Evaluation of gel chromatography for plasma lipoprotein fractionation

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ABSTRACT The fractionation of lipoproteins of normal and hyperlipidemic subjects on a column of 2% agarose was compared with ultracentrifugation and paper electrophoresis procedures. The following results were obtained. (*a*) Plasma lipoproteins were eluted successively from the column in the four overlapping peaks of chylomicrons, very low density lipoproteins, low density lipoproteins, and high density lipoproteins. (*b*) Very low density lipoproteins and high density lipoproteins (d > 1.063, containing nonlipoprotein proteins) showed continuous progressive changes in lipid composition as these fractions emerged, while low density lipoproteins showed a relatively constant lipid composition. (*c*) A discontinuous transition of lipid composition was observed when consecutive ultracentrifugal fractions were placed on the column. (*d*) The "trail" of pre-beta lipoprotein seen on paper electrophoresis was shown to consist of particles whose molecular sizes range between chylomicrons and pre-beta lipoproteins. A reverse relationship was observed between electrophoretic mobilities of "trail" components and their particle size. (*e*) Gel with an agarose content of 2% seemed to fractionate chylomicrons and very low density lipoproteins more effectively than other lipoprotein classes.

SUPPLEMENTARY KEY WORDS agarose gel · hyperlipidemia · thin-layer chromatography · paper electrophoresis · ultracentrifugation

To separate LP by gel chromatography, cross-linked dextrans have been used most commonly. However, the largest pore size available among such gels (Sephadex G-200) is relatively small for the fractionation of LP classes (1–3). However, gels of agarose (linear galactose polymers, the nonionic constituent of agar [4]) have much larger pores and have been shown to be more suitable for the separation of LP classes (5–9). Comparing 2%, 4%, and 6% agarose beads, 2% agarose offers better resolution of LP with larger molecular sizes such as chylomicrons and VLDL, while 4% and 6% agarose are most suitable for the separation of LDL from HDL (5, 6).

These data, however, are not sufficient to define the behavior of plasma or serum lipoproteins on an agarose gel column because of a lack of systematic analyses of lipid components in the eluates and inadequate comparisons of gel chromatography with the more traditional methods of separation, namely ultracentrifugation and paper electrophoresis.

The purpose of the present study was to evaluate the potential of agarose gel chromatography for plasma lipoprotein fractionation using the technique developed in this laboratory by Hanai, Wood, Michaels, and Kinsell (9).

METHODS

Preparation and Operation of Agarose Columns

A column with total bed volume of 450 ml (90–92 × 2.5 cm) was prepared with 2% agarose beads (Bio-Gel A 50m. 100–200 mesh; Bio-Rad Labs, Richmond, Calif.). The water-jacketed column (Sephadex Laboratory Col-

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); LP, lipoprotein(s); TC, total cholesterol; PL, phospholipid; TG, triglyceride; TL, total lipid; EDTA, ethylenediaminetetraacetic acid.

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umn, Type K 25/100, jacketed; Pharmacia Fine Chemicals Inc., Piscataway, N. J.) was filled with eluant and allowed to stand overnight. Nylon netting (400 mesh) was used as a filter at the bottom of the tube. The suspension of beads was pipetted into the column. After sedimentation, excess fluid was drawn off from the top of the column. In this manner the column was packed loosely to the top, and then the excess eluant was allowed to drain from the bottom. A suspension of beads was then pipetted on to the top of the column until the level of packed gel reached the desired height. A disc of nylon netting (400 mesh) was placed on top of the gel to prevent disturbance of the gel surface when samples were applied. The water in the jacket around the column was maintained at 17–18°C.

The EDTA-treated human plasma (see below) was layered on top of the agarose column; when the top of the sample disappeared into the bed, collection of eluate in 5-ml cuts was commenced using an automatic fraction collector. 0.2 M NaCl in water with 0.001 M EDTA (disodium salt), pH 7, was used as eluant, under a pressure head of 40–60 cm of water (9).

Column operating conditions were checked periodically with respect to reproducibility of elution volume of blue dextran-2000 (Pharmacia Fine Chemicals Inc.) and human albumin. As Werner reported, blue dextran-2000 produced two peaks (5). The Kd values (Kd = [Ve − Vo]/[Vt − Vo], where Ve, Vt, and Vo are, respectively, elution volume, total bed volume, and void volume of the column [10]) of human albumin and of the second peak of blue dextran-2000 were virtually constant.

The column could be used 20–25 times to fractionate samples including whole plasma, ultracentrifugal fractions of plasma, blue dextran-2000, and human albumin. The initial flow rate was about 35 ml/hr. When the flow rate fell to about 20 ml/hr, the column was repacked. Usually 12–20 hr were required for the fractionation of one sample.

Blood Samples

Blood from subjects who had been fasted overnight was collected in tubes containing EDTA (1.2 mg/ml). Plasma was prepared and stored at 4°C, and most analyses or fractionations were begun on the day of collection. LP fractions were separated using a Spinco Model L preparative ultracentrifuge with a 40.3 rotor (11). VLDL, LDL, and HDL were fractions with d < 1.006, d1.006–1.063, and d > 1.063, respectively. As the > d 1.063 fraction, referred to as HDL in the present study, was not fractionated further, it contained nonlipoprotein proteins. Ultracentrifugal fractions were applied to the column without dialysis. Usually 10–15 ml of whole plasma was applied. In the case of ultracentrifugal fractions the amount of the specific fraction obtained from 9–15 ml of whole plasma was used.

