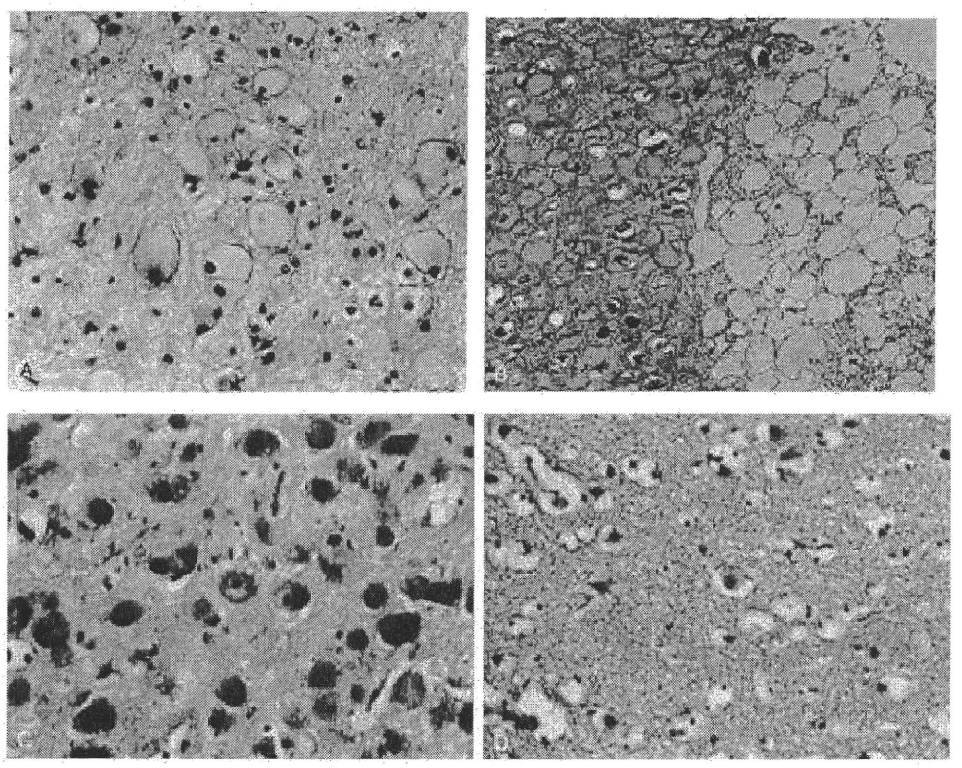
Light Microscopy

Cortical architecture is distorted, and the number of neurons is reduced.[16] Cytoplasm is distended in neurons and glial cells in all layers of the cerebral and cerebellar cortex, basal ganglia, brain stem, and spinal cord (Fig. 151-7 *A*). The nuclei are eccentrically placed and pyknotic. The cytoplasm contains finely granular material that stains pale pink with hematoxylin and eosin (HE) and periodic acid–Schiff (PAS) and occasionally stains with Luxol fast blue.[16] Many acid phosphatase–positive granules are seen in the distended neuronal perikarya, axons, and dendrites.[13] They are uniform in size and appear slightly larger than normal neuronal lysosomes. Frozen sections stained strongly PAS-positive in the cytoplasm of neurons in one patient[12] but did not stain with Sudan black B, Sudan IV, or PAS in another patient.[4] Acid mucopolysaccharide stains were positive in the original patient of Landing and colleagues.[4] Metachromatic material is not present. No cellular infiltrates or any significant gliosis is observed in the cortex. Astroglial infiltration and scant myelin were observed in the central white matter from an infantile patient.[4] Myelin stain suggests mild demyelination.[12]

Fig. 151-7:



Neuronal changes in GM1 gangliosidosis. A, Late infantile form, 5 years, male. Ballooning of neurons in the frontal cortex. HE stain, ×180. (Courtesy of Dr. Yoshio Morimatsu, Tokyo Metropolitan Institute of Neuroscience, Fuchu, Japan.) B, Infantile form, 5 years, male. Numerous MCBs in the cytoplasm of a Purkinje cell in the cerebellum. ×6300. (Courtesy of Dr. Yoshio Morimatsu, Tokyo Metropolitan Institute of Neuroscience, Fuchu, Japan.) C, Adult form. Head of

caudate nucleus. Almost all neurons are markedly distended with Luxol fast blue (LFB)–stained material within the cytoplasm. LFB-HE stain, ×1000. (Courtesy of Dr. Junichi Tanaka, Tokyo Jikei University School of Medicine, Tokyo, Japan.) D, Adult form. Cerebral cortex. Neurons are fairly well preserved in size, shape, number, and distribution. An occasional distended neuron is visible in the central field. LFB-HE stain, ×500. (Courtesy of Dr. Junichi Tanaka, Tokyo Jikei University School of Medicine, Tokyo, Japan.)

Lipofuscin has been described in G_{M1} gangliosidosis neurons.[13, 77, 130] Accumulation was remarkable in the patient with prolonged survival (onset, 2 years; autopsy, 17 years) reported by Lowden and colleagues.[77] This unusual longevity may have led to an increased amount of lipofuscin. Gaucher-like cells with numerous intracytoplasmic striations were found in a patient with juvenile G_{M1} gangliosidosis.[46] They were intensely positive for PAS stain and negative for various lipid stains.

Storage also was found in peripheral nerves[131] and astroglia.[130] Retinal neurons were investigated in one patient.[132] The ganglion cell layer is almost completely degenerated. Remaining neurons are strongly ballooned. Infiltration of glial cells with Luxol fast blue–positive material in the cytoplasm is remarkable. Optic atrophy corresponds to strong glial infiltration.

These histologic changes may be less severe in late infantile or juvenile patients, although the lesions are essentially the same as in infantile patients[43, 33] ; not all neurons are uniformly affected.

As decribed earlier, neuroimaging observations suggested a delay in myelination on the basis of persistently "immature" signal intensities.[110, 111] Subsequently, a semiquantitive evaluation of myelination was performed in two infantile G_{M1} gangliosidosis patients autopsied at 15 months of age.[134] Earlier-myelinating structures were comparable in development with that expected for postconceptional age, whereas later-myelinating structures were delayed. These data correlated well with the neuroradiologic diagnosis of myelination delay in both patients, suggesting that the metabolic defect has a primary influence on myelin development in addition to effects related to neuronal storage.

Electron Microscopy

The cytoplasm of neurons is filled with numerous concentrically arranged inclusion bodies, such as those in Tay-Sachs disease MCBs (see Fig. 151-7 *B*).[135] They are 0.5 to 3 μm in diameter,[12, 13] consist of parallel arrays of membranes usually arranged in a circumferential fashion, and replace largely normal cytoplasmic constituents. The membranes in the center often have an orientation roughly perpendicular to the outer membranes, which are circumferentially arranged. The periodicity is 50 to 60 Å,[12, 13] and the dimensions of the dense line and light band are 30 and 20 Å, respectively.[12] Splitting of the dense line is often seen. The core of the MCB is amorphous or polymorphic, with variable collections of vesicles, granules, and highly electron-opaque material. There is no distinct outer membrane enclosing it, but concentric layers of membranes limit each body.

These inclusion bodies are densely packed in the cell, and the small space between them is filled with mitochondria, smooth vesicles or membranous profiles, and ribosomes. They are also seen in axons and dendrites.[13] An MCB is an ordinary molecular complex and, in fact, was induced by a combination of gangliosides, cholesterol, phospholipids, cerebrosides, and casein hydrolysates.[136] MCBs are observed in the brain stems of affected fetuses. Inclusion bodies with other structures are also observed in glial cells

from late infantile patients.[12, 13] They are polymorphic, pleomorphic lipid bodies (PLBs), membranovesicular bodies (MVBs),[12, 137] or large, compact oval deposits.[16] MVBs may be a precursor of lipofuscin,[12] but they may not be the result of intracellular synthesis because their presence is related to phagocytic activity of these cells.[12, 13, 137] PLBs are surrounded by a limiting membrane and are composed of stacked or circularly layered lamellae with a granular substance of moderate electron opacity. MVBs are composed of stacks of circular arrangements of membranes. Large, compact oval deposits are bounded by broken single membranes and consist of irregularly accumulated curvilinear lamellae that blend into an amorphous matrix of moderate electron opacity.

