Cultured Skin Fibroblasts in Storage Disorders

An Analysis of Ultrastructural Features

E. Kamensky, MD, M. Philippart, MD, P. Cancilla, MD and
S. P. Frommes, BS

Electron microscopic studies were performed on cultured fibroblasts from patients with metachromatic leukodystrophy, Fabry's, Gaucher's, Niemann-Pick's (Type A and C), Sanfilippo's (Type A and B) disease, chondroitin-4-sulfate mucopolysaccharidosis, lipofuscinosis (Spielmeyer-Vogt's disease) and ceroid-lipofuscinosis (Batten's disease with curvilinear bodies). Specific cytoplasmic inclusions with a limiting membrane were identified in Fabry's disease, Niemann-Pick syndrome, chondroitin-4-sulfate mucopolysaccharidosis and Sanfilippo's Type B disease. In Fabry's disease, the lipid inclusions tended to form stacks of parallel and concentric membranes. In Niemann-Pick syndrome, the lipid inclusions were made of wavy, loosely packed membranes. In chondroitin-4-sulfate mucopolysaccharidosis and Sanfilippo B, the lysosomes were enlarged and contained a reticular matrix with little electron-dense material. No specific ultrastructural changes were observed in Gaucher's, Sanfilippo's (Type A) disease, metachromatic leukodystrophy (sulfatidosis) and Batten's disease (Am J Pathol 73:59–80, 1973).

THE STORAGE DISORDERS are characterized histologically by the appearance of a variety of cytoplasmic inclusions. These inclusion bodies are generally regarded as lysosomes which have retained substances because of the deficiency of a specific enzyme. Several specific enzyme deficiencies have been recognized in the sphingolipidoses and Hers has developed the concept of inborn lysosomal disorders as a base for understanding the protean family of diseases included in the general designation of the storage disorders.[1]

We have been utilizing the skin fibroblast system in metabolic studies of the sphingolipidoses, mucopolysaccharidoses and lipofuscinoses. Most ultrastructural studies of these disorders have been done directly on tissue obtained from the liver or nervous system. Since cultured skin fibroblasts have become a valuable system for metabolic studies [2] and

From the Departments of Pathology, Pediatrics, Neurology and Psychiatry, The Mental Retardation Center, The Neuropsychiatric Institute, Los Angeles, Calif.

Supported by funds from the Department of Mental Hygiene, State of California, The Mental Retardation Program, NPI, University of California at Los Angeles and Research Grants NB 06938, HD 04612, MCH 927, HD 00345, HD 05615 and NS 08185 from the National Institutes of Health.

Accepted for publication June 1, 1973.

Address reprint requests to Dr. M. Philippart, Mental Retardation Center, The Neuropsychiatric Institute, Los Angeles, CA 90024.

will be utilized more in the future, the present study was undertaken to examine the cells and their inclusions from cultures and to compare them with earlier studies of cells derived from other tissues.

Materials and Methods

Skin was obtained from the arm by punch biopsy. There were 16 patients with the conditions listed in Table 1. Several patients have been the subject of previous reports as indicated below. Thirteen fibroblasts lines were entirely processed in our laboratories. Two lines were received from Dr. E. Neufeld (one line each from Sanfilippo Type A and B) as nonfrozen subcultures in Eagle's Minimal Essential Medium containing 10% fetal calf serum; they were hand-carried from Washington, DC to Los Angeles. The third line (Niemann-Pick Type A), as a nonfrozen subculture in medium F 10 (Grand Island Biological Co) containing 15% of fetal calf serum, was air-mailed by Dr. J. Leroy from Belgium. Control biopsies were obtained from subjects who were investigated as possible heterozygotes but were found to have acid hydrolase levels within the normal range. Fibroblasts derived from the skin after no more than 3 or 4 subcultures, within 6 to 8 weeks after obtaining the skin biopsy, were grown up to confluence in plastic T-flasks (250 ml) with Eagle's Minimum Essential Medium containing 15% fetal calf serum and 0.6% glutamine. This is a well-established culture medium which we have successfully used for growing about 100 different fibroblasts lines. These lines, including those which are the subject of the present report, have been tested for acid hydrolase activity. For each line at least six of the following activities have been determined: arylsulfatase A and B,[3] methylumbelliferyl sulfatase,[4] α- and β-galactosidase,[5] α- and β-glucosidase,[5] α- and β-*N*-acetylglucosaminidase,[6] α- and β-*N*-acetylgalactosaminidase,[7] acid phosphatase,[5] hyaluronidase,[8] β-glucuronidase,[5] cerebrosidase,[9] lactosylceramide-β-galactosidase,[10] phosphodiesterases I and IV.[11,12] The metabolic turnover of all these fibroblasts lines has been extensively studied by following incorporation and decay of 1-^{14}C-acetate for at least 6 weeks after a 2-day pulse. Experiments with ^{14}C-labeled galactose, mannose, *N*-acetylmannosamine and *N*-acetylneuraminic acid, ^{32}P-phosphate, and ^{35}S-sulfate have also been conducted on most of these lines. No morphologic evidence for Mycoplasma contamination was ever encountered in short-term cultures used for the present study. Chromosome studies were not undertaken on most of our lines, but our standard short-term culture conditions are not known to induce chromosomal abnormalities.

Cell pellets were obtained by trypsinization and centrifugation, and were prefixed in 3% glutaraldehyde in phosphate buffer for 2 hours at 4 C, rinsed in the same buffer and then postfixed in 1% buffered osmium tetroxide for 2 hours at 4 C. After rapid dehydration in a graded series of ethanol solutions, the cells were embedded in Epon 812 and placed in an oven at 60 C for at least 2 days. The blocks were then cut with glass knives for thick (1 μ) sections which were stained with toluidine blue to select suitable areas for electron microscopy. Liver and brain tissue obtained by biopsy was fixed in 3% buffered glutaraldehyde and processed as described above. Ultrathin sections were obtained with an LKB ultramicrotome using a diamond knife, stained with uranyl acetate and lead citrate and examined with a Siemen's electron microscope.

Patient Data

The first patient with Fabry's Disease[13] was successfully transplanted for renal insufficiency. He was deficient in α-galactosidase and ceramide trihexosidase. Galactose turnover was impaired in fibroblasts cultured from his skin.[14] The

Table 1—Summary of Patients

Diseases	Number of patients	Abnormal electron microscopic features of possible diagnostic significance
Fabry	3	+
Sulfatidosis (metachromatic leukodystrophy)	1	—
Gaucher	1	—
Nieman-Pick		
Type A	2	+
Type C	1	+
Mucopolysaccharidosis		
Sanfilippo A	2	—
Sanfilippo B	1	+
Chondroitin-4-sulfate	1	+
Lipofuscinosis	2	+
Ceroid-lipofuscinosis	2	—

second patient with Fabry's disease came from a large, previously unreported family with affected males distributed among four generations. He was also deficient in α-galactosidase and had high levels of ceramide trihexosides in blood and urine.[15] The third patient, a female, was an obligatory carrier, being the daughter of an affected male and the niece of our first patient.[13] Her skin fibroblasts were cloned and two cell populations, with and without deficient α-galactosidase activity, were obtained.

The patient with sulfatidosis had an increased excretion of sulfatide, a deficiency of arylsulfatase A and an accumulation of sulfatide in a biopsied sural nerve.[15]

The patient with Gaucher's disease had Gaucher cells in her bone marrow, deficient β-glucosidase activity in her urine and cultured fibroblasts and increased glucosyl ceramide in her plasma.[15]

The family of the first patient with Niemann-Pick A has been the subject of a detailed report, including the autopsy of a sibling.[16] Cultured skin fibroblasts were markedly deficient in sphingomyelinase. The second patient with Niemann-Pick Type A excreted excessive amounts of sphingomyelin in his urine and had a severe deficiency of sphingomyelinase in his cultured fibroblasts, where an increased amount of sphingomyelin was also documented. The patient with Niemann-Pick Type C was diagnosed on the basis of foam cells in liver biopsy and bone marrow, normal urinary sphingomyelin, normal sphingomyelinase and abnormal phosphodiesterase activity in her skin fibroblasts.[17] The first patient with Sanfilippo Type A was diagnosed on the basis of an abnormal urinary excretion of heparan sulfate, absence of skeletal lesion, and normal activity of α-*N*-acetylglucosaminidase. The second patient with Sanfilippo Type A was studied by Dr. E. Neufeld who demonstrated a deficiency in heparan sulfate sulfatase activity in cultured fibroblasts.[18] Alpha-*N*-acetylglucosaminidase was normal in these cells. The patient with Sanfilippo Type B who was shown by Dr. Neufeld to have a normal heparan sulfate sulfatase activity was severely deficient in α-*N*-acetylglucosaminidase. Similar findings have been reported independently by O'Brien.[6]

The patient with chondroitin-4-sulfate mucopolysaccharidosis has been reported previously.[19] No enzyme deficiency has been detected, although the absence of cross-correction with authentic Hurler lines[20] might be interpreted as evidence for an α-L-iduronidase deficiency. The first patient with lipofuscinosis

(Spielmeyer-Vogt) was diagnosed by brain biopsy. An abnormal lipid turnover has been documented in the brain explant but not in fibroblast culture.[21,22] The second patient was a clinically affected sister. The two patients with ceroid-lipofuscinosis (Batten's Disease) were diagnosed by the demonstration of curvilinear bodies in a brain biopsy. No enzyme deficiencies have been detected so far in the patients with lipofuscinosis or ceroid-lipofuscinosis.

