

STUDIES CONCERNING THE PATHOGENESIS OF GAUCHER'S DISEASE

By BERTHA OTTENSTEIN, Ph.D., GERHARD SCHMIDT, M.D., AND
S. J. THANNHAUSER, M.D.

IN 1882 Charles Ernst Gaucher¹ described as "epithelioma primitif de la rate" the disease which now bears his name. In his case he found the splenic pulp entirely replaced by large cells and attributed this condition to a primary tumorous growth, epithelioma of the spleen. Collier² (1895) in England, and Picou and Ramond³ in France (1896) also regarded the condition as neoplastic. Bovaird⁴ (1900), reporting the first case in this country, called attention to the simultaneous appearance of these large cells in the liver as well as in the spleen and lymph nodes. He had commented on the familial character of the disease. In contrast to the current view that the disease was of tumorous nature, he believed that an unknown toxin caused hyperplasia of spleen, liver, and lymph nodes. Brill, Mandelbaum and Libman⁵ (1905) were the first to point out that the cells which characterized the disease were found, not only in the liver, spleen and lymph nodes, but appeared simultaneously in different parts of the skeleton. These authors suggested (1913) the name, "Gaucher's disease," to avoid the misleading term "primary idiopathic splenomegaly." Schlagenhauser⁶ (1906) considered that the condition was a systemic disease of the lymphhemopoietic tissue.

H. Lieb^{7, 8} (1924, 1925), in association with Epstein and Lorenz,⁹ isolated the substance which characterized the Gaucher cells and identified this substance as a cerebroside, namely kersin. It was believed that kersin, a galactosidocerebroside, was a constituent only of brain tissue and not of visceral organs. L. Pick¹⁰ assumed that kersin originated as a result of a general disturbance of intermediary lipid metabolism, accumulated in the blood, and was secondarily deposited and stored in the reticulum cells of the involved organs. He characterized Gaucher's disease "not as a reticulo-endothelial, reticular cell or histiocytomatotic disease, but as a histiocytic disease comparable to histiocytic storage as observed in vital staining or cholesterol feeding of animals with, however, elective participation of certain histiocytic forms."

In contrast to the conception of L. Pick,¹⁰ S. J. Thannhauser and co-workers¹¹ demonstrated that normal serum or serum of patients with Gaucher's disease did not contain measurable amounts of cerebroside. This observation was subsequently confirmed by Dvoracek and Pest,¹² and Bruckner¹³: Since cerebroside is not present in the serum of Gaucher's disease, Thannhauser¹⁴ concluded that cerebroside does not originate as a result of a general disturbance of the intermediary lipid metabolism but is synthesized and stored in the cells where they are found; i.e., in the Gaucher cells. This explanation of the pathologic formation of cerebroside places the metabolic disorder within the reticulum cells and histiocytes.

From the Research Laboratories of the Boston Dispensary and the Joseph H. Pratt Diagnostic Hospital and the Department of Medicine, Tufts College Medical School, Boston.

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An imbalance of the intracellular enzymes concerned with cerebroside formation and disintegration is assumed to be the fundamental defect in Gaucher's disease. Similarly in Niemann-Pick's disease an enzymatic intracellular disturbance presumably leads to increased sphingomyelin formation. An enzymatic deviation of the intracellular lipid metabolism is therefore considered as the etiology of these disorders.

This view is supported by the experiments of Thannhauser and Reichel¹⁵ and by Ottenstein, Schmidt and Thannhauser,¹⁶ who demonstrated that cerebrosidease is an intracellular enzyme which cannot be extracted from the tissue.

A further support for the view that the cerebroside in Gaucher's disease are synthesized and stored in the cells where they are found is evident from the discovery of an hitherto unknown group of cerebroside occurring exclusively in the Gaucher cells, namely a glucosidocerebroside. (Halliday, Deuel, Tragerman and Ward¹⁷ and Aghion¹⁸). In glucosidocerebroside, the carbohydrate group is glucose and not galactose as in kerafin. The findings of Halliday and co-workers have been confirmed by Klenk,¹⁹ Danielson, Hall and Everett²⁰ and by Polonovski.²¹

The question arises whether in Gaucher's disease an abnormal cerebroside, i.e., glucosidocerebroside, is formed exclusively or whether in different individual cases the type of cerebroside may vary and both cerebroside, galactosido- (kerafin) and glucosidocerebroside may be present together.