Paper Electrophoresis

Paper electrophoresis was performed by the method of Lees and Hatch (12). Eluates from the column were concentrated as follows before they were subjected to paper electrophoresis. Sephadex G-25 (coarse) was suspended in the eluates (1 g of Sephadex per 5 ml of eluate) and allowed to gel for 24 hr in a refrigerator at 4°C. The swollen gel with some of its water layer was transferred into a Boerner centrifuge tube (Arthur H. Thomas, Co., Philadelphia, Pa.) with 400 mesh nylon netting at the bottom. Concentrated eluates were recovered after the tubes were centrifuged at 2000 rpm for 10 min at 4°C. The eluates were concentrated approximately 8–10 times by carrying out the concentration procedure twice more.

Analytical Methods

The protein content and turbidity of the eluates were monitored by reading absorbance at 280 nm and 700 nm, respectively, with the realization that protein content is not correctly represented in the presence of turbidity. Three adjacent 5-ml cuts (in some cases two cuts) were pooled, and lipids were extracted by a slight modification of Carlson's method (13). Thin-layer chromatography on silica gel was carried out, and the lipids were visualized after charring with 50% sulfuric acid and heating to 250°C (14).

Total cholesterol was determined by the method of Zak, Dickenman, White, Burnett, and Cherney (15) after alkaline hydrolysis and precipitation by digitonin according to the method of Sperry and Webb (16). Lipid phosphorus was determined by a modification of the Fiske and Subbarow method (17). For determination of glycerides, a preliminary separation of phospholipid from glyceride was performed by a batch method using activated silicic acid and isopropyl ether (18). Saponification and estimation of glyceride–glycerol was accomplished by a combination of the methods of Carlson (13) and Hanahan and Olley (19). Triolein was used as a standard.

Calculations

Values for diffusion coefficient (Kd) of an eluted component were obtained from elution volume, total bed volume, and void volume of the column. Since successive 10- or 15-ml eluates were pooled as a single fraction, the median elution volume for this fraction was used in calculation of the Kd value. Kd values thus derived contained errors as great as ±0.03 units. The elution volume of the first peak of blue dextran-2000 was reasonably constant, but in some cases it did not coincide with

the first absorbance peak of plasma. As the significance of the difference in the elution volumes of these two peaks was not clear, the elution volume of the first peak of blue dextran was arbitrarily used as the void volume of the column. The first peak was produced by the elution of large particles of unhydrolyzed polysaccharide.

Phospholipid was expressed as lipid phosphorus × 25. Percentage contribution of individual lipids to TL of an eluted LP was calculated on the assumption that TL = TC + PL + TG.

RESULTS

The Effect of the Molar Concentration of Sodium Chloride on the Resolution of Plasma LP Classes

The elution patterns of plasma LP from a 2% agarose column were examined using varying concentrations of sodium chloride in the eluant. For this purpose, blue dextran-2000, human albumin, and 10 ml of plasma from a patient with type V hyperlipidemia (20) (TC, 235 mg/100 ml; PL, 334 mg/100 ml; TG, 969 mg/100 ml) were used as test materials. Samples were applied to the column separately and were eluted with 0.2, 0.4, 0.6, 0.8 M NaCl. In each case the eluant contained 0.001 M EDTA (disodium salt), pH 7. No significant effect on elution patterns or Kd values of specific lipid peaks was observed with these changes in electrolyte concentration when eluates were collected in 15-ml fractions. Since a high concentration of salt impedes the extraction of lipid from LP (21) and produces aggregation of protein, 0.2 M NaCl was used throughout the present study.

Recovery of Lipid from the Column

There were good recoveries of lipid components from the column: TC 97.5 ± 5.2% (mean ± SD), PL 94.7 ± 3.6%, and TG 97.9 ± 10.0%. Ranges of recoveries of TC, PL, and TG were 88.6–103.7%, 87.7–102.9%, and 89.0–115.6%, respectively. Average percentage recoveries of lipids and their standard deviations were calculated from 28 samples including whole plasma and ultracentrifugal fractions in which three lipid components, TC, PL, and TG were determined in each fraction.

Capacity of the Column

Normolipidemic and hyperlipidemic plasmas were chromatographed to determine the capacity of the column. In each case, 10, 15, and 20 ml of plasma were separately applied to the column. Although the application of larger amounts of plasma to the column tends to require a larger volume to elute the dominant lipid peaks, elution patterns of the lipid components and percentage compositions of fractions from 10-, 15-, and 20-ml loads from the same individuals were quite similar. However, in one separation (see Fig. 6*C* below) the column appeared to be overloaded with respect to the material of the second peak as evidenced by the high proportion of TG toward the end of the fraction. This indicates that the maximal amount of plasma which can be applied to the column depends on the lipid content of the specific fraction.

The Elution Pattern of Plasma LP from 2% Agarose Columns and its Relation to Ultracentrifugation

Fig. 1 shows the elution pattern for plasma of a patient with type V hyperlipidemia (20). Recoveries of TC, PL, and TG were 94.0%, 93.5%, and 98.5%, respectively. Measurement of absorbance at 280 nm and lipid analysis of the eluates showed four overlapping peaks (Fig. 1*A* and *B*). Material accounting for the marked turbidity of this plasma was eluted mostly in the first peak of the absorbance curve at 700 nm. The major lipid com-