Results

Normal Cultures

Fibroblasts derived from normal subjects were characterized in our tissue culture system by an irregular outline with thin and varying sized cytoplasmic processes covered by a cytoplasmic membrane. The cytoplasm contained typical organelles, including mitochondria, smooth and rough surfaced endoplasmic reticulum, ribosomal aggregates, Golgi substance and filaments (Figure 1). A variety of homogeneous or vacuolated dense bodies were occasionally present in small numbers in the cytoplasm (Figures 1 and 2). Most of the dense bodies were limited by a single membrane. The heterogeneity of the cytoplasmic inclusions and the complexity of some individual bodies were evident in this material. Since we were primarily interested in the appearance of these structures in storage disorders, the variability of these structures emphasized the need for careful comparisons between cells derived from patients with a disorder and those obtained from subjects without disease under strictly similar conditions. The nucleus was frequently irregular in outline and often contained a distinct nucleolus (Figure 1).

Fabry's Disease

In Fabry's disease, trihexosyl ceramide accumulates as a result of an α-galactosidase deficiency. Lipid inclusions with similar morphologic characteristics have been described in a variety of tissues.[23-25] The lipid aggregates were generally limited by a membrane and may vary in size from 0.1 to 10 μ in diameter. Variation in structure of the inclusions was present, but the most typical pattern consisted of parallel, concentric or interdigitating lamellae with a periodicity in the range of 40 to 50 Å. Such inclusions were present in skin biopsies directly processed for electron microscopy (Figure 3). Acid phosphatase activity was demonstrated in these structures.

In cultured fibroblasts from 3 patients with Fabry's disease we observed typical inclusions (Figure 4) similar to those found *in vivo* (Figure 3). The cytoplasmic bodies varied in size and shape, but they were generally surrounded by a unit membrane. They were composed

of stacks or circularly disposed lamellae with alternating dark and light bands with a regular period between 40 and 50 Å. The space between the lamellar aggregates contained electron-dense granular material.

In some cells, electron-lucent and vacuolated cytoplasmic bodies similar to those found in fibroblasts from normal subjects were commonly identified (Figure 5). The endoplasmic reticulum was more prominent than that observed in cells from normal subjects, and the cisternae were often dilated. Mitochondria and fibrils appeared normal. Cultured fibroblasts from a female carrier of Fabry's disease contained typical inclusions, but there were fewer inclusions in this instance than in male patients with the disease.

Metachromatic Leukodystrophy (Sulfatidosis)

In metachromatic leukodystrophy, lamellated or prismatic cytoplasmic inclusions have been described in a variety of tissues.[26-28] In the nervous system the inclusions may be composed of membrane-bound spherules organized into lamellar structures with a periodicity of around 60 Å. Resibois has analyzed the two types of inclusions and found average dimensions of 60 Å for the prismatic type and 75 Å for the leaflet type of inclusion.[29,30]

Cultured fibroblasts from a patient with metachromatic leukodystrophy contained large numbers of reticulated foamy vacuoles and several signet-ring and empty vacuoles (Figure 6). These structures were similar to those observed occasionally in fibroblasts from normal subjects. Structures identical to those described in tissues from patients with metachromatic leukodystrophy were not found.

Gaucher's Disease

Characteristic tubular structures have been described in a variety of organs in Gaucher's disease. The tubular elements measure up to 300 Å in diameter and are contained in cytoplasmic bodies which measure up to 2 μ in diameter.[31] Coarse clumps of chromatin applied to the inner surface of the nuclear membrane were a distinctive nuclear feature. There was frequent variability in the appearance of cytoplasmic structures such as mitochondria and the endoplasmic reticulum.

No characteristic cytoplasmic bodies were evident in our material. The nuclei of the fibroblasts were often folded and the nuclear membranes were irregular in outline, but coarse slumping of the chromatin along the nuclear membrane was not found, and the nucleoli appeared normal (Figure 7). The endoplasmic reticulum was sometimes dilated and contained a fine flocculent material (Figure 7).

Niemann-Pick's Syndrome

In Niemann-Pick's disease Type A (Crocker's classification [32]) lamellated structures have been found in many different cell types. In liver,[33] brain and spleen,[34] the cytoplasm was filled with loose concentric lamellar figures around an electron-lucent center.

In cultured fibroblasts from a clinical variant of Type A,[16] there were large numbers of cytoplasmic inclusion bodies which varied in size and shape and were surrounded by a unit membrane (Figure 8). Some of these structures had a granular content, but most of them contained loosely packed, wavy, concentric lamellar figures. The lamellae were often present at one pole of the inclusion, while larger inclusions were often filled with lamellae. Other lamellar inclusions were associated with a dense granular matrix and vacuolated and reticulated inclusions similar to those observed in cells from normal subjects were also observed.

Fibroblasts from another patient with Type A disease contained numerous lamellar inclusions. The folding of the limiting membrane surrounding the inclusion body and their fusiform shape suggested a rigidity of the closely stacked elongated structures within the inclusion (Figure 9). The number of lamellae varied in these structures and often groups of lamellae were separated by a finely granular matrix.

In a case of Niemann-Pick's disease Type C, the cytoplasmic inclusion bodies were very irregular in shape. The lamellar bodies were more compact than those seen in the first example of this disorder, and they were frequently found as a compact cluster of membranes within the boundaries of a vacuole (Figure 10). Foamy and reticulated inclusions were also present (Figure 10), and in some cells the Golgi substance and dilated cisternae of the endoplasmic reticulum were prominent.

Mucopolysaccharidoses

Sanfilippo's Disease

Two variants of this condition have recently been characterized.[6,18] numerous vacuoles within the cytoplasm. The vacuoles were lined by Examination of hepatocytes in 1 patient with Sanfilippo A revealed a single membrane and contained an electron-lucent finely reticulated matrix and a circumscribed electron-dense core (Figure 11). The cytoplasm of the liver cells contained abundant glycogen and the mitochondria were similar to those described in other examples of the mucopolysaccharidoses.[35-36]

In the cultured fibroblasts from this patient, mostly small vacuoles

with indistinct or ill-defined contents were found (Figure 12). There were no electron-dense circumscribed cores within these vacuoles. Much of the cytoplasm was obscured by dense aggregates of glycogen which were more extensive than any found in the other disorders or normal subjects (Figure 12), although biochemical analysis [37] of these lines did not reveal an increase in the glycogen level. Nonspecific cytoplasmic structures were encountered which included lipid and partly vacuolated dense bodies. The endoplasmic reticulum was dilated and contained a finely granular material (Figure 12).

In cultured fibroblasts from a patient with Sanfilippo B, there was abundant glycogen and numerous finely reticulated or vacuolated bodies with dense core regions (Figure 13 and inset).

Chondroitin-4-Sulfate Mucopolysaccharidosis

Electron micrographs of the liver in this patient [19] were indistinguishable from those described in the example of Sanfilippo's disease (Figure 11). The cytoplasm of the hepatocytes was vacuolated and the vacuoles were lined by a single membrane and contained finely reticulated material and electron-dense circumscribed cores.

The fibroblasts from this patient contained a variety of inclusion bodies none of which was similar to those described in the hepatocytes. The most distinctive inclusion body contained stacks of lamellated membranes often attached to one portion of the surrounding limiting membrane (Figure 14 and inset). Other inclusions were vacuolated with finely dispersed reticulated or granular material. Vacuoles were present in the Golgi region, and the endoplasmic reticulum was dilated and filled with granular material (Figure 14).

Lipofuscinosis (Spielmeyer-Vogt) and Ceroid-Lipofuscinosis (Batten's Disease)

Typical lipofuscin bodies with acid hydrolase activity were present in the neurons from a cerebral biopsy from this patient. The inclusions within the perikaryon were numerous and appeared in greater numbers than would be expected for the patient's age (Figure 15).

A large number of amorphous lipid inclusions and dense bodies with a finely granular matrix were distributed within the cytoplasm of the cultured fibroblasts. Although there was vacuolization of some of the dense bodies, no typical lipofuscin bodies or structures identical to the neuronal inclusions described above were found (Figure 16). Polyribosomes were present in the cytoplasm and mitochondria were unremarkable. Fibroblasts obtained from the affected sister of this patient contained numerous identical nonspecific inclusions. None of the

controls exhibited such a large number of lipid inclusions. Fibroblasts from 2 unrelated patients with ceroid-lipofuscinosis (Batten's disease) were indistinguishable from normal controls and thus they were clearly different from the two lipofuscinosis lines.

Discussion

The types of inclusion bodies found in the cytoplasm of cultured fibroblasts are similar to those reported in other cell types. They are usually limited by a unit membrane which is frequently underlined by a clear halo typical of lysosomes.[38] Except for glycogen granules, the occurrence of material lying free in the cytoplasm was infrequent. Acid hydrolase activity has been demonstrated within inclusion bodies from various storage disorders.[24,29,31,39] When the lysosomal matrix is markedly dilated, enzyme activity is only demonstrable near the limiting membrane.[35]

Culture conditions are known to have an influence on the ultrastructural features of cells. For example, the number of lysosomes may increase in older cells or diminish after the medium is changed.[40–42] Cells grown in a medium containing 50% human cord serum may have dilatation of the rough endoplasmic reticulum and an increase in the size and number of lysosomes.[43] The serum may supply needed nutrients to the culture, but it may also be potentially toxic when used in such large concentrations (50% rather than 15% in the present study). The difference between sufficient and excessive concentration of serum has not been established and may vary from one line to another. As a result, it was not possible to interpret the significance of the slight dilatation of the endoplasmic reticulum occasionally observed in our study. Lipids, such as cholesterol, in the serum have repressed lipid synthesis significantly in cultured fibroblasts [44] and brain explants.[21] Morphologic abnormalities detectable by the most sensitive technic presently available are still inexact. For example, subtle alteration in membrane structure involving targets for hormonal signal are not detectable and may well cause significant abnormalities of synthesis or catabolism.

