A NEW GLYCOLIPID IN TAY-SACHS BRAIN[≠]

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Two classes of glycolipids have thus far been identified in brain, namely, cerebrosides (lipids with equimolar ratios of sphingosine, fatty acid and galactose) and gangliosides, (complex water-soluble polymers containing sphingosine, fatty acid, hexose, hexosamine and sialic acid). Another class of glycolipids, intermediate in composition between these two, i.e. containing sphingosine, fatty acid, several moles of hexose per molecule and either hexosamine or sialic acid, has been found in erythrocytes (stroma) and spleen of various species (Klenk, 1959), but has not been found in brain. During the course of investigations on the nature and composition of gangliosides in Tay-Sachs brain, a chloroform-soluble, water-insoluble glycolipid containing sphingosine, fatty acid, hexose and hexosamine was found. It could not be detected in normal human brain.

Brain from a 22 month-old Tay-Sachs case was used for these studies. Autopsy had been performed immediately after death and tissues were frozen at -20° within 2 hours[≠≠]. 5-40 gm. samples were homogenized with 19 volumes of chloroform-methanol-water

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(64:32:4). The extract was filtered, shaken with 0.2 volumes of water and the phases separated. The lower phase was then washed twice with an equal volume of "ideal upper phase" (Folch et al., 1957) to completely remove gangliosides and other water-soluble material. The crude washed lower (chloroform) phase contained hexosamine at a concentration of about 1 μmole per gm. of wet tissue. It was evaporated to dryness, taken up in a small volume of chloroform and chromatographed on a column of silicic acid (Mallinckrodt), using 1-5 gm. of silicic acid per gm. equivalent of brain extract. The lipids were eluted in a gradient of increasing methanol concentration and eluent fractions were assayed for hexose, hexosamine and phosphate. The new glycolipid appeared immediately after the cephalins, at a methanol concentration of approximately 20%. Fractions containing both hexose and hexosamine were combined, evaporated in vacuo and the residue purified by either of the following two methods: a) direct crystallization from methanol; b) hydrolysis in 0.4 M methanolic KOH containing 10% water at 37° for 2 hours. After partitioning the hydrolysate between chloroform-methanol-water (2:1:0.75), the lower phase, containing the glycolipid, was rechromatographed on silicic acid and crystallized from methanol. Both procedures yielded substances identical in composition.

The following chemical analyses were performed: hexose (Radin et al., 1955), hexosamine (Boas, 1953), sialic acid (a. Bial's reaction and b. thiobarbituric acid assay (Warren, 1959), total nitrogen and phosphate. Sphingosine and fatty

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acids were determined by a modification of Carter's method (Carter et al., 1947). The glycolipid was hydrolysed in 1.2N methanolic HCl for 5 hours at 100° in a sealed tube. Fatty acid methyl esters were extracted with petroleum ether and hydrolysed in 1.5N NaOH in 85% methanol. The hydrolysate was acidified and the fatty acids extracted and titrated. The residue remaining after extraction of the fatty acid methyl esters was made strongly alkaline; sphingosine was extracted with ether, ashed and assayed with Nessler's reagent.

The molar composition of the new glycolipid may be seen in Table I. It was free of phosphate, had no sialic acid and con-

TABLE I

Relative Molar Composition of Glycolipid Isolated from Tay-Sachs Brain

Component	Moles
Sphingosine	1.0
Fatty acid	1.0
Hexose≠	3.0
Hexosamine	0.95
Sialic acid	0
Phosphate	0
Total N	2.15

≠ Calculated as galactose.

tained sphingosine, fatty acid, hexose and hexosamine in molar ratios of 1:1:3:1, respectively. It was further characterized by ascending paper chromatography in diisobutyl ketone-acetic acid-

water (Marinetti et al., 1957) and diisobutyl ketone-pyridine-water (Scringnar and Ferrans, 1960), having R_f values of 0.26 and 0.27 respectively, in these systems.

The glycolipid was found to be equally distributed between grey and white matter in Tay-Sachs brain. The hexosamine content (1 μmole per gm. wet weight of brain) of the glycolipid represents about 1/6 of the total lipid-extractable hexosamine, the remainder being present as ganglioside-hexosamine.

The main biochemical defect thus far described in Tay-Sachs disease involves gangliosides, which have been shown to accumulate in large quantity (Klenk, 1939) and in addition, appear to be altered chemically (Rosenberg and Chargaff, 1959; Berman and Gatt, unpublished observations). The new glycolipid here described is present in rather large quantities in Tay-Sachs brain and is absent from normal human brain. It therefore might be related metabolically to the pathological accumulation of gangliosides in this disease. Experiments to verify this possibility are now in progress.

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References

Boas, N.F., J. Biol. Chem., 204, 553 (1953).

Carter, H.E., Norris, W.P., Glick, F.J., Phillips, G.E., and Harris, R., J. Biol. Chem., 170, 269 (1947).

Folch, J., Lees, M., and Sloane-Stanley, G.H., J. Biol. Chem., 226, 497 (1957).

Klenk, E., Z. Phys. Chem., 262, 128 (1939).

Klenk, E., Ann. Rev. Biochem., 28, 39 (1959).

Marinetti, G.V., Erbland, J., and Kochen, J., Fed. Proc., 16, 837 (1957).

Radin, N.S., Lavin, F.B., and Brown, J.R., J. Biol. Chem., 217, 789 (1955).

Rosenberg, A., and Chargaff, E., A.M.A. J. Dis. Child., 97, 739 (1959).

Scrignar, C.B., and Ferrans, V.J., Fed. Proc., 19, 219 (1960).

Warren, L., J. Biol. Chem., 234, 1971 (1959).