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Multiple glycosidase deficiencies in a case of juvenile (type 3) Gaucher disease

(glucocerebrosidase/β-xylosidase/β-glucosidase/sphingolipidosis/glucocerebroside)

YU-BIN CHIAO*, GREGORY M. HOYSON*, STEPHEN P. PETERS*, ROBERT E. LEE†, WARREN DIVEN†, JEROME V. MURPHY‡, AND ROBERT H. GLEW*§

Departments of *Biochemistry and † Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, and ‡ Department of Neurology, Milwaukee Children's Hospital, Milwaukee, Wisconsin 53233

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ABSTRACT Biochemical investigations were performed on autopsy tissues obtained from an 11-year-old girl who died with the juvenile, subacute neuropathic form of Gaucher disease. In addition to the expected deficiency of glucocerebrosidase activity, extracts of both liver and kidney from this individual displayed a profound (≥90%) deficiency of "soluble" β-glucosidase, β-xylosidase, and β-galactosidase activities. Fibroblasts obtained from this individual also contained markedly reduced levels of β-xylosidase activity but normal levels of β-D-fucosidase and β-galactosidase activity. Because the soluble β-glucosidase, β-xylosidase, and a portion of the β-galactosidase activities from control human liver all cochromatographed on a gel filtration column of Sephadex G-200, it is suggested that these activities all reside in a single enzyme, analogous to the situation described in a number of nonhuman, mammalian tissues. This demonstration of multiple glycosidase deficiencies in addition to the deficiency of glucocerebrosidase in a case of subacute neuropathic Gaucher disease suggests that other biochemical aberrations, in addition to a deficiency of glucocerebrosidase, might contribute to pathology in some cases of Gaucher disease.

Gaucher disease is an autosomal recessive inborn error of metabolism characterized by the accumulation of glucocerebroside in lysosomes of reticuloendothelial cells of affected individuals. The glycosphingolipid accumulates as a result of a marked deficiency in glucocerebrosidase, the enzyme responsible for its degradation (1). The diagnosis of Gaucher disease can be made from clinical signs and symptoms together with microscopic examination of a bone marrow aspirate for typical "Gaucher cells" (2), or by the determination of glucocerebroside:β-glucosidase (EC 3.2.1.45) activity using either a fluorogenic substrate (3–5) or radiolabeled glucocerebroside (6) as substrate.

This sphingolipidosis presents most often in an adult, chronic, nonneuropathic form (type 1) (7) characterized by splenomegaly, hepatomegaly, anemia, thrombocytopenia, and erosion of the cortices of the long bones (8). Patients with this form of the disease display a wide variety of clinical severity: some individuals are devastated by the disease as early as the 3rd decade of life while others have relatively symptom-free lives for 7 or 8 decades. Although a lack of neurologic involvement is often cited as the distinguishing feature of the adult form of the disease, neurologic disorders in adults have been documented (9, 10). The second form of Gaucher disease is the rare, acute neuropathic or infantile form (type 2) (7), which is characterized by extensive neurologic involvement, hepatosplenomegaly, and death before age 2. Patients with juvenile or subacute neuropathic Gaucher disease (type 3) have central nervous system involvement, Gaucher cells in nonneural tissues (7), and survival after the 2nd year of life.

The biochemical basis that distinguishes the three clinical forms of Gaucher disease is unknown. Brady and King (8) have suggested that the severity of the disease is related to the degree of deficiency of glucocerebrosidase activity. However, a number of laboratories have not found a correlation between the degree of deficiency of glucocerebrosidase activity and the extent of pathology or organ involvement in various forms of Gaucher disease (11–13).

