Gaucher's Disease. I. Solubilization and Electrophoresis of β -Glucosidase from Leukocytes and Cultured Fibroblasts

FRANCIS Y. M. CHOY AND RONALD G. DAVIDSON

McMaster University Medical Center, Department of Pediatrics, Hamilton, Ontario, Canada

Summary

In order to determine unambiguously the presence of multiple forms of β -glucosidase in normal leukocytes and fibroblasts and the form(s) present in Gaucher's disease, we had to devise techniques to separate β -glucosidase from the lysosomal membrane in a catalytically active form and to visualize the isozymes electrophoretically. Separation of relatively stable enzyme from the membrane was not achieved until leukocyte and fibroblast suspensions were freeze-thawed consecutively 10 times. After Cellogel electrophoresis of normal leukocyte extracts, two distinct and discrete bands of β -glucosidase activity, which migrated toward the anode, were observed using the fluorogenic substrate 4-methylumbelliferyl- β -D-glucopyranoside. In patients with juvenile onset Gaucher's disease, all of the leukocyte β -glucosidase activity remained at the origin. The electrophoretic pattern of β -glucosidase from normal cultured skin fibroblasts showed only one fluorescent band of β -glucosidase activity which also migrated toward the anode, but with lesser mobility than the leukocyte bands. In fibroblast extracts of patients with Gaucher's disease, β -glucosidase activity could not be visualized after electrophoresis, even though prior to electrophoresis these extracts had 15-35% of normal activity.

In contrast to the enzyme from leukocytes and fibroblasts, β -glucosidase activity in the spleen and liver was easily released by homogenization. Only one band of β -glucosidase activity, more rapidly anodal than the leukocyte isozymes, was observed after electrophoresis. Enzyme activity in splenic crude homogenates from five patients with Gaucher's disease varied from 5.2-60.8% of normal. After electrophoresis, diminished activity of β -glucosidase with the same mobility as the normal enzyme was detected in the two spleen samples with highest activity.

Speculation

The electrophoretic behavior of leukocyte β -glucosidase from patients with juvenile onset Gaucher's disease suggests that the condition results from the synthesis of an altered enzyme, which is bound even more firmly to the lysosomal membrane than the normal enzyme. The abnormal membrane binding could interfere with the accessibility of the enzyme to the natural substrate and account for the gradual accumulation of glycosphingolipid in the patients.

Gaucher's disease is caused by an inherited deficiency of the lysosomal enzyme, glucocerebrosidase (glucosylceramidase, EC 3.2.1.45), which results in accumulation of glucocerebroside in histiocytes, especially in the spleen (13, 27). Chronic nonneuropathic forms and acute infantile neuropathic forms have been described (12).

Deficiency of glucocerebrosidase activity in adult onset

Gaucher's disease was first demonstrated by Brady *et al.* (7) in human spleen, using isotopically labeled glucocerebroside as substrate. This was confirmed by Patrick (22) using nonlabeled glucocerebroside, the natural substrate. With *p*-nitrophenyl- β -D-glucopyranoside, a chromogenic artificial substrate, Patrick also noted that deficiency of glucocerebrosidase activity coincided with decreased β -glucosidase activity (22). Ockerman (20) used the fluorogenic substrate, 4-methylumbelliferyl- β -D-glucopyranoside, to detect deficient β -glucosidase activity in liver and spleen of patients with Gaucher's disease and subsequently, deficient activity has been measured in circulating leukocytes (3, 18, 28), long-term cultured lymphocytes (28), and cultured skin fibroblasts (5, 28) with this fluorogenic substrate.

