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# Leukocyte β-Glucosidase in Homozygotes and Heterozygotes for Gaucher Disease

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SUMMARY

Human leukocytes contain at least two isozymes of 4-methylumbelliferyl-β-glucosidase acting optimally at pH 4.0 and 4.8; in Gaucher disease, only the former is deficient. Brief exposure of the leukocyte homogenate to pH 4.0 at room temperature results in irreversible inactivation of the pH 4.8 activity, while the activity at pH 4.0 remains unaffected. The more acidic isozyme is stimulated four- to fivefold by 0.2% sodium taurodeoxycholate (TDC) with a shift in the pH optimum to 5.0. The less acidic isozyme is completely suppressed in the presence of this detergent. Both leukocyte isozymes appear to be membrane-bound since gel filtration of Sephadex G-200 produces only one peak of activity located at the void volume, unlike in liver and kidney where a second peak also can be demonstrated. Heat inactivation analysis indicated that in controls, assayed in the absence of detergent, pH 4.0 activity is more thermostable than pH 4.8 activity. However, in Gaucher disease, the residual β-glucosidase at pH 4.0 is just as thermolabile as the unaffected pH 4.8 activity. Heat inactivation of the enzyme in the presence of TDC resulted in rapid loss of activity, suggesting a direct effect of the bile salt on the configuration of the enzyme decreasing its thermal stability. In the absence of detergent, acid β-glucosidase shows two $K_m$'s, one at 3.2 mM and another at 0.9 mM. In the presence of detergent, only the higher $K_m$ at 3.3 mM is obtained. In patients with Gaucher disease and in obligate carriers, the $K_m$ remains essentially unaffected while the $V_{max}$ shows the expected deficiency.

A reliable and reproducible selective assay technique has been developed for the diagnosis of Gaucher disease homozygotes and obligate heterozygotes

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and for the carrier screening of individuals at risk for this inherited disorder. The efficacy of this technique has been demonstrated by studying the activity in 42 controls, 26 patients, 32 obligate heterozygotes, and 23 healthy relatives of patients with Gaucher disease.

## INTRODUCTION

Gaucher disease is an autosomal recessively inherited disorder of sphingolipid metabolism characterized by hepatosplenomegaly, anemia, thrombocytopenia, and bone lesions with varying degrees of severity in different patients [1]. The chronic, adult, nonneuropathic form (type 1) is the most common and has a predilection for individuals of Ashkenazi Jewish ancestry. Much rarer are the infantile and fatal acute neuropathic form (type 2) and the juvenile or subacute neuropathic form (type 3), both of which involve the central nervous system [1]. Biochemically, this disease is characterized by a deficiency of cerebroside β-glucosidase [2], which results in the deposition of the glycolipid glucocerebroside in reticuloendothelial cells, particularly of spleen, bone marrow, and liver [1].

The enzymic deficiency in this disease has also been demonstrated with the use of the artificial substrates, *p*-nitrophenyl-β-D-glucoside [3] and 4-methylumbelliferyl (4MU)-β-D-glucoside [4]. Multiple forms of β-glucosidase active against these artificial substrates have been detected in a variety of human tissues, including liver [5–7], brain [8], spleen [9, 10], kidney [7], leukocytes, and fibroblasts [11]. Therefore, the diagnosis of Gaucher disease with the use of artificial substrates requires that assay conditions be carefully chosen so that only the specifically deficient isozyme in this disorder is measured. Beutler and Kuhl [12, 13] reported a bimodal pH-activity curve for human leukocyte 4MU-β-glucosidase with optima at pH 4.0 and 5.3. They found that in Gaucher disease, only the activity at pH 4.0 is severely depressed. On the basis of this observation, assay conditions have been developed for diagnosing Gaucher disease using leukocytes and fibroblasts [14–18]. However, these conditions are often not reliable nor reproducible for detecting heterozygous carriers of this genetic disease. Although use of the natural glycolipid substrate, glucocerebroside, would avoid ambiguity, the synthesis of this compound with a radioactive label in the glucose portion of the molecule is very difficult and expensive [17, 19, 20]. Consequently, the natural substrate is available in very few laboratories. If the glycolipid is labeled at positions other than the sugar residue, the assay procedure is a tedious one requiring chromatographic isolation of the product [7] and is unsuitable for the screening of large samples for diagnosis.

In this investigation, a detailed analysis of the properties of human leukocyte β-glucosidase was undertaken using the artificial substrate, 4MU-β-glucoside, enabling us to devise a substantially improved procedure for the diagnosis of Gaucher disease homozygotes and obligate heterozygotes and for carrier screening of individuals at risk for this inherited disorder. Part of this work has been presented at a meeting and published in abstract form [21].

## MATERIALS AND METHODS

Commercial sources supplied the following materials: 4MU-β-D-glucoside (Koch-Light Laboratories, Colnbrook, U.K.); crude sodium taurocholate (Pfanstiehl Laboratories, Waukegan, Ill.); pure sodium taurocholate, TDC, sodium glycocholate, and glycodeoxycholate (Calbiochem, Los Angeles, Calif.); and Triton X-100 and Scintiverse (Fisher Scientific Co., Medford, Mass.).

Leukocytes were isolated from freshly drawn heparanized blood by dextran sedimentation and homogenized by sonication in saline as described [22]. A stock substrate solution of 20 mM 4MU-β-D-glucoside was prepared by gentle warming under hot water. The pH activity was determined using citrate-phosphate buffer prepared by mixing 1 M citric acid and 2 M sodium phosphate (dibasic) in various proportions. The assay system contained 50 μl substrate, 10 μl buffer of indicated pH, leukocyte homogenate, detergents if mentioned, and water added to a final volume of 100 μl. Protein was determined by the method of Lowry et al. [23]. Unless indicated, protein concentration in the assay approximated 100 μg in the absence of detergent and 50 μg in the presence of detergent. Approximately twice this amount of protein was used for samples obtained from patients with Gaucher disease. After incubation at 37°C for 2 hrs, the reaction was stopped with 2 ml 0.25 M glycine-KOH buffer, pH 10.3, and fluorescence of the liberated 4MU measured in an Aminco-Bowman spectrofluorometer using an excitation wavelength set at 366 nm and an emission wavelength of 446 nm.

