Disc Electrophoresis–Immunodiffusion of Serum Proteins in Normal Human Gallbladder Bile[1] (34168)

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(Introduced by L. S. Goodman)

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The constituents of bile interfere with standard chemical methods of protein analysis, but the use of electrophoretic and immunological methods has produced considerable information about the proteins in bile. Electrophoresis separates the proteins for discovery and quantitation, while immunodiffusion provides sensitivity and identification with specific antisera. A number of serum proteins have been identified in bile (1, 2), and some authors reported the presence of additional proteins specifically present only in the biliary tract (3, 4). The high resolution of disc electrophoresis (DEP) combined with immunodiffusion should expand knowledge of the numbers and types of proteins in bile. The feasibility of combining these technics has been established with other proteins (5), but the combination has not yet been successful with bile. This communication presents the application of disc electrophoresis on polyacrylamide gel combined with immunodiffusion to normal human gallbladder bile and establishes the location of 12 specific plasma proteins among the total of 20 proteins found.

Materials and Methods. Ten normal bile samples were collected by aseptic gallbladder puncture shortly after opening the abdomen without prior manipulation of the gallbladder region in patients undergoing laparotomy for nonbiliary diseases. Samples were frozen immediately in sterile containers, then thawed for study.

Preliminary screening of bile for serum proteins was carried out by immunodiffusion after Ouchterlony (6) using 1% Behringwerke agar in 0.15 *M* phosphate buffer, pH 7.1, and specific antisera to human plasma proteins.[2] The agar slides were incubated at 37° for 24–48 hr.

Analytical disc electrophoresis was performed by the method of Davis and Ornstein after the modification of Clarke (7) in a Canalco apparatus,[3] except that a large pore 5% polyacrylamide gel was used. This gel provided optimum physical and chemical characteristics for the DEP of bile. In order to obtain discrete bands, the bile sample had to be in the pH range of 6.5–8.5. Sample loads of 10–100 μl were examined and it was found that 25 μl or 50 μl provided maximum sensitivity without overloading. Electrophoresis was continued until a standard distance (4.0 cm) was reached by bromphenol blue, which migrated at the front and was employed as a tracking dye. After electrophoresis, gels were stained for 2 hr in a protein fixative stain consisting of 0.1% amidoschwarz in 7% glacial acetic acid. Destaining of the clear zones of the gel between proteins was accomplished electrophoretically in the same apparatus using 15% glacial acetic acid. The stained gels were scanned on a Photovolt Densicord 542A densitometer fitted with a gel scanner. The logarithmic scale D-1 gave the best resolution when used in combination with a 610 mμ filter. The number of peaks recorded corresponded with

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[2] Hoechst Pharmaceuticals, Inc., Cincinnati, Ohio.

[3] Canal Industrial Corporation, Bethesda, Maryland.

TABLE I. Serum Proteins in Bile by Immunodiffusion.

Proteins	Bile samples									
	1	2	3	4	5	6	7	8	9	10
Albumin	+	+	+	+	+	+	+	+	+	+
α_1 Acid-glycoprotein	+	+	+	+	+	+	+	+	+	+
α_1 Antitrypsin	+	+	+	+	+	+	+	+	+	+
Gc-Globulin	+	+	+	+	+	+	+	+	+	+
Haptoglobin	+	+	+	+	+	+	+	+	+	+
IgA-Immunoglobulin	+	+	+	+	+	+	+	+	+	+
IgG-Immunoglobulin	+	+	+	+	+	+	+	+	+	+
β_2-Glycoprotein		+	+	+	+	+		+		+
IgM-Immunoglobulin		+		+	+	+	+	+		+
Prealbumin			+	+		+	+	+		+
Hemopexin		+	+	+	+	+				+
Transferin			+	+	+			+	+	
β-Lipoprotein	+	+				+	+			
Ceruloplasmin				+				+		
β_1 c–β_1 e Complement					+			+		
α_2-Macroglobin									+	
Fibrinogen					+					

the number of discs or bands visible on the gel (Fig. 1)

Disc electrophoresis–immunodiffusion was developed to identify individual bands of the DEP bile protein pattern. The DEP was repeated, gels were sliced in half longitudinally immediately thereafter, one-half of the gel was placed in the amido-schwarz stain fixative and the unstained half was placed flat side down on a feshly prepared agar slide, making certain there was no air trapped between gel and agar. The unstained gels on agar were placed in a humid chamber overnight at room temperature to allow protein to diffuse into the agar. Two 2-mm wide longitudinal troughs for antisera were cut in the agar 5 mm equidistant from the gel and parallel to it. The troughs were loaded with specific antisera and the slides were placed in a humid chamber at 37° where antigen–antibody reactions appeared in 12 hr and were optimum at 96 hr. DEP gels of pooled human serum and of normal bile were allowed to diffuse similarly against antisera to whole human serum[4] as controls and were examined with each batch of bile DEP–immunodiffusions against specific antisera. The stained half of each gel was superimposed on the imprint made by the half used for diffusion and areas of specific reactions were located.