When β -glucosidase activity in normal intact leukocytes was assayed, two pH optima at 4.0 and 5.3 were observed, suggesting the presence of isozymes (4). In patients with Gaucher's disease, enzyme activity was decreased at both pH optima, but more markedly at pH 4.0. With lysed cell homogenates of cultured skin fibroblasts, one pH optimum of β -glucosidase activity at 4.5 was observed in both the normal and Gaucher tissues (5). Subsequently, conflicting data have been published on the occurrence of multiple forms of β -glucosidase in human skin fibroblasts (16, 28) and on the deficiency in leukocytes (28). No unequivocal evidence for the existence of isozymes of β -glucosidase in leukocytes and cultured skin fibroblasts has been available, primarily because both leukocyte and fibroblast β -glucosidases are firmly membrane-bound enzymes that had not been adequately solubilized for further investigation, particularly by electrophoresis.

In order to determine unambiguously the presence of multiple forms of β -glucosidase in normal leukocytes and fibroblasts and the form(s) present in Gaucher's disease, we had to devise techniques to separate β -glucosidase from the lysosomal membrane in a catalytically active form and to visualize the isozymes electrophoretically.

MATERIALS AND METHODS

4-Methylumbelliferyl- β -D-glucopyranoside (4MUGP) and 4-methylumbelliferone were the products of Koch Light Co., Coinbrook, Bucks, England. Sodium taurocholate (grade A) was purchased from Calbiochem, La Jolla, CA. Citric acid, tris(2-amino-2-hydroxymethyl-1,3-propane-diol), sodium chloride, sodium azide, and sodium hydroxide (Baker's grade) were the products of J. T. Baker Chemical Co., Phillipsberg, NJ. Neuraminidase (no. N-3001, from *Clostridium perfringens*, type VI) was the product of Sigma Chemical Co., St. Louis, MO. Cell culture medium (Ham's F-10), fetal calf serum, L-glutamine, penicillin, streptomycin, and trypsin (0.25%, in balanced salt solution) were the products of Grand Island Biological Co., NY. Dextran T500 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Cellulose acetate gel (Cellogel) was purchased from Kalex Scientific Co., Manhasset, NY.

ISOLATION OF LEUKOCYTES

All glassware used in this procedure was siliconized in 1% siliclad solution (Clay Adams Co., NJ). Polycarbonate tubes were employed in all centrifugations.

The "Dextran gradient" method (2) was used in the isolation of human peripheral blood leukocytes. Twenty milliliters of fresh heparinized venous blood were added to and mixed gently with 80 ml of Dextran gradient solution, which contained 1.2% (w/v) Dextran T500, 0.88% sodium chloride (isotonic saline), and 0.02% sodium azide. After 30 min at room temperature, the bulk of the red cells had sedimented to the bottom. The leukocyte-rich supernatant fluid was then pipetted off and centrifuged at $2000 \times g$ for 20 min at 3° . The supernatant was discarded, and the pellet was resuspended in 5 ml of isotonic saline. The remaining red cells were lysed by exposure to five volumes of 0.2% (w/v) ice-chilled sodium chloride solution for 1 min. After centrifugation at $1000 \times g$ for 15 min, the hemoglobin and erythrocyte ghosts in the supernatant portion were removed by aspiration. Alternatively, the red cells could be removed by applying the cell suspension to a Ficoll-diatrizoate gradient, as suggested by Beutler and Kuhl (4). The isolated leukocytes were washed twice in isotonic saline, centrifuged at $600 \times g$ for 15 min at 3° , and then resuspended in 150 μ l of isotonic saline.

CELL CULTURE

Skin biopsies were obtained from two normal control donors and three patients with juvenile onset Gaucher's disease. The specimens were minced with a sterile scalpel, placed in F-10 medium supplemented with 20% fetal calf serum, 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 μ g/ml), and cultured at 37° in an atmosphere of 5% CO_2 in air. Confluent monolayer cultures of fibroblasts in 100-mm diameter culture dishes were rinsed several times with isotonic saline solution and harvested by scraping with a rubber policeman. The cell suspensions were centrifuged at $600 \times g$ for 15 min at 3° , washed twice in ice-chilled isotonic saline, and resuspended in isotonic saline at a final protein concentration of 50 mg/ml.