In the white matter, many degenerated axons are distended with abnormal mitochondria, lamellated dense bodies, and amorphous granular debris.[13, 137] Degeneration of myelin also is observed with or without accompanying axonal degeneration.[13]

MCBs in the retina are confined exclusively to the ganglion cells.[137] Their appearance is different from those in the brain; membranes are irregularly arranged, forming a water-silk pattern surrounded by amorphous material.

General Pathology

Gross Morphology

Marked hepatosplenomegaly is observed in infantile-onset patients, but later-onset patients show visceral organs of normal size. Generalized skeletal dysplasia is observed grossly in infantile-onset patients.

Light Microscopy

Histiocytes with distended cytoplasm filled with a finely granular material are observed in the liver, spleen, lymph nodes, thymus, lung, intestine, interlobular septa of the pancreas, and bone marrow.[4, 12, 13] Vacuolation also has been observed in the pituitary gland, thyroid glands, salivary glands, sweat glands, and vascular endothelial cells.[131, 137] They stain intensely with PAS in paraffin-embedded tissue. Alcian blue and toluidine blue stains are positive.[138] The nuclei are small and pyknotic and eccentric in location. Vacuolation has not been found in the islets of Langerhans, the adrenal cortex, or skeletal muscle.[131]

Renal glomerular epithelium is usually swollen,[4, 13] and this characteristic lesion is not found in any other lipid storage disease except Fabry disease.[36] The glomerular epithelial cells store large quantities of cytoplasmic material, giving the glomerulus a swollen appearance.[4] The storage material is very soluble in aqueous fixatives, and empty vacuoles remain after fixation for electron microscopy.[9, 35] Vacuoles also were found in renal tubules.[131] In freeze-dried tissue fixed with formaldehyde gas, the material in the vacuoles is retained and stains with hematoxylin, Alcian blue, PAS, and aldehyde fuchsin. This material also shows metachromasia with toluidine blue.[35]

Cardiac lesions are reported often. Mitral and tricuspid valves show a sparsely cellular connective tissue.[12, 84] Vacuolation of cardiac muscles has been observed.[38, 84, 131, 139] Foam cells were seen in the interstitial tissue, heart valves, subendocardial tissues, and adventitia of coronary arteries and large arteries, including the aorta.[131] Hepatic parenchymal cells are enlarged,[16] and foam cells are present in the portal triads and gallbladder mucosa.[131]

Tissues of a patient at autopsy revealed accumulation of a lipid with histochemical characteristics of gangliosides in the brain; this lipid was positive for Sudan black B and Bial stains and soluble in alcohol, hot water, chloroform-methanol, and pyridine. On the other hand, the storage material in liver was PAS- and Alcian blue–positive, Sudan black–negative, and could not be extracted by lipid solvents. This material was considered to be an acid polysaccharide different from ganglioside.[140]

Ocular pathology demonstrated two different lesions.[141] Foamy changes were present in the epithelial cells, histiocytes, and keratocytes of the cornea. Colloidal iron and Alcian blue stains are positive for mucopolysaccharides and become negative after treatment with β-galactosidase. Descemet membrane is normal. The retinal ganglion cells were markedly distended, especially in the macular region. They did not stain with HE or PAS. Electron microscopy revealed numerous MCBs in the cytoplasm. These findings, together with electron microscopic observations, suggest mucopolysaccharide storage in the cornea and ganglioside storage in retinal ganglion cells.[141]

Cultured skin fibroblasts contain vacuoles with fine granular material that show metachromatic stain with toluidine blue O when fixed in acetic acid.[48, 132, 142] Gaucher-like cells in the bone marrow from one patient were clearly distinguished from Gaucher cells[46]; in another patient, membrane-bound inclusions of various sizes were arranged in a mosaic pattern and filled with fibrillar material with a "crinkled paper" appearance.[17] Intermingled tubular structures are usually narrow compared with those in Gaucher cells.[46]

In late-onset patients, the histologic changes generally are less prominent, or patients may exhibit no evidence of visceral involvement.[133]

Electron Microscopy

Endothelial and mesangial cells in glomeruli, visceral and parietal epithelial cells of Bowman's capsule, and proximal tubule cells contain vacuoles that are filled with fine granular material or that are, on occasion, filled with amorphous osmiophilic material.[131] The cytoplasm of the macrophages in the liver and spleen appears to be divided into many small compartments. Each compartment is filled with interwoven bundles of many fine tubular structures that are different in size and shape from MCBs in the brain.[13, 17] Roels and colleagues[137] did not observe this tubular material in their patient.

Two types of inclusions are present in hepatocytes, polymorphic, dense membrane-limited bodies and cytoplasmic, multivacuolated, lysosome-like bodies (mainly in peribiliary canalicular regions).[16] The vacuoles contain thin filaments of low electron density that are arranged irregularly.[38, 119] Rarely, round lamellated bodies of various sizes are found.

Muscle fibers show vacuoles of various sizes containing fine granular material.[131] On the other hand, two types of inclusions have been observed in extramuscular cells, with nonspecific changes in muscle fibers.[143] These are moderately electron-dense and polymorphic material, probably corresponding to ganglioside accumulation, in Schwann cells of intramuscular nerves and vacuolar inclusions, probably containing polysaccharides, in perineurial cells, endothelium, pericytes of blood vessels, and muscle satellite cells. Electron-dense granules are seen in heart muscle, and amorphous, electron-dense bodies without clear substructure are present in endothelial cells and interstitial cells in the heart.[122]

The vesicles in the cells of the cornea consist of a single membrane and contain small amounts of finely granular material. Retinal ganglion cells show numerous MCBs composed of concentric and/or parallel lamellae bounded by a single membrane.[141]

Adult/Chronic G_{M1} Gangliosidosis

Central nervous system

Gross Morphology

The brain of the patient reported by Goldman and colleagues[69] was small but displayed only a slight degree of frontal cortical atrophy. The ventricular system was not dilated. Midbrain, pons, medulla, cerebellum, and spinal cord were grossly unremarkable. Gross abnormality was localized in the basal ganglia with firm consistency; the caudate nucleus, putamen, and globus pallidus were shrunken and somewhat yellow. In an autopsy case of adult G_{M1} gangliosidosis,[144] pathologic lesions were most prominent in the caudate nucleus and putamen and, to a lesser degree, in the amygdala, globus pallidus, and Purkinje cells. The other areas of the central nervous system were relatively spared. It was concluded that this selective neuronal involvement reflected a more active turnover of ganglioside G_{M1} in the affected areas than elsewhere in the central nervous system.

Light Microscopy

Neurons of basal ganglia show markedly swollen perikarya, the cytoplasm displaying an eosinophilic, slightly granular appearance with HE stain.[69] These changes are marked in the anterior putamen and head of the caudate nucleus (see Fig. 151-7 *C*). Neuronal loss and gliosis were observed in their superior part. The cell loss was greater in the more posterior parts of the nuclei. The cytoplasm of the enlarged neurons stains brightly with PAS with or without diastase but somewhat less well with Sudan black or oil red O. There is no intraneuronal storage in the cerebral cortex or other deep cerebral nuclei (e.g., thalamus, subthalamic nucleus, amygdala, hypothalamus, and mammillary body) (see Fig. 151-7 *D*). The cytoarchitecture is normal in several areas of the cerebral or cerebellar cortex. Neurons of the midbrain, pons, medulla, spinal cord, and dorsal root ganglia are all normal.