### *Heat Inactivation*

Leukocyte saline homogenates and water in a total volume of 30 μl were subjected to heat inactivation at the desired temperature for different time periods as indicated and then cooled in ice. Buffer (10 μl) of indicated pH, substrate (50 μl), and 2% TDC or water (10 μl) were added. The samples were incubated at 37°C and assayed as described earlier in this section. In some experiments, to study the effect of detergent on the course of heat inactivation, TDC was first added to the enzyme and the mixture then subjected to heat inactivation.

### *Gel Filtration*

Gel filtration was carried out at 4°C using 2.6 × 30 cm columns of Sephadex G-200 in 10 mM sodium phosphate buffer, pH 7.0, containing 10 mM sodium chloride. A flow rate of 18 ml/hr was established with a Buchler peristaltic pump. Fractions of 2.4 ml were collected for assay of β-glucosidase activity. Blue dextran (2 mg) was used to determine the column void volume (67–86 ml), whereas tryptophan (1 mg), detectable through its absorbance at 280 nm, served as an indicator of total column volume (206–276 ml).

*Leukocytes*. Sonicated homogenates of leukocytes (from 50 ml blood) in 2.5 ml elution buffer were prepared and spun at 10,000 *g* for 20 min in a Sorvall RC-2 refrigerated centrifuge. The supernatant was removed and the pellet suspended in 1 ml buffer, sonicated, and centrifuged. The pellet was again sonicated in 0.6 ml buffer and centrifuged. The combined 10,000 *g* extracts contained more than 80% of the β-glucosidase activity present in the initial homogenate. This extract, comprising approximately 25 mg protein, was applied to the Sephadex column. Fractions were collected in tubes containing bovine serum albumin (1 mg in 100 μl) to stabilize the enzyme fractions coming off the column and immediately assayed, five at a time, to prevent loss of activity resulting from standing in the cold room.

*Liver and kidney*. Approximately 700 mg of normal human liver and kidney were initially homogenized in 3 ml of the elution buffer using an all-glass hand homogenizer. The homogenates were then sonicated and centrifuged at 10,000 *g* in the same way as for the leukocytes. The kideny extract, containing about 25 mg protein, and liver extract, containing about 8 mg protein, were applied to the same Sephadex column on which the leukocyte extract was fractionated.

*Assay of column fractions*. The incubation mixture for column fractions contained 100 μl of each fraction, 20 μl citrate-phosphate buffer, pH 4.0 or 5.0 as described in RESULTS, 60 μl substrate solution, and 20 μl water or 2% TDC to give a final volume of 200 μl. The samples

were incubated at 37°C and assayed as indicated previously. Leukocyte samples without TDC were incubated for 4 hrs and with TDC for 1.5 hrs. Kidney samples without TDC were incubated for 2 hrs and with TDC for 15 min. Liver samples were incubated for 3 hrs both in the absence and in the presence of TDC.

*Leukocyte Glucocerebrosidase*

Glucocerebroside labeled with $^{14}C$ in the glucose moiety (250 cpm/nmole) was kindly provided by Dr. J. N. Kanfer. An aliquot containing 60 nmoles of the substrate in chloroform:methanol (C:M; 2:1, v/v) was taken to dryness under nitrogen. To this were added 10 μl citrate-phosphate buffer, pH 5.0, 10 μl 4% TDC, and enzyme, and then the final incubation volume was adjusted to 100 μl with water.

The range of protein concentration was kept between 200 and 300 μg. Following incubation for 3 hrs, the reaction was stopped with 5 ml C:M (2:1). After the addition of 0.1 ml carrier glucose (1 mg/ml) and 0.8 ml water, the contents of the tubes were vortexed and centrifuged at room temperature to clarify the two phases. The entire upper phase was transferred to another tube and washed with 2 ml chloroform. From the washed upper phase, 0.9 ml was removed and placed in a counting vial, taken to dryness under nitrogen, redissolved in 1 ml water, mixed with 15 ml Scintiverse, and counted in a liquid scintillation spectrometer. Substrate blanks without enzyme were routinely included.

## RESULTS

Leukocyte β-glucosidase assayed in the absence of any detergent shows two pH optima at 4.0 and 4.8 (fig. 1). After preliminary exposure to citrate-phosphate buffer (12.5 mM citrate), pH 4.0, for 5 or 10 min at room temperature, the shape of the pH curve is dramatically altered. The activity at pH 4.8 determined in experimental samples pretreated briefly with acid pH buffer is greatly diminished compared with controls treated with water instead of the acid buffer. Under these same conditions, the pH 4 activity is unaffected, suggesting the presence of at least two isoenzymes of β-glucosidase of which only the enzyme active at pH 4.8 is irreversibly inactivated by prior exposure to acid pH.

