Am J Hum Genet 32:670–680, 1980

Gaucher Disease. III. Substrate Specificity of Glucocerebrosidase and the Use of Nonlabeled Natural Substrates for the Investigation of Patients

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SUMMARY

A reproducible and convenient method for assaying glucocerebrosidase activity using the natural substrates has been developed. From the insoluble pellet fraction of cultured skin fibroblast homogenates, released glucose was measured enzymically using hexokinase coupled with the glucose-6-phosphate dehydrogenase (G6PD) and nicotinamide adenine dinucleotide phosphate (NADP) system. Optimal enzyme assay conditions required both Triton X-100 and sodium taurocholate, pH 4.8. Glucocerebrosidase activities from three patients with type 1 Gaucher disease were 17.5%, 15.8%, and 11.2% of normal (normal = 198 ± 14 nmol/hr per mg protein, n = 3). The first patient had normal β-glucosidase activity with the artificial fluorogenic umbelliferone substrate.

Interference with the accuracy of the glucose-dependent assay system by either glycolytic or gluconeogenic enzyme activities was not detected under these experimental conditions, and when substrates with long fatty-acid chain lengths (C = 22) were used, markedly decreased glucocerebrosidase activity occurred in both normal individuals and patients. The apparent K_m's for the natural substrates were 0.56 ± 0.05 mM for controls and 2.2–3.3 mM for Gaucher fibroblasts.

These data further support the hypothesis that a structurally altered and catalytically deficient enzyme is synthesized in patients with type 1 Gaucher disease and illustrate the value of the natural substrate in investigating patients.

Received October 1, 1979; revised January 18, 1980.

This investigation was supported, in part, by grant MT-5789 from the Medical Research Council of Canada and a research fellowship from the Ontario Mental Health Foundation to Dr. Choy.

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INTRODUCTION

Gaucher disease is an inherited metabolic disorder characterized by the appearance of large glucocerebroside-laden histiocytes in the spleen, liver, and bone marrow [1–3]. Chronic nonneuropathic (type 1), acute neuropathic (type 2), and subacute neuropathic (type 3) forms have been described [3, 4]. Deficient activity of the lysosomal enzymes glucocerebrosidase (glucosylceramidase, E.C.3.2.1.45) and β-glucosidase (β-D-glucoside glucohydrolase, E.C.3.2.1.21) has been demonstrated in tissues of patients with all three forms of Gaucher disease [5–15]. Although the artificial fluorogenic substrate, 4-methylumbelliferyl β-D-glucopyranoside (4MUGP), is a convenient and sensitive reagent for the diagnosis of Gaucher disease and detection of carriers [7–9, 12], the precision of this assay is impaired by the presence in tissues of other β-glucosidase(s) with hydrolytic activity toward the artificial but not the natural substrate [16–21]. Addition of detergent [19, 22] or lowering the pH of the assay system [20] is required to inhibit activity of these nonspecific β-glucosidases. The existence of variants of Gaucher disease with relatively high levels of residual β-glucosidase activity [21, 22] is an additional problem. Thus, assay procedures employing 4MUGP have diagnostic validity only if the assay conditions are carefully controlled so as to reflect activity of the relevant enzyme [15, 19, 20, 22]. Use of the natural substrate should minimize most of these problems, but preparation of glucocerebroside by organic synthesis or isolation from human tissues and subsequent radiolabeling are extremely laborious. Therefore, the natural substrate is not used in most clinical laboratories. Although a radiolabeling service is available commercially by special order, the cost is prohibitively high. Unlabeled glucocerebroside has been used as a substrate [6], but interpretation of assay results was complicated by the presence of tissue glycolytic enzymes that metabolize glucose released from glucocerebroside [5]. For these reasons, it seemed important to establish a reliable, yet convenient, procedure that utilizes the natural substrate without the above complications for the diagnosis of Gaucher disease and for studies of the nature of the enzymatic defect.

This paper presents a simple method for separating the cytosolic glycolytic enzymes from the membrane-bound glucocerebrosidase [23, 24] prior to assay, thereby making nonlabeled glucocerebroside a reliable substrate. In addition, we optimized conditions for glucocerebrosidase assay, using a sensitive coupling system to measure the amount of glucose produced enzymically. We also studied the substrate specificity of glucocerebrosidase with respect to the nature of the *N*-acyl long-chain fatty-acid component of glucocerebroside to further elucidate the nature of the enzyme defect in Gaucher disease.

