Studies of the Composition and Structure of Plasma Lipoproteins. Separation and Quantification of the Lipoprotein Families Occurring in the High Density Lipoproteins of Human Plasma†

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ABSTRACT: To account for the protein heterogeneity of the lipoprotein density classes of human plasma and to emphasize the essential role of apolipoproteins for the formation of lipoproteins, we have postulated that the plasma lipoprotein system consists of a mixture of lipoprotein families each of which is characterized by the presence of a single apolipoprotein or its constitutive polypeptides. To verify experimentally the existence of lipoprotein families we developed a procedure for the isolation and separation of lipoprotein families LP-A, LP-B, and LP-C from normal human high density lipoproteins. Treatment of high density lipoproteins with an immunoadsorber containing antibodies to LP-B resulted in complete removal of LP-B. The separation of LP-A from LP-C was accomplished either by the hydroxylapatite column chromatography or by fractional precipitation of LP-A with 33% polyethylene glycol. The characterization of lipoprotein families by disc electrophoresis and immunodiffusion indicated that the protein moiety of LP-A contained only the A-I and A-II polypeptides and the protein moiety of LP-C the C-I, C-II, and C-III polypeptides. The LP-B gave a positive immunoprecipitin reaction only with antibodies to LP-B. The lipid composition of LP-B was characterized by a higher per cent content of cholesterol ester and a lower relative content of phospholipid than those of the corresponding LP-A and LP-C families. The LP-B family was present only in the HDL_2 subfraction. The relative content of LP-A from female and male HDL_3 (94.5%; 94.3%) was higher than that from HDL_2 (90.0%; 82.5%). The HDL_3 from female and male donors had the same relative content of both LP-A (94–95%) and LP-C (5–5.2%). However, the HDL_2 subfraction from male subjects contained a higher percentage of LP-B (7.7%) and a lower percentage of LP-A (82.5%) than that from female subjects (2.1% LP-B and 87.5% LP-A). On the other hand, if taken as a single density class, the female HDL are characterized by a higher concentration of LP-A (442 mg/100 ml *vs.* 275 mg/100 ml) and LP-C (30 mg/100 ml *vs.* 20 mg/100 ml) than those of male donors. There seems to be no sex-linked difference in the HDL concentration of LP-B (4.8 mg/100 ml *vs.* 5.4 mg/100 ml). The separation of LP-A, LP-B, and LP-C from HDL represents the first experimental evidence for the postulated existence of lipoprotein families.

According to the most commonly used classification system based on hydrated densities as the differentiating criterion, five major polydisperse density classes (DeLalla and Gofman, 1954; Oncley, 1963; Ewing *et al.*, 1965, Alaupovic *et al.*, 1966) represent the basic physical-chemical entities of the plasma lipoprotein system. The well-recognized heterogeneity of each of these lipoprotein classes (Oncley, 1963; Ewing *et al.*, 1965; Nichols, 1967) with respect to particle size and hydrated density has been attributed to a great variation in the relative and absolute amounts of lipid constituents rather than protein moieties. However, the results of N-terminal amino acid analyses (Shore, 1957; Rodbell, 1958; Shore and Shore, 1962; Gustafson *et al.*, 1964; Bobbitt and Levy, 1965) and immunochemical studies (DeLalla *et al.*, 1957; Aladjem *et al.*, 1957; Scanu and Page, 1959; Ayrault-Jarrier *et al.*, 1963; Levy and Fredrickson, 1965; Gustafson *et al.*, 1966; Levy *et al.*, 1966; Alaupovic, 1968; Brown *et al.*, 1969; Scanu *et al.*, 1969; Lee and Alaupovic, 1970) demonstrated clearly that each major density class was heterogeneous also with respect to its protein moieties (apolipoproteins).

The human plasma lipoprotein system is characterized at the present time by three apolipoproteins:[1] apolipoprotein A (ApoA) is the major protein moiety of HDL[2] and VHDL, apolipoprotein B (ApoB) of VLDL and LDL, and apolipoprotein C (ApoC) of VLDL (Fredrickson, 1969; Alaupovic, 1971; Alaupovic *et al.*, 1972). It has been demonstrated that ApoA consists of two (Shore and Shore, 1968) and ApoC of three (Brown *et al.*, 1969) nonidentical polypeptides. The possible polypeptide heterogeneity of ApoB suggested on the basis of immunochemical evidence (Simons and Helenius, 1969; Lee and Alaupovic, 1970) or amino acid composition

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[1] At the present time, we are characterizing human plasma apolipoproteins by the following terminal amino acids of their constitutive polypeptides: apolipoprotein A (ApoA) consists of two nonidentical polypeptides A-I and A-II. The A-I polypeptide contains glutamine as the C-terminal and aspartic acid as the N-terminal amino acid; the A-II polypeptide is characterized by glutamine as the C-terminal and by a (as yet unknown) blocked N-terminal amino acid (Kostner and Alaupovic, 1971b). Apolipoprotein B (ApoB) is characterized by glutamic acid as the N-terminal and serine as the C-terminal amino acid (Shore, 1957). Apolipoprotein C (ApoC) consists of three nonidentical polypeptides C-I, C-II, and C-III. The C-I polypeptide has threonine as the N-terminal and serine as the C-terminal amino acid (Herbert *et al.*, 1971; McConathy *et al.*, 1972). The C-II polypeptide contains *N*-threonine and *C*-glutamic acid (Brown *et al.*, 1970), and the C-III polypeptide *N*-serine and *C*-alanine as the terminal amino acids (Brown *et al.*, 1969).

[2] Abbreviations used are: VLDL, very low density lipoproteins ($d < 1.006$ g/ml); LDL, low density lipoproteins (d 1.006–1.063 g/ml); HDL, high density lipoproteins (1.063–1.21 g/ml); VHDL, very high density lipoproteins ($d > 1.21$ g/ml).

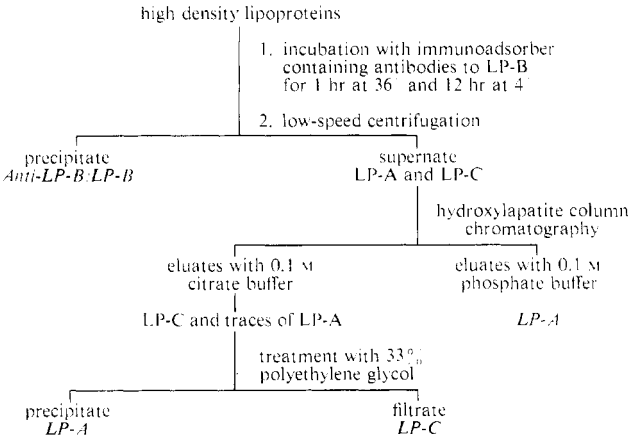

FIGURE 1: Procedure for the separation of lipoprotein families LP-A, LP-B, and LP-C from high density lipoproteins (*d* 1.073–1.22 g/ml).

of two ApoB subfractions (Shore and Shore, 1969; Kane *et al.*, 1970) has not yet been confirmed. Results of preliminary quantitative studies have already indicated (Gustafson *et al.*, 1966; Alaupovic, 1971; Alaupovic *et al.*, 1972) that the varying amounts of these three apolipoproteins in VLDL, LDL, and HDL may depend upon the dietary regimens as well as metabolic and clinical states of individual subjects. At the same time, the biochemical characterization of genetic disorders of lipoprotein transport (Fredrickson *et al.*, 1967) provided the most impressive evidence for the essential role of apolipoproteins in the formation of lipoprotein density classes. Disorders such as abetalipoproteinemia and Tangier disease are characterized primarily by the virtual absence or structural alteration of an apolipoprotein and only secondarily by the complete absence or decrease of a specific density class.