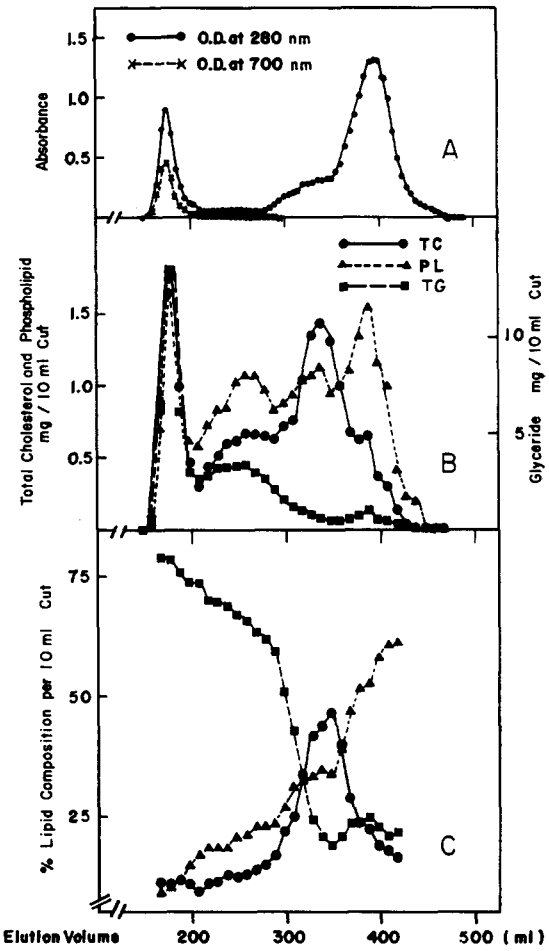

FIG. 1. Gel chromatography of whole plasma on 2% agarose column: 10 ml plasma from a 37-year-old male with type V hyperlipidemia. Plasma lipids: TC, 208 mg/100 ml; PL, 275 mg/100 ml; TG, 650 mg/100 ml. Eluates were diluted 1:10 for determination of absorbance at 280 nm (*A*). %TC + % PL + % TG = 100% (*C*).

ponent in this fraction was TG, comprising 78.9% of the TL (Fig. 1*B* and *C*). The 280 nm-absorbancy tracing of the second peak was low and wide, and the fractions from this region were slightly opalescent (Fig. 1*A*). Lipid analysis of the eluate in this region showed a rather broad peak with a maximum at an elution volume of 255 ml (Fig. 1*B*). The percentage content of TG decreased in successsive fractions from about 74% to 50% (Fig. 1*C*). Absorbancy at 280 nm revealed a shoulder (termed "the third absorbance peak") on the ascending limb of the main peak (Fig. 1*A*). The third lipid peak appeared with a maximum at an elution volume of 335 ml (TC, 44.2%; PL, 34.8%; TG, 21.0%) (Fig. 1*B* and *C*). The fourth and largest absorbance peak appeared with a maximum at an elution volume of 395 ml (Fig. 1*A*), while the maximum lipid concentration in this area slightly preceded the absorbancy peak (385 ml) (Fig. 1*B*). PL was the main lipid component in this area (Fig. 1*B*) with its contribution to total lipid increasing from 47.3 to 63.2% across the peak (Fig. 1*C*).

Fig. 2 shows the elution patterns of VLDL, LDL, and HDL from the plasma whose fractionation is shown in Fig. 1. The LP fractions were applied separately to the same column. The relationships among these fractions and the peaks obtained by gel chromatography of the whole plasma were as follows: (*a*) the first and second peaks of whole plasma were included in the VLDL (d < 1.006) fraction; (*b*) lipid analysis of the eluate showed that the third and fourth peaks in Fig. 1*B* coincided approximately with the LDL and HDL fraction, respectively; (*c*) the third absorbance peak (Fig. 1*A*) was included in the HDL (d > 1.063) fraction (Fig. 2*B*) and contained little lipid (Fig. 2*E*).

The lipid and absorbance peaks of ultracentrifugal fractions were eluted slightly earlier than the corresponding peaks from whole plasma and, as shown in the absorbancy tracing of HDL (Fig. 2*B*), their elution patterns were broader than those of the original plasma. This may have been caused by the salts in the ultracentrifugal fractions. The distribution of each lipid component of the ultracentrifugal fractions as a function of elution volume was similar to that of whole plasma. The percentage composition of individual lipids in the three ultracentrifugal fractions is shown in Table 1 for representative tubes obtained during the elution of each peak.

Fig. 3 shows the elution pattern of a normolipemic plasma sample. Recoveries of lipids were: TC, 101.6%; PL, 91.5%; and TG, 102.5%. Comparing Figs. 1 and 3, the absorbance peaks in Fig. 3*A* apparently correspond with the third and fourth peaks in Fig. 1*A*. The principal lipid constituent of the initial, small peak was TG (Fig. 3*C*). The second major TG peak (elution volume 297.5 ml, TC 38.8%, PL 30.1%, TG 31.2%) was not coincident with the peaks of TC and PL (elution volume 327.5

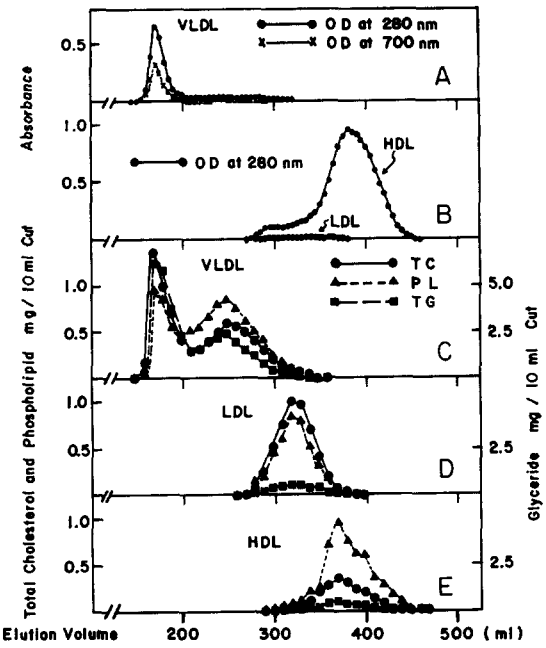

FIG. 2. Gel chromatography of VLDL (d < 1.006 fraction), LDL, and HDL (d > 1.063) applied separately to a 2% agarose column. Same individual as Fig. 1. Volume applied: VLDL, 3 ml; LDL, 6 ml; HDL, 18 ml. The amount of each ultracentrifugal fraction corresponds to 9 ml of original whole plasma. See legend to Fig. 1.

