Pathology of Pulmonary Disease, edited by Mario J. Saldana.
J. B. Lippincott Company, Philadelphia © 1994.

12

Pulmonary Involvement in Some Inborn Errors of Metabolism

Masazumi Adachi

The designation of inborn errors of metabolism applies to a group of inherited diseases characterized by specific enzymatic defects resulting in the accumulation of abnormal substances within cells of many visceral organs, including the lungs. They were discovered in the late nineteenth and early twentieth centuries by investigators such as Fabry, Gaucher, Niemann, Hunter, and Hurler, but it is only during the last three decades that their biochemical, genetic, and molecular bases have begun to unravel. The inborn errors of metabolism can lead to obliterative and destructive lesions of the airways, cystic changes of the lung parenchyma, interstitial or alveolar infiltrates, and vascular occlusion leading to pulmonary hypertension (see Display 12-1).

FABRY DISEASE

Fabry disease (*i.e.*, glycosphingolipid lipidosis) is transmitted by genes on the X chromosome that encode for α-D-galactosidase A (*i.e.*, Xq21.33q22).[1] Partial deletions, duplications, splice-junction defects, and point mutations have been identified. The clinical features are the result of the progressive accumulation of globotriaosylceramide in many visceral organs.[2]

Clinical Features

The clinical manifestations appear in childhood or adolescence and include severe pain and paresthesias in the extremities, angiokeratoma of the skin and mucosa, and hypohidrosis. The pain is characterized as a lightning or burning sensation in the fingers and toes extending to the palms and soles. Attacks of abdominal or flank pain may simulate appendicitis or renal colic. The telangiectases are symmetric, involve the superficial layers of the skin, do not blanch on pressure, and are progressive. The oral mucosa, conjunctivae, hips, back, thighs, buttocks, penis, and scrotum are most commonly involved. Pulmonary involvement ranges from obstructive disease of the airways to diffuse interstitial infiltrates. Roentgenologic findings include hyperinflation with bullous disease or bullous disease alone, with scintigraphic evidence of multiple ventilation and perfusion defects (Fig. 12-1).[3] Pulmonary function tests in older patients may reveal significant airflow obstruction, reduced diffusing capacity, and a reduction in the 25% Vmax values.[3] Pulmonary complications are a frequent cause of death. The clinical manifestations are also observed in isolated cases of heterozygous women who have an intermediate level of enzyme activity.

Pathology

The lungs of patients with Fabry disease are heavier than normal lungs. On cut sections, they are often congested and edematous. Microscopically, multiple inclusions are seen in the cytoplasm of alveolar epithelial cells (Fig. 12-2), smooth muscle of the bronchi, endothelial cells of capillaries and arterioles, and the smooth muscles of the arterioles. Affected cells exhibit distinct periodic acid-Schiff positivity (Color Fig. 12-1) and strong birefringency (Color Fig. 12-2). Electron microscopic studies show laminated inclusions with a periodicity of 50 to 60 nm in the endothelial and alveolar type II cells (Fig. 12-3). The periodicity of the inclusions differs from the variable periodicity of the lamellar bodies in the type II cells of the normal alveolus.

DISPLAY 12-1. INBORN ERRORS OF METABOLISM INVOLVING THE LUNGS

Fabry disease
Gaucher disease
Niemann-Pick disease
Mucopolysaccharidosis
GM_1 gangliosidosis
Sulfate lipidosis
Glycogen storage disease
Disorders of amino acid metabolism
Cystine storage disease

Biochemistry and Diagnosis

Fabry disease is caused by the defective activity of the lysosomal hydrolase α-D-galactosidase A.[4] The boys afflicted with this disorder can be identified by the demonstration of an increase in globotriaosylceramide (*i.e.*, trihexosyl ceramide) and by the assay of the hydrolase activity in the serum, leukocytes, tears, and cultured skin fibroblasts.

GAUCHER DISEASE

Gaucher disease (*i.e.*, glucosylceramide lipidosis) is characterized by the accumulation of glucosyl ceramide in various organs.[5] The primary enzyme defect is a deficiency of β-glucosidase.[6,7] The disease is transmitted as an autosomal recessive trait, and the Gaucher gene is located at the 1q21 locus of chromosome 1.[8]

Clinical Features

The disorder has been divided into three clinical types. Type 1, the adult form, is most common and usually occurs in Ashkenazi Jews. It is a chronic disorder that may start comparatively soon after birth and last into adulthood. It differs from the other types in its lack of neurologic manifestations. Type 2 is the acute form and occurs in infants with progressive neurologic deterioration. It occurs less frequently in Jewish families than type 1. Type 3 is the subacute variety, and it occurs in juveniles. It presents a more protracted course of neurologic involvement than type 2.

In type 1, pulmonary hypertension and severe pulmonary arteriosclerosis are occasionally observed. Some patients with type 1 die early in life of thrombocytopenia, severe anemia, and pulmonary infection. Hepatosplenomegaly and Gaucher cells in the bone marrow are regular features. The concentration of acid phosphatase in the serum is markedly increased. Repeated episodes of bone pain are common, as are fractures after minor trauma, sometimes leading to permanent deformity. Osteolytic changes are frequently observed by radiologic studies.

In type 2, the infants develop normally until 3 to 6 months of age. Thereafter, splenomegaly and lymphadenopathy become prominent, and Gaucher cells are found in the bone marrow. High levels of acid phosphatase in the serum sometimes occur as early as 3 months of age. Progressive psychomotor deterioration ensues, and death occurs within 2 years.

