Neuropathic Gaucher's Disease with Normal 4-Methylumbelliferyl- β -glucosidase Activity in the Liver

MISAO OWADA, TAKESHI SAKIYAMA, AND TERUO KITAGAWA⁽²⁵⁾

Department of Pediatrics, Nihon University School of Medicine, Tokyo, Japan

Summary

β -Glucosidase activity was measured in control subjects and in five patients with neuropathic Gaucher's disease. In three patients with Gaucher's disease, methylumbelliferyl- and *p*-nitrophenyl- β -d-glucopyranoside (4MU- and PNP- β -glucosidase) activity was almost normal in the liver but markedly reduced in the spleen and fibroblasts. In the other patients with Gaucher's disease 4MU- and PNP- β -glucosidase activity was also very much reduced in the liver, spleen, and fibroblasts.

DEAE-cellulose column chromatography with a chloride gradient elution of the liver extract from a control subject and from two patients with Gaucher's disease, exhibiting normal 4MU- and PNP- β -glucosidase activity, revealed the presence of two peaks of 4MU- and PNP- β -glucosidase activity (fractions 1 and 2). pH activity curves of β -glucosidases and K_m measured with 4MU- β -glucoside in fractions 1 and 2 from patients with Gaucher's liver were identical to those from the control liver. However, fractions 1 and 2 from infantile Gaucher's liver exhibited no activity measured with glucocerebroside whereas those from juvenile Gaucher's liver showed a considerable activity.

Glucocerebroside was greatly accumulated in the liver, even though an almost normal activity of 4MU- β -glucosidase was detected in three of the five patients studied.

Speculation

Neuropathic Gaucher's disease may be divided into two biochemical groups: one exhibits normal 4MU- β -glucosidase activity in the liver but low activity in the spleen and fibroblasts; the other exhibits low activity in the liver, spleen, and fibroblasts. The deficiency of 4MU- β -glucosidase in the liver may not account for the accumulation of glucocerebroside, and the deficiency of glucocerebroside- β -glucosidase activity probably existing within reticuloendothelial cells may be more essential for the accumulation.

Infantile or juvenile Gaucher's disease is a fatal lipidosis transmitted in an autosomal recessive manner, characterized by the accumulation of glucocerebroside in various tissues.

The activity of β -glucosidase, measured with glucocerebroside as a substrate, is known to be markedly deficient in several tissues, including spleen (3, 4, 19), liver (19, 21), skin fibroblasts (2, 8, 21), and white blood cells (9, 11), in patients with Gaucher's disease. β -Glucosidase activity has also been measured with synthetic substrates such as 4-methylumbelliferyl- β -d-glucopyranoside and *p*-nitrophenyl- β -d-glucopyranoside. A decreased activity of β -glucosidase in several tissues measured with artificial substrates has been demonstrated in patients with

Gaucher's disease (1, 2, 7, 8, 11, 17, 19). However, a high PNP- β -glucosidase activity in the liver of one patient has been reported by Patrick (19).

We report here five cases with infantile or juvenile Gaucher's disease. Three of these had considerable PNP- and 4MU- β -glucosidase activities in the liver but not in the skin fibroblasts and spleen and two had low β -glucosidase activities in the liver, spleen, and fibroblasts.

CASE REPORTS

CASE 1

The patient was a 5-month-old girl who showed retarded growth, marked hepatosplenomegaly, and neurologic signs. Liver biopsy was performed in order to confirm the diagnosis of Gaucher's disease. Marked infiltration of Gaucher cells was seen in the biopsy specimen and the accumulation of glucocerebroside was demonstrated by biochemical analysis. However, normal 4MU- β -glucosidase activity was found in the same biopsy specimen. This patient died at 6 months of age after development of signs of bulbar involvement.

CASE 2

The patient, a boy, was first noted to have feeding difficulty and marked hepatosplenomegaly when 6 months old. He died from bulbar involvement at 1 year of age. Considerable activity of 4MU- β -glucosidase was detected in the liver but not in the spleen and fibroblasts.

CASE 3

The patient, a boy, was first noted to have marked hepatosplenomegaly, strabismus, and retarded growth at 2 years of age. Bone marrow biopsy disclosed typical Gaucher cells. The accumulation of glucocerebroside was detected by thin layer chromatography in the liver and spleen. Considerable activity of 4MU- β -glucosidase was demonstrated in the liver but not in the spleen. Hepatosplenomegaly and neurologic signs increased steadily with age. At 5 years of age, the patient died from pneumonia.

