On the Isolation of Gangliosides

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Gangliosides in a high yield have been isolated on a small scale by partition chromatography on cellulose columns [1]. A disadvantage with the method is the low capacity. When larger amounts of lipids are applied to the column, part of the gangliosides is eluted together with the main bulk of other lipids. Other methods have therefore been tried. Reversed phase chromatography on hydrophobic kieselguhr did not separate gangliosides from the other lipids. Zone electrophoresis in a capillary system with pyridinium-formic acid buffer in 80 % methanol was suitable only for preparations containing 50 % or more of gangliosides. A good separation was achieved on anion exchange resins. Radin, Lavin and Brown [2] have in a recent work used the same type of resins for the separation of cerebrosides from other glycolipids.

Chloroform-methanol extracts of brain lipids were passed on a strong cation exchanger in hydrogen form (Amberlite IR-120 or Dowex 50-X8, 50—100 mesh). The solution was then percolated through a column packed with a weak anion exchange resin in hydroxyl form (Duolite A-7, 40—60 mesh) or through a strong one in bicarbonate form (Dowex 1, 50—100 mesh). Cerebrosides, cholesterols and part of the phospholipids passed through the column. The rest of the phospholipids, cerebroside sulphuric acid and gangliosides were taken up by the resin and eluted with 0.1—1.0 N formic acid in chloroform--methanol mainly in the order mentioned. The fractions containing gangliosides were evaporated to a small volume. Formic acid and contaminating lipids were removed by partition dialysis as described by Folch *et al* [3]. The anion exchange resins are capable to bind considerable amounts of gangliosides. A Duolite column of the dimensions 25 × 250 mm removed about 90 % of the gangliosides from a total lipid extract of one kilogram of fresh brains.

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Determination of Glycolipids in Plasma

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Only few quantitative estimations of the glycolipids in plasma have earlier been performed [1,2]. They have been based on the determination of the reducing power of a lipid extract before and after hydrolysis. The difference has been attributed to cerebroside sugar, but no attempt has been made to isolate the corresponding lipid. Large variations in the cerebroside content (0—167 mg/100 ml) have been reported. The methods used are unsatisfactory because other reducing substances than sugars are released during the hydrolysis. If gangliosides occur in addition to cerebrosides they will be included in the cerebroside value.

In the method worked out the lipid extract is purified through partition between chloroform and methanol-water. Cerebrosides will go into the chloroform phase, while gangliosides and low molecular contaminants will go into the methanol-water phase.

Experimental. Blood is drawn into a centrifuge tube containing sodium citrate. Plasma is separated from blood cells in a refrigerated centrifuge at 0° and lyophilized. The lipids are extracted with chloroform-methanol (2:1, v/v) under reflux boiling. After evaporation *in vacuo* the lipids are redissolved in the same solvent and quantitatively filtered down into a volumetric cylinder. 1/4 volume of 0.1 % sodium chloride in water is added and the cylinder is shaken thoroughly. On the resulting two phases hexose is determined in the chloroform phase with an orcinol method and sialic acid from gangliosides is determined in the methanol-water phase with Bial's reagent. The mean value of 8 normals was for cerebrosides 4.8 mg/100 ml (range 3.5—5.7) and for gangliosides 2.5 mg/100 ml (range 2.0—3.8). Cerebrosides were isolated from the chloroform phase by the method of Klenk and Leupold [3].

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