The diagnosis of a specific storage disorder cannot always be determined by an ultrastructural examination of cultured fibroblasts because, as this study has illustrated, specific inclusions may not be present in conditions such as Gaucher's disease and metachromatic leukodystrophy. This situation was not unexpected, since generally it is only cells which synthesize an undegradable substance that will present with enlarged lysosomes. Synthetic enzymes represent one of the bio-

chemical expressions of cell differentiation. Accordingly, the distribution of products such as hemoglobin or blood-group substances is restricted to a limited number of cells. On the other hand, lysosomal hydrolases are ubiquitous, although there may be differences in their concentrations in some instances.

This difference between synthetic and degrading enzymes may have an evolutionary advantage, since it would protect cells from being overwhelmed by exogenous undigestible material. Cells which synthesize only small amounts of a substrate might still be capable of degrading it properly by utilizing the residual activity of a genetically deficient enzyme. This may be the case in fibroblasts from patients with Gaucher's disease, which have the ability to synthesize glucosyl ceramide as well as complex derivatives of the lipid.[14] The lack of an ultrastructural abnormality in metachromatic leukodystrophy may be an example of the actual absence of an undegradable substrate because fibroblasts do not synthesize sulfatides from radioactive precursors.[14] Studies in which there is experimental overloading of tissue cultures may help to circumvent this problem.[36]

The type of inclusion observed in storage disorders may be classified as either electron-lucent or electron-dense. Combination of both electron-lucent and electron-dense material are also observed. Electron-dense inclusions can be amorphous, granular or lamellar in appearance. Different combinations of granules and lamellae are commonly observed with some indication that granules may be preliminary steps towards the lamellar formation [45] (Figure 4). The morphologic appearance of glycogen [46] might be misleading, however, as attested to by the lack of an increased glycogen content in cells from Sanfilippo disease (Figures 11–13). Further work is needed to characterize the observed granules. Lamellae have generally been associated with the presence of lipids, although it has been demonstrated that myelin lamellae maintained their morphologic appearance after lipid extraction.[47] Glycolipids such as trihexosyl ceramide give rise to rigid-appearing, sometimes crystal-like lamellae (Figures 3–5). GM_1 or GM_2 ganglioside storage has been associated with regular but less rigid lamellae.[45] Sphingomyelin is the only type of phospholipid storage presently known. A loose type of lamella has been repeatedly associated with that type of storage [33,34] (Figures 8–10). Neutral lipids such as triglycerides give rise to amorphous, moderately electron-dense inclusions [48] (Figure 16). Electron-lucent vacuoles have been typically observed in various types of mucopolysaccharidosis [49] (Figures 12, 13 and 14). Smaller and less numerous but similar vacuoles have been observed in normal controls

as well as in a variety of storage disorders. Since "metachromatic granules" [50] and increased mucopolysaccharide content on biochemical analysis [51] have been described in the same instances, it is reasonable to speculate that the large reticulated electron-lucent vacuoles might represent metachromatic granules containing increased amounts of mucopolysaccharide. As a corollary to this hypothesis, mucopolysaccharide might be normally involved in the lysosomal function.

Many of the ultrastructural features of the inclusion bodies in storage disorders are nonspecific and may represent an exaggerated appearance of normal organelles. This study indicates that, in some of the storage disorders, unique types of inclusion bodies may be found in fibroblasts as well as in other cell types such as in the liver and nervous system. Since storage substances tend to be chemically heterogeneous,[15] caution must be used before absolute values are given to ultrastructural features.

References

1. Hers HG: Inborn lysosomal diseases. Gastroenterology 48:625–633, 1965
2. Bearn AG: Cell culture in inherited disease with some notes on genetic heterogeneity. N Engl J Med 286:764–767, 1972
3. Baum H, Dodgson KS, Spencer B: The assay of arylsulphatases A and B in human urine. Clin Chim Acta 4:453–455, 1959
4. Guilbault GG, Hieserman J: Fluorometric substrate for sulfatase and lipase. Anal Chem 41:2006–2009, 1969
5. Van Hoof F, Hers HG: The abnormalities of lysosomal enzymes in mucopolysaccharidoses. Eur J Biochem 7:34–44, 1968
6. O'Brien JS: Sanfilippo syndrome: profound deficiency of alpha-acetylglucosaminidase activity in organs and skin fibroblasts from Type-B patients. Proc Natl Acad Sci USA 69:1720–1722, 1972
7. Callahan JW, Lassila EL, Den Tandt W, Philippart M. Alpha-N-acetylgalactosaminidase: isolation, properties and distribution of the human enzyme. Biochem Med 7:424–431, 1973
8. Margolis RU, Margolis RK, Santella R, Atherton DM: The hyaluronidase of brain. J Neurochem 19:2325–2332, 1972
9. Bowen DM, Radin NS: Cerebroside galactosidase: a method for determination and a comparison with other lysosomal enzymes in developing rat brain. J Neurochem 16:501–512, 1969
10. Bensaude I, Philippart M, Hildebrand J: Alpha-galactosidic configuration in ceramide trihexoside synthesized by rat spleen homogenate. Biochem Med 6:522–525, 1972
11. Brightwell R, Tappel AL: Subcellular distributions and properties of rat liver phosphodiesterases. Arch Biochem Biophys 124:325–332, 1968
12. Philippart M: Unpublished data
13. Philippart M, Franklin SS, Gordon, A: Reversal of an inborn sphingolipidosis (Fabry's disease) by kidney transplantation. Ann Intern Med 77:195–200, 1972

14. Philippart M: (^{14}C) galactose incorporation into skin fibroblast in glycolipid storage disorders (sulfatidosis, Fabry's, Gaucher's, and Hurler's disease). Proceedings of the Society for Pediatric Research, Eighty-first Annual Meeting, 1971, p 61
15. Philippart M: Glycolipid, mucopolysaccharide and carbohydrate distribution in tissues, plasma and urine from glycolipidoses and other disorders: complex nature of the accumulated substances, Glycolipids, Glycoproteins, and Mucopolysaccharides of the Nervous System. Edited by V Zambotti, G Tettamanti, M Arrigoni. New York, Plenum Publishing Corporation, 1972, pp 231–254
16. Martin JJ, Philippart, M, Van Hauwaert J, Callahan JW, Deberdt R: Neimann-Pick's disease (Crocker's Group A) with late onset and pigmentary degeneration similar to Hallervorden-Spatz syndrome. Arch Neurol 27:45–51, 1972
17. Callahan JW, Lassila EL, Philippart, M: Phosphodiesterases in normal and Niemann-Pick tissues. Trans Am Soc Neurochem 4:61, 1972
18. Neufeld EF, Barton RW, Cantz M, Derge JG, Hall CW, Kresse H, Scott JF: Deficiency of specific proteins in the inborn errors of mucopolysaccharide metabolism, Sphingolipids, Sphingolipidosis and Allied Disorders. Edited by B Volk, Aronson. New York, Plenum Press, 1972, pp 187–194
19. Philippart M, Sugarman GI: Chondroitin-4-sulfate mucopolysaccharidosis. Lancet 2:854, 1969
20. Neufeld E: Personal communication
21. Philippart M: (^{14}C) Incorporation into brain explants from lipofuscinosis and sulfatidosis. Third International Meeting of the International Society for Neurochemistry, Budapest, 1971, p 343
22. Philippart, M: Abnormal lipid turnover in cultured brain cells from lipofuscinosis, Fourth Symposium on Batten's Disease. In press
23. Sweeley CC, Klionsky B, Krivit, W, Desnick RJ: Fabry's disease: glycosphingolipid lipidosis, The Metabolic Basis of Inherited Disease, Third edition. Edited by JB Stanbury, JB Wyngaarden, DS Fredrickson. New York, McGraw-Hill Book Company, 1972, pp 663–682
24. Hashimoto K, Gross BG, Lever WF: Angiokeratoma corporis diffusum (Fabry): histochemical and EM studies of the skin. J Invest Dermatol 44:119–128, 1965
25. van Mullem PJ, Ruiter M: Fine structure of the skin in angiokeratoma corporis diffusum (Fabry's Disease). J Pathol 101:221–226, 1970
26. Terry RD, Suzuki K, Weiss M: Biopsy study in three cases of metachromatic leukodystrophy. J Neuropathol Exp Neurol 25:141–143, 1966
27. Fogelson MH, Gonatas, NK, Rorke, LB, Spiro, A: Oligodendroglial lamellar inclusions. Arch Neurol 19:150–155, 1968
28. Liu, HM: Ultrastructure of central nervous system lesions in metachromatic leukodystrophy with special reference to morphogenesis. J Neuropathol Exp Neurol 27:624–644, 1968
29. Resibois AE: Microscopic study of metachromatic leukodystrophy: lysosomal nature of the inclusions. Acta Neuropathol 13:149–156, 1969
30. Resibois A: Electron microscopic studies of metachromatic leucodystrophy. IV. Liver and kidney alterations. Pathol Eur 6:278–298, 1971
31. Hibbs RG, Ferrans VJ, Cipriano PR, Tardiff KJ: A histochemical and electron microscopic study of Gaucher cells. Arch Pathol 89:137–153, 1970

32. Crocker AC: The cerebral defect in Tay-Sachs disease and Nieman-Pick disease. J Neurochem 7:69–80, 1961

33. Volk BW, Wallace, BJ: The liver in lipidosis: an electron microscopic and histochemical study. Am J Pathol 49:203–225, 1966

34. Kamoshita S, Aron AM, Suzuki K, Suzuki S: Infantile Niemann-Pick disease: a chemical study with isolation and characterization of membranous cytoplasmic bodies and myelin. Am J Dis Child 117:379–394, 1969