Figure 2 shows the pH activity curves of leukocyte β-glucosidase in controls, in Gaucher disease carriers, and in Gaucher disease assayed with and without added 0.2% TDC. In the absence of the detergent (fig. 2*A*), the activity at pH 4.0 is considerably reduced in the disease and is intermediate in heterozygotes. No difference could be seen at pH 4.8 between carriers and controls, and only a small reduction was noticeable at this pH in the disease, confirming the earlier studies by Beutler and Kuhl [12] and further emphasizing the presence of at least two β-glucosidases in leukocytes. However, in the presence of TDC (fig. 2*B*), control leukocytes demonstrate only one pH optimum—5.0—with virtually no activity at pH 4.0. This activity is severely diminished in Gaucher disease and is intermediate in obligate heterozygotes, suggesting that, in the presence of bile salts, the pH 4.0 enzyme of figure 2*A*, which is the deficient enzyme in Gaucher disease, shifts its pH optimum to 5.0 with a concomitant four- to fivefold stimulation. The pH 4.8 activity which remains normal in Gaucher disease (fig. 2*A*) is completely suppressed when assayed in the presence of TDC.

The effects of various detergents on the leukocyte enzyme activity at pH 5.0 is shown in figure 3. Maximal stimulation representing a four- to fivefold increase in activity occurred with TDC at 0.2%. Pure taurocholate was optimally effective at a concentration of 0.6% but produced less stimulation than TDC. Glycocholate gave

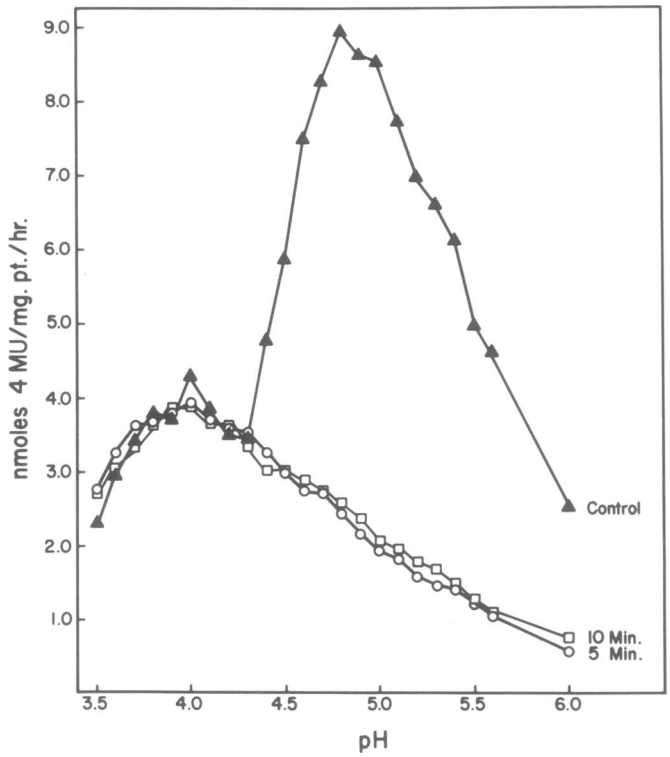


FIG. 1.—Leukocyte β-glucosidase activity as function of pH in control (▲——▲) and in preparations exposed to pH 4.0 at room temperature for 5 min (○——○) and 10 min (□——□).

results similar to pure taurocholate. With glycodeoxycholate present, enzyme activity was optimal at 0.2%, but this bile salt was a less effective stimulator than the detergents already mentioned and was progressively more inhibitory at higher concentrations. Crude taurocholate was less effective in stimulating enzyme activity and at higher concentration was inhibitory. Triton did not produce appreciable stimulation at any of the concentrations studied.

*Substrate Saturation Kinetics*

The substrate saturation kinetics of leukocyte β-glucosidase activity under different conditions was studied using nine concentrations covering the range from 0.2 mM to 10 mM in the final reaction mixture. The Lineweaver-Burk plots obtained are shown in figure 4. The activity assayed at pH 4.0 without TDC, shown earlier to be identical to the deficient activity in Gaucher disease, manifests two $K_m$'s (fig. 4*A*): a high value at 3.2 mM and another lower value at 0.9 mM. At pH 5.0 without detergent, there is an interplay of at least two different isozymes acting on the same substrate. One of these is the pH 4.0 enzyme which functions suboptimally at pH 5.0. Another has optimal activity at pH 5.0 and is unaffected in Gaucher disease. Here again, two $K_m$ values are obtained (fig. 4*C*)—one high and one low, but are different from those of the pH 4.0 enzyme shown in figure 4*A*. By destroying the activity of the second pH 5.0 enzyme by

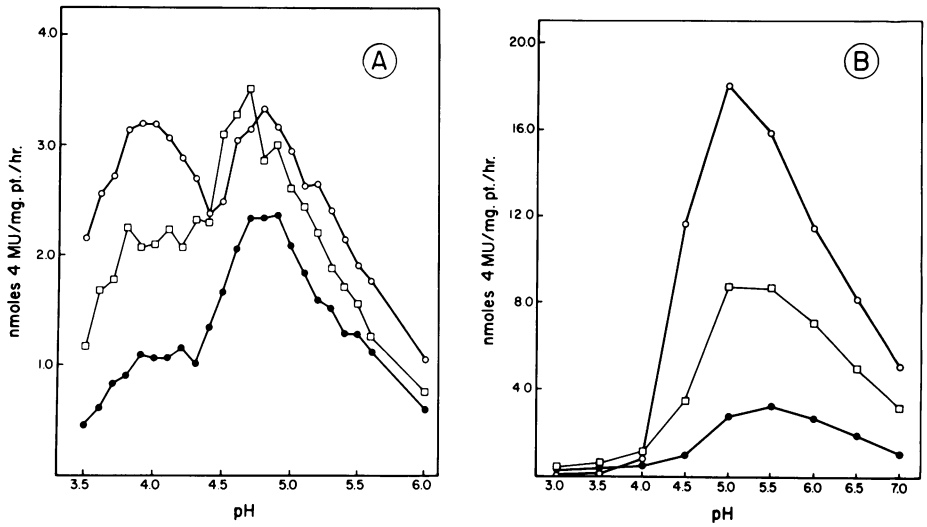


Fig. 2. —Comparison of pH activity curves for leukocyte β-glucosidase in control (○——○), obligate heterozygote (□——□), and patient with Gaucher disease (●——●) assayed in absence of detergent (*A*) and presence of 0.2% TDC (*B*).