Dilated proximal processes of medium-sized spiny neurons (meganeurites) were demonstrated on Golgi preparations of basal ganglia.[69] Axons arose from the distal part of the meganeurite. Dendrites were irregular in shape, with focal thickening and loss of spines. Purkinje cells showed focal swellings within their dendritic trees (megadendrites).

Electron Microscopy

Goldman and colleagues[69] described in detail the structure of inclusion bodies from a patient with adult G_{M1} gangliosidosis. Various intracytoplasmic inclusions were observed in neurons of the basal ganglia: (1) membranous cytoplasmic bodies with concentrically arranged lamellae surrounding a granular or lucent core, (2) pleomorphic inclusions, irregular in shape, containing elements of MCBs intermixed with granular material and short, curvilinear profiles, (3) large membrane-bound aggregates, and (4) membrane-bound collections of vesicular profiles or large, dense aggregates in astrocytes of the basal ganglia. Membranous inclusions were rare in Purkinje cells. The neuronal inclusions of the cerebellar cortex were small and membrane-bound, containing various membranous and vesicular profiles and granular material.

General Pathology

Light Microscopy

In the patient of Goldman and colleagues,[69] intracellular storage was observed in the reticuloendothelial system; vacuolated Kupffer cells or histiocytes were seen in the liver, spleen, bone marrow, lamina propria of the gastrointestinal tract, and glomerular epithelium of the kidney. The cytoplasm of these cells showed PAS-positive, diastase-resistant staining in a granular pattern. Lipofuscin was slightly increased in hepatic cells.

Rectal biopsy showed cytoplasmic granules in the rectal histiocytes. They were strongly or moderately positive for PAS and faintly positive for Sudan black B.[44, 58] Various lectin stains indicated the presence of acidic glycoconjugates containing abundant terminal β-galactose residues in histiocytes.[58]

Microscopic examination of the vertebral body in an infantile-onset patient[103] demonstrated an excessive accumulation of hyaline cartilage in the region of anterior notch deformity at the thoracolumbar junction. Columnar and palisading cartilaginous cells were detected along the posterosuperior portion of the body. This finding suggested an asymmetric growth process rather than a localized dysplasia.

Electron Microscopy

Kupffer cells contained numerous vacuoles of varying sizes bounded by a single membrane.[69] Some were empty, but others contained fibrillar material. In one patient, rectal biopsy showed abundant cytoplasmic inclusion bodies in autonomic neurons.[44]

Morquio B Disease

Dermis and epidermis were normal except for occasional extralysosomal U-shaped, round, or irregular lamellar inclusions in cutaneous nerves.[19] They were not enclosed by a limiting membrane and were comprised of lamellae with alternating light and dense lines. In another patient,[20] biopsy of bulbar conjunctiva revealed intracytoplasmic vacuoles limited by single-unit membranes containing fine fibrinogranular material typically seen in mucopolysaccharidoses.

STORAGE COMPOUNDS

The enzyme β-galactosidase has catalytic activities toward glycoconjugates containing a terminal β-galactosidic linkage. Three major groups of compounds have been identified in the cells from patients with β-galactosidase deficiency: ganglioside G_{M1} and its asialo derivative G_{A1}, glycoprotein-derived oligosaccharides, and keratan sulfate.

Glycosphingolipids

Gangliosides were discovered as a new group of glycolipids stored in Tay-Sachs disease,[145] and the monosialoganglioside G_{M1} was the first ganglioside to be analyzed for its structure. G_{M1}'s structure consisted of oligosaccharides and a hydrophobic ceramide moiety that were linked together by a glycosidic linkage[146]:

[IMAGE]

Gangliosides are normal components of cell plasma membranes that are concentrated in neuronal membranes, especially in the regions of nerve endings and dendrites. The hydrophilic oligosaccharide chain extends into the extracellular space. G_{M1} is the major ganglioside in brains of vertebrates.

At the membrane level, gangliosides display a broad capability of interactions.[147] They act as binding molecules for toxins and hormones and are also involved in cell differentiation and cell-cell interaction. G_{M1} is well known as a specific receptor of subunit B of cholera toxin. Long-term injection of G_{M1} selectively modulates serotonin receptors.[148, 149] Among various gangliosides, only G_{M1} was shown to be necessary to support synaptic transmission in Schaffer–collateral pyramidal cell synapses.[148]

Biosynthesis of gangliosides is catalyzed by a group of membrane-bound transferases. They are transported from the membranes to lysosomes, where they are degraded in a stepwise manner by acid hydrolases starting at the hydrophilic end of the molecules. In fact, the catabolic pathways were mostly studied on the basis of sphingolipid storage diseases. Sphingolipid hydrolases are bond-specific and degrade different glycoconjugates. Mutation of a gene coding for a lysosomal hydrolase results in its deficiency and causes intralysosomal accumulation of substrates. The water-insoluble lipid substrates form a complex with proteins, appearing as pathologic storage bodies in somatic cells.

The desialylated derivative of ganglioside G_{M1} (G_{A1}) is not the major component of cell membranes, and its function has not been fully determined. However, this compound also accumulates excessively in neuronal cells.[150]

Glycoproteins and Oligosaccharides

Glycoproteins are widely distributed macromolecules with a variety of functional implications. They are found in serum, urine, various secretions, intracellular and plasma membranes, and the extracellular spaces of connective tissue. Many enzymes and hormones are also glycoproteins. The protein portion is variable, and the carbohydrate portion consists mainly of sialic acid, galactose, mannose, *N*-acetylglucosamine, fucose, and *N*-acetylgalactosamine. Glucose, arabinose, and xylose are rarely found in glycoproteins.

Two major types of sugar–amino acid linkages are present: (1) *N*-acetylglucosamine linked to the amide nitrogen of asparagine (*N*-glycosidic linkage) and (2) *N*-acetylgalactosamine linked to the hydroxy group of serine or threonine (*O*-glycosidic linkage). β-Galactoside linkage is present in both molecular species. The structure and function of glycoproteins have been reviewed extensively.[151-154]

The *N*-linked oligosaccharide is first synthesized as a unique lipid intermediate, dolichol-oligosaccharide, by sequential addition of individual sugar residues to dolichol phosphate linkages, consisting of dolichol (Dol), phosphate (P), *N*-acetylglucosamine (GlcNAc), mannose (Man), and glucose (Glc):

[IMAGE]

This dolichol-linked oligosaccharide then is transferred cotranslationally to asparagine of the Asn-X-Ser/Thr sequence in nascent polypeptides within the lumen of the rough endoplastic reticulum (RER), and the biosynthesis continues and terminates in the smooth endoplastic reticulum (SER)/Golgi apparatus, where the oligosaccharide moiety is subjected to trimming and addition of other sugars to form complex-type oligosaccharides. For the biosynthesis of *O*-linked oligosaccharides, *N*-acetylgalactosamine is linked posttranslationally first to serine or threonine in the peptide, and then other sugars are added sequentially.

The degradation of glycoproteins occurs predominantly in lysosomes. Almost all lysosomal enzymes that catalyze the catabolism of glycoconjugates are glycoproteins themselves. Proteolytic enzymes first degrade the protein core of glycoprotein. The aspartylglucosamine linkage in the partial breakdown product (glycopeptide) then is hydrolyzed by the enzyme aspartylglucosaminidase. The free oligosaccharides are subjected to stepwise cleavage of monosaccharides by exoglycosidases from the nonreducing end of the carbohydrate chain. Hereditary β-galactosidase deficiency results in a defect of cleavage at the galactose moiety, and storage of galactose-containing oligosaccharides ensues.

Keratan Sulfate

Mucopolysaccharides are generally high-molecular-weight compounds with the repeating structure of disaccharides consisting of hexosamine and uronic acid. However, unlike other mucopolysaccharides, keratan sulfate does not contain uronic acid and consists of β-linked galactose (Gal) residues alternating with β-linked *N*-acetylglucosamine residues:

[IMAGE]

Both monosaccharides may be sulfated at their C-6 positions. Keratan sulfate exists in a proteoglycan linked with chondroitin sulfate. After proteolysis, free keratan sulfate chains are hydrolyzed stepwise by a series of exoenzymes, including galactose 6-sulfatase, β-galactosidase, glucosamine 6-sulfatase, and β-hexosaminidase A and B. Endoglycosidase activity toward keratan sulfate has not been described.