The purpose of this paper is an attempt to decide this question by a method that permits quantitative partition of cerebroside into galactosido- and glucosidocerebroside. This method is described in the present paper and applied to the analysis of organs of different cases of Gaucher's disease. It will be demonstrated that in individual cases the organs vary in the amount and type of cerebroside present.

Our investigations were carried out with the additional purpose of clarifying another question regarding the pathogenesis of Gaucher's disease. It is known that normal red blood cells contain a small amount of cerebroside. It might be inferred that the increase of cerebroside in Gaucher organs may have resulted from a primary accumulation of cerebroside in the red blood cells. Deposition of this material in the organs in Gaucher's disease could occur even though the serum did not contain appreciable amounts of this lipid.

It will be shown by the chemical analysis of red blood cells from normal individuals and from patients with Gaucher's disease that the cerebroside present are quantitatively and qualitatively the same. These findings seem to support the conception of Thannhauser and co-workers^{11, 14} that the large amount of cerebroside present in the organs involved in Gaucher's disease is due to their synthesis and storage in the cells where they are found.

MICRODETERMINATION OF CEREBROSIDES IN RED BLOOD CELLS AND IN TISSUES

PRINCIPLE

The characteristic group which distinguishes the cerebroside from most of the other lipids is their carbohydrate component. Since the carbohydrate group can be liberated quantitatively from the cerebroside by acid hydrolysis the estimation of the free sugars in hydrolysate total of the lipid fraction is at present the basis of all analytical procedures for the quantitative determination of cerebroside.

(Brückner,^{13a,b} Halliday, Deuel, Tragerman and Ward,¹⁷ Aghion,¹⁸ Klenk,¹⁹ Danielson, Hall and Everett,² Polonovski,²¹ and Brand and Sperry.²²

The conversion of the results of the sugar determination into cerebroside values, however, requires the application of certain precautions.

1. The crude alcoholic lipid extract contains in addition to the cerebroside noticeable amounts of nonlipid carbohydrates. The removal of the contaminating carbohydrates is achieved by extracting the final solutions of the lipids in chloroform repeatedly with an aqueous solution of trichloroacetic acid.

2. It was found by Brand and Sperry,²² as well as by us, that the customary hydrolysis of the lipids with sulfuric acid entails considerable losses due to destruction of carbohydrates, but that hydrochloric acid under certain conditions permits the quantitative recovery of the sugars.

3. It has recently been shown that the carbohydrate component of the cerebroside is not exclusively galactose, but at least under certain conditions in Gaucher's disease,¹⁷⁻²¹ a mixture of galactose and glucose is present. Since equal amounts of galactose and glucose have different reduction values, the correct conversion factor can only be established by separate determination of glucose and galactose in the hydrolysate. Our procedure for the quantitative partition of the sugars into galactose and glucose is based on the removal of the latter by yeast fermentation.

PROCEDURE

Extraction. The amount of material required for the determination varies according to its cerebroside content. The following amounts of various tissues have been found to be suitable for the determinations of cerebroside: 10 Gm. of washed red blood cells corresponding to approximately 40 cc. of oxalated blood, 5 to 10 Gm. of wet brain, 20 Gm. of visceral organs, 50 mg. of crystallized cerebroside mixtures. For the quantitative extraction of cerebroside the material is homogenized in the Waring Blendor with 20 to 30 volumes of 95 per cent alcohol. The suspension is refluxed for 30 minutes and filtered while still hot. The residue is re-extracted by refluxing with the same amount of 95 per cent alcohol. The combined extracts are evaporated to dryness under reduced pressure. The oily residue is refluxed with a mixture of 4 volumes of chloroform and 1 volume of ethanol, filtered while still hot and washed with the chloroform ethanol mixture.

