β-Glucosidase Assays in the Diagnosis of Gaucher’s Disease

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The description in 1965 of glucocerebroside:β-glucosidase as the enzymic defect in Gaucher’s disease stimulated considerable research interest and effort toward establishing rapid, reliable, and inexpensive enzymic assays for diagnostic purposes and carrier detection. Here, we consider some of the methods currently in use in which the substrate is the synthetic glucoside, 4-methylumbelliferyl-β-D-glucopyranoside, and leukocytes and fibroblasts are the sources of enzyme. We also consider the concepts of the “acid β-glucosidase” and multiple forms of β-glucosidase that have been proposed to explain the effectiveness of the fluorometric assays. Finally, we analyze the limitations of each method and discuss the difficulties involved in instituting heterozygote screening programs in the general population.

Additional Keyphrases: heritable disorders · screening · enzyme activity · cytosolic and lysosomal isoenzymes · glucocerebroside · sphingolipidoses

It has been more than 10 years since Beutler and Kuhl (1) first demonstrated the usefulness of the fluorogenic, nonphysiologic substrate 4-methylumbelliferyl-β-D-glucopyranoside (MUG)² for the diagnosis of Gaucher’s disease and carrier identification, using leukocytes as a source of glucocerebroside:β-glucosidase. During the past decade additional β-glucosidase assay conditions have been described and new substrates introduced for confirming the diagnosis of Gaucher’s disease on biochemical/ enzymic grounds. Despite the proven usefulness of these various assays for estimating the relative glucocerebroside content of leukocytes and fibroblasts, there is still considerable confusion regarding the basis for their effectiveness. The purpose of this report is to describe and compare the various leukocyte β-glucosidase assays used in the diagnosis of patients and detection of carriers of Gaucher’s disease and to relate why each is effective. To this end we analyze the concept of “acid β-glucosidase” and the question of multiple forms of β-glucosidase. Given the limitations of current methodologies, we discuss the problems that would be involved in screening for heterozygotes of Gaucher’s disease.

The Disease and Its Diagnosis

Gaucher’s disease is a familial inborn error of metabolism that follows an autosomal recessive mode of inheritance (2). It is one of the most common of the sphingolipidoses, a class of disorders in which sphingolipids accumulate within the cells of the reticuloendothelial system because of a deficiency of a catabolic enzyme. The primary enzymic defect in Gaucher’s disease is the profound lack of glucosylceramide:β-glucosidase (glucocerebroside) (3, 4).

Clinically, Gaucher’s disease appears in multiple forms (2). Most patients with Gaucher’s disease express the “type 1,” “adult,” or “chronic, non-neuropathic” form. These patients experience hepatosplenomegaly, anemia, thrombocytopenia, and erosion of the cortices of the long bones (5). The expression of these symptoms is highly variable; some individuals are devastated by the disease processes early in life, while others remain relatively unaffected for seven or eight decades. Usually individuals with type 1 disease are free of neurological involvement. However, with increasing frequency, we are seeing reports of persons with type 1 Gaucher’s disease who develop serious central nervous system disease later on in life, years after the initial diagnosis has been made.

In contrast, neurologic dysfunction is considered the hallmark of the second form of Gaucher’s disease, the “type 2,” “infantile,” or “acute neuropathic” form (2). This form often presents several months after birth and is characterized by such neurologic symptoms as muscle hypertonicity and persistent retroflexion of the head in addition to organomegaly and general failure to thrive. Death usually occurs before two years of age.

The third type of Gaucher’s disease is the “type 3,” “juvenile,” or “subacute neuropathic” form. These patients show a broad spectrum of clinical signs, generally including organomegaly and some neurologic dysfunction. Patients with type 3 Gaucher’s disease show a more protracted course of illness and usually survive longer than type 2 patients.

The preliminary diagnosis of Gaucher’s disease relies on the demonstration of organomegaly and anemia in individuals whose family history indicates a previous occurrence of Gaucher’s disease. Moreover, each of the three forms of Gaucher’s disease “breeds true”; that is, a case of neurologic disease appears only rarely in a pedigree characterized by the non-neurologic form of the disease (D.A. Wenger, personal communication).

Given a suspicion of Gaucher’s disease, the first stage in confirming the diagnosis historically involves examination of a bone-marrow aspirate for Gaucher cells. These histiocytes accumulate significant quantities of glucocerebroside and produce characteristic inclusion bodies within the cytoplasm. The unambiguous identification of Gaucher cells is sometimes difficult because several other storage disorders show similar cytoplasmic inclusions when examined with a light microscope (5). Furthermore, in some instances, it has been difficult to locate Gaucher cells in a bone-marrow aspirate, especially in individuals with mild symptomology (5). Secondly, serum acid phosphatase (EC 3.1.3.2) activity is determined, because Gaucher’s disease is also characterized by a marked increase in a type 5 isoenzyme of acid phosphatase (6).