MATERIALS AND METHODS

N-stearoyl dihydroglucocerebroside, *N*-palmitoyl dihydroglucocerebroside, *N*-lignoceroyl dihydroglucocerebroside, and glucose diagnostic reagent, which contains hexokinase (yeast, 800 U/liter), G6PD (yeast, 500 U/liter), adenosine triphosphate (ATP, 0.001 mol/liter), NADP (0.0002 mol/liter), and magnesium ions (0.002 mol/liter) in phosphate buffer, pH 7.0, were purchased from Sigma, St. Louis, Mo. Isocytophenoxypolyoxyethanol (Cutscum) was purchased from Fisher, Fair Lawn, N.J. Sources of all other reagents have been indicated [22].

All patients were believed to be of non-Jewish ancestry. Although clinical manifestations were highly variable, none showed any sign of neurological deterioration, and all would, therefore, be classified as type 1.

Skin fibroblasts were obtained from punch biopsies, cultured and harvested as described [10]. The harvested cells were suspended in deionized distilled water at a protein concentration of 5–7 mg/ml and subjected to sonication (Branson sonicator) at an output of 50 W for 10 seconds in ice. The lysed cell homogenates were then centrifuged at 48,000 *g* (or at other specified *g* values) for 90 min at 2°C. After centrifugation, the supernatant fluid was removed by a Pasteur pipette without disturbing the sediment. The residue pellet was resuspended in deionized water and mixed thoroughly so that an even suspension at a final protein concentration of 3–5 mg/ml was formed.

Glucocerebrosidase activity was assayed according to a method modified from Mueller and Rosenberg [25]. Twenty μl of enzyme preparation containing 60–100 μg of protein was added to 180 μl of dispersed mixtures consisting of: glucocerebroside (1 mM), sodium taurocholate (1.65% w/v for *N*-stearoyl dihydroglucocerebroside and 1.1% w/v for *N*-palmitoyl dihydroglucocerebroside), Triton X-100 (0.22% v/v), and citrate buffer (0.044 M), pH 4.8 or as specified (adjusted by the addition of 6 N NaOH). The reaction mixtures were incubated in sealed Pyrex tubes at 37°C in a shaking water bath for 3–6 hrs. The blank system contained a boiled enzyme preparation (100°C, 4 min) added to an identical amount of substrate mixture. The reaction was stopped by immersing the tubes in boiling water for 4 min. After cooling, the contents of each tube was transferred to an Eppendorf centrifuge tube, sealed, and centrifuged at 12,000 *g* (Brinkmann centrifuge, model 3200) for 5 min at 2°C. To a 160 μl aliquot of supernate, 0.8 ml of glucose reagent was added.* Any glucose present will be converted to glucose-6-phosphate by hexokinase in the presence of ATP and then coupled to the subsequent reduction of NADP [26] by G6PD. The glucose reagent mixtures were incubated at room temperature for 5 min for the reaction to go to completion. The amount of NADPH formed was measured as absorbance at 340 nm uv (Gilford spectrophotometer, model 250), and the amount of glucose present was calculated with respect to a glucose standard curve (0–60 nmol) calibrated under identical pH and detergent concentration. For kinetic studies, varied amounts of glucocerebroside were included in the reaction mixtures, and the reaction was stopped after 3 hrs of incubation.

To be certain that the amount of glucose produced and measured by our assay system was a true reflection of glucocerebrosidase hydrolytic activity in fibroblasts, we investigated other possible sources of production or degradation as follows: (1) for the detection of glucose-degradative enzymes, the amount of glucose normally produced over a 4-hr period from our normal controls was added to the enzyme reaction mixtures without the substrate, and the amount of glucose was determined at 0 hr, and at the end of the second and fourth hrs, respectively; (2) for the detection of gluconeogenesis, a similar experiment was conducted without the addition of glucose and substrate. Absorbance at 340 nm was measured at 0 hr, and at the end of the second and fourth hrs, respectively. β-Glucosidase activity was assayed according to our previous procedure [22]. Protein was measured by the method of Lowry et al. [27].

RESULTS

Effect of pH on Glucocerebrosidase Activity

The effect of pH on *N*-stearoyl dihydroglucocerebrosidase activity is shown in figure 1. Within the range of pH studied (3.6–6.0), glucocerebrosidase from both controls and patients appeared to be most active around pH 4.8. Enzyme activity levels from

* After centrifugation, samples for enzymic glucose assay should be withdrawn without disturbing the sedimented insoluble materials; otherwise, turbidity will result in artificially high readings. Because detergents reduce surface tension, addition of glucose reagents and transfer of samples to the cuvette must also be done carefully to avoid air bubbles and subsequent artificially high absorbance.

