Generalized Gangliosidosis: Beta-Galactosidase Deficiency

Abstract. *A profound deficiency (10- to 30-fold) of β-galactosidase activity was found in tissues (liver, spleen, kidney, and brain) from two patients with generalized gangliosidosis; this deficiency is demonstrated as a failure to cleave both* p-*nitrophenyl-β-*D-*galactopyranoside and ganglioside* GM_1 *labeled with* C^{14} *in the terminal galactose. We believe that this enzymic defect is responsible for the accumulation of ganglioside* GM_1 *and is the fundamental enzyme defect in generalized gangliosidosis.*

Generalized gangliosidosis is an inborn error of metabolism in which ganglioside GM_1 accumulates in large excess (*1*, *2*). The disease exhibits the following features: (i) progressive cerebral degeneration leading to death, usually within 2 years; (ii) accumulation of a glycolipid in neurons; in hepatic, splenic, and other histiocytes; and in renal glomerular epithelium; and (iii) the presence of skeletal abnormalities resembling those seen in Hurler's disease (*3*). The ganglioside stored in the brain has been isolated; it has a carbohydrate and fatty acid composition identical to that of the normal major monosialoganglioside, GM_1 (*1*). The sequence and the linkages of the oligosaccharides in the stored ganglioside have also been demonstrated to be identical to those of ganglioside GM_1 (*4*) {galactosyl-(1→3)-*N*-acetyl-galactosaminyl-(1 → 4)-[(2 → 3)-*N*-acetyl-neuraminyl]-galactosyl-(1→4)-glucosyl-(1→1)-[2-*N*-acyl]-sphingosine}.

Generalized gangliosidosis is a familial disease (*3*), probably transmitted in an autosomal recessive manner. The enzymic defect responsible for the accumulation of GM_1 is unknown. One likely possibility is a deficiency of a β-galactosidase which cleaves the terminal galactose from the oligosaccharide moiety of GM_1 (*1*, *5*). To test this possibility, we analyzed tissues from two patients with generalized gangliosidosis for the presence of this enzyme. The first patient was a boy who died at 8 months of age and was the patient we studied when we reported the GM_1 accumulation in this disease (*1*). The second patient was a boy who died at 2 years of age (*6*). We confirmed the diagnosis of generalized gangliosidosis in both patients by isolating gangliosides free from other lipids from gray matter and by demonstrating the accumulation of GM_1 by thin-layer chromatography (*1*). Portions of frozen liver and spleen were available from patient 1; portions of frozen brain, liver, spleen, and kidney were available from patient 2.

Tissues from both patients were assayed for β-galactosidase activity at *p*H 5.0, with *p*-nitrophenyl-β-D-galactopyranoside as the substrate (*7*). A deficiency of β-galactosidase activity was found in the visceral organs from both patients. β-Galactosidase activities in these organs (liver, spleen, and kidney) were nearly 20 times lower in the patients' tissues than in those from 15 control subjects (normal range in liver, 13.6 to 23.9 units; patient 1, 1.0 unit; patient 2, 0.8 unit). In cerebral gray matter, the β-galactosidase activities were approximately ten times lower in the patients (range in 19 controls, 9.3 to 23.7 units; patient 2, 1.8 units). This deficiency was specific for generalized gangliosidosis and was not found in patients with other sphingolipid storage diseases (diagnoses confirmed chemically) including Tay-Sachs disease (four patients), Niemann-Pick disease (one patient), metachromatic leucodystrophy (two patients), infantile cerebral Gaucher's disease (two patients), or a variety of other disorders.

Table 1. Ganglioside GM_1 β-galactosidase activity. Activity is expressed as galactose-C^{14} released (counts per minute) in 60 minutes by galactosidase, purified by the method of Gatt and Rapport (*7*), from 10 mg of tissue (wet weight) at 37°C. Each sample contained labeled GM_1 (4500 count/min, 276 mc/mmole) added to 0.1 ml of purified β-galactosidase in acetate buffer (*p*H 5.0) to make a final volume of 0.2 ml. Radioactive assays were done in duplicate. Nonradioactive GM_1 was added to all control tissue homogenates before β-galactosidase purification in concentrations equivalent to those found in the patients' tissues (0.5 percent of wet weight).

