STABILITY OF CHOLESTEROL IN ANIMAL BILE

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The study of the stability of cholesterol in the bile is of great interest because the deposition of cholesterol in the gall bladder leads to the formation of gallstones. Investigations [2-5] conducted by the paper electrophoresis method have shown that cholesterol exists in the bile in the composition of lipoproteins. However, very little is known of the composition of these lipoproteins, and it therefore is uncertain with what substances and in what proportions the cholesterol is linked in the lipoproteins.

The object of the present investigation was to isolate the lipoproteins from the bile, to determine their composition, and, hence, to identify the substances responsible for the stability of cholesterol in the bile.

EXPERIMENTAL METHOD AND RESULTS

Ox bile obtained the same day directly from an animal's gall bladder at the slaughterhouse was used in the investigation. The cholesterol concentration was determined by Sperry's method, the lipoid phosphorus level by incinerating the dry residue from a measured content of extract of bile lipids with H_2SO_4 and subsequent colorimetric estimation of the phosphorus by the Fiske-Subbarow method, and the acid content by the method of Reinhold and Wilson. The lipoproteins of the bile and the proteins were investigated by paper electrophoresis (veronal-acetate buffer, pH 8.6, voltage 150 V, current 12.5 mA, exposure 6 h; the proteinograms were stained with bromphenol blue solution and the lipoproteinograms by Canabrocchi's method with Sudan black).

The bile contains many other substances not bound to cholesterol. It was therefore, necessary to remove them from the bile while leaving in solution, as far as possible, all the cholesterol and cholesterol-bound substances. Preliminary experiments showed that the best method for this purpose was to use a 20% aqueous solution of lead acetate, which precipitates the mucins, the bile pigments, about 30% of the cholic acid, and only a very small part of the cholesterol and phospholipids. This precipitation was carried out as follows. Preliminary experiments with a small quantity of bile showed in what relative volumes of bile and 20% lead acetate solution complete precipitation of these substances took place without leaving an excess of the lead salt in solution (usually about 0.24 ml of lead acetate solution was needed for 1 ml of bile). After this ratio had been determined, the calculated volume of 20% lead acetate solution was added drop by drop to a measured volume of bile and the mixture was allowed to stand for 3 h at room temperature and then centrifuged. The same methods were then used to determine the content of cholesterol, lipid phosphorus, and cholic acid in the transparent supernatant, which contained no bile pigments or mucins. The results were then expressed in terms of the original bile, making allowance for dilution of the bile by addition of the lead acetate solution. The lipoproteins and proteins were investigated by the same methods but the solution was first filtered through Sephadex G-75 gel, as described in the literature [1].

The results given in Table 1 show that precipitation of the bile with lead acetate lowered the cholesterol and lipid phosphorus levels very little, but the cholic acid concentration fall by about 30%. Electrophoresis of the bile and of the solution obtained after precipitation of the bile with lead acetate showed that these substances occur as two lipoprotein fractions: one with a faster, the other with a slower rate of migration; the latter fraction was less prominent on electrophoresis of the solution than of the bile (see Fig. 1). It was also clear from electrophoresis that these lipoprotein fractions corresponded to the protein fractions.

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TABLE 1. Content of Cholesterol, Lipid Phosphorus, and Cholic Acid in the Bile, and in a Solution of the Same Bile after Precipitation with Lead Acetate (mean of 8 experiments)

Material investigated	Cholesterol (in mg%)	Lipid phosphorus (in mg%)	Cholic acid (in %)
Bile	34,3±1,24	36±5,21	6,16±0,88
Bile solution	30,0±1,48	31,9±4,03	4,52±0,63

Fig. 1. Electrophoresis of lipoproteins of bile (a) and of bile solution after precipitation with lead acetate (b) and concentration with ammonium sulfate (c).

The presence of proteins in the solution after precipitation of the bile with lead acetate was demonstrated by electrophoresis on paper, but it was impossible to determine them quantitatively because of their low concentration. The solution was therefore concentrated. A volume of 100 ml of the solution was saturated with ammonium sulfate and allowed to stand for 5 h at room temperature. The solution was then centrifuged, the supernatant was poured off, and the residue was dissolved in water and the volume made up to 25 ml. The content of cholesterol, lipid phosphorus, and cholic acid was determined in the concentrated solution thus obtained. The protein concentration in this solution could not be determined, for the reaction with Lowry's reagent was not completely identical with the reaction obtained with blood serum.

To remove traces of pigments and other substances interfering with the reaction from this solution it was filtered through Sephadex G-75 (medium), and the content of cholesterol, lipid phosphorus, cholic acid, and protein in the first fraction of this filtrate was determined by Lowry's method (using as the standard a solution of rabbit's blood serum in which the protein concentration had first been determined). The lipoproteins and proteins in this filtrate were also investigated by the same method. The results obtained are given in Table 2 and in Fig. 1.

Since the concentrated bile solution had been filtered through a hydrated Sephadex gel, the concentration of these substances was considerably reduced as a result of adsorption and dilution, and was very close to their concentration in the original bile.

Simultaneous investigation of the lipoproteins and proteins of the filtrate by the method of electrophoresis on paper revealed one fraction of lipoproteins which corresponded to the fastest migrating fraction of the biliary lipoproteins. This lipoprotein fraction evidently corresponded to the protein fraction of the same mobility incorporated in the lipoproteins.

From these results, it was possible to calculate the composition of the lipoproteins isolated from the bile, forming the greater part of the biliary lipoproteins. For each milligram of cholesterol in the lipo-

TABLE 2. Content of Protein, Cholesterol, Lipid Phosphorus, and Cholic Acid in a Concentrated Solution of Bile Lipoproteins and in the Same Solution after Filtration through Sephadex G-75, Medium (mean of 8 experiments)

Material investigated	Cholesterol (in mg%)	Lipid phosphorus (in mg %)	Cholic acid (in %)	Protein (in mg %)
Concentrated solution of biliary lipoproteins	98±0,65	123±3,1	15,8±0,59	—
The same after filtration	30,3±2,41	40,1±2,43	2,77±0,66	85,03±2,61

proteins there was 2.8 mg protein, 33.08 mg phospholipids (the conversion factor for lipid phosphorus was taken to be 25), and 94.4 mg cholic acid. These results show that, by comparison with the blood lipoproteins, the biliary lipoproteins contain very little protein but have a high content of phospholipids. The cholic acid content is also high. Consequently, these last two substances — phospholipids and cholic acid — play an important role in determining the stability of the cholesterol in the bile.

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