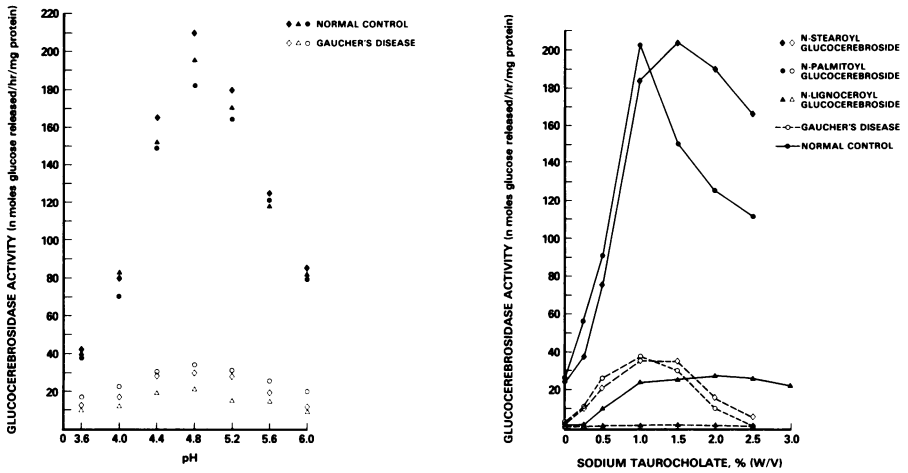


FIG. 1. (LEFT) —Effect of pH on glucocerebrosidase activity from normal control and Gaucher fibroblasts. Levels of enzyme activity were assayed from three controls and three patients with type 1 Gaucher disease. FIG. 2. (RIGHT) —Effect of detergents on *N*-palmitoyl, *N*-stearoyl, and *N*-lignoceroyl dihydroglucocerebrosidase activities from control and Gaucher fibroblasts. Cultured skin fibroblasts were from patient no. 2. Similar results were also observed from patients nos. 1 and 3.

patients were 17.5%, 15.8%, and 11.2% of the normal average. Enzyme activity from control fibroblasts decreased rapidly at pH's below 4.4 or above 5.2. Enzyme activity from fibroblasts of patients, however, appeared to be less sensitive to changes in pH.

When *N*-palmitoyl dihydroglucocerebroside was used as the substrate instead of *N*-stearoyl dihydroglucocerebroside, a similar pH effect was observed. Activity of *N*-palmitoyl dihydroglucocerebrosidase was 72% to 80% of *N*-stearoyl dihydroglucocerebrosidase when assayed under identical experimental conditions. With *N*-lignoceroyl glucocerebroside as substrate, activity of *N*-lignoceroyl dihydroglucocerebrosidase from normal fibroblasts was only 15% of *N*-stearoyl dihydroglucocerebrosidase. Activity of *N*-lignoceroyl dihydroglucocerebrosidase from Gaucher fibroblasts was undetectable.

Effect of Detergents on Glucocerebrosidase Activity

As shown in figure 2, when the concentration of Triton X-100 was held constant at .2%, optimal *N*-palmitoyl glucocerebrosidase and *N*-stearoyl glucocerebrosidase activities were achieved at 1% and 1.5% sodium taurocholate, respectively. Enzyme activity decreased steadily above these concentrations, presumably because of excess detergent. With lignoceroyl dihydroglucocerebroside, enzyme activity remained consistently low in up to 3% sodium taurocholate. Above 3%, there was a dramatic decrease in enzyme activity, most likely because of inactivation of enzyme by excess detergent. When the concentration of sodium taurocholate was held constant at 1%, suboptimal glucocerebrosidase activities (*N*-palmitoyl or *N*-stearoyl) were observed at concentrations of Triton X-100 either above or below .2%. In the absence of both detergents, no glucocerebrosidase activity could be detected from any of the fibroblast enzyme preparations.

The detergent, Cutscum, has been used in several glucocerebrosidase assay procedures [11, 21]. To determine whether this detergent is better in stimulating glucocerebrosidase activity, it was used instead of Triton X-100, according to the procedure of Ben-Yoseph and Nadler [21]. Enzyme activity was 90%–95% of that found with Triton X-100 as the detergent.

