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**Research Article**

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# Liquid Crystalline Lipid in the Plasma of Humans with Biliary Obstruction

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**ABSTRACT** Plasma lipoprotein characteristics were evaluated in a group of patients with obstructed biliary tracts. A 1:1 molar lecithin-free cholesterol liquid crystal phase was observed in the low density flotation region of these patients. The smectic nature of this mesophase was confirmed by electron microscopy, polarized microscopy, and low angle X-ray scattering. A small amount of protein was associated with these liquid crystals, some of which appeared to be components of normal very low and high density lipoproteins. The composition and physical properties of the very low and high density lipoproteins from these obstructed patients differed from normal as well. Aberrant apoprotein patterns were observed for both obstructed very low and high density lipoproteins. A  $\beta$ -electrophoretic migration was observed for a component of these two lipoprotein groups.

## INTRODUCTION

Patients with obstructive biliary disease often have substantially elevated plasma lipid levels (1). These large concentrations of plasma phospholipid and free sterol are ultracentrifugally isolated in the low density ( $S_r$  0–20) flotation fraction, but differ considerably from conventional low density lipoprotein (LDL)<sup>1</sup> (2, 3). The major lipid constituents of the low density fraction from these patients are phospholipid and free sterol

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<sup>1</sup>Abbreviations used in this paper: HDL, high density lipoprotein; LDL, low density lipoprotein; LPX, lipoprotein X; OLP, obstructed lipoprotein; SDS, sodium dodecyl sulfate; VLDL, very low density lipoprotein.

in contrast to sterol ester which predominates in normal LDL (4). Many of the peptides isolated in this low density fraction are not associated with normal LDL (5). It has also been reported (6) that the structure of this unusual low density lipid-protein complex has a bilayered coin-like configuration quite different from spherical LDL.

This report describes a series of patients with biliary obstruction who have liquid crystalline lipid in their plasma. The smectic nature of these liquid crystals was documented by polarized microscopy, low angle X-ray scattering, and electron microscopy. The liquid crystals were isolated from the conventional lipoproteins and the lipid and peptide composition and physical properties of this mesomorphous phase investigated. The very low density ( $S_r$  20–400, VLDL) and high density lipoproteins (HDL) from these patients also had compositions and physical properties differing from normal and these differences are described.

## METHODS

*Clinical.* Six patients with biliary obstruction were studied during inpatient visits and as outpatients at the Duke University Medical Center and Durham Veterans Administration Hospitals. Each patient had a liver biopsy and all had abdominal surgery to establish the cause of their obstructive biliary tract disease. Blood was obtained from these patients in the fasting state and was collected either in disodium ethylenediaminetetraacetic acid (EDTA), 1 mg/ml of whole blood, or without anticoagulants. Red cells were usually removed by centrifugating at 2000 rpm for 20 min in a Lourdes (Lourdes Instrument Co., Brooklyn, N. Y.) refrigerated centrifuge at 8°C. For some polarized microscopic, electron microscopic, and X-ray scattering studies the blood was maintained at 37°C and the plasma or serum rapidly isolated by centrifugation at that temperature.

*Lipoprotein isolation.* The plasma or serum was immediately subjected to preparative ultracentrifugation to isolate various lipoprotein fractions. To obtain an initial density of

1.006, these samples were diluted with 0.1 M, pH 7.4 Tris buffer or saline before centrifugation. After adjusting to this density, the VLDL fraction was isolated at the top of the tube after a  $1 \times 10^8$  *g*-min centrifugation. Another  $1 \times 10^8$  *g*-min ultracentrifugation was performed to insure the elimination of VLDL from the  $d > 1.006$  lipoproteins. After VLDL removal, the infranate was adjusted to density 1.063 either with D<sub>2</sub>O which had been made isotonic with NaCl or with KBr, and the low density lipoproteins recovered at the tube top after a  $1 \times 10^8$  *g*-min centrifugation. High density lipoproteins were prepared from the low density infranate by adjusting the density to 1.21 with KBr and centrifuging for  $2.5 \times 10^8$  *g*-min. Some of the isolated high density lipoproteins were readjusted to 1.063 with saline to insure the removal of LDL. All ultracentrifugations were performed in a Beckman L2-50 centrifuge (Beckman Instruments, Inc., Fullerton, Calif.) using a 50 rotor, usually at 8°C but occasionally at 32°C.

The ultracentrifugally prepared LDL fractions were chromatographed on a Sepharose 4B gel filtration system (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). A 100 × 2.5 cm column was equipped with flow adaptors and run in descending fashion at a rate of 6 ml/hr at 6°C using a 0.03 M Tris, 0.12 M NaCl pH 7.4 buffer containing 0.01% EDTA. Column fractions were monitored at 280 nm in a Beckman DU spectrophotometer (Beckman, Instruments, Inc.) and assayed for lipids and proteins.

