GAUCHER'S DISEASE: PATHOGENETIC CONSIDERATIONS BASED ON ELECTRON MISCROSCOPIC AND HISTOCHEMICAL OBSERVATIONS

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The clinical and histologic features of the disorder recognized as Gaucher's disease have been well documented. It has also been established that the deposits observed within the large reticuloendothelial cells morphologically pathognomonic of the disorder and designated as "Gaucher cells" contain the cerebroside, kerasin or its glucose analogue.[1] Uzman[2] has emphasized that the cerebroside is firmly bound within the cell in the form of a lipoprotein in which there is a marked tenacity between the lipid and protein moieties. He has concluded that this information militates against the pathogenetic possibility that a primary defect in specifically active glucocerebrosidase or galactocerebrosidase systems exists in Gaucher's disease. Nevertheless, there are many who consider this disorder to represent a disturbance of lipid metabolism within the affected reticuloendothelial cells. However, the precise mechanism or mechanisms involved remain to be elucidated. It has also been proposed that the large accumulation of intracellular cerebroside noted in Gaucher's disease may be derived from the serum as a result of a general disturbance in the intermediary metabolism of lipids. This concept has led to consideration of Gaucher's disease as a form of lipid storage disease, a designation not uncommonly used. Injections of cerebrosides and kerasin into experimental animals have revealed storage of this substance within cells of the reticuloendothelial system.[3,4] However, such experiments lack specificity since a variety of injected materials exhibit a similar fate and further, as emphasized by Thannhauser,[1] deposits of this nature appear to be only temporary whereas in the human disease they persist.

A recent study in our laboratory concerning the nature and pathogenesis of Whipple's disease,[5] a disorder also characterized by intracellular deposits within histiocytes, has revealed certain conceptual similarities between this condition and Gaucher's disease. Electron microscopy, as well as selected histochemical procedures, strongly indicated that Whipple's disease represented a disorder of cellular metabolism and that the locus of abnormality might reside within the mito-

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chondria. Because of this, it was considered worth while to examine by similar techniques the splenic tissue from a patient with Gaucher's disease. It was surprising to learn that except for the study of DeMarsh and Kautz,[6] who noted dense, elongated, crescent-shaped bodies within the Gaucher cell cytoplasm, and the brief note by Roos, Cottier and Rossi [7] indicating that storage began within the mitochondria of Gaucher cells, no information concerning the cellular ultrastructure was available.

Case Report

The patient, a 19-year-old Jewish woman, was first noted to have splenomegaly and hepatomegaly at 2 years of age. Bone marrow biopsy at that time disclosed typical Gaucher cells, but other laboratory examinations were within normal limits. She exhibited normal growth and development, and although splenomegaly was confirmed on numerous subsequent examinations, she was in good general health. The patient was married at the age of 18 and one year later became pregnant. This was accompanied by further enlargement of the spleen into the pelvis. Because of this a splenectomy was performed without event. The spleen weighed 1,750 gm. and measured 30 by 14 by 10 cm. The outer surface was smooth, glistening, purple and slightly bosselated. The cut surface was slightly convex, purple and firm, containing poorly defined grayish nodules measuring approximately 4 cm. in diameter.

The patient has remained in good general health during her pregnancy without notable hematologic or other alterations. She has no siblings, and inquiry into the family history failed to reveal other members with Gaucher's disease or other heritable disorders.

Methods of Pathologic Study

Immediately after surgical removal, blocks of spleen were fixed in absolute alcohol, 10 per cent neutral buffered formalin, Zenker-acetic acid fluid, or immediately quick-frozen on dry ice and stored at −20° C. until used. Some blocks fixed in formalin were subsequently treated with 2.5 per cent potassium dichromate for 48 hours before processing. A variety of enzymatic and tinctorial reactions, including the fluorescent antibody technique,[8] were performed on these tissues (Table I). One mm. cubes of splenic tissue were also fixed in 1 per cent osmic acid, dehydrated and embedded in Vestopal W. Ultrathin sections of these were prepared with a Porter-Blum microtome and were examined either stained with lead hydroxide [9] or unstained, by a Philips EM 100 electron microscope. Sections, 1 μ in thickness, were also prepared from the Vestopal blocks, stained with thionine and the periodic acid-Schiff (PAS) technique and examined by conventional microscopy.