SOLUBILIZATION OF β -GLUCOSIDASE FROM PERIPHERAL BLOOD LEUKOCYTES AND CULTURED FIBROBLASTS

Attempts were made to solubilize β -glucosidase by the use of Triton X-100 according to the methods of Ho *et al.* (14, 15). The effects of other detergents, such as sodium deoxycholate, sodium taurocholate (6), and lysolecithin (24) on the solubilization of β -glucosidase were investigated, as well as freeze-thawing three times and sonication. Although detergents are known to enhance the solubility of the enzyme, their presence in the cell extracts resulted in rapid diffusion and smearing across the surface of the cellogel due to a decrease in surface tension. Separation of relatively stable enzyme activity from the membrane was not achieved until leukocyte and fibroblast suspensions were freeze-thawed 10 consecutive times in an ethylene glycol dry ice bath and a cold water bath at 7° .

PREPARATION OF LIVER AND SPLENIC EXTRACTS

Autopsy specimens were obtained from the liver and spleen of two normal controls and three Gaucher patients, and portions of spleen were obtained after splenectomy from two additional patients. All patients with Gaucher's disease have the juvenile onset form. The specimens were frozen immediately and stored at -70° . All procedures were performed at 3° or in ice.

Samples of liver and spleen were minced into small pieces with surgical scissors, suspended in isotonic saline (15 g wet wt/100 ml) and disrupted in a Brinkman Polytron homogenizer (Physical Chemistry Institute, Lucerne, Switzerland) at 8000 rpm for 5 min at 30-sec intervals. The homogenates were then centrifuged at $40,000 \times g$ for 1 hr. Supernatant fluids were collected, assayed for β -glucosidase activity, and studied electrophoretically.

ENZYME ASSAY

β -Glucosidase was assayed at 37° according to a procedure modified from Beutler *et al.* (5). Ten microliters of enzyme preparation were added to a reaction mixture consisting of 30 μ l of 0.1 M citrate at the specified pH and 20 μ l of 1 mM 4MUGP. The assay system was incubated for 30–60 min and the reaction was stopped by the addition of 1.5 ml of 0.2 M glycine buffer, pH 10.5. Blank systems were also set up which contained the same reagents, except isotonic saline was substituted for the enzyme preparations. A calibration curve for 4-methylumbelliferone was also constructed. Fluorescence readings were made in an Aminco model J4-7439 fluorocolorimeter (American Instrument Co.) with a Wratten 2A primary filter and Corning 7-60 secondary filter.

CELLULOSE ACETATE GEL (CELLOGEL) ELECTROPHORESIS

PREPARATION OF CELLOGEL

Prior to use, the Cellogel sheet (17 x 18 x 0.25 cm) was washed thoroughly in distilled water to remove the methanol and then equilibrated by soaking in 0.025 M citrate–0.01 M Tris buffer, pH 7.2, for 2 hr at 3° . The gel sheet was then gently blotted dry between two pieces of Whatman No. 1 filter paper, mounted onto the plastic bridge with the porous surface upward, and placed horizontally in the electrophoresis tank (Chemetron model 200 electrophoretic tank).

PREPARATION OF SAMPLES

The leukocyte and fibroblast suspensions that had been freeze-thawed 10 consecutive times in an ethylene glycol dry ice bath were centrifuged at $40,000 \times g$ for 30 min at 3° . Homogenates from human liver and spleen, obtained at autopsy or biopsy, were centrifuged at the same speed and temperature for 1 hr. Samples from the supernatant portion (10–20 μ l) were applied to the gel with a micropipette in the form of thin streaks 12–14 mm long, about 10 mm apart, and 3 cm from the cathode side of the plastic support.

CONDITIONS OF ELECTROPHORESIS

A constant current of 12 ma and a DC voltage of 90 volts were applied for 2–3 hr at 3° . Electrophoresis was conducted in the same citrate–Tris buffer at pH 7.2.