35. Wallace BJ, Kaplan D, Adachi M, Schneck L, Volk BV: Mucopolysaccharidosis type III: morphologic and biochemical studies of two siblings with Sanfilippo syndrome. Arch Pathol 82:462–473, 1966

36. Van Hoof F, Hers HG: L'ultrastructure du foie dans certaines thesaurismoses. Rev Intern Hepatol 17:815–826, 1967

37. Angelini C, Engel AG, Titus JL: Adult acid maltase deficiency: abnormalities in fibroblasts cultured from patients. N Engl J Med 287:948–951, 1972

38. Daems WT, Van Rijssel TD: The fine structure of the peribiliary dense bodies of mouse liver tissue. J Ultrastruct Res 5:263–290, 1961

39. Tallman FJ, Brady RO, Suzuki K: Enzymic activities associated with membranous cytoplasmic bodies and isolated brain lysosomes. J Neurochem 18:1775–1777, 1971

40. Coelho-Maciera A, Garcia-Giralt E, Adrian M: Changes in lysosomal associated structures in human fibroblasts kept in resting phase. Proc Soc Exp Biol Med 138:712–718, 1971

41. Cirelli, E. Beitrag zur Ultrastruktur menslicher Fibroblasten *in vitro*. Acta Anat 76:25–34, 1970

42. Robbins E, Levine, EM, Eagle, H: Morphologic changes accompanying senescence of culture human diploid cells. J Exp Med 131:1211–1222, 1970

43. Brown CA, Draper, P: An electron-microscope study of rat fibroblasts infected with *Mycobacterium lepraemurium*. J Pathol 102:21–26, 1970

44. Bailey JM: Lipid metabolism in cultured cells. IV. Lipid biosynthesis in serum and synthetic growth media. Biochim Biophys Acta 125:226–236, 1966

45. Stern J, Novikoff AB, Terry, RD: The induction of sulfatide, ganglioside and cerebroside storage in organized nervous system culture.[18] pp 651–660

46. Baudhuin P, Hers HG, Loeb, H: An electron microscopic and biochemical study of type II glycogenosis. Lab Invest 13:1140–1152, 1964

47. Napolitano L, Lebaron F, Scaletti J: Preservation of myelin lamellar structure in the absence of lipidd J Cell Biol 34:817–826, 1967

48. Stein O, Stein Y: Electronmicroscopic autoradiography of ^{3}H-glycerol labeled lipid in ethanol induced fatty liver. Exp Cell Res 42:198–201, 1965

49. Kenyon KR, Quigley HA, Hussels, IE, Wyllie RG: The systemic mucopolysaccharidoses: ultrastructural and histochemical studies of conjunctiva and skin. Am J Ophthalmol 73:811–833, 1972

50. Dorfman A, Matalon, R: The mucopolysaccharidoses, The Metabolic Basis of Inherited Disease, Third edition. Edited by JB Stanbury, JB Wyngaarden, DS Fredrickson. New York, McGraw-Hill Book Company, 1972, pp 1218–1272

51. Matalon R, Dorfman A: Acid mucopolysaccharides in cultured human fibroblasts. Lancet 2:838–841, 1969

Acknowledgments

We thank Drs. G. Carpentier, Stanley S. Franklin, Arthur Gordon, Jules Leroy, Edward A. Loeb, Jean-Jacques Martin, Elizabeth F. Neufeld, Richard Schain, Gerald Sugarman and Larry L. Tibbles for referring patients or providing skin fibroblast cultures; Robert Sparkes and Mary Katz for cloning Fabry fibroblasts; Robert Neerhout for bone marrow examinations and W. R. Den Tandt for glycogen determinations.

Legends for Figures

Fig 1—Normal cultured fibroblast with typical organelles: mitochondria, smooth and rough endoplasmic reticulum, ribosomes, Golgi structure, various dense and vacuolated bodies. The nucleolus is apparent (Uranyl acetate and lead citrate, × 10,500).

Fig 2—Normal cultured fibroblast. The cytoplasm is filled with a large number of homogeneous or vacuolated dense bodies (Uranyl acetate and lead citrate, × 16,800).

Fig 3—Fibroblast from a skin biopsy of a patient with Fabry's disease. The cytoplasm is filled with lamellar inclusions, surrounded by a unit membrane, few foamy vacuoles. Outside of the cell, bundles of collagen fibers are seen (Uranyl acetate and lead citrate, × 64,800).

Fig 4—Cultured fibroblast from a patient with Fabry's disease. In the cytoplasm the inclusion bodies are surrounded by a unit membrane. These bodies are composed of stacks of parallel or concentric lamellae with alternating dark and light bands (Uranyl acetate and lead citrate, × 60,000).

Fig 5—Cultured fibroblast from a patient with Fabry's disease. In addition to characteristic inclusions shown in Figure 3, there are typical organelles and inclusions which are also similar but larger and more numerous than those found in normal cultured fibroblasts (Uranyl acetate and lead citrate, × 18,400).

Fig 6—Cultured fibroblasts from a patient with metachromatic leukodystrophy. Similar vacuoles with a reticulated, foamy matrix may be encountered in normal senescent fibroblasts. In the largest vacuoles present, the distended matrix becomes difficult to detect but almost never disappears entirely. A number of vacuoles have a signet-ring appearance (Uranyl acetate and lead citrate, × 11,500).

Fig 7—Cultured fibroblast from patient with Gaucher's disease. The nucleus appears normal; the endoplasmic reticulum is dilated and contains flocculent material (Uranyl acetate and lead citrate, × 27,600).

Fig 8—Cultured fibroblast from a patient with Niemann-Pick's disease, Type A. Inclusions surrounded by a unit membrane contain a granular core and loose, wavy, concentric lamellae (Uranyl acetate and lead citrate, × 32,000).

Fig 9—Cultured fibroblast from another patient with Niemann-Pick's disease, Type A. In addition to inclusions similar to those shown in Figure 8, another type was present. The elongated shape of these inclusion bodies suggests a rigidity of the lamellae which are separated by a granular matrix (Uranyl acetate and lead citrate, × 100,000).

Fig 10—Cultured fibroblast from a patient with Niemann-Pick's disease, Type C. This picture shows compact clusters of membranes within vacuoles, foamy reticulated inclusions and dilated endoplasmic reticulum (Uranyl acetate and lead citrate, × 64,400).

Fig 11—Liver biopsy of a patient with Sanfilippo's disease. In the hepatocytes, vacuoles surrounded by a unit membrane contain a fine reticular matrix with a circumscribed electron-dense core and scattered granules of varying density. Abundant glycogen and mitochondria with indistinct cristae are also recognizable (Uranyl acetate and lead citrate, × 11,500).

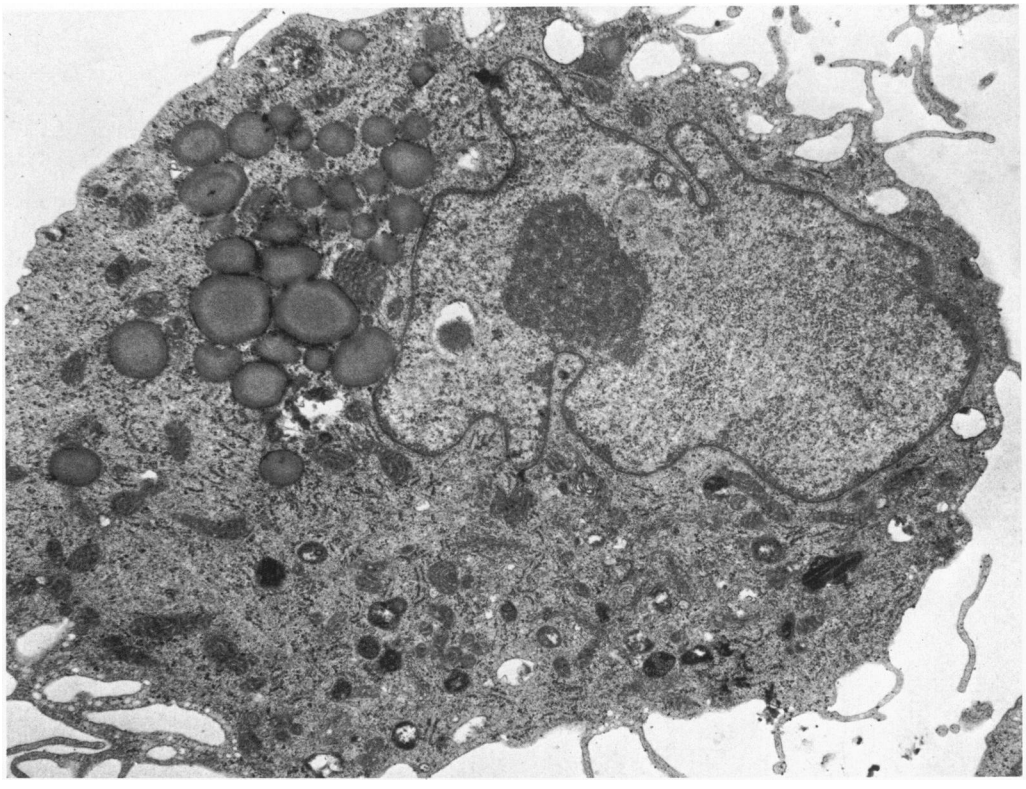
Fig 12—Cultured fibroblast from the same patient with Sanfilippo, Type A disease. Small vacuoles and dense glycogen clumps are present. The endoplasmic reticulum is dilated by a fine granular material (Uranyl acetate and lead citrate, × 11,500).

Fig 13—Cultured fibroblast from a patient with Sanfilippo, Type B disease. Abundant glycogen and numerous large reticular vacuolated bodies were found (Uranyl acetate and lead citrate, × 8800). **Inset**—Details from the same micrograph which shows the reticular bodies with a signet-ring appearance (Uranyl acetate and lead citrate, × 22,000).