Results

Histologic Observations

Sections of spleen prepared from formalin, post-chromed formalin, Zenker-acetic acid and alcohol fixed tissue revealed replacement of broad areas of splenic pulp by aggregates of large cells possessing a pale eosinophilic cytoplasm with faintly visible eosinophilic cytoplasmic striations characteristic of Gaucher cells (Fig. 1). Nuclei were round to ovoid and not infrequently eccentrically placed. Nuclear chromatin appeared condensed at the nuclear rim and nucleoli were not conspicuous.

Histochemical Observations

As indicated in Table I, the cytoplasm of Gaucher cells exhibited an intense PAS reaction in frozen sections as well as in tissue fixed in formalin and subsequently post-chromed. The reaction was less intense in tissues fixed in formalin, alcohol or Zenker's fluid. In all instances the PAS reaction was unaffected by antecedent diastase treatment. The positive PAS material appeared to be localized to the striations observed in conventionally stained hematoxylin and eosin preparations (Fig. 2) as well

TABLE I

GAUCHER CELLS
CYTOPLASMIC STAINING REACTIONS *

	Alcohol	Formalin	Form. PC †	Zenker's	Frozen section ‡
Hematoxylin and eosin	Eos. §	Eos.	Eos.	Eos.	Eos.
Mucopolysaccharides					
Periodic acid-Schiff	1+ ¶	2+	4+	2+	4+
Chloroform-methanol-PAS	1+	2+	4+	2+	4+
Pyridine-PAS	1+	2+	4+	2+	4+
Diastase-PAS	1+	2+	4+	2+	4+
Alcian blue	—	—	—	—	—
Thionine	O	O	O	O	O
Protein					
Danielli	3+	3+	3+	3+	3+
Ninhydrin-Schiff	2+	2+	2+	2+	2+
Lipid					
Peracetic acid-Schiff	—	—	—	—	—
Oil red O	—	—	±	—	2+
Chloroform-methanol-oil red O		—	±	—	—
Pyridine-oil red O		—	±	—	—
Luxol fast blue	—	—	—	—	—
Enzymes					
Acid phosphatase, diazo [11] and Gomori [10] methods					+
Alkaline phosphatase					—
Glucose-6-phosphate dehydrogenase [13]					±
Esterase					+
ATPase [12]					±
Glucose-6-phosphatase					—
Succinic dehydrogenase [14]					±
Cytochrome oxidase					—
Lipase					+

* Stains used are those cited by Lillie [10] unless otherwise indicated.
† Formalin, post-chromed.
‡ Fresh frozen tissue used for enzyme stains; frozen sections of wet formalin tissue used for others.
§ Eosinophilic.
¶ Staining intensity: O = orthochromatic; 1 to 4+ = increasing intensity.

as in cytoplasmic bodies noted by electron microscopy. The latter were evident in thin, 1 μ sections stained by the PAS method (Fig. 3). The oil red O stain for neutral lipid was found to be positive only in frozen sections and those prepared from post-chromed formalin-fixed tissue. The peracetic acid-Schiff reaction for unsaturated compounds was negative in all instances. The Gaucher cells revealed intense reactions for acid phosphatase (Fig. 4), lipase and nonspecific esterase, whereas only faint amounts of glucose-6-phosphate dehydrogenase and succinic dehydrogenase were present. The stain for the latter exhibited a globular configuration in many cells. The cytoplasmic striations also exhibited preferential localization of gamma globulin (Fig. 5). Fluorescein conjugates of anti-human albumin and fibrinogen as well as anti-rabbit gamma globulin were nonreactive.

