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THE LIVER IN LIPIDOSIS

AN ELECTRON MISCROSCOPIC AND HISTOCHEMICAL STUDY

BRUNO W. VOLK, M.D., AND BARBARA J. WALLACE, PH.D.

From the Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, N. Y.

Involvement of the liver is a characteristic phenomenon in several forms of lipidoses, for example, Niemann-Pick disease (NPD), Gaucher's disease (GD) and in the Hunter-Hurler syndrome. In other forms such as Tay-Sachs disease (TSD) and late infantile amaurotic idiocy (LIAI), histologic manifestations in the viscera are generally absent, although several cases of atypical infantile neurovisceral lipidoses have been reported.[1-5] The latter, however, appear to be dependent upon biochemical abnormalities different from those occurring in TSD.

Although many light microscopic studies exist of liver changes in the visceral forms of the lipidoses, there are relatively few electron microscopic investigations available. Studies of Hurler's disease have revealed the occurrence of large spherical membrane-bound storage bodies in hepatic cells and concomitantly, a dearth of peribiliary dense bodies.[6] In GD crescent-shaped Gaucher bodies with internal tubules have been described in reticuloendothelial cells and enlarged cisternae of the endoplasmic reticulum have been found in hepatocytes.[7] Unusual membrane-bound structures have also been described in the liver of a child with NPD.[8]

The present investigation was undertaken to examine, by electron microscopic and histochemical means, the liver alterations in children with NPD, GD, acid mucopolysaccharidosis (AMP) related to Hunter-Hurler's disease and TSD. It was intended to evaluate the visceral mor-

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phologic changes in these disorders and to compare their features, when feasible, with those previously described in the central nervous system.

Material and Methods

Needle biopsy specimens were procured from the liver in a 2-year-old boy with NPD, a 4-year-old girl with AMP, and a 20-year-old girl with GD. Similar specimens were obtained as controls from an 11-year-old patient with rheumatic fever, a 40-year-old with nonspecific reactive hepatitis and a 20-year-old boy with acute viral hepatitis. Liver biopsy was made at laparotomy in 5 children with TSD varying in age from 9 to 24 months. For the study of infantile GD liver tissue was obtained at necropsy from a 9-month-old girl.

Each biopsy specimen was quickly divided into 4 fragments. The first of these was minced and fixed in cold 1 per cent osmium for 90 minutes, dehydrated in ethanol and embedded in Epon for electron microscopic examination.

A second portion was cut into small blocks and fixed in 3 to 6 per cent gluteraldehyde buffered with 0.2M cacodylate buffer for 3 hours or in 4 per cent calcium formol overnight for enzyme histochemical studies. After fixation the tissue was washed in cold (0 to 4° C) 0.4M sucrose for 1 to 14 days. Blocks were then frozen in isopentane cooled by liquid nitrogen and stored in a liquid nitrogen refrigerator until sectioned in a cryostat. Acid phosphatase (AcPase) activity was demonstrated using a Gomori incubation solution with sodium β-glycerophosphate as substrate.

The third fragment, when sufficient tissue was available, was frozen without prior fixation for biochemical use. The remaining fragments were fixed in 10 per cent buffered formalin and embedded in paraffin for conventional histologic preparations. Necropsy tissue from the child with AMP was utilized for histochemical demonstration of acid mucopolysaccharide by means of a modification of the Hale staining method.[9] Necropsy tissue from the child with infantile GD was processed as described above for electron microscopy.

Results

Niemann-Pick Disease

Light Microscopy. In hematoxylin and eosin stained preparations hepatic parenchymal cells were vacuolated in appearance. Many large pale Niemann-Pick cells were scattered among them (Fig. 1). With AcPase preparations there was a lack of the typical peribiliary arrangement of stained lysosomes. Instead, the AcPase reaction product was distributed throughout the cytoplasm in a reticular pattern but with unstained areas in the network of the reticulum (Fig. 2).

Electron Microscopy. Parenchymal cells were filled with membrane-bound lobulated electron-lucent inclusions, most of which contained a few loosely arranged membranes (Fig. 3). Few typical lysosomes and peribiliary dense bodies were noted. Niemann-Pick cells were also filled with electron-lucent inclusions containing sparse parallel membranes (Fig. 4). Kupffer cells were enlarged and were filled with dense granular bodies, membrane-filled bodies and pale electron-lucent structures similar to those in parenchymal cells.

Acid Mucopolysaccharidosis

Light Microscopy. The hepatic cell cytoplasm was markedly vacuolated (Fig. 5). In preparations stained for acid mucopolysaccharides, positive staining was noted at the periphery of the vacuoles and in Kupffer cells as well (Fig. 6). Staining for AcPase yielded large stained cytoplasmic granules.

Electron Microscopy. Parenchymal cell cytoplasm contained numerous large pale membrane-bound vacuoles with a sparse granular substance and remnants of dense membranes (Fig. 7). Vacuoles were generally located near the bile canaliculi but also appeared elsewhere in the cytoplasm. No peribiliary dense bodies were noted in these cells either. Kupffer cells contained structures similar to those in parenchymal cells.

Ultrastructurally AcPase reaction product was localized at the periphery of the large cytoplasmic vacuoles as well as in the membranes (Fig. 8).

Gaucher's Disease

Light Microscopy. In both the 20-year-old patient and the 9-month-old child hepatic cells were unremarkable in hematoxylin and eosin preparations, but numerous large Gaucher cells were interspersed among the hepatocytes. These contained characteristic cytoplasmic striations (Fig. 9). AcPase activity was localized in large discrete granules in hepatic cells; these tended to be scattered at random in the cytoplasm rather than lined up along bile canaliculi. In Gaucher cells cytoplasm was diffusely stained but the striations stained more deeply than the surrounding cytoplasm (Fig. 10).

Electron Microscopy. The ultrastructural appearance of parenchymal cells in the juvenile patient was not unusual. There were numerous pale enlarged lysosomes but since the patient also suffered from viral hepatitis, no attempt was made to evaluate lysosomal alterations. Many Gaucher cells, however, contained large irregular bodies filled with hollow tubules (Fig. 11); in others there were large granular bodies with few tubules (Fig. 12). These often contained dense osmiophilic deposits. Numerous enlarged cisternae of endoplasmic reticulum were also evident. In the necropsy tissue from the child with infantile GD postmortem changes were confined largely to mitochondria and nuclei. Numerous pale membrane-bound vacuoles appeared in the cytoplasm of most hepatocytes (Fig. 13), while Gaucher bodies were absent. Other hepatocytes contained large dense bodies filled with coarse granular material and less dense homogeneous vacuoles (Fig. 14). Reticuloendothelial cells,

however, contained typical Gaucher bodies and also rough-surfaced endoplasmic reticulum filled with a dense granular material.

Tay-Sachs Disease

Light Microscopy. Hepatic cells appeared normal in hematoxylin and eosin stained preparations from all patients studied (Fig. 15). Sections stained for AcPase activity revealed the peribiliary localization of lysosomes characteristic of normal liver (Fig. 16). Similarly the Kupffer cells typically contained large amounts of reaction product.

Electron Microscopy. Most areas of the liver were quite unremarkable. Many membrane-bound granular bodies in liver cells lay primarily near the bile canaliculi and were considered to be typical lysosomes. In occasional sections, however, unusual membrane-filled structures were found near the canaliculi. These consisted most often of oval-shaped membrane-bound bodies containing parallel membranes, or of lipofuscin bodies with parallel or concentrically arranged membranous inclusions (Fig. 17). In one patient, in addition to the structures described, there were occasional large membranous cytoplasmic bodies closely resembling those found in the brain of children with TSD (Fig. 18). AcPase reaction product was found in typical lysosomes as well as in the atypical membrane-filled cytosomes along the canaliculi (Fig. 19).