Effects of Incubation Time and Protein Concentration

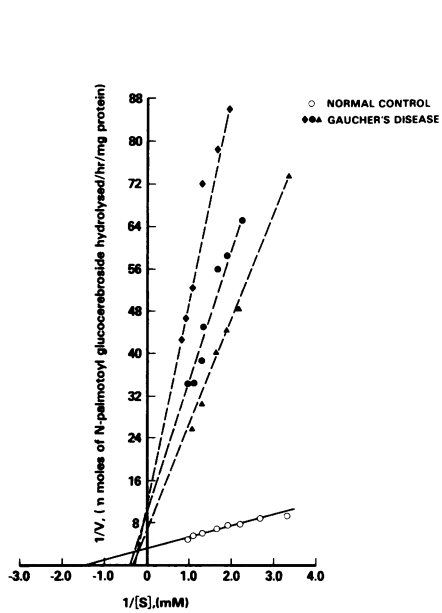
The amount of glucocerebrosides hydrolyzed at 37°C was directly proportional to the time of incubation up to 6 hrs, and to the amount of protein added from 60 to 100 μg.

Detection of the Presence of Glycolytic and Gluconeogenic Enzymes

Throughout the 4-hr period, there was no detectable degradation or increase of glucose. Also, the amount of endogenous glucose and glucose-6-phosphate, as reflected by NADPH, was assayed and found to constitute a negligible background reading at 340 nm (0%–2%) at 0 hr, which remained constant throughout the 4 hrs of incubation. This eliminated the possibility that any glucose formed resulted from endogenous gluconeogenesis rather than glucocerebrosidase hydrolytic activity.

Kinetic Data

The apparent Michaelis constants (K_m's) for both *N*-palmitoyl and *N*-stearoyl glucocerebrosidases from controls were calculated to be 0.56 ± 0.05 mM (figs. 3 and 4). Very different kinetics were observed for both *N*-palmitoyl and *N*-stearoyl



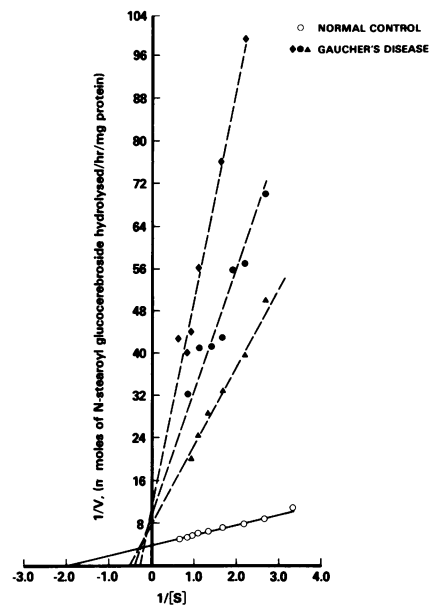


FIG. 3. (LEFT)—Lineweaver-Burk plot of *N*-palmitoyl dihydroglucocerebrosidase from control and Gaucher fibroblasts. FIG. 4. (RIGHT)—Lineweaver-Burk plot of *N*-stearoyl dihydroglucocerebrosidase from control and Gaucher fibroblasts.

glucocerebrosidases from patients with type 1 Gaucher disease. K_m values calculated ranged from 2.2 to 3.3 mM.

Enzyme Activity in Gaucher Fibroblasts Assayed with the Natural and Artificial Substrates

4MUGP β-glucosidase and *N*-stearoyl glucocerebrosidase activities from cultured fibroblasts of patients with type 1 Gaucher disease are shown in table 1. All patients were more deficient than controls in glucocerebrosidase activity. Patient 1 is an unusual case of Gaucher disease whose β-glucosidase activity level was within the normal range when assayed in the absence of sodium taurocholate [22].

The Use of Uncentrifuged Fibroblast Homogenates as Source of Enzyme

As described above, we have used the insoluble pellet fraction after centrifugation as the source of fibroblast enzyme for glucocerebrosidase activity assay to avoid glucose degradation by the soluble glycolytic enzymes. Since a high-speed centrifuge may not be available in some clinical laboratories, we also determined the feasibility of using uncentrifuged sonicated fibroblasts as the source of enzyme. Our enzyme assay system requires a pH of 4.8, and a relatively high concentration of detergents. Because these conditions are unfavorable for glycolytic enzyme activities [28], the glucose formed may not be readily metabolized. The results of these assays are shown in table 2. Deficient activities of glucocerebrosidase were still observed using unspun homogenates from these patients, although the specific activity of glucocerebrosidase may have been decreased because of the presence of soluble proteins which were normally removed after centrifugation.