prior exposure to pH 4.0 and then assaying at pH 5.0 (fig. 4*B*), two $K_m$ values closely resembling those of the pH 4.0 enzyme are obtained. The lower $V_{max}$ at pH 5.0 also suggests that these $K_m$'s arise from the same pH 4.0 enzyme (fig. 4*A*) acting suboptimally at pH 5.0. In the presence of TDC at pH 5.0, only the deficient enzyme in Gaucher disease is active, as shown earlier. Under these conditions, a single $K_m$ of 3.3 mM is obtained (fig. 4*D*). This $K_m$ is similar to the high $K_m$ found for the same enzyme when assayed at pH 4.0 in the absence of the bile salt. It is possible that in the presence of TDC, a mixed micelle is formed with the substrate so that only one $K_m$ results. The high $K_m$ seen in figures 4*A* and *B* for the same enzyme probably represents binding to the micellar form of the substrate present at high concentration, whereas the lower $K_m$ could represent binding to the monomeric species of the substrate.

*Gel Filtration*

Most human tissues contain at least two isozymes of β-glucosidase. These are separable by gel filtration on G-200 as shown for liver in figure 5*C* and kidney in figure 5*D*. Monitoring the column at pH 5.0 without bile salt, two peaks of enzyme activity are present, one at the void volume and another eluting later. If assay of β-glucosidase is done in the presence of 0.2% TDC, the peak at the void volume is stimulated and the second peak inhibited. Assays of the leukocyte column fractions at both pH 4.0 and pH 5.0 in the absence of detergent (fig. 5*A*) reveal only one peak at the void volume. The large pH 5.0 peak represents the enzyme component that is retained in Gaucher disease, while the small peak assayed at pH 4.0 represents the deficient enzyme in this disease. Assays with 0.2% TDC (fig. 5*B*) again disclose a single peak resulting from stimulation of the pH 4.0 enzyme and inhibition of the pH 5.0 enzyme of figure 5*A*. These elution characteristics suggest that the β-glucosidase isoenzyme, which is less

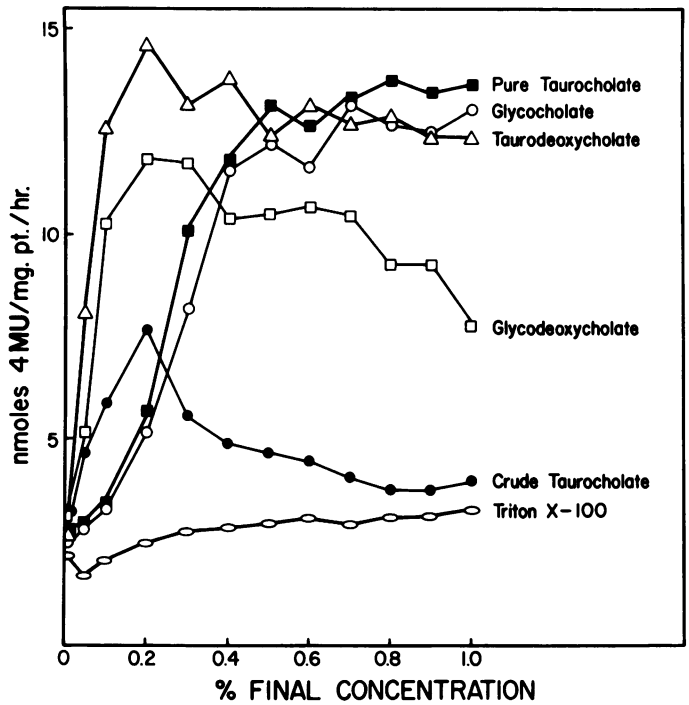


FIG. 3. —Effect of several detergents at varying concentrations on leukocyte β-glucosidase activity

acidic, inhibited by bile salt, and unaffected in Gaucher disease, is membrane-bound in leukocytes, while in soluble form in spleen and liver [9, 24] and separable by gel filtration. The more acidic β-glucosidase, which is stimulated by bile salt and the deficient enzyme in Gaucher disease, is particle-bound in all of the tissues examined.

*Heat Stability*

The thermal stability of the acid β-glucosidase at 45°C is markedly affected by the presence of TDC (fig. 6). In these experiments, the incubation mixture containing 10 μl pH 5.0 buffer, 10 μl 2% TDC, enzyme, and water in a final volume of 50 μl was kept at 45°C for the various time intervals indicated and then returned to an ice bucket. After the addition of 50 μl of substrate, the samples were incubated at 37°C for 2 hrs. In the controls, bile salt was omitted during inactivation at 45°C but added later along with the substrate and then incubated at 37°C for 2 hrs. As shown in figure 6, the presence of bile salt during thermal inactivation resulted in rapid and near total loss of enzyme activity at 45°C. However, in controls where the bile salt was added after exposure at 45°C, enzyme activity remained quite stable, suggesting that the bile salt exerts a direct effect on the membrane-bound enzyme, in addition to its possible contribution to the formation of mixed micelles with substrate. As the bile salt shifts the pH optimum of the enzyme and stimulates the activity fivefold, it also renders it unstable to heat at 45°C.