**Electrophoresis.** Whole plasma and isolated lipoprotein fractions were evaluated in a 1.0% agarose zonal electrophoretic system using a 0.05 M pH 8.6 sodium barbital buffer containing 0.035% EDTA. The gels were stained with fat red 7B stain. The apoproteins of delipidated lipoproteins were studied by polyacrylamide electrophoresis on a 10% gel using a technique similar to that of Weber and Osborn (7). The apoproteins were reduced and solubilized in a 1% mercaptoethanol, 1% sodium dodecyl sulfate 0.1 M pH 8.0 phosphate buffer. Peptide standards of known molecular weight were run with each determination. The gels were stained with Coomassie blue and destained by a methanol acetic acid mixture. Isolated peptide bands were occasionally electrophoresed out of the gels for immunochemical and N-terminal amino acid studies.

**Analytical ultracentrifugation.** Flotation velocity determinations were made on whole plasma and isolated lipoprotein fractions in a Beckman model E ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) by minor modifications of the method of DeLalla and Gofman (8). Samples were run in doubled sector cells in an AN-D

rotor (Beckman Instruments, Inc., Fullerton, Calif.). The samples were adjusted to 1.063 with isotopic D<sub>2</sub>O and run at 52,000 rpm and 26°C against a D<sub>2</sub>O saline blank. The schlieren displacement was determined and the flotation rate calculated from the standard relationship (9).

**Electron microscopy.** Whole plasma and isolated lipoproteins, both undiluted and diluted 8- to 20-fold, were studied by negative staining electron microscopy in an AEI microscope (AEI Scientific Apparatus, Inc., Elmsford, N. Y.). Copper grids of 200 mesh were coated with Formvar (Monsanto Co., St. Louis, Mo.) and carbon. A number of negative stains were used, including 2% sodium silicotungstate (pH 6.5), 2% potassium phosphotungstate (pH 6.8), and 1% uranyl acetate (pH 4.9). The sample was applied to the grid for 15-30 sec and the excess removed by touching to filter paper. A solution of ferritin was applied to the grid and the excess similarly removed with filter paper. Finally, the negative stain was applied and removed after 15 sec. Care was taken to be sure that the sample did not dry during the staining procedure. The stained sample was immediately viewed in the electron microscope.

**Polarized light microscopy.** A drop of sample was placed on an acetone- and water-cleaned microscope slide and a cover slip put over it. The slide was scanned at a 100-fold magnification and the birefringent structure confirmed at a magnification of 400-630. A quartz first order red compensator was interposed in the field to obtain the sign of birefringence.

**Low angle X-ray scattering.** Low angle X-ray studies were performed using an X-ray tube with a copper anode and a Kratky collimation system with automatic step scanner. The entrance slit was 120 μ, the counter slit 200 μ, and the distance from sample to counter slit 20.4 cm which gave a resolution in excess of 800 Å. Scattering curves were registered with a proportional counting tube. Monochromatization to 1.54 Å was achieved with a pulse height discriminator and an 8 μ nickel filter. The liquid samples were kept in Mark capillaries at 22°C. The samples were prepared in 0.03 M Tris, 0.12 M NaCl pH 7.4. The LDL fraction was studied as a 1% solution, while 5% solutions were used for the isolated lipid and the in vitro prepared lecithin cholesterol mixture. For preparation of the dry sample, small portions of the solution were applied to a cellulose acetate foil and allowed to dry. The foil gives no appreciable scattering in the range investigated. Correction for slit smearing was done with a computer program (10).

**Immunochemistry.** Antisera to normal human high density and low density lipoproteins were prepared in rabbits. Anti-

TABLE I  
Clinical Characteristics

| Patient | Diagnosis                        | Age | Sex | Cholesterol | Phospholipid | Triglyceride | Bilirubin* | Alkaline phosphatase* |
|---------|----------------------------------|-----|-----|-------------|--------------|--------------|------------|-----------------------|
|         |                                  | yr  |     |             | mg/dl        |              |            | IU                    |
| J. L.   | Bile duct carcinoma              | 52  | M   | 461         | 942          | 286          | 17.0       | 970                   |
| R. F.   | Pancreatic carcinoma             | 51  | M   | 465         | 866          | 212          | 34.0       | 1770                  |
| D. T.   | Post necrotic cirrhosis          | 62  | M   | 480         | 924          | 160          | 18.0       | 400                   |
| R. G.   | Secondary sclerosing cholangitis | 49  | F   | 720         | 1212         | 110          | 5.0        | 912                   |
| F. M.   | Primary biliary cirrhosis        | 42  | F   | 1220        | 2208         | 564          | 8.0        | 1136                  |
| J. B.   | Biliary stricture                | 56  | M   | 346         | 684          | 248          | 16.0       | 1218                  |

\* Total bilirubin upper limit of normal 1.4 mg/dl. Alkaline phosphatase upper limit of normal 113 IU.

TABLE II  
Relative Composition of Obstructed Low Density Fraction ( $S_f$  0-20)

| Patient | Protein* | Phospholipid | Cholesterol | Cholesteryl ester | Triglyceride |
|---------|----------|--------------|-------------|-------------------|--------------|
|         |          |              | %           |                   |              |
| R. G.   | 11.8     | 51.9         | 26.5        | 4.9               | 4.9          |
| J. B.   | 12.5     | 47.6         | 25.4        | 6.5               | 8.0          |
| D. T.   | 20.8     | 27.3         | 16.6        | 16.0              | 19.3         |
| R. F.   | 18.0     | 33.8         | 16.0        | 0.3               | 32.0         |
| J. L.   | 17.3     | 33.7         | 13.3        | 7.0               | 25.6         |
| Normal‡ | 21       | 22           | 8           | 37                | 11           |

\* Expressed as per cent of total weight.

