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## Constitution of Bile Proteins.* (25486)

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It is unlikely that the gallstone problem will be completely resolved before the biochemistry and physiology of bile are thoroughly understood. Unfortunately little is known about the nature of bile proteins even though several investigations concerning bile proteins have been reported(1,2,3,4,5,6). In this study various bile protein fractions are identified, classified and further characterized with respect to their physico-chemical properties.

*Materials and methods.* Female sheep weighing 60 to 100 lb and dogs weighing 40 to 50 lb were used. Gallbladder bile was obtained by puncture of gallbladder followed by cholecystectomy. Hepatic bile was obtained from T-tube inserted into the common duct. The long end of the T-tube was plugged with a glass rod and fixed under the skin between bile collections. Subsequent collections of bile from the T-tube were made by incising the skin under pentobarbital anesthesia and connecting a plastic tube to the long end of the T-tube. Bile samples were concentrated to approximatey 1/10 vol. by dialysis with the use of 50% (w/v) gum arabic solution in barbital buffer (pH 8.6, ionic strength 0.1)(7). Paper electrophoresis of concentrated bile samples was performed in barbital buffer of ionic strength 0.1, pH 8.6 for 15 hours at 200 volts and at 8°C using horizontal strip type apparatus(8). Approximately 0.1 ml of concentrated bile was used on filter paper strip (4.5 x 34.0 cm) of Whatman 3 MM filter paper. Since bile pigments and phospholipids are also stained with bromphenol blue and interfere with quantitative evaluation of bile protein fractions, filter paper strips were washed overnight in a mixture of ethanol: chloroform : concentrated hydrochloric acid (2:1:0.01). Proteins on filter paper strip were then stained with bromphenol blue(9). The stained filter paper strips were immersed into melted paraffin at approximately 60°C and color intensity measured with Photovolt densitometer model 525, using a filter of 570 mμ. The faster migrating A-fraction (see Results) was isolated electrophoretically as follows: A small amount of buffer was placed in a cellophane bag set in the anodal buffer vessel of paper electrophoresis apparatus. The anodal end of filter paper strip was placed in this buffer and the A-fraction was allowed to migrate into this buffer by means of electrophoresis and collected. Buffer was removed from the A-fraction as much as possible by dialysis and electrodialysis, then the A-fraction was concentrated by the ultrafiltration method of Mies(10). For paper chromatographic analysis of amino acids, the A-fraction was hydrolyzed in a sealed glass tube with 8 N hydrochloric acid at 100°C for 24 hours. An n-butanol : acetic acid : water

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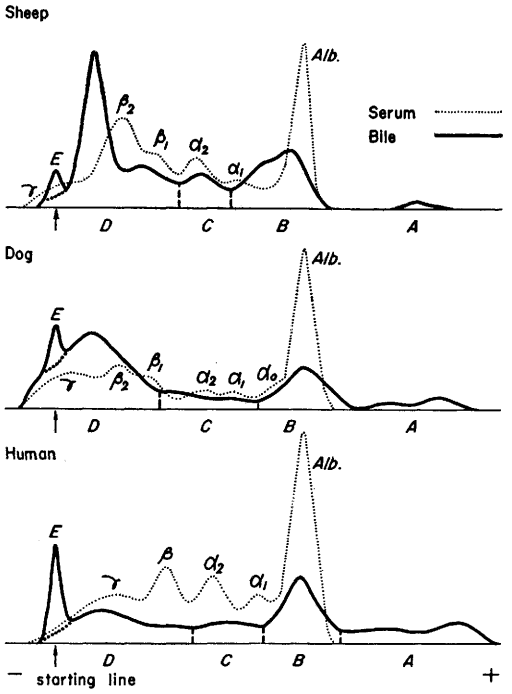


FIG. 1. Comparison of hepatic bile and serum protein fractions.

mixture (4:1:5) was used as solvent. A 0.2% solution of ninhydrin in water-saturated *n*-butanol was used to detect amino acids. For paper chromatographic analysis of carbohydrates, the A-fraction was hydrolyzed under reflux with 2 N hydrochloric acid for 12 hours. A phenol:water mixture (4:1) was used as solvent. An aniline hydrogen phthalate reagent was used to detect carbohydrates.

*Results.* The curves obtained from paper electrophoresis of the concentrated hepatic bile and serum are shown in Fig. 1. The paper electrophoretic patterns of bile and serum proteins differed among species, corresponding protein peaks were always present.

1. *Classification of bile proteins.* The bile curve might be divided into A-, B-, C-, D-, and E-fractions (Fig. 1). B-, C-, and D-fractions consisted of those proteins which migrated the same as serum protein fractions. The A-fraction migrated more rapidly than any protein fractions in serum. The E-fraction remained on starting line. Normal bile and bile from patients with gallstones contained all of these fractions.