Distribution of Glucocerebrosidase

Approximately 90%–95% of total enzyme activities (*N*-stearoyl glucocerebrosidase and 4MUGP β-glucosidase) were detected in the pellet fraction after centrifugation. The small amount of glucocerebrosidase and β-glucosidase activity detected in the supernatant fraction could be due to the presence of small pieces of broken lysosomal fragments formed during the sonication procedure. This assumption appears to be correct, as we detected less activity (0%–2%) of glucocerebrosidase and β-gluco-

TABLE 1

β-GLUCOSIDASE AND GLUCOCEREBROSIDASE ACTIVITY FROM CULTURED SKIN FIBROBLASTS OF NORMAL CONTROLS AND PATIENTS WITH TYPE 1 GAUCHER DISEASE

	β-GLUCOSIDASE		GLUCOCEREBROSIDASE	
	Specific activity	Relative %	Specific activity	Relative %
Controls (no. = 3)	176.7 ± 12.4	100.0	197.0 ± 14.0	100.0
Gaucher disease:				
1	131.2	74.0	34.5	17.5
2	42.0	23.8	31.2	15.8
3	51.1	28.9	22.0	11.2

NOTE.—Specific activity expressed as amount of product released in nmol/hr/mg protein.

TABLE 2

GLUCOCEREBROSIDASE SPECIFIC ACTIVITY FROM FIBROBLAST INSOLUBLE PELLETS AND HOMOGENATES FROM CONTROLS AND PATIENTS WITH TYPE 1 GAUCHER DISEASE

	Insoluble pellets	Homogenates
Controls (no. = 3)	197.0 ± 14.0	125.3 ± 18.2
Gaucher disease:		
1	34.5	26.8
2	31.2	26.2
3	22.0	19.7

NOTE. —Specific activity expressed as amount of glucose released in nmol/hr/mg protein.

sidase in the supernatant fraction after centrifugation at 150,000 *g* for 90 min. When the fibroblast homogenates were centrifuged at 10,000 *g* for 90 min, about 15% of total glucocerebrosidase activity was detected in the supernatant fraction.

DISCUSSION

We have developed a method that permits the use of nonlabeled glucocerebroside as a specific and reliable substrate for the diagnosis of Gaucher disease. This procedure, unlike previously documented procedures that utilized radiolabeled glucocerebroside [4, 5], bypasses the problems of organic synthesis and specific radioactive isotope labeling and, therefore, can be performed with relative ease in most clinical laboratories. Furthermore, this method of evaluating glucocerebrosidase activity by enzymic glucose assay requires much less time than methods that utilized glucocerebroside specifically labeled in the *N*-acyl moiety [4, 21]; additional two-phase partition or column chromatography is required by these methods to separate the mixtures of product and substrate before radioactivity can be determined [4, 21, 29].

Another advantage of using nonlabeled natural substrate is elimination of the isotope dilution effect [30]. Because of the relatively large amount of glucocerebroside that accumulates in tissues of Gaucher patients, specific determination of dilution of labeled substrate by endogenous glucocerebroside is required [5]. Otherwise, glucocerebrosidase activity will appear artificially low [5].

Our method of using the fibroblast insoluble pellet fraction as the source of enzyme is also justifiable since glucocerebrosidase is a lysosomal enzyme that remains insoluble after disruption of cells [23, 24, 31]. More than 90% of total enzyme activity was recovered in the pellet fraction with concomitant removal of the glycolytic enzymes in the supernatant fraction which may interfere with the assay. Similar high recovery of β-glucosidase activity in fibroblast insoluble pellets has been reported by other investigators after cell homogenization and high-speed centrifugation [25, 31].

Comparison of β-glucosidase and glucocerebrosidase activities (table 1) demonstrated that the natural substrate is a more reliable reagent for the diagnosis of Gaucher disease, especially when variants with relatively high residual β-glucosidase activity were encountered (table 1, patient no. 1) [21, 22]. Since the natural substrate, glucocerebroside, is far more complex in structure than the artificial umbelliferyl β-glucoside substrate, a structurally altered glucocerebrosidase in Gaucher disease

may, therefore, be more inefficient in hydrolyzing glucocerebroside. This altered enzyme may also be more labile in the presence of the sodium taurocholate [22] used in the glucocerebrosidase assay system, resulting in further lowering of activity. In addition, there may be other nonspecific β-glucosidases present in tissues with no activity toward the natural sphingolipid substrate [19]. Thus, the level of enzyme activity assayed from these tissues will be lower when the natural substrate is used.