‡ Average values for normal LDL from Oncley and Harvie (4).

sera to HDL were found to react only with high and low density lipoproteins. Immunoelectrophoresis and immunodiffusion were performed by micromethods (11).

**Chemistry.** For peptide evaluations the lipoproteins were delipidated by a previously described procedure (12). Three extractions were performed with 3/1 absolute ethanol and diethyl ether, followed by two washes with ether, all at 4°C, using a greater than 50 vol of solvent per volume of lipoprotein. For lipid studies the lipoproteins were extracted by a Folch technique (13) and for bile acid studies the extracts were made by the method of Weiner, Glasser, and Lack (14).

Lipids were separated by thin-layer chromatography. Neutral lipids were separated on a solvent system of petroleum ether, diethyl ether, and glacial acetic acid, 80:20:1. Chloroform, methanol, and ammonia water 65:25:5 was used for phospholipid separations and propionic acid, isoamyl acetate, water, and *n*-propanol 15:20:50:10 for the separation of conjugated bile acids (15). Appropriate standards were run with each thin-layer system, and the lipid bands scraped and eluted with 1:1 chloroform methanol, except for the bile acids which were eluted in ethanol.

Proteins were determined by the Lowry method (16). *N*-terminal amino acid analysis of peptide fractions were performed by a dansyl technique (17), using a polyamide thin-layer system to define the *N*-terminal residues (18). Phos-

pholipid determinations were obtained by the method of Ames and Dubin (19). Triglyceride quantitation was performed by an automated fluorometric procedure (20) and cholesterol by the method of Abell, Levy, Brodie, and Kendall (21), modified to determine smaller amounts of cholesterol. Cholic acid concentrations were obtained by the colorimetric method of Irvin (22) along with gas-liquid chromatography (23). Bile acids were methylated and trifluoroacetylated in preparation for gas-liquid chromatography. The samples were run on a QF 1 column at 270°C using 5 $\beta$ -cholanolic acid as an internal standard. Heparin and manganese precipitation of lipoproteins were performed by the method of Burstein and Samaille (24).

## RESULTS

This group of six patients had biliary obstruction at differing locations from a number of diseases. Each was icteric and had very high serum alkaline phosphatase levels during the time of study. All had substantially elevated plasma concentrations of cholesterol, and phospholipid levels which were about twice that of cholesterol (Table I). Most of these patients had elevated plasma triglyceride concentrations as well. The plasma lipid concentrations were relatively stable during the period of ob-

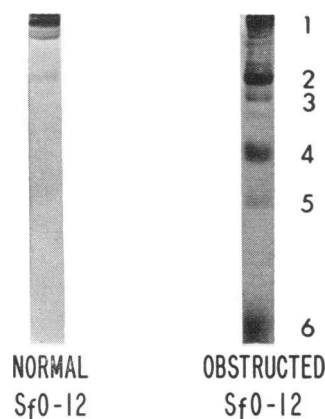


FIGURE 1 Normal and obstructed low density apoproteins evaluated by SDS-polyacrylamide electrophoresis. The protein bands are numbered and discussed in the text.

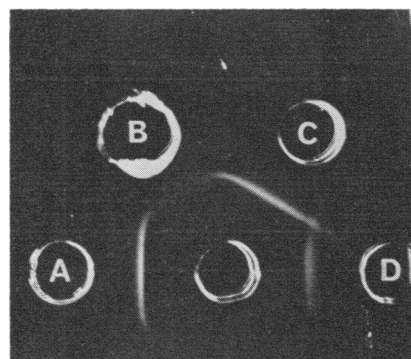


FIGURE 2 Immunodiffusion of obstructed low density apoproteins. Antisera to normal HDL and LDL in center well and the delipidated obstructed low density flotation fraction (A), polyacrylamide peptide band 5 (B), delipidated normal HDL (C), normal LDL (D).

