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Partition Chromatography of Brain Gangliosides on Cellulose

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The separation of the lipopolysaccharide ganglioside from a mixture of the naturally occurring brain lipids involves many difficulties and gives a low yield. Klenk [1] based his methods on solubility relations, including a separation of ganglioside from the last traces of sphingomyelin on aluminium oxide columns. Folch *et al.*[2] using partition dialysis were not able to obtain more than rather crude ganglioside. Trying to find a convenient method the author has explored the possibility of chromatographic separation on cellulose columns.

Gray matter from human brains was first extracted with boiling acetone and then in a Soxhlet apparatus with boiling chloroform: methanol 1 : 2 v/v. After removal of the solvent and re-solution in a small volume of watersaturated chloroform : methanol 8 : 1, the lipid extract was placed on the top of a column of cellulose packed as described by Hough *et al.*[3] The column was run with the solvent stated above. The solvent collected in 10 ml portions was removed and when no residue was obtained the solvent was changed to chloroform : methanol : water 3 : 9 : 1. Ganglioside determinations by the method of Klenk and Langerbeins [4] showed that ganglioside appeared as two bands; the first close behind the solvent front together with the bulk of the other lipids, the latter after the solvent had been changed. The hexosamine content was higher in the first ganglioside fraction. That is in good agreement with the fact that the hexosamine content of ganglioside is reduced by reprecipitations with chloroform: methanol.

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An Attempt to Crystallise β-Lactoglobulin from Human Milk Whey

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In contrast to the fairly well known proteins of bovine milk whey the whey proteins in human milk have been subject to very few investigations. Wegelin [1] recently reported about the occurrence in human whey of a component with approx. the same electrophoretic mobility as bovine β-lactoglobulin at pH 6.8. This component amounted to about 10 % of the total whey protein and does not seem to have been isolated so far.

An attempt to isolate the possible β-lactoglobulin in human milk whey has recently been made in this laboratory as part of an investigation of the non-casein proteins of human milk.

Pooled human milk was freed from fat by centrifugation and dialysed against frequently changed distilled water at a temperature of + 3—4° C for 48 hours. The casein was precipitated at 30° C and pH 4.6 without dilution of the milk. The completely clear whey was treated with anhydr. sodium sulphate according to Palmer [2] or ammonium sulphate according to Sörensen [3]. In both cases the resulting precipitate of "lactalbumine" appeared to be faintly salmon coloured. This colour has been observed in all preparations. After redissolving in water this fraction was dialysed against distilled water for a long time and the solution adjusted to pH 5.2. In this step crystals of β-lactoglobulin are obtained when the initial material is bovine whey. Despite repeated preparations it has not been possible to obtain any crystals in the corresponding human fraction. Paper electrophoresis of the red coloured dialysed protein solution reveals three components of which the one with the lowest mobility appeared as a faintly red coloured spot on the paper strips. None of the components seems to be identical with bovine β-lactoglobulin. The properties of this red coloured protein fraction are under investigation.

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