The most specific method for the detection of an increase in type 5 acid phosphatase activity in serum of persons with Gaucher’s disease is that described by Chambers et al. (7), in which 4-methylumbelliferyl phosphate is the substrate. The
assay is performed at pH 6 in the presence of 2-mercaptoethanol, 3.0 mol/L. This method is significantly more selective and sensitive than procedures in which p-nitrophenyl phosphate or thymolphthalein phosphate is the phosphatase substrate. A review (8) of the use of serum acid phosphatase determinations in the presumptive diagnosis of Gaucher’s disease has appeared elsewhere.

Both the fluorometric and colorimetric procedures have their limitations, the principal one being that an unremarkable serum acid phosphatase value is not uncommon in someone with proven Gaucher’s disease. Although an increase in serum acid phosphatase activity has been seen in most patients with Gaucher’s disease (9) when assays are performed under optimum conditions, acid phosphatase activity can also be increased in several other disease states (8, 10); an increase in type 5 acid phosphatase isoenzyme activity in serum is not specific for Gaucher’s disease.

The definitive biochemical test for the diagnosis of Gaucher’s disease is the demonstration of the deficiency of tissue glucocerebrosidase. Although any one of a variety of tissues could be used to demonstrate the glucocerebrosidase deficiency, leukocytes or fibroblasts are the most readily available tissues and therefore the most commonly used source of enzyme. With the methods currently available, glucocerebroside activity cannot be measured in serum or urine.

Glucocerebrosidase activity can be measured by using the authentic glycolipid substrate (Figure 1), radiolabeled in either the glucose (11) or the long-chain fatty acid moiety (12, 13). However, methods that involve radiolabeled authentic substrate are relatively time-consuming, require expensive equipment, and are not readily applicable to the usual clinical laboratory setting. Consequently, various procedures in which nonspecific artificial glucosides (Figure 2) are substrates have been developed to avoid some of the operational problems involved in using the authentic substrate. In the following discussion we review some of the more common assays involving nonphysiologic substrates (Table I) applied to leukocytes and cultured fibroblasts for confirming the diagnosis of Gaucher’s disease and for heterozygote detection.

**Leukocyte β-Glucosidase Assays**

**“Acid β-Glucosidase” Assay**

Encouraged by the observation of Ockerman and Kohlin (14) that extracts of liver and spleen from patients with Gaucher’s disease were deficient in their capacity to hydrolyze glucose from MUG (Figure 2), Beutler and Kuhl (1) in 1970 used this fluorogenic substrate in developing a technique to estimate the relative glucocerebrosidase content of human leukocytes. They were the first to show that the specific enzymic lesion of Gaucher’s disease could be demonstrated by using nonphysiologic β-D-glucosides as substrates and leukocytes as sources of enzyme. Assays at pH 4.0, a condition far below the pH optimum of glucocerebrosidase (15), allowed optimum differentiation between leukocytes of patients with Gaucher’s disease and those of control subjects. The “acid β-glucosidase” assay, carried out in sodium acetate buffer, 4.4 mmol/L, pH 4.0, requires 0.5 to 1.0 mmol of MUG per liter. Nearly all investigators who study Gaucher’s disease or other lysosomal storage disorders isolate leukocytes by the rapid, simple, and inexpensive dextran settling procedure (15).

In their first publication (16) describing this assay, Beutler and Kuhl correctly identified seven out of seven subjects with adult Gaucher’s disease; however, for two out of eight (25%) obligate heterozygotes, “acid β-glucosidase” values were in the control range.

Although the “acid β-glucosidase” assay has proven useful in identifying patients and some carriers of Gaucher’s disease in some laboratories (1, 16), we (17) and others (11, 18) have found the “acid β-glucosidase” assay generally unsatisfactory in identifying heterozygotes for Gaucher’s disease when leukocytes serve as the source of enzyme.

In a more recent study (19) involving extracts of leukocytes from 22 normal individuals, 11 obligate heterozygotes, and five patients with type 1 Gaucher’s disease, we found that seven of 11 obligate carriers had β-glucosidase values within the control range when analyzed by the “acid β-glucosidase” procedure of Beutler and Kuhl (1). Two of these 11 carriers (18%) had enzyme activities within one standard deviation (SD) of the control mean. That is, on the basis of assigning 1 SD below the control mean as the maximum value of the heterozygote group of specimens or as the lower limit of the control range, some obligate heterozygotes were classified as noncarriers. We have also found that this “acid β-glucosidase” procedure produces the greatest degree of dispersion about the means of each group of samples (1 SD = 21.4% of the mean for the control sample and 1 SD = 37.6% of the mean for the carrier sample), thus accounting for the high degree of overlap between the control and heterozygote groups with this assay. A large dispersion about the mean β-glucosidase value for the control group of specimens is undesirable because the greater the standard deviation, the more extensive will be the overlap.