Removal of 6-sulfate on the galactose moiety is necessary for β-galactosidic hydrolysis by β-galactosidase. Specific diseases are known to result from deficiency of one of these four enzymes: galactose 6-sulfatase for Morquio A disease, β-galactosidase for Morquio B disease, glucosamine 6-sulfatase for Sanfilippo D disease, and β-hexosaminidase for G_{M2} gangliosidosis. However, keratan sulfate does not accumulate in G_{M2} gangliosidosis because hexosaminidase S is not deficient and is able to hydrolyze both β-GlcNAc and β-GlcNAc-6S in this disease.[155]

Keratan sulfate is one of the natural substrates for acid β-galactosidase and is a part of proteoglycan. Two types of keratan sulfate, I and II, have been distinguished.[156] The former is linked to protein via the *N*-glycosidic linkage between *N*-acetylglucosamine and the amide group of an asparagine residue[157]; it is a major component of the cornea. The latter is linked to protein via *O*-glycosidic linkages between *N*-acetylgalactosamine (a minor component) and the hydroxyl group of threonine/serine residue[158]; it is a component of skeletal tissues.

Storage Material in β-Galactosidosis

Ganglioside G_{M1} and Asialo G_{M1} (G_{A1})

Storage of ganglioside G_{M1} has been the most prominent observation for G_{M1} gangliosidosis since the first reports by Jatzkewitz and Sandhoff,[6] Jatzkewitz and colleagues,[159] O'Brien and colleagues,[5] and Ledeen and colleagues.[160] Extensive analytical studies were subsequently performed by Suzuki and colleagues.[13, 150] The stored G_{M1} has the same fatty acid composition, sugar composition and sequence, and glycosidic linkages as normal G_{M1}. An enormous increase in G_{M1} and, to a lesser extent, in G_{A1} has been found in brains from G_{M1} gangliosidosis patients; the total amount of ganglioside sialic acid is increased three- to fivefold in the cerebral gray matter, and G_{M1} is the major component in distribution (75–80 percent of total ganglioside sialic acid; control values, 15–20 percent). The total increase in G_{A1} is 4- to 20-fold in the gray matter, but no definite increase is observed in the white matter. G_{M1} constitutes about one-third of the dry weight of pure isolated MCBs. Storage of G_{M1} was detected by specific binding of the cholera toxin B subunit,[161] immunoassay,[62, 99] and radioassay,[69] as well as by conventional biochemical analysis. Lysocompounds of G_{M1} and asialo G_{M1} were reported to be increased in the brains of patients with infantile, late infantile, and adult G_{M1} gangliosidosis.[162] These cytotoxic lysosphingolipids may be of pathogenetic significance in G_{M1} gangliosidosis.

Visceral organs also show an abnormal increase in G_{M1}.[37, 150] A normal ganglioside pattern was reported in the liver of a patient with late infantile G_{M1} gangliosidosis.[45] An increase in G_{M1} was observed in erythrocytes from a patient with G_{M1} gangliosidosis,[163] but lipid-bound sialic acid was normal in erythrocytes and highly increased in another late infantile patient.[45] G_{M1} was apparently normal in fibroblasts from one patient.[40] No definite conclusion was made for the ganglioside pattern in fibroblasts from G_{M1} gangliosidosis patients.[142]

The extent of G_{M1} storage in the brain is not significantly different between infantile and late infantile/juvenile patients.[36, 37, 150, 164] Accumulation of G_{M1} is detected only in the caudate nucleus and putamen in adult or chronic patients.[76]

Abnormalities of Other Lipids

Glucosylceramide and lactosylceramide are increased in the brain in G_{M1} gangliosidosis as well as in G_{M2} gangliosidosis. White matter shows chemical manifestations of myelin breakdown, including low proteolipid protein, low total lipid, and the presence of esterified cholesterol.[45, 150] The decrease in major cerebral lipids (e.g., cholesterol, phospholipids, cerebroside, and sulfatide) was more remarkable in an infantile-form patient than in a juvenile-form patient.[164] In addition, other unusual neutral sphingolipids were found only in the infantile-form patient. They are tentatively identified as fucolipids consisting of glucose, galactose, *N*-acetylglucosamine, and fucose (1:2:1:1 and 1:3:2:2); lacto-*N*-fucopentaose II (lacto-*N*-difucooctanose); and lacto-*N*-fucopentaose III.[165]

Glycolipids are normal in visceral organs, but a large amount of cholesterol glucuronide was found in a juvenile-form patient.[166]

Oligosaccharides and keratan sulfate

Galactose-containing oligosaccharides and undersulfated keratan sulfate and its partial degradation derivatives have been demonstrated in urine and liver from β-galactosidase-deficient patients.[150, 167, 168]

Keratan sulfate and keratan sulfate-derived oligosaccharides

Vacuoles in visceral organs of G_{M1} gangliosidosis patients contain highly water-soluble compounds, suggesting the presence of compounds other than lipids. A mucopolysaccharide was detected in large amounts in the livers and spleens of G_{M1} gangliosidosis patients.[150, 169] It was tentatively identified as keratan sulfate on the basis of its behavior in the preparative procedure, composition (almost equimolar galactose and hexosamine), and electrophoretic and thin-layer chromatographic mobilities. The presence of very soluble sialomucopolysaccharide, which contained small amounts of galactosamine, mannose, and fucose, in addition to galactose and glucosamine, also was reported.[150, 169] Results of further characterization of this compound have not been reported.

Wolfe and colleagues[48] detected two types of undersulfated keratan sulfate–like mucopolysaccharides in the urine of a late infantile patient. One type was precipitated by CPC and migrated on Sepharose electrophoresis like standard cartilage keratan sulfate. The other was not precipitated by CPC and was free of uronic acid. The sugar composition closely resembled the material from the liver of another autopsy patient. Urinary mucopolysaccharides were markedly increased in an infantile patient,[94] but the preparation contained significant amounts of uronic acid and galactosamine, major constituents of chondroitin sulfate.

The mucopolysaccharides accumulating in the liver of a patient with infantile G_{M1} gangliosidosis contained galactose and glucosamine as the major carbohydrate constituents, with small amounts of sulfate, sialic acid, and galactosamine.[167] Threonine was found in the keratan sulfate fraction.[170] Mucopolysaccharides were not precipitated by CPC. The structure was similar to the skeletal form of keratan sulfate. However, sulfate was extremely low in content in the mucopolysaccharide from the G_{M1} gangliosidosis liver as compared with normal human skeletal keratan sulfate.

