A Lipoprotein Characterizing Obstructive Jaundice.

I. Method for Quantitative Separation and Identification of Lipoproteins in Jaundiced Subjects

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ABSTRACT Three immunochemically and electrophoretically distinct lipoproteins, LP-A, LP-B, and LP-X,[1] were isolated from the low density lipoprotein fraction (1.006–1.063 g/ml) in plasma from patients with biliary obstruction by a separation procedure which combines ultracentrifugation, heparin precipitation, and ethanol fractionation. This method, here described, permits the quantitative determination of individual plasma lipoprotein families on the basis of their protein moieties, rather than on the basis of their lipid moieties or density.

The chemical composition of the unique lipoprotein, LP-X, was similar to that of an abnormal lipoprotein, OLP, isolated by Russ et al. (29) and by Switzer (30). In obstructive jaundice plasma, the combined LP-X and LP-B accounted for 98% and the LP-A for only 2% of the total protein content of the LDL fraction.

This study indicates that the plasma lipoprotein pattern in obstructive jaundice is characterized by (a) a decreased concentration of HDL, (b) an increased concentration of LDL, and (c) the presence in the LDL fraction of varying amounts of a specific lipoprotein, LP-X, immunochemically and chemically distinct from LP-A and LP-B. LP-X, with its characteristically high content of unesterified cholesterol and phospholipids, is primarily responsible for the unusual protein and lipid content of the LDL fraction.

Screening tests in 61 patients with various forms of jaundice indicated that a characteristic immunoelectro-

[1] The hydrated densities and electrophoretic mobilities of serum lipoproteins provide the basis for the two most commonly used classification systems, each density and (or) electrophoretic class consisting of a heterogeneous, polydisperse system of particles. Chemical and immunochemical studies of lipoproteins isolated on the basis of density or of electrophoretic mobility have revealed heterogeneity in respect to protein moieties. To formulate a chemical rather than an operational classification system, it has been proposed (1) that the specific protein moieties (apolipoproteins) be utilized as criteria for the differentiation of lipoprotein families. This classification system defines three polydisperse lipoprotein families characterized by the presence of apolipoproteins A, B, and C, in addition to simpler albumin–fatty acid and albumin-lysolecithin complexes, and recognizes heterogeneity with respect to particle size while providing for protein homogeneity. Its usefulness in defining the lipoprotein pattern in obstructive jaundice will be readily recognized.

Abbreviations based on the operational classifications of lipoproteins. VLDL, very low density lipoproteins, lipoproteins of d < 1.006 g/ml (S$_f$ > 20); LDL, low density lipoproteins, lipoproteins of d = 1.006–1.063 g/ml (S$_f$ 0–20); HDL, high density lipoproteins, lipoproteins of d = 1.063–1.210 g/ml; α$_1$-LP, α-lipoproteins, lipoproteins with an electrophoretic mobility of α$_1$-globulins; β-LP, β-lipoproteins, lipoproteins with an electrophoretic mobility of β-globulins.

Abbreviations based on the chemical classification of lipoproteins. LP-A, lipoproteins characterized by the presence of apolipoprotein A; LP-B, lipoproteins characterized by the presence of apolipoprotein B; LP-X, lipoproteins occurring in obstructive jaundice and characterized by the presence of apolipoprotein X; Apo-A, apolipoprotein A, protein moiety of LP-A; Apo-B apolipoprotein B, protein moiety of LP-B; Apo-X, apolipoprotein X, protein moiety of LP-X. Each apolipoprotein is recognized by its specific immunochemical characteristics and by such chemical properties as amino acid composition and terminal amino acids.

phoretic precipitin arc between plasma samples and purified antibodies to LP-X was observed only in patients with obstructive jaundice. This simple immunochemical test may represent a valuable new tool in the differential diagnosis of obstructive and nonobstructive jaundice.

INTRODUCTION

It has been recognized for a long time that liver disorders are frequently accompanied by marked changes in plasma lipid concentrations. Flint (2) observed, more than a century ago, an increased blood cholesterol concentration in patients with obstructive jaundice. Subsequent studies correlating plasma lipids with various liver diseases (3–14) demonstrated dramatic changes in plasma lipids and lipoproteins in patients with intra- or extrahepatic biliary obstruction.

Plasma lipid changes in subjects with biliary obstruction are characterized by increased concentrations of unesterified cholesterol and phospholipid resulting in an increased free: total cholesterol ratio and a diminished cholesterol: phospholipid ratio.

Kunkel and Ahrens (15) and Kunkel and Slater (16) correlated increased plasma lipid and β-globulin concentrations and were first to suggest (8, 17) that the hyperlipoproteinemia and increased serum phospholipid concentrations in biliary obstruction were related. Several authors have proposed (17–20) that phospholipids increase the stability of lipoproteins and, therefore, their capacity for cholesterol binding. Pierce and Gofman (21), McGinley, Jones, and Gofman (22), and Gofman (23) first described the marked increase of the LDL fraction as a change characteristic of the ultracentrifugal lipoprotein pattern in patients with obstructive jaundice. Several investigators (13, 24–27) demonstrated that the increased concentration of LDL is accompanied by a decreased concentration of HDL. Eder, Russ, Pritchett, Wilber, and Barr (13) found an increased concentration of serum lipoproteins in Cohn fractions IV–VI (28). Russ, Raymunt, and Barr (29) established the presence of an "abnormal" lipoprotein in Cohn fraction VI. Recently, Switzer (30) described the presence of an abnormal low density lipoprotein ("obstructive" lipoprotein) which failed to react with antibodies to LDL. Antibodies produced with this "obstructive" lipoprotein, however, did react occasionally with normal serum. The amino acid composition and the immunochemical characteristics of this lipoprotein differed slightly from those of apolipoproteins A and B. Burstein and Caroli (31), utilizing serum from patients with obstructive jaundice, found that a lipoprotein ob-

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[a] For nomenclature and abbreviations used in the text, see footnote 1.

tained by methods used for precipitating LDL[2] showed no reaction with antibodies to LDL. Although several investigators (32–34) demonstrated immunochemically the presence of LP-A in the LDL fraction, no quantitation of LP-A in the LDL fraction has been reported. Fredrickson, Levy, and Lees (35) suggested that the increased concentration of LDL in patients with biliary obstruction is caused by a shift of LP-A from the HDL into the LDL fraction. However, this suggestion has not yet been supported by experimental evidence.

This paper is the initial report of our studies on the isolation and characterization of plasma lipoproteins in patients with jaundice. It sets forth a method for the separation of various plasma lipoproteins, including an abnormal lipoprotein in the LDL fraction, and describes the distribution, qualitative and quantitative, of lipoprotein families within the density range 1.006–1.063 g/ml. As a result of these studies an immunoelectrophoretic method for the differential diagnosis of obstructive and nonobstructive jaundice has been proposed.