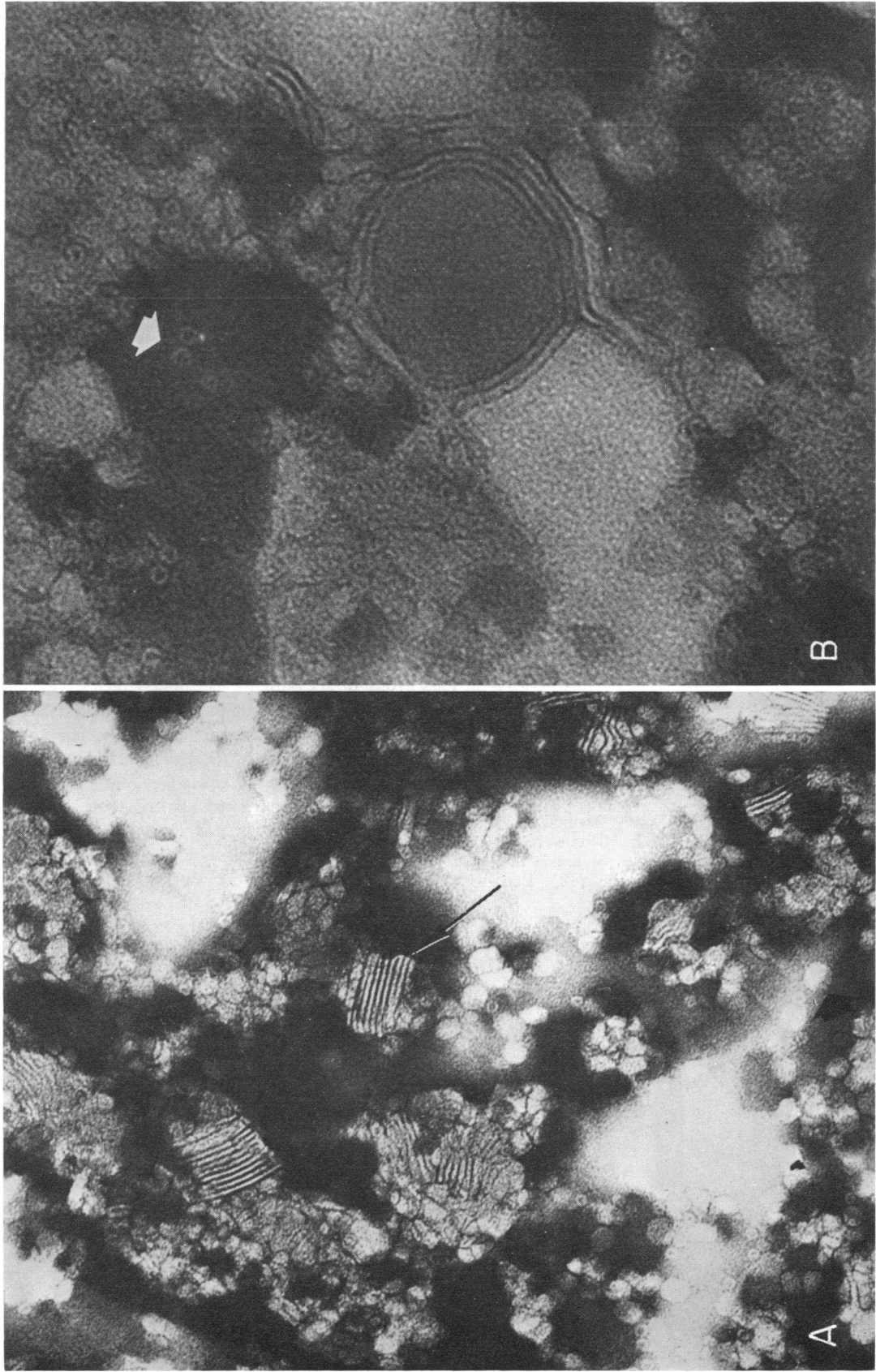


FIGURE 3 (A) Potassium phosphotungstate (2%) stained LDL fraction of F. M. ( $\times 200,000$ ). Short arrow identifies spherical LDL, and long arrow mesophasic lipid. (B) Same as A. Arrow indicates ferritin  $\times 450,000$ .

servation for all patients except F. M. (Table I), who had a variable plasma cholesterol concentration with intermittent elevations as high as 1800 mg/dl.

Nearly all of the plasma lipid from these obstructed individuals was isolated in the low density ( $S_r$  0–20) flotation fraction. The composition of this low density material differed from that of normal human LDL. All (Table II) had relatively more free cholesterol and phospholipid and less protein than conventional LDL (4), and three (Table II, D. T., R. F., and J. L.) had more triglyceride. A difference was noted between the apoprotein contents of this low density fraction and that of normal LDL. On an SDS 10% polyacrylamide gel (Fig. 1) normal LDL contained a large molecular weight apoprotein which did not enter this gel. A 3% gel was necessary to demonstrate migration of a single large apoprotein. A single apoLDL protein with a molecular weight of 250,000 was seen for normal LDL by Sepharose 4B chromatography in a 6 M guanidine buffer system.<sup>2</sup> All of the patients with obstructive biliary disease had a more complex LDL peptide pattern on the SDS 10% polyacrylamide system (Fig. 1). Aside from apoLDL five other peptides have been consistently identified in the obstructed low density fraction. Band 5, with a mol wt of 28,000, was immunochemically identical to apoHDL (Fig. 2). Band 2 of mol wt 68,000 was immunochemically identical to albumin. Band 6 had a mol wt of 8500, similar to the D peptides described by Brown, Levy, and Fredrickson (12), and the same *N*-terminal amino acid residues, serine and threonine. Bands 3 and 4 are unidentified, but normal VLDL have peptides of similar molecular size.