Although fibroblast homogenates can be used for glucocerebrosidase assay, the following should be noted: (1) the background blank reading will be higher because of the presence of soluble endogenous glucose and glucose-6-phosphate in the cytosol, which, subsequently, increases the concentration of NADPH; (2) the specific activity of glucocerebrosidase will be decreased because of the presence of additional cytosolic proteins; and (3) excess endogenous glucocerebroside accumulated in Gaucher fibroblasts was sometimes found as a thin surface layer of lipid after sonication and centrifugation at 48,000 *g* or more, as with our patient no. 3. This lipid material was subsequently removed together with the supernatant fluid. If cell homogenates were being used, the presence of this excess glucocerebroside, and possibly polyglycosylceramides [32], would elevate the substrate concentration and create inconsistent assay conditions between Gaucher patients and controls.

Our data indicate that both *N*-palmitoyl and *N*-stearoyl dihydroglucocerebrosides are suitable in vitro substrates for glucocerebrosidase activity assay. These glucocerebrosides were hydrolyzed significantly by glucocerebrosidase from normal fibroblasts. Since the difference between these glucocerebrosides lies solely in the aliphatic *N*-acyl moiety which determines the extent of its hydrophobicity and solubility, the amount of detergent in our enzyme assay mixtures may critically affect the various *N*-acyl glucocerebrosidase activities. For example, the less hydrophobic *N*-palmitoyl dihydroglucocerebroside may require a lower concentration of detergent for optimal enzyme activity (1% sodium taurocholate, fig. 2), whereas the more hydrophobic *N*-stearoyl dihydroglucocerebroside may require a higher concentration (1.5% sodium taurocholate, fig. 2). On the other hand, the relatively small amount of hydrolysis by normal fibroblast glucocerebrosidase on *N*-lignoceroyl dihydroglucocerebroside could be due to: (1) excess hydrophobicity and insolubility of this substrate under our experimental conditions (by virtue of the lignoceric acid present in the glucocerebroside) and (2) stearic hindrance created by lignoceric acid at the catalytic site of glucocerebrosidase, as lignoceric acid possesses a longer chain length (C = 22) than either palmitic acid (C = 16) or stearic acid (C = 18). Glucocerebrosidase activity from fibroblasts of patients with type 1 Gaucher disease was consistently deficient toward the *N*-palmitoyl and *N*-stearoyl dihydroglucocerebroside substrates (10%–16%), and activity toward *N*-lignoceroyl dihydroglucocerebroside was undetectable. Since the sensitivity limit of our assay system requires the presence of 1 nmol of glucose, we should still be able to detect traces of activity from these cells if the ratio of hydrolytic activity between *N*-stearoyl dihydroglucocerebroside and *N*-lignoceroyl dihydroglucocerebroside is 1.00:0.15, as in our controls. Therefore, our data suggest that glucocerebrosidase from Gaucher fibroblasts is more deficient or ineffective in hydrolyzing glucocerebrosides with longer fatty-acid chain lengths (around C = 22). This hypothesis is supported by the results of analysis of glucocerebrosides from Gaucher spleens by other investigators

[33–35]. A higher proportion of fatty acids averaging 22 atoms [33] and a lower proportion of unsaturated fatty acids of shorter chain lengths [34, 35] have been found in glucocerebroside of Gaucher spleens than in those from controls. However, our suggestion remains speculative until glucocerebrosidase from the relevant tissues of patients and controls can be purified and characterized in detail in regard to substrate specificity, affinity, and other properties, including the nature of the lipids present. A recent study [31] indicated that the addition of G_{M1} ganglioside to fibroblast homogenates resulted in a 16-fold increase in V_{max} of 4MUGP β-glucosidase in type 1 Gaucher disease and a three-fold increase in normal cells but no change in K_m. It will be interesting to observe the effect of G_{M1} ganglioside on glucocerebrosidase activity from these cells, specifically with different *N*-acyl moieties in the glucocerebroside substrate molecule.

In a study by Mueller and Rosenberg [25], glucocerebrosidase and β-glucosidase from human cultured skin fibroblasts were solubilized, partially purified, and characterized. The enzyme specific activity, as well as the K_m value of glucocerebrosidase from normal control fibroblasts, were lower than ours. These discrepancies could be due to different sources of substrate and different experimental conditions. The natural substrate, glucocerebroside, employed in their enzyme assay system was purified from the total lipid extract of a Gaucher spleen [33], the *N*-acyl moiety of which could be quite different from ours. A partially purified enzyme preparation was also used in their study. These may account for the differences in K_m value (0.18 mM vs. 0.56 mM). Furthermore, phosphate buffer (5 mM) was used in their enzyme assay and gel filtration chromatography. Since phosphate ion is a potent inhibitor of human glucocerebrosidase activity [36], this may partially explain the difference in enzyme activity.