Type 3 patients have a more protracted course of neurologic

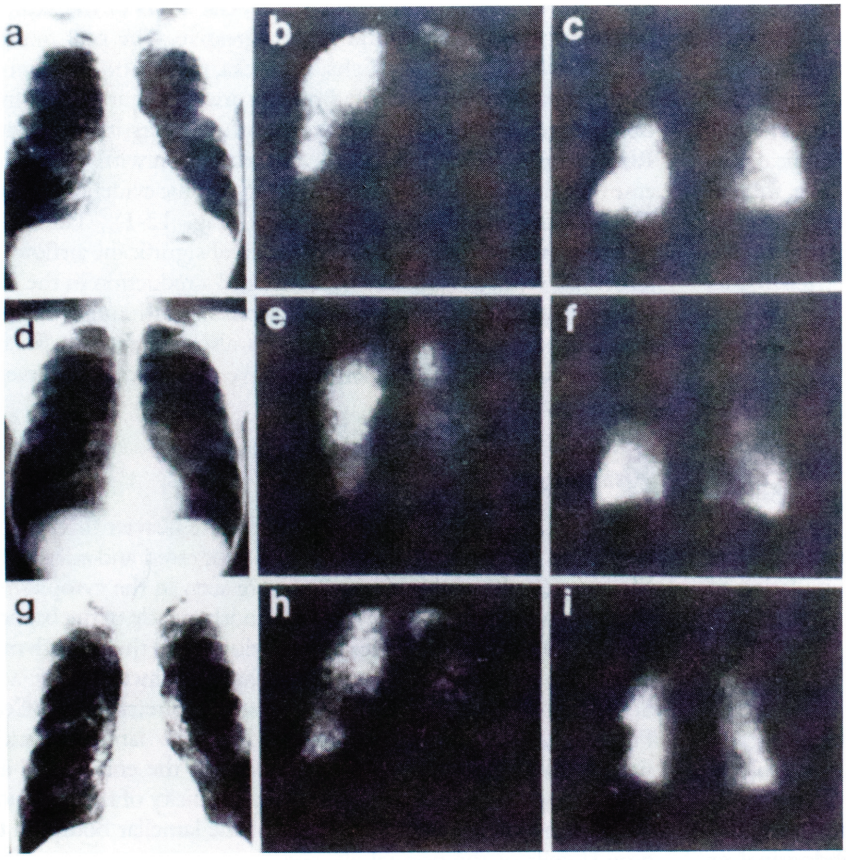

FIGURE 12-1. Chest roentgenograms and ventilation and perfusion scans of three patients with Fabry disease. (**A**) In the first patient, the chest roentgenogram demonstrated hyperinflation and bullae. (**B**) The ventilation image after 3 minutes of washout revealed marked retention in the upper right lung field, corresponding to the bullae seen on the chest roentgenogram. (**C**) The perfusion scan showed bilateral defects, particularly in regions of ventilation abnormalities. (**D**) In the second patient, the chest roentgenogram demonstrated hyperinflation. (**E**) The ventilation image after 3 minutes of washout demonstrated an inhomogeneity of tracer clearance, predominantly on the right side. (**F**) The perfusion scan showed defects in the region of ventilatory abnormalities. (**G**) In the third patient, the chest roentgenogram demonstrated hyperinflation with bullae formation. (**H**) The ventilation image after 3 minutes of washout showed marked retention in the right lung field, corresponding to bullae. (**I**) The perfusion scan demonstrated bilateral defects, particularly in regions of altered ventilation. (From Rosenberg DM, Ferrans VJ, Fulmer JD, et al. Chronic airflow obstruction in Fabry's disease. Am J Med 1980;68:898.)

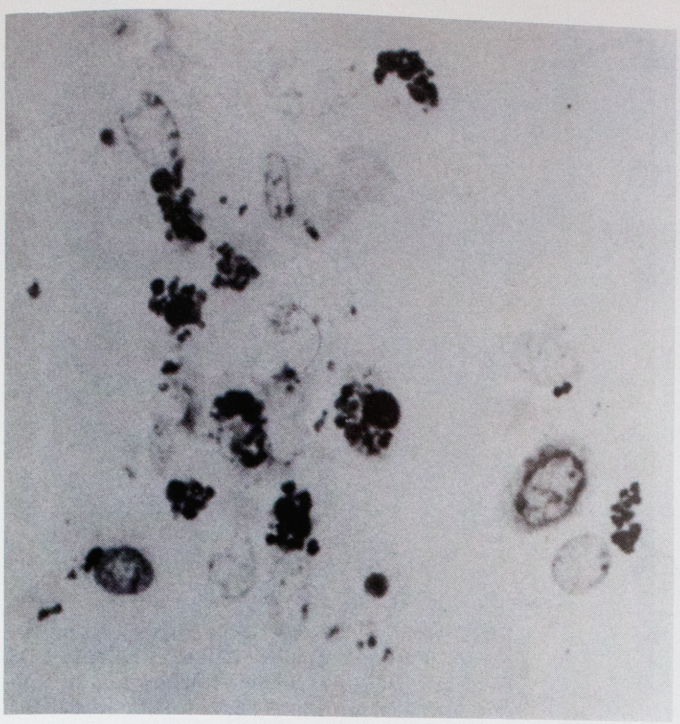

FIGURE 12-2. Light micrograph of a 0.5-μm-thick plastic section of cells brushed from the airway epithelium of a patient with Fabry disease. Darkly stained inclusion bodies, characteristically found in this disease, are in the cytoplasm of many of the epithelial cells. (Alkaline toluidine blue stain; original magnification ×800; from Rosenberg DM, Ferrans VJ, Fulmer JD, et al. Chronic airflow obstruction in Fabry's disease. Am J Med 1980;68:898.)

changes. The patients display splenomegaly and a slowly progressive hepatomegaly. Pulmonary infiltration is often revealed on radiologic examination of these children. However, the typical reticular pattern is rarely observed. Osteolytic lesions are frequent. About one half of the cases have been reported from four interrelated families from the Province of Norrbotten in northern Sweden. The mode of inheritance is also consistent with an autosomal recessive trait.