CASE 4

The patient was a 5-month-old girl who showed hepatosplenomegaly, spasticity, opisthotonus, and feeding difficulty. These signs progressed steadily with age. At 1 year 3 months of age, she died from bulbar palsy. At autopsy, 4MU- β -glucosidase activity was markedly decreased in the liver and spleen.

CASE 5

The patient, a boy, was first noted to have typical signs of infantile Gaucher's disease at 6 months of age. He died from pneumonia at 10 months. At autopsy, 4MU- β -glucosidase activity was very low in the liver, spleen, and fibroblasts.

EXPERIMENTAL PROCEDURES

LIPID ANALYSIS

The specimens were kept frozen until analysis at -20° . Lipid was extracted from the tissues with chloroform-methanol (2:1, v/v), and fractionated by the method of Folch *et al.* (5). The lipid was dissolved in a small amount of chloroform and subjected to silicic acid column chromatography. Glucocerebroside was eluted with chloroform-methanol (9:1, v/v) as described by Makita *et al.* (15), and estimated by weighing or by measurement of hexose content.

For analysis of fatty acid composition, fatty acid methyl ester of purified glucocerebroside was prepared by methanolysis with $\text{BF}_3\text{-CH}_3\text{OH}$ (140 g BF_3 /liter methanol), according to the method of Morrison and Smith (16). Gas-liquid chromatography was carried out on a column with 6% polydiethylene glycol succinate on Chromosorb WAW (80–100 mesh) at 190° using a Shimadzu GC-5A type universal gas chromatograph equipped with a hydrogen flame ionization detector.

CULTURED SKIN FIBROBLASTS

Skin fibroblasts obtained from skin biopsies were cultured in F10 medium supplemented with 15% fetal calf serum. The pH was adjusted by an atmosphere of 5% CO_2 in air. Cells were cultured for 4–6 weeks after the second or third subculture. Then the cells were collected, washed with physiologic saline solution, and homogenized in distilled water. β -Glucosidase activity in fibroblasts was estimated on whole homogenates.

PREPARATION OF TISSUE MATERIALS FOR ENZYME ASSAYS

The spleen and liver tissue used in these studies were obtained by biopsy or at necropsy. The tissues were homogenized in 9 volumes of water with a glass homogenizer. The homogenates were centrifuged at $8,000 \times g$ for 20 min. The activity of β -glucosidase was assayed on samples of the supernatant and particulate fraction.

ENZYME ASSAYS

The standard assay method of glucocerebroside- β -glucosidase was as follows. (6) An emulsion of glucocerebroside, containing 1 mg glucocerebroside and 1 mg sodium cholate in 1 ml of 0.1 M citrate phosphate buffer at pH 4.6, was freshly prepared; 0.2 ml of this emulsion was added to 0.1 ml fibroblast homogenate or tissue sample to form the assay mixture.

The mixture was incubated for 6 hr at 37° with occasional shaking, and the reaction was stopped by heating for 3 min at 100° . After the addition of hexokinase, glucose-6-phosphate dehydrogenase, and NADP to the incubated test solutions, liberated glucose was estimated by a modified Lowry's method (14), in which the amount of NADPH formed was measured on a fluorometer fitted with exciting wavelength at 340 nm and emitting wavelength at 448 nm.

PNP- AND 4MU- β -GLUCOSIDASE

β -Glucosidase activity with PNP- or 4MU- β -glucoside as a substrate was estimated by the method which has been used by Patrick (19) and others (18, 20), with a slight modification.

For the assay of β -glucosidase, the incubation mixture contained 5 μ l 10 mM PNP- β -glucoside or 2 mM 4MU- β -glucoside, 50 μ l 0.1 M citrate buffer (pH 4.6), and 100 μ l tissue sample or

fibroblast homogenate. The mixture was incubated for 3 hr at 37° , and the reaction was stopped by the addition of 1–3 ml 0.25 M glycine-NaOH buffer (pH 10.6) to the mixture. The liberated *p*-nitrophenol or 4-methylumbelliferon was estimated by a fluorometer or spectrophotometer.