Control Cases

In control patients without lipidoses (for example, rheumatic fever, nonspecific reactive hepatitis, acute viral hepatitis) unusual cellular inclusions were occasionally found. Pale vacuole-like structures with dense cores frequently appeared near bile canaliculi (Fig. 20), but membrane-filled bodies such as those observed in TSD were not encountered, nor were the inclusions of control cases similar to the abnormal vacuole-like structures found in NPD, AMP or GD.

Discussion

The cytosomes in hepatic cells in NPD were morphologically similar to those previously described in the brain[10] and presumably were composed largely of sphingomyelin. In lymph node[11] and rectal mucosa[12] in patients with NPD, inclusion bodies filled with concentric membranes similar to those in cerebral neurons in TSD have been described. Membranous structures were found in the liver also but were much less common than the vacuole-like cytosomes. There was no evidence to indicate that the substances stored in these various organs differed, thus, the striking morphologic variations in cytosomes could relate to different metabolic stages of the same stored substance.

It is suggested that the abnormal cytosomes in NPD develop from

peribiliary dense bodies which incorporate the lipid and attempt to degrade it. This seems likely since no typical peribiliary dense bodies (or lysosomes) were found in NPD parenchymal cells. Sufficient biopsy tissue was not available for an adequate study of the fine structural localization of hydrolytic enzymes. It is significant, however, that stained peribiliary dense bodies were not found in conventional sections stained for AcPase activity. Moreover, it is likely that the reticular cytoplasmic deposit of reaction product (Fig. 2) was the result of its deposition around the periphery of cytosomes. Localization of thiolacetate esterase activity at the margin of similar vacuole-like cytosomes has been demonstrated electron microscopically in Purkinje cells in a patient with NPD.[13]

In AMP the appearance of liver cytosomes was quite different from that in neurons[10] but resembled those observed in rectal mucosa.[12] Moreover, the histochemical properties of the neuronal inclusions differed from those of the visceral inclusions in this disorder. In the brain the stored substance in neurons was sudanophilic[10] and apparently lipid in nature; in the viscera it was not sudanophilic, but stained with a modification of the Hale method. It, therefore, appears to be an acid mucopolysaccharide.

In AMP cytosomes appeared to develop from peribiliary dense bodies. As in NPD, no typical lysosomes were noted along bile canaliculi. Moreover, the AcPase reaction product was manifest at the periphery of the vacuoles as well as on the membrane fragments they contained (Fig. 6). Since acid mucopolysaccharide has been shown in these structures, it is suggested that this material collected in the peribiliary dense bodies altering them and causing them to form the large vacuoles seen in AMP. Similar vacuoles have been described in the liver of children with gargoylism[6,14] and have been compared to the altered hepatic lysosomes which appear following injection of dextran or Triton® WR-1339. Vacuoles containing acid mucopolysaccharide have also been described in other viscera in children with Hurler's disease.[15]

The apparent development in NPD and AMP of inclusion bodies derived from the lysosome system does not necessarily indicate that these are inborn lysosomal disorders, i.e., diseases due to a genetic defect of lysosomal hydrolases. It may merely mean that mucopolysaccharide or sphingomyelin deposited as the result of an enzyme defect elsewhere in the cell secondarily accumulated in otherwise normal lysosomes.

The large rod-shaped bodies in Gaucher cells have been described in various viscera.[7,12,16–18] Marked AcPase activity as well as nonspecific esterase and arylsulfatase activities in these cells have suggested that the unusual cytosomes noted might be lysosomal in origin.[17,19] Some investigators have felt, however, that there was evidence of their devel-

opment from mitochondria.[17,20] In our preparations and those of other investigators [18] no forms transitional between Gaucher bodies and mitochondria were observed. In earlier studies upon the ultrastructural histochemical features in a lymph node from a patient with GD, AcPase reaction product observed within the bodies indicated that they were of lysosomal nature.[12] Some investigators have suggested that kerasin accumulation in Gaucher cells is the result of pinocytotic or phagocytic activity rather than endogenous synthesis.[16,18] The appearance of numerous large vacuoles in parenchymal cells as well as the unusually dense and enlarged cisternae of endoplasmic reticulum in Gaucher cells suggests the possibility of endogenous synthesis of the stored material and the consequent formation of abnormal cytosomes. The difference in appearance of cytosomes in hepatic cells and in reticuloendothelial cells is difficult to interpret. It may reflect different responses in the two cell types to the stored lipid or may even indicate a quantitative difference in the lipid.

The membrane-filled cytosomes encountered in this study have not been described in the liver cells in TSD; they may reflect a systematic involvement in this disorder also. Typical concentric membranous cytoplasmic bodies found in neurons were observed in the liver in only one patient and in occasional lipofuscin bodies. Oval structures with parallel membranes found in hepatic cytosomes in this study have often been observed in cerebral glial cells and in interstitial cells in autonomic ganglia and occasionally in cerebral neurons as well.[12] It is suggested that the membrane-filled bodies contain the Tay-Sachs ganglioside which ultimately appears in membrane-filled lipofuscin bodies. We were not able to identify clear transitions between typical peribiliary dense bodies and membrane-filled cytosomes but some forms suggestive of transitions were seen. Large numbers of abnormal hepatic granules have been described in children with TSD [6,14] but these differed morphologically from the granules observed in our patients.

The occurrence of abnormal hepatic cell inclusions in TSD supports the biochemical studies of Svennerholm who demonstrated increased ganglioside levels in the liver of children with this condition.[21] Our studies, however, have failed to indicate whether the Tay-Sachs ganglioside in the liver was due to direct involvement of this organ as part of the systemic process, or whether ganglioside was only secondarily deposited in the liver.

Summary

Electron microscopic and histochemical studies were performed on biopsy specimens of the liver in children with Niemann-Pick disease (NPD), acid mucopolysaccharidosis (AMP) and Tay-Sachs disease

(TSD), and in a patient with juvenile Gaucher's disease (GD). A necropsy case of infantile GD was also studied.

No peribiliary dense bodies were found in NPD but numerous large lipid bodies were scattered throughout the cytoplasm. The peribiliary bodies were also lacking in AMP but vacuole-like cytoplasmic inclusions containing acid phosphatase (AcPase) reaction product were observed in hepatocytes. Gaucher bodies were found in both the juvenile and infantile forms of GD. In the infantile variety hepatocytes contained pale vesicles, not observed in the older patient. In TSD unusual membrane-filled cytosomes in hepatic cells occasionally resembled the membranous cytoplasmic bodies found in neurons. These also exhibited AcPase activity. It is suggested that the storage material accumulates in hepatic peribiliary dense bodies resulting in the formation of altered lysosomes.