Tsay and colleagues[171, 172] determined the structure of "keratosulfate-like" oligosaccharide from the liver of a patient with infantile G_{M1} gangliosidosis. It was identified as an octahexosyl glycopeptide, probably representing the desulfated linkage region of skeletal keratan sulfate with a structure as follows:

[IMAGE]

The amount of urinary keratan sulfate in G_{M1} gangliosidosis is less than that for Morquio B disease patients.[21] Keratan sulfate excretion is markedly increased in the patients with Morquio B disease[19]; it constituted 31 and 26 percent of total mucopolysaccharides in two patients.[115, 118, 119] Chondroitin sulfate, which is often observed in Morquio A disease, is not increased.[115, 119] Undersulfated keratan sulfate was detected in the CPC-nonprecipitable fraction of one patient.[20]

Glycoprotein-Derived Oligosaccharides

A large number of galactose-containing oligosaccharides have been found in the livers[172, 173] and urine[174-182] of G_{M1} gangliosidosis patients and the urine of a Morquio B disease patient.[183] They are heterogeneous, consisting of widely ranging molecular weights.[184] Abnormal oligosaccharide bands were visualized on thin-layer chromatography in an infantile G_{M1} gangliosidosis patient.[21] The most abundant material is an octasaccharide.[173, 185, 186] The octasaccharide band structure indicates a block in glycoprotein catabolism, not related to keratan sulfate, at the step of β-galactosidic cleavage subsequent to hydrolysis by endo-*N*-acetylglucosaminidase (sugar-peptide linkage), neuraminidase (sialic acid), and a-fucosidase (fucose). Some of the bands are detected in both liver and urine. They appear to have derived from incomplete degradation of erythrocyte stromal glycoproteins[173] or immunoglobulins.[174]

Neutral oligosaccharides were increased in the urine from adult patients[187]; the pattern was identical to that reported in type 2 G_{M1} gangliosidosis.[44, 56, 57] However, the pattern was indistinguishable from that in normal control urine.[21] Two major oligosaccharides were isolated from the urine of a patient with adult G_{M1} gangliosidosis.[182] The first was one of the most common urinary oligosaccharides found in type 1 and type 2 patients, but the amount was much less than that in a type 2 patient. The second oligosaccharide had not been described previously in G_{M1} gangliosidosis. Hepatic glycoproteins were normal in the liver of an autopsied patient.[76]

Four types of oligosaccharides have been identified in the tissues and urine of cats affected with G_{M1} gangliosidosis.[188-190] Oligosaccharide analysis of the liver from Beagle dogs with G_{M1} gangliosidosis revealed carbohydrate sequences nearly identical to those in oligosaccharides stored in the human disease.[189] However, the Beagle carbohydrate sequences differ from the human compounds in that they contain two glucosamine residues at the reducing terminus instead of one.[188] There also may be differences between humans and dogs in glycoprotein metabolism or structure.

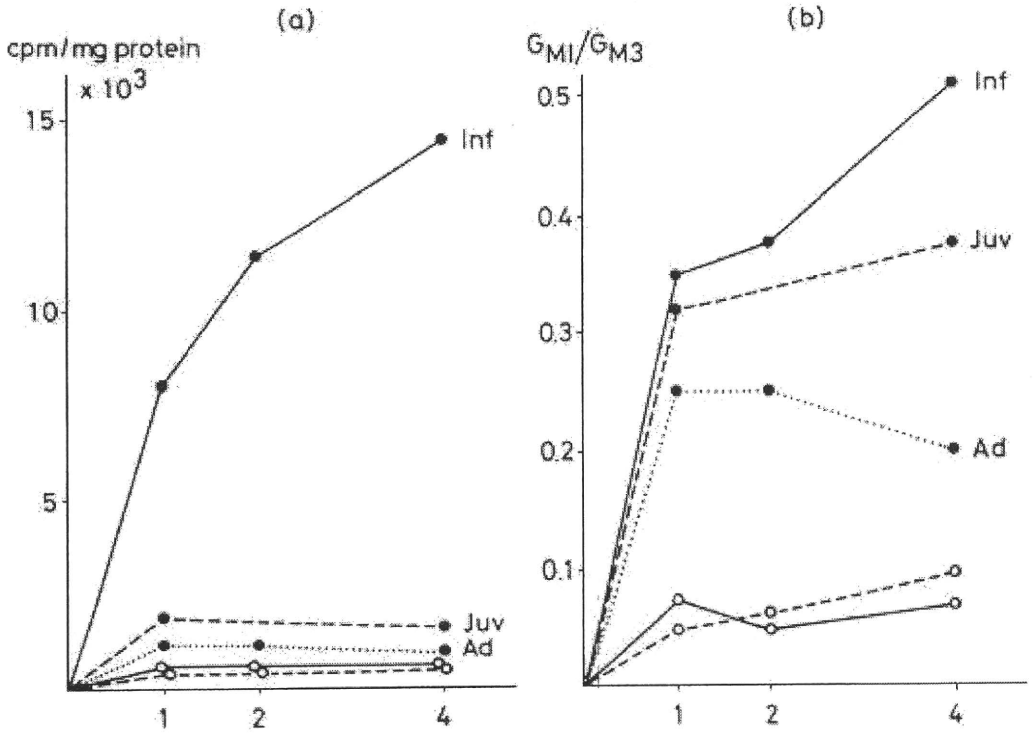
Asparagine-linked sugar chains of sphingolipid activator protein 1 were identified in normal and G_{M1} gangliosidosis livers.[191] Eight degradation products from complex-type sugar chains were present in the normal liver; the sugar chains were of sialylated or nonsialylated monoantennary to tetra-antennary complex type in G_{M1} gangliosidosis, different from those in normal liver.

Oligosaccharide excretion is increased in Morquio B disease.[117] The oligosaccharide pattern is similar to that in G_{M1} gangliosidosis,[115, 118, 119] but the intensity of all bands is considerably less for Morquio B patients.[119] In another report,[21] the patterns were different from those for G_{M1} gangliosidosis. Six different oligosaccharides have been identified from the urine of a patient with Morquio B disease.[183] Three oligosaccharides were identical to those reported for G_{M1} gangliosidosis, and the other three were novel oligosaccharides in this patient.

Correlation of phenotype and substrate storage

The degree of storage of G_{M1} does not correlate with the age of onset or severity of phenotypic expression, at least for infantile and late infantile patients.[13, 37, 150] In an experiment using cultured human fibroblasts, a relative increase in G_{M1} was demonstrated in three different forms of G_{M1} gangliosidosis[54] (Fig. 151-8). Gangliosides in the cells were radiolabeled with [^{14}C]galactose in the culture medium. G_{M3} was the major ganglioside in control cells, and a relative increase in G_{M1} was observed in the cells from patients. The degree of increase was inversely correlated with the age of onset.

Fig. 151-8:



Storage of GM1 in fibroblasts with GM1 gangliosidosis. Gangliosides were labeled with [14C]galactose, and the radioactivity in the GM1 and GM3 fractions was determined. Values are expressed as (a) GM1/protein and (b) GM1/GM3 ratio. ◦, controls; •, patients. Inf = infantile form; Juv = juvenile form; Ad = adult form. (Reproduced from Suzuki et al. 54 Used by permission.)

The amounts of total urinary oligosaccharides are the highest in type 1 (infantile) G_{M1} gangliosidosis; the amounts for type 2 patients were approximately one-tenth those for type 1 patients.[177] Oligosaccharides with long outer chains (Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1 or longer) were reported not to be detectable in the urine from type 2 patients,[177] but most of the oligosaccharides were later found to be present in the latter.[179] Results of comparative quantitative analysis of urinary oligosaccharides have not been reported between G_{M1} gangliosidosis and Morquio B disease patients, although some oligosaccharides are present in both phenotypes.[175, 177, 179, 183]

ENZYME DEFECT

Lysosomal Acid β-Galactosidase

Two lysosomal enzymes are known for hydrolysis of terminal β-linked galactose at acidic pH in various glycoconjugates. One is G_{M1} β-galactosidase (EC 3.2.1.23), catabolizing ganglioside G_{M1}, G_{A1}, lactosylceramide, asialofetuin, galactose-containing oligosaccharides, and keratan sulfate (G_{M1} β-galactosidase). The other genetically different enzyme is galactosylceramidase (galactocerebrosidase;

EC 3.2.1.46), catabolizing galactosylceramide, galactosylsphingosine, lactosylceramide, and monogalactosyl diglyceride. Hereditary deficiency of the latter results in a neurometabolic disease called *globoid cell leukodystrophy* (see Chap. 147).