Electron Microscopy

The cytoplasm of the Gaucher cells was characterized by the presence of variable but frequently numerous round, ovoid and irregular, elongated bodies, measuring 0.5 to 3 μ (Fig. 6). They were most often surrounded by a single, smooth limiting membrane although on rare occasion the latter structure appeared duplicated, having a periodicity of approximately 125 Å. The internal structure of the cytoplasmic bodies was characterized by varying numbers of moderately osmiophilic, smooth-walled tubules measuring approximately 250 to 725 Å in diameter. Occasionally, altered but still clearly recognizable mitochondria could be identified within such bodies, whereas in other instances one had the impression that transitions between mitochondria and the bodies had occurred (Figs. 7 and 8). Indeed, mitochondria in these cells not infrequently exhibited varying degrees of alteration and often the organelles were noted adjacent to the limiting membrane of the cytoplasmic bodies described above as well as within invaginations of these structures (Fig. 9). The endoplasmic reticulum in the Gaucher cells appeared as large lacunas or tubules exhibiting moderate numbers of ribonucleoprotein (RNP) particles within their limiting membranes. Other vacuoles with smooth limiting membranes were less frequent. Moderate numbers of RNP particles were also observed throughout the cytoplasm of Gaucher cells. Well-defined Golgi structures were not evident. The cytoplasmic rim of the Gaucher cells was, for the most part, regular and smooth, and apposition between adjacent cells appeared to be linear. The nuclei were often eccentrically located and exhibited a double limiting membrane. They were frequently surrounded by a thin lacuna separated from the cytoplasm by a single, smooth limiting membrane. The nuclear particles were frequently condensed in aggregates in the region of the nuclear membrane.

Discussion

Correlation of the electron microscopic features of Gaucher cells with those observed by light microscopy in conventional as well as thin (1 μ) sections clearly indicated that the cytoplasmic striations, long considered pathognomonic of such cells, corresponded to the tubule-containing cytoplasmic bodies noted by electron microscopy. Further, the localization of the PAS-reactive material and protein to these structures strongly suggested that they contained the cerebroside-protein complex of Gaucher's disease. A similar suggestion has been proposed by DeMarsh and Kautz [6] from their comparison of unstained sections examined by phase microscopy and those studied by electron microscopy. It appears worthy of note that the positive PAS reaction was found to be only moderately intense within Gaucher cells after fixation in 10 per cent neutral formalin, Zenker's fluid or absolute alcohol although more intense when the formalin-fixed tissue was suitably post-chromed or frozen sections were examined. The moderate reactivity noted in formalin-fixed preparations has been experienced by Pearse,[11] but is unlike that reported by Morrison and Hack [15] who observed a brilliant staining with this technique. Our observations suggest that the divergent results might, in large part, be attributable to loss of the cerebroside which may have been extracted during alcohol, Zenker's fluid or formalin fixation. However, it was also apparent from the failure to remove reactive components from sections, except the neutral lipid in frozen sections, by the various lipid extractives employed that at least a large part of the cerebroside appeared to be firmly bound to protein as indicated biochemically.[2] The relatively weak reaction observed in frozen sections stained by oil red O is similar to the experience of others and may reflect the lack of significant quantities of neutral lipid in the deposits. The failure to observe unsaturated components within the Gaucher cells by the peracetic acid-Schiff reaction appeared to be related to the saturated nature of lignoceric and palmitic acids, the principal fatty acids of kerasin.[16]

Information concerning the derivation of the cytoplasmic bodies observed in the Gaucher cells would appear highly significant in relation to the pathogenesis of this disorder. The presence of microvesicles beneath the cytoplasmic border suggested to DeMarsh and Kaust [6] the possibility that these cells were engaged in the incorporation of lipoprotein within their substance. This interpretation would tend to support the concept that the cerebroside of Gaucher's disease was of extracellular origin; this, as indicated previously, is a commonly held view. However, we have not observed unusual numbers of submembranous vesicles in any of the many Gaucher cells examined. Moreover, in no instance were

bodies or other structures comparable to those observed within Gaucher cells noted in extracellular sites although particular attention was directed to these locations. On the other hand, our interpretations of the electron micrographs in this single example of Gaucher's disease strongly suggest that the cytoplasmic bodies constituting an outstanding cytologic feature may be endogenously derived in the reticuloendothelial elements *per se.*

Certain morphologic features of the bodies indicate that they are either directly derived from transformations of mitochondria or that the latter participate in their formation. The recognition of apparent transitions between normal-appearing mitochondria and the cytoplasmic bodies were thought to exist, and the possibility that the tubular elements characteristic of the bodies represented altered mitochondrial cristas did not appear too unreasonable. The occasional presence of a double membrane about the bodies is in keeping with this interpretation. On the other hand, from the morphologic evidence available, it cannot be denied that the bodies could have been derived from preformed cytoplasmic structures exhibiting a capacity for the encapsulation and subsequent lysis of mitochondria. The presence of mitochondria adjacent to and lying within invaginations of the bodies and the presence of altered and apparently ruptured mitochondria within them might be considered indications of a pathogenetic relationship.