PARTIAL PURIFICATION OF β -GLUCOSIDASE IN LIVER

Liver homogenates (20%, w/v) were prepared in 0.01 M sodium phosphate buffer (pH 6.0), using a Potter Elvehjem homogenizer. The homogenates were centrifuged at $8,000 \times g$ for 20 min, and precipitates were discarded.

To the supernatant, solid ammonium sulfate was added slowly with stirring to 70% saturation. After 1 hr the precipitates were collected by centrifugation and dissolved in 0.01 M sodium phosphate buffer (pH 6.0). This fraction was dialyzed against 0.01 M sodium phosphate buffer (pH 6.0) for 16 hr. About 10 ml extract, containing up to 200 mg protein, was applied on a DEAE-cellulose (Brown lot no. 2890) column (15×120 mm).

A linear NaCl gradient up to a final concentration of 0.1 M was introduced for elution at $0\text{--}4^{\circ}$, and 3.5 ml of each fraction were collected for protein determination and enzyme assay. The protein of each fraction was determined by the colorimetric method of Lowry *et al.* (13) or by the absorbance at 280 nm; the β -glucosidase activity was assayed at pH 5 with 4-MU- β -glucoside as a substrate.

RESULTS

GLUCOCEREBROSIDE IN TISSUES

Glucocerebroside content of the liver and spleen in the patients with Gaucher's disease showed a great increase, as shown in Table 1. (One case could not be measured.) Glucocerebroside isolated from tissues of patients with Gaucher's disease contained a larger proportion of $\text{C}_{22:0}$ fatty acids as shown in Table 2. However, no particular difference in its composition among the patients measured (three patients) was observed.

HYDROLYSIS OF β -GLUCOSIDES BY SOLUBLE AND PARTICULATE FRACTION OF TISSUES FROM NEUROPATHIC GAUCHER'S DISEASE AND CONTROL SUBJECTS

The activity of β -glucosidase was detected in the soluble and particulate fraction of the tissue obtained by centrifugation at $8,000 \times g$. Hence, in later experiments, β -glucosidase activity was estimated on both fractions of the liver and spleen and on the whole homogenate of the skin fibroblasts.

The activity of 4MU- or PNP- β -glucosidase in the soluble and particulate fractions of the liver was within normal range or slightly low in cases 1, 2, and 3, whereas the activity in the soluble fraction of the spleen and fibroblasts homogenate was quite deficient and the activity in the particulate fraction of the spleen was about 20–40% of normal in the same subjects. The activities

Table 1. Glucocerebroside contents of tissues from control subjects and patients with Gaucher's disease (milligrams per g wet tissue)

	Spleen	Liver
Case 1	21.0	10.3
Case 2	23.2	16.2
Case 3	25.6	18.5
Case 4	33.8	17.3
Control subjects	0.9–1.5 ¹	0.2 ²

¹ Normal values of glucocerebroside in spleen from Suomi and associates (22).

² Normal values of total neutral glycolipids in liver from Kwitterovich and associates (12).

Table 2. Fatty acid compositions of glucocerebroside from tissues of patients with neuropathic Gaucher's disease

	Fatty acids											
	14:0	16:0	17:0	18:0	18:1	19:0	20:0	21:0	22:0	23:0	24:0	24:1
Non-Gaucher's spleen												
A ¹	0.6	21.6	0.4	5.8	5.1	0.5	3.3	0.7	14.3	8.7	19.2	15.2
B ¹	0.8	33.1	0.9	9.4	7.4	1.5	2.2	1.0	11.0	4.6	10.3	17.9
Gaucher's spleen												
Case 1	0.7	22.7	0.2	7.8	²	1.2	5.4	²	31.1	5.6	20.1	²
Case 3	0.5	31.3	0.2	4.7	0.2	2.4	4.0	0.1	24.1	2.7	20.7	8.7
Case 4	0.3	23.3	0.1	5.4	²	0.7	6.1	²	27.7	5.9	23.7	6.1
Gaucher's liver												
Case 1	0.3	20.6	0.3	7.2	²	0.6	5.9	0.3	25.8	8.4	26.6	4.0
Case 3	1.4	28.5		13.3	1.8	2.1	7.7	²	27.7	6.0	11.5	²
Case 4	0.7	18.8		5.2	0.2	3.9	5.1	0.3	28.3	7.1	27.0	3.4