METHODS

Blood samples were obtained from patients with hemolytic jaundice, lupoid hepatitis, infectious hepatitis, cholangiolitic hepatitis, Laennec's cirrhosis, biliary cirrhosis, and various types of intra- and extrahepatic biliary obstruction. The diagnoses were established by the standard laboratory tests, liver biopsy, surgical procedures, and, in some cases, by autopsy. Blood was usually drawn 10 hr after a meal, but never less than 4–5 hr after. It was collected for preparative isolation of lipoproteins into plastic bags containing 2.2% sodium citrate (Fenwall Laboratories, Inc., Morton Grove, Ill.), and the plasma was recovered by low speed centrifugation. Blood samples used only for the immunological tests were drawn into standard test tubes, allowed to clot for 2 hr at 37°C, and the serum separated by centrifugation.

Isolation and fractionation of lipoproteins. The isolation scheme is outlined in Fig. 1. The VLDL lipoproteins were obtained by layering plasma samples under equal volumes of d = 1.0055 g/ml buffer solution (1.42 g of anhydrous disodium phosphate, 7.27 g of NaCl, and 0.1 g of disodium ethylenediaminetetraacetate (EDTA) in 1 liter of solution adjusted to pH 7.0 with 1 N HCl) and centrifuging in the Type Ti 50 rotor of the Spinco model L-2 ultracentrifuge for 22 hr at 105,000 g and 4°C.

The top fraction (d < 1.006 g/ml) containing the VLDL was removed by a tube-slicing technique, resuspended in 3 volumes of buffer solution, and centrifuged under identical conditions. This washing procedure was repeated three times to eliminate traces of albumin.

The bottom fraction (d > 1.006 g/ml) was further fractionated by heparin precipitation, according to a slight modification of the procedure of Burstein and Samaille (36, 37). 4 ml of 5% heparin solution and 5 ml of 1 M manganese chloride solution were added to 100 ml of the fraction d > 1.006 g/ml and the mixture allowed to stand for 30 min at room temperature. The precipitated lipoproteins were removed by low speed centrifugation and the filtrate set aside for ultracentrifugal isolation of HDL. The precipitated lipoproteins were then dissolved in a mixture of 1 ml of 10% sodium citrate and 0.5 ml of 20% NaCl, diluted with

0.005 M Tris buffer to a volume of 100 ml, and precipitated by adding 2.5 ml of 2 M magnesium chloride solution. This procedure was repeated two times, and the final precipitate was dissolved as described above. The solution was dialyzed first for 36 hr against 9% NaCl and then for 24 hr against 5% barium chloride at 4°C. The heparin-barium precipitate was removed by low speed centrifugation, and the supernatant portion was dialyzed for 48 hr against 0.9% NaCl.

For further fractionation with ethanol (13, 28, 29), 5 volumes of cold (−5°C) ethanol acetate buffer (19% ethanol, pH 8.5, ionic strength 0.04) was added in small portions to 1 volume of cold (1°C) dialyzed supernatant portion. The mixture was stirred for 30 min at −5°C and centrifuged at low speed. The precipitate corresponding to Cohn fractions I–III was dissolved in 2 M NaCl and dialyzed, first for 48 hr against 9% NaCl, and then for additional 48 hr against 0.9% NaCl. The remaining solution, containing Cohn fractions IV–VI, was concentrated *in vacuo* to ½ of its original volume and dialyzed for 48 hr against 0.9% NaCl. Both fractions (Cohn I–III and IV–VI) were centrifuged at solution density of 1.063 g/ml for 44 hr at 105,000 g and 4°C. The upper layers of both fractions, removed by tube slicing, contained lipoproteins within the density range 1.006–1.063 g/ml. In some instances the fractions IV–VI were further separated into Cohn fraction IV + V and VI (28).

The filtrate remaining after the first heparin precipitation was dialyzed as previously described, adjusted to 1.063 g/ml density, and centrifuged for 22 hr at 105,000 g. The top layer was discarded, and the infranatant portion was centrifuged at a solution density of 1.210 g/ml for 44 hr at 105,000 g. The top layer contained HDL (1.063–1.210 g/ml).

In certain cases plasma samples were fractionated into VLDL, LDL, and HDL by the standard sequential preparative ultracentrifugation (25, 38).

All fractions were characterized by protein and lipid analyses and by determination of electrophoretic and immunochemical properties.

Immunological methods. The immunochemical properties of lipoproteins were studied by double diffusion (39) and immunoelectrophoresis (40) in 1% agar or 1% agarose gels employing barbital buffer, pH 8.6, ionic strength 0.05. Plates were developed and stained as previously described (41, 42). Rabbit anti-human sera α_1-LP, β-LP, albumin, whole human serum, and 7S γ-globulin (Behringwerke AG, Marburg an der Lahn, Germany) were used. The antibodies to β-LP present in the rabbit antiserum gave a single precipitin line, and the antibodies to α_1-LP gave, in most instances, two precipitin lines with whole serum from normal subjects.

The antibodies to α-LP and β-LP showed no reaction with human serum albumin.

Rabbit antisera to LP-X were prepared in this laboratory. The LP-X fractions (protein concentration 10 mg/ml), showing no reaction with antibodies to α-LP, β-LP, and (or) albumin, were mixed with an equal volume of Freund's adjuvant and administered intraperitoneally in a single injection. The rabbits were bled by heart puncture after 2 wk, and the antisera examined by immunodiffusion and immunoelectrophoresis formed precipitin lines with LP-X and albumin. Some antisera, drawn 3 months after immunization, showed a precipitin line with LP-B and an additional line in the γ-globulin region with whole serum. The antisera were absorbed with equivalent amounts of appropriate antigens determined by a serial micro dilution method. The mixture was incubated for 3 hr at 37°C, allowed to stand for 12 hr at 4°C, and the precipitated antigen-antibody complex was sedimented by low speed centrifugation for 30 min. The supernatant fraction contained purified antiserum which reacted only with LP-X.

Electrophoresis. Paper electrophoresis was performed according to the method of Lees and Hatch (43). Electrophoresis of lipoprotein fractions was carried out also in 1% agar and 1% agarose gels by the method of Grabar and Williams (40) employing barbital buffer, pH 8.6, ionic strength 0.05. The plates were fixed for 45 min in a solution of acetic acid-ethanol-water (5:70:25, v/v), washed for 8 hr in distilled water, and dried at room temperature. They were stained for protein and lipid with Amidoblack 10B and Oil Red O, respectively.

