

Beta-Glucosidase Activity as a Diagnostic Index of Gaucher's Disease

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SUMMARY

Gaucher's disease is a disorder characterised by accumulation of glucocerebroside in the cells of the reticulo-endothelial system due to the deficit of β -glucosidase. The lack of one iso-enzyme of β -glucosidase can be detected in the lymphocytes of the homozygotes and a severe deficit is found in the heterozygotes of the disease. We have determined the enzymatic activity in normal and homozygous subjects with Gaucher's disease and investigated relatives and descendants of one of the homozygous patients, among whom 3 carriers were detected.

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The clinical aspects of Gaucher's disease were described in 1882 by Phillippe Gaucher,¹ but the underlying biochemical disturbance has been elucidated only fairly recently. Aghion,² in 1934, recognised the compound which accumulates in the spleen as a glucocerebroside. A major advance was achieved in 1965 by Brady *et al.*³ Using isotopically labelled glucocerebroside, they showed that the spleen of 11 Gaucher patients had a markedly decreased capacity to hydrolyse the glycosidic linkage of the glucocerebroside. Lack of the hydrolysing enzyme, beta-glucosidase, in the spleen, has been confirmed recently by many other investigators. Patrick,⁴ using non-labelled glucocerebroside, found decreased activity in the spleen of four patients. Brady *et al.*, investigating brain,⁵ leucocytes^{5,6} and skin fibroblasts⁷ from patients with Gaucher's disease, observed lowered enzymatic activity in all the tissues.

In 1968, Öckermann and Kohlin⁸ demonstrated a decrease in β -glucosidase activity in the spleen and liver of Gaucher patients as measured on an artificial substrate, 4-methylumbelliferyl- β -D-glucopyranoside. This substrate offers many advantages; it is commercially available and it is hydrolysed to give 4-methylumbelliferone, which is highly fluorescent, thereby giving the assay enhanced sensitivity.

Although this method was successful in diagnosing the disease, results were unpredictable for heterozygotes. This disability was remedied in 1970 when Beutler and Kuhl⁹ demonstrated that the pH activity curve of the lymphocytic enzyme of Gaucher patients differed from that of normal subjects. Two maxima, at pH 4,0 and pH

5,3, were observed in normal individuals, whereas enzyme activity in a Gaucher patient was undetectable at pH 4,0, and was less at pH 5,3. Lymphocytes appear, therefore, to contain at least two iso-enzymes of β -glucosidase, one with a pH optimum at approximately 4,0, the other with an optimum at approximately pH 5,3. The two iso-enzymes are decreased in Gaucher's disease, although to different degrees. Carriers of the disease exhibit a normal or sub-normal activity at pH 5, but a significant decrease in activity at pH 4.

An 'acidic β -glucosidase' has been detected in fibroblasts and amniotic cells by Beutler *et al.*¹⁰ Fibroblasts did not display the typical two-peak pH optimum curve obtained with lymphocytes, but a single pH optimum at approximately 4,0 - 4,5. Heterozygotes of the disease had markedly lowered 'acidic β -glucosidase' enzymatic activity in cultures of their fibroblasts. These results were confirmed with some modifications by Wan Ho *et al.*¹¹

In this article we report investigations on β -glucosidase activity of peripheral lymphocytes.

MATERIALS AND METHODS

The method proposed by Beutler and Kuhl⁹ was followed, with minor modifications.

Twenty millilitres of venous blood were collected in a bottle containing 6,6 ml of 5% w/v dextran in 3% w/v sodium citrate and 0,85% w/v sodium chloride. The mixture was gently shaken and, after aspiration of air bubbles, the bottle was kept at 37°C for 20 - 30 minutes. Red blood cells settled out and the supernatant was collected and centrifuged at 100 g for 10 minutes. The supernatant fluid, containing most of the platelets, was discarded and the pellet of leucocytes and remaining red blood cells was diluted in 2 ml of isotonic sodium chloride. Separation of lymphocytes from the granulocytes and few remaining red blood cells was accomplished by using isopycnic centrifugation with Ficoll diatrizoate,¹² 72 ml of Ficoll (Pharmacia; 9 g/100 ml of water) and 30 ml of sodium diatrizoate (Winthrop; 35% w/v), giving a final density of 1,08. This reagent is light-sensitive but can be stored for one month in a dark bottle at 4°C. The cellular suspension was layered on the surface of 2,5 ml of Ficoll diatrizoate and horizontal centrifugation was performed for 20 minutes at 400 g. Under these conditions, granulocytes and red cells appear at the bottom of the centrifuge tube and lymphocytes at the interface between the Ficoll and the saline. The lymphocytes were collected, washed twice with isotonic sodium chloride (0,85% w/v) and suspended in a final volume of 0,2 ml of sodium chloride.