¹ Fatty acid compositions of glucocerebroside in non-Gaucher's spleen obtained by Suomi and associates (22).² Not measurable.Table 3. β -Glucosidase activities in tissues and fibroblasts from control subjects and from patients with Gaucher's disease¹

Fractions		Glucocerebroside- β -glucosidase		4 MU- β -glucosidase		PNP- β -glucosidase, pH 4.6
		pH 4.6	6.0	pH 4.6	pH 6.0	
Gaucher's liver						
Case 1	Soluble	0.28	0.33	18.20	22.10	15.5
	Particulate	0 ²	0	15.00	15.80	³
Case 2	Soluble	0	0.19	10.27	12.80	³
	Particulate	0	0	12.45	7.55	³
Case 3	Soluble	1.00	0.65	9.47	14.60	14.5
	Particulate	0.41	0.93	6.68	5.86	³
Case 4	Soluble	0.32	0	0.55	0.64	1.2
	Particulate	0	0	3.48	2.17	³
Case 5	Soluble	0	0	0.80	0.80	³
	Particulate	³	³	³	³	³
Control liver	Soluble	2.86 $\pm$ 1.45 (n = 2)	2.99 $\pm$ 0.66 (n = 2)	19.7 $\pm$ 3.4 (n = 5)	27.8 $\pm$ 0.2 (n = 2)	18.0 $\pm$ 4.2 (n = 4)
	Particulate	1.58 $\pm$ 0.61 (n = 2)	3.66 $\pm$ 0.50 (n = 2)	14.6 $\pm$ 0.9 (n = 2)	18.0 $\pm$ 4.2 (n = 2)	³
Gaucher's spleen						
Case 1	Soluble	0.10	0.11	0.61	0.81	0.9
	Particulate	0	0	1.70	1.60	³
Case 2	Soluble	0	0	0.29	0.44	³
	Particulate	0	0.07	4.55	1.81	³
Case 3	Soluble	0	0	0.21	0.31	0.7
	Particulate	0	0.13	2.50	1.67	³
Case 4	Soluble	0.12	0	0.60	0.82	0.9
	Particulate	0	0	0.50	0.70	³
Case 5	Soluble	0	0	0.50	0.70	³
	Particulate	³	³	³	³	³
Control spleen	Soluble	1.09 $\pm$ 0.20 (n = 2)	0.51 $\pm$ 0.07 (n = 2)	5.1 $\pm$ 3.0 (n = 2)	3.0 $\pm$ 0.7 (n = 2)	7.5 $\pm$ 1.7 (n = 2)
	Particulate	2.89 $\pm$ 0.16 (n = 2)	2.34 $\pm$ 0.27 (n = 2)	11.2 $\pm$ 1.8 (n = 2)	5.1 $\pm$ 0.3 (n = 2)	³
Gaucher's fibroblasts						
Case 1	Whole homogenate	0.92	4.62	³	³	4.4
Case 2	Whole homogenate	³	³	4.63	2.78	³
Case 3	Whole homogenate	0.83	0.78	³	³	6.3
Case 4	Whole homogenate	³	³	³	³	³
Case 5	Whole homogenate	³	³	2.81	2.66	³
Control fibroblasts	Whole homogenate	19.60 $\pm$ 6.84 (n = 3)	29.68 (n = 1)	51.66 $\pm$ 6.95 (n = 4)	15.93 $\pm$ 1.64 (n = 3)	65.6 $\pm$ 16.8 (n = 7)

¹ The activity was expressed as liberated glucose, 4-methylumbelliferon (4MU- β -glucosidase) or *p*-nitrophenol (PNP- β -glucosidase) in nanomoles per mg protein per hr.² Not measurable.³ Not measured.

of PNP- and 4MU- β -glucosidase in the soluble and particulate fractions of the liver as well as of the spleen were lower in *cases 4* and *5*.

The activity of β -glucosidase in the liver and spleen at pH 4.6 and 6.0 measured with glucocerebroside as a substrate was low in all patients with infantile Gaucher's disease (type II), but the activity in the soluble and particulate fractions of the liver was only slightly low, 25–45% of normal, in *case 3* with juvenile Gaucher's disease (type III, Table 3).