Lipid and protein analyses. Esterified and unesterified cholesterol was determined according to the method of Sperry and Webb (44). Values for cholesterol esters were calculated as cholesteryl linoleate (mol wt 649.1). Triglycerides were determined by the method of Van Handel and Zilversmit (45) and lipid phosphorus according to the method of Gerlach and Deuticke (46). The factor 25 was used to convert lipid phosphorus to phospholipid. Protein was determined by the method of Lowry, Rosenbrough, Farr, and Randall (47).

RESULTS

Plasma lipids of patients with jaundice. Results of the plasma lipid analyses in patients with jaundice of different etiologies are presented in Table I. The most marked changes in the lipid concentrations occurred in

TABLE I

The Plasma Lipid Composition in Patients with Jaundice

Clinical diagnosis	Total cholesterol	Unesterified cholesterol / Total cholesterol	Phospholipid	Total cholesterol / Phospholipid	Triglyceride	Number of patients
	mg/100 ml		*mg/100 ml*		*mg/100 ml*	
Hemolytic jaundice	132	0.30	186	0.71	119	1
Infectious hepatitis	118 ±12	0.52 ±0.006	237 ±71	0.50 ±0.04	164 ±15	11
Laennec's cirrhosis	105 ±11	0.40 ±0.05	146 ±55	0.72 ±0.08	96 ±10	9
Biliary cirrhosis	247 ±15	0.64 ±0.07	723 ±60	0.34 ±0.05	136 ±4	5
Extrahepatic biliary obstruction	315 ±31	0.75 ±0.03	839 ±92	0.38 ±0.03	167 ±20	12
Normal range*	180–230	0.25–0.35	150–280	0.70–1.10	50–150	

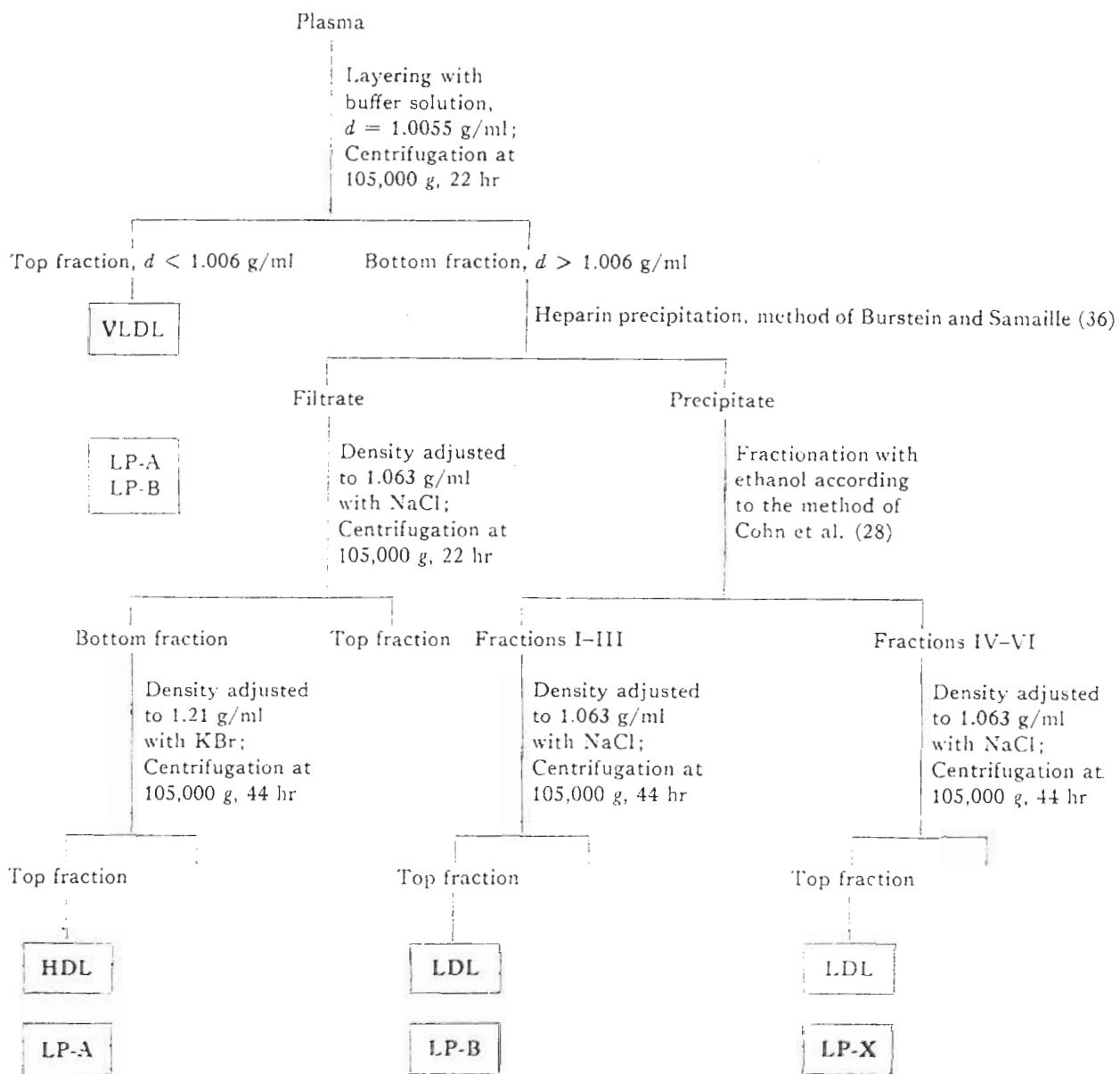* Values for normal range are taken from a study by Furman, Howard, Lakshmi, and Norcia (48).

Plasma

Layering with
buffer solution,
$d = 1.0055$ g/ml;
Centrifugation at
105,000 g, 22 hr

Top fraction, $d < 1.006$ g/ml   Bottom fraction, $d > 1.006$ g/ml

VLDL

Heparin precipitation, method of Burstein and Samaille (36)

Filtrate   Precipitate

LP-A
LP-B

Density adjusted
to 1.063 g/ml
with NaCl;
Centrifugation at
105,000 g, 22 hr

Fractionation with
ethanol according
to the method of
Cohn et al. (28)

Bottom fraction   Top fraction   Fractions I–III   Fractions IV–VI

Density adjusted
to 1.21 g/ml
with KBr;
Centrifugation at
105,000 g, 44 hr

Density adjusted
to 1.063 g/ml
with NaCl;
Centrifugation at
105,000 g, 44 hr

Density adjusted
to 1.063 g/ml
with NaCl;
Centrifugation at
105,000 g, 44 hr

Top fraction   Top fraction   Top fraction

HDL   LDL   LDL

LP-A   LP-B   LP-X

FIGURE 1 Procedure for the isolation of plasma lipoprotein fractions from patients with obstructive jaundice. See footnote 1 in Abstract for explanation of abbreviations in this and all subsequent figures.

the plasma of patients with obstructive jaundice. As expected, the increased cholesterol concentration was confined to unesterified cholesterol, resulting in an increased ratio of unesterified: total cholesterol. Since the elevation of phospholipid concentration in these patients was relatively greater than that of cholesterol, the cholesterol: phospholipid ratio was low. In contrast, the cholesterol and phospholipid concentrations were low or normal in patients with nonobstructive jaundice. Triglyceride concentrations were normal or only slightly increased in all groups of patients. These results are in excellent agreement with those reported in the literature (13, 14, 26).