Pathology

The lungs have Gaucher cells in alveolar capillaries (Fig. 12-4), perivascular spaces, or in alveoli, interfering with gas exchange.[9] Globoid lesions in pulmonary arterioles and numerous marrow emboli with Gaucher cells are also seen. The spleen, liver, and lymph nodes are markedly enlarged. Gaucher cells are prominent in the red pulp of the spleen, the sinusoids and medullary portions of the lymph nodes, sinusoids of the liver, and bone marrow.

Gaucher cells are the histologic hallmark of this disease. They are round or polygonal (Fig. 12-5) and 20 to 80 μm in diameter. The cytoplasm has many fibrils of various sizes and an appearance of striation. The cells are derived from the reticuloendothelial system.

Ultrastructurally, the Gaucher cells contain cytoplasmic inclusion bodies (Fig. 12-6), which are pleomorphic structures surrounded by a single limiting membrane. The inclusions, called Gaucher bodies, contain tubular structures 120 to 250 nm in diameter, each of which contains 10 to 12 fibrils in a characteristic arrangement. The inclusion bodies are derived from the cisternae

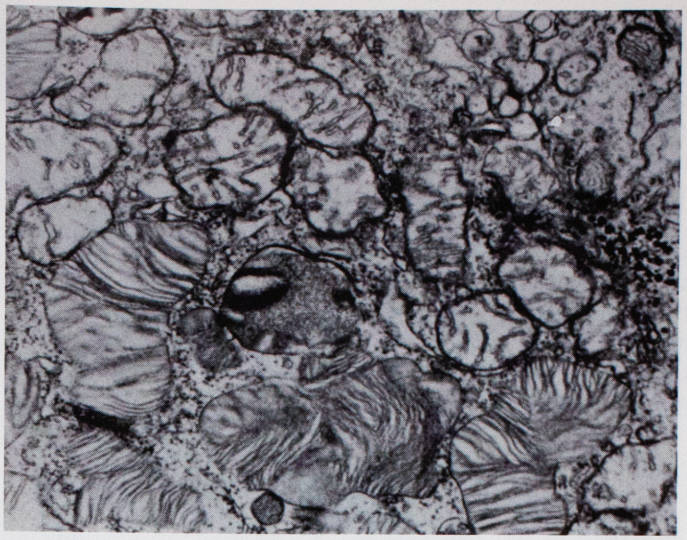

FIGURE 12-3. Laminated intracellular inclusions, also called "zebra" bodies, as shown by electron microscopy, are characteristic of Fabry disease. (Uranyl acetate stain; courtesy of Joyce Bruce, M.D., Miami, FL.)

of the endoplasmic reticulum. Acid phosphatase preparations show reaction granules within the Gaucher bodies indicating their lysosomal character.

Biochemistry

Patients with these three types of Gaucher disease show a markedly increased glucose-1-ceramide, occasionally exceeding 100 times the normal concentration. The enzyme defect in Gaucher disease is a deficiency of β-glucosidase which catalyzes the cleavage of glucose from glycosyl ceramide. Bone marrow transplantation in severe Gaucher disease has been successful in restoring β-glucosidase in mononuclear leukocytes and plasma with complete engraftment of the normal donor cells. Enzyme replacement treatment for lysosomal hydrolase deficiencies, such as Gaucher disease, remains experimental.

Diagnosis

The diagnosis of this disorder is confirmed by identification of Gaucher cells in bone marrow smears and assays of β-glucosidase in leukocytes or cultured fibroblasts. One gram of fresh-frozen tissue from liver or spleen can be examined for the determination of glycosyl ceramide and activity of β-glucosidase. Biopsy tissues from these organs should be studied histologically and electron microscopically.

NIEMANN-PICK DISEASE

The Niemann-Pick (*i.e.*, sphingomyelin-cholesterol lipidosis) group of inherited metabolic disorders is characterized by excessive accumulation of sphingomyelin-cholesterol in the cells of visceral organs.

Clinical Features

The group can be divided into two broad types according to the cause, and each type is subdivided into three clinical forms.[10] Type

