METABOLISM OF GLUCOCEREBROSIDES

II. EVIDENCE OF AN ENZYMATIC DEFICIENCY IN GAUCHER'S DISEASE

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Although there is abundant evidence for the accumulation of excessive amounts of glucocerebrosides in the reticuloendothelial cells of the spleen, liver and bone marrow in Gaucher's disease (Halliday, et al., 1940; Rosenberg and Chargaff, 1958; Agranoff, et al., 1962), studies from this laboratory revealed no abnormality in their formation (Trams and Brady, 1960). The latter observation prompted the hypothesis that a deficiency in one or more enzymes involved in the catabolism of glucocerebrosides was responsible for the metabolic defect. More specifically, cleavage of glucose from the glucocerebroside molecule was postulated as the site of enzymatic deficiency (Brady, 1963), a suggestion which has received support from the recent work of Philippart and Menkes (1964). In order to test this hypothesis, the level of glucocerebroside-cleaving enzyme was determined in human spleen tissue obtained at operation for various conditions and contrasted with the level found in spleens from patients with Gaucher's disease.

Materials and Methods: Glucocerebroside labeled in carbon 1 of the D-glucose portion of the molecule was synthesized (Brady, Kanfer and Shapiro, 1965). With the use of this labeled substrate, an enzyme was demonstrated in rat and human spleen tissue which catalyzed the cleavage

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of the glucosidic bond of the glucocerebroside molecule resulting in the formation of glucose and N-acyl sphingosine (ceramide) (Brady, et al., 1965). The enzyme was found in the 100,000 x g supernatant solution and was purified 82-fold by conventional fractionation procedures with ammonium sulfate, ethanol, and acetone.

In the present experiments, the activity of the glucocerebroside-cleaving enzyme in the 100,000 x g supernatant preparation was assayed by measuring the formation of water-soluble radioactive products using glucose-1-^{14}C cerebroside as substrate. The incubation mixtures contained 100 umoles of potassium phosphate buffer, pH 7.0, 275 mumoles of glucose-1-^{14}C cerebroside (121,000 c.p.m.), 1.6 mg of Cutscum, and the 100,000 x g supernatant solution of spleen tissue in a final volume of 1.0 ml. The vessels were stoppered and incubated for 14 hours at 37°. The reaction was stopped by the addition of 0.34 ml of 10% perchloric acid. The insoluble material was removed by centrifugation, and the supernatant solution was neutralized with 2.5 N KOH. The $KClO_4$ was removed by centrifugation, and 19 volumes of chloroform-methanol, 2:1, were added to the supernatant solution forming a single phase. To this solution were added 2 ml of water; the suspension was clarified by centrifugation; the aqueous phase was decanted; and 5 ml of aqueous "theoretical upper phase" (Folch et al., 1957) were equilibrated with the chloroform phase. The aqueous extracts were combined, and an aliquot was taken for radioactivity determination. Under these conditions, the glucocerebroside-cleaving enzyme activity was found to be proportional to the amount of enzyme solution employed.

In order to correct for dilution of the labeled substrate by relatively large endogenous levels of glucocerebrosides in Gaucher's spleen preparations, freshly-prepared glucocerebroside-cleaving enzyme from rat spleen tissue was incubated with glucocerebroside-^{14}C alone and in combination with the preparations from human spleen tissue. The re-

sults from such an experiment are shown in Table I. If no dilution of the labeled substrate had occurred, one would have expected 6780 c.p.m.

TABLE I

DETERMINATION OF GLUCOCEREBROSIDE-CLEAVING ENZYME

The conditions of incubation and recovery of water-soluble radioactive products are described in the text.

Part	Source and volume of enzyme solution	Radioactivity in combined aqueous phase
		c.p.m. *
A	Gaucher's spleen supernatant, 1.0 ml	300
B	Rat spleen supernatant, 1.0 ml	13,560
C	0.5 ml of A + 0.5 ml of B	5,440

The Gaucher's spleen used in these experiments was obtained from the Henry Ford Hospital, Detroit.

* Total c.p.m. less boiled enzyme control (35-39 c.p.m.).

in the aqueous phase of Part C due to the glucocerebroside-cleaving activity of the rat enzyme preparation. Since 150 c.p.m. were contributed by the enzyme from the Gaucher's spleen preparation, 5440 - 150 = 5,290 c.p.m. could actually be attributed to the activity of the rat enzyme preparation. Therefore, the value found in Part A was multiplied by a factor of 1.28 (6780/5290) to correct for endogenous dilution of substrate. Such corrections were made in each of the human spleen preparations examined. In the present experiments, the activity of the glucocerebroside-cleaving enzyme is expressed with respect to the amount of protein in 1 ml of the soluble enzyme preparation. The results obtained with 4 non-Gaucher's and 3 Gaucher's human spleen preparations are shown in Table II.

TABLE II

LEVEL OF GLUCOCEREBROSIDE-CLEAVING ENZYME IN HUMAN SPLEEN PREPARATIONS

The conditions of incubation and procedure for correcting for endogenous dilution of the labeled substrate are described in the text.

Condition of patient			Activity of glucocerebroside-cleaving enzyme	
			Radioactivity in aqueous phase	% of non-Gaucher's activity
			c.p.m. per mg of protein	%
Congenital hemolytic anemia	F	76	93.8	-
Congenital spherocytosis	M	4	72.1	-
Idiopathic thrombocytopenic purpura	F	37	77.0	-
Congenital hemolytic anemia	F	14	67.4	
Average specific activity			77.6 ± 11.5*	
Gaucher's disease	F	33	13.9	17.9
" "	F	3	10.9	14.0
" "	M	13	8.4	10.8
Average specific activity			11.1 ± 2.24*	

* Standard deviation

It is apparent from these data that there is a pronounced diminution of the activity of the glucocerebroside-cleaving enzyme in the spleens obtained from patients with Gaucher's disease. It seems reasonable that this metabolic lesion may account for the accumulation of glucocerebrosides in Gaucher's disease.

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