Although absolute demonstration of the derivation of the cytoplasmic bodies in Gaucher cells has not been made with certainty, there nevertheless does appear sufficient evidence to warrant the conclusion that mitochondria are either directly or indirectly related to their formation, a view also expressed by Roos and co-workers[7] in their brief note concerning the ultrastructure of Gaucher's disease. Similar structures identified as lysosomes have been depicted by Ashford and Porter[17] in the livers of rats perfused with glucagon. These investigators considered that each lysosome was a portion of cytoplasm which exhibited a marked predilection for the endogenous lysis of mitochondria. They suggested the possibility that the lysosomes represented portions of cytoplasm, including mitochondria, which by their hydrolytic action might provide products for use in a re-oriented cellular function. In keeping with the identification of these bodies in Gaucher cells as lysosomes is the histochemical demonstration of their cytoplasmic acid phosphatase activity.

Acid phosphatase and esterase activities have been observed in histiocytes in a variety of situations.[18] These enzymes have been thought to be concerned with the digestion or hydrolysis of engulfed material or, as indicated above, of the cells' own constituents under pathologic as well as physiologic conditions.[19] The latter consideration offers support to

the contention that the cerebroside in Gaucher cells may be produced endogenously. The relationship of the acid phosphatase content of the Gaucher cells and elevated levels of this enzyme in the serum of patients with the disorder[20] is at present unclear. Whether the serum elevation reflects a spillage from tissue deposits or is a manifestation of the fundamental abnormality in the disorder, as has been suggested,[21] remains to be demonstrated.

It is of interest that the characteristic cells in Whipple's disease exhibit enzymatic reactions similar to those in Gaucher cells.[5] Indeed, electron microscopic examination of the former has revealed cytoplasmic bodies (corresponding to the sickleform particles observed with conventional microscopy) which have the same configuration and general internal structure as those we have observed in Gaucher's disease, except that the tubular structures in the bodies have a significantly greater osmiophilia. It appears noteworthy in this regard that the sickleform bodies in Whipple's disease are characterized by a neutral mucopolysaccharide or mucoprotein content more intensely colored by the PAS reaction than in the Gaucher cells. The chemical difference in the stored substance might well explain the ultrastructural distinctions observed in the two disorders. In both instances the cytoplasmic bodies exhibit a significant relationship to mitochondria.

The etiologic implications of the gamma globulin localization within the cytoplasmic bodies of the Gaucher cells are unclear. Pertinent serologic and immunologic studies in this disorder are assuredly warranted.

Summary

Electron microscopy of the spleen from a 19-year-old woman with Gaucher's disease has disclosed round, ovoid and irregular cytoplasmic bodies within the pathognomonic cells of this disorder. These bodies correspond to the cellular striations observed by conventional microscopy and appear to contain the cerebroside of Gaucher's disease. The ultrastructural features suggest either a transition between the mitochondria and the cytoplasmic particles or the incorporation of the mitochondria into preformed bodies. This points to an intracellular defect in Gaucher's disease, with the mitochondria appearing to play a significant pathogenetic role. The existence of hydrolytic enzymes, particularly acid phosphatase, in the Gaucher cells adds some weight to this concept.

The significance of the preferential localization of gamma globulin within Gaucher cells, as noted by fluorescent antibody techniques, is unclear.

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[*Illustrations follow*]