The pH activity curves of 4MU- β -glucosidase in the liver, spleen, and fibroblasts from the control subjects and from the patients with Gaucher's disease were plotted in the range of pH 3.0–8.0 in 0.025 M citrate buffer (Fig. 1). The pH optimum of 4MU- β -glucosidase from the soluble fraction of the control subject's liver was at 6.0–7.0, whereas the pH optimum of the enzyme from the spleen, fibroblasts, and the particulate fraction of the liver in normal subjects was at 4.5–5.0.

The activity of 4MU- β -glucosidase between pH 4.0 and 7.0 was markedly decreased in the fibroblast and the supernatant fraction of the spleen from patients with Gaucher's disease. Although the activity of 4MU- β -glucosidase between pH 4.0 and 7.0 was very low in liver from *cases 4* and *5*, pH activity curves of this enzyme were noted to be normal in both of the soluble and particulate fractions of the liver from *cases 1, 2*, and *3*.

SEPARATION AND PROPERTIES OF β -GLUCOSIDASE IN LIVER

The liver extracts obtained from the control subjects and from the patients with Gaucher's disease (*cases 2* and *3*) were fractionated in a DEAE-cellulose column with a chloride gradient elution (Fig. 2). Although β -glucosidase was separated into at least two peaks (fractions 1 and 2), the elution patterns of the control and of the Gaucher's liver were almost identical. β -Glucosidase in fractions 1 and 2 represented about 40-fold purification compared with that in the $8,000 \times g$ supernatant fraction.

Fig. 1. The pH activity curves of β -glucosidase in the liver, spleen, and fibroblasts from a control subject and from the patients with Gaucher's disease. An activity was measured with 4-methylumbelliferyl- β -glucopyranoside. The specific activity is expressed in nanomolar concentration of substrate cleaved per mg protein in 1 hr. Liver, $\circ$ — $\circ$; spleen, $\bullet$ — $\bullet$; fibroblasts, Δ — Δ .

Fig. 2. Chloride gradient elution from DEAE-cellulose column of liver β -glucosidase. The column (15×120 mm) was equilibrated with 0.01 M phosphate buffer, pH 6.0, and enzyme was eluted with NaCl gradient (in the same buffer) up to 0.1 M. Each 3.5-ml fraction was collected. 4-methyl umbelliferyl- (4MU-) β -glucosidase and protein were measured as described in the text. $\circ$ — $\circ$: β -glucosidase activity with 4MU- β -glucoside (emission 365/450); $\bullet$ — $\bullet$: protein was determined by the absorbance at 280 nm.

The pH activity curves of 4MU- β -glucosidase in fractions 1 and 2 obtained from control and from Gaucher's liver gave a broad peak; the optimum was approximately 4.5–5.0 and 6.0–6.5, respectively. The pH activity curves of the enzyme in fractions 1 and 2 from Gaucher's liver were essentially similar to those from the control (Fig. 3). K_m values of 4MU- β -glucosidase in fraction 1 from control and from Gaucher's liver were 10.7 , 9.8 , and 10.6×10^{-5} M, respectively, whereas in fraction 2 they were 16.2 , 24.3 , and 22.7×10^{-5} M. No significant difference in K_m values measured with 4MU- β -glucoside between the control subject and the patients with Gaucher's disease was apparent (Fig. 4).

However, the ratios of the specific activity of glucocerebroside- β -glucosidase to 4MU- β -glucosidase in fractions 1 and 2 obtained from Gaucher's liver were significantly low, compared with those from the control, as shown in Table 4.

Fractions 1 and 2 from infantile Gaucher's liver (*case 2*) exhibited almost no detectable activity measured with a natural substrate whereas those from juvenile Gaucher's liver (*case 3*) showed an activity of about 25–45% of normal.

Fig. 3. pH activity curves of partially purified liver 4-methylumbelliferyl- β -glucosidase, fractions 1 and 2, from a control subject and from patients with Gaucher's disease. The liver extracts were fractionated with a chloride gradient elution from DEAE-cellulose column as described in the text. The activity was expressed as liberated 4-methylumbelliferone in nanomolar concentration per mg protein per hr. Control: ●—●; Gaucher's disease: case 2, ○—○; case 3, □—□.