 β -GLUCOSIDASE ACTIVITY STAINING

After the run the gel was blotted gently between two pieces of Whatman No. 4 filter paper to remove any excess buffer and immersed in the developer solution consisting of 0.5 mM 4MUGP in 0.05 M citrate, pH 5.3, at 37° for 1 min. Excess buffered substrate was removed by blotting. The gel was then placed horizontally in a sealed moist chamber and incubated at 37° from 1–2 hr in the dark.

After incubation, the gel was immersed in a 0.2 M sodium glycinate stopping buffer, pH 10.3, for 30 sec at room temperature. Visualization of fluorescence, which indicated β -glucosidase activity, occurred during long wave UV light.

RESULTS

Initial attempts to solubilize membrane-bound β -glucosidase by detergents were unsatisfactory, as the solubilized enzyme was extremely unstable and more than 90% of activity was lost overnight, even when the enzyme preparation was stored at -70° . The loss of solubilized β -glucosidase activity cannot be prevented by removing the detergents by dialysis or by Bio Beads SM-2 (19).

When a leukocyte or fibroblast suspension was freeze-thawed 10 consecutive times in an ethylene glycol dry ice bath and a cold water bath at 7° , up to 30% of total β -glucosidase activity could be detected in the $40,000 \times g$ supernatant fraction. This activity was stable for at least 1 week when the enzyme preparation was

stored at -70° . Consequently, this multiple freeze-thawing procedure became the method of choice for the liberation of membrane-bound β -glucosidase activity.

Figure 1, lane 1, shows the results of cellogel electrophoresis of normal leukocyte extracts. Two distinct and discrete bands of β -glucosidase activity which migrated toward the anode were observed. There was no change in the electrophoretic mobility or activity of β -glucosidase when the solubilized leukocyte enzyme, before centrifugation, was preincubated with neuraminidase for 3 hr at 37° under the conditions described by Alhadeff *et al.* (1).

Fig. 1. β -Glucosidase activity from normal (lane 1) and Gaucher (lane 2) leukocyte extracts after cellogel electrophoresis. 0 represents the origin or line of application and the signs indicate the anode and cathode.

Apparently sialic acid, if present in the enzyme molecule, does not contribute to the electrophoretic mobility or catalytic activity.

Figure 1, lane 2, depicts the electrophoretic pattern of β -glucosidase in leukocyte extracts from a patient with juvenile onset Gaucher's disease. All of the β -glucosidase activity remained at the origin, and there was no trace of the two isozymes seen in normal leukocytes. The same pattern was observed in leukocyte extracts from four additional patients with juvenile Gaucher's disease.

Figure 2 depicts β -glucosidase activity from extracts of normal spleen and liver. In contrast to the enzyme from leukocytes and fibroblasts, β -glucosidase activity in the spleen and liver was easily released by homogenization. More than 80% and 50% of total activities were detected in the supernatant of the liver and spleen, respectively. Only one band of β -glucosidase activity, which migrated toward the anode, was observed after electrophoresis. The electrophoretic mobility of β -glucosidase from liver and splenic homogenates appeared to be identical and more rapidly anodal than the leukocyte isozymes. There was no change in the mobility or fluorogenic activity of the enzyme if the samples were freeze-thawed 10 consecutive times, as in the preparation of the leukocyte extracts. Preincubation with neuraminidase also had no effect on the mobility or activity of liver or spleen enzyme.

β -Glucosidase activity in the Gaucher spleen homogenates and extracts was assayed and results are shown in Table 1. Activities

Table 1. β -Glucosidase activity in splenic crude homogenates and supernatants from normal controls and patients with Gaucher's disease

	Homogenate ¹	% Relative activity	Supernatant ¹	% Relative activity
Normal control				
1	9.42	100	14.4	100
2	8.89	94.4	13.57	94.2
Gaucher's disease				
1	2.70	28.7	1.51	10.5
2	2.31	24.5	0.94	6.5
3	1.51	16.0	0.83	5.2
4	4.57	48.5	4.40	30.6
5	4.79	50.8	8.76	60.8

¹ The specific activity is expressed as nanomoles of 4MUGP liberated per hr per mg of protein. All assays were run in duplicate which agree within 10%. Relative activity of sample 1 of normal control is designated as 100%.

