On the Dissolving Power of the Bile for Cholesterol

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The fractional extraction procedure on lyophilized bile described by Isaksen 1 gives the following separation of the main components: cholesterol is found in the ethyl ether extract, lecithin and part of the bile salts in the subsequent chloroform extract and the rest of the bile salts in the following ethanol extract. The constituents of the chloroform appear in rather constant proportions, 1 mole lecithin per 3 moles bile salts. This system is soluble in water and has the power to dissolve cholesterol. Thus, it seemed to be of value to interpret the importance of lecithin for the dispersion of the bile cholesterol. Since the works of Andrews et al. 2 the bile salts have been known as the only components of bile of interest in that respect.

Experiments on human bile: Normal bladder bile were lyophilized and extracted as described earlier 1. Part of an ether extract was mixed with an equivalent part of either the chloroform or the ethanol extract, the solvents evaporated in vacuo and the residue taken up in a phosphate buffer (pH 6.0, 7.0 or 8.0) at 37°C. A quite clear solution was obtained with the chloroform but not with the ethanol residue. Addition of bile salts to the ethanol extract in amount equal to that in the chloroform extract was without influence. This must mean that the magnitude of the bile salts in normal bile is not sufficient to keep the actual amount of cholesterol in solution, in contrast to the reports of Andrews 2. Moreover, as lecithin and bile salts are the only components found in the chloroform (except bile pigments) the two seem to be obligatory components in the solving system. The minimal relation of lecithin bile salts system to cholesterol for complete solution was titrated out and found to be 11/1—12/1, it means a molar relation lecithin: bile salts: cholesterol = 2:6:1.

Experiments on artificial systems: Pure lecithin and bile salts were mixed in different proportions and tested for their power to dissolve added cholesterol with pH and temperature conditions as above. The best weight relation was found to be about 35/65 (i.e. 1 mole lecithin per 3 moles bile salts). The deoxycholic acids were slightly superior to the cholic acids, but no significant difference between tauro and glyco conjugates was found.
An Attempt to Crystallise $\beta$-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 recently reported about the occurrence in human whey of a component with approx. the same electrophoretic mobility as bovine $\beta$-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 $\beta$-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 $+3-4^\circ$C for 48 hours. The casein was precipitated at $30^\circ$C and pH 4.6 without dilution of the milk. The completely clear whey was treated with anhydrous sodium sulphate according to Palmer or ammonium sulphate according to Sörensen. 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 $\beta$-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 $\beta$-lactoglobulin. The properties of this red coloured protein fraction are under investigation.

1. Wegelin, E. On the proteins of milk whey (Diss.) Utrecht (1932).

Acta Chem. Scand. 8 (1954) No. 6