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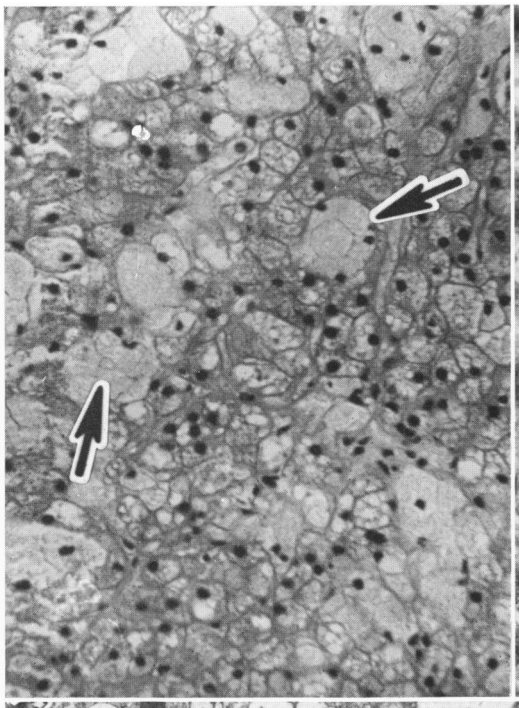
LEGENDS FOR FIGURES

FIG. 1. Niemann-Pick disease. Hepatocytes are vacuolated; scattered among them are groups of pale staining Niemann-Pick cells (arrows). Hematoxylin and eosin stain. × 280.

FIG. 2. Niemann-Pick disease, specimen stained for the demonstration of acid phosphatase activity. Discretely stained lysosomes are not evident along bile canaliculi. Instead, the reaction product is scattered with a network-like pattern throughout the cytoplasm. × 280.

Inset: Acid phosphatase distribution is shown in a Niemann-Pick cell at high magnification. × 9,600.

FIG. 3. Niemann-Pick disease. Large electron-lucent lipid inclusions (L) containing some membranes appear in the cytoplasm of hepatocytes. Numerous dense mitochondria are visible but there are no peribiliary dense bodies or typical lysosomes. Bile canaliculus (B). × 4,320.



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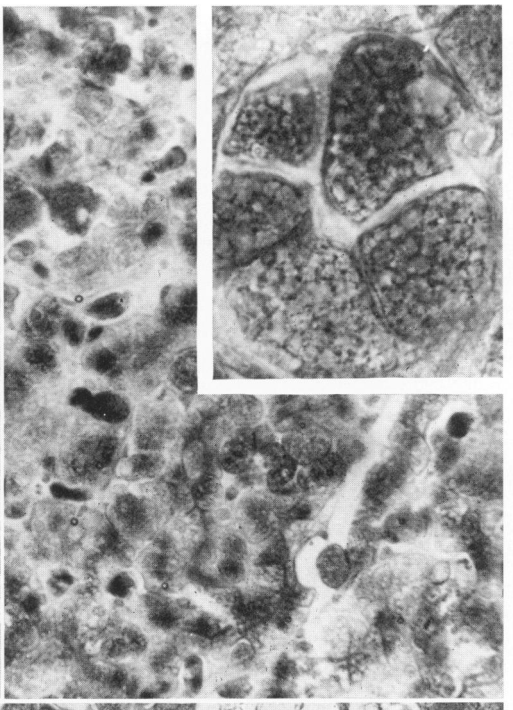

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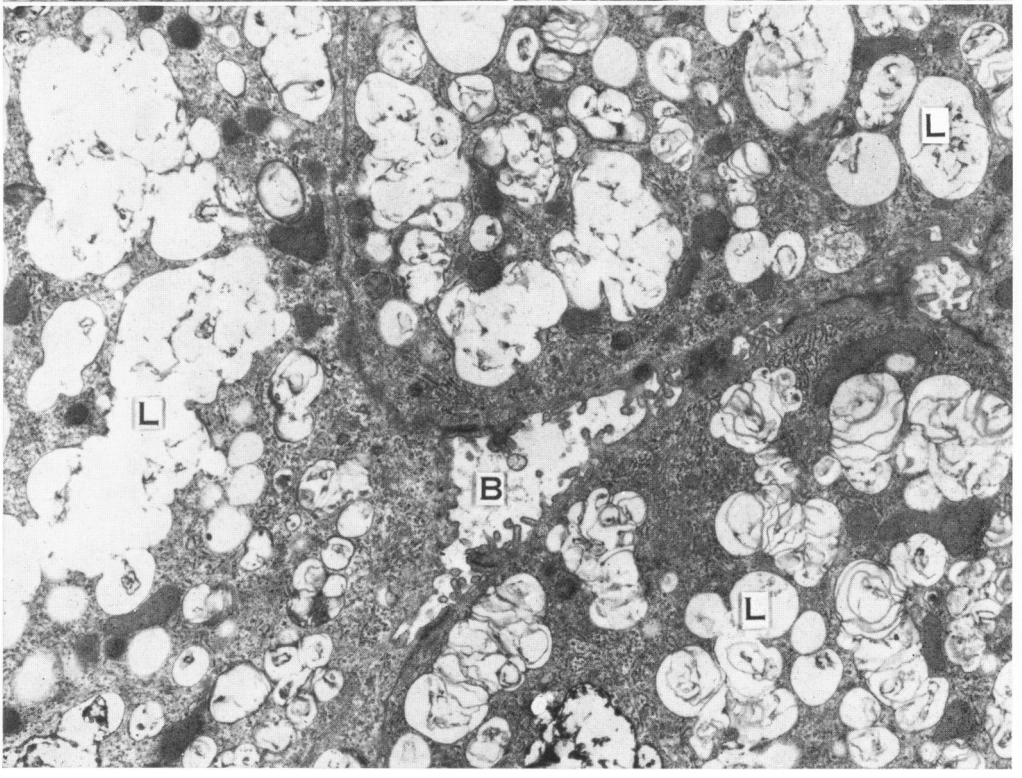

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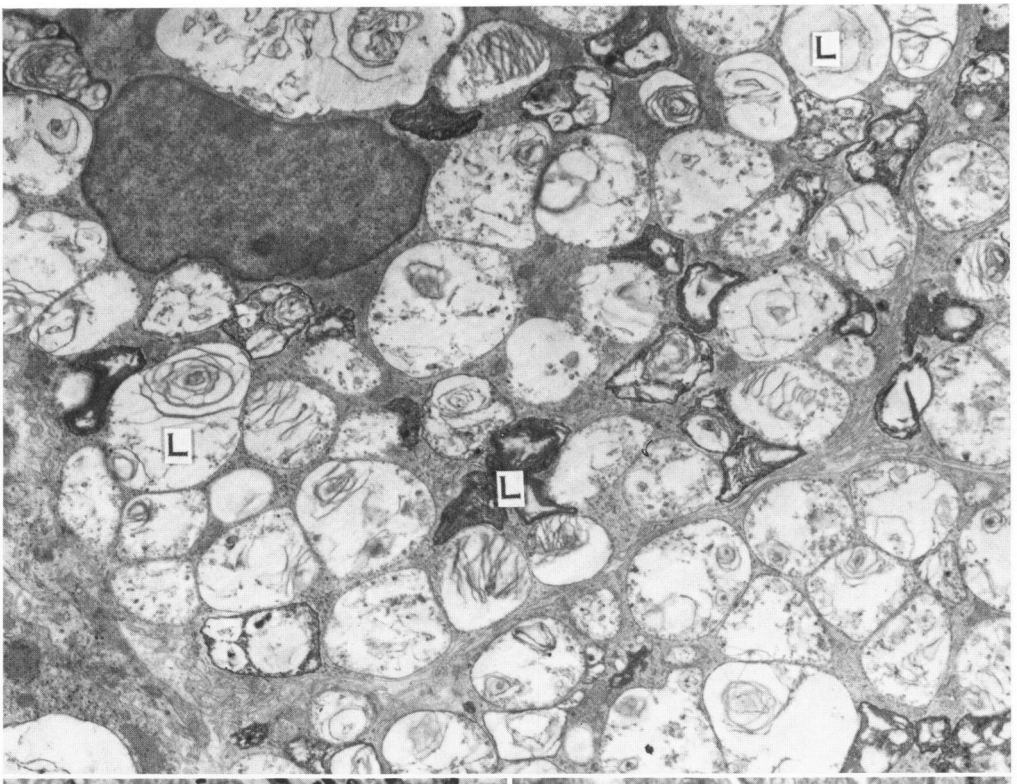
FIG. 4. Niemann-Pick cell, from the same liver shown in Figure 3. The cytoplasm is filled with pale and dense lipid inclusions with membranes (L). × 3,210.