Fig. 2. β -Glucosidase activity from normal spleens (lanes 1-3) and livers (lanes 4 and 5) after electrophoresis.

Fig. 3. β -Glucosidase activity from normal (lanes 1 and 2) and Gaucher (lanes 3 and 4) fibroblast extracts after electrophoresis.

Fig. 4. pH study on β -glucosidase activity from normal and Gaucher fibroblast extracts. Each point on normal curve represents the average of two individuals and the other three curves represent three patients with Gaucher's disease.

from Gaucher samples 1-3 were more deficient than the other two, especially in the supernatant fractions (5.2-10.5% of normal). No β -glucosidase activity was detected in these samples after electrophoresis, even if the quantity of aliquots applied was doubled. Diminished activity of β -glucosidase with same mobility as the normal enzyme was detected in Gaucher samples 4 and 5. There was no correlation between the relative activity of the enzyme and the clinical severity of the disease.

Figure 3 depicts the electrophoretic pattern of β -glucosidase in cultured skin fibroblasts. In lanes 1 and 2 are extracts from normal

controls showing only one fluorescent band of β -glucosidase activity which migrated toward the anode, but with lesser mobility than the leukocyte bands. Lanes 3 and 4 contained fibroblast extracts from two patients with juvenile Gaucher's disease. β -Glucosidase activity could not be visualized in these samples nor in a sample from a third patient after electrophoresis. On the other hand, prior to electrophoresis these extracts had 15-35% of normal activity, as shown in the pH activity curves of Figure 4.

DISCUSSION

The major obstacle to qualitative studies of β -glucosidase in patients with Gaucher's disease has been the difficulty in dissociating the enzyme from the lysosomal membranes of either peripheral blood leukocytes or cultured fibroblasts. This paper presents techniques for solubilizing β -glucosidase activity in a relatively stable form by multiple consecutive freeze-thawing and for visualizing enzyme activity after electrophoresis in cellulose acetate gel, as introduced previously (9). Data presented by other investigators (4, 28) had suggested the existence of two isozymes of leukocyte β -glucosidase; our electrophoretic observation provides further confirmation.

Although β -glucosidase activity has been assayed for the diagnosis of Gaucher's disease in various tissues, including the liver (17, 20), spleen (7, 14, 15), peripheral blood leukocytes (3, 4, 18), and cultured skin fibroblasts (5, 16), the latter two are the most useful sources since they can be obtained with ease from patients. Furthermore, cultured fibroblasts are useful as a source of enzyme for biochemical and genetic studies. Because of the firm binding of β -glucosidase to the lysosomal membrane, either intact cells or lysed cell homogenates were being used in the assay procedure (3-5, 16). Such preparations are, of course, not suitable for electrophoresis. Sonication and/or the usual three to five consecutive freeze-thawing failed to solubilize more than 10% of the total enzyme activity. But freeze-thawing 10 times yielded maximal release of enzyme from membrane, averaging 30% of total activity. Combinations of physical procedures with chemical techniques, such as detergents, either caused enzyme instability or so reduced the surface tension of the extract that the preparation rapidly diffused over the gel. In addition, Turner *et al.* (28) reported that in the presence of detergents, the cell specificity of β -glucosidase was no longer observed in leukocytes.