The qualitative and quantitative changes in the lipoprotein patterns of patients with obstructive jaundice were studied utilizing the fractionation procedure (Fig. 1) developed in this laboratory which permits the isolation of immunochemically and electrophoretically homogeneous lipoprotein fractions.

Qualitative composition of lipoprotein fractions. The immunochemical characterization of lipoprotein fractions
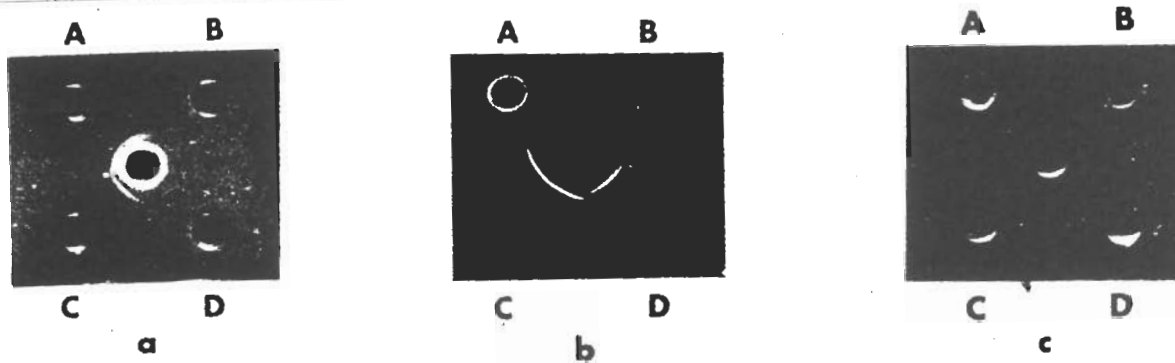
FIGURE 2 Immunodiffusion patterns of lipoprotein fractions. Central wells contain (a) VLDL, (b) LDL, and (c) HDL. Outer wells contain antibodies to LP-A (A), albumin (B), LP-B (C), and LP-X (D).

obtained from patients with obstructive jaundice by standard sequential preparative ultracentrifugation showed that VLDL consisted of LP-A and LP-B (Fig. 2, pattern a). The HDL fraction contained only LP-A (Fig. 2, pattern c). The LDL showed not only the presence of LP-A and LP-B but also a lipoprotein which was immunochemically different from LP-A and LP-B (Fig. 2, pattern b). This lipoprotein was designated LP-X.

The lipoproteins obtained by heparin precipitation of a VLDL-free (d > 1.006 g/ml) plasma sample (Fig. 1) contained only the LP-B and LP-X (Fig. 3, pattern b). The filtrate consisted of LP-A. Thus, in contrast to the LDL fraction obtained by ultracentrifugation (Fig. 3, pattern a), the heparin precipitation yielded an LDL fraction which was free of LP-A.

The two different low density lipoproteins, LP-B and

LP-X, were separated by cold ethanol fractionation (Fig. 1). The immunodiffusion pattern (Fig. 4, pattern a) shows that the heparin-precipitated LDL fraction used as a starting material for ethanol fractionation consisted of LP-B and LP-X (two precipitin lines with antibodies to LP-B and LP-X, one of which is identical with that of purified LP-B). Cohn fractions I–III showed the presence of only LP-B (Fig. 4, pattern b), and Cohn fractions IV–VI, the presence only of LP-X (Fig. 4, pattern c). It was established by further fractionation that LP-X occurred in Cohn fraction VI. This purified LP-X fraction was utilized for the immunization of rabbits.

Immunochemical and electrophoretic characterization of LP-X. Plasma from a patient with obstructive jaundice (WSOJ) gave single precipitation lines with antibodies to LP-B, LP-X, and albumin, and two precipitin
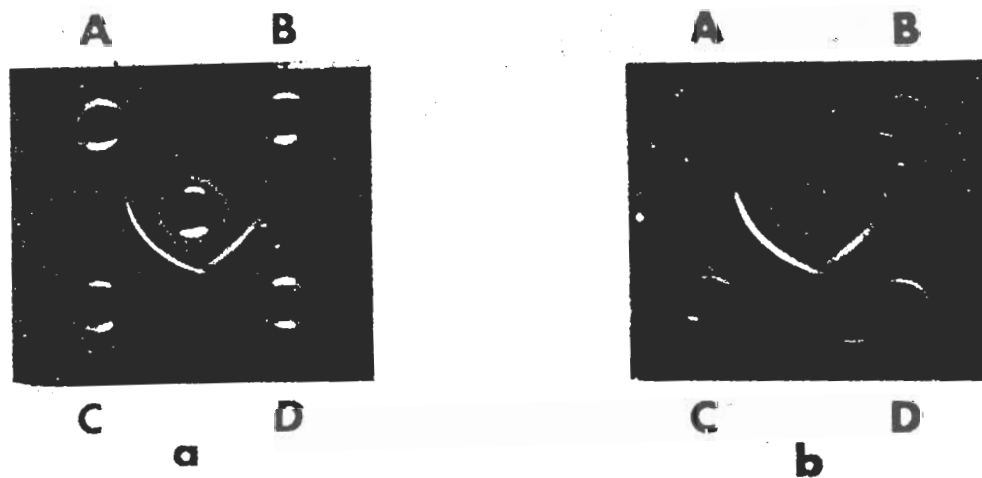