To account for the protein heterogeneity of the density classes and to emphasize the essential role of apolipoproteins for the formation of lipoproteins, we have postulated that the plasma lipoprotein system consists of a mixture of individual lipoprotein families (Alaupovic, 1968, 1971; Alaupovic *et al.*, 1972) each of which is characterized by the presence of a single apolipoprotein or its constitutive polypeptides. In contrast to the operational concept based on five major density classes, the chemical concept currently recognizes, as a first approximation, three lipoprotein families: lipoprotein family LP-A characterized by ApoA, lipoprotein family LP-B by ApoB, and lipoprotein family LP-C by ApoC. Although this classification system recognizes that varying concentrations of each lipoprotein family may exist in most, if not all, segments of the density spectrum, it establishes apolipoproteins as the sole determinants of lipoproteins as definable chemical entities.

Since this conceptual view had evolved from studies on the immunological detection and terminal amino acid analyses of apolipoproteins in various density classes, it became necessary to verify experimentally the existence of lipoprotein families by their actual isolation from a density class. This paper describes a procedure for the isolation and separation of LP-A, LP-B, and LP-C families from normal human high density lipoproteins and provides the first experimental evidence for the postulated existence of lipoprotein families.

Experimental Section

Isolation of Lipoprotein Density Classes. To remove the VLDL and LDL, the solution density of individual plasma specimens was adjusted to 1.073 g/ml by adding solid NaBr. This solution density corresponds to solvent density of approximately 1.063 g/ml (Lee and Alaupovic, 1970). The mixture was centrifuged in the No. 50 rotors of the Spinco Model L or L-2 ultracentrifuges at 140,000*g* for 24 hr, and the top layer containing both VLDL and LDL was removed by a tube-slicing technique. The solution density of bottom fraction was raised to 1.22 g/ml by the addition of solid NaBr. The mixture was then centrifuged at 140,000*g* for 40 hr. The top layer accumulating in the upper 2.0 cm of each tube was removed by the Spinco tube-slicer and washed two to three times by recentrifugation under identical experimental conditions to eliminate traces of albumin. Immunodiffusion tests showed that second washing procedure yielded albumin-free high density lipoproteins. To isolate the HDL_2 and HDL_3 subfractions, the solution density of HDL preparation was lowered to 1.105 g/ml by diluting the lipoprotein solution with distilled water. The ultracentrifugation of this solution at 140,000*g* for 48 hr resulted in the formation of two distinct yellow layers which were removed separately by a tube-slicing technique. In contrast, when the density of HDL preparation was adjusted to 1.125 g/ml, a value suggested originally by DeLalla and Gofman (1954) as the solution boundary between the HDL_2 and HDL_3 subfractions, there was no sharp separation between the two diffuse lipoprotein layers. However, if HDL_2 is to be isolated from plasma by sequential preparative ultracentrifugation after removal of LDL, a sharp separation of HDL into subfractions will occur at the solution density 1.125 g/ml, because under these experimental circumstances the solution density includes also density contributed by the plasma proteins. Therefore, *d* 1.105 g/ml was selected as the solution density only when HDL_2 and HDL_3 were separated from the isolated HDL. Both layers were recentrifuged once at *d* 1.105 g/ml. The upper layer consisted of HDL_2 subfraction, the lower layer of HDL_3 subfraction. The purified HDL preparation and its subfractions were dialyzed exhaustively against 0.15 M NaCl, adjusted to pH 7.6 with 1 M Na-citrate buffer, and stored in the presence of 1 mg of NaN_3/ml and 0.1 mg of EDTA/ml at 4°.

Separation of Lipoprotein Families. The separation of lipoprotein families LP-A, LP-B, and LP-C from HDL, HDL_2, or HDL_3 preparations was carried out according to the procedure outlined in Figure 1. To remove the LP-B, the dialyzed HDL preparations were treated with an immunoadsorber containing antibodies to LP-B. The preparation of the immunoadsorber, the incubation sequence, and the removal of adsorbed LP-B were performed according to the procedure described by Kostner and Holasek (1970). The subsequent separation of LP-A from LP-C was accomplished either by hydroxylapatite column chromatography or by 33% polyethylene glycol precipitation.

HYDROXYLAPATITE COLUMN CHROMATOGRAPHY. Five to ten milliliters of the LP-B-free HDL, HDL_2, or HDL_3 solutions (10 mg/ml), dialyzed exhaustively against 0.1 M phosphate buffer (pH 6.8), were applied to a column (20 × 1.2 cm) of hydroxylapatite (Bio-Gel HT, Bio-Rad Laboratories, Richmond, Calif.) equilibrated with the same buffer. The 5-ml fractions were collected at a flow rate of 2 ml/hr for a total chromatography time of 50–60 hr at room temperature. The elution pattern was monitored by absorbancy at 280 nm and by double diffusion with antibodies to LP-A and LP-C. The eluates with

0.1 M phosphate buffer contained only the LP-A. The adsorbed LP-C was then eluted with 0.1 M Na-citrate buffer (pH 7.0). The separation of LP-A from LP-C was accomplished also by the following batch-wise procedure. Ten milliliters of the LP-B-free HDL solutions (10 mg/ml) in 0.1 M phosphate buffer (pH 6.8) were mixed with 3.0 g of wet hydroxylapatite and stirred gently for 1 hr at 6°. The hydroxylapatite was removed by centrifugation at 12,000*g* for 10 min. Identical treatment of the filtrate with hydroxylapatite was repeated twice. After the third treatment, the filtrate contained only the LP-A. The LP-C was eluted from the combined hydroxylapatite adsorbents with 0.1 M sodium citrate buffer (pH 7.0). The LP-A and LP-C fractions were dialyzed either against 0.15 M NaCl or 0.01 M Tris buffer, pH 8.2, and concentrated by vacuum dialysis.

33% POLYETHYLENE GLYCOL PRECIPITATION. The LP-B-free HDL solutions (10–15 mg/ml), dialyzed exhaustively against 0.15 M NaCl (pH 7.6–8.5 adjusted with 0.1 M NaOH), were mixed with the 33% (w/v) aqueous polyethylene glycol (mol wt approximately 6000) in a ratio of 1 ml of lipoprotein/0.6 ml of polyethylene glycol. The mixture was stirred gently for 5 min. If the lipoprotein concentration was higher than 10 mg/ml, some LP-A precipitated immediately. Then, 0.2 ml of 5 M NaBr was added for each milliliter of lipoprotein solution, and the stirring was continued for 15 min. The precipitated LP-A was removed by low-speed centrifugation, dissolved in 1 ml of 0.1 M $(NH_4)_2CO_3$, and dialyzed against 0.15 M NaCl. The precipitation procedure was repeated twice. The filtrate after the last precipitation of LP-A contained LP-C and only trace amounts of LP-A (the LP-A represented less than 5% of the total protein content of filtrate).