Our electrophoretic studies of β -glucosidase from leukocyte extracts demonstrated the presence of two anodally migrating isozymes with activity toward the fluorogenic substrate, 4MUGP, the existence of which was suggested by other biochemical ap-

proaches (4, 28). In patients with juvenile onset Gaucher's disease, the two isozymes found in normal individuals are absent and in their place is an altered enzyme which did not migrate through cellogel. This may mean that the mutant enzyme possesses the isoelectric pH of the gel buffer system, or that the enzyme has remained membrane-bound and the enzyme-membrane complex is too large to migrate through the matrix of the gel. In order to investigate these possibilities, electrophoresis was conducted at pH values from 5.7–8.0. Under such conditions, the two isozymes from normal leukocyte extracts were always detected, with different rates of migration depending on the pH. β -Glucosidase from Gaucher leukocyte extracts, however, was observed to remain at the origin each time. This clearly indicated that β -glucosidase in Gaucher leukocytes is altered in such a way as to result in binding more firmly to the membrane than the normal enzyme, eliminating the possibility that the presence of the two isozymes in normal leukocyte extracts is due to artificial pH dissociation under our experimental conditions. The aberrant binding may result in decreased accessibility of the enzyme to the natural glycosphingolipid substrate and lead to its accumulation during the life of the patient with Gaucher's disease. This may also at least partially explain the fact that glucocerebrosidase and β -glucosidase activity are always detectable in the tissues of these patients (3–5, 16, 20), and that β -glucosidase activity may be as high as 60% of normal (Table 1, sample 5) despite the enzyme's ineffectiveness in preventing the more or less gradual accumulation of the substrate. This relatively high β -glucosidase activity could result from genetic and biochemical heterogeneity, or the presence in some tissues of a soluble β -glucosidase which is not involved in Gaucher's disease (8, 15). Furthermore, our assay method, unlike that of most investigators, did not contain sodium taurocholate because we found that it selectively inhibited the β -glucosidase activity of Gaucher tissues (10).

It should also be noted that when leukocytes are used as the source of β -glucosidase, they should be isolated immediately from heparinized fresh blood. Clumping of the leukocytes occurs rapidly and results in diminished yield of enzyme extraction, as well as problems with the quantitative assay (4).

In extracts of cultured skin fibroblasts from normal individuals, only one band of β -glucosidase activity was detected. The existence of only one isozyme in fibroblasts is supported by data from our pH activity studies, which exhibit one pH optimum around 4.5 in both the cell homogenates and extracts (Fig. 4), and by a recently published report (28). Of potential importance to the eventual understanding of the nature of the defect in this disease is the observation that β -glucosidase activity in Gaucher fibroblast extracts was diminished beyond detection after electrophoresis, in spite of relatively abundant activity in the sample prior to electrophoresis (15–35% of normal). This could be accounted for by the presence of a stabilizing or activating factor(s) essential for β -glucosidase activity in Gaucher fibroblasts, which is separated from the enzyme during electrophoresis. The presence of such a "Gaucher factor" has been reported (14) and isolated (25) from human spleen.

In contrast to the β -glucosidase in leukocytes and fibroblasts, the major form of this enzyme in the liver and spleen is relatively soluble and can be released readily by simple homogenization. After electrophoresis, one discrete band of β -glucosidase activity with greater mobility toward the anode than that of either leukocyte or fibroblast enzyme was observed. Thus, although the enzymes from the liver and spleen showed identical migration rates in cellogel (Fig. 2), it is evident that highly tissue-specific β -glucosidases exist.

Recently, the reliability of using the fluorogenic substrate, 4MUGP, and visceral tissues as the source of enzyme in the diagnosis of Gaucher's disease had been studied by Owada *et al.* (21). It was reported that in neuropathic Gaucher's disease, normal β -glucosidase activity was detected in the liver of one group of patients, whereas deficient activity was detected in another group (21). Therefore, the deficiency of 4MUGP- β -glucosidase in liver (and possibly in spleen) may not account for the accumulation of

glucocerebroside, and the deficiency of glucocerebroside- β -glucosidase activity, probably existing within the reticuloendothelial cells, may be more essential for the accumulation (21).