Fig. 4. Michaelis constant of purified liver β -glucosidase. β -Glucosidase was measured with 4-methylumbelliferyl- β -glucoside as a substrate.

Table 4. Ratio of specific activities of glucocerebroside- β -glucosidase (GC- β -gluc) to 4-methylumbelliferyl- β -glucosidase (4MU- β -gluc) of partial purified liver β -glucosidase

	pH	Control	Gaucher's case 2	Gaucher's case 3
GC- β -gluc/4MU- β -gluc in fraction 1	4.5	0.22	0.01	0.06
	6.0	0.25	0.02	0.09
GC- β -gluc/4MU- β -gluc in fraction 2	4.5	0.05	0	0.02
	6.0	0.07	0	0.03

DISCUSSION

Examination of the lipid in Gaucher's tissues revealed a large excess of glucocerebroside, similar to the values found by Suomi *et al.* (22). The fatty acid composition of glucocerebroside from the patients with Gaucher's disease in our studies was also similar to that described in Suomi's report (22). The accumulation of glucocerebroside in the liver and spleen was identical in quality and quantity among the three patients measured whereas 4MU- β -glucosidase in the liver of one patient was deficient and of two was normal.

A decreased activity of glucocerebroside- β -glucosidase, 4MU- β -glucosidase, and PNP- β -glucosidase in the spleen and fibroblast from the patients with neuropathic Gaucher's disease was a constant phenomenon as reported earlier (2-4, 7, 8, 11, 17-19).

Low activities of glucocerebroside- β -glucosidase, 4MU- β -glucosidase, and PNP- β -glucosidase in the liver from patients with Gaucher's disease have been reported by Öckerman *et al.* (7, 17, 18) and by others (21). However, an increased activity of PNP-

β -glucosidase in the liver from one patient with Gaucher's disease was reported by Patrick (19). According to his report, a liver biopsy specimen possessed 30% of the activity of cerebroside- β -glucosidase found for the control but twice the PNP- β -glucosidase activity.

Recently, Kanfer *et al.* (10) reported that two of three infantile Gaucher's cases had normal activity of liver 4MU- β -glucosidase in the soluble fraction and one showed no detectable hydrolysis.

Such cases of both high and low 4MU- or PNP- β -glucosidase activity in Gaucher's liver could be explained by our results, as follows. Neuropathic Gaucher's disease may be divided into two biochemical groups. In the first group, 4MU- and PNP- β -glucosidase activity is almost normal in the liver but markedly reduced in the spleen and fibroblasts. In the second group, the activity is markedly low in the liver, spleen, and fibroblasts.

Three patients in the present study were in the first group: two of them represented a typical course of an acute infantile type (type II); the other patient showed a subacute onset and symptoms of type III. The remaining two cases were in the second group and they represented typical symptoms of the infantile type (type II). Glucocerebroside- β -glucosidase activity in the liver and spleen was low in all patients with infantile Gaucher's disease but the activity in the liver was slightly low in the patient with juvenile type. It is possible that the clinical phenotypes in type II and type III were related to the type of the genetic mutation of glucocerebroside- β -glucosidase but not to the heterogeneity of 4MU- β -glucosidase in liver.

Our results suggest that β -glucosidase in tissue consists of more than two subunits, and one subunit may be the same in the enzymes of the liver, spleen, and fibroblasts and subject to inactivation by mutation in cases 4 and 5.

However, the other subunit, or subunits, is different in β -glucosidase of the liver and spleen or fibroblasts, and inactivated in the spleen and fibroblasts but not in the liver from case 1, 2, and 3.

Hultberg and Öckerman (7) reported that the activity of 4MU- β -glucosidase in the soluble fraction of control human liver homogenates could be fractionated into two peaks by gel filtration, but that the kinetic parameters (pH dependence and K_m) of β -glucosidase activity in two peaks were very similar when measured with 4MU- β -glucoside. In our studies, 4MU- β -glucosidase in the liver was separated into two peaks by DEAE-cellulose column chromatography. Two separated enzymes revealed different pH activity curves, substrate specificities, and K_m , obtained using 4MU- β -glucoside. A substrate affinity for glucocerebroside in the purified enzyme of fractions 1 and 2 from Gaucher's liver was significantly lower than that from control liver. No difference, however, was found between partially purified liver 4MU- β -glucosidase from the control subject and that from patients with Gaucher's disease with respect to pH dependence or K_m .