Kanfer *et al.* (14) have put forth an alternate explanation to account for the difference between infantile and adult Gaucher disease. In addition to having the deficiency in particulate, membrane-associated glucocerebrosidase, homogenates of brain and spleen but not liver from two infantile, neuropathic cases of Gaucher disease were found to be profoundly deficient in a second, soluble β-glucosidase (EC 3.2.1.21). The soluble β-glucosidase, which is distinct from particulate glucocerebrosidase, appears not to be deficient in the spleen of adults with type I Gaucher disease (14, 15). The soluble β-glucosidase probably represents the aryl β-glucosidase described by a number of investigators that will hydrolyze 4-methylumbelliferyl-β-D-glucopyranoside (16, 17) and steroid β-glucosides (14).

Ockerman has reported several cases of Gaucher disease of unspecified clinical type in which the patients, in addition to being deficient in particulate glucocerebrosidase, also appeared to be deficient in the soluble aryl β-glucosidase (18, 19). An extract of liver from one of these patients with Gaucher disease that was profoundly deficient in soluble β-glucosidase activity was also markedly deficient in soluble β-xylosidase activity (18); however, in this report the type of Gaucher disease that served as a source of enzyme was not indicated nor was specific activity of β-xylosidase compared in crude homogenates of control and Gaucher tissue. In the more recent study (19), homogenates of liver from three children with an unspecified type of Gaucher disease were shown to be profoundly deficient in β-D-fucosidase activity; information concerning the β-xylosidase content of these tissues was not provided.

Recently, Owada *et al.* (20) described four cases of acute neuropathic and one case of subacute neuropathic Gaucher disease in which the activity of aryl β-glucosidase was markedly deficient in extracts of spleen and fibroblasts. However, a deficiency in *liver* aryl β-glucosidase was found in only two of these patients and both had the acute neuropathic form of Gaucher disease.

Glew *et al.* (17) have shown that a soluble aryl β-glucosidase from rat kidney cortex has broad specificity in that the exten-

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§ To whom all correspondence should be addressed.

sively purified enzyme will hydrolyze β-galactosides, β-xylosides, and α-L-arabinosides, as well as β-glucosides. It appears that this soluble aryl β-glucosidase that is deficient in certain patients with acute or subacute neuropathic Gaucher disease as described by Kanfer *et al.* (14), Ockerman and colleagues (18, 19), and Owada *et al.* (20) is analogous to the broad-specificity aryl hydrolase described by Glew *et al.* (17). If this is true, patients with a deficiency of soluble β-glucosidase activity should also be deficient in soluble β-xylosidase and β-galactosidase activities.

In the present report we describe the results of biochemical investigations of extracts of liver, kidney, and fibroblasts from a patient with subacute neuropathic Gaucher disease. These tissues are shown not only to be glucocerebrosidase deficient, but also greater than 90% deficient in the activities of soluble β-glucosidase, β-xylosidase, and β-galactosidase.

MATERIALS AND METHODS

Case Description. At birth, the patient was a 5-pound, 10-ounce (2.6 kg) female who was the product of an 8-month pregnancy. She was noted to have a protuberant abdomen at 10 mo of age. At 2 yr of age strabismus on the left side and marked hepatosplenomegaly were evident. A bone marrow biopsy performed at 3 yr of age revealed the presence of Gaucher cells and splenectomy performed at that time yielded a 745-g spleen. Spasticity and opisthotonic posturing were noted at 3¾ yr and a seizure disorder began at age 4 yr. Immediately prior to her death at 11 yr of age, seizures were frequent, respirations were labored, and swallowing was poor.

The glucocerebroside content of the patient's liver was 12.1 mg/g wet weight, which is similar to the values reported by others (7). Two years prior to her death, leukocyte glucocerebrosidase activity, determined according to the method of Peters *et al.* (21), was 1.11 nmol/mg per hr (controls 6.88–9.10, mean 8.00), and her mother's activity was 4.61 nmol/mg per hr. This case therefore conforms to the generally accepted definition of type 3, subacute neuropathic Gaucher disease (7).