Electron micrographs of the LDL from these patients revealed two types of structure. Spherical 200–250 Å particles identical to the morphology of normal LDL (25) were seen along with smectic liquid crystals (Fig. 3A). These lamellar structures had a number of different organizations. Some appeared as stacked layers (Fig. 3A), virtually identical to the appearance of biliary lipid described by Howell, Lucy, Pirola, and Bouchier (26). Others appeared as lamellar whorls (Fig. 3B) similar to liposomes and some had the stacked-coin, bilayer vesicle appearance described by Hamilton, Havel, Kane, Blau-rock, and Sata (6). The latter structure had a width varying from about 80 to 100 Å and diameters varying from 300 to 500 Å. The periodicity of the stacked layers and whorls (Fig. 3A and B) varied between 45 and 55 Å. The lamellar whorls had a wide range of diameters varying from about 300 to 10,000 Å. These liquid crystal structures were readily identified in fresh diluted plasma, and were not affected by the type of negative stain, or the temperature of preparation.

<sup>2</sup> Smith, R., J. Dawson, and C. Tanford. Manuscript in preparation.

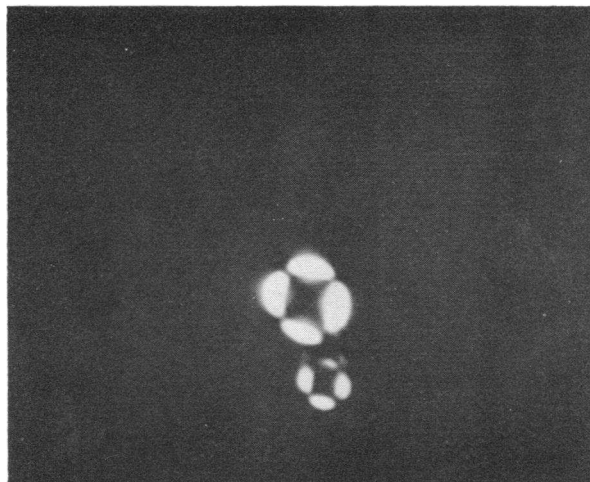


FIGURE 4 Polarized microscopic observation of whole plasma from patient R. F.  $\times 1200$ .

Polarized microscopy provided confirmation of the smectic structure observed in plasma by electron microscopy. Small birefringent "maltese" crosses were seen in the isolated LDL of these patients when viewed with crossed polarizers (Fig. 4). The same structures were seen in freshly obtained plasma viewed immediately at 37°C. The sign of birefringence of these structures obtained by the interposition of a quartz first order red compensator was observed to be positive. This birefringence was not observed for any of 30 normal LDL's including a child with homozygous Type II hyperlipoproteinemia.

Low angle X-ray scattering data of the LDL's from these obstructed patients revealed three flat maxima (Fig. 5). These Bragg spacings  $D$ , which are also seen with normal LDL are theoretically consistent with spherical particles of approximately uniform electron density having a diameter of about 180 Å. It was therefore necessary to free the liquid crystalline structures of

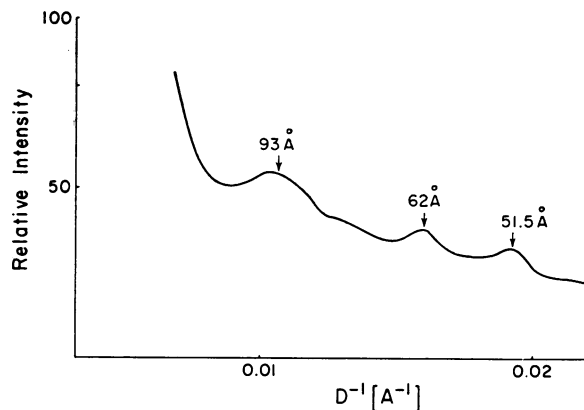


FIGURE 5 Low angle X-ray scattering pattern of the obstructed LDL fraction from patient R. G.

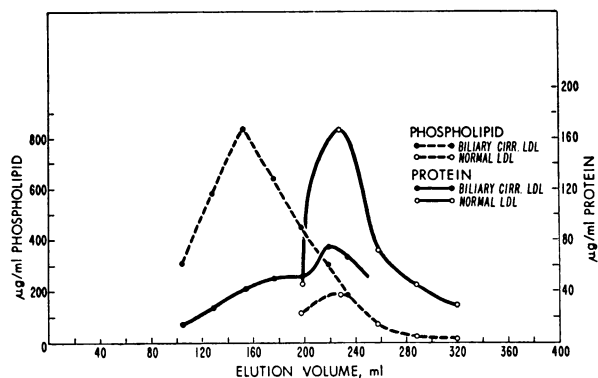


FIGURE 6 Lipid and protein elution pattern of obstructed and normal low density lipoproteins chromatographed on Sepharose 4B.

the spherical LDL for adequate low angle X-ray studies of the unusual lipid. The lamellar structures were isolated from the spherical LDL particle by gel filtration chromatography on Sepharose 4B (Fig. 6). The lamellar structures were found near the void volume where most of the phospholipid distributed. These column fractions were somewhat more turbid than the whole low density fraction, possibly reflecting some aggregation of the lamellar structures. Spherical LDL chromatographed at the protein peak where normal LDL distributed on this column.