FIGURE 3 Immunodiffusion patterns of LDL. Central wells contain (a) LDL isolated by ultracentrifugation, and (b) lipoproteins isolated by heparin precipitation. Outer wells contain antibodies to LP-A (A), albumin (B), LP-B (C), and LP-X (D).
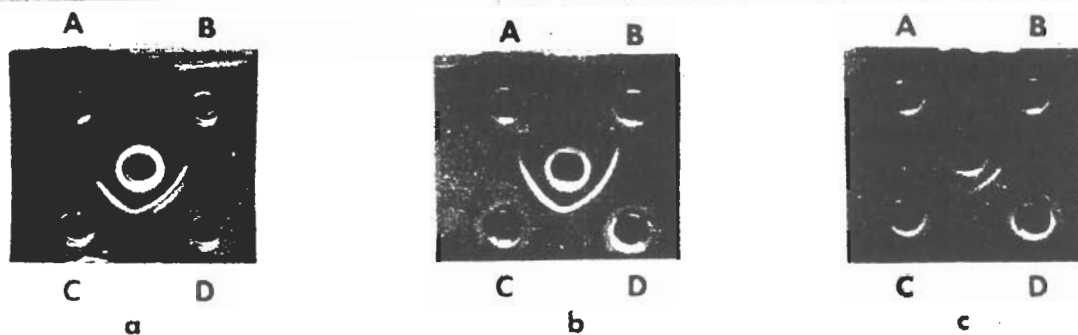
FIGURE 4 Immunodiffusion patterns of lipoprotein fractions. Central wells contain (a) lipoproteins isolated by heparin precipitation, (b) Cohn fractions I–III obtained from heparin-precipitated lipoproteins, and (c) Cohn fractions IV–VI obtained from heparin-precipitated lipoprotein. Outer wells contain antibodies to LP-A (A), albumin (B), LP-B (C), and LP-B + LP-X (D).

lines with antibodies to LP-A (Fig. 5). The anti-LP-X serum showed no reaction with LDL, HDL, or albumin. Fig. 6 shows that LP-X reacted only with antibodies to LP-X (pattern a), and that anti-LP-X serum reacted only with LP-X (pattern b). These latter experiments were performed with various concentrations of antigens and antibodies. Immunoelectrophoretic patterns showed that LP-X gave no reaction with antibodies to normal whole serum, albumin, LP-A, or LP-B. The immunodiffusion pattern in Fig. 6 a shows clearly that LP-X is not identical with γ-globulins.

Fig. 7 shows the difference between the electrophoretic mobility of LP-X in agar and in agarose gel. Under

identical experimental conditions. LP-X moved on agar gel toward the cathode, and on agarose gel toward the anode. Fig. 8 shows the difference between the electrophoretic patterns on agar and agarose gels of serum from a normal subject and from a patient with obstructive jaundice. The LP-X band was sharply separated from the LP-B band only by agar gel electrophoresis. The LP-X moved as a narrow band toward the cathode and was stained with Oil Red O. In contrast, on agarose gel, LP-B and LP-X migrated with the same mobility into the β-globulin region. The presence of weakly stained bands in the α₁-globulin region corresponds well with the low concentration of HDL characterizing patients
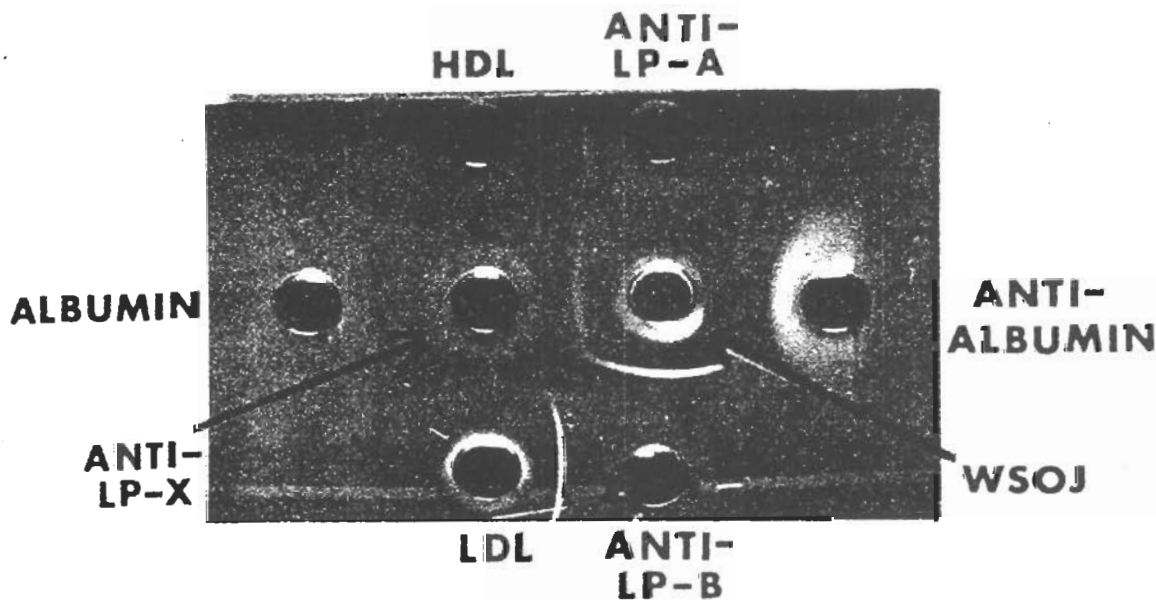

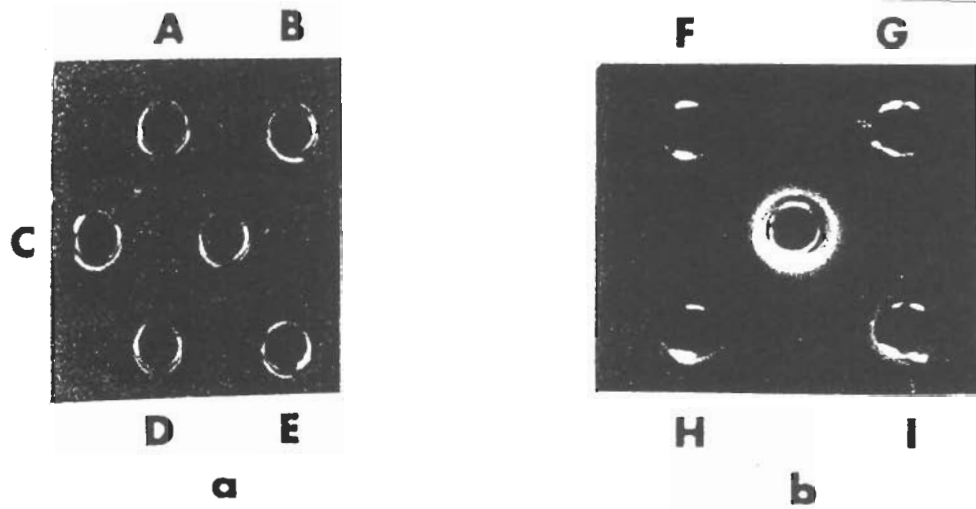FIGURE 5 Immunodiffusion pattern of whole serum of a patient with extrahepatic obstruction (WSOJ).