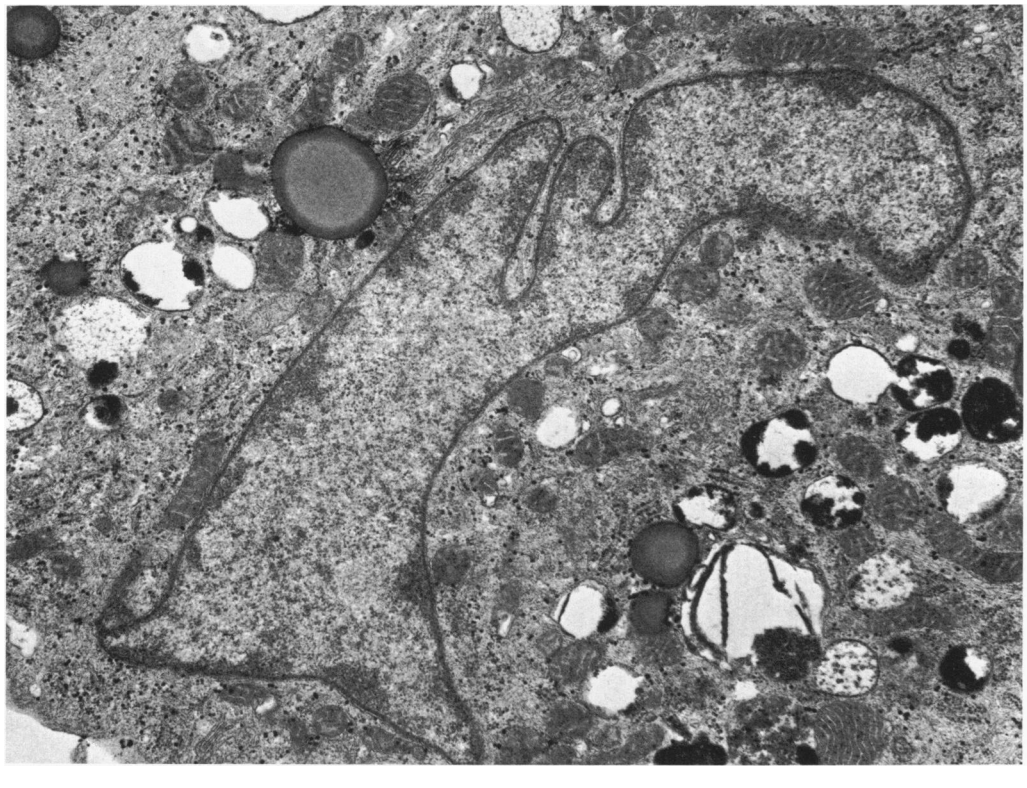
Fig 14—Cultured fibroblast from a patient with chondroitin-4-sulphate mucopolysaccharidosis. The cytoplasm is filled with lamellar or granular inclusions. The Golgi structure is vacuolated, and the endoplasmic reticulum is dilated and filled with a granular material (Uranyl acetate and lead citrate, × 12,000). **Inset**—Detail showing the lamellar body, and vacuoles in the golgi structure (Uranyl acetate and lead citrate, × 15,500).

Fig 15—Brain biopsy from a patient with lipofuscinosis. This picture demonstrates lipofuscin bodies with acid phosphatase activity (Uranyl acetate and lead citrate, × 25,600).

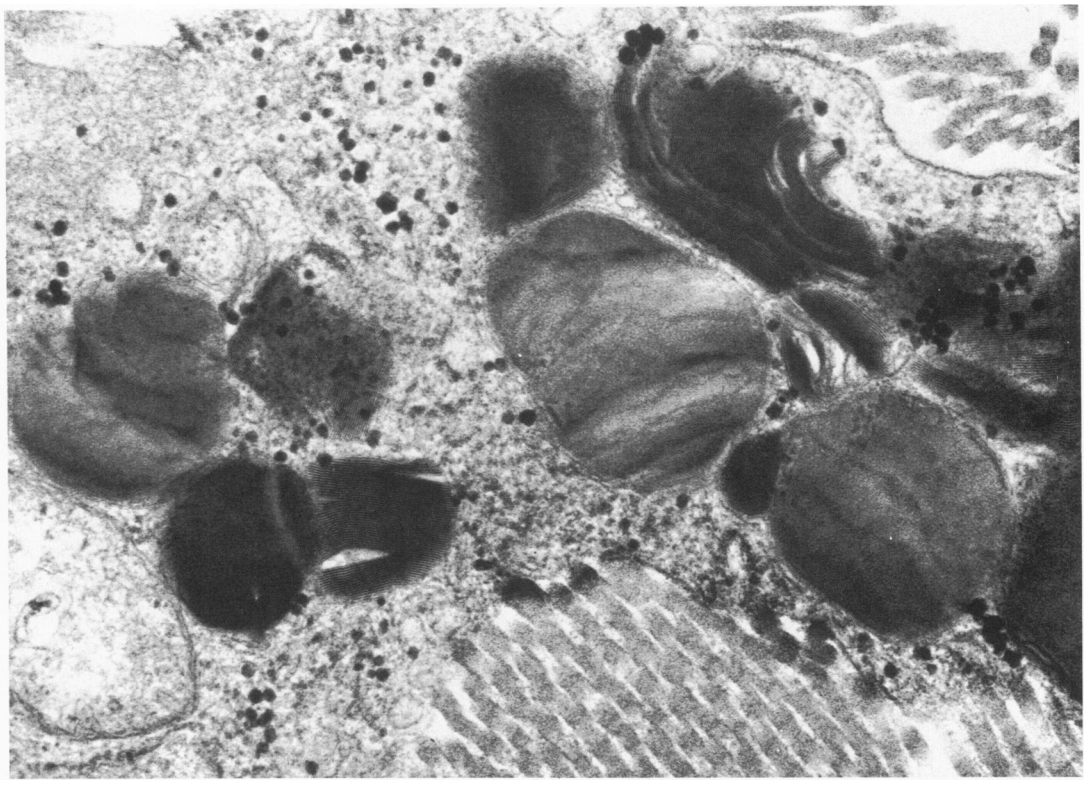
Fig 16—A cultured fibroblast from the same patient as Figure 15. Tightly packed triglyceride-like inclusions and dense bodies with a granular matrix are seen (Uranyl acetate and lead citrate, × 28,800).