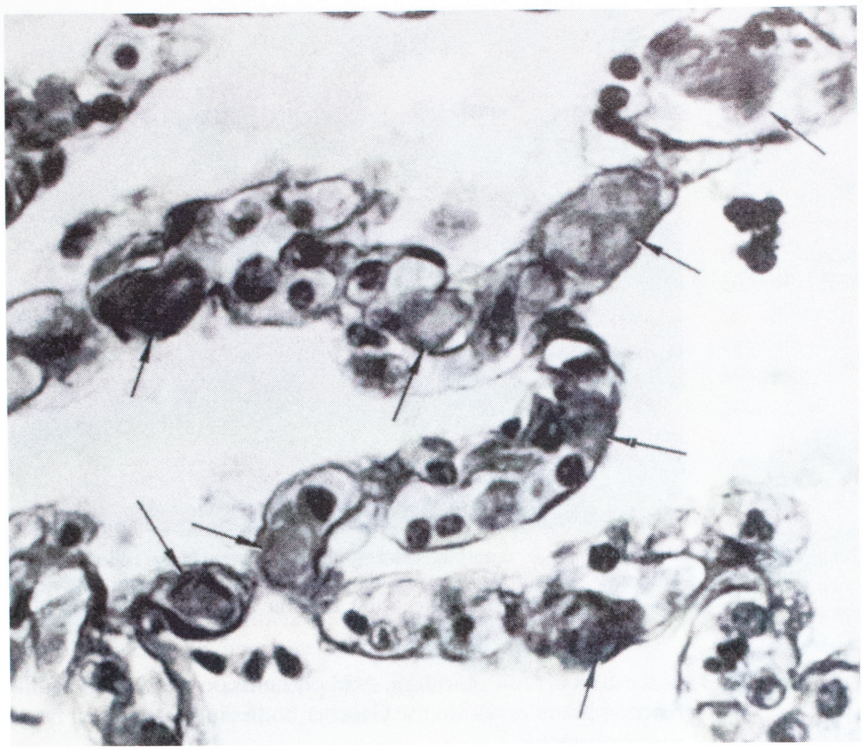

FIGURE 12-4. Gaucher cells (*arrows*) occlude the pulmonary alveolar capillaries. (From Frederickson DS, Sloan HR. Glucosyl ceramide lipidosis: Gaucher's disease. In: Stanbury JB, Wyngaarden JB, Frederickson DS, eds. The metabolic basis of inherited disease. 4th ed. New York: McGraw-Hill, 1978:731.)

I is the disorder characterized by sphingomyelinase deficiency (*i.e.*, primary sphingomyelin storage disease).

Type IA, originally described as type A, is the acute form of type I and characterized by massive accumulation of sphingomyelin in the viscera, including the lungs and nervous system, in early infancy. Type IA occurs in 75% of the lipidoses.[11] Type IS is the subacute form of type I, originally described as types B and F, with or without involvement of the central nervous system. Type IC is the chronic form, which discloses the first signs and symptoms in adults; it was originally described as type E, and it is characterized by low levels of sphingomyelinase in all tissues.

Type II includes the cases with uncertain primary enzyme defects and secondary sphingomyelin storage in the tissues. Type IIA is the acute form of type II, characterized by hepatosplenomegaly and psychomotor symptoms and appearing between the early stages of infancy and 2 years of age. Children die by the age of 8 years, usually of respiratory infection. Type IIS is the subacute form of type II, originally described as types C and D. The onset of the disease varies from infancy to 18 years of age. Children have progressive psychomotor deterioration, with storage of foam cells and often with sea blue histiocytes and hepatosplenomegaly. Some patients have vertical supranuclear ophthalmoplegia, seizures, and

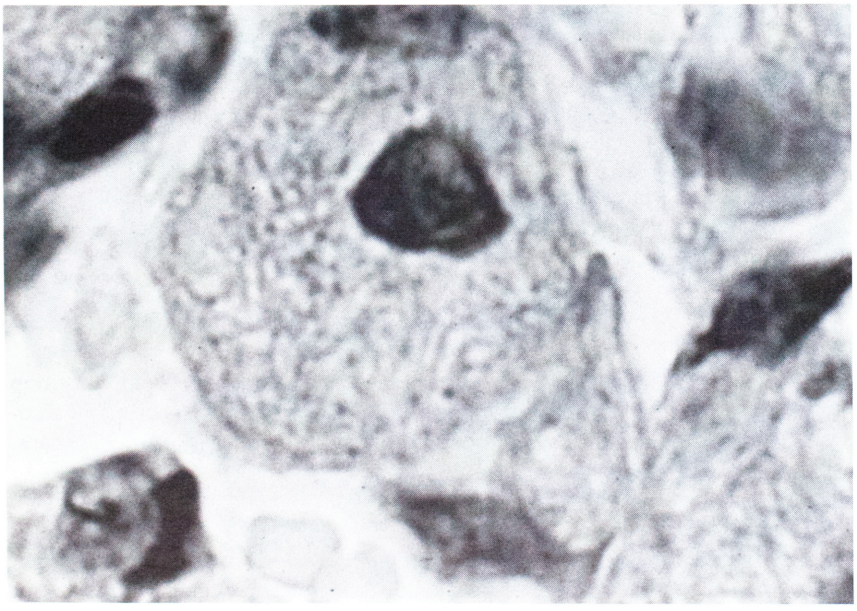

FIGURE 12-5. A Gaucher cell from an infant with Gaucher disease contains numerous fibrils that give the cytoplasm a striated appearance. (H & E stain; original magnification ×2180.)

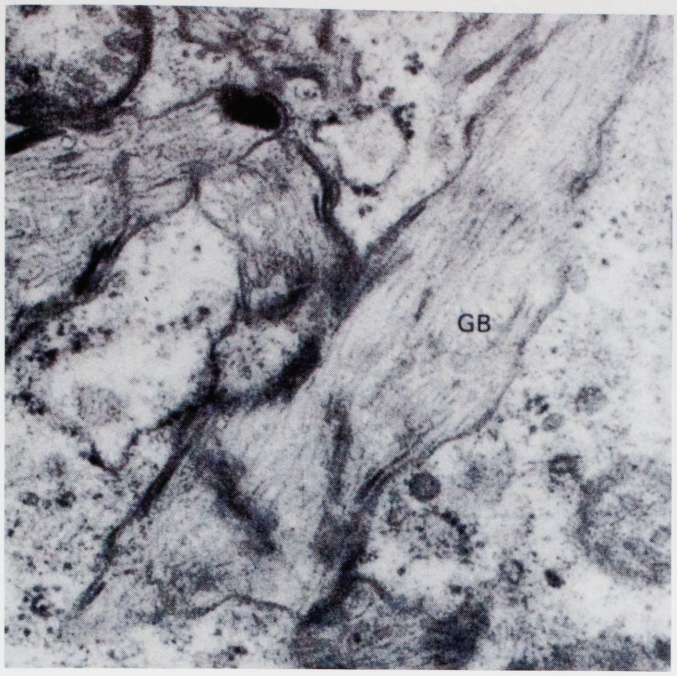

FIGURE 12-6. The ultrastructure of a portion of a Gaucher cell exhibits a pleomorphic Gaucher body (GB) with tubular structures. (Uranyl acetate stain; original magnification × 43,000; from Fishman AP, ed. Pulmonary diseases and disorders. 2nd ed. vol. 1. New York; McGraw-Hill, 1988:867.)

jaundice. The patients originally described as type D were from southwestern Nova Scotia, and jaundice affected about 50% of the patients during infancy.[12] These patients develop hepatosplenomegaly by 6 years of age and progress to seizures and profound psychomotor deterioration. They die in their teens of a combination of pneumonia and cachexia. Type IIC, originally described as type E, is the chronic form of a neurovisceral storage disease with its onset in adulthood. The visceral organs and brain contain twofold to fourfold elevations of sphingomyelin and cholesterol, respectively, and have normal or elevated sphingomyelinase activities.