Age	Diagnosis	Enzyme activity
	Cerebral gray matter	
3 yr	Cardiac anomaly	46
10 yr	Ataxia-telangiectasia	23
59 yr	Adenocarcinoma of lung	25
81 yr	Myocardial infarction	36
2 yr	Generalized gangliosidosis	1
	Liver	
2 days	Meconium peritonitis	77
1 mo	Gastroenteritis	73
1 mo	Multiple congenital anomalies	52
2 yr	Lymphangioma	65
32 mo	Chronic renal disease	51
8 mo	Generalized gangliosidosis	4
2 yr	Generalized gangliosidosis	2

The accumulation of GM_1 in the patients' tissues was not responsible for the very low β-galactosidase activity. This was demonstrated in two ways. First, GM_1, which was soluble in the buffers used, was added to brain and liver homogenates from control subjects in amounts equivalent to those found in generalized gangliosidosis. A slight diminution of β-galactosidase activity was found in the GM_1-enriched homogenates of control liver (Fig. 1) and a somewhat larger diminution (10 to 15 percent) was found in gray matter. The enzyme activities in the GM_1-enriched control tissues were still approximately 15 times higher than those in the patients' tissues. Second, liver homogenates from controls and from the patients were mixed in equal proportions, and β-galactosidase activity was assayed under conditions when the rate of hydrolysis was nearly linear (Fig. 1). In the mixed samples from both patients, the rate of hydrolysis was very close (85 percent) to the average of the rates for the controls' and patients' tissues. These results are consistent with simple enzyme dilution alone and indicate that, if an endogenous inhibitor of β-galactosidase is present in the patients' tissues (be it GM_1 or other compounds), the degree of inhibition is not sufficient to explain the 20-fold lowered activity in the patients' tissues.

Acid phosphatase (*p*H 5.0) and β-glucosidase (*p*H 5.0) (*7*) activities were also assayed in the spleen and liver tissues of both patients and in five control subjects. The activities of both enzymes in generalized gangliosidosis fell within the range of the control values.

To determine whether the β-galactosidase deficiency in generalized gangliosidosis could be responsible for the GM_1 accumulation, we determined β-galactosidase activity using GM_1 as substrate. Radioactive GM_1, exclusively labeled with C^{14} in the terminal galactose, was prepared with the $GM_2 \rightarrow GM_1$ uridine diphosphate (UDP)-galactosyl transferase system of Kaufman, Basu, and Roseman (*8*). Ganglioside GM_2 (*9*), isolated from the brain of a child who died from Tay-Sachs disease, was incubated with UDP-galactose-C^{14} in the presence of a particulate fraction from chick embryo brain prepared according to Kaufman *et al.* (*8*). Galactose-C^{14}, UDP-galactose-C^{14}, radioac-

tive GM_1, and nonradioactive GM_2 were products of the reaction. Radioactive GM_1 was purified both by dialysis and by preparative thin-layer chromatography on plates coated with silica gel and developed in a mixture of propanol and water (7:3) (*10*). After purification, radioactive GM_1 had an R_F in this system identical to that of authentic GM_1 and was free from contamination with galactose, UDP-galactose, GM_2, or the asialo derivative of GM_1. The assay for β-galactosidase activity involved estimating the amount of galactose-C^{14} and radioactive GM_1 by preparative paper chromatography [unimpregnated paper, propanol, and water (7:3); R_F of GM_1, 0.9; R_F of galactose, 0.5] and determining radioactivity in a scintillation counter. When preparations of β-galactosidase purified from brain or liver (*7*) were used, the radioactivity of the galactose-C^{14} released from GM_1 was equal to the loss of radioactivity of GM_1. Although the product of the enzymic hydrolysis was not isolated and characterized here, Gatt (*11*) has shown it to be GM_2.

In generalized gangliosidosis, the rate of cleavage of the terminal galactose from GM_1 was markedly diminished in homogenates of liver tissue. In control livers after 60 minutes of incubation, 8 to 11 percent of the GM_1 added was hydrolyzed, whereas the hydrolysis of GM_1 was almost negligible in the liver homogenates from both patients. The difference in activity between controls and patients was 40-fold. Addition of nonradioactive GM_1 to control homogenates in amounts equivalent to those present in the patients' tissues caused only a slight (10 percent) depression of enzymic activity. In the homogenates, the radioactivity of the galactose released was much lower than the loss of radioactive GM_1. This can be explained by conversion of galactose-C^{14} to other compounds and further metabolism by the homogenates.

To obviate this difficulty, we used the method of Gatt and Rapport (*7*) to partially purify β-galactosidase from brain and liver. This procedure resulted in a 15- to 20-fold purification of β-galactosidase from both controls' and patients' tissues. Once again, the rate of cleavage of galactose-C^{14} from GM_1 was markedly lower in generalized gangliosidosis when partially purified preparations of β-galactosidase from liver (Fig. 2) or cerebral gray matter were used (Fig. 3). When preparations of β-galactosidase from control tissue and the patients' tissue were mixed in equal proportions, the rate of galactose hydrolysis was very close to the average of the two rates, both with preparations from liver (Fig. 2) and cerebral gray matter (Fig. 3). Assays of purified β-galactosidase preparations from a number of control subjects demonstrated that activity for GM_1 was diminished 33-fold (patient 2) in brain, and 16-fold (patient 1) and 32-fold (patient 2) in liver (Table 1). One objection to the use of purified β-galactosidase preparations is that the enzyme may not fractionate the same in the diseased tissues. This objection is removed by the demonstration that galactosidase activity (for both GM_1 and *p*-nitrophenyl-β-D-galactopyranoside) is deficient to about the same extent in whole tissue homogenates.