Low angle X-ray scattering studies of the isolated lamellar lipid revealed spacings consistent with the first

two orders of an ordered lamellar structure having a 72 Å periodicity (Fig. 7A). This was similar to the X-ray scattering data obtained from an in vitro prepared dispersion of lecithin and cholesterol at about the same lipid concentration (Fig. 7B). This 72 Å periodicity was considerably greater than that determined electron microscopically. However, when the structures were dried in a fashion similar to their preparation for electron microscopy, their long spacing decreased to 50 Å close to the periodicity seen with negative staining electron microscopy. A similar decrease in spacing with decreasing water content was observed for in vitro lecithin cholesterol dispersions by Lecuyer and Dervichian (27) and our scattering pattern for the dried lipid resembled their result for the anhydrous 1:1 molar mixture.

The lamellar structures contained predominantly phospholipid and free cholesterol in a molar ratio of about 1:1 (Table III) with only a small amount of protein, triglyceride, and cholesteryl ester. The phospholipid was 91% lecithin, 6.5% sphingomyelin, and 2.5% lysolecithin in the one patient where these determinations were performed. The proteins associated with the liquid crystalline lipid were bands 2 through 6 described previously in Fig. 1. Most of the low density fraction bile acids were isolated with these liquid crystals, but comprised less than 2% of their mass.

The mean flotation peak of normal LDL varies between  $S_r$  5 and 8 (28), whereas in this group of obstructed patients it varied between  $S_r$  9 and 14. When plasma phos-

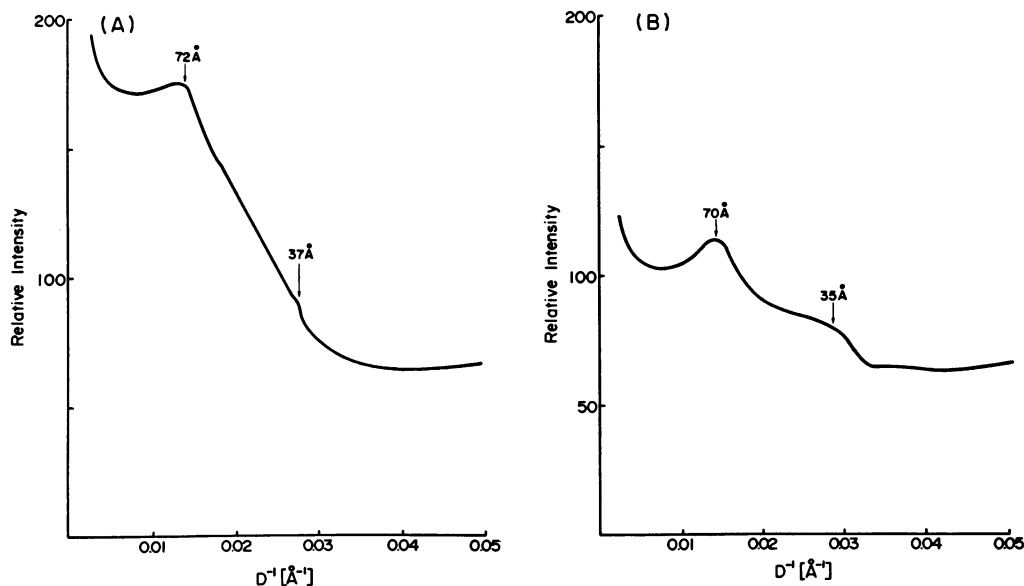


FIGURE 7 (A) Low angle X-ray scattering pattern of the isolated mesophase from the low density fraction of patient R. G. These curves have not been corrected for slit smearing effect. (B) Low angle X-ray scattering pattern of an in vitro prepared 1:1 molar lecithin cholesterol mesophase at a concentration similar to that of R. G.

TABLE III  
Relative Composition of Liquid Crystal Fraction

| Patient | Protein* | Phospholipid | Cholesterol | Cholesteryl ester | Triglyceride |
|---------|----------|--------------|-------------|-------------------|--------------|
|         |          |              | %           |                   |              |
| F. M.   | 3.0      | 59.8         | 30.9        | 1.3               | 4.9          |
| R. G.   | 4.8      | 60.0         | 32.5        | 2.9               | 3.0          |
| E. F.   | 3.0      | 61.0         | 32.7        | 2.1               | 1.3          |

\* Expressed as per cent of total weight.

pholipid and cholesterol concentrations increased, a second peak at  $S_r$  18–22 became apparent (Fig. 8A). With higher lipid concentrations, proportionately more of the LDL occurred in the more rapidly floating peak. Antisera to normal LDL precipitated much of the first peak, but did not effect the second peak (Fig. 8B). Heparin and manganese precipitated both peaks.