G_{M1} β-galactosidase is often designated simply as β-galactosidase, and we follow this nomenclature in this chapter. Artificial substrates are currently used for simple and sensitive assays of this enzyme. These include 4-methylumbelliferyl-β-D-galactopyranoside (fluorogenic) and *p*-nitrophenyl-β-D-galactopyranoside (chromogenic). However, more sensitivity is often required for the diagnostic purpose, using small amounts of clinically available samples. Some techniques have been reported for single-cell assays[192] and for microassays using microtiter plates,[193, 194] HPLC,[195] and chemiluminescence.[196]

β-Galactosidase is present in a variety of human tissues and body fluids. The enzyme activity is higher in systemic organs, including fibroblasts and lymphocytes, than in the central nervous system. The enzyme has been studied in human liver,[197-204] brain,[205, 206] skin fibroblasts,[193, 207-211] kidney,[212] urine,[213] placenta,[214] and leukocytes.[215, 216]

The optimal pH of β-galactosidase is approximately 4.5. It is thermolabile and activated by chloride ions[217, 218]; its activity is inhibited in the presence of mucopolysaccharides[219, 220] and partially restored by the addition of CPC[219, 220] or chloride ions.[221] Specific inhibitors of β-galactosidase have been developed, including *N*-bromoacetyl-β-galactosylamine,[222] β-D-galactopyranosylmethyl-*p*-nitrophenyltriazene,[223] *N*-substituted (β-D-galactopyranosylmethyl) amines,[224] and diazomethyl-β-D-galactopyranosyl ketone.[225] β-D-Galactopyranosylmethyl-*p*-nitrophenyltriazene was used to establish an in vitro model of G_{M1} gangliosidosis.[226]

In human fibroblasts, β-galactosidase is synthesized as a high-molecular-mass precursor of 84 to 85 kDa that is C-terminally processed to the 64-kDa mature enzyme.[227, 228] After processing, the monomeric-form protein aggregates to a homomultimer of approximately 700 kDa. The released approximately 2-kDa proteolytic fragment remains associated with the 64-kDa chain after partial proteolysis of the precursor, and it is copurified with the catalytically active enzyme in mouse liver, Madin-Darby bovine kidney, and normal human fibroblasts (but not human fibroblasts with β-galactosidosis or galactosialidosis[229]). This result suggests that the C-terminal fragment is an essential domain of the catalytically active enzyme of the two-subunit molecule. Mutations in G_{M1} gangliosidosis in the C-terminal part of the enzyme molecule may have a special significance.

Multiple forms of β-galactosidase have been separated on the basis of molecular mass or electrophoretic mobility. Molecular weights of β-galactosidases purified from human liver are 65, 150, and 700 kDa, probably representing monomeric, dimeric, and multimeric forms, respectively.[200, 202, 205] Immunologic studies suggested that the high-molecular-weight form is a multimeric aggregate of the monomer.[199] Furthermore, a multifunctional glycoprotein (protective protein/cathepsin A) is necessary for aggregation of β-galactosidase in vivo.[209, 227] The half-life of aggregated β-galactosidase is approximately 10 days.[230] In the absence of protective protein/cathepsin A, the β-galactosidase precursor is subjected to an abnormal trimming, precluding its normal processing and expression of catalytic activity.[231]

β-Galactosidase is a glycoprotein containing 7.5 to 9 percent carbohydrate.[198, 201, 203] Frost and colleagues[201] isolated a dimeric form of β-galactosidase from human liver; it contained high amounts of acid and neutral amino acids and low amounts of basic and sulfur-containing amino acids.

β-Galactosidase is a soluble lysosomal enzyme with a high content of complex-type oligosaccharides and a low content of oligomannoside-type oligosaccharides.[203] A calculation indicated three carbohydrate chains per enzyme molecule on average.[203]

β-Galactosidase cleaves substrates by formation of a galactosylated amino acid intermediate (the catalytic nucleophile), with subsequent release of the galactose and retention of the β-configuration. This mechanism predicts that at least two acidic residues are involved in the catalytic mechanism. Two amino acids have been suggested as candidates in the catalytic mechanism: Glu268[232] and Asp332.[233] On the other hand, a molecular modeling analysis suggested two glutamic acid residues (131 and 188) as candidates for molecular interaction with the substrate at the active site of the enzyme (Sakakibara and colleagues, unpublished data). The W273L mutation is expected to play an essential role in hydrolysis of keratin sulfate because it is the only mutation strictly associated with Morquio B disease.

Natural Substrates for β-Galactosidase

Sphingolipids

Hydrolysis of G_{M1} has been studied for various tissues and cells, including human leukocytes,[234, 235] human fibroblasts,[235] human liver,[198, 205, 234, 236] human placenta,[237] rabbit brain,[237] and rat brain.[238] Some reports have discussed the spectrum of substrate specificity.[239-242] In vitro enzyme studies demonstrated that the only substrate common to the two β-galactosidases, G_{M1}-cleaving enzyme and galactosylceramide-cleaving enzyme, is lactosylceramide.[241, 242] The terminal galactose in most sphingolipids is cleaved by G_{M1} β-galactosidase, except for galactosylceramide and galactosylsphingosine. Lactosylceramide is hydrolyzed by both enzymes.[207, 240, 242] The enzymic hydrolysis of liposome-integrated lactosylceramide is significantly dependent on the structure of its lipophilic aglycon moiety with increasing length of its fatty acyl chain.[243] However, in the presence of detergents, the degradation rate is independent of the acyl chain length.

Bile salts have been added into the enzyme assay mixture for stimulation of hydrolysis of G_{M1} in vitro; this stimulation occurs probably by a detergent effect on the substrate and an interaction between bile salts and the enzyme molecule.[244] Shiraishi and colleagues[244] reported that the hydrolysis of G_{M1} by β-galactosidase was greatly enhanced by the addition of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin or α-cyclodextrin in the assay mixture. They concluded that these compounds stimulated the hydrolysis by formation of an inclusion complex between G_{M1} and cyclodextrin without enzyme-protein interaction.

Carbohydrates

β-Galactosidase is also involved in the degradation of keratan sulfate and glycoprotein-derived oligosaccharides. A few enzymatic studies have been reported.[21, 245] More evidence for β-galactosidase's role in carbohydrate degradation has accumulated from analysis of the storage material in urine or livers from β-galactosidase-deficient patients, as described earlier.

Protective Protein/Cathepsin A

Protective protein is a glycoprotein associated with β-galactosidase and neuraminidase in the lysosome, stabilizing the former and activating the latter.[209, 227, 246, 247] It is synthesized in human fibroblasts as a 54-kDa precursor that is processed to a mature form, a heterodimer of 32- and 20-kDa subunits held together by a disulfide bond, and aggregates with monomeric β-galactosidase to form a high-molecular-weight complex involving neuraminidase.[209, 227, 228, 248-250]

Some recent studies reveal that this is a multifunctional enzyme protein with serine esterase activities, acid carboxypeptidase at pH 5.6, esterase at pH 7.0, and C-terminal deamidase at pH 7.0.[251, 252] It also has cathepsin A–like activity.[253] A genetic defect of protective protein results in combined deficiency of β-galactosidase and neuraminidase (galactosialidosis)[254, 255] (see Chap. 152). A defect in multimerization of β-galactosidase has been reported in fibroblasts from galactosialidosis patients; the monomeric enzyme is rapidly degraded by thiol proteases, and the enzyme activity is restored by their inhibitors.[209, 230, 256, 257] Protease inhibitors are also effective for preventing rapid degradation of exogenous *Aspergillus oryzae*β-galactosidase in human fibroblasts.[258]

Activator Protein

Some water-soluble hydrolases need nonenzymic, low-molecular-weight factors (activator proteins) for degradation of sphingolipids in the lysosome (see Chap. 134). They act as physiologic detergents that facilitate enzyme-substrate interactions. Sphingolipid activator protein 1 (SAP-1), or saposin B, is required for in vivo cleavage of ganglioside G_{M1} by β-galactosidase.[259, 260]

