhere and taken up by the Gaucher cells. If the latter situation obtains, it is reasonable to postulate some method of active transport of the substance across the Gaucher cell surface membrane. The molecule is so large that it would diffuse only very slowly and the lipoprotein is concentrated inside the cell against a gradient. Incorpo-
ration of the lipoprotein, or an immediate precursor, into microvesicles, in a man-
ner similar to that postulated in other cells, could be the mechanism involved.
This would account for the many small vesicles seen near the cell border in
Gaucher cells.

Summary

The large cells characteristically found in the bone marrow and other organs
in Gaucher’s disease have been reinvestigated with the electron microscope, as
well as with phase contrast and polarizing microscopes and by standard staining
methods. The cytoplasm was filled with a number of dense elongate or crescent
shaped bodies. Each of these fibrils was in turn seen with the electron microscope
to be bounded by a single dense limiting membrane, and to contain a homo-
geous appearing matrix in which were embedded numerous tubular-appearing
subunits. These tubular elements measured approximately 130 Å in diameter,
and were of very great length. It is suggested that these submicroscopic tubular
structures could represent the molecular keratin or lipoprotein units known from
biochemical and histochemical evidence to be present in Gaucher cells.

Some details about the appearance of the ectoplasm in spread cells, and the
area near the cell border in cells fixed in situ, are reported. Electron micrographs
revealed a complex cell border which was extended into many small pseudopodia
and ridge-like projections. Numerous microvesicles about 600 Å in diameter were
found in the area near the cell membrane. This is taken as possible evidence of
transport of some substance across an active cell membrane. An attempt is made
to correlate these morphologic findings with the clinical and biochemical findings
of ourselves and others on Gaucher’s disease.

Summary in Interlingua

Le grande cellulas que es characteristicamente incontrate in le medulla ossee e
altere organos de patientes con morbo de Gaucher ha essite re-investigate per
medio del microscopio electronic e etiam per medio del microscopios a contrasto
de phase e polarisante e per methodos standard de coloration. Le cytoplasma
esseva plenate de un numero de dense elongate o crescentiforme corpores. Le
microscopio electronic monstrava que omne iste fibrillas esesva individualmente
delimitate per un dense membrana unic e que illos contineva un matrice de aere
homogeneity in que reposava numeroso subunitates de apparentia tubular. Iste
elementos tubular habeva diametros de circa 130 Å durante que lor longor esseva
considerable. Es stipulate le possibilitate que iste submicroscopic structuras
tubiforme representa le unitates de cerasina o lipoproteina que es presente in
cellulas de Gaucher secundo lo que ha essite demonstrate per medios biochimic
e histochimic.

Es reportate certe detalios in re le apparentia del ectoplasm in cellulas de
frottis e del area vicin al margine de cellulas fixate in situ. Micrographia elec-
tronic revelava un complexe margine cellular que se estendeva in numerose
parve pseudopodios e projectiones crestiforme. Multe microvesiculas de circa
600 Å in diametro esseva trovate in le area vicin al membrana cellular. Isto
indica possiblemente que il occurre hic le transporto de alicun substantia a
transverso un active membrana cellular. Es interprendite le tentativa de correla-
tionar iste constataiones morphologic con le datos clinic e biochimic colligite per nos e per alteres in le manipulation de morbo de Gaucher.

REFERENCES

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QUIN B. DEMARSH and JEAN KAUTZ