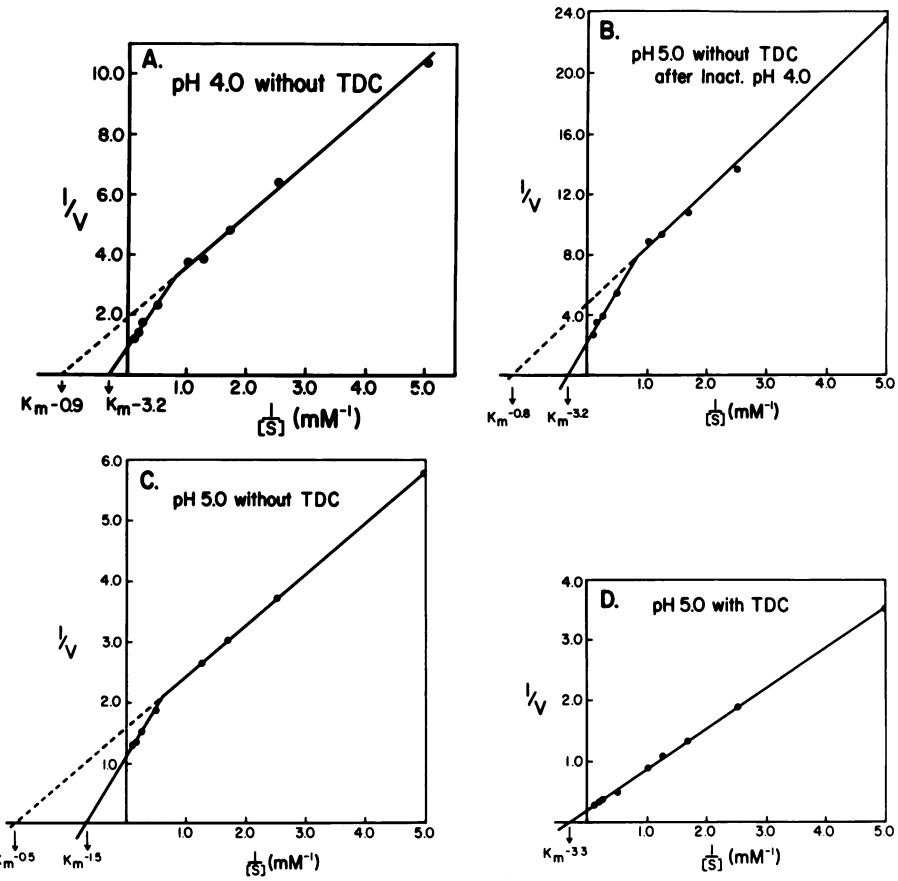


FIG. 4. —Lineweaver-Burk plots for leukocyte β-glucosidase assayed under different conditions as stated in figure.

The thermal stability of β-glucosidase enzymes at 52.5°C is shown in figure 7. After heat inactivation was performed as outlined in METHODS, the activity was determined at the indicated pH with and without 0.2% TDC. β-Glucosidase activity in controls assayed at pH 4.0 without TDC is more thermostable than at pH 4.8 (fig. 7*A*). However, in comparison with control activity at pH 4.0, the residual β-glucosidase in Gaucher disease at this pH is thermolabile. Increased heat lability of acid β-glucosidase in Gaucher disease compared with controls has also been observed by Mueller and Rosenberg [25] in cultured fibroblasts. This difference in the thermostability of the deficient enzyme in Gaucher disease demonstrable in the absence of bile salt is not evident when assayed in the presence of TDC (fig. 7*B*). Thermolability of the residual acid β-glucosidase in Gaucher disease might result from an abnormality in the membraneous association of the mutant enzyme, but this abnormality is not evident when assayed in the presence of a detergent that potentially affects membrane properties.