Detection of a significant activity measured with glucocerebroside in the partially purified enzyme from juvenile Gaucher's liver might be a pronounced finding to explain a late onset and subacute symptoms in this type of Gaucher's disease.

From our results it might be suggested that the deficiency of 4MU- β -glucosidase within the hepatic cells may not account for the accumulation of glucocerebroside, and the deficient activity of glucocerebroside- β -glucosidase probably existing within reticuloendothelial cells, *e.g.*, Kupffer cells, may be more essential for the accumulation.

CONCLUSION

The activities of β -glucosidase in the spleen and skin fibroblasts, measured with glucocerebroside and PNP- or 4MU- β -glucoside as substrates, were very much reduced in five patients with neuropathic Gaucher's disease. However, when PNP- or 4MU- β -glucoside was used as a substrate, three of the five patients exhibited almost normal β -glucosidase activity in the

soluble and particulate fraction of the liver, whereas the other two patients were deficient in such an activity. Partially purified- β -glucosidase from acute infantile Gaucher's liver exhibited no activity measured with glucocerebroside as a substrate whereas those from juvenile Gaucher's liver showed considerable activity. These results suggest the possibility that a new type of genetic heterogeneity may be involved in neuropathic Gaucher's disease.

REFERENCES AND NOTES

1. Beutler, E., and Kuhl, W.: The diagnosis of the adult type of Gaucher's disease and its carrier state by demonstration of deficiency of β -glucosidase activity in peripheral blood leukocytes. *J. Lab. Clin. Med.*, **76**: 747 (1970).
2. Beutler, E., Kuhl, W., Trinidad, F., Teplitz, R., and Nadler, H.: β -Glucosidase activity in fibroblasts from homozygotes and heterozygotes for Gaucher's disease. *Amer. J. Hum. Genet.*, **23**: 62 (1971).
3. Brady, R. O., Kanfer, J. N., and Shapiro, D.: Metabolism of glucocerebroside II. Evidence of an enzymatic deficiency in Gaucher's disease. *Biochem. Biophys. Res. Commun.*, **18**: 221 (1965).
4. Brady, R. O., Kanfer, J. N., Bradley, R. M., and Shapiro, D.: Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher's disease. *J. Clin. Invest.*, **45**: 1112 (1966).
5. Folch, J., Lees, M., and Sloane Stanley, G. H.: A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**: 497 (1957).
6. Handa, S., and Yamakawa, T.: Biochemical study in cat and human gangliosidosis. *J. Neurochem.*, **18**: 1275 (1971).
7. Hultberg, B., and Öckerman, P. A.: β -Glucosidase activities in human tissues findings in Gaucher's disease. *Clin. Chem. Acta*, **28**: 169 (1970).
8. Hultberg, B., Sjöblad, S., and Öckerman, P. A.: 4-Methylumbelliferyl- β -glucosidase in cultured human fibroblasts from controls and patients with Gaucher's disease. *Clin. Chem. Acta*, **49**: 93 (1973).
9. Kampine, J. P., Brady, R. O., Kanfer, J. N., Feld, M., and Shapiro, D.: Diagnosis of Gaucher's disease and Niemann-Pick disease with small samples of venous blood. *Science*, **155**: 86 (1967).
10. Kanfer, J. N., Raghavan, S. S., Mumford R. A., Labow R. S., Williamson D. G., and Layne D. S.: Deficiency of steroid β -glucosidase in Gaucher disease. *Biochem. Biophys. Res. Commun.*, **67**: 683 (1975).
11. Klibansky, C., Hoffmann, J., Pinkhas, J., Algom, D., Dintzman, M., Ben-Bassat, M., and de Vries, A.: Leukocyte glucocerebroside deficiency diagnostic in adult Gaucher's disease with negative bone marrow biopsy. Some properties of the enzyme in leukocytes and spleen. *Eur. J. Clin. Invest.*, **4**: 101 (1974).
12. Kwitterovich, P. O., Sloan, H. R., and Fredrickson, D. S.: Glycolipids and other lipid constituents of normal human liver. *J. Lipid Res.*, **11**: 322 (1970).
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265 (1951).
14. Lowry, O. H., Passonneau, J. V., Hasslberger, F. X., and Schulz, D. W.: Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J. Biol. Chem.*, **239**: 18 (1964).
15. Makita, A., Suzuki, C., Yoshizawa, Z., and Konno, T.: Glycolipids isolated from the spleen of Gaucher's disease. *Tohoku J. Exp. Med.*, **88**: 277 (1966).
16. Morrison, W. R., and Smith, L. M.: Preparation of fatty acid methyl esters and dimethylacetates from lipid with boron fluoride-methanol. *J. Lipid. Res.*, **5**: 600 (1964).
17. Öckerman, P. A.: Identity of β -glucosidase, β -xylosidase and one of the β -galactosidase activities in human liver when assayed with 4-methylumbelliferyl- β -D-glycosides studies in case of Gaucher's disease. *Biochim. Biophys. Acta*, **165**: 59 (1968).
18. Öckerman, P. A., and Kohlin, P.: Tissue acid hydrolase activities in Gaucher's disease. *Scand. J. Clin. Lab. Invest.*, **22**: 62 (1968).
19. Patrick, A. D.: A deficiency of glucocerebroside in Gaucher's disease. *Biochem. J.*, **97**: 17c (1965).
20. Robinson, D., Price, R. G., and Dance, N.: Separation and properties of β -galactosidase, β -glucosidase, β -glucuronidase and N-acetyl- β -glucosaminidase from rat kidney. *Biochem. J.*, **102**: 525 (1967).
21. Schneider, E. L., Ellis, W. G., Brady, R. O., McCulloch, J. R., and Epstein, C. J.: Infantile (type 11) Gaucher's disease: In utero diagnosis and fetal pathology. *J. Pediatr.*, **81**: 1134 (1972).
22. Suomi, W. D., and Agranoff, B. W.: Lipids of the spleen in Gaucher's disease. *J. Lipid Res.*, **6**: 211 (1965).
23. We are grateful to Dr. H. Maesaka, Kanagawa Children's Medical Center, Kanagawa, and Dr. J. Sugiura, Department of Pediatrics, Nagoya University School of Medicine, Nagoya, who made the diagnosis of the patients as Gaucher's disease and referred them to us.
24. This work was supported in part by grants from the Ministry of Education in Japan for research on inborn errors of metabolism, 1974-1975.
25. Requests for reprints should be addressed to: T. Kitagawa, M.D., Department of Pediatrics, Nihon University School of Medicine, 1-8 Kanda-Surugadai, Chiyoda-ku, Tokyo (Japan).
26. Received for publication September 9, 1975.
27. Accepted for publication October 14, 1976.