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**Fig. 1. Reactants and products of the glucocerebrosidase reaction**

**Fig. 2. Structure of two nonphysiologic β-glucosidase substrates and their reaction products**
between heterozygote and control values. Statistical evaluation of our results suggests that nearly one-fourth (23.6%) of all true heterozygotes would show "acid β-glucosidase" values within the control range and would therefore be misclassified as noncarriers.

"pH 5.5 Taurocholate" Assay

Identity of the "acid β-glucosidase." Until Peters et al. (15) reported on the results of incorporation of the bile salt sodium taurocholate into the fluorometric (MUG) β-glucosidase assay, most investigators could detect heterozygotes and diagnose Gaucher's disease with assays involving MUG only if the pH of the incubation medium was 4.0–4.3 (16, 20). That one should use any β-glucoside as substrate at pH 4.0 to compare the relative glucocerebroside contents of crude extracts of tissues was always puzzling to us because glucocerebrosidase in extracts of leukocytes (15), liver (21), spleen (22), brain (23), fibroblasts (24), and placenta (25) exhibits optimum activity in the pH range 4.5–5.5. Furthermore, even when extensively purified preparations of lysosomal glucocerebrosidase are assayed with MUG, maximum activity is seen at or above pH 5, not in the pH range 4.0–4.3. The often repeated justification for this discrepancy between the pH optimum of glucocerebroside (approximately pH 5.5) and the need to use an acidic (pH 4.0–4.3) assay medium (in the absence of any bile salt) to detect heterozygotes was that leukocytes contain two β-glucosidase isoenzymes, one with a pH optimum of approximately 4.0 and the other with an optimum near pH 5.5. For some time it was widely held that the β-glucosidase isoenzyme missing or inactive in Gaucher's disease was the one with an optimum at pH 4.0 and that this isoenzyme was actually glucocerebrosidase. The impetus to ascribe Gaucher's disease to a deficiency in "acid β-glucosidase" arose from Beutler and Kuhl's finding that, in the pH range 3.0–6.5, leukocytes from healthy controls exhibited a double-peak curve for enzyme activity vs pH (17, 18). The enzyme activity at pH 4.0 was affected to a much greater degree in Gaucher's disease than was the more prominent activity peak at pH 5.0–5.5.

However, there is at least one other simple explanation for the discrepancy in the pH optimum of lysosomal glucocerebrosidase and the pH at which "acid β-glucosidase" assays are effective in confirming the diagnosis of Gaucher's disease. Assume that there are two β-glucosidase isoenzymes in leukocytes, one of which is actually glucocerebrosidase, which utilizes both glucocerebroside and MUG as substrates, whereas the second β-glucosidase cleaves only MUG and not glucocerebroside. We believe that the two prominent leukocyte β-glucosidase isoenzymes both have pH optima in the vicinity of pH 5.0–5.5. Moreover, we propose that the second leukocyte β-glucosidase is a particulate isoenzyme of broad specificity and is very similar in terms of substrate specificity and inhibitor sensitivity to the prominent cytosolic β-glucosidase of liver and spleen (26). The broad-specificity β-glucosidase utilizes a wide range of substrates including 4-methylumbelliferyl derivatives of β-D-glucose, β-D-galactose, β-D-xylene, α-L-arabinose, and β-D-fucose (28). Thirdly, we suggest that although in every case of Gaucher's disease there is a profound deficiency in lysosomal glucocerebrosidase, most tissues (including leukocytes) of such cases are not deficient in β-glucosidase activity that is attributable to the broad-specificity isoenzyme.

On the basis of these three considerations, we propose the following explanation for the effectiveness of the pH 4.0 fluorometric (MUG) assay in diagnosing Gaucher's disease and detecting heterozygotes when leukocytes are the source of enzyme. Although the pH optima for both β-glucosidase isoenzymes are in the range 5.0–5.5, the activity of lysosomal glucocerebrosidase extends well down into the acid range (especially when the assay medium is supplemented with a bile salt), such that at pH 4.0 the β-glucosidase isoenzyme corresponding to glucocerebrosidase will catalyze hydrolysis of MUG; glucocerebrosidase activity at pH 4.0 is still about 40% of that measured at pH 5.5 (Figure 3). In contrast, the broad-specificity β-glucosidase is characterized by a sharper pH curve on the acid side, such that in the region near pH 4.0 the enzyme is essentially inactive. Thus, the diminished "acid β-glucosidase" activity observed with leukocytes from persons with Gaucher's disease or from heterozygotes does not represent a specific deficiency of some β-glucosidase isoenzyme with a pH optimum of pH 4.0; instead, the successful application of the "acid β-glucosidase" assay to diagnose Gaucher's disease reflects the need to use artificial substrates at a pH where the broad-specificity β-glucosidase does not demonstrate activity (i.e., at pH ≤4.0). In short, there is probably no "acid β-glucosidase" enzyme as such in human leukocytes.