FIGURE 6 Immunodiffusion patterns of LP-X. Central wells contain (a) LP-X, and (b) rabbit anti-LP-X serum. Outer wells in pattern a contain antibodies to LP-A (A), albumin (B), γ-globulin (C), LP-B (D), and LP-X (E). Outer wells in pattern b contain LP-A (F), albumin (G), LP-B (H), and LP-X (I).

with obstructive jaundice. On paper electrophoresis LP-X migrated toward the anode with a mobility slightly less than that of LP-B (Fig. 9).

The affinity of all immunoprecipitin lines and electrophoretic bands of LP-X for lipid and protein stains indicates clearly its lipoprotein nature. Studies characterizing the chemical nature of the protein present in LP-X are in progress.

Distribution and per cent composition of lipoprotein fractions in patients with obstructive jaundice. Table II shows the per cent distribution of protein and lipid components in three major lipoprotein fractions obtained by preparative ultracentrifugation or heparin precipitation. Most of the total cholesterol, phospholipid, and protein, and a high percentage of the triglyceride are concentrated in the LDL fraction. That portion of LDL corresponding to LP-X varied widely in different patients. It is likely that the concentration of LP-X de-
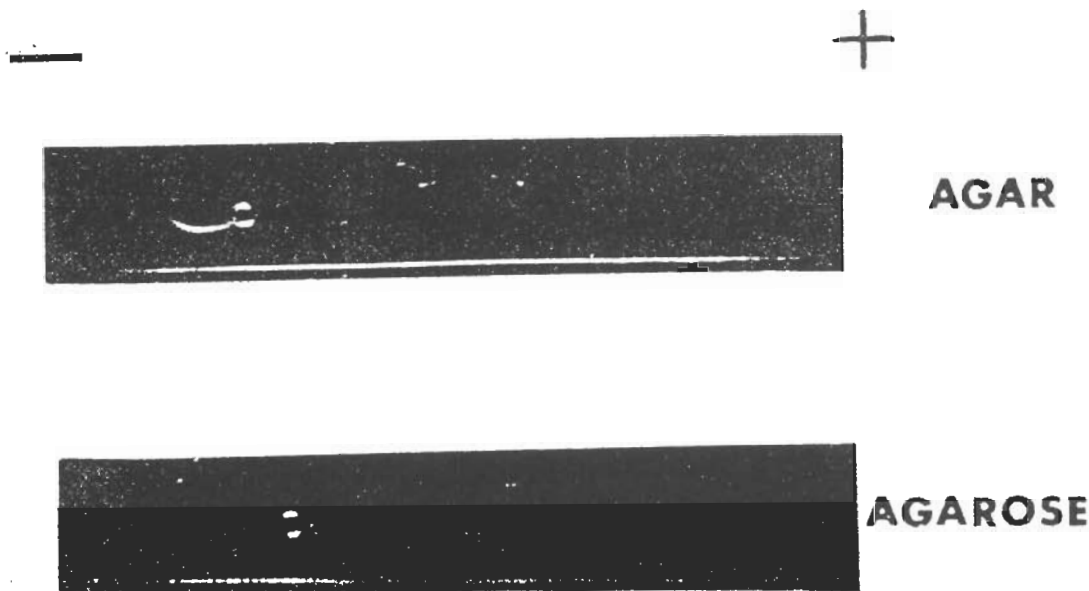

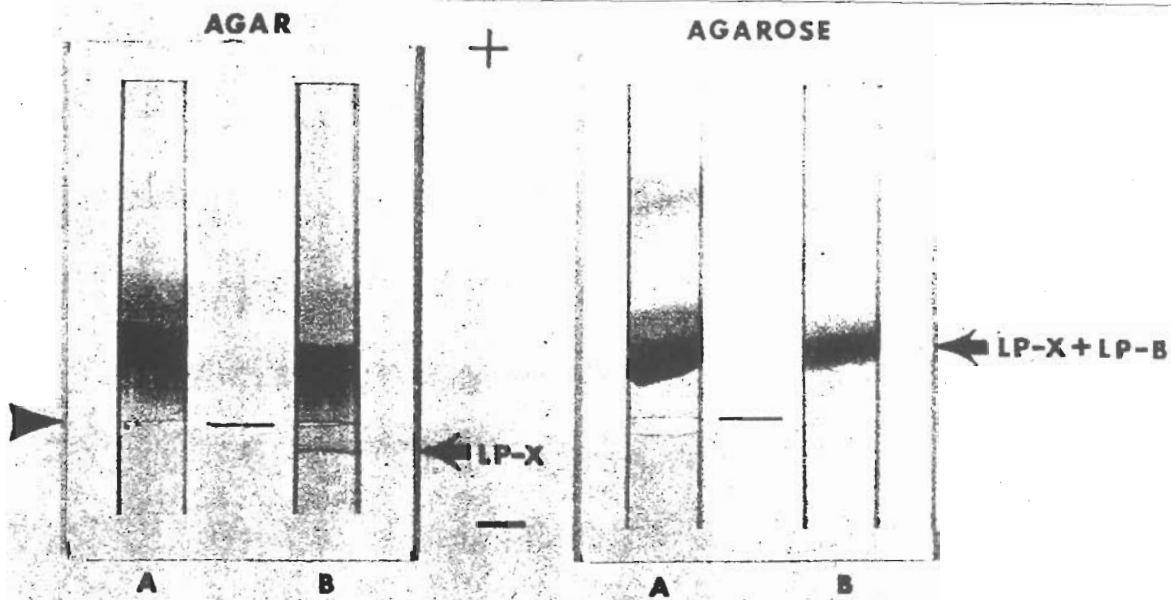FIGURE 7 Immunoelectrophoretic patterns of LP-X in 1% agar and 1% agarose.

FIGURE 8 Electrophoretic patterns of whole serum in 1% agar and 1% agarose. Normal serum (A) and serum of a patient with obstructive jaundice (B). Patterns were stained with Oil Red O.

pends on the degree and (or) duration of biliary obstruction.

The per cent composition of lipoprotein classes is presented in Table III. The values for the VLDL and HDL are nearly normal (27, 38, 48, 49) with the possible exception of an increased phospholipid and a decreased cholesterol ester content of HDL. The LDL fraction showed increased unesterified cholesterol: cholesterol ester and phospholipid: protein ratios. The analysis of the two low density lipoproteins, LP-B and LP-X, revealed that the protein-lipid composition of the whole LDL fraction, and thus of whole serum, is attributable primarily to the presence of LP-X. The composition of LP-B is nearly normal, with the possible exception of a slightly decreased amount of cholesterol ester and increased content of unesterified cholesterol (38, 49, 50). An unusually high content of phospholipid (66.5%) and a low content of protein (5.8%) represent the unique characteristics of the chemical composition of LP-X. The phospholipid: protein ratio of LP-X is almost 12 times that of LP-B. 93% of the cholesterol present in LP-X was unesterified. It has been demonstrated on the basis of these studies that the per cent composition of LP-X differs significantly from that of either LP-A or LP-B. The composition of LP-A and LP-B as isolated by these methods from jaundiced plasma differs somewhat from the composition of those obtained from normal plasma.