Legends for Figures

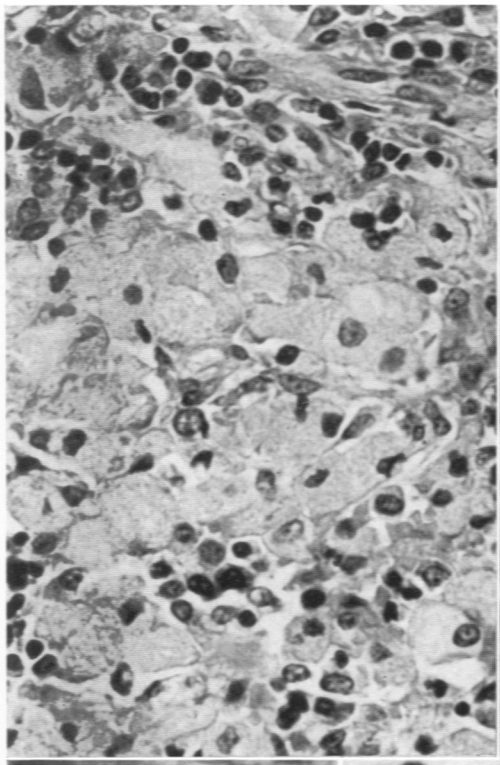
FIG. 1. Aggregates of Gaucher cells with faintly visible cytoplasmic striation. Hematoxylin and eosin stain. × 250.

FIG. 2. Gaucher cells in the spleen exhibit PAS-reactive substance (dark gray) confined principally to cytoplasmic striations. Periodic acid-Schiff (PAS) stain. × 680.

FIG. 3. Gaucher cells in a thin (1 μ) section prepared from Vestopal block and stained by the PAS technique. The cytoplasmic bodies are reactive and correspond to those observed by electron microscopy as depicted in Figure 6. × 1,100.

FIG. 4. Intense acid phosphatase activity in Gaucher cells. Gomori method. × 425.

FIG. 5. Section of spleen stained with a fluorescein conjugate of anti-human gamma globulin. Fluorescence (appearing white) indicating gamma globulin is evident principally in cytoplasmic bodies. × 400.



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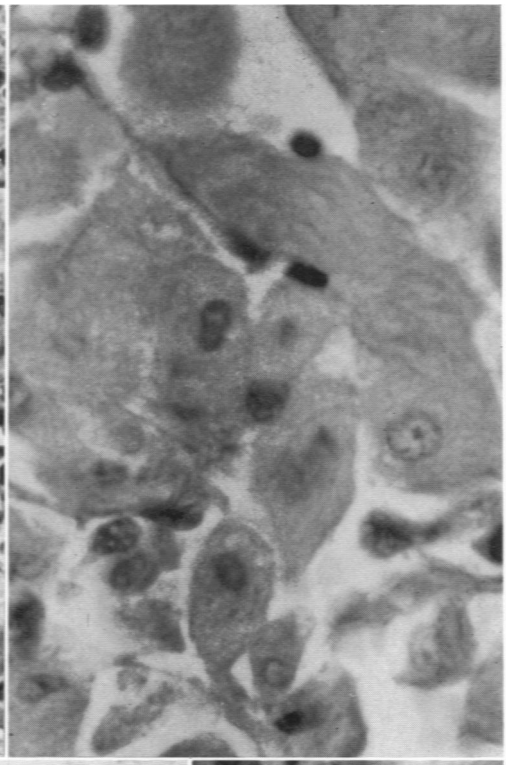

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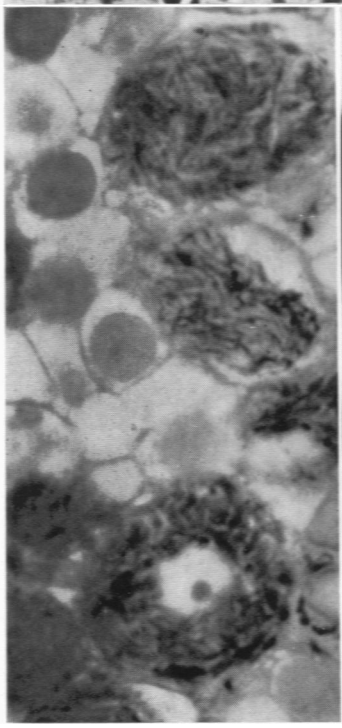

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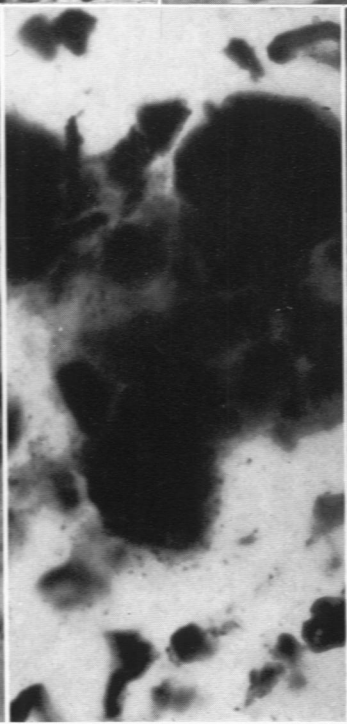