FIG. 5. Acid mucopolysaccharidosis. Hepatic cells are vacuolated. Hematoxylin and eosin stain. × 280.

FIG. 6. The same liver as that seen in Figure 5, stained for acid mucopolysaccharides. Positive staining occurs at the periphery of cytoplasmic vacuoles (small arrows) and in Kupffer cells (large arrows). Rinehart's stain. × 280.



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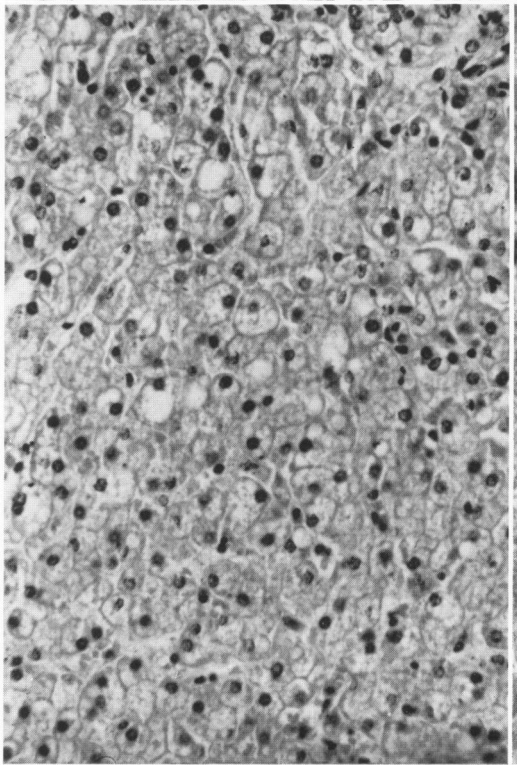

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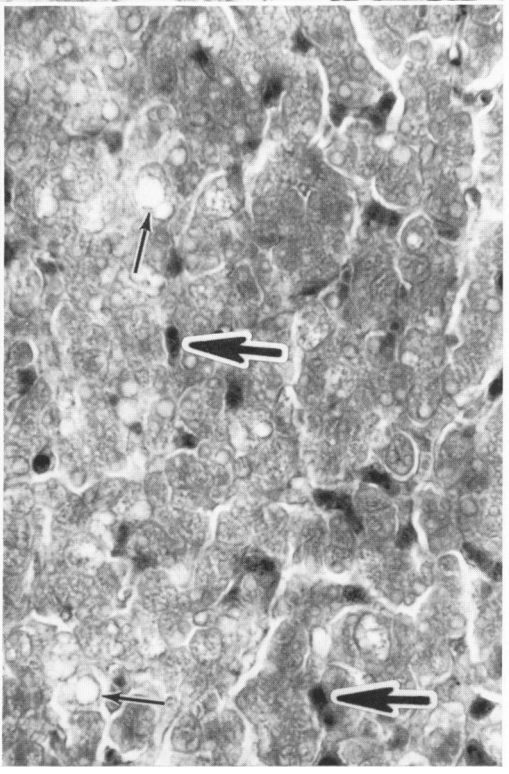

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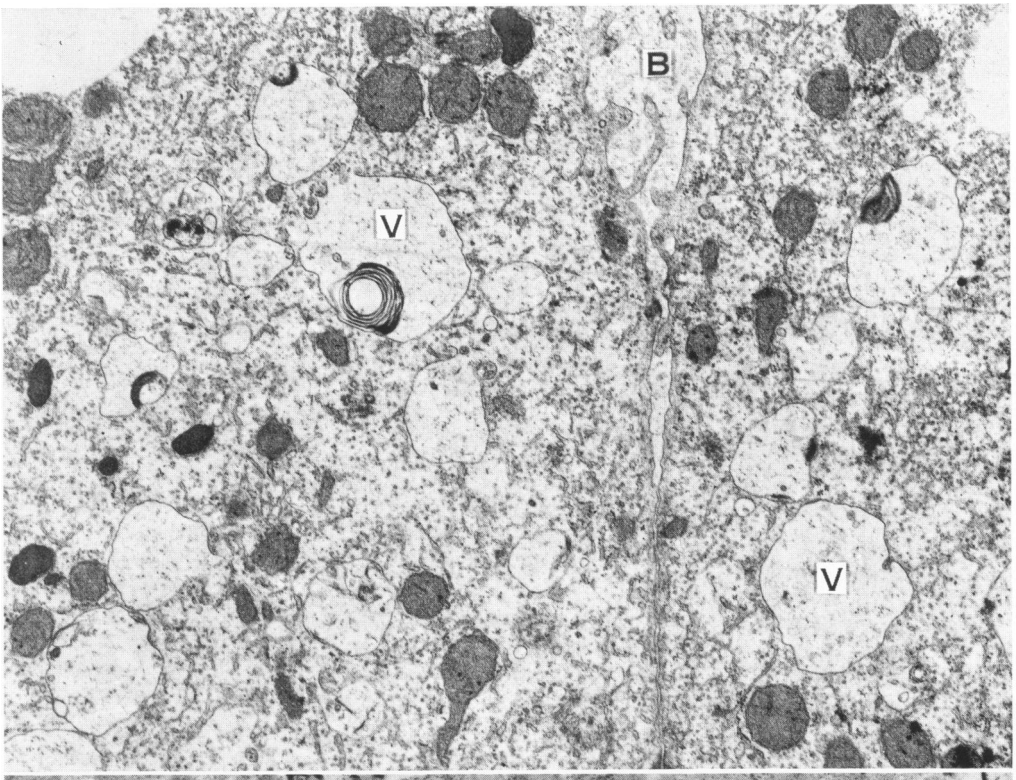
FIG. 7. The same liver as that illustrated in Figure 5. Membrane-bound vacuoles (V) appear in the hepatic cell cytoplasm. No typical lysosomes or peribiliary dense bodies are seen. Bile canaliculus (B). × 6,600.

FIG. 8. The same liver as that seen in Figure 5, but fixed in gluteraldehyde and stained for acid phosphatase activity. The reaction product is localized at the periphery of vacuoles (V) and on the internal vacuolar membranes. × 8,640.