Removal of nonlipid carbohydrates. The combined extracts and washings are transferred to a 250 cc. centrifuge tube and stirred mechanically with four volumes of 2 per cent trichloroacetic acid for ten minutes. After separation of the emulsion by centrifugation the aqueous top layer is siphoned off and discarded. The extraction with trichloroacetic acid is repeated at least twice until a microdetermination of the sugar (Somogyi²³) in an aliquot of the aqueous layer shows the absence of reducing carbohydrates.

Hydrolysis. The chloroform ethanol extract is transferred to a 60 cc. centrifuge tube and brought to dryness at room temperature by an air current which is bubbled through the solution. The residue is suspended in 15 cc. of an aqueous, 1.5 N, solution of hydrochloric acid by means of a glass rod. The hydrolysis is carried out on a boiling water bath for sixty minutes. The centrifuge tube is covered with a loosely fitting glass bulb. The cooled hydrolysate is brought to a volume of 25 cc., sharply centrifuged and filtered.

Removal of free acid by Amberlite. Prior to the sugar determination, the free acid is removed from the hydrolysate by absorption on Amberlite (analytical grade IR 4B). (The neutralization cannot be carried out by the addition of alkali since the presence of salts interferes with the subsequent fermentation.) For this purpose an aliquot of the hydrolysate is stirred mechanically with the resin (2 Gm. per 10 cc. hydrolysate*), for twenty minutes until the color of congo red paper is not changed by a drop of the supernatant.

All controls are brought to a volume of 10 cc. and incubated for three hours at room temperature. After centrifugation, the reduction is determined in an aliquot of the supernatant according to Somogyi.²³ The mixture is brought to a volume of 10 cc. in a volumetric flask and fermented at room temperature for 3 hours. After centrifugation, the nonfermentable sugar is determined in 5 cc. of the supernatant according to Somogyi.²³

* Control experiments with known glucose solutions have shown that analytical grade Amberlite does not absorb any glucose.

In a second aliquot of 4 cc. of the Amberlite supernatant the total carbohydrates are determined according to Somogyi without fermentation.

Calculation. The titration value F obtained in the fermented aliquot represents galactose and is accordingly multiplied by the factor 0.20 in order to obtain the amount of galactose in milligrams. The difference between F and between the titration value T obtained with the unfermented aliquot represents glucose and is multiplied by the factor 0.14 (Somogyi).

In order to calculate the amount of cerebrosides the galactose, respectively the glucose values are multiplied by the factor 4.6.

Determination of galactose and of total carbohydrates. An aliquot of 8 cc. of the Amberlite supernatant is mixed with 1 cc. of a 10 per cent suspension of fresh baker's yeast* (Fleischmann).

Analyses of the cerebrosides in normal serum and Gaucher serum showed:

<i>Normal serum</i>		<i>Gaucher serum</i>	
Galactosidocerebrosides	Glucosidocerebrosides	Galactosidocerebrosides	Glucosidocerebrosides
Traces	0	Traces	0

The analyses were made on 30 cc. samples of serum of three normal individuals, as well as on 30 cc. samples of two patients with Gaucher's disease (case Ke, 14 years old and case Le, 24 years old).

These figures confirm the analytical findings of Thannhauser and co-workers,¹¹ Brückner,^{13a} Dovracek and Pesta¹² indicating that the serum of Gaucher's disease does not contain measurable amounts of cerebrosides.

TABLE 1.—ANALYSIS OF RED BLOOD CELLS IN NORMAL INDIVIDUALS AND PATIENTS WITH GAUCHER'S DISEASE

Normal R.B.C. Per cent of desiccator-dried weight		Gaucher R.B.C. Per cent of desiccator-dried weight	
Galactosidocerebrosides	Glucosidocerebrosides	Galactosidocerebrosides	Glucosidocerebrosides
0.189	0	0.200 (case Ke) 0.220 (case Le)	0 (case Ke) 0 (case Le)

The analyses of red blood cells, washed with saline, were made in three normal individuals from an average of 9.8 Gm. of dried material, the samples being dried in a desiccator at room temperature.