In an effort to provide some support for the view that the "acid β-glucosidase" is an artifact and that leukocytes contain two principal pH 5.0–5.5 β-glucosidase isoenzymes, both of which utilize MUG as substrate but only one of which is ly-
sosomal glucocerebrosidase, we performed a reconstitution experiment using glucocerebrosidase and broad-specificity \( \beta \)-glucosidase, both from human liver. We mixed various proportions of glucocerebrosidase derived from liver membranes with cytosolic, broad-specificity \( \beta \)-glucosidase. The broad-specificity \( \beta \)-glucosidase catalyzes the hydrolysis of MUG but not glucocerebrosidase, has a pH optimum in the range pH 5.5–6, is inhibited by sodium taurocholate, and is insensitive to conduritol B epoxide. Conduritol B epoxide (CBE), a potent, irreversible inhibitor of glucocerebrosidase, does not affect the activity of the broad-specificity \( \beta \)-glucosidase that is deficient in leukocytes of patients with Gaucher’s disease. Originally synthesized by Legler (27), CBE is the 1,2-epoxide of myoinositol. Dr. Norman Radin of the University of Michigan, who has since revised the synthesis (28), generously provided the CBE used in these studies. Glucocerebrosidase also hydrolyzes MUG over a broad pH range with optimum activity at pH 5.0–5.5, and is activated by bile salts but profoundly inhibited by CBE. Given the sensitivity of leukocyte \( \beta \)-glucosidase activity to CBE at pH 5.5 with MUG substrate, we estimate that of the total \( \beta \)-glucosidase activity in leukocytes from healthy controls, 25% is accounted for by the broad-specificity \( \beta \)-glucosidase, with lysosomal glucocerebrosidase accounting for the other 75% of the activity. We mixed the two enzymes in proportions approximating those that would occur in leukocytes from controls, heterozygotes, and patients with Gaucher’s disease (Table 2). The “control” leukocyte preparation contained 100 units (nmol/h per milligram of protein) of glucocerebrosidase for every 30 units of the broad-specificity \( \beta \)-glucosidase. The “heterozygote” sample contained only half as much glucocerebrosidase (50 units) as the “control” but the same 30 units of broad-specificity \( \beta \)-glucosidase. Finally, the specimen representing leukocytes from a patients with adult type 1 Gaucher’s disease was formed by mixing 15 units of glucocerebrosidase (representing a patient with 15% of normal glucocerebrosidase activity) with 30 units of broad-specificity \( \beta \)-glucosidase.

We analyzed three different reconstituted enzyme preparations by several of the \( \beta \)-glucosidase assay techniques used to diagnose Gaucher’s disease and to detect carriers when leukocytes serve as the source of enzyme, and compared the assays for their ability to confirm the “diagnosis” of Gaucher’s disease and to detect the “heterozygote” (Table 2). Note that in the absence of detergent at pH 5.5, the fluorometric \( \beta \)-glucosidase assay failed to demonstrate the profound deficiency of \( \beta \)-glucosidase activity in the “patient” specimen (49.4% of control). Using the pH 4.0 “acid \( \beta \)-glucosidase” assay procedure of Beutler and Kuhl (1), we could identify the “patient” (29.1% residual activity) and the “heterozygote” (60.7% of the “control” activity), a result consistent with reports (11–21) that the mean leukocyte activity of \( \beta \)-glucosidase in the obligate carrier population is usually 55–60% of that observed for the control, rather than the theoretical value of 50%. Note also that the absolute \( \beta \)-glucosidase values obtained were five- to 10-fold lower than those observed when assays were performed at pH 5.5.

When a bile salt was incorporated into the pH 5.5 assay, as in the methods of Peters et al. (15) or Daniels et al. (19), the \( \beta \)-glucosidase activity of the “patient’s” leukocytes was 21.7–25.6% of the control activity and the “heterozygote” value was nearly 50% of the control values. Also, the absolute difference between values for control and patient in the assays that contained bile salt was greater than with MUG alone, whether at pH 5.5 or 4.0, illustrating the value of incorporating the bile salt into the \( \beta \)-glucosidase assay.

Inclusion of bile salts. The strategy of using a bile salt in the fluorometric leukocyte \( \beta \)-glucosidase assay for Gaucher’s disease was conceived when we observed that the broad-specificity \( \beta \)-glucosidase of human liver was markedly inhibited by sodium taurocholate, whereas lysosomal glucocerebrosidase was activated by this agent (15). When leukocytes served as a source of enzyme, incorporating sodium taurocholate (5–20 g/L) into the assay medium allowed us to

![Graph](image-url)
confirm the diagnosis of Gaucher’s disease and to detect most obligate heterozygotes (15). We refer to this particular assay as the “pH 5.5/taurocholate” assay or the “taurocholate β-glucosidase” assay. It is performed at pH 5.5 in 0.2 mol/L sodium acetate buffer containing 6 g of sodium taurocholate per liter.