ml, TC 44.7%, PL 35.0%, TG 20.3%) (Fig. 3*B*), which contrasts with the results shown in Fig. 1. The concentration peaks of lipids eluted towards the end of the fractionation (Fig. 3*B*) showed a pattern similar to that in Fig. 1*B*.

A thin-layer chromatogram of successive fractions from the plasma separation depicted in Fig. 3 is shown in Fig. 4.

TABLE 1 LIPID COMPOSITION OF LIPOPROTEINS IN REPRESENTATIVE ELUTION VOLUMES DURING GEL CHROMATOGRAPHY OF ULTRACENTRIFUGAL FRACTIONS

	Fraction	Elution Volume	TC	PL	TG
		ml	*% of total**		
Subject 1 (Fig. 2)					
VLDL	1st peak	160–170	15.6	10.9	73.4
	Between 1st and 2nd peak	190–200	13.3	15.2	71.5
	2nd peak	240–250	15.1	22.1	62.8
		280–290	19.6	28.2	52.2
LDL	Peak	310–320	42.0	34.9	23.1
HDL	Peak	360–370	21.0	55.3	23.7
Subject 2 (Fig. 5)					
VLDL	1st peak	170–185	25.5	15.3	59.1
	Between 1st and 2nd peak	200–215	23.3	19.7	56.9
	2nd peak	290–305	32.2	28.4	39.4
LDL	Peak	320–335	48.6	38.1	13.3
HDL	Peak	395–410	27.2	63.9	8.9

* Total lipid is considered to be the sum of TC, PL, and TG.

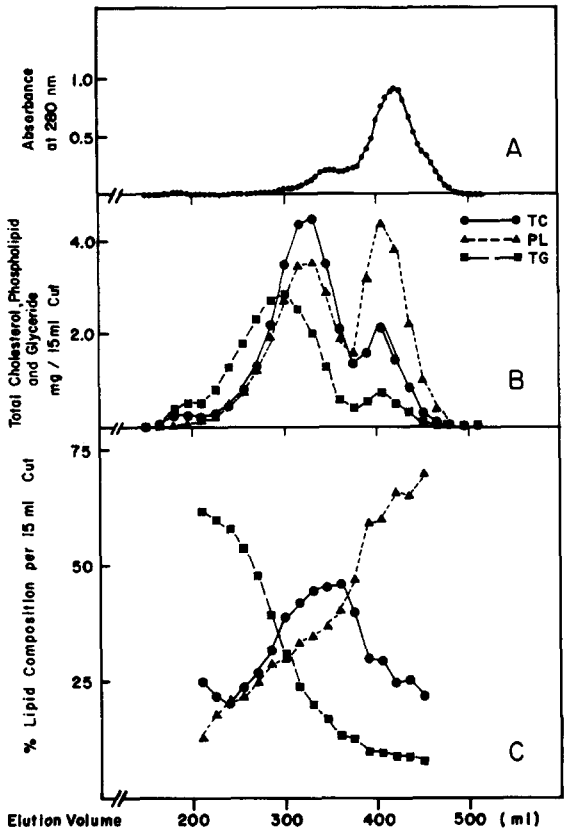

FIG. 3. Gel chromatography of whole plasma on 2% agarose column: 15 ml of plasma from a 55-year-old normolipemic male with angina pectoris. Plasma lipids: TC, 207 mg/100 ml; PL, 260 mg/100 ml; TG, 145 mg/100 ml. Eluates were diluted 1:15 for the determination of absorbance at 280 nm.

The elution pattern of lipids is indicated by the relative densities of lipid spots. The free fatty acids present in the later fractions were bound to albumin which is eluted in this area. The absence of trailing on the chromatogram suggests that little degradation of lipids occurred during processing.

Fig. 5 shows the gel chromatogram of VLDL, LDL, and HDL ultracentrifugal fractions of this plasma. Fractionation was as above (Fig. 2). Comparing Figs. 3*B*, 5*D*, and 5*E*, the second TG peak and the second peaks of TC and PL in Fig. 3*B* had the same elution volume as VLDL (Fig. 5*D*) and LDL (Fig. 5*E*), respectively. The Kd values of the second lipid peak of VLDL and the lipid peak of LDL were 0.27 and 0.53 in Fig. 2, and 0.42 and 0.54 in Fig. 5, respectively. The difference between the elution patterns in the area of VLDL and LDL of Fig. 3*B* and those of Fig. 1*B* seems to be attributable to clear differences in the Kd values of VLDL in these two subjects. The percentage composition of individual lipids in the three ultracentrifugal fractions is shown in Table 1 for representative tubes obtained during the elution of each peak.

The following observations may be made from Figs. 1 and 3: (*a*) in each case, as the fractionation proceeded, the proportion of TG decreased sharply in the area between VLDL and LDL; (*b*) the proportion of PL increased gradually initially, and there was a rapid increase between LDL and HDL; (*c*) TC increased from the chylomicron area to a peak of concentration in the LDL region, after which it declined rapidly across the HDL area.