A soluble enzyme with activity toward both the fluorogenic and chromogenic artificial substrates, but not the natural substrate, has been reported to be present in the human spleen (14) and liver (26). In Gaucher's disease, activity of this soluble enzyme was either undiminished (15, 26), moderately diminished (22), or severely diminished (17). It had also been reported that a deficiency of β -glucosidase only existed in the "membrane-bound" but not the soluble form in splenic specimens of patients with adult Gaucher's disease (14). In our studies using 4MUGP as the substrate, β -glucosidase activity in splenic extracts from patients with Gaucher's disease was either moderately or severely deficient in both the lysed cell homogenates and supernatant fractions (Table 1). When sufficient activity for visualization after electrophoresis remained (as in two of our five specimens), the mobility was indistinguishable from normal. The exact physiologic role of this soluble enzyme remains unclear, and elucidation of its role and these discrepancies await the purification of β -glucosidase to homogeneity and further studies of this enzyme from normal to Gaucher tissues with both the artificial fluorogenic and natural substrates (11, 23).

REFERENCES AND NOTES

1. Alhadeff, J. A., Miller, A. L., Wenger, D. A., and O'Brien, J. O.: Electrophoretic forms of human liver α -L-fucosidase and their relationship to fucosidosis (Mucopolysaccharidosis F). *Clin. Chim. Acta*, 57: 307 (1974).
2. Avila, J. L., and Convit, J.: Heterogeneity of acid phosphatase activity in human polymorphonuclear leukocytes. *Clin. Chim. Acta*, 44: 21 (1973).
3. Beutler, E., and Kuhl, W.: Detection of the defect of Gaucher's disease and its carrier state in peripheral blood leukocytes. *Lancet*, 1: 612 (1970).
4. Beutler, E., and 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: 747 (1970).
5. Beutler, E., Kuhl, W., Trinidad, F., Teplitz, R., and Nadler, H.: β -glucosidase activity in fibroblasts from homozygotes and heterozygotes for Gaucher's disease. *Am. J. Hum. Genet.*, 23: 62 (1971).
6. Blonder, E., Klibansky, C., and DeVries, A.: Effects of detergents and choline-containing phospholipids on human spleen glucocerebrosidase. *Biochem. Biophys. Acta*, 431: 45 (1976).
7. Brady, R. O., Kanfer, J. N., and Shapiro, D.: Metabolism of glucocerebrosides. II. Evidence of enzymatic deficiency in Gaucher's disease. *Biochem. Biophys. Res. Commun.*, 18: 221 (1965).
8. Broadhead, D. M., and Butterworth, J.: The diagnosis of Gaucher's disease in liver using 4-methylumbelliferyl- β -D-glucopyranoside. *Clin. Chim. Acta*, 75: 155 (1977).
9. Choy, F. Y. M., and Davidson, R. G.: Gaucher's disease: An electrophoretic technique for the detection of β -glucosidase in human leukocytes and cultured fibroblasts. *Pediatr. Res.*, 11: 454 (1977).
10. Choy, F. Y. M., and Davidson, R. G.: Gaucher disease. II. Studies on the kinetics of β -glucosidase and effects of sodium taurocholate in normal and ceramidotonic tissues. Manuscript in preparation.
11. Dale, G. L., and Beutler, E.: Enzyme replacement therapy in Gaucher's disease: A rapid high yield method for purification of glucocerebrosidase. *Proc. Natl. Acad. Sci. USA*, 73: 4672 (1976).
12. Fredrickson, D. S.: In J. Stanburg, J. B. Wyngarden, and D. S. Fredrickson: *Metabolic Basis of Inherited Disease*, p. 565 (McGraw-Hill, New York, 1966).
13. Halliday, N., Denel, Jr., H. J., Tragerman, L. J., and Ward, W. E.: On the isolation of glucose-containing cerebroside from spleen in a case of Gaucher's disease. *J. Biol. Chem.*, 132: 171 (1940).
14. Ho, M. W.: Identity of "acid" β -glucosidase and glucocerebrosidase in human spleen. *Biochem. J.*, 136: 721 (1973).
15. Ho, M. W., and O'Brien, J. S.: Gaucher's disease: Deficiency of "acid" β -glucosidase and reconstitution of enzyme activity *in vitro*. *Proc. Natl. Acad. Sci. USA*, 68: 2810 (1971).
16. Ho, M. W., Seck, J., Schmidt, D., Veath, M. L., Johnson, W., Brady, R. O., and O'Brien, J. S.: Adult Gaucher's disease: Kindred studies and demonstration of a deficiency of acid β -glucosidase in cultured fibroblasts. *Am. J. Hum. Genet.*, 24: 37 (1972).
17. Hultberg, B., and Ockerman, P. A.: β -glucosidase activities in human tissues. Findings in Gaucher's disease. *Clin. Chim. Acta*, 28: 169 (1970).
18. Klibansky, G. H., Hoffman, J., Zaizov, R., and Matoth, Y.: Gaucher's disease, chronic adult type: A comparative study of glucocerebroside and methylumbelliferyl-glucopyranoside cleaving potency in leukocytes. *Biomedicine*, 20: 24 (1974).
19. Mumford, R. A., Raghavan, S. S., and Kanfer, J. N.: Hydrolytic and transglucolytic activities of a partially purified calf brain β -glucosidase. *J. Neurochem.*, 27: 943 (1976).
20. Ockerman, P. A.: Identity of β -glucosidase, β -xylosidase and one of the β -galactosidase activity on human liver when assayed with 4-methylumbelliferyl-

