THE CHEMICAL STRUCTURE OF NORMAL HUMAN BRAIN AND TAY-SACHS GANGLIOSIDES[x]

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According to the usual definition gangliosides are a family of glycosphingosides with a complex carbohydrate moiety in which sialic acid is one of the components. The homogenity of isolated gangliosides varies according to the preparative methods employed, and although a number of chemical structures have been proposed in different laboratories the exact structure of any ganglioside has not been proved.

In our laboratory a quantitative isolation procedure for total brain gangliosides has been elaborated and by chromatography on silicic acid columns the total gangliosides have been separated into mono- and disialogangliosides (Svennerholm and Raal, 1961; Svennerholm, 1962). About 90 % of the monosialogangliosides isolated from normal human brain consist of a compound with the molar ratio: Ceramide - hexose - N-acetylgalactosamine - N-acetylneuraminic acid = 1:3:1:1. The disialogangliosides are more complex and at thin-layer chromatography on silica gel G (Merck) three disialogangliosides are separated using 1-propanol - water 3:1, v/v as solvent

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for 3 hours at 22°. Their migration relative to the major monosialoganglioside are for disialoganglioside a, R_{mG}=0.80 (R_F= 0.30), disialoganglioside b, R_{mG} = 0.70 and disialoganglioside c, R_{mG} = 0.58.

Action of neuraminidase on gangliosides. The mono- and disialogangliosides were incubated with crystalline neuraminidase (sialidase) isolated from Vibrio cholerae[1] for eight days in an acetate buffer of pH 6.2 (Bernheimer and van Heyningen, 1961) at 37°. Every other day increasing concentrations of fresh neuraminidase were added. During the first two days there was 1 unit neuraminidase per 10 µg sialic acid and in the final solution 50 units neuraminidase per 10 µg sialic acid. The enzymic hydrolysis was followed by thin-layer chromatography and quantitative analysis for free sialic acid by the thiobarbituric acid assay (Warren, 1959). The monosialogangliosides did not undergo any hydrolysis. All three disialogangliosides were converted to the same monosialoganglioside under the liberation of one mole of sialic acid in the following order: disialoganglioside c, a and b. The sialic acid liberated (47% of total) was crystallized and showed to have the same X-ray diffraction pattern as authentic N-acetylneuraminic acid (Abrahamsson, Fischmeister and Svennerholm, 1961). The monosialoganglioside formed had the same composition and the same R_F-values on paper chromatography and thin-layer plates of silica gel as the normally occurring major monosialoganglioside.

Partial degradation of monosialoganglioside. The major monosialoganglioside was transferred into the free acid form by

[1] The neuraminidase was kindly supplied by Professor H. Schultze Behring-Werke, Marburg/Lahn, Germany.

passage through a column with a strong cation exchange resin (Dowex-50 X8) in hydrogen form. It was then heated on a boiling water bath for 1 hour, transferred to dialysis tubing and dialysed against distilled water. From the dialysate N-acetylneuramic acid, isolated by the resin method (Svennerholm, 1956) was crystallized in 50 per cent yield. Besides sialic acid there were minor amounts of galactose, N-acetylgalactosamine and a disaccharide.

The inner solution was then hydrolysed with 0.1 N hydrochloric acid in a boiling water bath for 1 hour. After renewed dialysis the inner solution was lyophilized and the material obtained separated on a silicic acid column in four different ceramide-saccharides with chloroform-methanol 4:1, v/v as eluting solvent. The yield of ceramide-saccharides in per cent of hydrolysed ganglioside and their composition are recorded in Table I.

Table I.

Material	Normal monosialoganglioside		Tay-Sachs ganglioside	
	Yield in %	Sugars Molar ratio	Yield in %	Sugars Molar ratio
Ceramide				
- monosaccharide	3.3	Glucose	4.5	Glucose
- disaccharide	12.4	Glucose 1 Galactose 1	18.5	Glucose 1 Galactose 1
- trisaccharide	3.2	Glucose 1 Galactose 1 Galacto-samine 1	18.0	Glucose 1 Galactose 1 Galacto-samine 1
- tetrasaccharide	25.4	Glucose 1 Galactose 2 Galactosamine 1	0	

It can be seen that the sequence of the sugars in this ganglioside seems to be - glucose - galactose - N-acetylgalactosamine - galactose. This is contrary to the previously proposed constitutional formula for this ganglioside (Klenk and Gielen, 1960) in which the galactosamine was assumed to be in the end position of the asialoganglioside.