Pathology

Foamy cells in pulmonary septa and alveoli (Fig. 12-7) are relatively consistent features. They are as large as 90 μm in diameter and contain one or two nuclei and numerous fine cytoplasmic vacuoles. Because the vacuoles seen in routine sections are partly soluble during histologic preparations, frozen sections are required for histochemical analysis (Fig. 12-8). They are positive with modified acid hematein, phosphomolybdic acid plus stannous chloride, and mercury diphenylcarbazone methods for sphingomyelin.[13] Ultrastructurally, the foamy cells are filled with round to oval cytoplasmic bodies (Fig. 12-9) that are 0.05 to 5 μm in diameter and contain loosely arranged membranous structures. The bodies' lysosomal reactions indicate their true nature.

Biochemistry

Type I patients have a marked accumulation of sphingomyelin in the viscera, but type II patients have only slight or moderate accumulation of sphingomyelin. The cholesterol levels in the viscera with type I disease are elevated, are not as massive as for sphingomyelin, and may be secondary to the primary storage of sphingomyelin. In type II, the viscera have moderate elevations of cholesterol. The metabolic defect of type I is a deficiency of lysosomal sphingomyelinase, which degrades sphingomyelin to ceramide and phosphocholine. In type II Niemann-Pick disease, lysosomal sphingomyelinase activity is often normal in tissues and may be normal or reduced in cultured fibroblasts. A decrease in cholesterol esterification in cultured fibroblasts has been demonstrated in some patients.[14]

Diagnosis

The diagnosis of type I is confirmed by the analysis of sphingomyelinase activity in leukocytes, fibroblasts, or tissue biopsy specimens. Type II patients may have partially decreased sphingomyelinase activity or deficiencies in cholesterol esterification.[14]

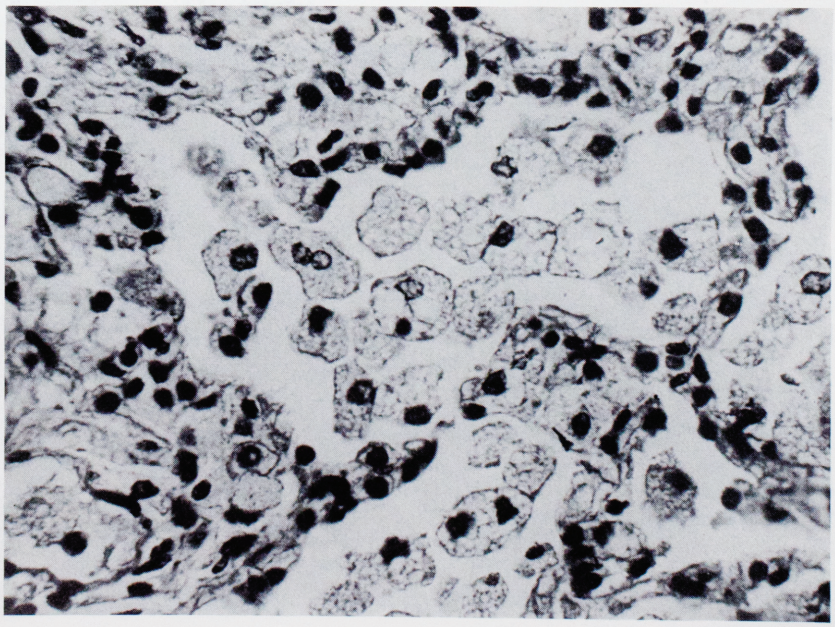

FIGURE 12-7. The histologic section of the lung of a child with Niemann-Pick type IA disease shows foamy cells in the alveoli. (H & E stain; original magnification ×640; from Fishman AP, ed. Pulmonary diseases and disorders. 2nd ed. vol. 1. New York; McGraw-Hill, 1988:867.)

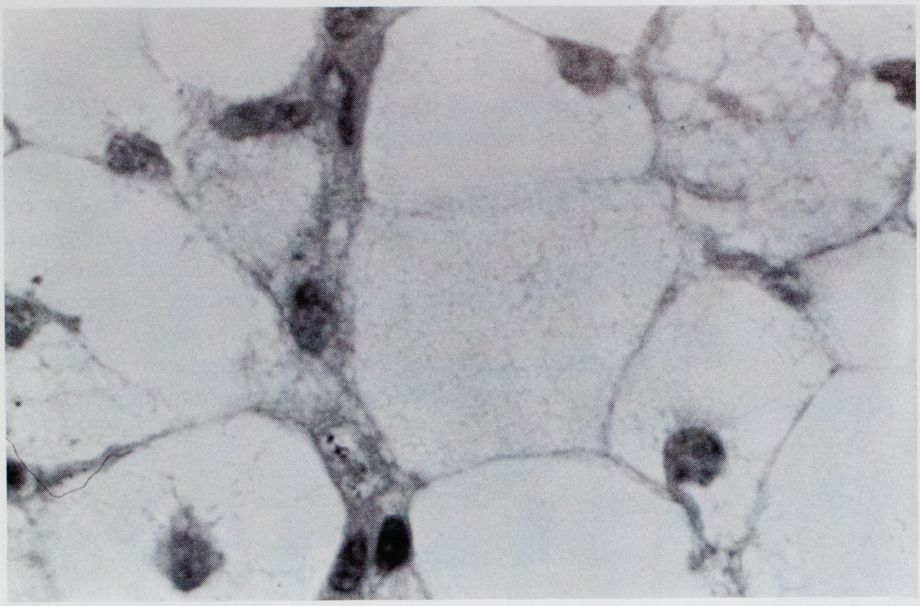

FIGURE 12-8. Foamy cells in a patient with Niemann-Pick disease. Most of the intracytoplasmic lipids have been washed away during the usual histologic processing. (H & E stain; original magnification ×1000; contributed by the editor.)