The sensitivity of our assay system enabled us to conduct kinetic studies on glucocerebrosidase from fibroblasts of both the controls and Gaucher patients. In contrast to previous pH studies that used the artificial substrate and yielded inconsistent data regarding the existence of isozymes of β-glucosidase in fibroblasts [8, 9], use of the natural substrate in this study revealed one symmetrical peak (fig. 1). This is further evidence of one form of glucocerebrosidase in fibroblasts of both normal individuals and patients with type I Gaucher disease. The results of our experiments using varying concentrations of the detergents Triton X-100 and sodium taurocholate indicate the importance of detergent concentration in the assay system and the differing substrate specificities of normal and mutant glucocerebrosidase with respect to the *N*-acyl moiety of the substrate (fig. 2). Data from our present (figs. 3 and 4) and previous [22] kinetic studies once again support the hypothesis by other investigators [37, 38] that the underlying molecular defect of type 1 Gaucher disease involves a structurally altered and catalytically deficient enzyme. In view of the clinical and biochemical heterogeneity of this metabolic disorder, it will be interesting to characterize and compare glucocerebrosidase from different tissues between the normal and mutant enzyme, and among the mutant enzymes from patients with the different clinical types of Gaucher disease to elucidate the physiological role of glucocerebrosidase in the balance and maintenance of normal cerebral lipid metabolism.

REFERENCES

1. HALLIDAY N, DENEL HG JR, TRAGERMAN LJ, WARD WE: On the isolation of a glucose-containing cerebroside from spleen in a case of Gaucher's disease. *J Biol Chem* 132:171–180, 1940
2. ROSENBERG A, CHARGAFF E: A reinvestigation of the cerebroside deposited in Gaucher's disease. *J Biol Chem* 233:1323–1326, 1956
3. BRADY RO: in *The Metabolic Basis of Inherited Disease*, 4th ed, edited by STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, New York, McGraw-Hill, 1978, pp 731–746
4. PETERS SP, GLEW RH, LEE RE: Gaucher's disease, in *Practical Enzymology of the Sphingolipidoses*, 1st ed, edited by GLEW RH, PETERS SP, New York, Alan R. Liss, 1977, pp 71–100
5. BRADY RO, KANFER JN, SHAPIRO D: Metabolism of glucocerebrosidase. II. Evidence of enzymatic deficiency in Gaucher's disease. *Biochim Biophys Acta* 18:221–225, 1965
6. PATRICK AD: A deficiency of glucocerebrosidase in Gaucher's disease. *Biochem J* 97:17c, 1965
7. BEUTLER E, KUHL W: The diagnosis of the adult type of Gaucher's disease and its carrier state in peripheral blood leukocytes. *J Lab Clin Med* 76:745–755, 1970
8. BEUTLER E, KUHL W, TRINIDAD F, TEPLITZ R, NADLER HL: β-glucosidase activity in fibroblasts from homozygotes and heterozygotes for Gaucher's disease. *Am J Hum Genet* 23:62–66, 1971
9. HO MW, SECK J, SCHMIDT D, ET AL.: Adult Gaucher's disease: kindred studies and demonstration of a deficiency of acid β-glucosidase in cultured fibroblasts. *Am J Hum Genet* 24:37–45, 1972
10. CHOY FYM, DAVIDSON RG: Gaucher's disease. I. Solubilization and electrophoresis of β-glucosidase from human peripheral blood leukocytes and cultured skin fibroblasts. *Pediatr Res* 12:1115–1120, 1978
11. KLIBANSKY GH, HOFAAN J, ZAIAOV R, MATOTH Y: A comparative study of glucocerebroside and methylumbelliferylglucopyranoside cleaving potency in leukocytes. *Biomedicine* 20:24–30, 1974
12. OCKERMAN PA: Identity of β-glucosidase, β-xylosidase and one of the β-galactosidase activity in human liver when assayed with 4-methylumbelliferyl β-D-glycosides: studies in cases of Gaucher's disease. *Biochim Biophys Acta* 165:59–62, 1968
13. PETERS SP, LEE RT, GLEW RH: A microassay for Gaucher's disease. *Clin Chim Acta* 60:391–396, 1975
14. CHIAO YB, HOYSON GM, PETERS SP, ET AL.: Multiple glycosidase deficiencies in a case of juvenile (type 3) Gaucher disease. *Proc Natl Acad Sci USA* 75:2448–2452, 1978
15. WENGER DA, CLARK C, SATTLER M, WHARTON C: Synthetic substrate β-glucosidase activity in leukocytes: a reproducible method for the identification of patients and carriers of Gaucher's disease. *Clin Genet* 13:145–153, 1978
16. BROADHEAD DM, BUTTERWORTH J: The diagnosis of Gaucher's disease in liver using 4-methylumbelliferyl-β-D-glucopyranoside. *Clin Chim Acta* 74:155–161, 1977
17. HO MW, O'BRIEN JS: Gaucher's disease: deficiency of acid β-glucosidase and reconstitution of enzyme activity in vitro. *Proc Natl Acad Sci USA* 68:2810–2813, 1971
18. OWADA M, SAKIYAMA T, KITOGAWA T: Neuropathic Gaucher's disease with normal 4-methylumbelliferyl-β-glucosidase activity in the liver. *Pediatr Res* 11:641–646, 1977
19. PETERS SP, COYLE P, GLEW RH: Differentiation of β-glucocerebrosidase from β-glucosidase in human tissues using sodium taurocholate. *Arch Biochem Biophys* 175:569–582, 1976
20. TURNER BM, BERATIS NG, HIRSCHHORN K: Cell-specific differences in membrane β-glucosidase from normal and Gaucher cells. *Biochim Biophys Acta* 480:422–449, 1977
21. BEN-YOSEPH Y, NADLER HL: Pitfalls in the use of artificial substrates for the diagnosis of Gaucher's disease. *J Clin Pathol* 31:1091–1093, 1978