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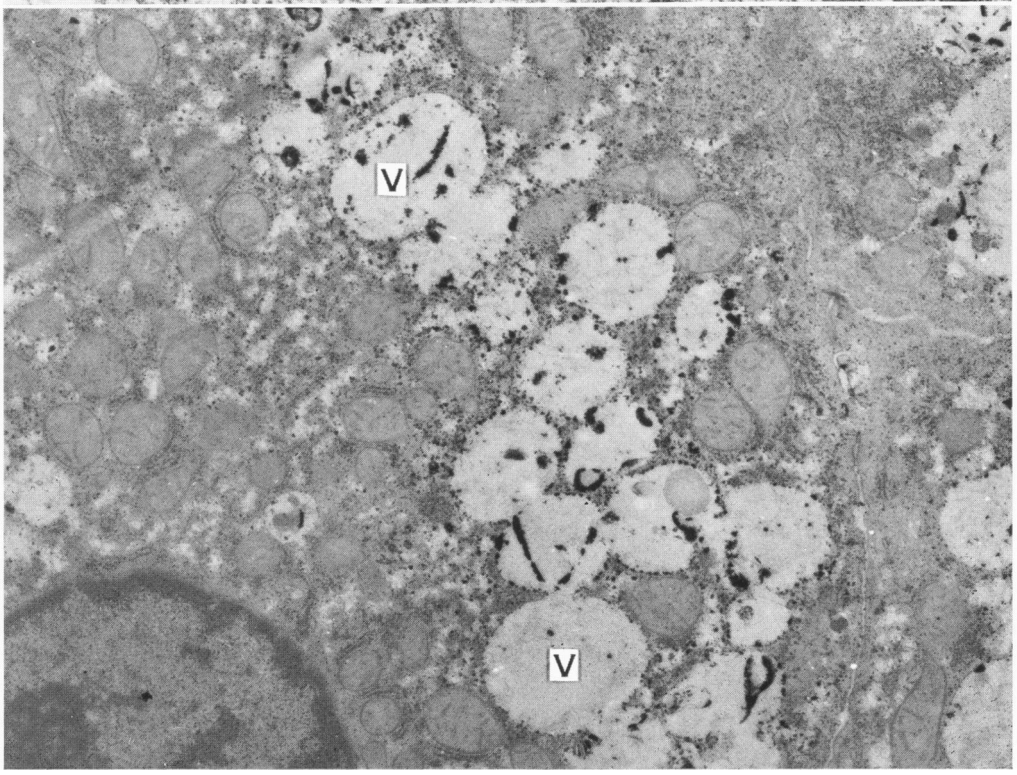

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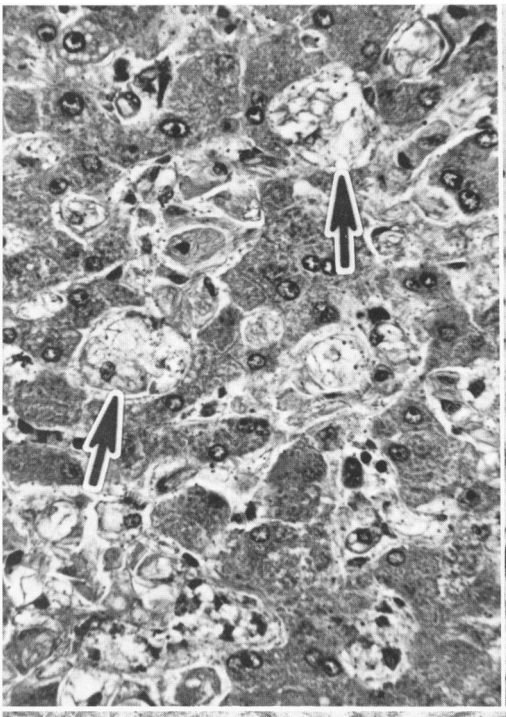
FIG. 9. Gaucher's disease, 20-year-old patient. Hepatocytes are unremarkable but numerous Gaucher cells (arrows) are scattered among them. Hematoxylin and eosin stain. × 375.

FIG. 10. Section from the patient with juvenile Gaucher's disease, stained for the demonstration of acid phosphatase activity. Large discrete lysosomes are scattered at random in the cytoplasm of hepatocytes (small arrows). The dense diffuse staining in Gaucher cells (large arrow) is more prominent in the cytoplasmic striations. × 375.

FIG. 11. The same liver as that shown in Figure 9. Numerous tubule-filled Gaucher bodies (G) are evident in the cytoplasm. × 21,600.



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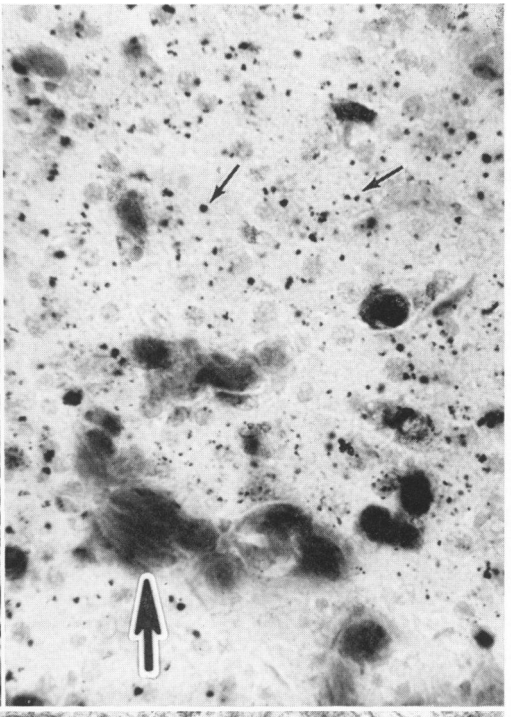

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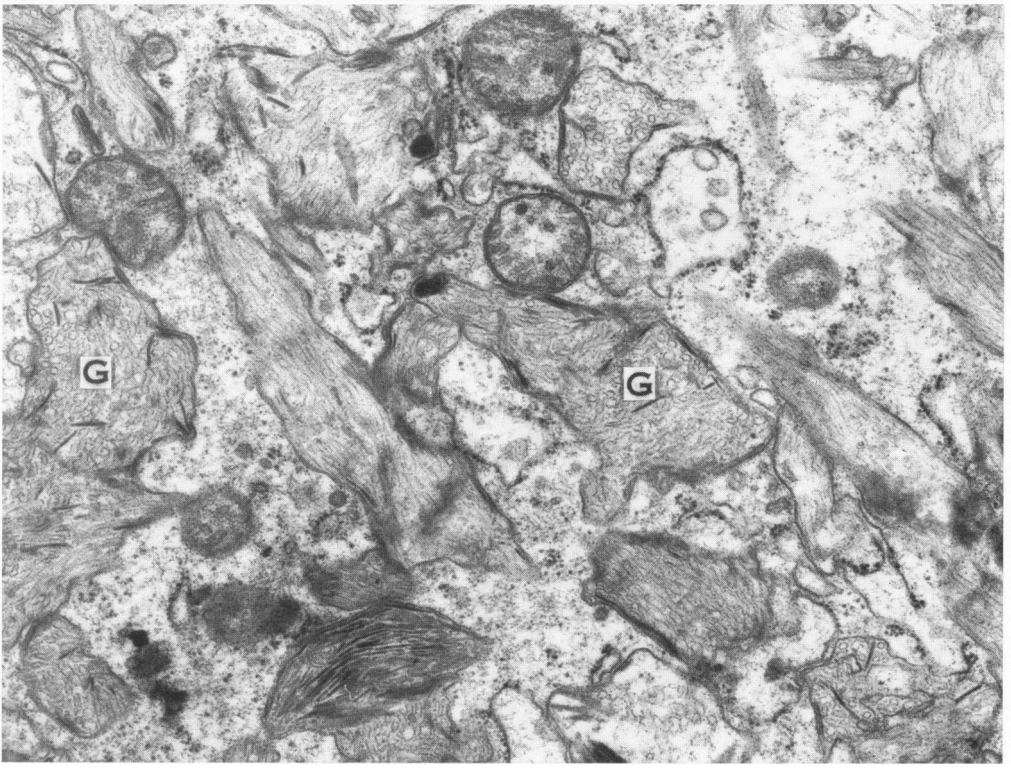