Source of Enzymes. All procedures were performed at 1°–4°. Liver and kidney samples (1 g) were minced, suspended in 7 ml of distilled water, and homogenized with 10 passes of a Potter–Elvehjem homogenizer. The crude homogenate was centrifuged at 100,000 × *g* for 60 min, and the resulting supernatant served as the source of soluble glycosidases.

Enzyme Assays. Glucocerebrosidase was assayed using *N*-[^{14}C]stearoylglucocerebroside as substrate as described elsewhere (21, 22). Unless otherwise indicated, the standard assays for β-glucosidase, β-xylosidase, β-galactosidase, α-L-arabinosidase, and α-mannosidase used the fluorogenic 4-methylumbelliferyl derivatives of the appropriate glycoside as substrate and contained: 2.0 mM substrate, 0.2 M sodium acetate (pH 5.5), and appropriate amounts of enzyme (usually 0.05–0.15 mg of protein) in a final volume of 0.1 ml. Acid phosphatase was assayed in 0.2 M sodium acetate buffer (pH 5.0) with 4.0 mM 4-methylumbelliferyl phosphate as substrate. Hexosaminidase was assayed in 0.08 M citrate/phosphate buffer (pH 4.4) containing 0.01% bovine serum albumin with 5.0 mM 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside as substrate. Incubations were carried out at 37° for 30–120 min with gentle shaking and fluorescence was determined as previously described (5, 21). β-D-Fucosidase was estimated using *p*-nitrophenyl-β-D-fucoside (2 mM) as substrate in a 0.1-ml incubation medium that contained 0.2 M sodium acetate (pH 5.5); after 3.5 hr at 37°, the reaction was terminated by the addition of 0.9 ml of glycine/ammonium hydroxide buffer (0.3 M, pH 10.5) and liberated *p*-nitrophenol was estimated spectrophotometrically (ϵ_{410}, 14,300 M^{-1} cm^{-1}).

One unit of enzyme activity is defined as the amount of enzyme that cleaves 1 nmol of sugar from the appropriate substrate per hr at 37°.

Protein Determination. Protein was estimated by the method of Lowry *et al.* (23) with bovine serum albumin as a standard.

Fibroblast Cultures. Fibroblasts were grown from skin obtained by biopsy or at autopsy in Eagle's minimum essential medium modified to contain 0.22% (wt/vol) $NaHCO_3$, 1% (wt/vol) nonessential amino acids, 10% (vol/vol) virus-screened fetal calf serum, and antibiotics. Cells were grown at 37° in an atmosphere of 95% air/5% CO_2. As soon as growth of primary cultures was well established, fibroblasts were subcultured into 250-ml Falcon flasks. When fully confluent, cells were removed by trypsinization, centrifuged at 250 × *g* for 10 min, and washed twice in phosphate-buffered saline, pH 7.4.

RESULTS

Multiple Glycosidase Deficiencies in Gaucher Liver and Kidney. As was described for leukocytes, glucocerebrosidase activity was also markedly deficient in liver and kidney from this patient with subacute neuropathic Gaucher disease. Her liver contained 115 units of glucocerebrosidase activity per g wet weight (control, 798 units/g); her kidney was similarly deficient in this activity.

Table 1 shows the content of soluble hydrolases obtained from aqueous extracts of control and Gaucher liver and kidney. The activities of β-glucosidase, β-xylosidase, and β-galactosidase are all markedly (≥90%) deficient in both Gaucher liver and kidney. α-L-Arabinosidase is deficient in liver but not kidney, while the control enzyme activities α-mannosidase, acid phosphatase, and hexosaminidase are either normal or elevated. However, the β-D-fucosidase content of Gaucher liver and kidney was not markedly reduced when compared with control tissues; β-D-fucosidase activity was 2-fold elevated in Gaucher kidney and diminished by only 20–30% in Gaucher liver.