The mesophase low density lipid has an electrophoretic mobility equivalent to normal LDL (Fig. 9). Obstructed VLDL ( $S_r$  20–400) has a  $\beta$ -electrophoretic migration similar to obstructed or normal LDL (Fig. 9). With higher plasma triglyceride levels an  $\alpha_2$ -migrating VLDL was seen in addition to the  $\beta$ -band. The mean flotation rate of the obstructed VLDL was about 30 in two patients where this was evaluated. The slower flotation rate and the  $\beta$ -migration of obstructive VLDL resemble the VLDL properties in familial Type III hyperlipoproteinemia (29). The HDL from these patients had both a  $\beta$ -migrating and an  $\alpha_1$ -migrating component (Fig. 9). The VLDL from these obstructed patients contained rela-

tively less triglyceride (Table IV) and more protein and phospholipid than usual (4). The obstructed VLDL peptide pattern (Fig. 10) lacked some of the smaller molecular weight peptides found in normal VLDL. HDL from these patients also had an unusual composition (Table IV) with relatively less protein and more phospholipid and free cholesterol. In addition to the major 28,000 mol wt normal apoHDL peptide, other apoproteins were found in obstructed HDL (Fig. 10). The prominent rapid migrating peptide of obstructed HDL had a molecular size (8500) and *N*-terminal amino acid content, serine and threonine, identical with the *D* peptides of normal VLDL (12). The unusual physical properties and composition of the VLDL and HDL from this group of patients may reflect the presence of some smectic mesophase which has occasionally been seen by electron microscopy in the VLDL and HDL fractions of these patients.

## DISCUSSION

The unusual nature of plasma lipid transport in patients with biliary obstruction has been repeatedly documented (1–3, 5). Although most of the lipid is isolated in the LDL flotation fraction ( $S_r$  0–20) it has neither the lipid

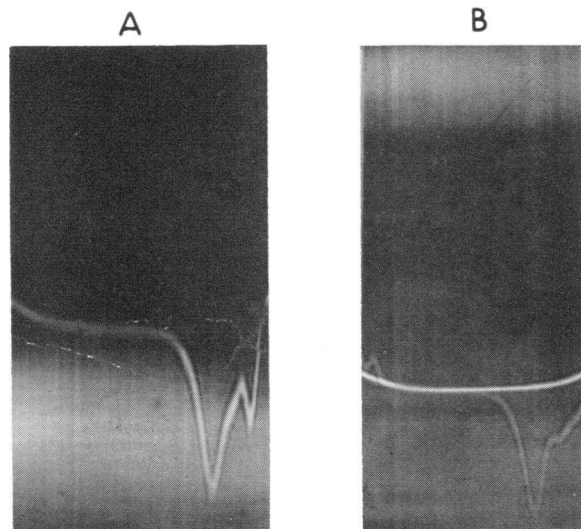


FIGURE 8 (A) Flotation velocity pattern of patient F. M. Flotation direction is toward the left of the figure. (B) Same pattern after treatment with antisera to normal low density lipoproteins.

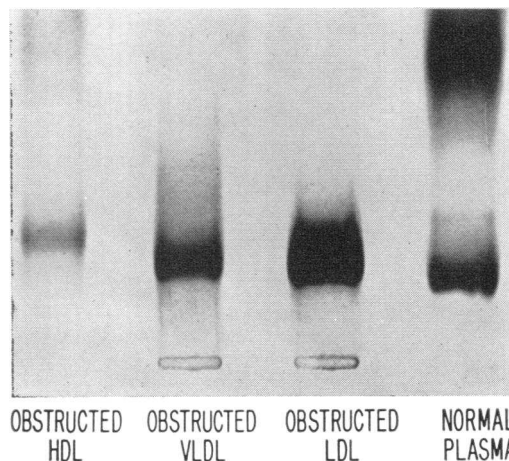


FIGURE 9 Lipoprotein electrophoretic mobility of obstructed VLDL, LDL, and HDL from patient D. T. compared with normal plasma lipoprotein electrophoresis.



TABLE IV  
A. Relative Composition of the Obstructed Very Low Density Fraction ( $S_f$  20-400)

| Patient | Protein* | Phospholipid | Cholesterol | Cholesteryl ester | Triglyceride |
|---------|----------|--------------|-------------|-------------------|--------------|
|         |          |              | %           |                   |              |
| R. G.   | 14.8     | 18.3         | 3.7         | 8.0               | 55.1         |
| R. F.   | 14.3     | 19.6         | 8.3         | 4.5               | 53.4         |
| D. T.   | 14.3     | 29.0         | 14.6        | 17.8              | 24.3         |
| J. B.   | 16.3     | 33.7         | 16.3        | 7.0               | 26.6         |
| Normal‡ | 8        | 19           | 7           | 13                | 51           |

| B. Relative Composition of Obstructed High Density Lipoproteins |      |      |      |      |     |
|---|------|------|------|------|-----|
| R. G.   | 39.4 | 42.7 | 6.2  | 6.4  | 5.2 |
| R. F.   | 24.3 | 45.7 | 21.7 | 0    | 8.3 |
| D. T.   | 28.1 | 36.7 | 14.7 | 12.4 | 8.1 |
| Normal‡   | 50   | 22   | 3    | 14   | 8   |

\* Expressed as per cent of total weight.

‡ Average values for normal VLDL and HDL from Oncley and Harvie (4).

nor peptide composition of normal LDL (30). The ultracentrifugal (2) and electrophoretic (5) behavior of this low density fraction also differed from normal LDL.