These results demonstrate an extraordinary deficiency of β-galactosidase activity in generalized gangliosidosis. The deficient (or defective) enzyme is an acid hydrolase known to be localized in lysosomes. The enzyme cleaves a variety of substrates other than ganglioside GM_1 (*7, 11*), and the accumulation of substances other than glycolipids (*12*) in tissues from patients with generalized gangliosidosis may be due either to the relative lack of specificity of the enzyme, or to structural alterations of a group of β-galactosidases with similar structures. Although the absence of neuraminidase could also account for the GM_1 accumulation, the fact that asialo-GM_1 also accumulates in generalized gangliosidosis (*13*) argues against a neuraminidase deficiency. Furthermore, a deficiency of β-galactosidase activity would result in the accumulation of both GM_1 and its asialo derivative, since both compounds serve as substrates (*11*). For these reasons,

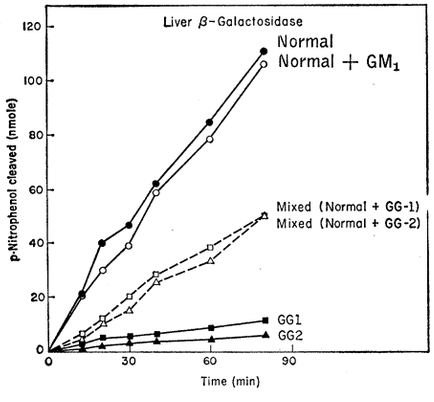

Fig. 1. β-Galactosidase activity of liver. Assay conditions are those of Gatt and Rapport (*7*), except that the tissues were homogenized in 90 volumes of sucrose in ethylenediamine tetraacetate instead of 9 volumes. (*Normal*) Liver tissue from a boy who died at 2 days of age from meconium peritonitis; (*Normal + GM_1*) ganglioside GM_1 added to give a concentration of 0.5 percent of the wet weight; (*GG-1*) 8-month-old patient with generalized gangliosidosis; (*GG-2*) 2-year-old patient with generalized gangliosidosis; (*Mixed*) mixtures of equal proportions of normal and the patients' homogenates.

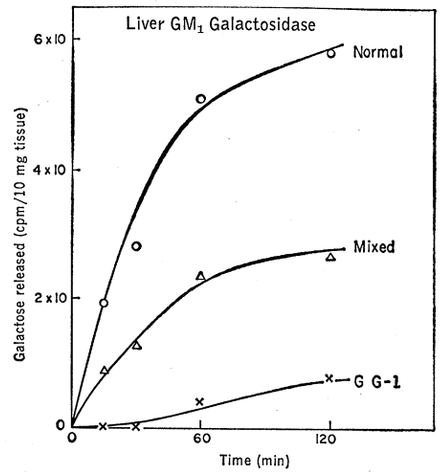

Fig. 2. Galactosidase activity for GM_1 determined with partially purified β-galactosidase from liver. Assay conditions are given in the legend for Table 1; (*Normal*) 3-year-old boy who died from congenital heart disaese; (*GG-1*) 8-month-old patient with generalized gangliosidosis; (*Mixed*) mixture of equal proportions of the normal and patients' β-galactosidase preparations.

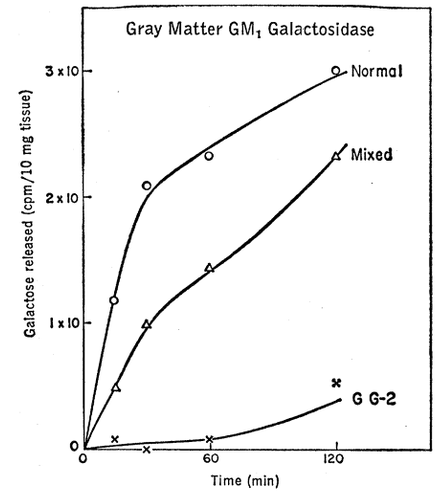

Fig. 3. Galactosidase activity for GM_1 determined with partially purified β-galactosidase from cerebral gray matter. Assay conditions are given in the legend for Table 1; (*Normal*) 32-month-old boy who died from chronic renal disease; (*GG-2*) 2-year-old patient with generalized gangliosidosis; (*Mixed*) mixture of equal proportions of the normal and patients' β-galactosidase preparations.

we believe that the deficiency of β-galactosidase is the fundamental enzymic defect in generalized gangliosidosis (*14*). Assays of tissues and body fluids for β-galactosidase activity should provide a means for determining both homozygotic patients and heterozygotic carriers of the abnormal gene.