2. *Nature of A- and E-fractions of bile proteins.* A- and E-fractions were unique to bile insofar as no comparable fractions were seen in serum (Fig. 1). The A-fraction was present in fresh bile in significant amounts, diminishing after concentration of the bile. Conversely, the E-fraction was not prominent in electrophoresis of fresh bile but became prominent in electrophoresis of concentrated bile. The E-fraction was insoluble in barbital buffer (pH 8.6, ionic strength 0.1), so that it did not migrate and remained on the starting line during electrophoresis. The E-fraction, as well as the A-fraction, did not combine with bromphenol blue at pH 8.6, but combined with bile pigments. From these facts, it was assumed that the E-fraction consisted of proteins of the A-fraction denatured in process of concentration.

Paper chromatography of the hydrolysate of the A-fraction separated as described under Methods, revealed the presence of various amino acids, hexose and hexosamine. The black precipitate remaining after hydrolysis was readily soluble in anhydrous ethanol, ether and chloroform. It was slightly soluble in 50% ethanol and was insoluble in water. The A-fraction was stained with Sudan black B. The precipitate therefore was assumed to be composed mainly of lipids. Furthermore, the A-fraction was not heat coagulable. It was therefore assumed that the A-fraction

TABLE I. Constitution of Bile Proteins.

| | Type of bile | No. of cases | Bile protein fractions (%)* A | B | C | D | E |
|---|---|---|---|---|---|---|---|
| Sheep | Gallbladder bile | 6 | 2.1 ± 1.7 | 42.8 ± 1.4 | 25.3 ± 4.3 | 25.3 ± 4.9 | 4.5 ± 2.9 |
| | Hepatic " | 4 | 2.8 ± 1.8 | 42.4 ± 2.1 | 14.0 ± 2.1 | 36.2 ± 6.5 | 4.6 ± 2.1 |
| Dog | Gallbladder " | 6 | 4.6 ± 1.9 | 5.1 ± 3.0 | 6.1 ± 2.1 | 72.0 ± 5.9 | 12.3 ± 4.1 |
| | Hepatic " | 5 | 15.7 ± 2.7 | 17.8 ± 5.2 | 8.3 ± 2.8 | 54.5 ± 4.3 | 3.8 ± 1.6 |
| Human | Gallbladder " † | 1 | 43.0 | 35.3 | 4.4 | 15.5 | 1.8 |

* Mean ± S.D. (except human gallbladder bile). † Cholelithiasis.

contained a mucoprotein and a lipoprotein.

3. *Nature of B-, C- and D-fractions.* Unlike the A- and E-fractions, mobilities of B-, C- and D-fractions were comparable to serum protein fractions, and were thus assumed to be similar to the serum proteins.

The B-fraction migrated at a rate similar to that of serum albumin. However, the evidence for this similarity also came from other sources than their similar mobilities. For instance, it was reported that bilirubin in serum was combined with albumin and $\alpha$-globulin (12,13,14,15) and bile pigments likewise combined with the B-fraction. Furthermore, Kunkel and Tiselius(9) reported that bromphenol blue combined only with serum albumin when it was added to serum prior to electrophoresis at pH 8.6. Similarly, bromphenol blue migrated with the B-fraction when it was added to bile prior to electrophoresis at pH 8.6.

*Summary.* 1. Proteins detectable by paper electrophoresis of concentrated bile were classified into A-, B-, C-, D- and E-fractions. 2. The A-fraction migrated more rapidly than any protein fractions in serum. 3. The B-fraction was similar to serum albumin as evidenced by its similar mobility to serum albumin and affinity for bile pigments and bromphenol blue. 4. C- and D-fractions had mobilities similar to serum globulins. 5. The E-fraction was probably a denatured A-fraction, and became prominent only after concentration of bile, associated with a decrease in the A-fraction. 6. Normal and pathological human bile, the bile of sheep and dog contain all these protein fractions. 7. Values of bile protein fractions varied considerably according to species and type of bile.

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## ACTH Releasing Factor Active in the Guinea Pig.* (25487)

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The hypothalamus produces a chemical mediator concerned with ACTH release(1). It is presumably transported by way of neurohypophyseal tracts into the posterior pituitary gland where it is stored(2). There is now much evidence which points to presence of a substance in extracts of posterior pituitary that is capable of causing ACTH release(3,4,5); apparently this is related to the hypothalamic agent. Certain investigations lead to the belief that this substance is vasopressin (6,7,8,9). However, powerful evidence suggests that it differs from vasopressin(10,11,12). This communication presents the results which demonstrate that vasopressin

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