We have shown previously (21) that although the conditions of homogenization employed in the present study solubilize only minimal glucocerebrosidase activity, they solubilize most of the aryl β-glucosidase activity in the tissue. In fact, under the conditions of assay and homogenization employed in the present report, approximately 85% of the total β-glucosidase and β-xylosidase in liver, and 60% of the total β-glucosidase and β-xylosidase in kidney were soluble.

A deficiency of β-glucosidase and β-xylosidase activities was also present in fibroblasts obtained from this patient with subacute neuropathic Gaucher disease (Table 2). However, no deficiency of β-galactosidase or β-D-fucosidase activity was observed in fibroblasts.

Properties of Soluble β-Glucosidase in Control and Gaucher Liver. The pH optimum of the soluble β-glucosidase of normal human liver was between pH 5 and 6. The very low level of residual soluble β-glucosidase activity in the Gaucher liver had its greatest activity at pH 5.5. The K_m (4-methylumbelliferyl-β-D-glucopyranoside) for the soluble β-glucosidase of control liver was 0.11 mM; the K_m for the residual soluble β-glucosidase from the Gaucher liver was 0.24 mM. The soluble β-glucosidase activities in liver and kidney from control and Gaucher tissue were all equally sensitive to heat inactivation at 45° ($t_{1/2}$ = 95 ± 5 min).

Gel Filtration Chromatography of Soluble Glycosidase Activities from Control and Gaucher Liver and Kidney. To

Table 1. Soluble glycosidase activities in Gaucher and control liver and kidney

Enzyme	Activity, units/g wet weight of tissue							
	Gaucher liver		Control* liver		Gaucher kidney		Control* kidney	
	Absolute	% of control†	Average	Range	Absolute	% of control†	Average	Range
β-Glucosidase	42	0.8	5,017	2,201–11,000	82.2	7.2	1,150	820–1,760
β-Xylosidase	3.5	0.3	1,330	577–2,380	5.9	2.1	279	191–451
β-Galactosidase	783	6.2	12,600	7,740–16,596	831	11.2	7,420	3,460–11,300
α-L-Arabinosidase	855	12.8	6,670	3,950–11,000	1,330	65.9	2,020	1,565–2,250
β-D-Fucosidase	840	74.3	1,130	280–1,210	509	220	232	166–324
α-Mannosidase	2,050	108	1,900	970–3,140	3,450	110	3,140	2,705–4,040
Acid phosphatase	183,000	173	106,000	95,760–119,000	80,200	56.2	143,000	116,740–185,300
Hexosaminidase	894,000	344	260,000	216,000–304,230	548,000	151	363,000	327,000–415,000

* Control liver and kidney were obtained at autopsy from four different individuals.
† Values obtained for the Gaucher extract expressed as percent of the average of the controls.

obtain information concerning the molecular properties of the residual hydrolase activities in the soluble fraction of liver and kidney in this case of subacute neuropathic Gaucher disease, we subjected the high-speed supernatant fractions to gel filtration chromatography on a column of Sephadex G-200 (Fig. 1). The soluble β-glucosidase, β-xylosidase, and α-L-arabinosidase activities from control liver all cochromatographed as a single peak on the gel filtration column (Fig. 1*B*). An apparent molecular weight of 55,000–60,000 was determined for these activities. The residual β-glucosidase activity in the supernatant from Gaucher liver was so low that only baseline activity could be detected when fractions from the gel filtration column were assayed for activity (Fig. 1*A*).

Residual α-L-arabinosidase activity in the extract of Gaucher liver, however, chromatographed as two prominent and well-resolved species. The larger form of α-L-arabinosidase eluted in the void volume of the column (molecular weight > 500,000) and the smaller form eluted with an approximate molecular weight of 125,000. It appears, therefore, that the soluble α-L-arabinosidase of the Gaucher liver is not only reduced in activity but is also considerably larger in size than the predominant species of α-L-arabinosidase of control liver.