Relationship Between Gel Chromatography, Ultracentrifugation, and Paper Electrophoresis

Fig. 6 shows the elution pattern for plasma from a male patient with type V hyperlipidemia. The absorbance curves at 280 nm and 700 nm showed peaks similar to those of Fig. 1. Lipid analysis of the eluates showed three peaks with elution volumes of 167.5 ml (TC 18.8%, PL 9.3%, TG 71.8%); 257.5 ml (TC 18.9%, PL 18.7%, TG 62.4%); and 407.5 ml (TC 21.0%, PL 54.8%, TG 24.3%) (Fig. 6*B*). Gel chromatography of VLDL ($d < 1.006$), LDL, and HDL ($d > 1.063$) fractions were performed in a manner similar to the previous case. The elution patterns are shown in Fig. 7.

From Figs. 6 and 7 it is clear that the LDL peak was hidden in the broad and large second peak of VLDL. Fig. 8 shows the pattern of paper electrophoresis of the pooled, concentrated eluates, whole plasma, and the ultracentrifugal fractions. The elution volumes correspond to those in Fig. 6. Paper electrophoretograms of the fractions of elution volume 310–325 ml and 325–340 ml showed both pre-beta and beta LP while those with elution volumes of 340–355 ml and 355–370 ml showed only the beta LP band. These relationships showed that LDL was concealed under the descending limb of a dominant pre-beta LP peak and did not appear as a peak on the gel chromatogram of whole plasma.

Paper electrophoretograms of fractions eluted between 160 and 235 ml showed LP with increasing mobility. From the pattern of paper electrophoresis of the VLDL fraction (Fig. 8B), these LP seemed to be the trail of pre-beta LP. This agrees with the suggestion of Smith (22) and Lees and Fredrickson (23), that the molecular sizes of particles making up the trail range between those of chylomicrons and the pre-beta LP. These findings indicate an inverse relationship between size and electrophoretic mobility on paper for these particles.

The relationships among gel chromatography, ultracentrifugation, and paper electrophoresis of LP are summarized in Table 2.

DISCUSSION

The results obtained in the present studies strongly suggest that LP are fractionated on the basis of molecular

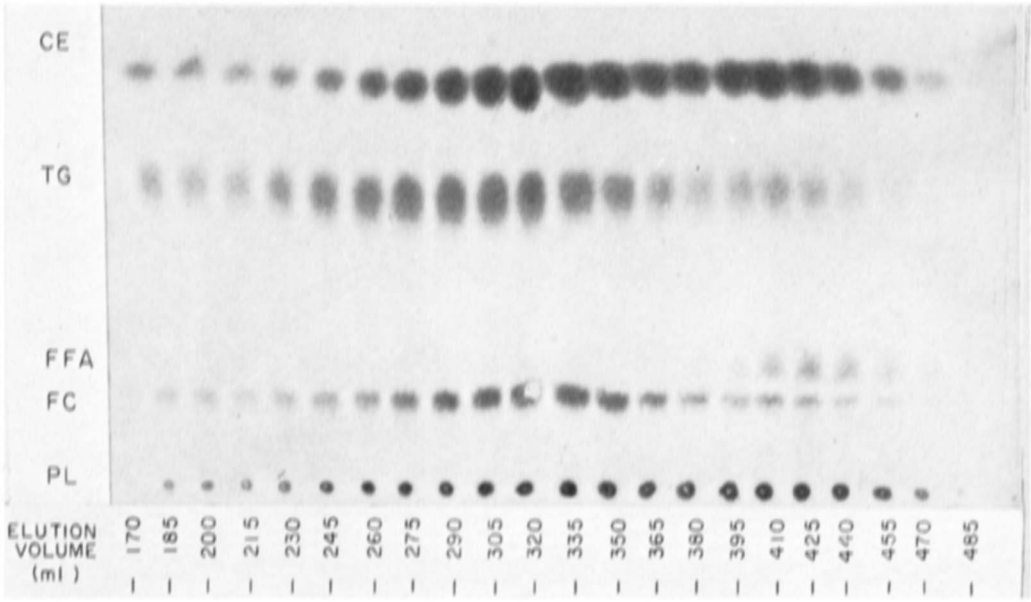

FIG. 4. Thin-layer chromatogram of fractions shown in Fig. 3. CE, cholesterol esters; TG, triglycerides; FFA, free fatty acids; FC, free cholesterol; PL, phospholipids.

size by gel chromatography on an agarose column. LP fractions showed the expected changes in lipid composition as elution progressed. The direct relationship between electrophoretic mobility of the trail of pre-beta LP and elution volume shows that the trailing is a function of particle size. These large particles in the trail have physical and chemical properties which are intermediate between chylomicrons and pre-beta LP (22–25). 2% agarose seems most effective for fractionation of LP with particle sizes ranging from chylomicrons to VLDL, as shown by the paper electrophoretograms and the wide VLDL peak with continuously changing lipid composition.