22. CHOY FYM, DAVIDSON RG: Gaucher's disease. II. Studies on the kinetics of β-glucosidase and the effects of sodium taurocholate in normal and Gaucher tissues. *Pediatr Res* 14:54–59, 1980
23. HO MW: Identity of "acid" β-glucosidase and glucocerebrosidase in human spleen. *Biochem J* 136:721–729, 1973
24. PENTCHEV PG, BRADY RO, HIBBERT SP, GAL AE, SHAPIRO C: Isolation and characterization of glucocerebrosidase from human placental tissue. *J Biol Chem* 348:5256–5261, 1973
25. MUELLER OT, ROSENBERG A: β-Glucosidase hydrolase activity of normal and glucosylceramidotic cultured human skin fibroblasts. *J Biol Chem* 252:825–829, 1976
26. BARTHELMAI W, CZOK R: Enzymatische Bestimmunger der Glucose in blut, liquor and harn. *Klin Wochenschr* 40:585–591, 1962
27. LOWRY DH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275, 1951
28. SCRUTTON MC, UTTER MF: The regulation of glycolysis and gluconeogenesis in animal tissue. *Annu Rev Biochem* 37:249–302, 1968
29. HYUN JC, MISRA RS, GREENBLATT D, RADIN SN: Synthetic inhibitors of glucocerebroside β-glucosidase. *Arch Biochem Biophys* 166:382–389, 1975
30. ARONEFF S: *Techniques of Radiochemistry*. Ames, Iowa State College Press, 1956
31. MUELLER OT, ROSENBERG A: Activation of membrane-bound glucosylceramide: β-glucosidase in fibroblasts cultured from normal and glucosylceramidotic human skin. *J Biol Chem* 254:3521–3525, 1979
32. BARTON NW, ROSENBERG A: Metabolism of glucosyl [^{3}H]ceramide by human skin fibroblasts from normal and glucosylceramidotic subjects. *J Biol Chem* 250:3966–3971, 1974
33. KUSKE TT, ROSENBERG A: Quantity and fatty acyl composition of the glycosphingolipids of Gaucher spleen. *J Lab Clin Med* 80:523–529, 1972
34. SUOMI W, AGRANOFF BW: Lipids of the spleen in Gaucher's disease. *J Lipid Res* 6:211–219, 1965
35. KOERNER TAW JR, CARY LW, LI SC, LI YT: Carbon 13 NMR spectroscopy of a cerebroside. Proof of the β-pyranosyl structure of D-glucosylceramide. *J Biol Chem* 254:2326–2329, 1979
36. PETERS SP: Studies on the enzymology of Gaucher disease. Ph.D. thesis, Univ. of Pittsburgh, School of Medicine, 1976
37. PENTCHEV PG, BRADY RO, BLAIR HE, BRITTON DE, SORRELL SH: Gaucher disease: isolation and comparison of normal and mutant glucocerebrosidase from human spleen tissue. *Proc Natl Acad Sci USA* 75:3970–3973, 1978