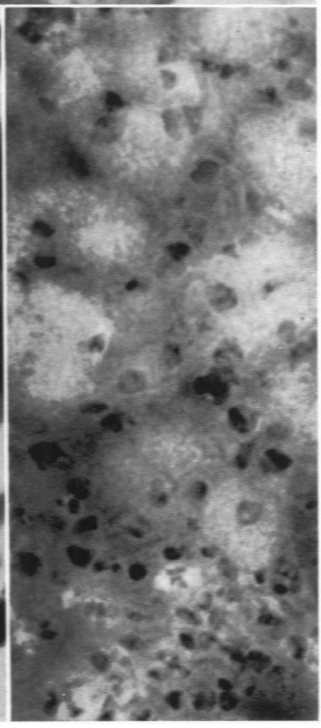

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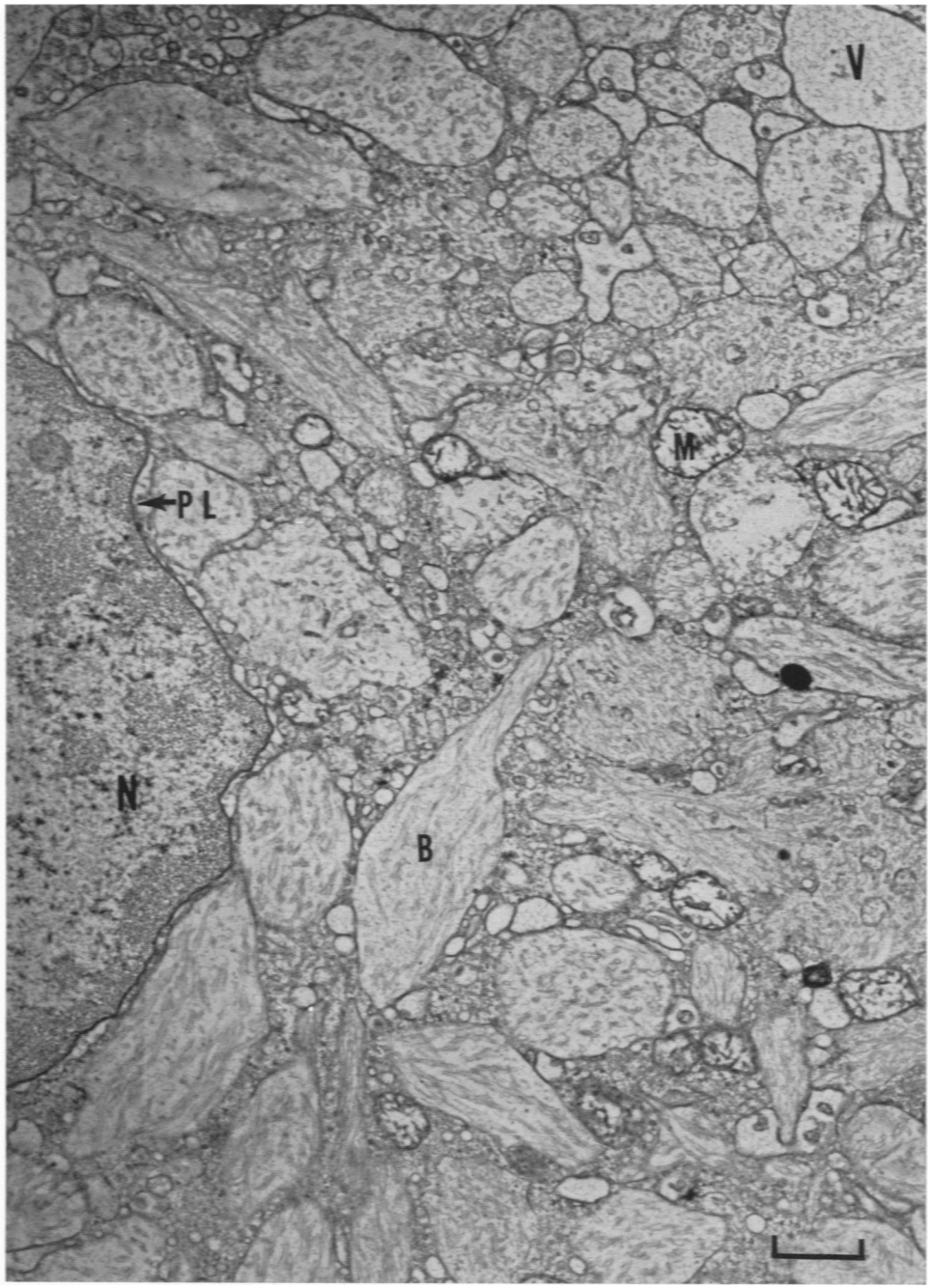
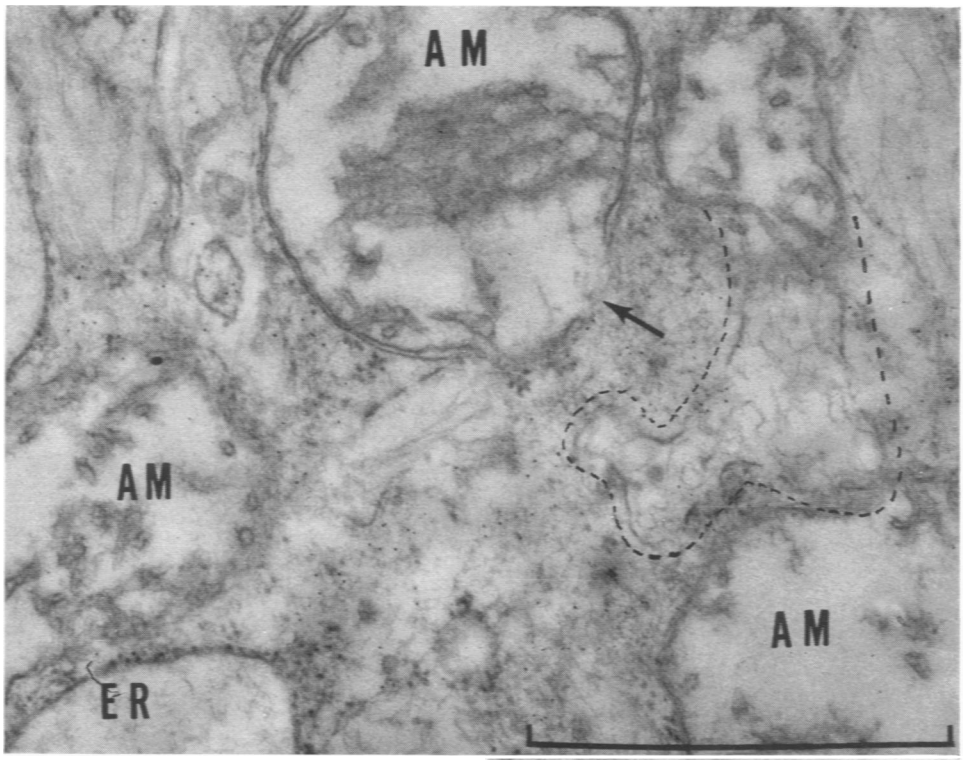


FIG. 6. A portion of a Gaucher cell, low power electron micrograph. The nucleus (N) is eccentric and exhibits clumped nuclear particles and a slightly serrated nuclear rim. The latter appears surrounded by a thin, clear lacuna or space (PL). The cytoplasm contains many elongated, spindle and sometimes crescentic bodies (B) with an internal tubular structure. Mitochondria (M) and vacuoles (V) of variable size are also evident. × 12,880.