Copyright © 1977 International Pediatric Research Foundation, Inc.

Printed in U.S.A.

Pediat. Res. **11**: 646-648 (1977)

Liver pregnancy
newborn trace elements
placenta

Trace Elements (Zinc, Cobalt, Selenium, Rubidium, Bromine, Gold) in Human Placenta and Newborn Liver at Birth

D. ALEXIOU,¹⁸⁹ A. P. GRIMANIS, M. GRIMANI, G. PAPAÉVANGELOU, E. KOUMANTAKIS, AND C. PAPADATOS

Second Pediatric Clinic of the University of Athens: Demokritos Nuclear Research Center Chemistry Division, Athens, Greece

Summary

Concentrations of zinc, cobalt, selenium, rubidium, bromine, and gold have been determined by neutron activation analysis in 18 placental and 6 liver tissue samples at birth. Their respective mean concentrations $\pm$ standard deviations (parts per million) were 58 ± 10 , 0.060 ± 0.036 , 1.90 ± 0.41 , 14.0 ± 3.5 , $26.9 \pm$

14.3 , and 0.31 ± 0.14 in placenta, and 651 ± 257 , 0.156 ± 0.077 , 4.52 ± 1.96 , 9.2 ± 3.7 , 11.2 ± 4.1 , and 0.12 ± 0.06 in liver tissue. Thus, we observed that the mean concentrations of essential trace elements (zinc, cobalt, and selenium) were significantly higher in liver than in placenta, whereas the nonessential trace elements (rubidium, bromine, and gold) were found in significantly higher concentrations in placenta than in liver tissue.