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Lactate Dehydrogenase Isozymes in Parthenogenetic Teiid Lizards (Cnemidophorus)

Abstract. *Heterozygosity occurs at the lactate dehydrogenase* b-*locus in two diploid parthenogenetic species of* Cnemidophorus. *Each such species produces two different B subunits, one of which is also found in two sexual species and in two triploid parthenogenetic species; the other occurs also in a third sexual species. Interspecific hybridization between sexual species carrying different* b-*alleles and producing different B subunits may be responsible for the heterozygosity at the lactate dehydrogenase* b-*locus in diploid parthenogenetic* Cnemidophorus.

Interspecific hybridization has been implicated in the origin of all natural instances of vertebrate parthenogenesis. Morphological considerations have fostered the speculation that *Poecilia formosa,* a diploid gynogenetic teleost, resulted from a cross between *P. sphenops* and *P. latipinna* (*1*). The suspected hybrid origin of triploid gynogenetic ambystomatid salamanders has been recently documented by biochemical evidence (*2*). Hybridization has been hypothesized for the origin of a triploid gynogenetic teleost, *Poeciliopsis* Cy (*3*), and of parthenogenetic species of the lizard genus *Lacerta* (*4*).

Lowe and Wright (*5*) proposed hybrid origins for parthenogenetic species of *Cnemidophorus* on the basis of karyotypic evidence that haploid chromosome complements from distinct sexual species were combined in certain parthenogenones. The evidence of hybridization was strongest in *C. neomexicanus*; its diploid chromosome complement could be partitioned into distinct haploid sets matching closely the haploid chromosomes of *C. tigris* and *C. inornatus,* two sympatric sexual species with quite different chromosome morphologies.

Our preliminary electrophoretic analysis of a variety of proteins from three sexual and four parthenogenetic species of *Cnemidophorus* supports the proposed hybrid origin of *C. neomexicanus* and suggests a similar hybrid origin for a second diploid parthenogenetic species, *C. tesselatus,* class E (*5, 6*).

Lizards used by us were captured alive in southern New Mexico and western Texas during August 1967. Enzyme studies were performed on various tissues fresh from the lizard body or after storage in a freezer. Glass-ground tissue homogenates containing 25 mg of tissue per milliliter of gel buffer were centrifuged at 40,000g for 30 minutes. Samples of the supernatant were subjected to electrophoresis on vertical starch gel and analyzed for specific enzyme activity according to reported techniques (*7*). A twofold dilution of the prescribed (*7*) gel buffer was used, with a voltage gradient of 6 volt/cm for 15 to 16 hours. Serum and red-cell proteins were examined by reported procedures (*8*), with substitution of agarose electrophoresis (*9*) in the separation of serum proteins.

All lizards contributing tissues to this study are deposited in the Museum of Comparative Zoology, Harvard University; identification of the species has been confirmed (*10*). In the case of the lactate dehydrogenase (LDH) patterns, at least three individuals from each of the seven species have been analyzed without detection of infraspecific differences.

Malate dehydrogenase isozymes from the seven species exhibited indistinguishable electrophoretic mobilities; likewise, hemoglobin and serum albumin showed no differences. Lactate dehydrogenase isozymes, however, showed differences: in each tissue studied, the seven species were divided among three distinct electrophoretic patterns (Figs. 1–3).

In most vertebrates, LDH isozymes arise from the association of two different subunits, A and B, to form five possible tetramer combinations: A_0B_4(LDH-1); A_1B_3(LDH-2); A_2B_2-(LDH-3); A_3B_1(LDH-4); and A_4B_0-(LDH-5) (*11*). Genetic studies have shown that the synthesis of A and B subunits is under the control of structural genes at two distinct loci: *a* and *b* (*12–14*). It is assumed that these principles apply to LDH isozymes of the species we discuss.

Each of three sexual species, *C. tigris, C. gularis,* and *C. inornatus,* and of two triploid parthenogenetic species, *C. uniparens* and *C. exsanguis,* produces no more than the predicted five isozymes in tissues where both A and B subunits are developed (Figs. 1 and 2). These species differ in that the fastest band, corresponding to LDH-1 (A_0B_4), of *C. tigris* (No. 1 in Figs. 1 and 2) exhibits greater mobility than the fastest band in each of the other four species (Nos. 3, 5, 6, and 7 in Figs. 1 and 2). Variation in LDH-5 (A_4B_0) mobility among the species studied has not been detected. The relative mobility of the individual bands in electrophoretic patterns from the above five species supports the interpretation that these species synthesize similar A subunits, but that *C. tigris* produces a variant B subunit that we shall call B′.

Two diploid parthenogenetic species, *C. neomexicanus* and *C. tesselatus* (Nos.