In the case of the extract of Gaucher kidney (Fig. 1*C*), residual β-glucosidase activity chromatographed along with the hemoglobin standard, whereas the α-L-arabinosidase activity was again found close to the void volume of the column, in fractions suggesting an apparent molecular weight of 120,000. We do not know if these higher molecular weight forms of soluble α-L-arabinosidase found in this Gaucher tissue represent new and qualitatively distinct isoenzymes, or if they are the result of some defect in the metabolism of the α-L-arabinosidase that is normally present in control tissues. Because human liver has been shown to contain two species of α-L-arabinosidase that can be resolved by chromatographic means (19), the residual α-L-arabinosidase activity present in extracts of the Gaucher tissues may represent a form of the enzyme that is not decreased in Gaucher disease.

Soluble β-xylosidase activity is essentially absent in both

Table 2. Glycosidase activities in Gaucher and control fibroblasts

Enzyme	Activity, units/mg protein			
	Gaucher fibroblasts		Control fibroblasts*	
	Absolute	% of control†	Average	Range
β-Glucosidase	8.16	14.2	57.6	45.7–79.0
β-Xylosidase	0.33	4.5	7.27	4.67–9.12
β-Galactosidase	293	91.3	321	265–361
β-D-Fucosidase	39.4	99.5	39.6	33.1–51.2
α-L-Arabinosidase	75.6	86.8	87.1	78.8–91.7
Acid phosphatase	1140	83.8	1360	1300–1420
Hexosaminidase	401	106	378	279–451

Fibroblasts were grown and harvested as described in *Materials and Methods*, suspended in saline to a protein concentration of 0.5–6 mg/ml, and then sonicated. Hydrolase assays were performed with the appropriate 4-methylumbelliferyl substrates. β-Glucosidase, β-xylosidase, and α-L-arabinosidase assays contained 3.0 mM substrate, 0.2 M sodium acetate buffer (pH 5.5), and appropriate amounts of enzyme. Hexosaminidase and β-galactosidase assays were similar except they contained only 1.0 mM substrate. Acid phosphatase assays contained 3.0 mM substrate but were performed in 0.2 M sodium acetate buffer, pH 4.5.
* Three different normal lines of fibroblasts served as controls.
† Values obtained for the Gaucher fibroblasts expressed as percent of the average of the controls.

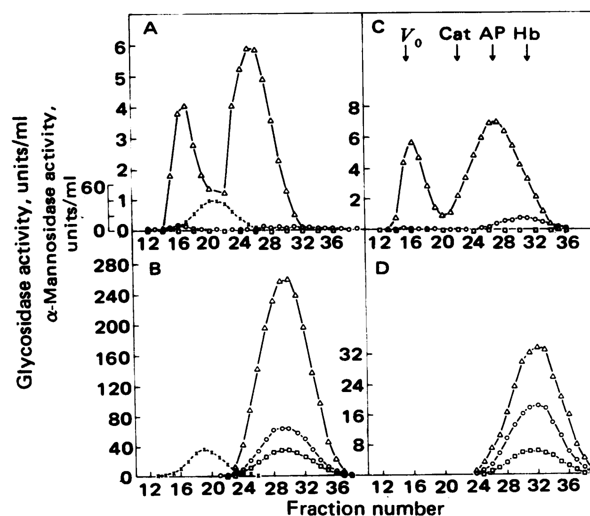

FIG. 1. Gel filtration chromatography of various soluble glycosidases from Gaucher and control liver and kidney. High-speed supernatant fractions (1 ml) were added onto a Sephadex G-200 column (1.2 × 40 cm) and eluted with 10 mM sodium phosphate buffer (pH 7.0) containing 10 mM 2-mercaptoethanol. Fractions (1.2 ml) were collected and assayed for various glycosidase activities. (*A*) Gaucher liver; (*B*) control liver; (*C*) Gaucher kidney; (*D*) control kidney. Δ, α-L-Arabinosidase; O, β-glucosidase; □, β-xylosidase; ×, α-mannosidase. Hb, human hemoglobin (64,500 daltons); AP, *Escherichia coli* alkaline phosphatase (80,000 daltons); Cat, beef liver catalase (240,000 daltons); V_0, void volume.