The analyses of Gaucher red blood cells, washed with saline, were made in case Ke, 14 years old, on 7.7 Gm. dried substance, in case Le, 24 years old, on 8.6 Gm. dried substance.

The figures in table 1 demonstrate that the content and the type of cerebroside in the red cells of Gaucher's disease do not differ from those found in normal red blood cells. It is important to note that both in the normal and Gaucher's disease, only galactosidocerebrosides were found in the red cells even when glucosidocerebrosides were accumulated in the Gaucher cells of the spleen, as in case Ke (corresponding to Case 1 in table 2).

The organs of cases 1 and 2 were from children of 6 to 10 years of age; cases 3 and

* With each series of analyses the following additional control determinations are carried out: (a) 1 cc. of the washed yeast suspension is mixed with water. (b) 1 cc. of the washed yeast suspension is mixed with a known amount of glucose. (c) 1 cc. of the washed yeast suspension is mixed with a known amount of galactose.

TABLE 2.—ANALYSES OF SPLEENS IN 4 CASES OF GAUCHER'S DISEASE BY ISOLATION

Crystalline cerebrosides from Gaucher spleen	Total cerebrosides in per cent of dry weight	Galactosidocerebrosides in per cent of dry weight	Glucosidocerebrosides in per cent of dry weight	Ratio galactocerebrosides: glucosidocerebrosides
Gaucher spleen 1.....	8.3	Negligible	8.3	0:8.3
Gaucher spleen 2.....	11.3	Negligible	11.3	0:11.3
Gaucher spleen 3.....	15.8	8.6	7.2	1.2:1
Gaucher spleen 4*..... (See below)	21.6	12.0	9.6	1.3:1

Case 4.—Analyses by Analytic Determination

	Total cerebrosides in Gm. per 100 Gm. tissue	Galactosidocerebrosides in Gm. per 100 Gm. tissue	Glucosidocerebrosides, in Gm. per 100 Gm. tissue	Ratio galactocerebrosides: glucosidocerebrosides
Gaucher spleen (Tissue).....	29.4	14.4	15.0	1:1
Normal spleen (Tissue).....	0.4-0.6	0.4-0.6	Negligible	—

* In case 4 the galactosidocerebrosides and glucosidocerebrosides were also determined directly in the organ.

TABLE 3.—Case 1: Higgins, E.; aged 7 months

Diagnosis: Generalized Infantile Gaucher's Disease. Organs Preserved in Formaldehyde, Received October, 1945, from Dr. Robb-Smith, Radcliffe Infirmary, Department of Pathology, Oxford, England

Organ	Chemical analysis		
	Galactocerebrosides Gm. per cent of dry weight	Glucocerebrosides Gm. per cent of dry weight	Galactocerebrosides In normal human organs Gm. per cent of dry weight
Brain.....	2.6	Negligible	4.0-6.0 (1.0)*
Spleen.....	3.2	"	0.1-0.5
Liver.....	2.3	"	0.05-0.15
Lungs.....	6.8	"	0.1-0.6
Heart.....	2.3	"	0.24
Bonemarrow.....	1.3	"	—
Kidney.....	3.1	"	0.1-0.7
Intestine I.....	3.2	"	—
Intestine II.....	2.8	"	—
Pancreas.....	3.1	"	—

* Cerebrosides in the brain of a one day old child.

4 from adults. In cases 1, 2, and 3 the cerebrosides were isolated in crystalline form and the analyses were made for galactosido- and glucosidocerebrosides.

In case 4* the galactosidocerebrosides and glucosidocerebrosides were determined

* We are indebted to Dr. Karl Singer, Michael Reese Hospital, Chicago, for sending this spleen of an adult Gaucher's disease.

directly in the organ as well as after isolation of the crystalline cerebroside mixture.

The analytic figures of normal human and animal spleen are charted in table 2.