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Assay was performed with 20 microlitres of 0,2M acetate buffer (pH 4,0 or 5,3), 50 microlitres of methylumbelliferyl- β -D-glucopyranoside (1 mM), and 20 microlitres of lymphocyte suspension. After incubation for 60 minutes at 37°C, the reaction was terminated by adding 3 ml of 0,2M glycine buffer, pH 10,7. The tubes were centrifuged to sediment the cells and the supernatant fluid was read in an Hitachi fluorometer at excitation wavelength 365 nm and emission wavelength 450 nm. A standard curve of 4-methylumbelliferone (Sigma Chemicals) in glycine buffer (pH 10,7) and final concentration varying from 0,025 to 0,5 nanomoles/ml was prepared for each determination.

All assays were performed in duplicate, blanks for reagents and lymphocytes being included. Enzyme activity, in international micro-units per 10^7 lymphocytes, was calculated by means of the following expression, where χ is the number of nanomoles/ml as read from the standard curve:

$$\text{Beta glucosidase activity (in micro-units}/10^7 \text{ lymphocytes)} = \frac{\chi \times 3,09^a \times 10^7 \times 1\,000^d}{\text{No. lymphocytes}^b \times 60^c}$$

where: a = volume of assay mixture in the cuvette

b = number of lymphocytes in 20 microlitres

c = time of incubation (60 minutes)

d = factor for conversion of mili-units to micro-units.

One international milli-unit is the enzymatic activity which hydrolyses 1 nanomole of substrate per minute.

RESULTS

Normal individuals: The β -glucosidase activity of lymphocytes in 12 healthy subjects was determined. At pH 4,0 values ranged between 44 and 127 micro-units/ 10^7 lymphocytes. (These figures are similar to Beutler's findings in 20 normal subjects—38 to 80 micro-units.)

In an attempt to determine the influence of contaminating platelets, β -glucosidase activity was determined with or without low-speed centrifugation in 2 normal subjects. Findings were as follows:

Subject	β -glucosidase (micro-units) (pH 4)	
	Platelets removed	Platelets not removed
1	74	154
2	64	78

Haemolysed specimens appeared to give falsely-low values of enzymatic activity—the grossly haemolysed sample from subject 2 gave a value of 15 micro-units, while the unhaemolysed sample was 64 micro-units.

Patients with Gaucher's disease: Three adults with Gaucher's disease were investigated and findings were as follows:

Patient	β -glucosidase (micro-units)	
	pH 4	pH 5,3
1	7	16
2	11	34
3	13	19

Obligate heterozygotes: The β -glucosidase activity of one obligate heterozygote (the daughter of patient 1) was found to be 21 micro-units at pH 4 and 95 micro-units at pH 5,3.

Family study: Seven members of patient 1's family, including 2 brothers, 3 sisters and a granddaughter, were studied. Two additional heterozygotes, a brother and the granddaughter, were discovered. The β -glucosidase activity found in various members of the family is shown below:

	β -glucosidase (micro-units) (pH 4,0)
Patient 1	7
His brother	103
His brother (heterozygote)	37
His sister	65
His sister	112
His sister	56
His daughter (heterozygote)	21
His granddaughter (heterozygote)	27

DISCUSSION

Since Beutler and Kuhl's first report in 1970,⁹ extensive studies relating to the detection of homozygotes and heterozygotes in Gaucher's disease have been made. Determination of β -glucosidase activity in lymphocytes was undertaken, since lymphocytes are readily available and easy to collect. However, at least 20 ml of blood was needed to provide sufficient lymphocytes for the assay. We observed that removal of platelets is an important and critical step in the procedure. After low-speed centrifugation of citrated plasma, many lymphocytes remained in the supernatant fluid with the platelets. Although some of the lymphocytes were lost, this procedure was preferred to those which involved the defibrination of blood by the use of glass beads or wooden sticks. Defibrination by any mechanical means greatly increased the risk of haemolysis. After repeated determinations of β -glucosidase activity on specimens of blood from one individual, the assay was found to be consistent. At pH 4,0, the β -glucosidase activity of lymphocytes from normal individuals has been found to be at least 44 micro-units/ 10^7 lymphocytes and between 7 and 13 micro-units in homozygotes of Gaucher's disease. The values for the heterozygotes range between 21 and 37 micro-units. These figures agree with those of Beutler and Kuhl⁹: normal 38-86 micro-units; homozygotes 3-8 micro-units; and obligate heterozygotes 18-40 micro-units. Assay of β -glucosidase is the only method, at present, capable of detecting carrier states in Gaucher's disease. Gaucher cells are absent in carriers and there is no increase in serum acid phosphatase.

Beutler *et al.*¹⁰ and Wan Ho *et al.*¹¹ reported observations on β -glucosidase activity in skin fibroblasts. Lymphocytes are not stable in blood samples, and as yet no practical method has been devised for the storage and transport of blood samples. However, fibroblasts do not deteriorate as rapidly as lymphocytes, and the determination of

enzyme activity in fibroblasts appears to be as sensitive as that in lymphocytes. Probably, therefore, fibroblasts will prove to be more amenable to study than lymphocytes, and facilitate still further the biochemical diagnosis of Gaucher's disease.

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