Saposin B is a 9-kDa glycoprotein that stimulates degradation of sulfatides[261-263] and trihexosylceramide[264] by arylsulfatase A and α-galactosidase, respectively. Saposin B and three other thermostable proteins (saposins A, C, and D) are derived from a single high-molecular-weight precursor (prosaposin) by proteolytic processing.[265-267] Saposin B forms water-soluble complexes with both lactosylceramide and G_{M1}, and these complexes are recognized by β-galactosidase as optimal substrates in the same mode.[243] A synthetic peptide corresponding to a predicted α-helix of saposin B, spanning the amino acid residues 52 to 69, has been suggested to play a major role in the recognition and binding to G_{M1} by this activator protein.[268]

Mutant β-Galactosidase

Residual Enzyme Activity

A genetic defect of β-galactosidase in humans is responsible for G_{M1} gangliosidosis and Morquio B disease. A profound loss of enzyme activity against natural substrates (G_{M1} and G_{A1}) and artificial substrates has been found in infantile and juvenile G_{M1} gangliosidosis patients (Table 151-4). Fewer than 5 percent of the control values are found in liver and brain,[205] as well as almost total loss of activity in leukocytes and fibroblasts.[23, 235, 269, 270] Patients with adult-form G_{M1} gangliosidosis have higher residual β-galactosidase activity (5–10 percent of control values).[23, 55, 63] β-Galactosidase activity toward a radiolabeled trisaccharide derived from shark cartilage keratan sulfate was low in fibroblasts from G_{M1} gangliosidosis patients.[271]

Table 151-4 Residual β-Galactosidase Activity in Fibroblasts from Patients with β-Galactosidosis

		G_{M1} Gangliosidosis[a]				
Substrate	**Data**	**Infantile**	**Juvenile**	**Adult**	**Morquio B disease[a]**	**Reference**
4MU*		0.07–1.3	0.3–4.8	1.8–8.9	1.5–12.3	19, 23, 55, 115, 119, 120, 270–272
G_{M1}	A[a]		4.6–9.4		2.9–25	17, 19, 115, 272
	B[b]	0.1–0.6	0.6–2.0	1.1–4.4		272

4MU = 4-methylumbelliferyl β-galactopyranoside; G_{M1} = ganglioside G_{M1}.

[a] Enzyme activity: percent of control mean activity per protein (min – max).

[b] Enzyme activity: percent of control mean activity per amount of cross-reacting material (CRM) (min – max)

Morquio B disease patients show β-galactosidase activity (see Table 151-4) that is 5 to 10 percent of control values[115] or less than 5 percent.[120] Residual enzyme activity in fibroblasts was variable toward three different substrates.[19] It was 7 percent for G_{M1}, 1.4 percent for asialofetuin, and 3.5 percent for 4-methylumbelliferyl β-galactoside. It exhibited normal thermostability and had a normal optimal pH. The K_m for *p*-nitrophenyl β-D-galactoside was fivefold higher than normal.[115, 119, 273] Mutant β-galactosidase in Morquio B disease had no detectable affinity toward either keratan sulfate or oligosaccharides isolated from patient urine,[21] but a high residual activity of the mutant enzyme was found toward G_{M1} in the presence of partially purified G_{M1} activator protein.[274] G_{M1} activator thus stimulated G_{M1} hydrolysis by the Morquio B mutant enzyme but did not stimulate keratan sulfate hydrolysis.[274]

In general, serum β-galactosidase activity is low in G_{M1} gangliosidosis, particularly after long-term clotting of the whole blood.[275] In the patients with juvenile G_{M1} gangliosidosis with homozygous R201C mutation, however, serum enzyme activity was normal and only relatively low, even after long-term clotting,[276] showing a pattern similar to that in galactosialidosis. The R201C mutation is known to produce a mutant enzyme protein with a defect in molecular interaction with protective protein/carboxypeptidase,[277] which is probably a common molecular mechanism for the β-galactosidoses caused by this mutation and for galactosialidosis.

The activities of lysosomal enzymes other than β-galactosidase are usually normal or increased at most three- to fourfold in plasma from patients with β-galactosidase deficiency. However, markedly increased activities of plasma lysosomal enzymes that were comparable with those seen in I-cell disease were found in fibroblasts from a patient with a single deficiency of β-galactosidase, thus excluding the diagnosis of I-cell disease.[277] The reason for this unusual observation is not known.

Substrate loading in culture cells

Uptake and degradation of G_{M1} and G_{A1} in cultured fibroblasts were studied in patients with β-galactosidase deficiency after lipid loading in the culture medium.[278-281] In infantile G_{M1} gangliosidosis, the loaded substrates were hardly hydrolyzed and remained in the cells on any day of culture. However, fibroblasts from adult G_{M1} gangliosidosis and Morquio B disease patients hydrolyzed the substrates at nearly normal rates. The in situ metabolism of G_{M1} and G_{A1} may be normal, even though in vitro β-galactosidase activities are very low. This result seems compatible with the findings that G_{M1} and G_{A1} do not accumulate in somatic cells of patients with these clinical types of β-galactosidosis, except in basal ganglia.

Mutant Enzyme

Somatic cell hybridization studies showed that the different forms of G_{M1} gangliosidosis and Morquio B disease are based on different mutations in the same gene on chromosome 3.[21, 55, 282-284] Previous immunologic studies using antisera against purified human β-galactosidase demonstrated the presence of normal amounts of CRM in liver and fibroblasts from infantile and adult form G_{M1} gangliosidosis patients.[137, 285] Normal amounts of CRM were found in cells from juvenile G_{M1} gangliosidosis patients,[199] but a reduction or increase was reported when compared with controls.[272, 286] Abnormal electrophoretic migration, a high K_m, and a high antigenic activity per unit of catalytic activity were found in one patient.[254]

In one study, the enzyme protein was labeled in vivo in fibroblasts, immunoprecipitated, and fractionated by sucrose-density-gradient centrifugation.[210] An 85-kDa precursor was normally synthesized in infantile and adult G_{M1} gangliosidosis cells, but more than 90 percent of the enzyme was degraded at one of the early steps in the posttranslational processing. The residual enzyme was a 64-kDa mature form in adult G_{M1} gangliosidosis with normal catalytic properties and reduced aggregation to multimers. A contradictory result was obtained in another study using a precursor-specific antibody.[287] An immunoprecipitation analysis revealed that the precursor protein primarily accounted for the residual enzyme activity in fibroblasts from an adult G_{M1} gangliosidosis patient and that the mature protein accounted for the activity in fibroblasts from a juvenile G_{M1} gangliosidosis patient. In Morquio B disease, the mutation of the enzyme did not interfere with posttranslational processing or intralysosomal aggregation.[210] The mature mutant W273L gene product showed a total loss of affinity toward a synthetic substrate, although its precursor protein was measurable, with relatively high K_m compared with the wild-type enzyme.[288]

Phosphorylation of precursor β-galactosidase was reported to be defective in both infantile- and adult-form G_{M1} gangliosidosis fibroblasts.[210] The impairment of phosphorylation could be due to conformational changes of the precursor, resulting in secretion into culture medium instead of compartmentalization into lysosomes. An immunoelectron-microscopic study demonstrated the precursor form of the enzyme in the RER and Golgi apparatus, but the enzyme molecule was not detected in lysosomes.[289]

Characterization of mutant gene products

The following results have been observed by expression of mutant genes in G_{M1} gangliosidosis fibroblasts, followed by intracellular turnover analysis of the mutant enzyme protein.[290]

The expression product of the mutant gene W273L (Table 151-5), commonly found in Morquio B disease, is sorted to lysosomes, aggregated with protective protein, and stabilized. A stable mature enzyme has been detected in previous biologic and immunologic studies on Morquio B disease patients, although their genotypes were not known.[21, 210, 245] This observation was further confirmed by another experiment using three different forms of human β-galactosidase antibody: a high-molecular-weight multienzyme complex, a recombinant 84-kDa precursor, and a 64-kDa tryptic product of the precursor (an analogue of the mature form enzyme).[291] Immunoprecipitation and immunostaining studies demonstrated normal patterns in Morquio B disease, except that the residual enzyme activity was markedly reduced. Formation of a complex in the lysosome (β-galactosidase, neuraminidase, and protective protein) may be related to catabolism of G_{M1}; this substrate is almost normally hydrolyzed in fibroblasts derived from Morquio B patients.[25]