The figures in table 3 and table 4 demonstrate that even in the organs of siblings with generalized infantile Gaucher's disease the amount and the type of galactosido- and glucosidocerebrosides may vary greatly. The intracellular metabolic disorder in Gaucher's disease is thus not solely confined to the formation of an abnormal glucosidocerebroside, since in one of the examined siblings, mainly galactosidocerebrosides and in the other sibling galactosido- and glucosidocerebrosides in varying amounts were found in the organs.

TABLE 4.—Case 2: Higgins, Timothy; aged 5½ months (Sibling of Case 1)

Diagnosis: Generalized Infantile Gaucher's Disease. (Organs received from Dr. Robb-Smith, Radcliffe Infirmary, Oxford, England)

Organ	Chemical Analysis				
	Galactocerebrosides; per cent of dry weight	Glucocerebrosides; per cent of dry weight	Ratio galactocerebrosides to glucocerebrosides	Total cerebrosides; per cent of dry weight	Galactocerebrosides in normal human organs; per cent of dry weight
Spleen.....	0.44	0.95	1:2.1	1.44	0.1-0.5
Liver.....	0.28	0.05	5.6:1	0.33	0.05-0.15
Brain.....	3.25	0		3.25	4.0-6.0*
Lung.....	0.61	0.43	1.4:1	1.04	0.1-0.6
Kidney.....	0.29	0.25	1.2:1	0.54	0.1-0.7
Pancreas.....	0.09	0.05	1.8:1	0.14	0.1-0.3
Thymus.....	0.30	0.30	1.0:1	0.60	—
Suprarenal.....	0.20	0.09	2.2:1	0.29	—

* Cerebrosides in the brain of a one day old child.

DISCUSSION

The above findings (table 2) demonstrate that the spleen of two children with Gaucher's disease contained glucosidocerebrosides almost exclusively, thus confirming the observations of Halliday, Deuel, Tragerman and Ward,¹⁷ Aghion,¹⁸ Klenk,¹⁹ Danielson, Hall and Everett,²⁰ and Polonovski.²¹

In cases 3 and 4, however, both galactosido- and glucosidocerebrosides were present. These findings are the first demonstration that both types of cerebrosides may be increased in the organs of patients with Gaucher's disease.

The fact that the galactosidocerebrosides and the glucosidocerebrosides may be found together indicates that the increased formation of cerebrosides within the Gaucher cells is not a complete deviation from the synthesis of the normal galactosidocerebrosides. The occurrence of the abnormal glucosidocerebrosides exclusively within the Gaucher cells lends support to the theory that these cerebrosides are built and stored in the Gaucher cells.

The alternating occurrence of galacto- and glucosidocerebrosides is especially evident in the cases of 2 siblings with generalized infantile Gaucher's disease. In the 7 months old infant, mainly galactosidocerebrosides were present with only

traces of glucosidocerebrosides, while in the 5½ months old infant both types were increased in all organs examined. It is probable that there is no fundamental difference in the chemical intracellular disorder of the generalized infantile and of the adult Gaucher's disease.

It was formerly believed that the disordered enzymatic metabolism occurred in the *milieu interieur* of the organism and was the result of the enzyme content of the secretions of glandular structure. The concept that enzymes exist bound to the cellular structure is relatively new (Desmoenzymes Willstaetter).

Embryonic reticulum cells and histiocytes have apparently a greater variety of enzymatic possibilities than mature cells. This seems especially true in the case