The efficacy of this method for detecting heterozygotes for Gaucher’s disease was recently compared with that of the “acid β-glucosidase” method of Beutler and Kuhl (1). From the same population of subjects as described earlier (22 normal controls, 11 obligate carriers, five patients), only one of 11 (9%) of the carriers of Gaucher’s disease fell within the control range of β-glucosidase values. The predicted degree of overlap of heterozygote values into the control range was 12.3%, or about one-half of that of the procedure of Beutler and Kuhl. Compared with the results obtained by the “acid β-glucosidase” assay, the degree of dispersion about the means for these two populations by our method was also less (18.8 vs 21.4% for the controls and 19.5 vs 37.6% for the heterozygotes), indicating that the range of observed β-glucosidase values is much narrower for the bile-salt-dependent procedure.

Another advantage of the pH 5.5/taurocholate procedure is sensitivity. The diagnosis of Gaucher’s disease can be based on a very small (0.3 mL) sample of venous blood (29), which is important when young children or infants are to be evaluated.

Definition of “control range” in heterozygote testing. Perhaps the most critical aspect of heterozygote testing is the definition of the “control” range, particularly its lower limit. Rather than resorting to intuition in assigning an individual to the control or heterozygote category, one must utilize objective criteria.

One approach is to assume that the lowest β-glucosidase value for a control population of leukocyte donors marks the lower limit of the control range. However, there are two problems with this approach. First, one can never be sure that the so-called control group does not contain individuals who are in fact true carriers of Gaucher’s disease. Secondly, when one utilizes a large sample size for the control group (e.g., 100 different specimens), a certain number of leukocyte preparations from controls will fall clearly in the range of values delimited by obligate heterozygotes. Thus, taking the β-glucosidase values of one or several “low controls” will result in the incorrect assignment of many obligate heterozygotes to the “control” category.

An alternative approach that we have found useful has been to define the control range objectively on the basis of the standard deviation; we now set the lower limit of the control range by subtracting 1 SD from the control mean. In the past, most investigators (including ourselves) have not clearly and directly indicated how they defined the control range; this point should be given more consideration in future reports.

Review of the various bile-salt-dependent assays. In 1978, Wenger et al. (18) described a very similar sodium taurocholate-dependent assay (see Table 1) that has proven to be effective in their laboratory and in ours (17) for identification of patients and carriers of Gaucher’s disease. In their hands, the modified “taurocholate β-glucosidase” assay successfully identified 12 of 12 patients with Gaucher’s disease. However, the results they obtained with leukocytes from obligate heterozygotes were not as good, underscoring the problem of carrier detection for this storage disease. They reported that two out of 12 (16%) obligate heterozygotes overlapped with the lower end of the control range. From our experience, we concur with their statement (18) that attempts to identify patients and carriers of Gaucher’s disease by using fluorogenic substrates at pH 4.0 or 5.3 without detergents, or in the presence of various amounts of Triton X-100, are unreliable.

In the same report, they also point out that the reproducibility of the assay is improved by the use of pure sodium taurocholate.

In a comparative study performed recently in our laboratory (19), we found that the “taurocholate β-glucosidase” methods of Wenger et al. (18) and Peters et al. (15) yielded very similar results; however, for a significant number of obligate heterozygotes, leukocyte β-glucosidase values fell within the control range, regardless of which β-glucosidase assay was used. We believe that regardless of which of the currently available β-glucosidase assays is used—even the glucocerebrosidase assay—there is a significant overlap (at least 10%) of heterozygote and control ranges.

In 1980, Svennerholm et al. (30) described a modified sodium taurocholate-dependent β-glucosidase assay that is performed at a relatively acid pH (see Table 1). Because the broad-specificity β-glucosidase (which does not act on glucocerebroside) is inactive at pH 4, the sole reason for including sodium taurocholate in the assay is apparently to stimulate lysosomal glucocerebrosidase. The pH 4.1/taurocholate method clearly distinguished leukocytes from Gaucher’s disease homozygotes from controls and heterozygotes; however, there was considerable overlap between the control group and the heterozygote population (three out of 16 men, 11 out of 16 women, all parents of Gaucher patients). By this procedure the coefficient of variation was 16% for the controls (men) and about 12% for the heterozygotes (men); and the mean specific activity of β-glucosidase exhibited by the heterozygotes was about 63% of the control means (both sexes).

