From this evidence we conclude that treatment of Cohn's fraction I + II + III with concentrated salt solutions brings about the loss of some lipid components from the β-lipoproteins, with the formation of substances of higher density (compare Hayashi, Lindgren and Nichols[8]). Furthermore, it seems highly probable that the 'high-density β-lipoproteins', which were originally observed[9] during the sub-fractionation of I + II + III, were also artefacts formed in this way. It also follows that the different results obtained by the ultracentrifugal and chemical techniques for separating lipoproteins must be ascribed to deficiencies in the methods and not to the presence of lipoproteins of bizarre properties. It may also be observed that the 'high-density β-lipoprotein' was suggested by Lindgren and Nichols[10] to be evidence for different lipoprotein spectra in fraction I + II + III and in the low-density group. This postulate now appears to be superfluous, at least in the case of normal plasma.

These experiments suggest that, although the Cohn fractionation technique does not itself cause damage to the lipoproteins, it can potentiate unstable characteristics which become manifest during subsequent treatment. It is therefore not a good starting point for the preparation of intact lipoproteins, but may be a useful route to the preparations deficient in lipids which are required for investigations of lipoprotein structure.

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Glycolyl-neuraminic Acid in Ox Brain Gangliosides

In this communication we report the presence of glycolyl-neuraminic acid in sialic acids from ox brain gangliosides. Our method of analysis consisted of the following six steps: (i) gangliosides were extracted from ox brain grey matter and purified according to a method which we have previously described[1]; (ii) gangliosides so obtained were hydrolysed in 0·1 N sulphuric acid at 80° C for 2 h, to split off sialic acid; (iii) sialic acid was purified on 'Dowex' 2 × 8 column as acetate according to Svennerholm[2], and desalted on 'Amberlite *IR*-120'; (iv) the solution was dried in vacuum and the residue was hydrolysed in 1 N sulphuric acid for 5 h at 100° C to obtain glycolic acid[3]; (v) glycolic acid was purified on 'Dowex' 2 × 8 column as acetate and eluted with NaCl 2 M; (vi) the glycolic acid was identified by the Eegriwe reaction[4] and by gas-chromatography.

The following procedure was used for the gas-chromatographic analysis: glycolic acid was extracted from the 2 M NaCl solution by chloroform–methanol 7 : 3 (v/v).

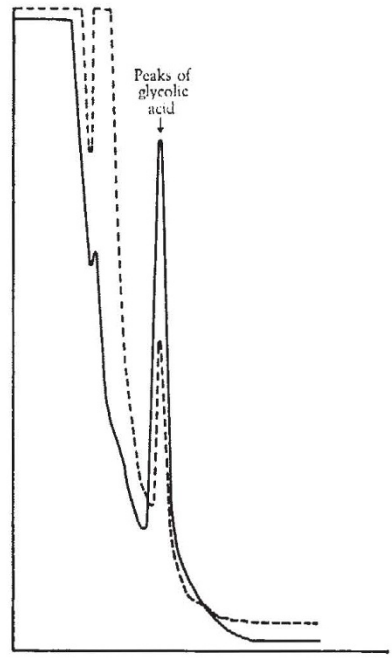

Fig. 1. Gas chromatogram of propylated glycolic acid. Solid line, pure glycolic acid (propylated); broken line, glycolic acid from ganglioside sialic acid (propylated)

The solution was dried in vacuum and the glycolic acid contained in the residue was propylated by propanol saturated with HCl at 70° C for 10 min[3]. A standard of pure glycolic acid (propylated in the same way) and the solution under analysis were then gas-chromatographed[3]. Good correspondence was obtained between the peaks of pure and extracted glycolic acid, as shown by the gas-chromatograms given in Fig. 1. The amount of glycolyl-neuraminic acid was calculated from the glycolic acid content (obtained by the Eegriwe reaction method) and found to represent 2–4 per cent of the total sialic acids contained in ox brain gangliosides.

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Precipitation of Pertussis Immunogen with Benzathene and Proflavine

Two years ago I described a method for obtaining soluble preparations of pertussis mouse protective antigen (immunogen) by lysing the bacterial cells with sodium deoxycholate[1]. I now wish to report the partial purification of these extracts, using the organic bases benzathene and proflavine as precipitating agents. In a typical experiment with the former, deoxycholate lysate of pertussis derived from a cell concentration of 320 opacity units (OU) per ml. was treated dropwise at *p*H 7·4–7·5 at room temperature with a 14 per cent (w/v) solution of benzathene (dibenzylethylene diamine acetate). The mixture was shaken continuously and its optical density monitored until maximum density, indicative of maximum