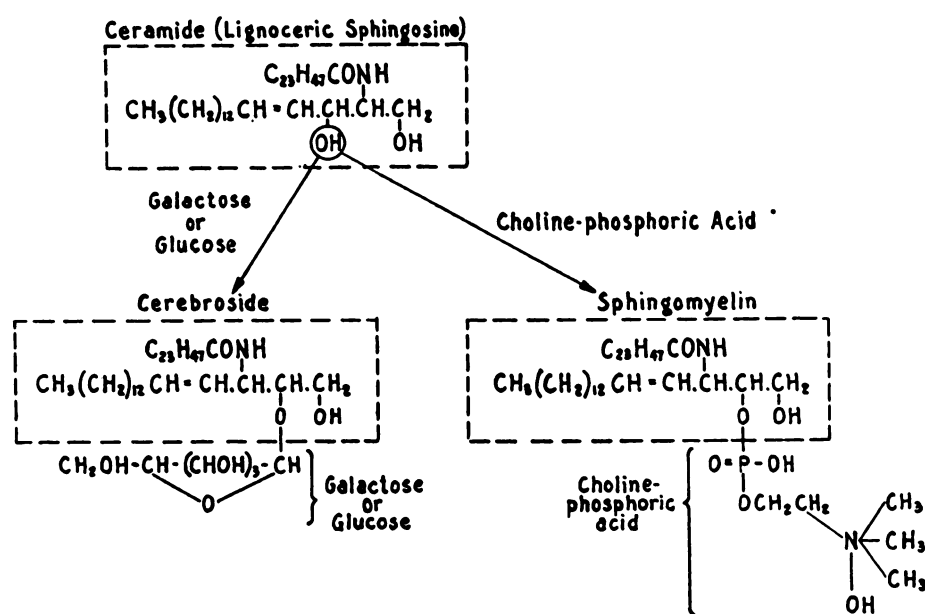


FIG. 1.—CEREBROSIDE AND SPHINGOMYELIN FORMATION FROM CERAMIDE

of the lipid substances such as cerebroside, sphingomyelin and cholesterol. In Gaucher's disease the capacity to synthesize cerebroside in increased amounts is, in our opinion, a perpetuation of the embryonic enzymatic potentialities of certain reticulum cells and histiocytes. The suggested physiology and pathology of the enzymatic metabolism of cerebroside and sphingomyelin is pictured in figure 1.

The ceramides isolated and identified by Thannhauser and Frankel²⁴ are common intermediaries of the metabolism of both cerebroside and sphingomyelin. Enzymatic glucosido-formation with glucose or galactose leads to the formation of cerebroside, while sphingomyelin results by esterification with phosphorylcholine. In the case of Gaucher's disease cerebroside is formed apparently from the ceramides in abundance, while the intermediate disintegration of cerebroside to ceramide is diminished.

SUMMARY

1. The serum of normal individuals and of patients with Gaucher's disease does not contain cerebroside in measurable amounts. Cerebroside in Gaucher's disease are increased only in those organs containing abundant numbers of cells characteristic of the disease.

2. Normal red blood cells contain approximately 0.19 per cent cerebroside. The cerebroside in red blood cells is a galactosidocerebroside.

3. Red blood cells in Gaucher's disease do not differ quantitatively and qualitatively in their cerebroside content from the red blood cells of normal individuals.

4. In four different cases of Gaucher's disease separate determinations of splenic galactosido- and glucosidocerebroside were made. The spleen of two adults showed mainly glucosidocerebroside and only traces of galactosidocerebroside, while the analysis of the spleen of two other adults showed that galactosido- as well as glucosidocerebroside may be accumulated simultaneously in Gaucher cells. These findings are of importance, since it is demonstrated that the deviation of the cerebroside metabolism in Gaucher cells not only results in the formation of an abnormal glucosidocerebroside, but also may lead to the increased formation of the normal galactosidocerebroside, kersasin. It is demonstrated that the relative proportions of galactosido- and glucosidocerebroside in Gaucher cells may differ considerably in individual cases.

5. The organs of infantile siblings with "generalized infantile Gaucher's" disease were analyzed. The organs of one infant showed mainly galactosidocerebroside while in the organs of the other sibling both kinds of cerebroside, glucosido- as well as galactosidocerebroside, were present.

6. The findings reported in this paper lend support to the theory that Gaucher's disease is the result of a deviation of the intracellular metabolism of reticulum cells and histiocytes. The cerebroside are not transported by the serum or by the red blood cells and secondarily deposited in the cells involved, but are formed and stored in the reticulum cells and histiocytes where they are found.

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