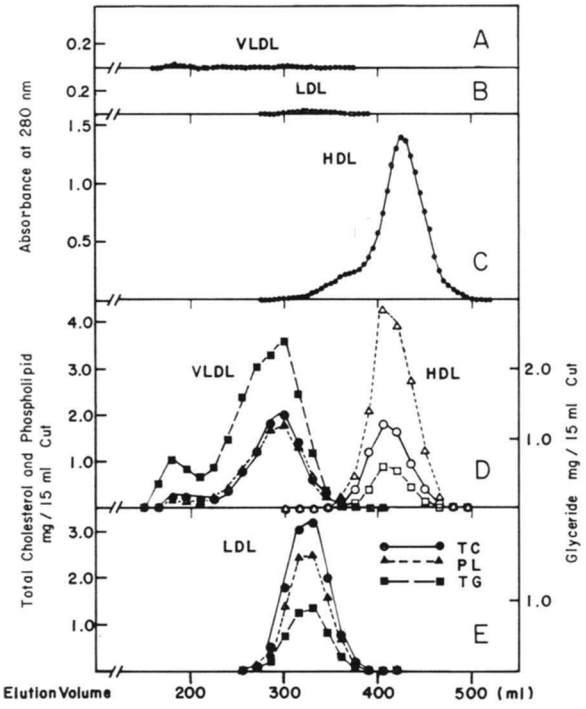

FIG. 5. Gel chromatography of VLDL (d < 1.006 fraction), LDL, and HDL (d > 1.063) applied separately to a 2% agarose column. Same individual as Fig. 3. Volume applied: VLDL, 5 ml; LDL, 5 ml; HDL, 20 ml. The amount of each ultracentrifugal fraction corresponds to 15 ml of original whole plasma. Eluates were diluted 1:10 for the determination of absorbance at 280 nm.

Werner (5) and Hanai et al. (9) reported reasonably complete recoveries (more than 90%) of TL, TC, and radioactivity (cholesterol-^{14}C), while Kalab and Martin (8) and Akanuma and Glomset (26) experienced lower recoveries which they ascribed to residual sulfated polysaccharide in their agarose preparations. In the present studies good recoveries of lipid constituents were obtained, confirming that LP are not appreciably adsorbed to the agarose beads.

TABLE 2 RELATIONSHIP BETWEEN GEL CHROMATOGRAPHY, ULTRACENTRIFUGATION, AND PAPER ELECTROPHORESIS

Gel Chromatography		Paper Electrophoresis (Staining for Lipids)	Ultracentrifugation
Absorption Curve	Lipid Analysis		
1st Peak	1st Peak	Chylomicrons (Origin)	VLDL (d < 1.006)
Between 1st and 2nd Peak		Trail of pre-beta lipoprotein	
2nd Peak	2nd Peak	Pre-beta lipoprotein	
	3rd Peak	Beta lipoprotein	LDL (d 1.006–1.063)
3rd Peak		?	HDL (d 1.063–1.21) *plus* nonlipoprotein proteins
4th Peak	4th Peak	Alpha lipoprotein	

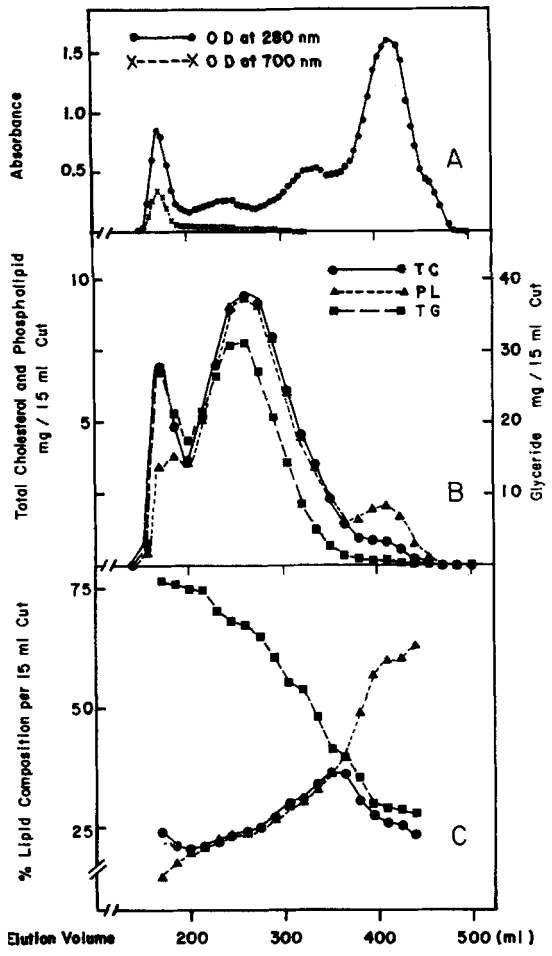

FIG. 6. Gel chromatography of whole plasma on a 2% agarose column: 15 ml plasma from a 46-year-old male with type V hyperlipidemia. Plasma lipids: TC, 550 mg/100 ml; PL, 554 mg/100 ml; TG, 1622 mg/100 ml. Eluates were diluted 1:15 for determination of absorbance at 280 nm.