Much of the lipid in this flotation fraction was unreactive to antisera against normal LDL. Switzer (30), using this property, isolated a lipoprotein from the obstructed low density fraction which was mostly phospholipid and free cholesterol, and contained only small amounts of triglyceride and cholesteryl ester, and about 5% protein. The amino acid composition of this obstructed lipoprotein (OLP) differed from that of normal LDL, and more closely resembled normal VLDL. Seidel, Alaupovic, Furman, and McConathy (5), by means of

ethanol fractionation of the low density fraction were able to isolate a lipoprotein with a protein and lipid content similar to that of OLP. The lipoprotein, called lipoprotein X (LPX), was found to contain albumin and apolipoprotein C, a phospholipid protein complex which had been observed in normal VLDL.

The present study, the report of Picard and Veissiere (31), and that of Hamilton et al. (6) describe the chromatographic isolation of a fraction from the low density flotation region of obstructed patients with a composition similar to OLP and LPX. Hamilton described albumin and peptides of normal VLDL as the major protein constituents of this unusual lipoprotein. A number of proteins were found on the mesophase in the present study, including the 28,000 mol wt apoHDL peptide, peptides with the molecular size and the *N*-terminal characteristics of the VLDL *D* peptides (12), albumin, and two uncharacterized proteins which have molecular sizes comparable to VLDL components. The obstructive VLDL appeared to contain relatively less of these smaller molecular weight peptides than normal VLDL, possibly indicating their loss to the mesophase.

Hamilton et al. (6), by means of electron microscopy and low angle X-ray scattering, described the cholestatic lipoprotein to be a coin-shaped, bilayer vesicle. Similar structures were seen in this patient group in addition to stacked lamellar and whorled lamellar liquid crystals. Polarized microscopic observations confirmed the presence of a smectic mesophase in fresh plasma where a birefringence identical to that of lecithin cholesterol dispersions (32) was observed. The positive sign of birefringence is similar to that seen for lecithin liposomes (33), and indicates that the phospholipid fatty acid chains are predominantly normal to the lamellar plane.

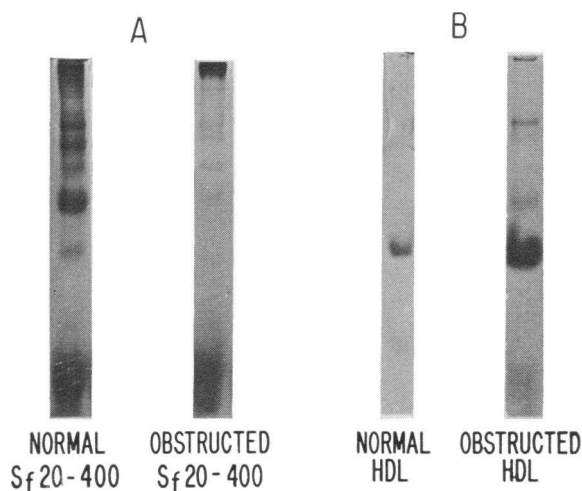


FIGURE 10 Normal and obstructed (A) VLDL and (B) HDL apoproteins evaluated by SDS-polyacrylamide electrophoresis.

The lamellar periodicity of this plasma lipid noted by low angle X-ray scattering and electron microscopy is also consistent with the view of the fatty acids being normal to the lamellar plane.

The presence of a mesophase lipid dispersion in the plasma of biliary obstructed patients possibly accounts for the isolation of albumin and some of the VLDL peptides in the low density flotation fraction. Lecithin mesophases have been observed to bind both albumin (34) and many of the peptides of VLDL.<sup>3</sup> The binding of these peptides to lecithin cholesterol liquid crystals could lead to their isolation in the low density ( $S_r$  0-20) flotation region, because of the hydrated density of this mesophase. The alterations in flotation behavior and electrophoretic mobility of the obstructed low density fraction are also possibly due to the presence of this mesophase. Plasma lipoprotein properties similar to those found in biliary obstruction have in part been reproduced by in vitro additions of a lecithin cholesterol mesophase to normal plasma.<sup>4</sup>

The similarity in electron microscopic structure between some of the obstructed plasma lipid and biliary lipid (26) suggests a biliary origin for the plasma lipid of these patients. However, patients with lecithin cholesterol acyl transferase defects, who most probably derive none of their plasma lipid from a biliary source, have a somewhat similar plasma lipid configuration (35). This smectic appearance may only reflect the presence of high plasma phospholipid and free cholesterol concentrations. In biliary obstructed dogs,<sup>5</sup> rising plasma phospholipid and free cholesterol concentrations were observed at the same time biliary concentrations of these lipids fell, making it likely that the plasma lipid increment reflects refluxed biliary lipid. Only the lecithin and cholesterol fractions of biliary lipid appreciably partitioned in the plasma of these obstructed dogs.

Bile acids do not occur in obstructed plasma at anywhere near their biliary concentration and could not maintain plasma lecithin and cholesterol in a micellar dispersion similar to bile. However, plasma proteins disperse lipid mesophases (31), and could function in plasma analogous to biliary bile acid. The plasma lecithin cholesterol acyl transferase (LCAT) enzyme would also be anticipated to influence the structure and composition of the lecithin cholesterol mesophase. This mesophase is an active substrate (36) for the plasma lecithin cholesterol acyl transferase system and lysolecithin produced from this reaction induces the formation of coin-

shaped vesicles (37) identical to those seen in these patients.

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