The yield of ceramido - trisaccharides is low which indicates that a disaccharide is released during the hydrolysis. This was confirmed by the analysis of the second dialysate. It contained galactose and very little galactosamine-HCl but the major component was a ninhydrin positive, Ehrlich negative, reducing disaccharide. There was also present a second disaccharide, which gave the same reactions but had a somewhat higher R_F-value in paper chromatography with pyridin - containing solvents. After hydrolysis of a mixture of the two disaccharides with 2 N hydrochloric acid for 3 hours at 100°, galactose and D-galactosamine-HCl were obtained. No other sugar was detected. After reduction with potassium borohydride before the hydrolysis, galactose, galactosamine-HCl, dulcitol and D-galactosaminol were identified. This finding is a further support for the assumption that there is an unbranched carbohydrate chain in the gangliosides and that galactosamine is situated between the two galactose residues.

Klenk and Gielen (1961) have indicated the following sugar derivatives in permethylated gangliosides: 2,3,6 - trimethylglycopyranose, 2,3,4,6 - tetramethylgalactopyranose, 2,4,6 - trimethylgalactopyranose, and 4,6 - dimethyl-2-desoxy-2-amino-galactopyranose. These data together with the results obtained at the partial degradation of gangliosides are consistent with the structure given below (Fig. I). The junction of N-acetylneuraminic acid is still unknown.

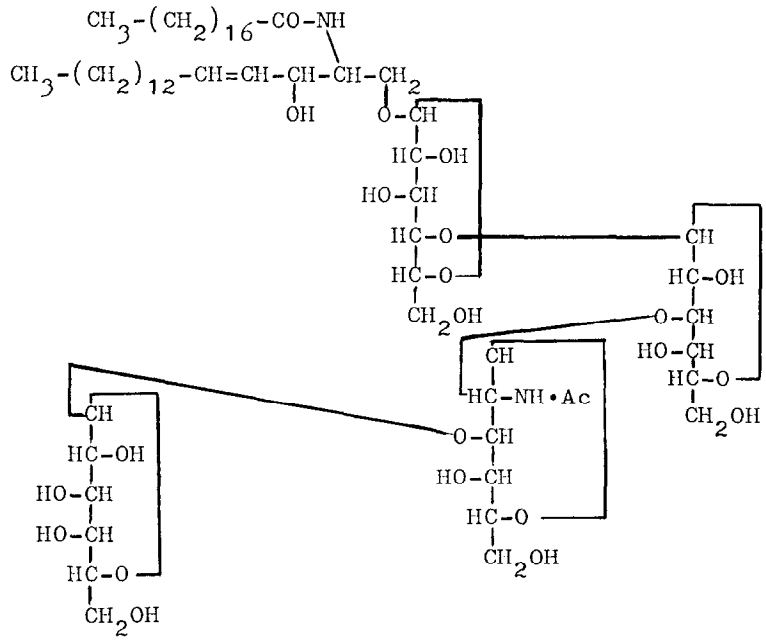

Fig. I.

The gangliosides in Tay-Sachs disease. The abnormal ganglioside accumulated in the brain of a patient with infantile amaurotic idiocy was separated from the normal ones by column chromatography on silicic acid (Svennerholm and Raal, 1961). It constituted about 90 % of the total gangliosides. At thin layer chromatography on silica gel the R_{mG}-value was 1.33 in propanol - water 3:1, v/v and 1.77 in chloroform - methanol - water 60:35:8, v/v. The molar ratio of its components was ceramide - hexose - N-acetylgalactosamine - N-acetylneuraminic acid = 1:2:1:1. Thus it contained one mole hexose less than the normal monosialoganglioside. It was quite resistant to enzymic hydrolysis with neuraminidase. At hydrolysis and isolation of the ceramide - saccharides the neutral ceramide - tetrasaccharide was lacking (Table I). The three other ceramide - saccharides had identical composition and R_F-values of those from the normal gangliosides. Therefore, Tay-Sachs ganglioside

has probably the same structural formula as the normal monosialoganglioside but is lacking the galactose in end position.

This abnormal ganglioside has also been identified in normal brains. In infant brains this ganglioside constituted less than 1%, but in brains of senile persons more than 5% of total gangliosides. This suggests that this ganglioside cannot undergo normal metabolic breakdown.

SUMMARY

Normal human brains contain mono- and disialogangliosides. The latter can be converted to the major monosialoganglioside by enzymic hydrolysis with neuraminidase. Partial acid degradation of the monosialoganglioside has given four neutral ceramide - saccharides which have been isolated and characterised. The ganglioside accumulated into the brain in Tay-Sachs disease has been shown to lack the galactose in end position.

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