Gaucher liver and kidney and we could not detect this activity in fractions from the gel filtration column (Fig. 1 *A* and *C*).

The α-mannosidase activities in the supernatant fractions from liver and kidney of both control and Gaucher extracts all chromatographed in a nearly identical manner on the Sephadex G-200 column. The amounts of activity contained in the most active fractions as well as their elution positions were similar (Fig. 1 *A* and *B*).

Because there are at least three different species of soluble β-galactosidase in normal human tissues (18), it was not surprising that the β-galactosidase profile from the Sephadex G-200 column appeared as a broad peak covering much of the included volume of the column (Fig. 2). The β-galactosidase activity in the high-speed supernatant fraction from control liver fractionated into a minor component that appeared in the void volume and a second more prominent, relatively broad peak of β-galactosidase activity that eluted in the region of the hemoglobin standard. The residual β-galactosidase activity (approximately 5% of control) in the corresponding extract of Gaucher liver eluted as a single peak with an apparent molecular weight of 135,000. Chromatography of the kidney extracts on sephadex G-200 yielded results essentially the same as those shown in Fig. 2 for the extracts of Gaucher and control liver (data not shown).

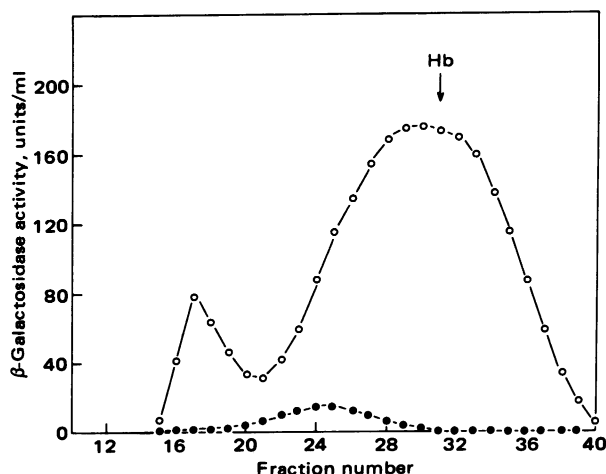

FIG. 2. Gel filtration chromatography of soluble β-galactosidase from Gaucher and control liver. The column is the same as that described in Fig. 1. ○, β-Galactosidase from control liver; ●, β-galactosidase from Gaucher liver.

DISCUSSION

This report describes the biochemical abnormalities observed in a patient with subacute neuropathic Gaucher disease. In addition to the expected deficiency in glucocerebrosidase activity, liver and kidney from this individual were also deficient in soluble β-glucosidase, β-xylosidase, and β-galactosidase activities. The finding of this deficiency in kidney, as well as liver, is, in our opinion, noteworthy. While liver is involved in the disease process of Gaucher disease and accumulates large quantities of glucocerebrosidase, kidney is generally not considered to be involved in Gaucher disease. Therefore, this aryl hydrolase deficiency occurs in uninvolved (kidney) as well as involved (liver) visceral organs. The finding of a significant deficiency of both β-glucosidase and β-xylosidase in fibroblasts from this patient also suggests that this deficiency is a relatively consistent feature of this patient's tissues. The finding of a normal level of β-galactosidase in fibroblasts from this patient but not in liver or kidney suggests that the aryl hydrolase may not be identical in all body tissues, or that the quantity of various β-galactosidases differs in different tissues. Our results suggest that very little of the β-galactosidase activity in fibroblasts is due to the β-galactosidase activity associated with the broad-specificity aryl β-glucosidase enzyme.