MUCOPOLYSACCHARIDOSES

The mucopolysaccharidoses (MPS) are a group of genetic diseases manifested by abnormal tissue deposits of acid mucopolysaccharides (*i.e.*, glycosaminoglycans) that are the result of a deficiency of lysosomal enzymes. Eight major forms or variants of the disease have been described. However, MPS V is now classified as MPS IS (*i.e.*, Scheie syndrome), because the deficiency of α-L-iduronidase is identical to that of other subtypes of MPS I.[15] MPS VIII was reclassified to the type of sulfatide lipidoses (*i.e.*, metachromatic leukodystrophy) that is caused by multiple sulfatase deficiency (MSD). MPS II is transmitted as an X-linked recessive pattern. All other forms are autosomal recessive.

Clinical Features

The most severely affected patients, except for those with MPS IS, commonly have respiratory involvement, particularly obstruction of the airways. Most children with MPS have recurring respiratory tract infections. Obstructive pulmonary disease and pneumonia are the usual causes of death.

Pathology

The airways are obstructed by the narrowed trachea, thickened vocal cords, and enlarged tongue. MPS I, II, and III show histologic alterations in the lungs caused by abnormal deposits of cells called clear cells, gargoyle cells, or balloon cells. They are large, oval, or polygonal, 20 μm in diameter, and contain a pale central nuclei. Histochemical studies demonstrate a metachromatic reaction with toluidine blue and positive granules with Alcian blue. The histologic changes in other forms are less well documented. Ultrastructural examination reveals round or oval inclusions.

Biochemistry

The clinical manifestations, type of enzyme deficiency, and glycosaminoglycan affected have been summarized by Neufeld and

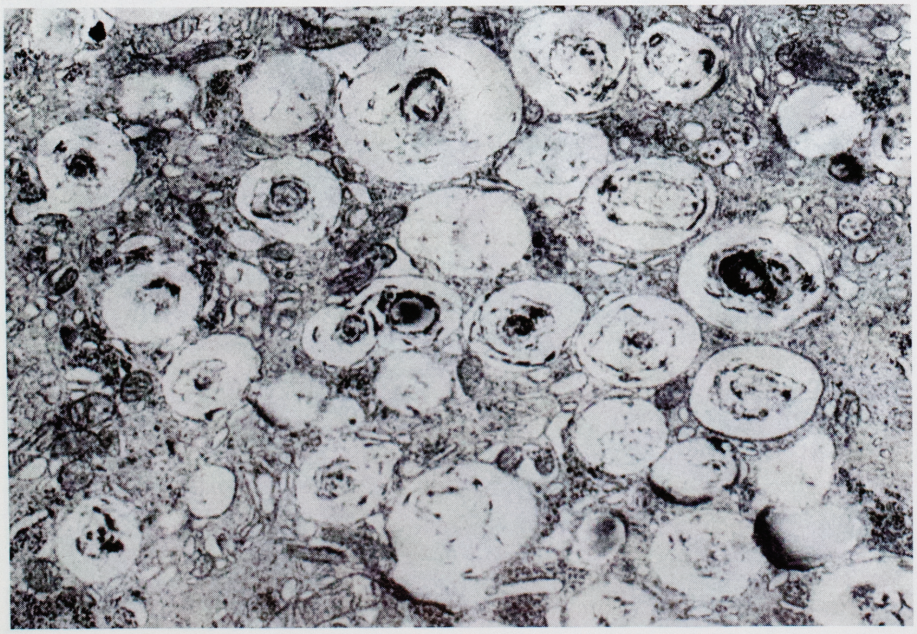

FIGURE 12-9. Ultrastructure of a portion of a foamy cell from a child with Niemann-Pick type IA disease shows cytoplasmic inclusion bodies that are membrane bound and contain loosely arranged membranous structures. (Original magnification × 7200; from Fishman AP, ed. Pulmonary diseases and disorders. 2nd ed. vol. 1. New York; McGraw-Hill, 1988:867.)

Muenzer.[15] The increased urinary excretion of dermatan sulfate and heparan sulfate are observed in MPS I and II, and MPS VII produces chondroitin 4-sulfate and chondroitin 6-sulfate in addition to the other two sulfates in the urine.

Diagnosis

Simple enzyme assays are available for the diagnosis of MPS, using skin fibroblasts, leukocytes, or serum. The diagnosis can be established only by measuring urine mucopolysaccharides with precise identification of the excreted substances.

GM_1 GANGLIOSIDOSIS

Three subtypes of GM_1 gangliosidosis are recognized: infantile, juvenile, and adult. All are transmitted as autosomal recessive traits.

Clinical Features

The infantile form is generalized and accompanied by bone involvement and psychomotor retardation manifested shortly after birth. During the early stage, the lungs are unremarkable. After 6 months, radiologic abnormalities are similar to those seen in cases of Hurler disease. Foamy cells are demonstrated in smears of the bone marrow. The clinical manifestations of the juvenile type is later onset of milder bone abnormalities and progressive motor and mental deterioration. The average life span varies from 3 to 10 years. The adult form has a juvenile onset of progressive cerebellar dysarthria and slow but progressive motor and intellectual impairment. Patients survive to adulthood.