The presence of LP-A could be demonstrated only in the LDL fraction obtained by preparative ultracentri-

fugation. To determine the per cent content of LP-A, the LDL fractions were isolated by ultracentrifugation, and the LP-B and LP-X were precipitated by heparin, in two studies involving two subjects. The immunochemically identified LP-A was then determined quantitatively in the filtrate. The LP-A content was only 2% of LDL fraction, based on protein determination. Varying proportions of LP-B and LP-X accounted for the major part (98%) of the LDL fraction in patients with obstructive jaundice.

Presence of LP-X in plasma of patients with various forms of jaundice. The LP-X could be detected by immunochemical techniques in patients with obstructive jaundice, but not in plasma of normal, fasting subjects (Fig. 10). A survey of 61 patients with various forms of jaundice (Table IV) indicated the occurrence of LP-X in 38 patients in whom obstructive jaundice of varying degree had been demonstrated by various other tests. In three patients with Laennec's cirrhosis in whom LP-X was demonstrated, the presence of an obstructive component was confirmed later by liver biopsy. In patients in whom extrahepatic biliary obstruction was corrected by surgery, LP-X could not be detected 10-14 days postoperatively. In those patients in whom LP-X could not be demonstrated, evidence of an obstructive component to the jaundice could not be demonstrated by other tests. It is not known, as yet, what degree or duration of obstruction is requisite to the production of LP-X in detectable amounts.
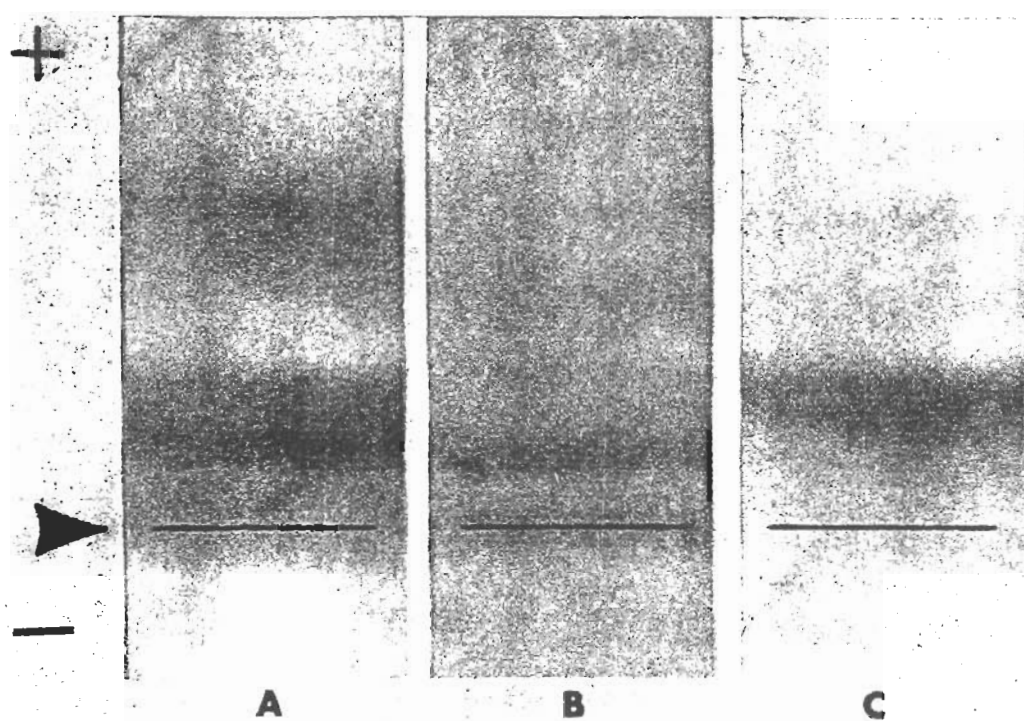
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FIGURE 9 Paper electrophoretic patterns of whole serum (A), LP-X (B), and LP-B (C) from a patient with obstructive jaundice.

DISCUSSION

Isolation of three immunochemically and electrophoretically distinct lipoproteins, LP-A, LP-B, and LP-X, from the low density lipoprotein fraction (1.006–1.063 g/ml) in patients with biliary obstruction may now be achieved by this separation procedure which combines ultracentrifugation, heparin precipitation, and ethanol fractionation. The unique lipoprotein, designated LP-X, isolated from the low density lipoprotein fraction of

plasma from patients with obstructive jaundice, has a protein and lipid composition similar to that of an abnormal lipoprotein (OLP) isolated from the same density fraction by Switzer (30) and a lipoprotein found in Cohn fraction VI by Russ et al. (29). Our studies demonstrate that the increased concentration of LDL in obstructive jaundice is due primarily, if not exclusively, to the content of LP-X in Cohn fraction VI.

Reports (32–35) that LP-A is present in the LDL

TABLE II

Per Cent Distribution of Serum Lipids and Lipoprotein Protein in Major Lipoprotein Fractions from Four Patients with Extrahepatic Obstructive Jaundice

Density classes	Total cholesterol	Phospholipid	Triglyceride	Protein
	%	%	%	%
VLDL < 1.006	2–6	2–8	20–40	3–10
LDL* 1.006–1.063	90–95 (30–50)‡	81–95 (50–80)‡	54–78 (5–15)‡	75–92 (20–60)‡
HDL 1.063–1.21	1–7	3–14	3–9	3–15

See footnote 1 in Abstract for explanation of abbreviations in this and all subsequent Tables.
* This fraction was obtained by heparin precipitation and, therefore, contains only LP-B and LP-X.
‡ Per cent attributable to LP-X.