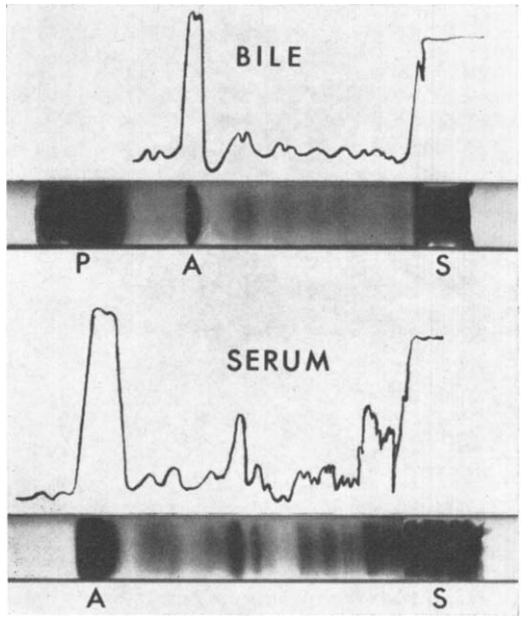

FIG. 1. Densitometry of stained disc-electrophoresis gels of bile and serum: (P), pigment front; (A), albumin; (S), stacking gel origin. Thirteen proteins are separated in bile. Note albumin migrates slower in bile than in serum.

Results and Discussion. Immunodiffusion. Studies of the proteins in bile indicated that

[4] Hyland Laboratories, Los Angeles, California.