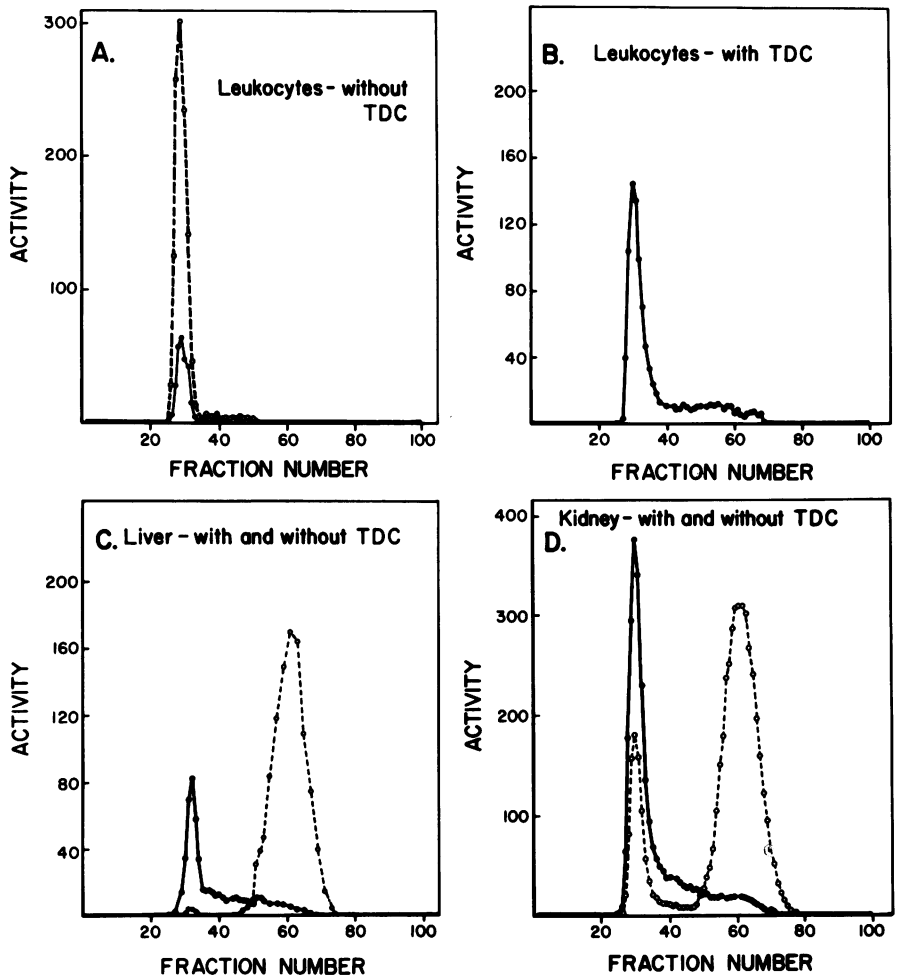


FIG. 5.—Gel filtration pattern of β-glucosidase from leukocytes, liver, and kidney assayed under different conditions. Total activity of each fraction in units of fluorescence at 446 nm is plotted against fraction number. *A*, Leukocyte enzyme assayed without TDC at pH 4.0 (●——●) and pH 5.0 (○---○); *B*, leukocyte enzyme assayed at pH 5.0 in presence of TDC; *C*, liver enzyme assayed at pH 5.0 in absence of detergent (○---○) and in presence of TDC (●——●); *D*, kidney enzyme assayed at pH 5.0 in the absence of detergent (○----○) and in presence of TDC (●——●).

*Stability in Freezer Storage*

Sonicated leukocyte homogenates were checked for the stability of β-glucosidase during storage for different periods in the −20°C freezer. After 3 weeks and 8 weeks of storage of the homogenate, thawing and resonication results in marked reduction in activity at pH 4.0 and 4.8 assayed in the absence of TDC. But the same samples show no change in activity when assayed at pH 5.0 in the presence of bile salt (table 1). Freeze-thaw and resonication seem to affect the membrane-bound β-glucosidase activity in the absence of detergent, whereas the bile salt restores its normal activity,

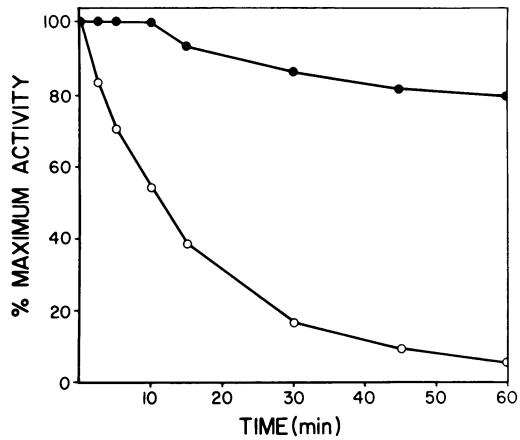


FIG. 6. — Leukocyte β-glucosidase activity following thermal inactivation at 45°C for different time intervals in presence of TDC (○——○) and in absence of TDC (●——●). Final determination of enzyme activity was made with detergent present in reaction mixture.

suggesting that it is undesirable to perform diagnostic assays for carriers and affected individuals in the absence of bile salt because of the apparent loss of activity by freeze-thaw of the stored enzyme and aging of the enzyme preparation. Such artificial loss of activity can be avoided by performing the β-glucosidase assay with fresh sonicates in the presence of bile salt.

The $K_m$ and $V_{max}$ of β-glucosidase in controls, carriers, and the disease state assayed in the presence of TDC is shown in table 2. It is obvious that the $K_m$ is unaffected in carriers and diseased patients, while the $V_{max}$ does reflect the expected deficiency.

The final optimal assay conditions established for diagnosing Gaucher disease and its carrier state are given in table 3. The reaction is linear with respect to the amount of protein used and time of incubation. The efficacy of this diagnostic technique is demonstrated in figure 8. The mean β-glucosidase activity in 42 controls was 18.29 nmoles/mg protein per hr. In 26 patients with the disease, the mean specific activity was 2.54, which represents about 14% residual activity. In 32 obligate heterozygotes, the mean specific activity was 10.02, amounting to 55% of mean control activity. There is no overlap of the obligate heterozygotes with either controls or diseased patients. Even if we allow 1.8 times the standard deviation observed beyond the mean heterozygote value, only one out of 32 obligates fell outside the maximum limit of 14 nmoles/mg protein per hr. Even this single carrier with specific activity of 14.5 fell just below the lowest control value of 15 nmoles/mg protein per hr. Thus, the present assay technique is suitable for carrier detection in unknown subjects with 97% accuracy, with only 3% falling into an inconclusive group defined by a specific activity in the 14–15 nmoles/mg protein per hr range.

In applying this procedure to healthy relatives of patients with Gaucher disease, we diagnosed 18 subjects as potential carriers, and five as normal controls. The relationship of these 23 people to patients with Gaucher disease, the expected frequency and number of heterozygotes in each category of relationship, and the actual