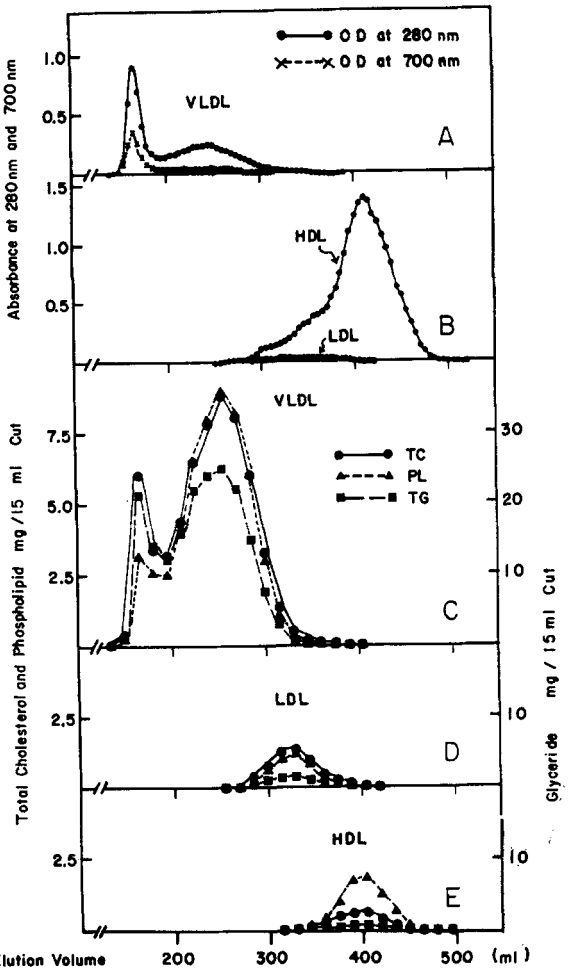

FIG. 7. Gel chromatography of VLDL (d < 1.006 fraction), LDL, and HDL (d > 1.063) applied separately to a 2% agarose column. Same individual as Fig. 6. Volume applied: VLDL, 10 ml; LDL, 5 ml; HDL, 20 ml. The amount of each ultracentrifugal fraction corresponds to that contained in 15 ml of original whole plasma.

Our findings suggest that 2% agarose gel provides a satisfactory column material for fractionation of plasma lipoproteins, and fulfills the following criteria (27): (*a*) a suitable pore size for fractionation of LP; (*b*) absence of irreversible adsorption of plasma LP; and (*c*) a solid and relatively noncompressible framework to maintain a reasonable flow rate.

As shown in Figs. 1*C*, 3*C*, and 6*C*, the relative amounts of TG in the first lipid peak are lower than those reported for chylomicrons obtained from plasma by ultracentrifugation (14, 28). On the other hand, the first lipid peak obtained on the agarose column from an analysis of plasma from normolipemic individuals who had been given fat-loads had a lipid composition quite similar to that of chylomicron fractions obtained by ultracentrifugation.[1] The paper electrophoretogram of the first lipid peak shown in Fig. 6*A* (Fig. 8*A*, elution volume 160–175 ml) shows that 2% agarose columns exclude materials with slow mobility on paper electrophoresis, in addition to chylomicrons which remain at the origin on paper. The relatively low content of TG as compared with chylomicrons in the first lipid peak may be attributable to the presence in this fraction of two kinds of particles with slow and zero mobility on paper electrophoresis. The general pattern of LP lipid composition observed during gel chromatography in the present study is quite similar to that obtained by ultracentrifugation (28–30).

It has been reported that LDL are a morphologically (31) and chemically (32) homogeneous group. This is confirmed by the relative narrowness and symmetry of the lipid peak of this fraction (Fig. 2*D*, 5*E*, and 7*D*) as well as by the compact bands on the electrophoretograms compared with the more diffuse bands of VLDL and HDL components. It is well known that VLDL are mor-

[1] Sata, T. Unpublished observation.