The procedure of Svennerholm et al. (30) represents the only acid (pH 4.1) β-glucosidase assay that includes detergents; however, comparing its results with those by the method of Beutler and Kuhl (1) indicates that the presence of sodium taurocholate and Triton X-100 apparently does not increase the sensitivity of this “acid β-glucosidase” assay: mean ± 1 SD β-glucosidase values (nmol/h per milligram of protein) were controls, 3.74 ± 0.43; heterozygotes, 2.38 ± 0.50; Gaucher’s disease homozygotes, 0.83 ± 0.22.

Concurrently with the report by Svennerholm et al. (30), we reported (31) the results of a novel bile-salt-dependent β-glucosidase assay involving CBE. Using this CBE-dependent β-glucosidase assay and liver as a source of enzyme, we were able to confirm the diagnosis of Gaucher’s disease in five of five cases. We recently extended this study to examine the usefulness of this procedure with leukocytes as a source of enzyme (19). Because the standard taurocholate-dependent fluorometric assay measures glucocerebrosidase plus whatever residual nonspecific β-glucosidase activity is not inhibited by sodium taurocholate, the addition of CBE to the standard assay completely inhibits glucocerebrosidase activity such that the resulting β-glucosidase measurement determines only the activity of the residual broad-specificity β-glucosidase. Therefore, subtracting the results of fluorometric assays performed in the presence of CBE from those obtained in the absence of CBE provides a measure of relative glucocerebrosidase activity (31). When leukocytes were the source of enzyme, this assay correctly identified five of five Gaucher’s disease patients and 10 of 11 obligate carriers of the disease. The degree of dispersion (1 SD) about the mean β-glucosidase values for the control population (19.7% of mean) and heterozygote population (19.7% of mean) was comparable with that reported for the “pH 5.5/taurocholate” procedure of Peters et al. (15), but the absolute difference between the means of the two populations was slightly larger (7.3 vs 6.9 nmol/h per milligram of protein). Both of these factors contribute to decreasing the degree of overlap between the heterozygote and control ranges. Using a standard statistical
evaluation, we predicted (19) a 5% overlap of heterozygote values into the control range for the CBE-dependent β-glucosidase assay and a 12.3% overlap for the “pH 5.5/taurocholate” procedure.

Finally, Raghaven et al. (11) have described another variation of the bile-salt-dependent fluorometric assay that also helps confirm the diagnosis of Gaucher’s disease and detects heterozygotes. More importantly, they present convincing evidence for the presence of two distinct β-glucosidase isoenzymes in leukocytes. They argue that in leukocytes both β-glucosidases occur as particulate, membrane-bound enzymes, but only one is deficient in leukocyte homogenates in Gaucher’s disease. They claim that this deficient isoenzyme corresponds to glucocerebrosidase and exhibits a pH optimum of 4.0 when assays are performed in the absence of detergent and 5.0 when some bile salt is included in the assay medium. The bile salt they found to be most effective in stimulating glucocerebrosidase:β-glucosidase was sodium taurodeoxycholate; 2 to 10 g of sodium taurodeoxycholate per liter stimulates this particular β-glucosidase isoenzyme more than fivefold.

These same investigators confirm the observation of Peters et al. (15) that bile salts markedly inhibit the activity of the second β-glucosidase present in leukocytes, the one that is not deficient in Gaucher’s disease. Unlike the bile-salt-activated isoenzyme, which is stable at pH 4.0, this second β-glucosidase is rapidly inactivated by brief (5-min) exposure to pH 4.0. Because the β-glucosidase isoenzyme that is not deficient in persons with Gaucher’s disease is inhibited by taurodeoxycholate, Raghaven et al. (11) point out that inactivation of this enzyme by prior exposure of the leukocyte homogenate to pH 4.0 is not necessary when the assay is being used to detect heterozygotes or to confirm the diagnosis of Gaucher’s disease. They reiterate the principle first recognized by Peters et al. (15) that the presence of bile salt in the β-glucosidase assay for Gaucher’s disease accomplishes two things: it stimulates the acid β-glucosidase that is identical to glucocerebrosidase while inhibiting the second, broad-specificity isoenzyme, thereby making the assay specific for the diagnosis of Gaucher’s disease.

Raghaven et al. (11) show that leukocyte glucocerebrosidase has a stronger affinity for the natural substrate (i.e., glucocerebroside) than for the artificial substrate, but that the maximum hydrolytic rate for the lipid substrate is only about one-half of that obtained with MUG. Thus, the greater specificity for the artificial substrate and the greater sensitivity of the fluorometric procedure permit one to perform the β-glucosidase assay with only about 10% as much leukocyte protein as that needed for assays with radiolabeled glucocerebrosidase as the substrate. Commenting on the choice of procedures, Raghaven et al. (11) conclude that

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 . . . for assaysing the deficient enzyme in Gaucher’s disease offers advantages in screening large numbers of individuals at risk for this genetic disorder.