Traces of polyethylene glycol were removed from LP-A by passing the LP-A, dissolved in 0.1 M $(NH_4)_2CO_3$, and dialyzed against 0.15 M NaCl, over a Sephadex G-75 column (50 × 2.5 cm) equilibrated and eluted with 0.15 M NaCl. The LP-A fractions were combined and concentrated by vacuum dialysis.

The filtrate containing the LP-C, polyethylene glycol, and NaBr was dialyzed exhaustively against 0.01 M Tris-HCl buffer (pH 8.2). Since polyethylene glycol could not be dialyzed, there was a severalfold increase in the volume of inner dialysate. To eliminate polyethylene glycol, the dialyzed filtrate was chromatographed over a DEAE-cellulose (Cellex D, 0.38 mequiv/g capacity, Bio-Rad, Richmond, Calif.) column (10 × 1.2 cm) which had been washed several times with 1 M HCl–1 M NaOH–distilled water and equilibrated with 0.01 M Tris buffer (pH 8.2). After the entire filtrate had been applied, the column was eluted with 100 ml of 0.01 M Tris buffer to remove the polyethylene glycol. Under these conditions, the LP-C remained adsorbed on the column. The LP-C was eluted with 1 M NaCl. The monitoring of 5-ml fractions by absorbancy at 280 nm indicated a single sharp elution peak of LP-C. The combined LP-C fractions were dialyzed against 0.15 M NaCl (pH 7.4) and concentrated by vacuum dialysis.

Quantitative Determination of Lipoprotein Families. The recovery of lipoprotein families was based on the protein content of washed, albumin-free HDL_2 and HDL_3. The per cent composition of individual lipoprotein families in HDL_2 and HDL_3 was estimated from the protein content of the isolated LP-A, LP-B, and LP-C as determined by the method of Lowry *et al.* (1951) and using human serum albumin as the standard. The amount of LP-B was established by determining the protein concentrations of the starting HDL_2 preparation before and after treatment with the LP-B immunoadsorber as well as that of LP-B eluted from the immunoadsorber with 0.2 M glycine-HCl buffer (pH 2.4). With a freshly prepared immunoadsorber about 20% of the firmly attached LP-B could be eluted only at a much lower pH value. However, with a reused immunoadsorber the recovery of LP-B eluted at pH 2.4 was over 95%. The calculation of LP-A and LP-C contents was based on the measurement of protein concentrations of these two lipoprotein families separated by hydroxylapatite column chromatography only and not by the polyethylene glycol precipitation. The combined protein contents of the phosphate buffer (LP-A) and citrate buffer (LP-C) eluates accounted for 95–98% of the total protein of LP-B-free HDL_2 or HDL_3 applied onto the hydroxylapatite column. Trace amounts of LP-A representing less than 5% of the protein content of citrate buffer eluates were neglected in calculating the recoveries of LP-A and LP-C. Compositional data presented in Tables II–IV were based on the 95% recovery of lipoprotein families separated by immunoadsorption and hydroxylapatite column chromatography. The conversion factor for calculating the lipoprotein values was based on the per cent protein content of each lipoprotein family.

Immunological and physical-chemical characterizations of LP-A and LP-C were performed only with preparations which had been separated and purified by hydroxylapatite column chromatography and 33% polyethylene glycol precipitation.

Ultracentrifugal Analyses. The determination of the observed sedimentation rate of LP-C was carried out in a Spinco Model E ultracentrifuge equipped with a phase-plate schlieren diaphragm and an automatic temperature control unit. Plate measurements were made with a Nikon microcomparator (Nikon Co., Japan). The observed sedimentation coefficient, s_{obsd}, and flotation rate, $F_{1.20}$, were calculated as outlined by Schachman (1957).

Polyacrylamide Gel Electrophoresis. The polyacrylamide gel electrophoresis was performed in a Canalco Model 6 unit according to the procedure by Davis (1964). Electrophoresis was carried out at 4 mA/tube employing Tris-glycine buffer (pH 8.6). The acrylamide monomer concentration was 7% (w/v). Gels were stained for protein with Amido Black 10B or Coomassie Brilliant Blue.

Immunological Methods. Immunological properties of lipoprotein families were studied by double diffusion (Ouchterlony, 1953) and immunoelectrophoresis (Grabar and Williams, 1955) in 1% agar (Special Agar Noble, Difco Laboratories, Detroit, Mich.) or 1% agarose (Seakem Agarose, Bausch & Lomb, Inc., Rochester, N. Y.) employing barbital buffer (pH 8.6), ionic strength 0.05.

Rabbit antisera containing antibodies to human albumin and γ-globulin, respectively, were purchased from Behring Diagnostics, Inc., Woodburry, N. Y. The antibodies to LP-A were prepared by immunizing two goats (15–20 kg) with purified LP-A preparation isolated from human plasma HDL_3 by hydroxylapatite column chromatography. The LP-A solutions, containing 20 mg of LP-A dissolved in a mixture of 5 ml of 0.15 M NaCl and 5 ml of complete Freund's adjuvant, were injected intramuscularly four times at intervals of 10 days. The presence of antibodies to LP-A was demonstrated 2 weeks after the fourth injection. Animals were bled (250 ml of blood) from the jugular vein at weekly intervals. The rabbit antisera to LP-B and an abnormal low density lipoprotein (LP-X) isolated from patients with obstructive jaundice were prepared and characterized as described earlier (Lee and Alaupovic, 1970). The antiserum to LP-C was prepared by injecting rabbits intraperitoneally with a single dose of a mixture of equal volumes of partially de-

TABLE I: Characterization of Antisera.

Antisera	Animal	Antigens[a]										
		LP-A	LP-B	LP-C	LP-X	A-I	A-II	C-I	C-II	C-III	Albumin	γ-Globulin
LP-A	Goat	+	−	−	−	+	+	−	−	−	−	−
LP-B	Rabbit	−	+	−	−	−	−	−	−	−	−	−
LP-C	Rabbit	−	−	+	+	−	−	+	+	+	−	−
LP-X	Rabbit	−	−	+	+	−	−	+	+	+	−	−
A-I	Sheep	+	−	−	−	+	−	−	−	−	−	−
A-II	Sheep	+	−	−	−	−	+	−	−	−	−	−
Albumin	Rabbit	−	−	−	−	−	−	−	−	−	+	−
γ-Globulin	Rabbit	−	−	−	−	−	−	−	−	−	−	+

[a] Each + sign represents a distinct immunoprecipitin line obtained in 1% agar or agarose double-diffusion experiments.

lipidized LP-C (protein concentration 5 mg/ml of 0.15 M NaCl) and complete Freund's adjuvant. The partially delipidized LP-C was prepared according to a previously described procedure (Alaupovic *et al.*, 1969). The rabbits were bled by heart puncture, and the antibodies were detected 2 weeks after the injection. The A-I and A-II polypeptides of apolipoprotein A were prepared according to the procedure described by Kostner and Alaupovic (1971a). Monospecific antisera to these two polypeptides were prepared by immunizing sheep with two successive intraperitoneal injections of 10 mg of antigen dissolved in 5 ml of 0.1 M $(NH_4)_2CO_3$ and 5 ml of complete Freund's adjuvant. The presence of antibodies to A-I and A-II polypeptides was observed 3 weeks after the second injection. The C-I, C-II, and C-III polypeptides were prepared and characterized according to the procedures by Alaupovic *et al.* (1969) and Brown *et al.* (1969). The characterization of antisera examined by immunodiffusion and immunoelectrophoresis is shown in Table I. Antiserum to LP-A consisted of antibodies to A-I and A-II polypeptides. Antibodies to LP-B showed no reaction with either LP-A or LP-C. The LP-C and LP-X antisera formed distinct immunoprecipitin lines with C-I, C-II, and C-III polypeptides but gave no reaction with either LP-A or LP-B. The antibodies to A-I polypeptide gave no reaction with A-II polypeptide and *vice versa*. The antisera to albumin and γ-globulin reacted positively only with their corresponding antigens.