Density classes		Cholesterol ester	Unesterified cholesterol	Phospholipid	Triglyceride	Protein	Phospholipid protein
		%	%	%	%	%	
VLDL <1.006		12.8 (16.2)	7.4 (6.0)	15.2 (17.9)	55.5 (51.8)	9.5 (7.1)	1.60 (2.5)
	LP-B	27.3	13.9	26.5	5.9	26.4	0.94
LDL‡ 1.006–1.063		15.9 (39.4)	19.4 (7.5)	38.8 (23.1)	6.5 (9.3)	19.4 (20.7)	2.0 (1.1)
	LP-X	2.4	22.4	66.5	2.9	5.8	11.5
HDL 1.063–1.21		11.0 (18.5)	4.5 (2.3)	38.0 (26.9)	2.3 (4.6)	43.7 (47.7)	0.88 (0.56)

* The values in parentheses represent per cent composition of lipoprotein fractions in normal subjects (38).
‡ This fraction was obtained by heparin precipitation, and contains, therefore, only LP-B and LP-X; the values represent mean values of four preparations.

fraction in obstructive jaundice have been confirmed by utilizing the herein described separation procedures, although we found no immunochemical evidence of LP-A in the heparin-precipitable portion of LDL. This portion accounted for 98% of the total LDL protein. The assumption by Fredrickson, et al. (35) that an increased concentration of LDL is caused by a shift of LP-A of higher hydrated densities into a region of lower hydrated densities (1.006–1.063 g/ml), therefore, is not supported, other than qualitatively, by our experimental evidence.

Our quantitative studies indicate that the lipoprotein pattern in obstructive jaundice is characterized by (a) a decreased concentration of HDL, (b) an increased concentration of LDL, and (c) the presence in the LDL fraction of varying amounts of a specific lipoprotein, LP-X, immunochemically and chemically distinct from LP-A and LP-B. Since the latter lipoproteins are of an almost normal chemical composition, the third lipoprotein, LP-X, with its characteristically high content of unesterified cholesterol and phospholipids, is primarily
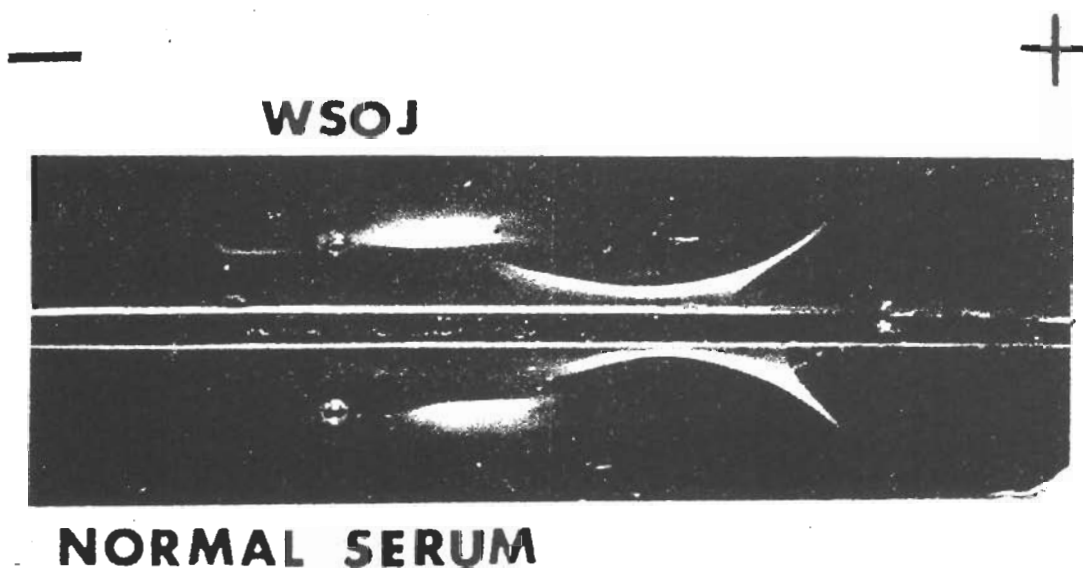

FIGURE 10 Immunoelectrophoretic patterns of whole serum on agar. The upper pattern contains whole serum of a patient with obstructive jaundice (WSOJ) and the lower pattern whole serum of a normal subject. The central trough contains a mixture of antibodies to albumin, LP-A, LP-B, and LP-X.

responsible for the unusual protein and lipid composition of LDL and for the well documented changes in the concentration of plasma lipids in obstructive jaundice.

Switzer reported (30) that the abnormal lipoprotein (OLP), prepared by a procedure utilizing immunoprecipitation, contained γ-globulin. The isolation of a purified LP-X without the use of an immunoprecipitation technique thus represents an additional advantage of the new frachtionation procedure.

Screening tests in 61 patients with various forms of jaundice showed that an immunoprecipitin reaction between plasma samples and purified antibodies to LP-X appeared to be limited to those with obstructive jaundice demonstrable by other techniques. Absorbed anti-LP-X serum did not react with whole plasma from fasting normal subjects or with plasma from patients with nonobstructive jaundice. In this respect our findings differ somewhat from those of Switzer (30) who detected, occasionally, precipitin lines between anti-OLP and normal whole serum. Although it is essential to continue with an extensive testing program, supplemented with appropriate clinical observations and liver biopsies, this simple immunochemical method utilizing purified anti-LP-X serum may represent a valuable new tool in the differential diagnosis of obstructive and nonobstructive jaundice. Though it is not yet possible to correlate the concentration of LP-X with the degree or duration of biliary obstruction or the extent of the derangement of the serum lipids, indications are that such relationships exist.

It is relevant at this point to inquire regarding the origin and nature of LP-X. Is LP-X a normally occurring lipoprotein? If so, why is it not detectable in normal serum? Why does it accumulate in obstructive jaundice? Is it synthesized in liver or elsewhere? What is the nature of its protein moiety? It has been shown (51, 52) that the intestine synthesizes, in addition to LP-A

and LP-B, some as yet unidentified lipoproteins which may play an important role in the secretion and transport of exogenous fat. Alaupovic, Furman, Walraven, Falor, and Newman (53) have found that the presence of apolipoprotein C coincided with the peak triglyceride concentration in chyle and was still noted in subsequent chyle samples over a period of several additional hours. It could not be detected in chyle or serum samples from fasting subjects or in postprandial samples when the meal consisted of medium chain triglycerides or sucrose. Thus, LP-X may be an intestinal lipoprotein similar to lipoproteins containing apolipoprotein C, catabolized rapidly in the liver and, therefore, largely undetectable in normal, fasting plasma but accumulating in the plasma when, as a result of liver derangement, the rate of degradation is decreased. Although very little is known about lipoprotein catabolism in general, and the effect of bile salts on this process in particular, it has been reported (54, 55) that bile acids inhibit lipoprotein lipase, one of the enzymes participating in the degradation of lipoproteins. We would like to suggest, therefore, as a working hypothesis, that LP-X originates in the intestinal mucosa where it participates in the process of fat absorption and transport. In obstructive jaundice, the increased concentrations of bile acids inhibit certain catabolic reactions of LP-X which, in its partially delipidized form, or as a newly formed lipoprotein entity, accumulates in the plasma LDL fraction. Whether the protein moiety of LP-X is similar to or identical with apolipoprotein C remains to be answered.

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