With their assay conditions (see Table 1) Raghaven et al. achieved remarkable separation of 42 controls from 32 obligate heterozygotes. In the obligate heterozygote group the mean leukocyte β-glucosidase specific activity was 55% of the mean of the control group, and 1 SD of values for controls and heterozygotes was 12% and 22% of the mean, respectively. They saw no overlap whatsoever of obligate heterozygotes with either controls or patients with Gaucher’s disease. This is one of the few reports of carrier testing in Gaucher’s disease in which a relatively large number of subjects were involved but no overlap of heterozygotes and controls was observed and the coefficient of variation for the control group was less than 15%. The results obtained with this particular assay are perhaps the best reported to date and should encourage other investigators to evaluate its usefulness in diagnosing Gaucher’s disease and heterozygote detection. However, it is important for others to verify the ability of this new taurodeoxycholate-dependent β-glucosidase assay to distinguish absolutely heterozygotes and controls, because screening programs should not be attempted if the overlap of heterozygote values into the control range is as frequent and extensive as that seen by most other investigators (11–21, 30, 31).

We are highly confident that all of the various fluorogenic assays that involve some bile salt will demonstrate the profound deficiency of lysosomal β-glucosidase in extracts of leukocytes. In fact, most investigators (11–21, 30, 31) have found the fluorogenic assay to be as useful as those involving the authentic substrate, glucocerebrosidase, in identifying cases of Gaucher’s disease. However, from the published results reviewed herein, approximately 5 to 20% of results for controls and heterozygotes will be misclassified in most laboratories when either the fluorometric or glucocerebrosidase-dependent β-glucosidase assays are used for carrier detection.

Other Assays

The preceding discussion presents only a sample of the fluorometric, diagnostic methods proposed during the past decade. Diagnostic assays involving use of radiolabeled glucocerebrosidase suffer from the same problem of overlap of the control and heterozygote enzyme activity ranges, despite the increased specificity associated with the use of the authentic substrate (13, 30). To illustrate the universality of this problem, we briefly describe a recently reported β-glucosidase assay procedure with a novel substrate.

In 1980, Johnson et al. (32) used a new chromogenic β-glucosidase substrate (Figure 2), 2-hexadecanoylamino-4-nitrophenyl-β-D-glucopyranoside (HNGlc), with fibroblasts from controls and patients and carriers of Gaucher’s disease as the source of enzyme (see Table 1 for other details). The data they present do not support their conclusion that HNGlc may be useful for reliable detection of carriers of Gaucher’s disease. Not only did fibroblasts from patients with the disease exhibit relatively high residual β-glucosidase activity (28% of mean control activity), but also, more disturbingly, the mean β-glucosidase value for the obligate heterozygotes they tested was 77% of the control mean. No data are available on the use of HNGlc with leukocytes, which is the most widely used source of enzyme for carrier detection.

Physical Resolution of β-Glucosidase Isoenzymes

Although a number of investigators have isolated both glucocerebrosidase (33–36) and the broad-specificity β-glucosidase (26, 36–38), the use of physical separation techniques to increase the specificity of diagnostic methods has been limited. Electrophoretic techniques (36, 39, 40) are available that resolve glucocerebrosidase and β-glucosidase activities in crude extracts of spleen, liver, kidney, fibroblasts, and leukocytes and qualitatively demonstrate the glucocerebrosidase deficiency in Gaucher’s disease, but these methods have not been used diagnostically. None of these methods are quantitative, thereby excluding their use for carrier screening. Selective inactivation by heat (41) and pH (11, 42) of the broad-specificity β-glucosidase before assay with MUG has been proposed as a possible method for improving heterozygote detection. These methods are subject to the same limitations as the procedures in which reaction mixtures are chemically manipulated to improve differentiation of controls and heterozygotes. Perhaps as our understanding of the enzymology of Gaucher’s disease advances, the physical sepa-
Screening for Heterozygotes for Gaucher’s Disease

Given the plethora of β-glucosidase assays available and the limitations of each, how reliable would a general screening program for Gaucher’s disease be at this time? Several factors indicate that mass screening could be useful. First, Gaucher’s disease appears to have several ethnic predilections; type 1 cases appear most frequently in the Ashkenazi Jewish population, type 2 among blacks, and type 3 among a select Swedish population. Secondly, within certain of these populations, the disease has a relatively high incidence; for example, the frequency of Gaucher’s disease among Ashkenazi Jews has been reported to be as high as 1:5000 to 1:10 000. Nonetheless, we would discourage the implementation of general screening at the present time of any population, Jewish or otherwise, to determine the carrier status of persons with respect to Gaucher’s disease, primarily because currently there is no biochemical/ enzymatic method that reliably identifies all carriers of Gaucher’s disease. Stated simply, the β-glucosidase assays in use today suffer from an unacceptable degree of overlap of results for control and heterozygote samples; furthermore, using various diagnostic methods, including some that involve authentic glucocerebrosidase as substrate and fibroblasts as a source of enzyme, we have encountered instances where parents of children with Gaucher’s disease exhibit “control” values for glucocerebrosidase activity. In short, the degree of misclassification of obligate heterozygotes is unacceptably high, ranging from 5 to 37% for all of the currently available β-glucosidase assays.