Analytical Procedures. Protein and lipid analyses of plasma samples and lipoprotein preparations were performed according to the procedures described previously (Alaupovic *et al.*, 1966).

Results

Immunochemical Characterization of HDL. The HDL and its HDL_2 and HDL_3 subfractions isolated from individual male and female donors displayed a remarkable immunochemical heterogeneity when tested with antibodies to LP-A, LP-B, and LP-C (Figure 2). The HDL (Figure 2, pattern a) reacted with anti-LP-A, anti-LP-B, and several separate antisera to LP-C. The typical nonidentity reactions between the precipitin line of LP-A and those of LP-B and LP-C on one hand, and between LP-B and LP-C lines on the other, showed clearly the antigenic individuality of each of these lipoprotein families. The expected fusion of LP-C lines with antibodies to LP-C, LP-X, and VLDL indicated the antigenic identity of ApoC from HDL, VLDL, and LP-X (Alaupovic *et al.*, 1969). The immunodiffusion patterns of HDL subfractions showed clearly that LP-B family is present only in HDL_2 (Figure 2, pattern b). However, LP-A and LP-C occurred in both HDL_2 and HDL_3 subfractions (Figure 2, patterns b and c). Both subfractions gave negative reactions with antibodies to albumin and γ-globulin.

Separation of Lipoprotein Families. Various attempts to separate the three immunochemically distinct lipoprotein families of HDL resulted in the development of a fractionation procedure outlined in Figure 1. The fractionation procedure was monitored by immunodiffusion and 7% polyacrylamide gel electrophoresis. The relatively small amount of LP-B family was removed by treating the HDL preparations with an immunoadsorber which contained antibodies to LP-B. To isolate the intact LP-B, the charged immunoadsorber was first washed with 0.15 M NaCl and distilled water, and then the adsorbed lipoproteins were eluted with 0.2 M glycine-HCl buffer at pH 2.4 and 0°. After dialysis against 0.15 M NaCl at 4°, the LP-B gave a positive immunoprecipitin reaction only with antibodies to LP-B. Upon 7% polyacrylamide gel electrophoresis, the LP-B remained as a single band at the junction of stacking and separating gels (Figure 3, pattern a).

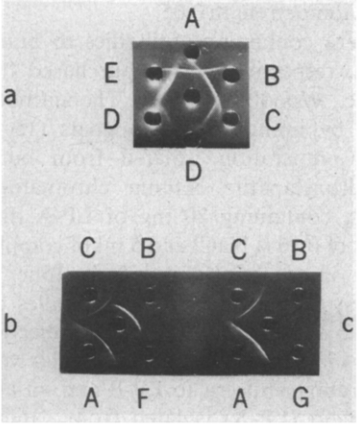

FIGURE 2: Immunodiffusion patterns of HDL (pattern a), HDL_2 (pattern b, left side), and HDL_3 (pattern c, right side). Outer wells contain antibodies to LP-A (A), LP-B (B), LP-C from VLDL (C), LP-X (D), VLDL (E), albumin (F), γ-globulin (G).

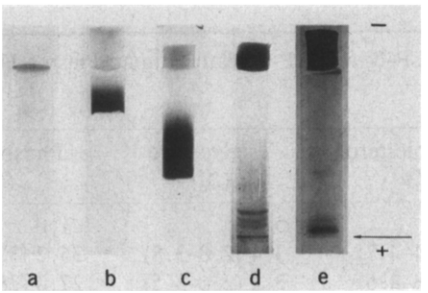

FIGURE 3: Polyacrylamide gel electrophoretic patterns of LP-B from HDL_2 (a), LA-P from HDL_2 (b), LP-A from HDL_3 (c). LP-C from HDL_3 (d and e). Patterns a–d were obtained in gels of 7% and pattern e in a gel of 3.5% acrylamide. The amounts of protein applied were as follows: a, 50 μg; b, 80 μg; c, 100 μg; d and e, 50 μg. The arrow indicates the dye front. Patterns a, b, and c were stained with Coomassie Brilliant Blue and patterns d and e with Amido Black 10B. Line in the middle portion of pattern e represents an artifact.

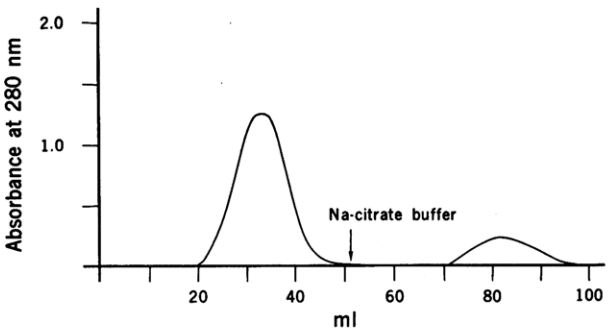

FIGURE 4: Separation of LP-A and LP-C families by hydroxylapatite column chromatography.

Although the separation of LP-A and LP-C was achieved either by adsorption chromatography on hydroxylapatite columns or by differential precipitation with 33% polyethylene glycol, a combination of both methods (Figure 1) gave the most satisfactory results. The LP-B-free HDL or its subfractions HDL_2 and HDL_3 were placed on hydroxylapatite columns and the LP-A and LP-C were separated by a discontinuous elution with two different buffers as shown in Figure 4. The LP-A was eluted with 0.1 M phosphate buffer and the LP-C by 0.1 M Na-citrate buffer. Small amounts of LP-A still present in LP-C eluates were then removed by precipitation with 33% polyethylene glycol (Figure 1). The electrophoretic pattern of an LP-B-free HDL preparation on 7% polyacrylamide gel was characterized by the typical fast-moving bands of LP-C and slower migrating bands of LP-A. The efficiency of the fractionation procedure was illustrated clearly by the electrophoretic patterns of separated LP-A fractions from HDL_2 (Figure 3, pattern b) and from HDL_3 (Figure 3, pattern c) which were characterized by the absence of fast-moving LP-C bands. On the other hand, the electrophoretic pattern of LP-C fraction isolated from the filtrate after precipitation of LP-A with 33% polyethylene glycol displayed (Figure 3, pattern d) only the fast-migrating bands of LP-C. Immunochemically, the separated LP-A fractions reacted positively only with antibodies to LP-A and the LP-C fractions only with antibodies to LP-C. To isolate solely the LP-A fraction by hydroxylapatite column chromatography of HDL, the preceding step involving immunoadsorption of LP-B was not necessary, since LP-B adsorbed on hydroxylapatite was eluted with 0.1 M Na-citrate, but not with 0.1 M phosphate buffer. The 33% polyethylene glycol treatment resulted in coprecipitation of LP-B and LP-A. If not removed by repeated ultracentrifugations of HDL preparations, traces of albumin were found in the Na-citrate eluates from hydroxylapatite column or in the LP-A fraction precipitated by 33% polyethylene glycol. Each of the separated lipoprotein families floated at solution density 1.21 g/ml after ultracentrifugation at 105,000*g* for 24 hr.