- β -D-glycosides: Studies in cases of Gaucher's disease. *Biochem. Biophys. Acta*, 165: 59 (1968).
21. Owada, M., Sakiyama, T., and Kitagawa, T.: Neuropathic Gaucher's disease with normal 4-methylumbelliferyl- β -glucosidase activity in the liver. *Pediatr. Res.*, 11: 641 (1977).
 22. Patrick, D. A.: A deficiency of glucocerebrosidase in Gaucher's disease. *Biochem. J.*, 97: 170 (1965).
 23. Pentchev, P. G., Brady, R. O., Hibbert, S. R., Gal, A. E., and Shapiro, D.: Isolation and characterization of glucocerebrosidase from human placental tissue. *J. Biol. Chem.*, 248: 5256 (1973).
 24. Perumal, A. S., and Robins, E.: Arylsulphatases in human brain: Purification and characterization of an insoluble arylsulphatase. *J. Neurochem.*, 21: 459 (1973).
 25. Peters, S. P., Coyle, P., Coffee, C. J., and Glew, R. H.: Purification and properties of a heat-stable glucocerebrosidase activating factor from control and Gaucher spleen. *J. Biol. Chem.*, 252: 563 (1977).
 26. Peters, S. P., Coyle, P., and Glew, R. H.: Differentiation of β -glucocerebrosidase from β -glucosidase in human tissues using sodium taurocholate. *Arch. Biochem. Biophys.*, 175: 569 (1976).
 27. Rosenberg, A., and Chargaff, E.: A reinvestigation of the cerebrosides deposited in Gaucher's disease. *J. Biol. Chem.*, 233: 1323 (1956).
 28. Turner, B. M., Beratis, N. G., and Hirschhorn, K.: Cell specific differences in membrane β -glucosidase from normal and Gaucher cells. *Biochem. Biophys. Acta*, 480: 442 (1977).
 29. We wish to thank Dr. J. A. Lowden of the Hospital for Sick Children, Toronto, for generously supplying us with cultured fibroblasts, blood samples, and splenic tissue from patients with Gaucher's disease. We also thank Mrs. D. Handcock for her technical assistance and Dr. P. L. Y. Chang for her advice and help with the electrophoresis and cell culture.
 30. Mailing address: Dr. F. Y. M. Choy, McMaster University Medical Center, Department of Pediatrics, Room 3N17, 1200 Main Street West, Hamilton, Ontario, Canada, L8S 4J9.
 31. This research was supported by the Medical Research Council of Canada Grant MA-5789.
 32. Received for publication September 6, 1977.
 33. Accepted for publication March 13, 1978.