Pathology

Grossly, the lungs are normal in appearance. The liver, spleen, and kidneys are increased in size and weight. Microscopically, the lungs have foamy cells in the alveoli and septa. These foamy histiocytes are also observed in many visceral organs. Histochemically, the cytoplasmic material consists of complexed proteolipid compounds. Ultrastructurally, the foamy cells in the lungs and the other organs have membrane-bound bodies that contain a slightly electron-dense material mixed with fine granules (Color Fig. 12-3).

Biochemistry and Diagnosis

Deficiency in β-galactosidase causes GM_1 ganglioside accumulation in various visceral organs and the nervous system. The storage of GM_1 gangliosides in the viscera and nervous system is prominent in the infantile form but less so in the juvenile and adult forms. The disorder can be confirmed by analysis of β-galactosidase activity in leukocytes, urine, and skin. In heterozygotes, intermediate levels of the enzyme can be demonstrated.

SULFATIDE LIPIDOSIS

Sulfatide lipidosis (*i.e.*, metachromatic leukodystrophy) is an autosomal recessive disorder that can appear in seven forms: congenital, late infantile, early juvenile, late juvenile, adult, a rare type of MSD, and cerebroside sulfate activator deficiency.[16] It is characterized by accumulation of galactosyl sulfatide (*i.e.*, cerebroside sulfate) in the nervous system and, to a lesser degree, by lactosyl sulfatide in the visceral organs, including the lungs.

Clinical Features

The late infantile form is the most common, and the onset of symptoms is between the ages of 12 and 18 months. Flaccid weakness and hypotonia of the extremities is associated with progressive psychomotor deterioration. The final stage lasts a few months to several years. The early and late juvenile forms present between the ages of 4 and 12 years, and a rare adult form may begin at any time between the midteens and the seventh decade. The earliest signs of these forms are a gait disturbance. The clinical manifestations reflect striking alterations in the nervous system, from flaccid weakness of the extremities to progressive psychomotor deterioration. The MSD form has symptoms similar to those of the late infantile form and MPS with pulmonary obstructive disease.

Pathology

There is no gross abnormal change of the visceral organs. Microscopically, metachromatic inclusion bodies are observed in histiocytes of the interalveolar septa but not in the alveolar spaces or in the pulmonary vessels. Ultrastructural studies show that the cytoplasmic inclusions consist of lamellar structures and irregular whorls.

Biochemistry

The late infantile, juvenile, and adult forms have markedly increased concentrations of cerebroside sulfatide in the brain and viscera because of the deficiency of arylsulfatase A and, to a lesser degree, of arylsulfatase B. Patients with the MSD form accumulate cerebroside sulfatides, mucopolysaccharides, and steroids because of deficiencies of arylsulfatases A, B, and C, steroid sulfatase, and mucopolysaccharide sulfatases.[17]

Diagnosis

The most important diagnostic procedure is the determination of arylsulfatase activity in leukocytes or cultured skin fibroblasts. Analysis for sulfatases in the urine is rapid and simpler but less reliable.

GLYCOGEN STORAGE DISEASE

Among the major groups of glycogen storage disorders, Pompe disease (*i.e.*, type II glycogenosis) most frequently is associated with cardiopulmonary disturbances.[18]

Clinical Features

In most patients with Pompe disease, the onset of symptoms is before the age of 2 years. The clinical manifestations are poor motor activity, respiratory difficulties, and cardiac failure. Chest x-ray films may show complete opacity secondary to atelectasis. The disease is transmitted by an autosomal recessive trait.

Pathology

A massive accumulation of glycogen granules is observed in the cytoplasm of the parenchymal cells of most organs (see Color Fig. 12-3). The lungs have alveolar foamy cells filled with glycogen deposits. The deposits occur in smaller amounts in cartilage cells, mucus, and bronchial epithelial cells. Ultrastructural studies show membrane-bound lysosomal bodies filled with glycogen granules in the affected cells.

Biochemistry and Diagnosis

Tissue accumulation of glycogen in Pompe disease is the result of a deficiency of α-glucosidase.[19] The diagnosis can be established by the demonstration of increased glycogen concentration in tissues or deficiency of α-glucosidase.

DISORDERS OF AMINO ACID METABOLISM

Maple syrup urine disease (*i.e.*, leucinosis or branched chain ketonuria) is the only disorder to produce occasional pulmonary disturbances among the various types of amino acid metabolic disorders.

Clinical Features

The affected infants develop respiratory distress within the first week of life. They often become apneic and require respiratory assistance. Severe psychomotor deterioration and episodes of seizures ensue during the course of the disease. These children usually die of intercurrent infections within the first year. With the help of a synthetic diet, some patients survive until 13 years of age. Intermediate types of clinical features are also observed between 2 months to 40 years of age that are associated with intermediate levels of chemical accumulation and enzyme activity.[20] The classic type is transmitted by an autosomal recessive pattern.

Pathology

The correlation between clinical and pathologic features of apnea, stupor, and frank coma are complicated with secondary effects of hypoxia, acidosis, and hypoglycemia. Despite severe clinical symptoms in early life, autopsy shows gross changes only in the brain, which exhibits microcephaly and microgyria. Histologic studies show a deficiency of myelin sheaths, presumably a result of the reduced synthesis of proteolipids.

Biochemistry and Diagnosis

Three amino acids (*i.e.*, leucine, isoleucine, and valine) accumulate because of the deficiency of branched chain α-keto acid dehydrogenase.[21] The maple syrup odor of the urine can be detected within the first week of life. The diagnosis should be verified by analysis of the amino acids and keto acids in blood and urine.