Partial Characterization of the LP-A Family. The LP-A preparations isolated from HDL_2 and HDL_3 subfractions reacted with antibodies to ApoA polypeptides, but not with antibodies to either LP-C, LP-B, or albumin (Figure 5, patterns a and b). Positive immunoprecipitin reactions between these two preparations and antibodies to either A-I or A-II polypeptides indicated no difference in the *qualitative* polypeptide composition of LP-A isolated from either the lower or higher density ranges of HDL. On agarose immunoelectrophoresis, the LP-A preparations from HDL_2 and HDL_3 gave single precipitin arcs of identical mobilities with antibodies to A-I and antibodies to A-II polypeptides (Figure 6, patterns a and b). Single electrophoretic bands of both LP-A preparations in agarose gels (Figure 7, patterns b and c) were stainable with the protein as well as lipid stains. In contrast to the agarose electrophoresis, the 7% polyacrylamide gel electrophoresis showed (Figure 3, patterns b and c) that the LP-A from HDL_2 had a slower migration rate than that from HDL_3. The difference in the mobilities and relatively broad bands of these two LP-A samples indicated a possible quantitative difference in the corresponding polypeptide composition and/or the polydisperse character of the entire LP-A family. The LP-A preparations from HDL_2 and HDL_3 exhibited single, symmetrical peaks in the analytical ultracentrifuge with average flotation rates of $F_{1.20}$ 6.4 and $F_{1.20}$ 2.5, respectively. Detailed physical–chemical characterization of

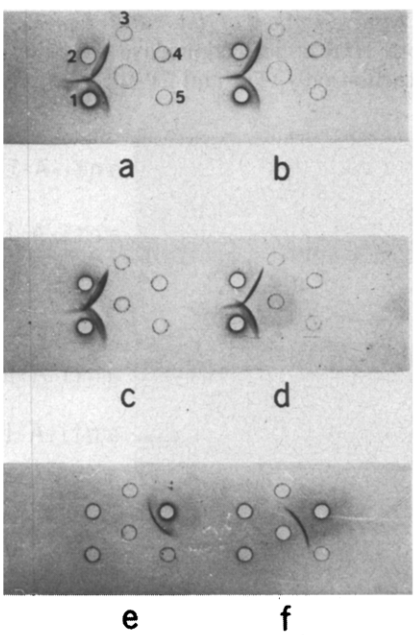

FIGURE 5: Immunodiffusion patterns of LP-A from HDL_3 (a), LP-A from HDL_2 (b), ApoA from HDL_3 (c), ApoA from HDL_2 (d), ApoB from HDL_2 (e) and LP-B from HDL_2 (f). In all six patterns the outer wells contained antibodies to A-II polypeptide (1), A-I polypeptide (2), LP-C (3), LP-B (4), albumin (5). Precipitin lines were stained with Amido Black 10B.

TABLE II: Per Cent Chemical Composition of Lipoprotein Families LP-A, LP-B, and LP-C from Human Plasma High Density Lipoproteins.

Lipoprotein Family	Protein (%)	Cholesterol Ester (%)	Free Cholesterol (%)	Triglyceride (%)	Phospholipid (%)
LP-B[a] (HDL_2)	39.5	25.1	6.8	5.1	21.0
LP-A[b] (HDL_2)	40.1 (39.5–42.0)	13.1 (12.0–14.4)	4.4 (3.4–4.7)	3.4 (2.8–3.8)	35.2 (33.0–37.5)
LP-A[b] (HDL_3)	56.1 (54.7–57.8)	9.4 (8.4–10.5)	2.2 (1.8–2.6)	3.2 (2.6–3.5)	27.0 (26.1–27.5)
LP-C[a] (HDL)	51.0	15.2	1.5	5.4	26.0

[a] Figures for LP-B and LP-C represent values of single samples. [b] Expressed as per cent of sum of individually determined protein and lipid values. Figures for LP-A represent mean values of four separate samples.

LP-A will be described in a forthcoming publication (Kostner and Alaupovic). The chemical composition of LP-A samples was characterized by an increased content of protein and a decreased content of phospholipid with increasing density (Table II). In that respect, the LP-A preparations differed very little from the corresponding HDL subfractions (Scanu and Granda, 1966). The gradual change in the relative contents of protein and lipid constituents of otherwise immunochemically identical macromolecules represents another indication for the polydispersity of LP-A family. Protein moieties of both LP-A preparations gave no reaction with antibodies to either LP-B, LP-C, or albumin. The presence of characteristic A-I and A-II bands (Alaupovic *et al.*, 1972) on 7% polyacrylamide gel (Figure 8) and the positive immunoprecipitin reactions with their corresponding monospecific antibodies (Figure 5, patterns c and d) indicate clearly that the protein moiety (apolipoprotein A) of LP-A consists of these two nonidentical polypeptides. However, the possible presence of the polymorphic forms of these two polypeptides in individual LP-A preparations has not been excluded by the results of this study.

Partial Characterization of the LP-B Family. The LP-B isolated from HDL_2 subfraction gave a positive immunoprecipitin reaction only with anti-LP-B serum prepared with an LP-B sample from normal human LDL (Figure 5, pattern f). On agarose immunoelectrophoresis, the LP-B exhibited a single precipitin arc with antibodies to LP-B (Figure 6, pattern c). The electrophoretic behavior of LP-B from HDL_2 was identical with that of a similar LP-B preparation from LDL; they were both retained at the junction of stacking and separating gel in 7% polyacrylamide gel (Figure 3, pattern a), but moved as single bands into the β-globulin position on 3.5% polyacrylamide (Figure 9) and 0.5% agarose (Figure 7, pattern d) gels. The lipid:protein ratio of LP-B (Table II) differed very little from that of LP-A isolated from HDL_2 subfraction. However, these two lipoprotein families had different contents of cholesterol ester and phospholipid. Reflecting the lipid composition of LP-B subfractions of lower density ranges (Lee and Alaupovic, 1970), the LP-B had a higher percentage of cholesterol ester and a lower percentage of phospholipid than the corresponding LP-A. To identify the protein moiety, the LP-B was totally delipidized and the water-insoluble protein was dissolved in 0.05 M Tris-HCl buffer containing 0.9% sodium dodecyl sulfate (pH 8.2). A positive reaction between the solubilized protein moiety and antibodies to LP-B, but not with antibodies to either ApoA polypeptides, LP-C, or albumin (Figure 5, pattern e), indicated apolipoprotein B as the sole protein moiety of LP-B family. Although treatment of HDL_2 with an LP-B immunoadsorber may precipitate not only LP-B family, but also the polymorphic Lp(a) lipoprotein, the failure to observe a post-β band on 3.5% polyacrylamide gel (Figure 9) suggests either the presence of electrophoretically undetectable amounts or the actual absence of Lp(a) lipoprotein in this

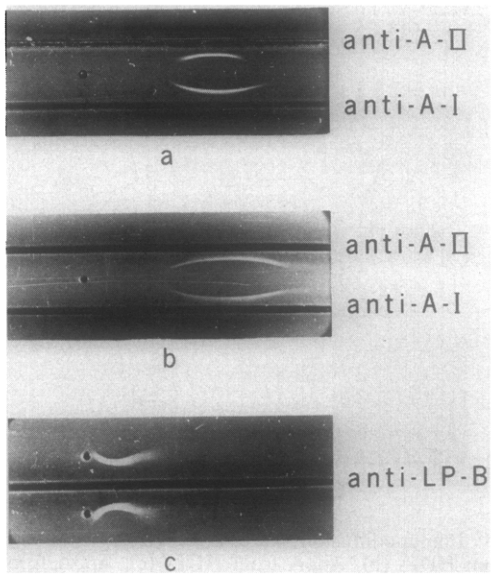

FIGURE 6: Immunoelectrophoresis patterns in 1% agarose gel of LP-A from HDL_2 (a), LP-A from HDL_3 (b), and LP-B from HDL_2 (c).