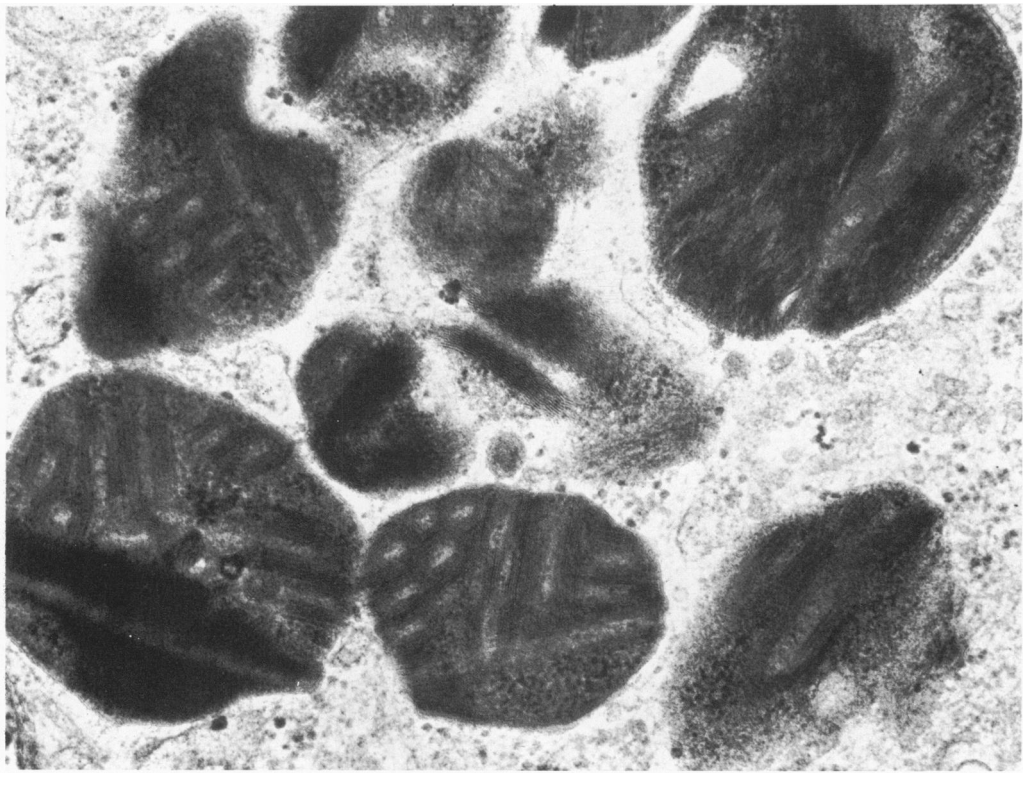
1



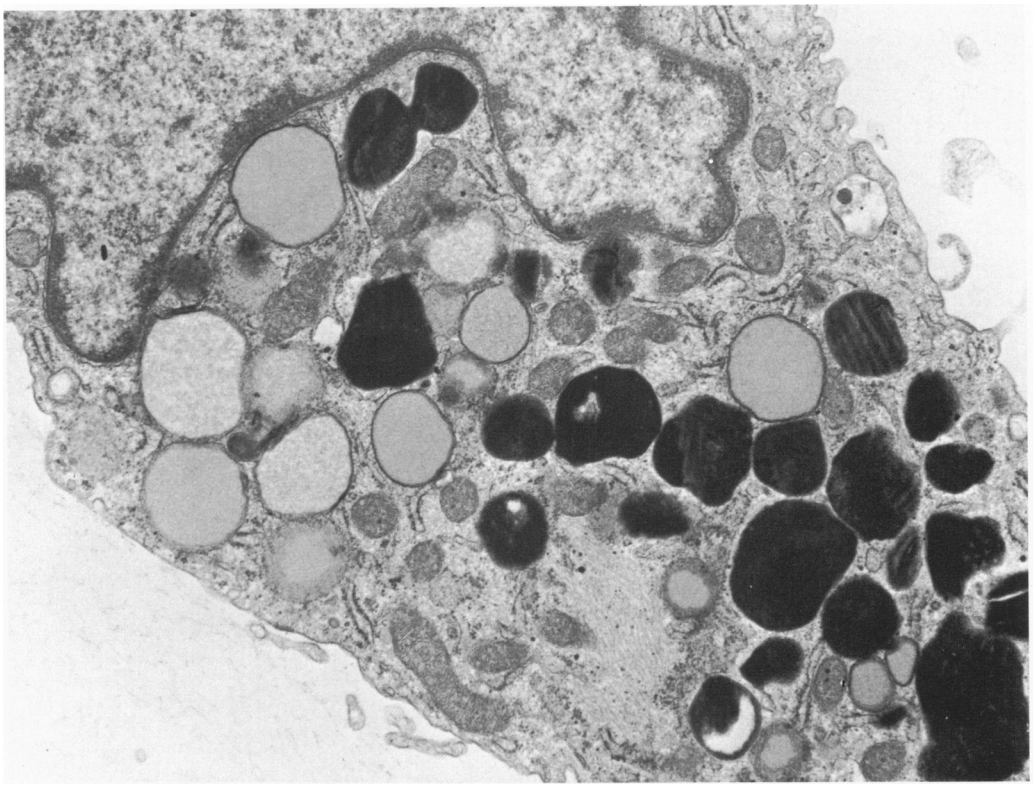
2



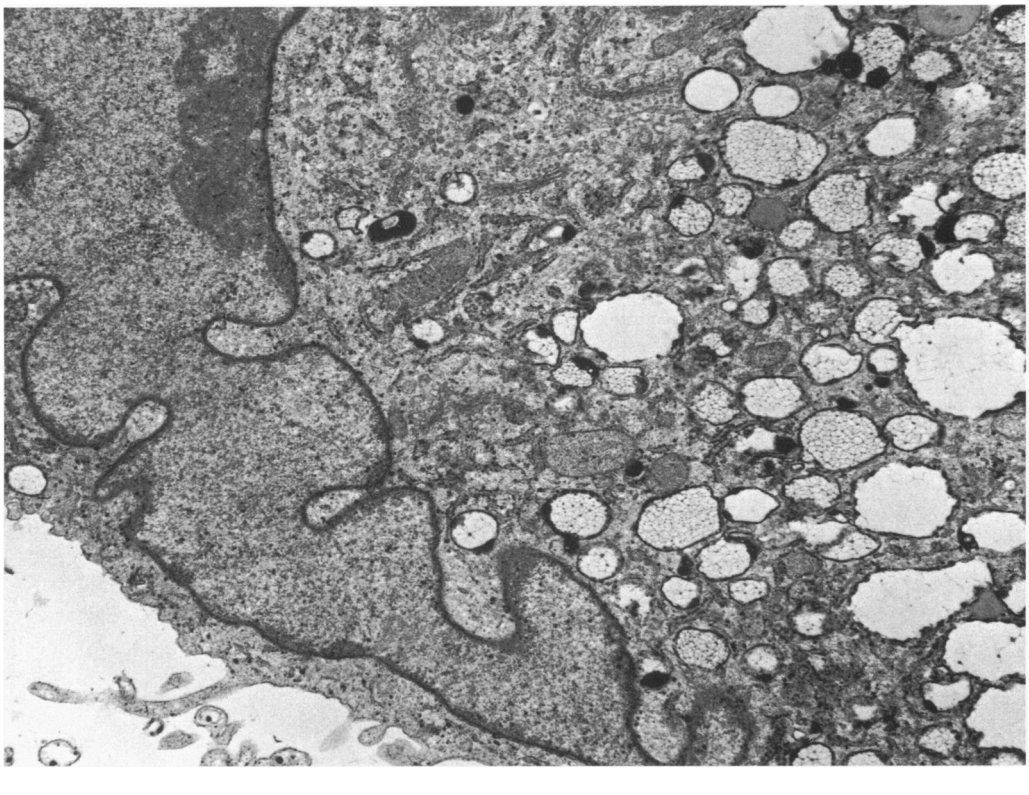
3



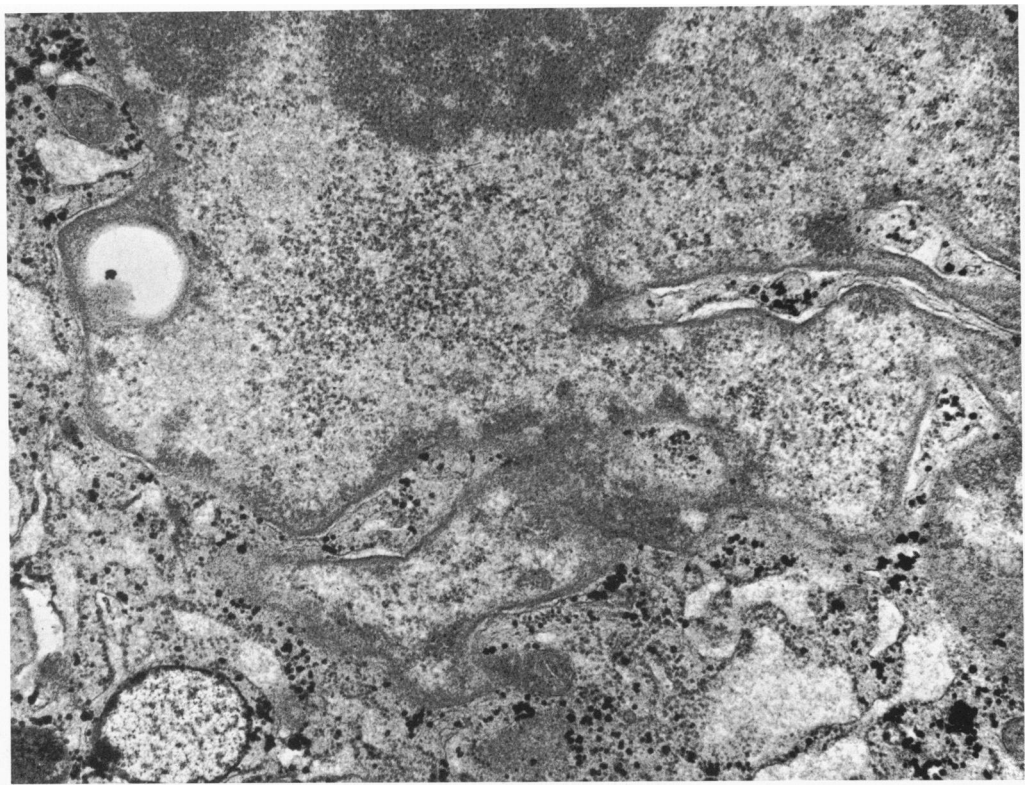
4