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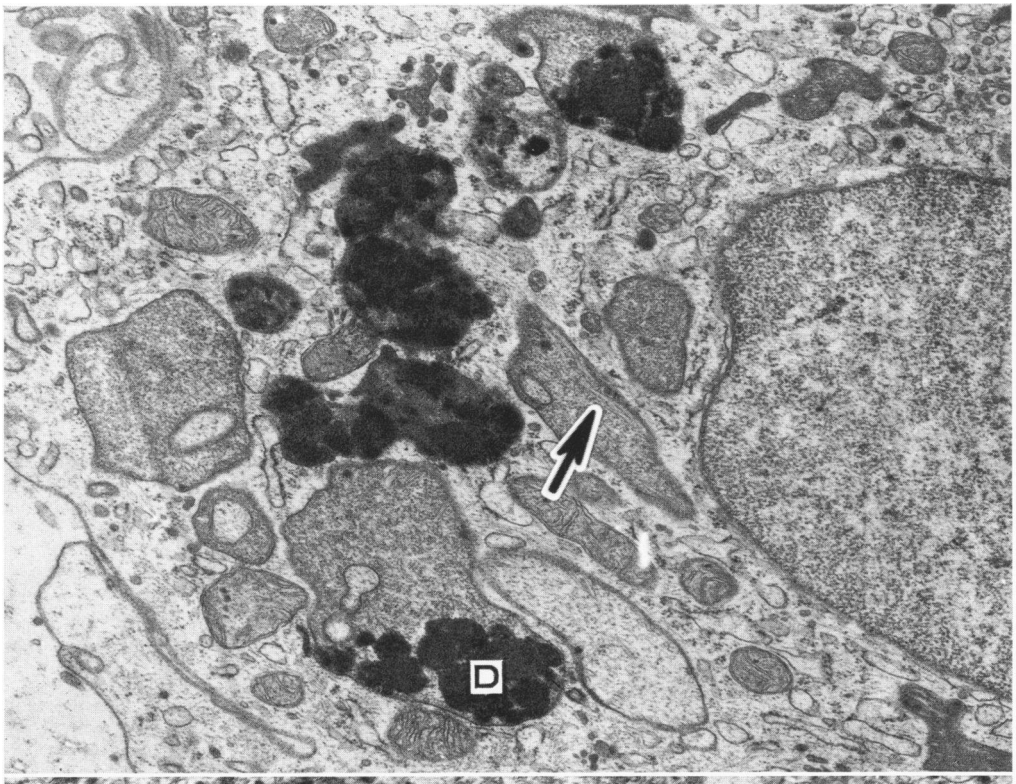
FIG. 12. A Gaucher cell from the same liver as that shown in Figure 9. Gaucher bodies are filled with granules. Occasional tubules (arrow) appear in some structures. Dense osmiophilic deposits (D) are also evident in some inclusions. × 12,300.

FIG. 13. A hepatocyte in the liver of a 9-month-old child with Gaucher's disease. The cytoplasm is filled with large membrane-bound vesicles (V). Gaucher bodies are absent. × 24,400.



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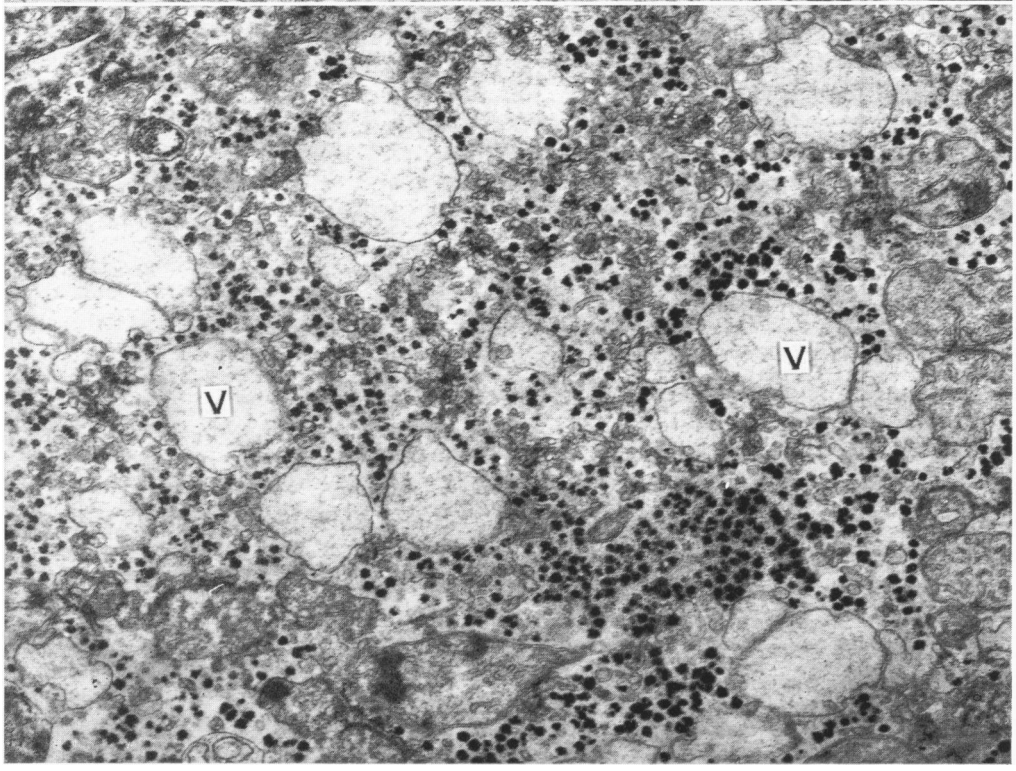

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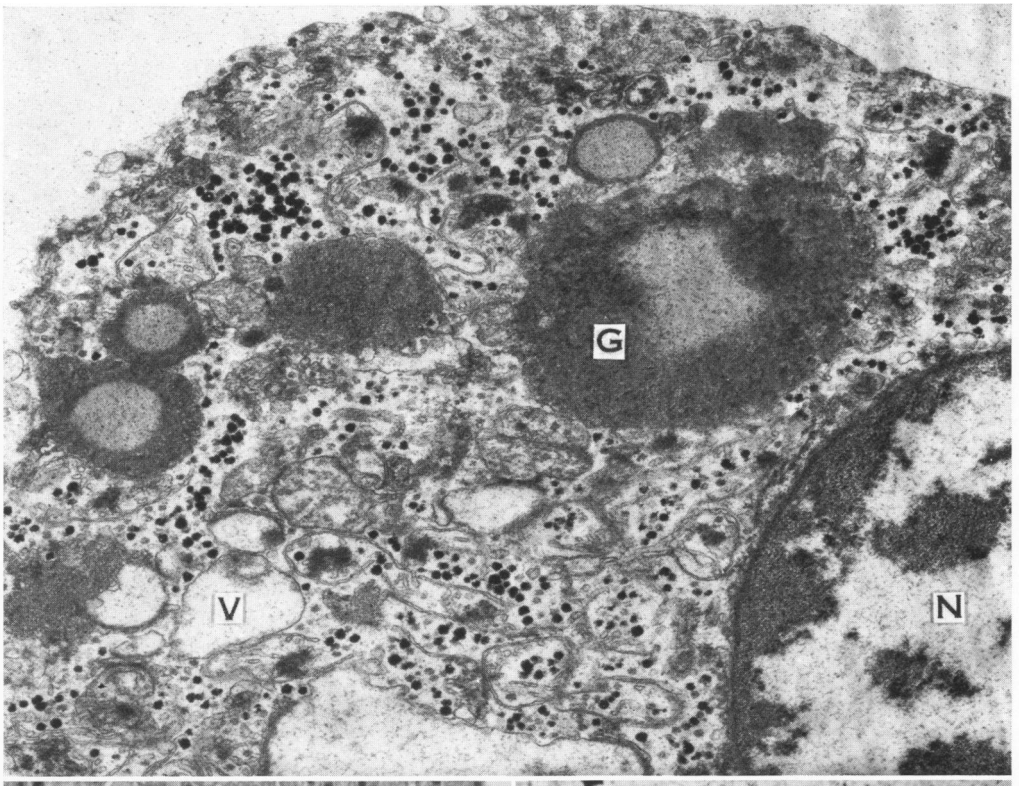
FIG. 14. A hepatocyte from the same liver as that seen in Figure 13. The cytoplasm contains vesicles (V) as well as large coarsely granular bodies (G). Gaucher bodies are absent. Nucleus (N). × 12,300.