Given the fact that as many as one out of three true carriers of Gaucher’s disease may be incorrectly classified by results of β-glucosidase assays on leukocyte extracts, the usefulness of applying these enzyme assays to cases where no previous history of Gaucher’s disease exists should be seriously questioned. The ethical and legal problems stemming from such uncertainties intrinsic to carrier screening have been discussed by others.

Given the large degree of overlap between β-glucosidase values for heterozygote and controls that exists at the present time, two significant adverse outcomes are likely to occur if widespread screening for carriers of Gaucher’s disease is implemented. First, true carriers of the disease whose leukocyte β-glucosidase values fall in the control range will be falsely reassured that their children will not have the disease, whereas, in fact, they and their carrier spouses are at risk for having a child with Gaucher’s disease. The second untoward circumstance that can occur when a noncarrier of the disease is incorrectly designated a heterozygote is that individuals falsely labeled as carriers are likely to needlessly alter their plans for childbearing on the basis of a rather unreliable test for Gaucher’s disease heterozygotes.

With regard to the use of fluorometric β-glucosidase assays to detect heterozygotes for Gaucher’s disease, we draw the following conclusions. First, under the proper assay conditions the synthetic fluorogenic substrate MUG is as effective a reagent as authentic glucocerebrosidase. Under optimum conditions of neutral detergent, bile salt, and pH, the results of fluorometric procedures are highly reproducible. Pure reagents, particularly the bile salt, enhance reproducibility. Second, at least 15 samples (i.e., leukocytes or fibroblasts) are necessary to define adequately the mean and SD of the control population. If leukocytes are used as the source of enzyme, the cells should be disrupted just before the assays are performed. Extracts of cells prepared by sonication rapidly lose activity upon storage. Although some authors advocate the separation of leukocytes into the granulocyte, lymphocyte, and monocyte fractions before assay, this appears to be unnecessary. Third, one should use two different procedures to estimate the relative glucocerebrosidase content of leukocyte or fibroblast extracts. This internal check of results can reduce the chances of misassigning an individual because of a faulty assay. Because current procedures all rely on the same chemical properties of the β-glucosidase enzymes, multiple determinations do not increase specificity. Redundant assays minimize the chance that a procedural error goes undetected and leads to misclassification of an individual.

Also, one needs to be aware of environmental factors that could alter the interpretation of a result: illness, pregnancy, and the use of oral contraceptives have been suggested as causes for aberrant results.

On the basis of our own experience and a review of the literature, we have made the following conclusions regarding the biochemical diagnosis of Gaucher’s disease and heterozygote detection:

1. The determination of serum acid phosphatase is not a definitive test for Gaucher’s disease. Even with the most reliable assay for the prominent type 5 acid phosphatase isoenzyme, which is increased in this disease, 5% of patients with Gaucher’s disease will have serum acid phosphatase values in the control range. Furthermore, patients with other sphingolipidoses may also have an increase in serum acid phosphatase activity. Obligate heterozygotes have unremarkable acid phosphatase values.

2. Any of a number of the fluorometric β-glucosidase assays involving MUG as the glucocerebrosidase substrate allow accurate diagnosis of Gaucher’s disease when leukocytes or fibroblasts are the source of the enzyme. The β-glucosidase assays that include a bile salt and are performed at pH 5.5 are preferred over the “acid β-glucosidase” assays, being four- to fivefold more sensitive and indicating a more extensive deficiency of β-glucosidase activity. The taurocholate-dependent β-glucosidase assays support high specific activities; are sensitive, inexpensive, simple, and rapid; and reproducibly estimate relative leukocyte glucocerebrosidase activity.

3. A serious problem involved in Gaucher’s disease carrier detection is the high rate of misclassification of individuals. Under the best fluorometric assay conditions, at least one out of 20 (5%) members of the true carrier population will be given a false-negative assignment and about one out of eight (13%) noncarriers will be informed they are Gaucher’s disease heterozygotes; this means that nearly 20% of the people tested will be falsely classified. Until more effective and discriminatory assays are available that would substantially reduce the extent of overlap between the control and heterozygote populations, we would discourage screening for heterozygotes beyond families with known incidence of Gaucher’s disease. Heterozygote testing within families with a history of Gaucher’s disease is acceptable, given the limitation that as many as one out of five individuals will probably be falsely assigned to either the control or heterozygote category. The ethical and legal implications of incorrectly assigning a high proportion of counselees to the heterozygote group should be considered before undertaking screening programs for Gaucher’s disease.

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References