CYSTINE STORAGE DISEASE

Cystine storage disease (*i.e.*, Lignac-Fanconi disease, cystinosis) produces widespread pathologic changes and is transmitted in an autosomal recessive mode.

Clinical Features

Clinical manifestations usually appear between 6 and 12 months of age and consist of dehydration, acidosis, vomiting, electrolyte imbalance, marked photophobia, hypophosphatemic rickets, and dwarfism. Because of progressive renal failure, patients require dialysis or transplantation at 6 to 12 years of age.

Pathology

Cystine crystals accumulate in the lungs, kidneys, bones, lymph nodes, spleen, and liver. There is no cellular reaction, and pulmonary function tests are not altered. The deposits may be mistaken for calcium, but they are negative by von Kossa stain. The cystine is water soluble and is best fixed in absolute alcohol. In tissues, the deposits are birefringent and form clumps of radiating needlelike crystals when treated with concentrated sulfuric acid and phosphotungstic acid. The crystals in the lungs are mainly within the peribronchial and periarterial connective tissue cells and in the alveolar septa.

Biochemistry and Diagnosis

The disorder is caused by defective lysosomal transport of the amino acid cystine.[22] The most direct method of diagnosis is measurement of the leukocyte cystine content.[23] The typical crystalline keratopathy can be demonstrated by ophthalmologic examination. Cystine can also be demonstrated in hair by infrared spectroscopy and by the characteristic birefringent crystals in a conjunctival biopsy sample, rectal mucosal biopsy sample, or bone marrow aspiration sample.

REFERENCES

1. Yang HM, Niebuhr E, Norby S, Lund T, Schwartz M. Subregional localization of anonymous Xg DNA probe by deletion mapping. Cytogenet Cell Genet 1988;46:722.
2. Clarke JTR, Knaack J, Crawhall JC, Wolfe LS. Ceramide trihexosidosis (Fabry's disease) without skin lesions. N Engl J Med 1971; 284:233.
3. Rosenberg DM, Ferrans VJ, Fulmer JD, et al. Chronic airflow obstruction in Fabry's disease. Am J Med 1980;68:898.
4. Desnick RJ, Sweeley CC. Fabry's disease: defective α-galactosidase a. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. The metabolic basis of inherited disease. 5th ed. New York: McGraw-Hill, 1983:906.
5. Suomi WD, Agranoff BW. Lipids of the spleen Gaucher's disease. J Lipid Res 1965;6:211.
6. Patrick AD. A deficiency of glucocerebrosidase in Gaucher's disease. Biochem J 1965;97:17C.
7. Brady RO, Kanfer JN, Shapiro D. Metabolism of glucocerebrosides. II. Evidence of enzymatic deficiency in Gaucher's disease. Biochem Biophys Res Commun 1965;18:221.
8. Ginns EI, Choudary PV, Tsuji S, et al. Gene mapping and leader polypeptide sequence of human glucocerebrosidase: implication for Gaucher's disease. Proc Natl Acad Sci USA 1985;82:7101.
9. Fredrickson DS, Sloan HR. Glucosyl ceramide lipidosis: Gaucher's disease. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. The metabolic basis of inherited disease. 4th ed. New York: McGraw-Hill, 1978:731.

10. Spence MW, Callahan JW. Sphingomyelin-cholesterol lipidoses: the Niemann-Pick group of diseases. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic basis of inherited disease. 6th ed. New York: McGraw-Hill, 1989:1655.
11. Fredrickson DS, Sloan JB. Sphingomyelin lipidoses: Niemann-Pick disease. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. The metabolic basis of inherited disease. 3rd ed. New York: McGraw-Hill, 1972:783.
12. Tibbles JAR, Welch JP. Clinical and genetic data on twelve new cases of type D Niemann-Pick disease. Pediatr Res 1972;6:643.
13. Adachi M, Volk BW. Methodology: histochemistry. In: Volk BW, Schneck L, eds. The gangliosidoses. New York: Plenum, 1975: 249.
14. Pentchev PG, Comly ME, Kruth HS, et al. A defect in cholesterol esterification in Niemann-Pick disease (type C) patients. Proc Natl Acad Sci USA 1985;82:8247.
15. Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic basis of inherited disease. 6th ed. New York: McGraw-Hill, 1989:1565.
16. Kolodny EH. Metachromatic leukodystrophy and multiple sulfate deficiency: sulfatide lipidosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic basis of inherited disease. 6th ed. New York: McGraw-Hill, 1989:1721.
17. Basner R, von Figura K, Glossl J, Klein U, Kresse H, Mlekusch W. Multiple deficiencies of mucopolysaccharide sulfatases in mucosulfatidosis. Pediatr Res 1979;13:1316.
18. Pompe JC. Over idiopatische hypertrophie van het hart. Ned Tijdschr Geneeskd 1932;76:304.
19. Hers HG. α-Deficiency in generalized glycogen storage disease (Pompe's disease). Biochem J 1963;86:11.
20. Danner DJ, Elsas LJ II. Disorders of branched chain amino acid and keto acid metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic basis of inherited disease. 6th ed. New York: McGraw-Hill, 1989:671.
21. Dancis J, Hutzler J, Levitz M. Metabolism of the white blood cells in maple syrup urine disease. Biochem Biophys Acta 1960;43:342.
22. Jonas AJ, Greene AA, Smith ML, Schneider JA. Cystine accumulation and loss in normal, heterozygous, and cystinotic fibroblasts. Proc Natl Acad Sci USA 1982;79:4442.
23. Schneider JA, Bradley K, Seegmiller JE. Increased cystine in leukocytes from individuals homozygous for cystinosis. Science 1967; 157:1321.