FIG. 15. Liver in a child with Tay-Sachs disease. Hepatic cells are normal in appearance. Hematoxylin and eosin stain. × 280.

FIG. 16. Liver in a child with Tay-Sachs disease, stained for acid phosphatase activity. The reaction product is localized to Kupffer cells (large arrow) and lysosomes (small arrows). The latter exhibit the characteristic normal peribiliary localization. × 280.



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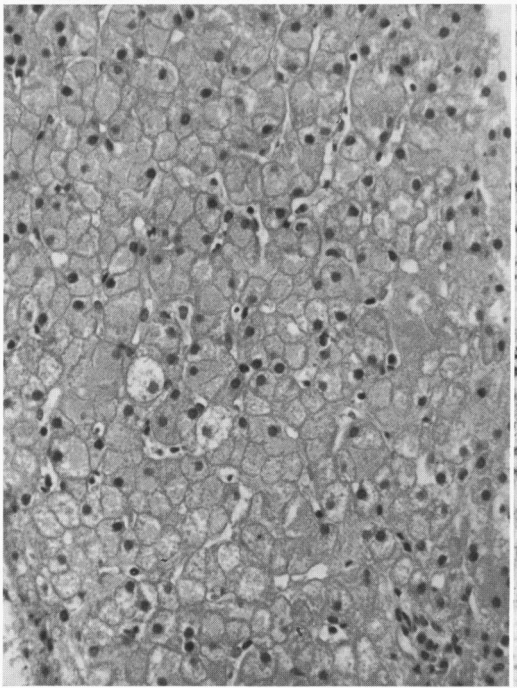

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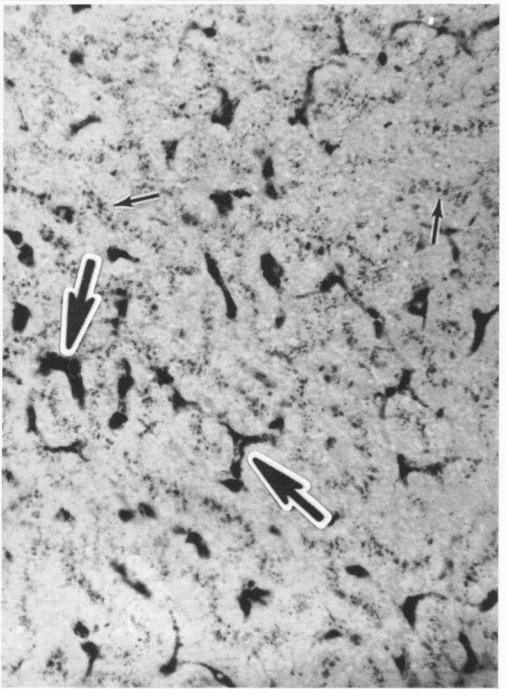

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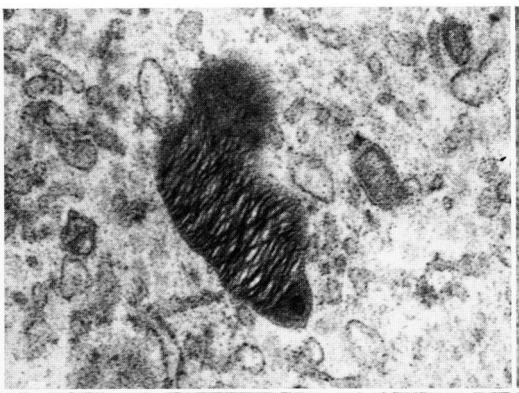
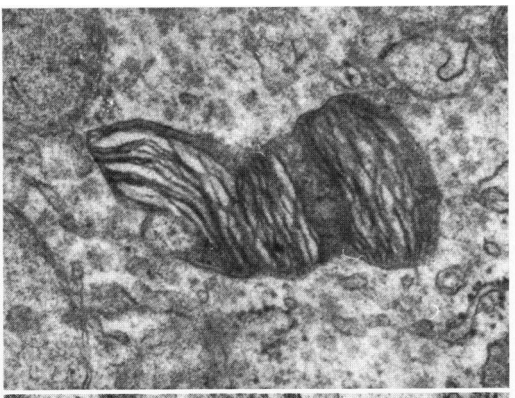
FIG. 17. Atypical pericanalicular membrane-filled structures in hepatocytes from children with Tay-Sachs disease. a) and b) Elongated structures exhibit parallel membranes. × 30,600. c) Concentrically laminated inclusions are similar to the membranous cytoplasmic bodies in neurons. × 14,350. d) A lipofuscin body contains concentric membranes. × 28,800.

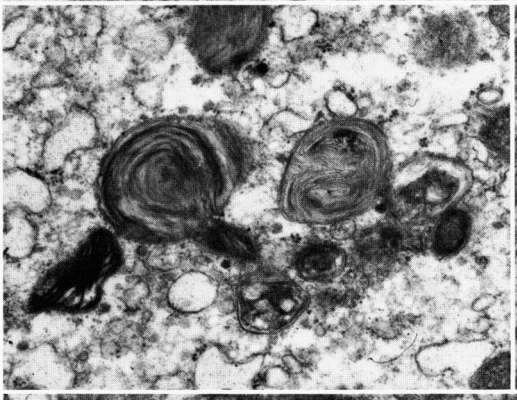
FIG. 18. Large membranous structures are occasionally encountered in the hepatocytes of a patient with Tay-Sachs disease. The endoplasmic reticulum is dilated. × 13,325.



