ISOLATION AND CHARACTERIZATION OF THE MAIN SPLENIC GLYCOLIPIDS IN GAUCHER'S DISEASE: EVIDENCE FOR THE SITE OF METABOLIC BLOCK.*

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Gaucher's disease is a metabolic disorder characterized by the storage of glycolipids within the reticuloendothelial system of various organs, principally the spleen. Using chromatographic methods we have been able to isolate cerebrosides and gangliosides from spleens of three children and one adult with pathologically verified Gaucher's disease, and to identify their chemical composition.

The numerous structural resemblances between the gangliosides and cerebrosides deposited in the organ strongly suggest that the two glycolipids are metabolically interrelated, and that the enzymatic lesion in this condition is localized within the degradative pathway of red cell gangliosides.

Isolation and Identification of Cerebrosides: Spleens were washed free of blood following the procedure of Rosenberg (1). The lipids were extracted with chloroform-methanol (2:1), washed, and fractionated by successive passages through florisil, DEAE cellulose, and finally silicic acid (2,3,4). Eluates were collected automatically, and fractions pooled on the basis of thin-layer chromatographic (TLC) analysis.

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The amount of pure cerebrosides obtained ranged between 23% and 40% of the total splenic lipids. Based on the hexose concentrations of the initial lipid extracts, recovery of the cerebrosides was virtually quantitative. In a normal adult spleen, cerebrosides could not be detected by thin-layer chromatography.

Purified Gaucher cerebrosides were free of phosphorus, hexosamine, and N-acetylneuraminic acid (NANA). On TLC in chloroform-methanol (85:15), they migrated ahead of the galactocerebrosides prepared from normal brain. The chromatographic properties were unchanged by either mild alkaline hydrolysis (5), or mild acid hydrolysis (6). The latter procedure released about 1% aldehydes as measured by ultraviolet absorption of their 2,4-dinitrophenylhydrazones.

Cerebroside hexose was determined by anthrone, orcinol, and glucose oxidase, and the identity of the sugar verified by gas-liquid chromatography (GLC) of the trimethylsilane derivatives of the methyl-glycosides (7). The total hexose concentration in the four samples of Gaucher cerebrosides was found to be 21.2%, 22.2%, 19.0%, and 21.4% respectively. In all instances glucose constituted 95% or more of the hexose moiety.

Fatty acid composition varied little from case to case. 16:0, and 22:0 constituted more than 50% of the total. 24:0 (9-31%), 18:0 (3-11%), and 24:1 (3-14%) were also found. The latter was the only unsaturated acid found in significant amounts. 20:0 and 23:0 were present in trace amounts. The mean fatty acid chain length was 21. Hydroxy acids were not present in appreciable amounts.

The sphingosine moiety of the Gaucher cerebrosides was subjected to periodate oxidation according to the procedure of Sweeley and Moscatelli (8), and the aldehydes obtained identified by GLC. As in cerebrosides isolated from brain, 18:1 sphingosine was the principal base.

Isolation and Identification of Gangliosides: The NANA content of Gaucher spleens (0.32% to 0.59% of the total lipids) as measured by the orcinol

method (9), was markedly increased over that of normal spleen (0.04% of total lipids).

Two neuraminic acid containing lipids (Gangliosides A and B) were isolated. These substances, present in much smaller amounts in normal spleen and in that instance comprising the only detectable glycolipid, were chromatographically different from both the major gangliosides of normal cerebral grey matter and the Tay-Sachs ganglioside.

Since the composition of aqueous and organic phases appeared identical on TLC, and as no more than one-fourth of the total NANA content of the lipid extract passed into the aqueous phase upon washing, the organic phase was used as the starting material for the isolation of gangliosides. In the initial step the glycolipids were purified by elution from florisil with methanol. Contaminating fatty acids and phospholipids were removed by passage through DEAE cellulose from which gangliosides were eluted with methanol. Final purification was accomplished by fractional elution from silica with chloroform-methanol (2:1). The initial tubes contained ganglioside A, which was also the faster moving of the two glycolipids on TLC (chloroform-methanol-10% ammonium hydroxide 120:70:16). Subsequent tubes contained a mixture of Gangliosides A and B.

1.54 mg of Ganglioside A, a white crystalline powder, were used for analysis. Galactose, glucose and NANA were present in molar ratios of 1.00:1.18:0.88. The lipid was essentially hexosamine free, with but a minimal amount (0.02 mg) being present. GLC of the trimethylsilane derivatives of the methylglycosides confirmed the molar ratior of 1:1 for glucose and galactose.

2.16 mg of Ganglioside A were hydrolyzed for five hours in methanol and con. hydrochloric acid (5:1). The molar ratio of fatty acid esters isolated was 1.04:1.00 with respect to glucose. The fatty acid composition, determined by GLC resembled that of the Gaucher cerebrosides, with 22:0 (23%), 24:0 (19%), 18:0 (15%), 24:1 (12%), 16:0

(8%), 23:0 (8%), 20:0 (7%), and 18:1 (5%), as principal constituents. The average chain length was 21. The sphingosine moiety was analysed by means of GLC of the long-chain aldehydes, as was done for the Gaucher cerebrosides. Again 18:1 was the principal sphingosine base. This finding contrasts with that for cerebral gangliosides in which C 20 sphingosine is the characteristic base (10).

Hydrolysis of the slower moving Ganglioside B was carried out on less than 1 mg of the compound freed of Ganglioside A. The mean chain length of the fatty acids obtained was 19.6, and the concentration of 16:0 (22%) was considerably larger than in Ganglioside A. 22:0 and 18:0 constituted the other major fatty acids. Hexose and sphingosine analyses could not be carried out.

Aside from cerebrosides and gangliosides, only small amounts of other glycolipids were detected. These have been tentatively identified as cytosides and sulfatides, and their isolation is in progress.

Discussion: From its composition, Ganglioside A is similar to Ganglioside GM 3 isolated by Svennerholm (11), and is probably identical with the hexosamine-free ganglioside recovered recently from the brains of individuals with Gargoylism (12).

It is by far the principal neuraminic acid component in both normal and Gaucher spleens, and its accumulation is responsible for the increased NANA content of the diseased tissue. Its storage and its chemical similarity to Gaucher cerebroside point to a metabolic interrelationship, the former probably serving as precursor of the latter. One may speculate that the metabolic defect in Gaucher's disease is localized to the degradative pathway of the red cell glycolipids (13). This is summarized in Figure 1.

Thus Gaucher's disease would represent the outcome of an enzymatic lesion at Step 4, the conversion of ceramide-glucose to ceramide.

A variant of Gaucher's disease reported by one of us (14), in which a cytoside was stored in spleen (1), might well represent an

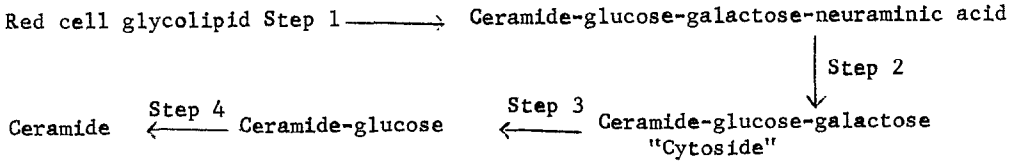

Figure 1

Schematic outline of the postulated degradative pathway of red cell glycolipids.

enzymatic defect localized to Step 3, the site of cleavage of cytoside to ceramide-glucose.

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