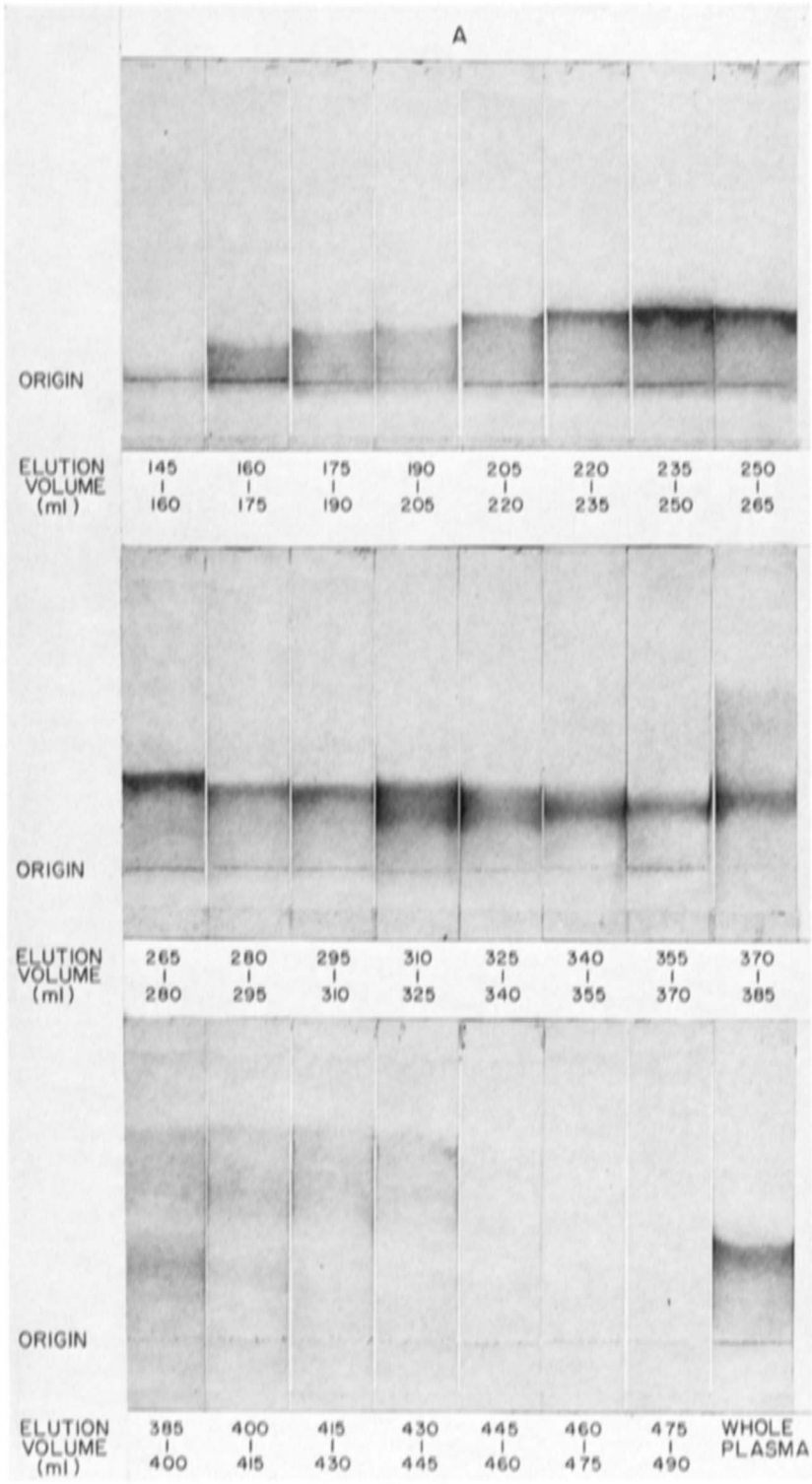

FIG. 8

phologically and chemically heterogeneous (32, 33). The Kd value of the second TG peak in whole plasma was quite variable in different subjects, suggesting different mean diameters of VLDL particles. As a general tendency the Kd values of the second TG peak of the plasma of normal subjects and patients with type II hyperlipidemia were greater than those of patients with types IV or V hyperlipidemia. This suggests that the average particle size of VLDL in types IV and V hyperlipidemia is larger than that seen in type II hyperlipidemia and in normals. The variability of electrophoretic mobility of the pre-beta band on both cellulose acetate (34) and agarose gel (35)

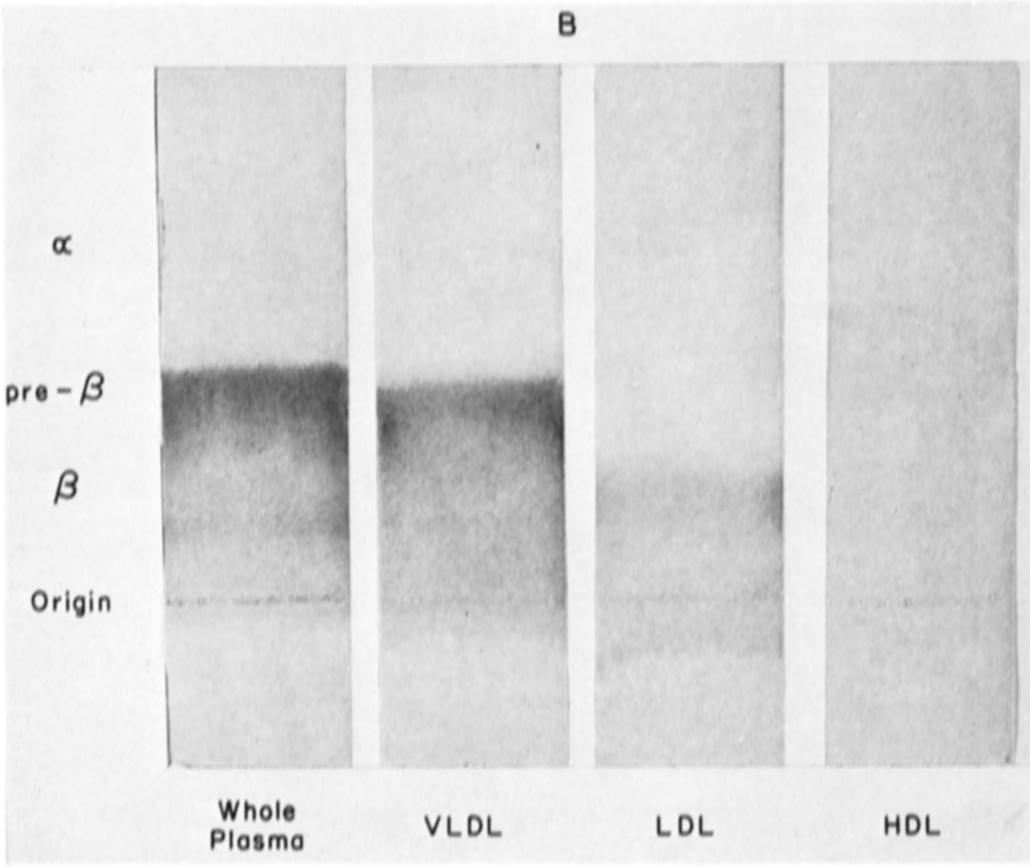

FIG. 8. (*A*) Paper electrophoretogram of column eluates from fractionation shown in Fig. 6. Eluates were concentrated approximately 10 times using Sephadex G-25, before being applied to the paper. (*B*) Paper electrophoretogram of whole plasma and ultracentrifugal fractions. Same plasma as that used in Figs. 6, 7, and 8*A*.

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is almost certainly related to the difference in mean molecular size of VLDL in different individuals. The relationship between molecular size of VLDL and plasma TG value seems to be explained by the concept presented by Fredrickson, Levy, and Lees (36).

The third absorbance peak consists of at least two components, one of which is LDL; the other component has a density greater than 1.063 and contains little lipid. Subsequent studies have suggested that this material contains mainly fibrinogen.[2] In the present study, HDL was not separated from the $d > 1.21$ fraction so that plasma nonlipoprotein protein, free fatty acids, and lysolecithin were also present.

Detailed subfractionation of $d < 1.006$ LP would be a formidable task by available ultracentrifugal procedures (33, 37, 38); however, the combination of preparative ultracentrifugation and chromatography on 2% agarose gel seems to provide a practical approach to this problem.

[2] Sata, T., and R. J. Havel. Unpublished data.

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