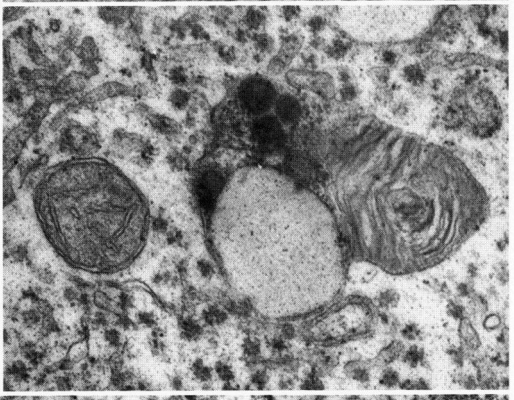
17a



17b



17c



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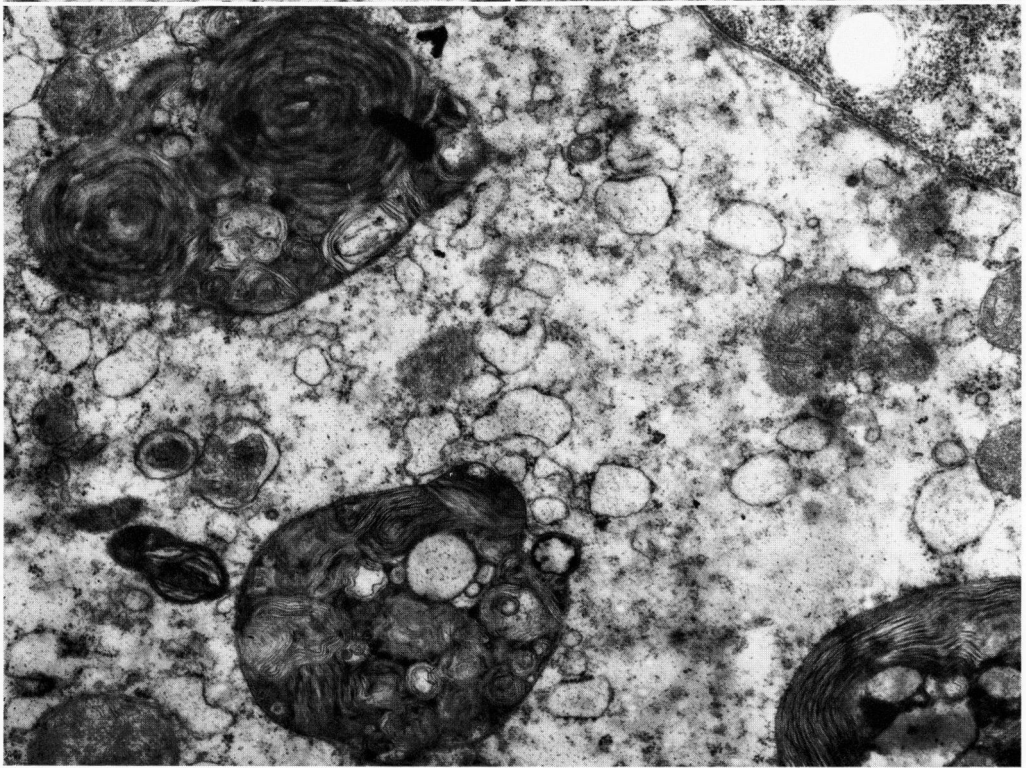

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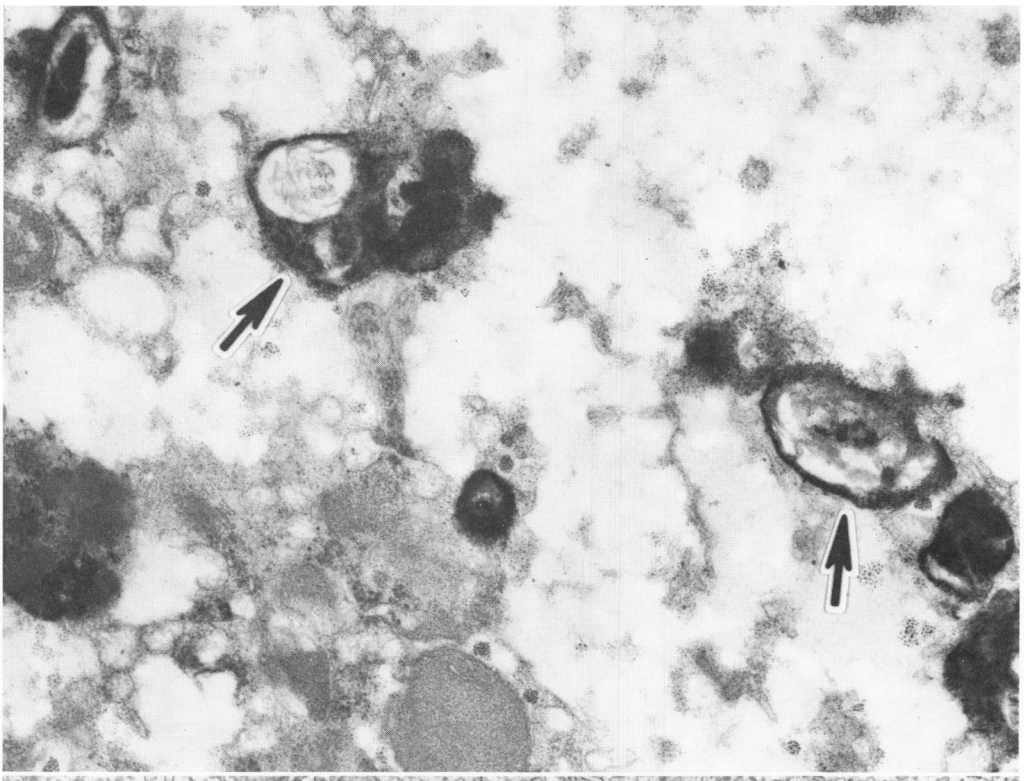
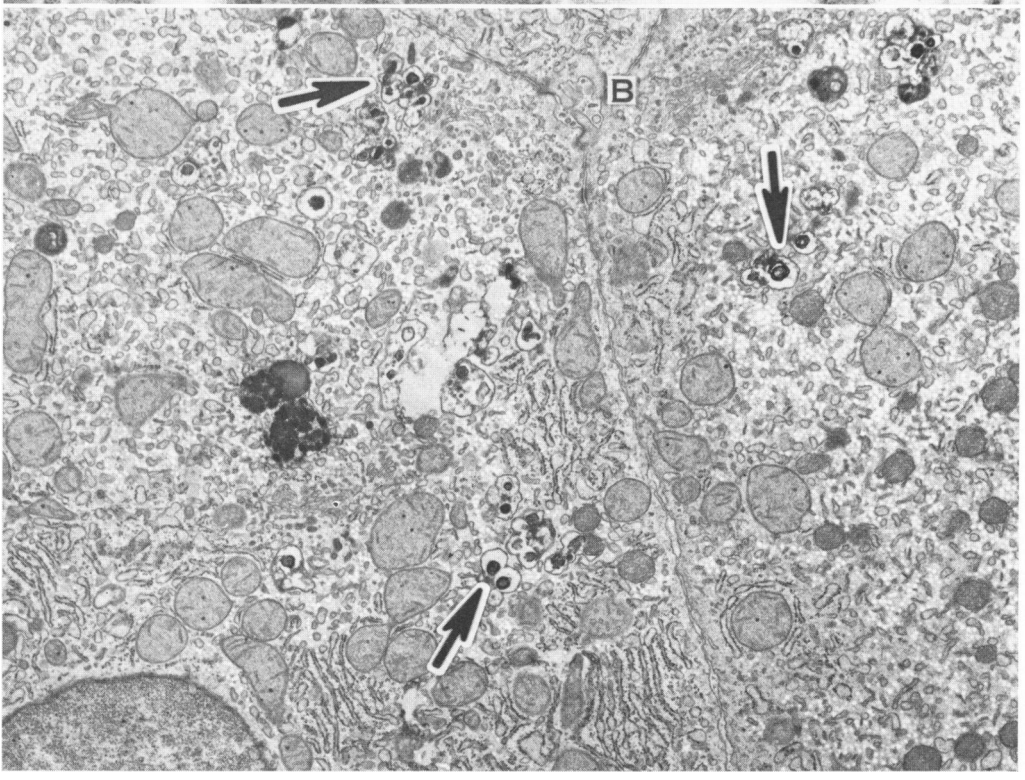
FIG. 19. Atypical membranous pericanalicular bodies appear in the liver of a child with Tay-Sachs disease, stained to demonstrate acid phosphatase activity. The reaction product appears in typical lysosomes as well as in membrane-containing structures (arrows) suggesting that they too are of lysosomal nature. × 25,200.

FIG. 20. Liver, child with rheumatic fever. Numerous pericanalicular bodies (arrows) are evident near the bile canaliculi. There are no structures resembling those observed in the lipidoses. Bile canaliculus (B). × 5,040.



19



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