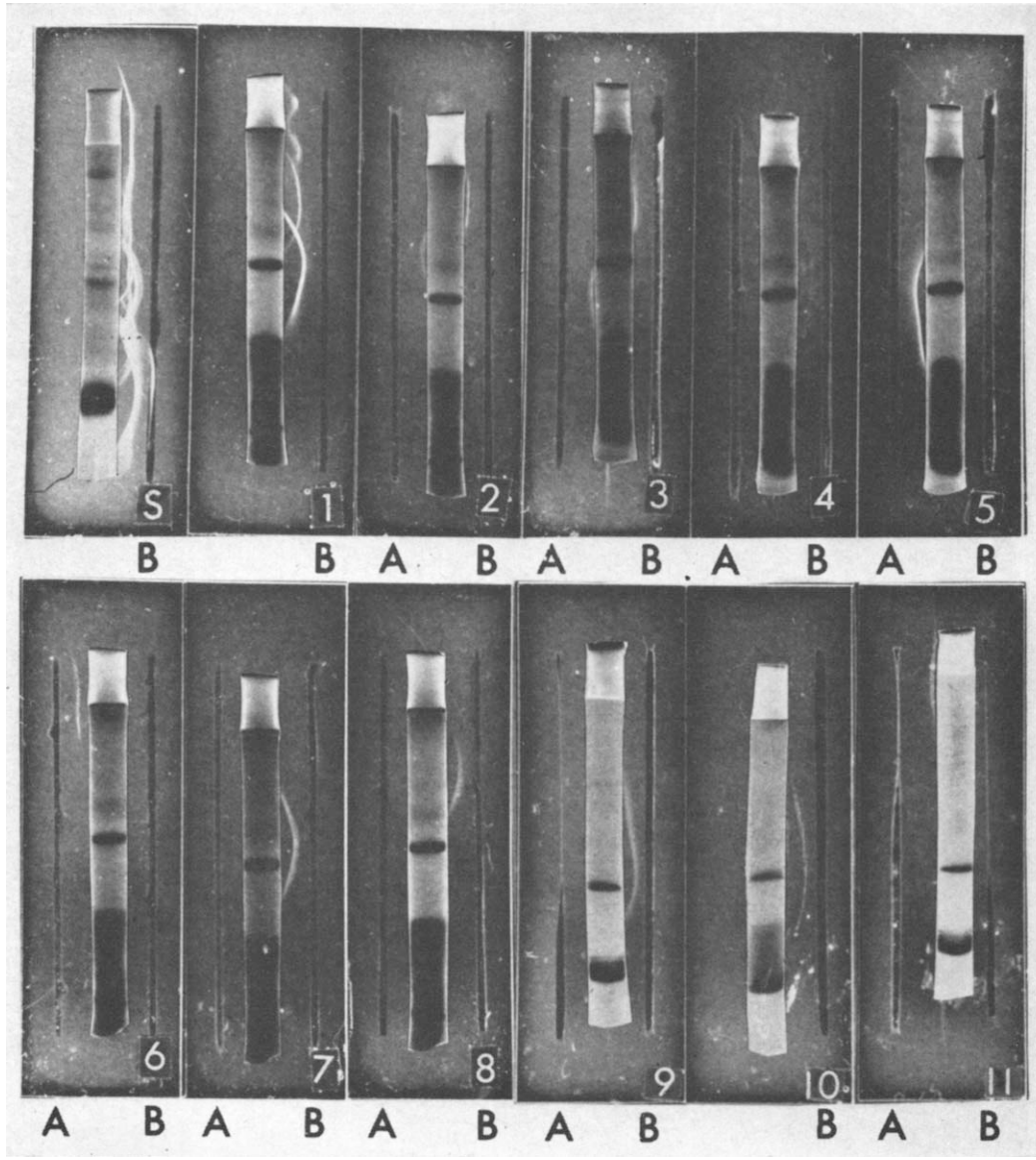

FIG. 2. Serum proteins identified and located by disc electrophoresis-immunodiffusion: (S), serum (pooled, normal) vs. antiserum to whole human serum; (1) bile vs. antiserum to whole human serum; (2–11), biles vs. univalent antisera to single human serum proteins as follows: (2A), transferrin; (2B), β_2-glycoprotein; (3A), prealbumin; (3B), haptoglobin; (4B), Gc-globulin; (5A), albumin; (6A), IgA-immunoglobulin; (7B), α_1-antitrypsin; (8B), hemopexin; (9B), α_1-acid-glycoprotein; (10B), β-lipoprotein and (11A), IgG-immunoglobulin.

most of the proteins present are antigenically the same as those found in serum. Those established heretofore with immune techniques (2, 3, 4, 8) were prealbumin, albumin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein, haptoglobin, α_2-macroglobin, α_2-lipoprotein, transferrin, fibrinogen, two components of complement, IgG, IgA, and IgM immunoglobulins, and ceruloplasmin. In addition to these, we have found 4 other serum proteins in the biles examined using the Ouchterlony immunodiffusion technique. Ta-

ble I shows seven of the proteins identified were common to all 10 bile samples, namely albumin, α_1-acid glycoprotein, α_1-antitrypsin, Gc-globulins (group-specific components), haptoglobin, IgA-immunoglobulin, and IgG-immunoglobulin. Two proteins, β_2-glycoprotein and IgM-immunoglobulin were common to 7 of the 10 biles, and 6 biles contained prealbumin and hemopexin. Transferrin was identified in 5 bile samples. β-Lipoprotein, a protein rarely reported in bile, was present in 4 of the 10 samples.

Analytical gel. A pooled serum representing some 200 normal sera has been examined repeatedly with remarkable reproducibility on 5% polyacrylamide gel, yielding 28–31 bands staining with amido-schwarz. Repeated electrophoresis on individual biles gave similar reproducibility but fewer bands. The number of fractions, excluding the front running pigment band, ranged from 8 to 20 with a mean of 12 (Fig. 1). The reproducibility from bile to bile was not uniform despite the fact that all samples were run to a common bromphenol blue front. The varying concentrations of pigments and other bile constituents probably affected the migration of individual biles. It was not possible under these conditions to establish a standard normal DEP pattern for normal human gallbladder bile by R_f measurements, but Fig. 1 illustrates a typical gel with its densitometric tracing. Relative positions of major fractions were consistent and recognizable from sample to sample.

DEP–immunodiffusion. The high resolution achieved with polyacrylamide gel electrophoresis and the difficulty in comparing individual bands in the bile pattern to individual bands in the control serum pattern made the identification of individual fractions other than albumin only tentative. By combining the sensitive technique of DEP with that of immunodiffusion, however, it was possible to locate and identify 12 serum proteins. Figure 2 shows the location of these in the disc-electropherograms. Other proteins present were not identifiable because of either low concentration or nonidentity with proteins against which the antisera had been produced. The latter possibility was taken as preliminary evidence for the presence in bile and location in the electrophoretic pattern of proteins, which are distinct from serum proteins and are derived from the biliary tract itself (9, 10). Thus, this technique offers the advantages of separating the individual proteins present, identifying them, and locating their position in the electrophoretic pattern.

Summary. Immunodiffusion of bile against specific antisera revealed 17 serum proteins in 10 normal gallbladder bile samples. Seven were common to all 10 samples. Five others were present in at least 50% of the biles. Analytical disc electrophoresis gave a mean of 12 bands staining with amido-schwarz with a range of 8–20. Because of the variability of bile patterns no uniform DEP pattern for normal gallbladder bile could be established, although relative relationships of major bands usually were recognizable. By use of DEP–immunodiffusion it was possible to identify and locate 12 specific serum proteins in the polyacrylamide electrophoretic patterns of bile.

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