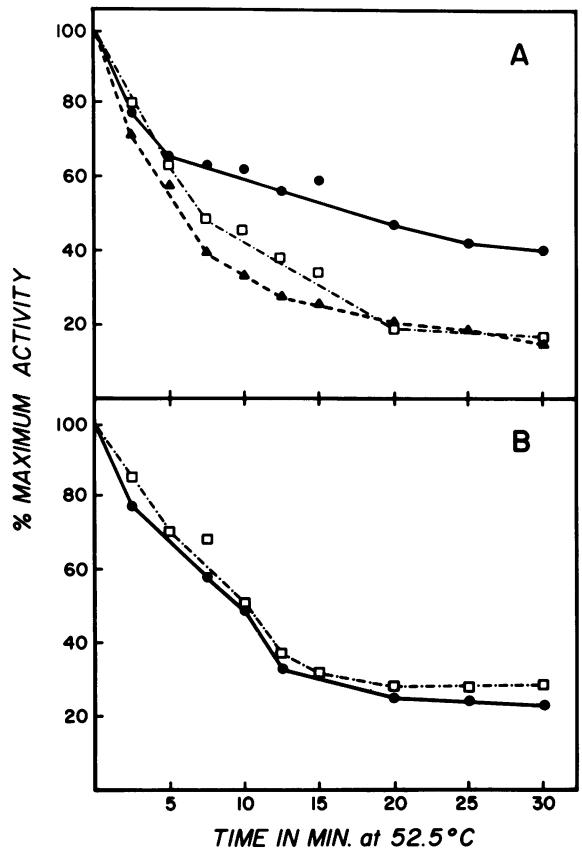


FIG. 7.—Thermal stability of β-glucosidase in absence of detergent at 52.5°C. *A*, Assay performed in absence of TDC in control at pH 4.0 (●——●) and pH 4.8 (▲---▲), and in Gaucher disease at pH 4.0 (□ · - · - · □); *B*, assay performed in presence of 0.2% TDC at pH 5.0 in same control (●——●) and Gaucher disease patient (□ · - · - · □).

number observed are shown in table 4. These results demonstrate the potential reliability of this assay technique for carrier screening of individuals at risk for the Gaucher disease trait.

TABLE 1

STABILITY OF LEUKOCYTE β-GLUCOSIDASE IN FREEZER STORAGE

| | SPECIFIC ACTIVITY (NMOLES/MG PROTEIN/HR) | | |
|---|---|---|---|
| | WITHOUT TDC | | WITH TDC |
| | pH 4.0 | pH 4.8 | pH 5.0 |
| First sonicate | 4.57 | 5.68 | 21.2 |
| Second sonicate (after 3 wks) | 3.02 | 2.69 | 21.8 |
| Third sonicate (after 8 wks) | 2.26 | 2.12 | 20.2 |

NOTE.—Enzyme activity was assayed in absence and in presence of 0.2% TDC as outlined in METHODS.

TABLE 2

$K_m$'s OF LEUKOCYTE β-GLUCOSIDASE DETERMINED IN PRESENCE OF 0.2% TDC

| Sample nos. | $K_m$ (mM substrate) | $V_{max}$ (nmoles 4MU/mg protein/hr) |
|---|---|---|
| Controls: | | |
| 1 | 4.0 | 20.0 |
| 2 | 4.0 | 17.3 |
| 3 | 4.0 | 20.0 |
| 4 | 3.3 | 23.0 |
| Heterozygotes (type 1): | | |
| 1 | 2.9 | 10.2 |
| 2 | 3.6 | 10.6 |
| 3 | 3.3 | 11.9 |
| Homozygotes (type 1): | | |
| 1 | 4.0 | 2.0 |
| 2 | 3.3 | 2.7 |
| 3 | 3.3 | 2.1 |

NOTE. —$K_m$'s determined as outlined in METHODS.

## DISCUSSION

The presence of two particulate 4MU-β-glucosidases in leukocytes, only one of which is the deficient enzyme in Gaucher disease, requires a selective assay technique for the enzymatic diagnosis of this disease and its carrier state. As shown by Turner et al. [26] and confirmed in this study, the unaffected enzyme is irreversibly inactivated by exposure to pH 4.0, so that the assay technique of Beutler and Kuhl which utilizes pH 4.0 [13] measures only the activity affected in Gaucher disease. We find it particularly important to use freshly prepared leukocytes for their assay at pH 4.0, as freeze-thawed and stored leukocytes yield an artifactual loss of enzyme activity affecting proper diagnosis. Furthermore, the substrate concentration used by Beutler and Kuhl [13] is too low to be saturating. The unreliability and insensitivity of their assay at pH 4.0 can be overcome by utilizing optimal amounts of the anionic detergent TDC at pH 5.0, together with concentrations of substrate that are saturating.

Normal 4MU-β-glucosidase activity has been reported in the leukocytes of one patient [27] and liver of several patients [27, 28] with Gaucher disease. However,

TABLE 3

ASSAY CONDITIONS FOR β-GLUCOSIDASE IN LEUKOCYTES

| | Amount | Final concentration |
|---|---|---|
| Substrate: | | |
| 4MU-β-D-glucopyranoside (20 mM) | 50 μl | 10 mM |
| Buffer: | | |
| Citrate-phosphate (1 M in citrate), pH 5.0 | 10 μl | 0.1 M in citrate |
| Bile salt: | | |
| TDC (2%) | 10 μl | 0.2% |
| Enzyme: | | |
| Leukocyte homogenate in saline | 10—60 μg protein | ... |

NOTE. —Distilled water to total incubation volume of 100 μl; incubation: 2 hrs at 37°C.