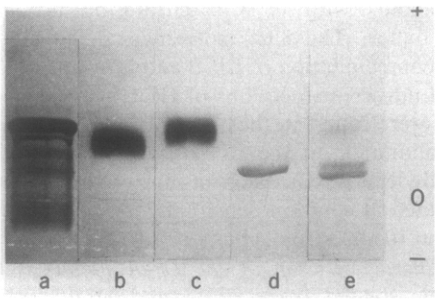

FIGURE 7: Agarose gel (0.5 %) electrophoresis of whole serum (a), LP-A from HDL_3 (b), LP-A from HDL_2 (c), LP-B from HDL_2 (d), whole serum (e). Patterns a–d were stained with Amido Black 10B and pattern e with Sudan Black. Patterns b–c were also stainable with Sudan Black. O, origin. Electrophoresis was carried out according to the procedure by Kostner *et al.* (1971).

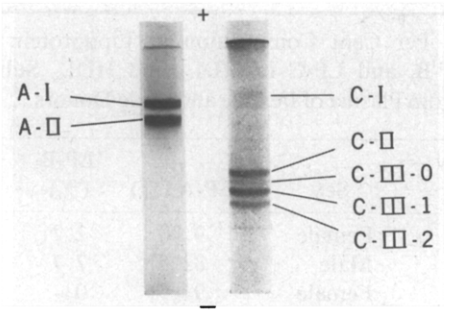

FIGURE 8: Polyacrylamide gel (7%) electrophoresis of totally delipidized LP-A (left pattern) and totally delipidized LP-C (right pattern) from HDL. The protein loads were approximately 100 μg. Both patterns were stained with Coomassie Brilliant Blue.

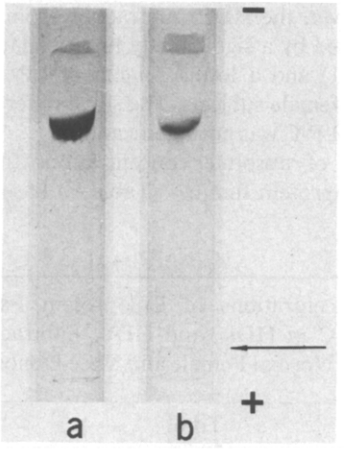

FIGURE 9: Polyacrylamide gel (3.5%) electrophoresis patterns of LP-B from LDL (*d* 1.027–1.055 g/ml) (a) and LP-B from HDL_2 (b). The protein loads were 120 μg in pattern a and 50 μg in pattern b. Both patterns were stained with Amido Black 10B.

particular LP-B preparation. In Lp(a)-positive individuals, however, both lipoprotein species have been detected (Simons *et al.*, 1970; Utermann and Wiegandt, 1970; Ehnholm *et al.*, 1971).

Partial Characterization of LP-C Family. Due to relatively small amounts of LP-C in HDL_2 and HDL_3 subfractions, studies on the partial characterization of LP-C were performed with a sample isolated from the whole HDL. Figure 10 shows that LP-C gave a single immunoprecipitin line with antibodies to either LP-C or LP-X, but none with antibodies to either γ-globulin, albumin, LP-A, or LP-B. There was a distinct difference between the electrophoretic mobility of

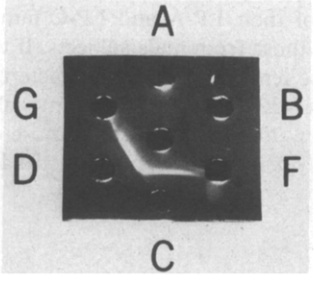

FIGURE 10: Immunodiffusion pattern of LP-C. Outer wells contain antibodies to LP-A (A); LP-B (B); LP-C (C); LP-X (D); albumin (F); γ-globulin (G).

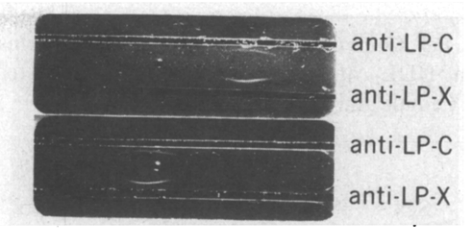

FIGURE 11: Immunoelectrophoresis of LP-C in 1% agarose gel (upper pattern) and 1% agar gel (lower pattern).

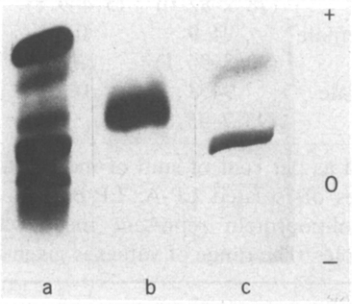

FIGURE 12: Agarose gel (1%) electrophoresis of whole human serum (pattern a stained with Amido Black 10B), LP-C (pattern b stained with Sudan Black) and whole human serum (pattern c stained with Sudan Black). Electrophoresis was carried out for 60 min employing barbital buffer, pH 8.4, ionic strength 0.1. 0, origin.

LP-C in agar and in agarose gel (Figure 11). Under identical experimental conditions, LP-C moved as a single precipitin arc in both gels; however, on agar gel it migrated slightly toward the cathode and on agarose gel toward the anode. On agarose gel electrophoresis, the LP-C migrated into the α_2-globulin region (Figure 12). The affinity of LP-C band for Sudan Black (Figure 12), Oil Red O, and Amido Black 10B indicated clearly its lipoprotein nature. Despite the appearance of a single electrophoretic band on agar or agarose gels, the LP-C was dissociated by electrophoresis on 7% polyacrylamide gel into one slow-moving band and three fast-migrating bands (Figure 3, pattern d). In contrast to the fast-moving bands, the slow-migrating band showed a very poor affinity for the protein stain; all bands were stainable lightly with Oil Red O. On 3.5% polyacrylamide gel, the LP-C migrated as a single band just slightly behind the dye front (Figure 3, pattern e). The ultracentrifugal analysis of LP-C showed a single, symmetrical peak (Figure 13) with s_{obsd} 9.5 S at *d* 1.0056 g/ml. Its relatively broad sedimenting boundary indicated most probably the presence of a variety of LP-C mole-

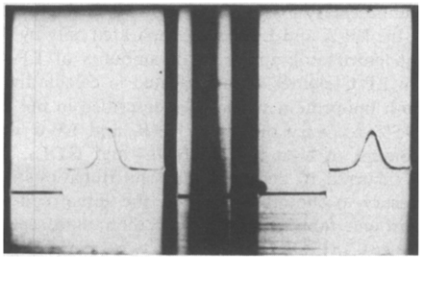

FIGURE 13: Schlieren pattern of LP-C. The lipoprotein sample (6.8 mg/ml) was dissolved in a mixture of 0.15 M NaCl and 0.01 M $(NH_4)_2CO_3$. The solvent density was 1.0056 g/ml. Photographs were taken at 24 and 32 min and a phase-plate angle of 65° after reaching a rotor speed of 56,100 rpm. Sedimentation is from left to right.