Table 151-5 Intracellular Turnover of Mutant β-Galactosidase in β-Galactosidosis

Phenotype	Mutation	Ethnic origin	Molecular defect
G_{M1}-gangliosidosis			
Infantile	R482H	Italian	Biosynthesis[a]
	R208C	American	
Juvenile/adult	R201C	Japanese	Complex formation[b]
	R201H	Caucasian	
Adult	I51T	Japanese	Transport[c]
	T62M	Caucasian	
Morquio B	W273L	Caucasian	Substrate specificity[d]
	Y83H	Japanese	

[a] ***Defect in protein biosynthesis.***

[b] ***Molecular interaction between b-galactosidase and protective protein/cathepsin A.***

[c] ***Intracellular transport of the b-galactosidase protein to the lysosome.***

[d] ***Altered substrate specificity of the mutant enzyme.***

The product of R201C, a common mutation in late infantile/juvenile G_{M1} gangliosidosis, is sorted to the lysosome but not aggregated with protective protein.[290] The mature enzyme is rapidly degraded in the lysosome as in galactosialidosis with protective protein/cathepsin A gene mutations.

The product of I51T, a common mutation in adult/chronic G_{M1} gangliosidosis among Japanese patients, is not phosphorylated. Sorting of the mutant enzyme to lysosomes is disturbed at the Golgi apparatus, and only a small amount of the mutant enzyme reached the lysosome.[290] Endogenous protective protein/cathepsin A may stabilize the mutant enzyme. The phosphorylation defect was present at the Golgi apparatus in a mutant cell strain of adult G_{M1} gangliosidosis,[211] and the inactive precursor was secreted in the culture medium. The amount of the mutant enzyme molecule is reduced,[181, 210, 289] but most of the enzyme activity is in the lysosome. The mutant enzyme is as stable as wild-type β-galactosidase.[292] Further details of the molecular defect are not known.

Correlation of phenotype and enzyme defect

Quantitative and qualitative studies have been performed in each clinical subtype of β-galactosidosis. Residual enzyme activities toward synthetic substrates or ganglioside G_{M1} were not clearly correlated with the severity of clinical manifestations in infantile-, late-infantile-, or juvenile-form patients.[17, 37, 215] On the other hand, more residual enzyme activity was found in type 2 patients than in type 1 patients.[54, 208] Adults with G_{M1} gangliosidosis show higher residual enzyme activities than the patients with other clinical forms.[23, 54, 55] Residual β-galactosidase activity is also relatively high in Morquio B patients. Direct comparison of the data from different laboratories with different enzyme sources and different substrates is difficult, but the amount of residual enzyme activity seems to have an inverse correlation with the age of onset or clinical severity. No data have been demonstrated for further comparative characterization of mutant enzymes between adult G_{M1} gangliosidosis and Morquio B disease.

Differences in optimal pH, thermostability, and electrophoretic mobility also have been reported.[208, 270, 293, 294] Two clinical forms (infantile and juvenile) occurred in a single family, and different isoelectric focusing patterns were elicited,[295] but further molecular analysis was not performed.

Neutral β-Galactosidase

An enzyme was found in human liver with β-galactosidase activity at neutral pH. It is different from the acid enzyme and cleaves synthetic substrates with both aryl β-galactoside and β-glucoside linkages but not G_{M1} or asialofetuin.[296] Antibodies against acid and neutral β-galactosidases do not crossreact with each other.[272, 297] In G_{M1} gangliosidosis liver, the neutral β-galactosidase activity is normal[272] or high.[298] This enzyme activity shows a bimodal distribution in human liver with a high-activity group and a low-activity group (10 percent of the high-activity group).[299] No clinical symptoms have been described even in individuals with extremely low enzyme activity.

MOLECULAR GENETICS OF β-GALACTOSIDASE DEFICIENCY

Molecular Genetics of β-Galactosidase

Gene assignment

The structural gene coding for human acid β-galactosidase was initially assigned to chromosome 22[300] and subsequently to chromosome 3.[283, 284, 301, 302] Actually, two loci, one on chromosome 3 and one on 22, were required for the full gene expression.[303] Using human-mouse or human-hamster hybrids and anti-β-galactosidase antibody, the structural gene for β-galactosidase was assigned to chromosome 3p21-3q21[304] and 3cen-3pter.[305] Hybridization of the β-galactosidase cDNA probe to human-mouse somatic cell hybrids revealed that the β-galactosidase gene is located in the 3p21-3pter region.[306] Finally, fluorescence in situ hybridization recently confirmed the localization at 3p21.33.[307] Several data indicate that the second locus is actually on chromosome 20 and that it codes for protective protein/cathepsin A

(see Chap. 152).

β-Galactosidase cDNA

A 2.4-kb full-length cDNA for human placental β-galactosidase was isolated and designated GP8.[22] Expression of the functional molecule in transfected COS (CV-1 transformed by an origin-defective SV40) cells confirmed that the clone GP8 encoded a functional sequence, a protein of 677 amino acids, including a putative signal sequence of 23 amino acids and 7 potential asparagine-linked glycosylation sites.

Two additional 2.4- and 2.0-kb clones were isolated independently.[306, 308] The amino acid sequence of the 2.4-kb clone was corrected at position 10 [Pro(CCT) → Leu(CTT)] and position 200 to 201 [Leu(CTC)Ala(GCG) → Leu(CTG)Arg(CGC)]. Sequence analysis of apparently normal subjects and patients with G_{M1} gangliosidosis or Morquio B disease revealed that Leu10 is most common, but Pro10 is also found in fewer than 10 percent of the subjects analyzed. The expression products of the cDNAs GP8 (with Pro10) and GPN (with Leu10) exhibited almost the same catalytic activity.[23] Pro10 is probably a neutral polymorphism.

The other, shorter clone of 2.0 kb is a product of alternative splicing.[308] Exons 2, 3, and 5 are skipped. The band corresponding to the 2.0-kb cDNA was hardly visualized by northern blot analysis. Its expression product was not active toward a fluorogenic substrate, 4-methylumbelliferyl β-galactoside, and failed to be sorted to lysosomes. Alternative splicing also has been observed for other lysosomal enzymes, but its physiologic significance is unknown.[309]

A full-length cDNA clone for mouse β-galactosidase was isolated on the basis of homology with the human gene.[310] The degree of similarity between the human and mouse enzymes was nearly 80 percent in the amino acid sequence, and five of seven putative glycosylation sites in the human sequence are conserved.

cDNA expression

Full-length cDNAs were expressed in COS-1 cells to characterize the gene products.[22, 308, 310, 311] Transient expression led to an increase in β-galactosidase activity up to fivefold 3 days after transfection, and precursors of 84 and 88 kDa were detected by immunoprecipitation from the extract of the transfected cells. The 88-kDa precursor, secreted from transiently transfected[308] or stably transformed[311] COS cells, was efficiently taken up and processed to the 64-kDa mature form by fibroblasts derived from patients with G_{M1} gangliosidosis.[311] No increase in the enzyme activity was detected in COS-1 cells transfected with mutant genes; the endogenous β-galactosidase activity in the host cells was much higher than the residual mutant enzyme activity. In this system, newly synthesized gene products are overexpressed, the endogenous protective protein is relatively deficient, and the enzyme molecule is degraded rapidly.

In another experiment, the fibroblasts derived from a patient with infantile G_{M1} gangliosidosis, expressing no β-galactosidase activity, were transformed by adenovirus-SV40 recombinant virus.[23] They were transfected with mutant cDNAs inserted into the pCAGGS expression vector.[312] Detection of the residual enzyme activity expressed by the mutant β-galactosidase gene is possible in this system.