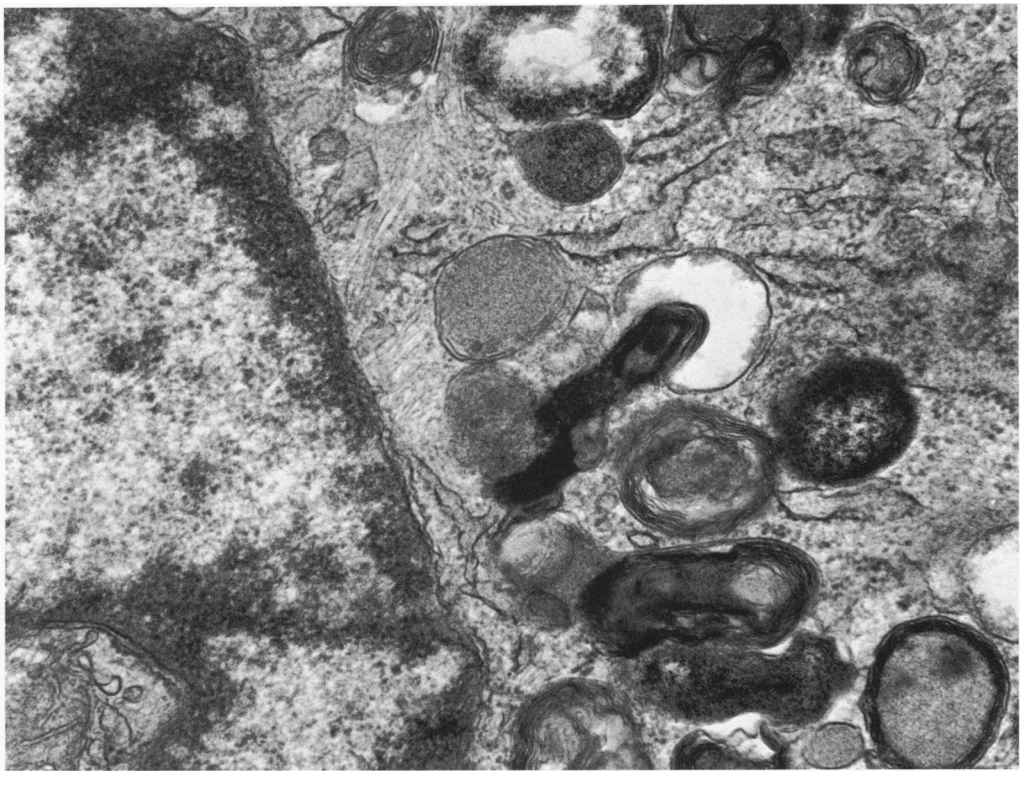
5



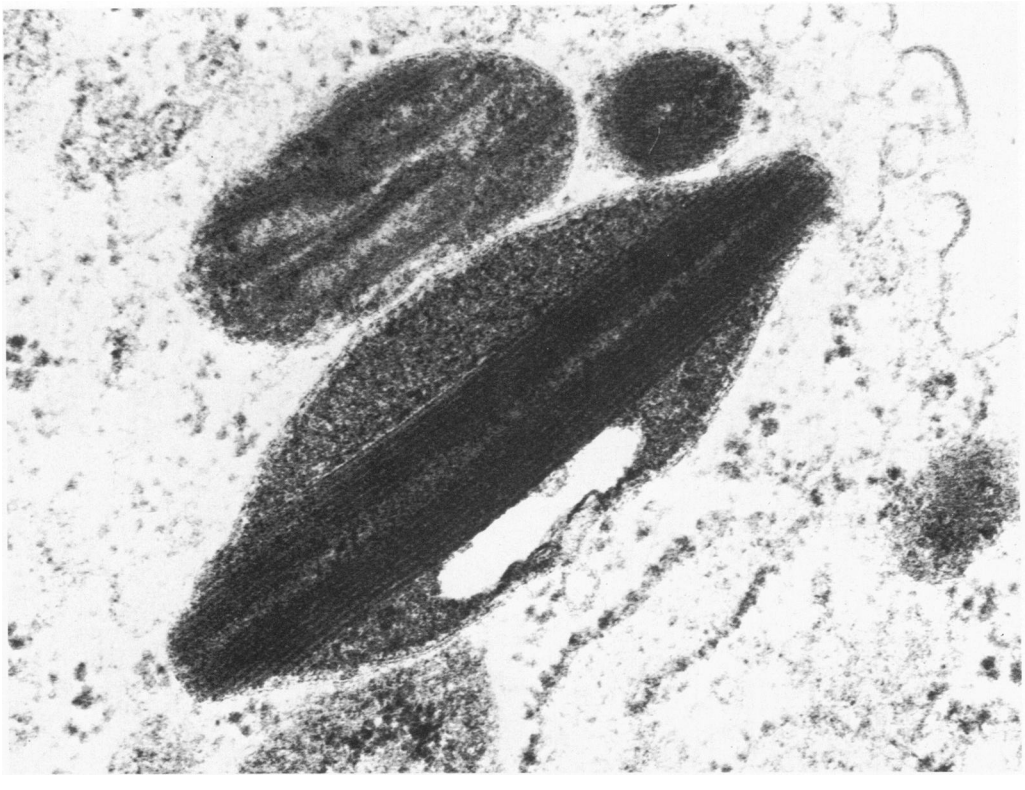
6



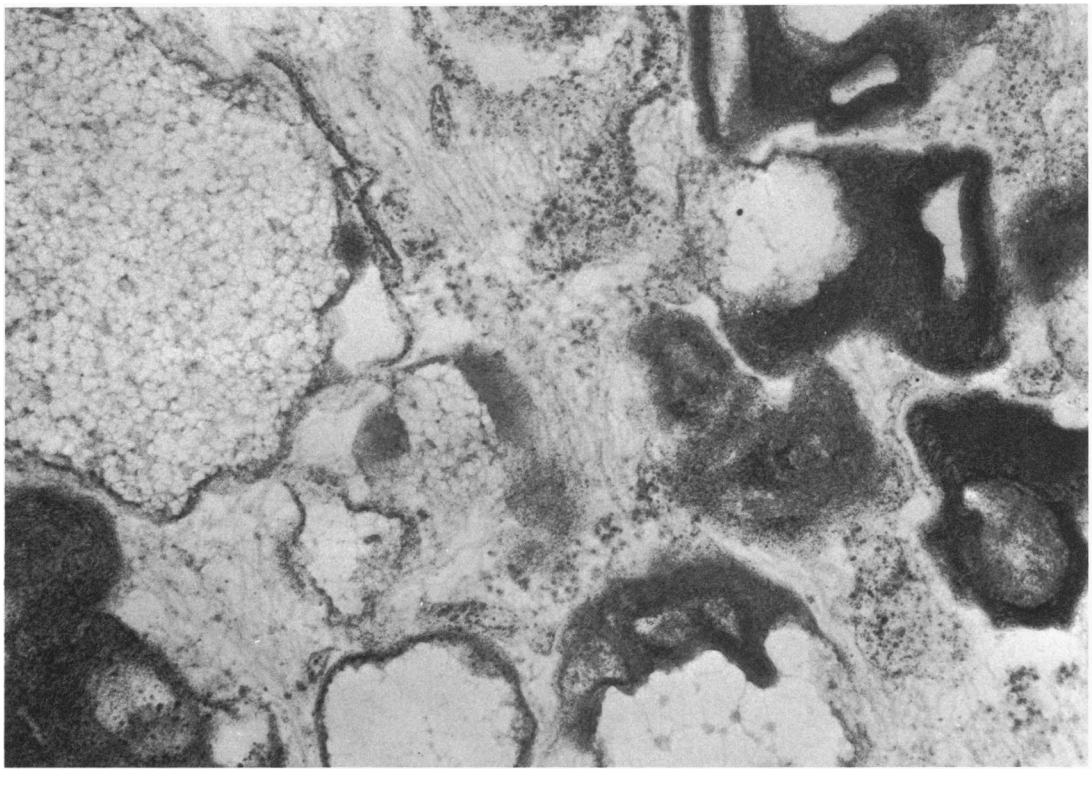
7



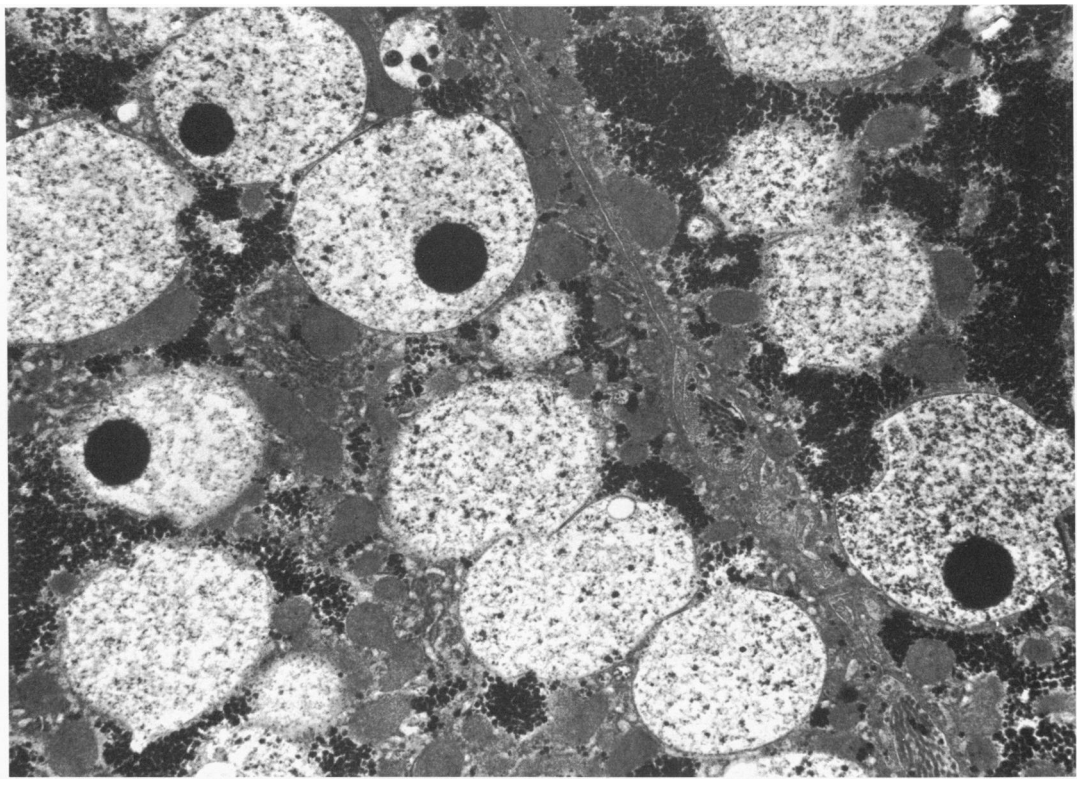
8