TABLE III: Per Cent Composition of Apolipoproteins A, B, and C in HDL_2 and HDL_3 Subfractions Isolated from Plasma of Female and Male Donors.[a]

Density Class	Sex	Apolipoprotein Families		
		ApoA (%)	ApoB (%)	ApoC (%)
HDL_2	Female	87.5 (86.6–88.4)	2.1 (1.8–4.1)	9.7 (8.4–11.6)
HDL_2	Male	80.3 (78.2–82.0)	7.4 (5.4–9.8)	12.2 (10.8–13.8)
HDL_3	Female	94.9 (93.8–96.1)	0	5.0 (3.8–6.2)
HDL_3	Male	94.8 (93.7–95.8)	0	5.2 (4.2–6.3)

[a] Expressed as per cent of sum of individually determined protein values of isolated LP-A, LP-B, and LP-C. Figures for each apolipoprotein represent mean values of three separate samples. The range of values is given in parentheses.

TABLE IV: Per Cent Composition of Lipoprotein Families LP-A, LP-B, and LP-C in HDL_2 and HDL_3 Subfractions Isolated from Plasma of Female and Male Donors.

Density Class	Sex	LP-A (%)	LP-B (%)	LP-C (%)
HDL_2	Female	9.00	2.2	7.8
HDL_2	Male	82.5	7.7	9.8
HDL_3	Female	94.5	0	5.5
HDL_3	Male	94.3	0	5.7

cules of differing sizes and hydrated densities. Qualitative thin-layer chromatography of the lipid extract of LP-C disclosed the presence of phospholipid, triglyceride, and esterified and unesterified cholesterol as the major lipid constituents. The quantitative composition of LP-C (Table II) with its almost equal content of protein and total lipid is very similar to those of typical high density lipoprotein preparations in general.

The 7% polyacrylamide gel electrophoresis (Figure 8) of the totally delipidized LP-C showed the typical appearance of one slow-migrating band of C-I polypeptide and four fast-migrating bands of C-II and C-III polypeptides. The presence of additional polymorphic forms of C-I and C-III polypeptides has been observed in some ApoC preparations, but further studies are necessary for their chemical characterization. Totally delipidized LP-C preparations gave positive immunoprecipitin reactions only with antibodies to LP-C.

Composition of Lipoprotein Families LP-A, LP-B, and LP-C in HDL_2 and HDL_3.[3] Results of the per cent composition of apolipoproteins (Table III) and lipoprotein families (Table IV) in two HDL subfractions corroborated the results of immunochemical analyses by demonstrating that LP-B was present only in the HDL_2 subfraction. Although the LP-A was the major lipoprotein family in both HDL subfractions, its relative content was higher in HDL_3 (94.3%; 94.5%) than in HDL_2 (82.5%; 90.0%). The HDL_3 from female and male donors had the same relative content of both LP-A and LP-C families. However, the HDL_2 subfraction from male subjects was characterized by a significantly higher relative content of LP-B ($p < 0.01$) and a lower content of LP-A ($p < 0.005$) than that from female subjects. The small difference in the per cent content of LP-C was not significant.

Calculations of absolute concentrations (mg/100 ml of plasma) of lipoprotein families (Table V) based on the data for total HDL_2 and HDL_3 concentrations (Ewing *et al.*, 1965) showed that female and male subjects had very similar concentrations of LP-B (4.8 and 5.4 mg/100 ml) in HDL_2. Similarly, the HDL_3 subfractions from both females and males contained the same amounts of LP-A and LP-C. However, since females have three times as much HDL_2 as males, the concentrations of their LP-A and LP-C families were three times as high as those from male subjects. If taken as a single density class, the female HDL are characterized by a higher amount of LP-A (422 mg/100 ml *vs.* 275 mg/100 ml) and LP-C (30 mg/100 ml *vs.* 20 mg/100 ml) than those of males. There seems to be no sex-linked difference in the HDL concentrations of LP-B.

TABLE V: Concentrations of Lipoprotein Families LP-A, LP-B, and LP-C in HDL_2 and HDL_3 Subfractions Isolated from Plasma of Normal Female and Male Donors.

Density Class	Sex	Total Lipoprotein[a] (mg/100 ml)	LP-A (mg/100 ml)	LP-B (mg/100 ml)	LP-C (mg/100 ml)
HDL_2	Female	219.0	197.1	4.8	17.1
HDL_2	Male	70.0	57.8	5.4	6.9
HDL_3	Female	238.0	224.9	0	13.1
HDL_3	Male	230.0	216.9	0	13.1

[a] Mean values for the normal female and male HDL_2 and HDL_3 subfractions were taken from Ewing *et al.* (1965).

Discussion

The separation of high density lipoproteins into three immunochemically and chemically defined LP-A, LP-B, and LP-C families provided not only a procedure for the isola-

[3] To avoid unnecessary losses of lipoproteins in the present compositional studies, the LP-A and LP-C were separated only by the hydroxylapatite column chromatography. Small amounts of LP-A (less than 5%) present in LP-C eluates were neglected in calculating the relative contents of both lipoprotein species. As described in the Experimental Section, the 95% recovery of LP-A, LP-B, and LP-C was based on the protein content of albumin-free HDL_2 and HDL_3. Due to lipoprotein losses incurred by repeated ultracentrifugations of HDL subfractions necessary to eliminate albumin, the actual concentrations of all three lipoprotein families were lower than those calculated from data by Ewing *et al.* (1965). However, in two separate experiments in which the HDL subfractions were washed only once, the values for the concentrations of lipoprotein families were comparable (±5%) to those presented in Table V. Since the repeated ultracentrifugations of HDL had no effect on the per cent composition of lipoprotein families, we concluded that the manipulation losses affected equally all three lipoprotein species.

tion and quantification of these lipoprotein species but also the experimental support for a conceptual view which regards plasma lipoproteins as a system of polydisperse lipoprotein families each of which is characterized by the presence of a single, distinct apolipoprotein or its constitutive polypeptides (Alaupovic, 1971; Alaupovic *et al.*, 1972). According to this chemical concept an apolipoprotein is defined as a protein which binds neutral lipids and phospholipids to form a soluble, polydisperse lipoprotein family. Since the human lipoprotein system consists of at least three major apolipoproteins (ApoA, ApoB, and ApoC) characterized by distinct chemical, immunological, and lipid-binding characteristics, we have postulated accordingly the existence of three major lipoprotein families. The actual isolation of LP-A, LP-B, and LP-C families has confirmed their predicted existence as separate entities of plasma high density lipoproteins. The first evidence for the existence of lipoprotein families was provided by studies on the isolation of an abnormal plasma low density lipoprotein from patients with obstructive jaundice (Seidel *et al.*, 1969). By employing a procedure which combined ultracentrifugation, heparin precipitation, and ethanol fractionation, the low density lipoproteins were fractionated into LP-A and LP-B families and an abnormal lipoprotein family designated as LP-X. The separation of the polymorphic Lp(a) lipoprotein from LP-A and LP-B by either the hydroxylapatite column chromatography (Utermann and Weigandt, 1969) or Sepharose 4B gel filtration (Ehnholm *et al.*, 1971) of human HDL_2 represents another example of a successful isolation of and evidence for the existence of lipoprotein species characterized primarily by their specific apolipoproteins as a differentiating chemical criterion.