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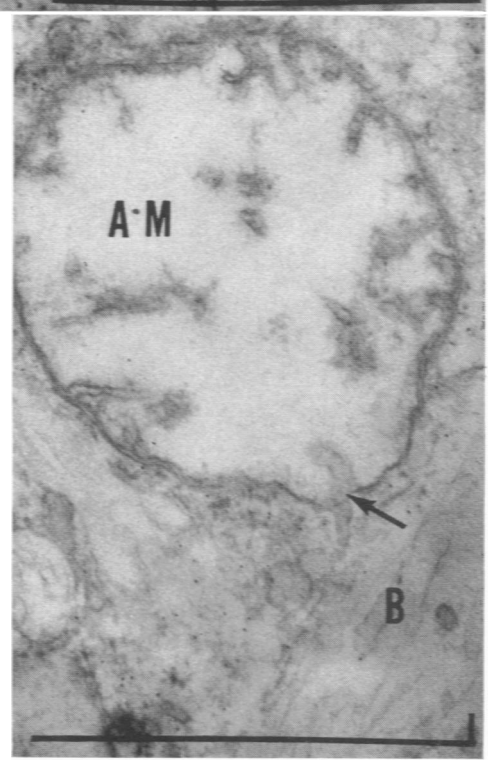

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FIG. 7. Gaucher cell. The apparent transition between altered mitochondria (AM) and cytoplasmic bodies is shown. The arrow indicates the site of rupture in one of the mitochondria, and the dotted line encircles a portion of a mitochondrion assuming the appearance of a cytoplasmic body. The rough endoplasmic reticulum (ER) is also evident. × 63,800.

FIG. 8. An altered mitochondrion (AM) in a Gaucher cell exhibits extrusion or incorporation of a tubular structure from a cytoplasmic body (B) at arrow. × 55,100.

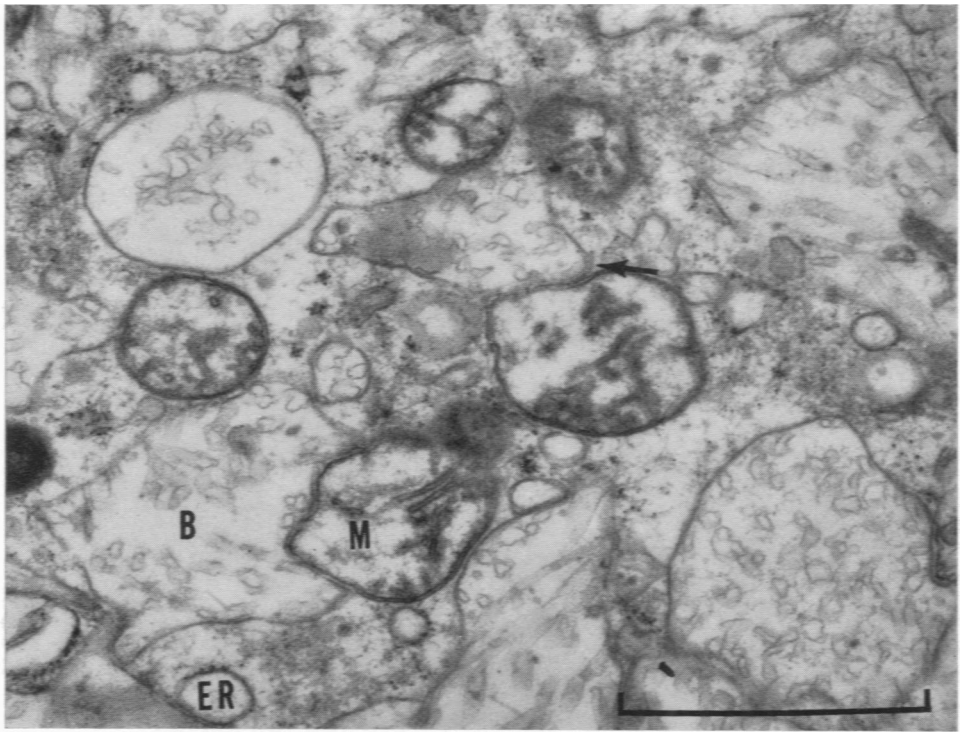

FIG. 9. Gaucher cell. Alterations in mitochondria (M) and cytoplasmic bodies (B) with tubular internal structure are manifest. Suggestions of transition between mitochondria and the bodies are shown. The mitochondrion (M) appears to lie in an invagination of the body (B). The arrow indicates an apparent double membrane at one point of a cytoplasmic body. Endoplasmic reticulum (ER) is of the rough variety. Stained by lead hydroxide method. × 43,700.