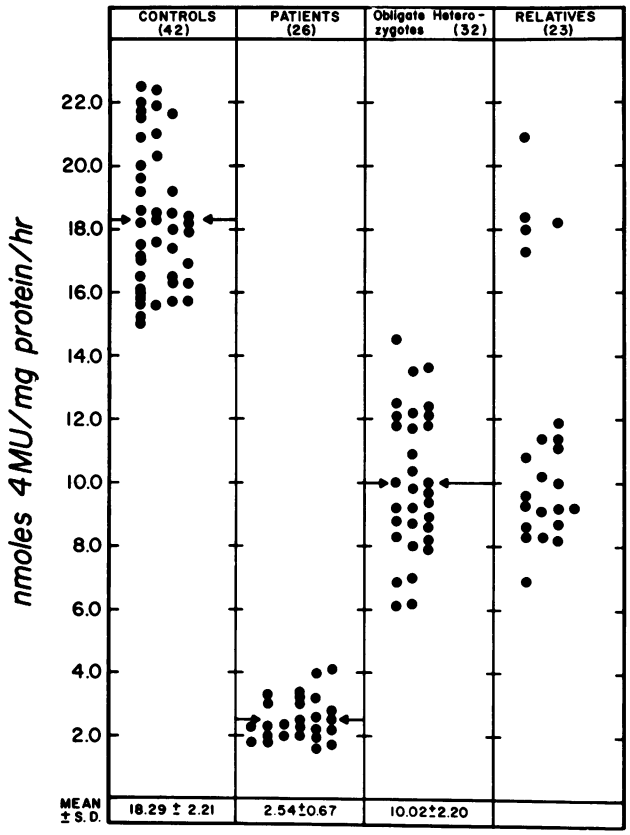


FIG. 8.—Leukocyte β-glucosidase activity assayed according to conditions given in table 3 in 42 controls, 26 patients with Gaucher disease, 32 obligate heterozygotes, and 23 healthy relatives of patients. *Arrows* in each column indicate mean value for that group.

sufficient experimental details have not been published to judge whether the authors of these reports used methods for the assay of acid β-glucosidase activity that would eliminate interference by other uninvolved β-glucosidase isozymes known to be present in high levels in these tissues. Butterworth and Broadhead [29] have clearly shown that the measurement of liver acid β-glucosidase activity for the diagnosis of Gaucher disease requires a selective assay procedure to eliminate the interfering particulate and soluble enzyme components.

It has been shown that taurocholate stimulates particulate acid β-glucosidase in tissues [7, 10, 30], while it inhibits the soluble isozyme [7]. As reported by Peters et al. [7], the leukocyte β-glucosidase that is active at pH 4.0 and is the deficient enzyme in Gaucher disease has been found in this study also to be markedly stimulated by TDC with a shift in the pH optimum. Since the unaffected β-glucosidase isozyme is suppressed by TDC, inactivation of this enzyme by prior exposure of the leukocyte homogenate to acid pH 4.0 is not necessary. Thus, the presence of this detergent serves

TABLE 4

CARRIER PROBABILITY AMONG RELATIVES OF GAUCHER PATIENTS

| Relationship to patient | No. | Carrier probability among healthy members | Expected carriers | Observed carriers |
|---|---|---|---|---|
| Sibling | 14 | 66.67% | 9.33 | 12 |
| Aunt or uncle | 2 | 50% | 1 | 2 |
| Grandchildren | 2 | 50% | 1 | 1 |
| Niece or nephew | 4 | 33.33% | 1.33 | 3 |
| First cousin | 1 | 25% | 0.25 | 0 |
| Total | 23 | ··· | 12.91 | 18 |

the dual advantage of stimulating the acid β-glucosidase and inhibiting the other isozyme which makes the assay specific for the diagnosis of Gaucher disease.

Leukocyte cerebroside β-glucosidase, which is regularly assayed in this laboratory, requires 0.4% TDC for optimal hydrolysis of [$^{14}$C]glucocerebroside, while 0.2% is sufficient for maximal stimulation of 4MU-β-glucoside hydrolysis. The apparent $K_m$ for glucocerebroside is 0.3 mM with a $V_{max}$ of 9.7 nmoles/mg protein per hr. Thus, the enzyme exhibits a much stronger affinity for the natural substrate than the artificial substrate, but the maximal hydrolytic rate for the sphingolipid is about one-half of what has been obtained with the 4MU-β-glucoside. The higher hydrolytic rate seen with the synthetic substrate, coupled with the sensitivity of the fluorometric technique for analysis of the product formed, enable the assay to be performed with only a tenth of the amount of leukocyte protein required to demonstrate the hydrolysis of glucocerebroside. Drawbacks to routine diagnostic use of the natural substrate assay are the relative scarcity and high cost of the radioactively labeled sphingolipid and the amount of time required to complete the assay. The simplicity, speed, and selectivity of the artificial substrate technique described here for assaying the deficient enzyme in Gaucher disease offers advantages in screening large numbers of individuals at risk for this genetic disorder.

The use of crude bile salt as employed in the procedure of Peters et al. [18] should be avoided, since it is inhibitory and thus renders the assay unreliable. Both we and Peters et al. [7] have observed progressive inhibition of leukocyte 4MU-β-glucosidase by varying concentrations of crude taurocholate. However, the kinetic data reported by Peters et al. [7] indicated no change in the $V_{max}$ whether the assay was done in the absence or presence of 0.6% crude taurocholate. Pure taurocholate, as reported here, consistently stimulates enzyme activity. We preferred pure TDC as it gives better stimulation at a much lower concentration compared with pure sodium taurocholate. Wenger et al. [31, 32] have published a procedure using a mixture of pure taurocholate and Triton X-100 for the assay of leukocyte β-glucosidase with 4MU-β-glucoside as the substrate. The $K_m$ value which they reported is in agreement with our data, but the substrate concentration they used is insufficient to be saturating. The values they obtained for leukocyte β-glucosidase are about half of what we found using a saturating substrate concentration in our assay system. Svennerholm [33] also used a mixture of taurocholate and Triton for measuring leukocyte β-glucosidase activity in Gaucher

disease. The substrate concentration in this method also is far below the level necessary for enzyme saturation. This may be responsible for the reported inadequate separation of heterozygotes from controls and patients. It is necessary to perform enzyme assays at saturating substrate concentration under zero-order-kinetics to compare levels of activity among various samples.

The leukocyte fraction employed in this study contained a mixture of mononuclear and polymorphonuclear cells. Since monocytes are enriched in acid β-glucosidase activity [34], the total specific activity of a Gaucher heterozygote with monocytosis could be in the normal range. This factor must be considered in evaluating the results of carrier screening for individuals at risk for the Gaucher disease gene.

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