Application of separation procedures such as precipitation with antibodies or organic solvents to lipoproteins of low hydrated densities ($d < 1.109$ g/ml) resulted in isolation of products containing mixtures of three lipoprotein families (Alaupovic, 1971). Similarly, Levy *et al.* (1966) observed that treatment of VLDL with antibodies to LP-B caused a simultaneous precipitation of LP-A and LP-B. These findings indicate that lipoprotein families exist above a certain critical density range (d 1.019–1.030 g/ml) as distinct, separable entities and below that range as weak associations. Extraction of VLDL by nonpolar solvents (Gustafson, 1965; Brown *et al.*, 1969) removes the neutral lipids and liberates lipoprotein families in the form of their phospholipid–protein residues. These residues can be separated by electrophoretic, ultracentrifugal, and immunological techniques into corresponding partially delipidized LP-A, LP-B, and LP-C (Gustafson *et al.*, 1966; Alaupovic *et al.*, 1969; Brown *et al.*, 1969). Results of immunochemical studies showed that each of these partially delipidized lipoprotein families contained only a single characteristic and specific protein moiety.

According to the present lipoprotein family concept, the recognized lipoprotein polypeptides are not considered automatically as apolipoproteins proper, *i.e.*, proteins capable of forming their own lipoproteins. Thus, the A-I and A-II polypeptides are regarded as integral structural constituents of apolipoprotein A, and C-I, C-II, and C-III of apolipoprotein C. This assumption is strengthened, if not verified, by the present results which have shown that the isolated LP-A family contains A-I and A-II polypeptides and LP-C family C-I, C-II, and C-III polypeptides. Recent results of two independent studies with monospecific antibodies to A-I and A-II polypeptides demonstrated that at least 90% of high density lipoproteins precipitated by either of these two antisera contained both polypeptides as components of their protein moiety (Albers and Aladjem, 1971; Kostner and Alaupovic, 1971a). There was also a small amount of another lipoprotein species which contained only A-I polypeptide. Whereas these findings demonstrate clearly a qualitatively identical polypeptide composition of LP-A molecules throughout most, if not all, of the high density spectrum, the differences between LP-A from HDL_2 and HDL_3 in the chemical composition, sedimentation rates, hydrated densities, and electrophoretic behavior on 7% polyacrylamide gels indicate that LP-A family represents a system of macromolecular distributions.

The isolated LP-C behaved in the agar or agarose gel immunoelectrophoresis and on 3.5% polyacrylamide gel electrophoresis as a single, homogeneous entity. It contained all three typical ApoC–polypeptides (Figure 8). However, despite the fact that each of these polypeptides, in their delipidized forms, has a different migration rate in agar or agarose gel, the LP-C migrated under identical experimental conditions as a single band. Similarly, on immunodiffusion it gave a single immunoprecipitin line even with antisera which contained antibodies to all three ApoC–polypeptides. Our preliminary experiments with monospecific antibodies indicated that all three ApoC–polypeptides were required for the formation of a high density LP-C (Kostner and Alaupovic, 1971a). In contrast to its electrophoretic behavior on agar and 3.5% polyacrylamide gels, the LP-C sample dissociated on 7% polyacrylamide gel into one slow-moving and three fast-moving bands similar in their migration rates to those of delipidized ApoC–polypeptides. One can only speculate whether these bands represent dissociation products of a very fragile and complex LP-C structure or distinct lipoproteins characterized by the presence of a single specific apolipoprotein. If clustered phospholipid–polypeptide units represent the structural framework of native lipoprotein species or families, the weak linkages between these elementary structural components may be disrupted during electrophoresis in a molecular sieve such as 7% polyacrylamide gel and an otherwide single lipoprotein entity may appear as multiple bands of dissociated phospholipid–polypeptide residues. It is conceivable that a similar dissociation of lipoprotein families LP-A and LP-C may also occur during prolonged ultracentrifugation or other laboratory manipulations of native lipoproteins. This may possibly be the explanation for the observed conversion of one HDL form into another (Levy and Fredrickson, 1965) as well as for the presence in the very high density lipoproteins of a lipoprotein species with A-I polypeptide as the only protein moiety (Albers and Aladjem, 1971).

It has been demonstrated independently by two groups of investigators (Utermann and Wiegandt, 1969; Ehnholm *et al.*, 1971) that immunochemically reactive LP-B in HDL_2 consists of a mixture of the normal LP-B and its polymorphic Lp(a) form. These two lipoprotein species can be separated preparatively by hydroxylapatite or Sepharose 4B chromatography or distinguished analytically by 3.75% polyacrylamide gel electrophoresis. Although it is apparently not possible to separate the LP-B and Lp(a) lipoprotein bands by 7% polyacrylamide gel electrophoresis, the difference in the chemical composition between the LP-B preparation isolated in this study and Lp(a) lipoprotein isolated by Simons *et al.* (1970) suggested that lipoproteins precipitated from HDL_2 by antibodies to LP-B consist of both lipoprotein species. Furthermore, an examination of all published 3.75% polyacrylamide gel patterns of individual HDL_2 samples revealed the presence of normal LP-B band even in cases characterized by the absence of Lp(a) lipoprotein (Garoff *et al.*, 1970;

Simons *et al.*, 1970, Utermann and Weigandt, 1970). We consider on the basis of this evidence that lipoproteins precipitated from HDL_2 by immunoadsorber to LP-B consist either of normal LP-B family or of a mixture of LP-B and varying amounts of the Lp(a) variant. Since Lp(a) lipoprotein shares the common antigenic determinant(s) with LP-B, it is classified according to the lipoprotein family concept as a subfamily of LP-B. Similarly, since the protein moiety of LP-X consists of ApoC–polypeptides and albumin (Alaupovic *et al.*, 1969), the LP-X is classified as a subfamily of LP-C (Alaupovic, 1971).

The present separation procedure was applied to a study of the per cent composition and absolute concentrations of three major lipoprotein families in the high density lipoprotein class of normal men and premenopausal normal women. Results of this study have shown clearly that the LP-A family comprises 90% of the total high density lipoprotein class of both female and male subjects. Although there is no sex-linked difference in the per cent composition of LP-A (91.6% in males and 92.3% in females), LP-B (1.8% and 1.1%, respectively), and LP-C (6.7% and 6.6% respectively), the entire high density lipoprotein class from female donors contains approximately 50–60% higher concentrations of LP-A (422.0 mg/100 ml) and LP-C (30.0 mg/100 ml) than the corresponding density class from male subjects (275.0 mg/100 ml of LP-A and 20.0 mg/100 ml of LP-C). The significantly higher lipoprotein concentration of female HDL_2 subfraction is the obvious factor responsible for this difference. It is hoped that similar distribution studies with either normal subjects maintained on different dietary regimens or with patients with various types of hyperlipoproteinemias may indicate the possible metabolic significance of this sex-linked difference in particular and contribute to a better understanding of the lipid transport process in general.

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