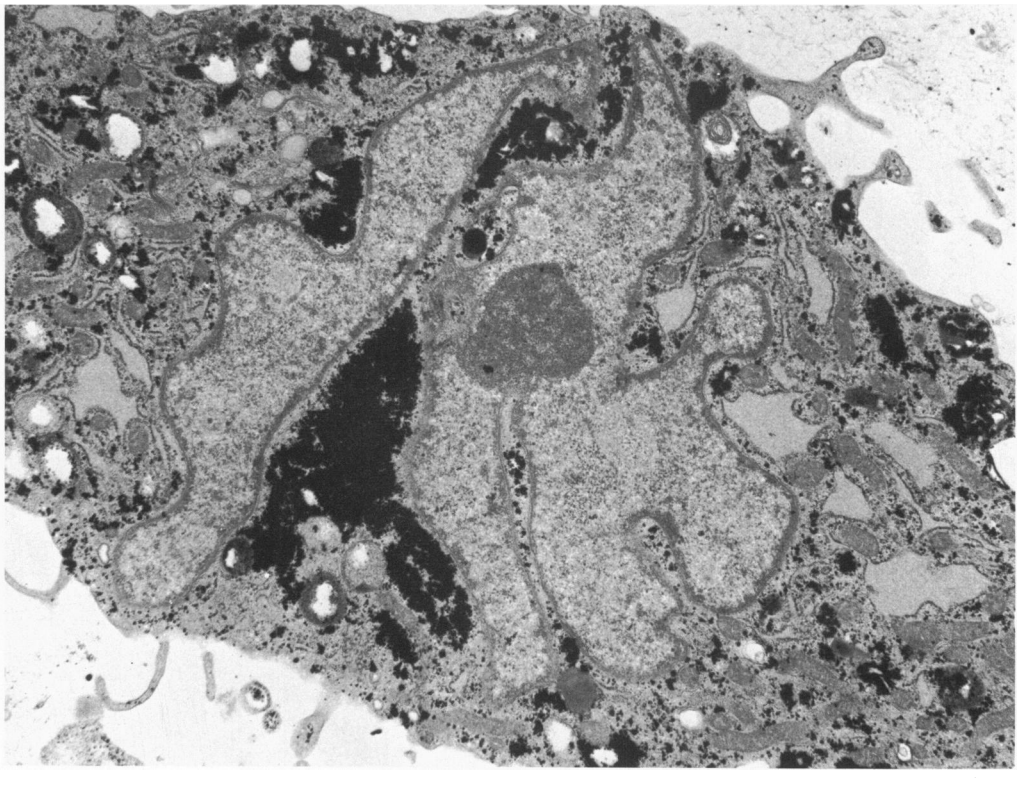
9



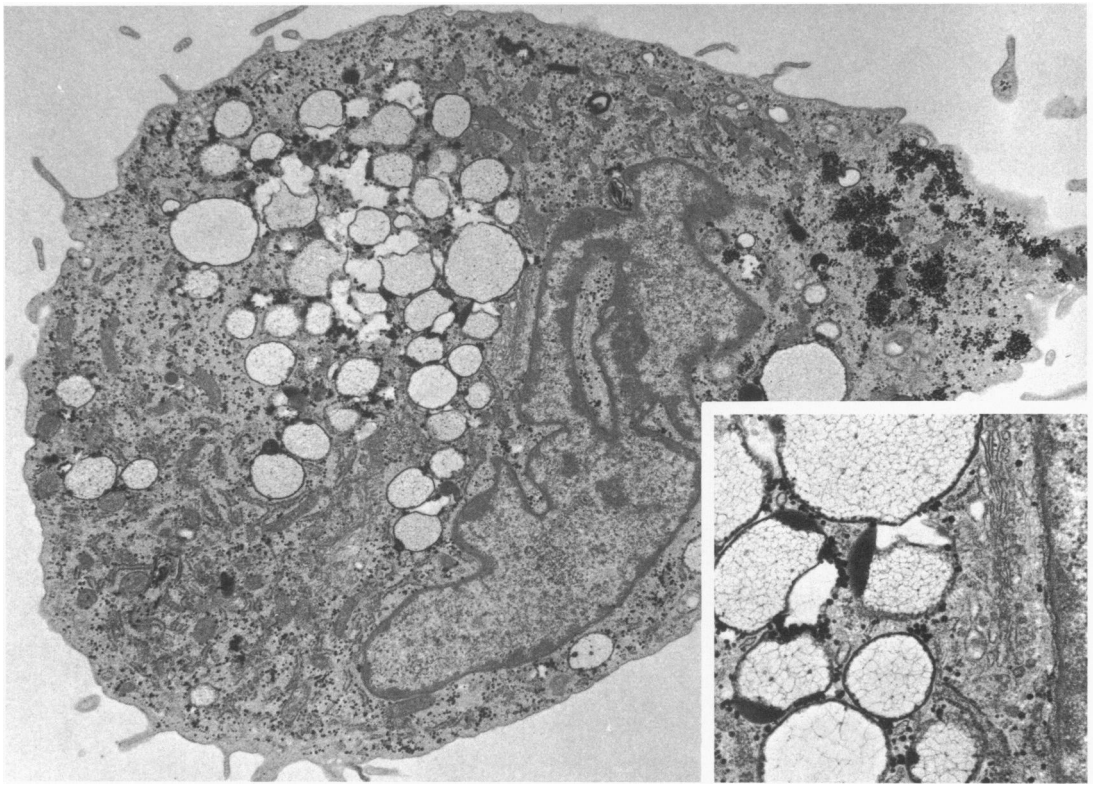
10



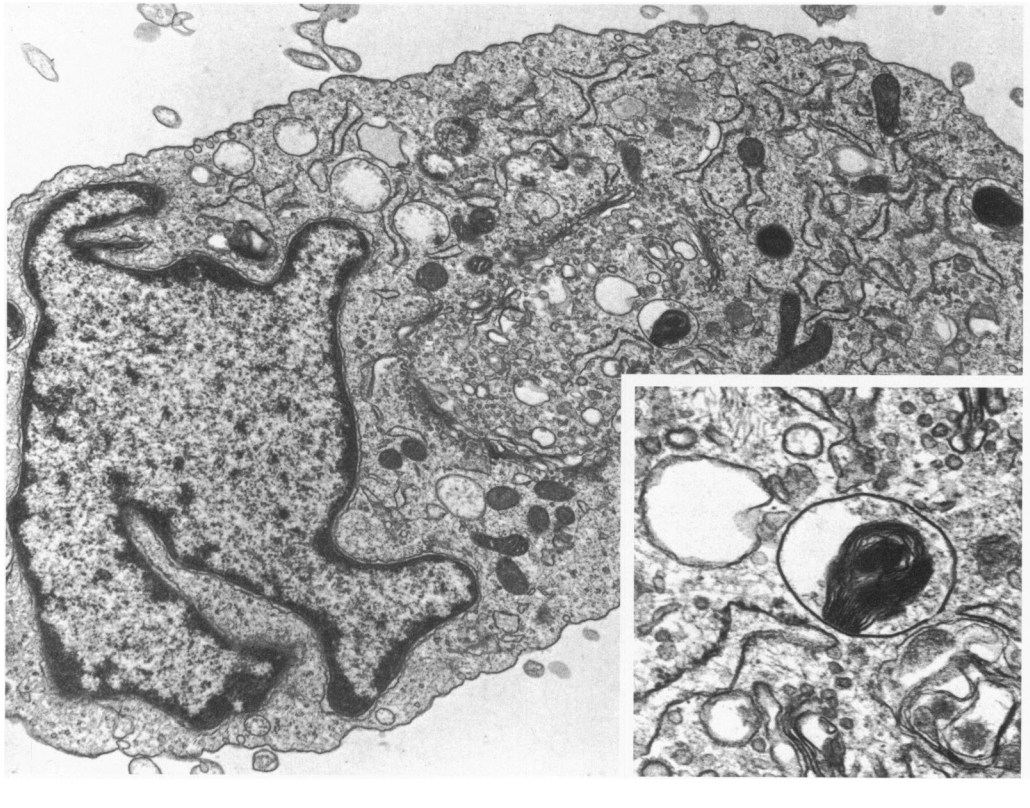
11



12

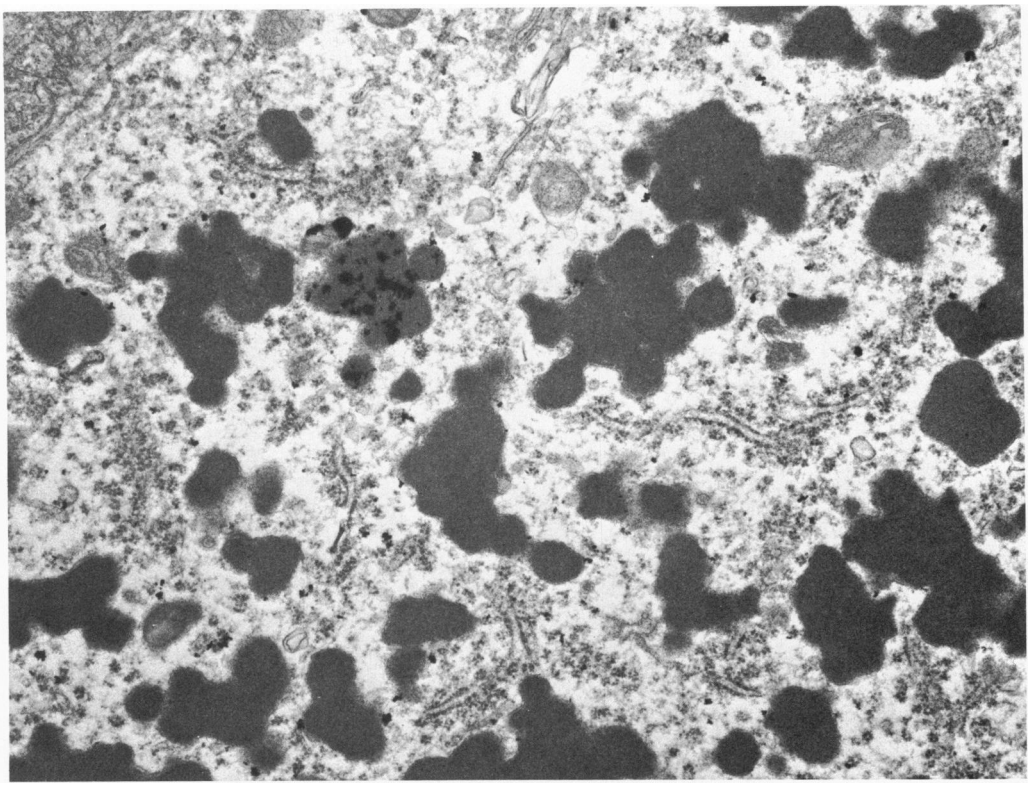


13



14

15



16