Studies using nonhuman mammalian tissues have demonstrated that most tissues contain a soluble aryl glycosidase with multiple enzymatic activities (16). For example, a purified glycosidase from the cytosol of rat kidney cortex has been shown to have β-glucosidase, β-galactosidase, β-xylosidase, and α-L-arabinosidase activities (17). In general, tissues from this individual with subacute neuropathic Gaucher disease lack these same enzymatic activities. However, the data that we present contain two observations inconsistent with this idea. First, a deficiency of a α-L-arabinosidase is present in liver but not in kidney or fibroblasts. However, examination of extracts of these tissues by chromatography on gel filtration columns reveals that the residual Gaucher α-L-arabinosidase is qualitatively different (it is larger) than that extracted from control tissue. Second, β-galactosidase activity from control tissues does not actually cochromatograph with the same apparent molecular weight as control β-glucosidase and β-xylosidase activities. However, at least three β-galactosidases are known to exist in human tissues (18). A reasonable hypothesis is that human tissues contain a soluble, broad specificity aryl hydrolase, and that this patient with subacute, neuropathic Gaucher disease is deficient in its activity. Because most mammalian tissues contain multiple β-glucosidases (21) and β-galactosidases (18), evaluation of the status of this broad-specificity enzyme that is deficient in the patient that we have described appears to be most effectively done using a β-xyloside substrate.

The finding that β-D-fucosidase activity was not profoundly diminished in liver, kidney, and fibroblasts from this neurologic case is consistent with our observation that 500-fold purified preparations of the aryl β-glucosidase of human liver lacks β-D-fucosidase activity.

The cause of this deficiency in soluble aryl hydrolase activity is obscure. Biochemical aberrations in Gaucher disease range from the primary genetic abnormality, the deficiency of glucocerebrosidase activity (1), to clearly secondary changes, such as the elevation of acid phosphatase activity observed in Gaucher spleen (24). Where on this continuum the aryl hydrolase deficiency falls is not known. However, the finding of this deficiency in an organ lacking pathology (kidney) and in fibroblasts suggests that this deficiency may be closely related to the primary genetic deficiency of glucocerebrosidase activity. If, as appropriate tissues become available, we find intermediate β-D-xylosidase values in heterozygous parents of xylosidase-deficient persons with Gaucher disease, it is tempting to speculate that such cases may be deficient in glucocerebrosidase and aryl β-glucosidase activities because of a defect in a subunit protein that is shared by the two enzymes.

Just as the cause of this aryl hydrolase deficiency is obscure, its consequences are also a mystery. Although it has been suggested that this enzyme hydrolyzes steroid-β-D-glucopyranosides (14), the accumulation of these steroid conjugates has not been demonstrated in Gaucher disease. No other natural substrates have been identified for the aryl hydrolase. The possibility should be entertained that important natural substrates for this enzyme might include galactosides or xylosides as well as glucosides. For example, it is conceivable that this enzyme might function in the metabolism of mucopolysaccharides (i.e., in xylosyl-*O*-serine hydrolysis). Because Gaucher fibroblasts have been shown to contain increased quantities of mucopolysaccharides (25), and because mucopolysaccharides

can inhibit glucocerebrosidase activity (26), a reduction in the activity of the soluble aryl glucosidase could potentiate the glucocerebrosidase deficiency observed in some patients with Gaucher disease. Investigators with access to patients with this form of Gaucher disease are encouraged to evaluate tissues and body fluids for increased amounts of galactosides and xylosides, as well as glucosides other than glucocerebroside.

Is the deficiency of this hydrolase related to the neurological complications seen in patients with the neuropathic forms of Gaucher disease? The finding of a deficiency of this enzyme rather consistently in spleen but only occasionally in liver in patients with neuropathological Gaucher disease (14, 20) provides no clear-cut answer to this question.

Clearly, further biochemical investigations on tissues from a wide variety of patients with diverse clinical presentations of Gaucher disease are required as we try to relate clinical pathology to biochemical aberrations.

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