Plasma Lipoproteins in Familial Lecithin:Cholesterol Acyltransferase Deficiency

FURTHER STUDIES OF VERY LOW AND LOW DENSITY LIPOPROTEIN ABNORMALITIES

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ABSTRACT Plasma lipoproteins of $d < 1.006$ g/ml, d 1.006–1.019 g/ml, and d 1.019–1.063 g/ml from patients with familial lecithin: cholesterol acyltransferase deficiency yielded abnormal subfractions upon being separately filtered through 2% agarose gel. A subfraction that emerged with the void volume and contained unusually large amounts of unesterified cholesterol and phosphatidylcholine was present in each lipoprotein group, and in each group this subfraction was less prominent in the nonlipemic plasma of one patient than in the lipemic plasma of other patients. A subfraction containing smaller lipoproteins also was present in each lipoprotein group. These lipoproteins were of the same size as normal lipoproteins of the corresponding density, but contained abnormally small amounts of cholesteryl ester. The lipoproteins of 1.019–1.063 g/ml contained abnormal components of intermediate molecular weight as well as large and small abnormal components similar to those described previously. The intermediate components were more prominent in the nonlipemic plasma but were easily recognized in the hyperlipemic plasma as a peak of S_f 20–30 in the analytical ultracentrifuge. Also they could be recognized, upon electron microscopy of the lipoproteins of d 1.019–1.063 g/ml, as particles 340–1000 Å in diameter.

The data suggest that related large, abnormal particles pervade the patients' very low and low density lipoproteins, and that the large particles are affected by, but are not dependent on, the lipemia that frequently accompanies the disease. The smaller very low and low density lipoproteins appear to be counterparts of lipoproteins present in normal plasma. Their abnormal composition is compatible with the possibility that lecithin: cholesterol acyltransferase normally decreases the triglyceride and phosphatidylcholine and increases the cholesteryl ester of very low density and low density plasma lipoproteins in vivo.

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INTRODUCTION

Human plasma contains an enzyme that can catalyze formation of cholesteryl esters by transferring fatty acids from the lecithin (phosphatidylcholine [PC])[1] to the unesterified cholesterol (UC) of high density lipoproteins (HDL). Although this lecithin: cholesterol acyltransferase (LCAT) appears to form most of the cholesteryl esters (CE) of human plasma lipoproteins (1, 2), its physiological role remains to be clarified. Thus, it is not clear why more than two-thirds of the plasma cholesterol is esterified nor why most of the

[1] *Abbreviations used in this paper:* CE, cholesteryl ester; HDL, high density lipoprotein; LCAT, lecithin: cholesterol acyltransferase; LDL, low density lipoprotein; PC, lecithin; PCMPS, *p*-chloromercuriphenylsulfonate; S, sphingomyelin; TG, triglyceride; UC, unesterified cholesterol; VLDL, very low density lipoprotein. K_d, the distribution coefficient for a solute eluted upon gel filtration $= V_e - V_o / V_i$, where V_e is the elution volume of the solute and V_o and V_i are determined from the elution volumes of tobacco mosaic virus and H^3_2O, respectively.

TABLE I

Plasma Lipoprotein Lipids and Protein of Patients L. G., I. S., and M. R.

Patient and lipoprotein class	UC	PC	S	CE	TG	Protein
			μmol/ml plasma			*μg/ml plasma*
d < 1.006 g/ml						
Normal*	0.084±0.074	0.077±0.008	0.011±0.011	0.066±0.060	0.220±0.223	—
L. G.	1.891	1.281	0.103	0.398	3.73	332
I. S.	2.43	1.71	0.173	0.414	3.51	382
M. R.	0.233	0.186	0.016	0.101	0.331	81
d 1.006–1.019 g/ml						
Normal	0.020±0.008	0.010±0.005	0.002±0.001	0.026±0.009	0.009±0.003	—
L. G.	0.176	0.144	0.012	0.022	0.158	78
I. S.	0.270	0.205	0.018	0.032	0.249	62
M. R.	0.113	0.095	0.009	0.042	0.099	59
d 1.019–1.063 g/ml						
Normal	0.627±0.134	0.336±0.088	0.153±0.039	1.37±0.366	0.071±0.012	653
L. G.	1.74	1.15	0.096	0.039	0.258	254
I. S.	6.96	4.53	0.524	0.101	0.685	342
M. R.	1.73	1.21	0.128	0.164	0.261	391
d 1.063–1.25 g/ml						
Normal	0.514±0.085	1.074±0.151	0.177±0.035	1.460±0.260	0.052±0.009	2074
L. G.	0.728	0.636	0.060	0.017	0.090	390
I. S.	0.576	0.529	0.060	0.018	0.100	—
M. R.	0.748	0.669	0.067	0.053	0.078	411

* Plasma lipoprotein lipids ±SD from six normal females, aged 22–35. Data from Glomset, Norum, and King, 1970. Recoveries for normal and patient lipoproteins averaged 93.4% for all lipids.

esters should be formed in the plasma. One possibility is that the enzyme plays a role in cholesterol transport (1–3) by altering the structure and fate of plasma lipoproteins. However, experiments designed to test this possibility in vitro have been hampered by the fact that most of the cholesterol of freshly prepared plasma lipoproteins is already esterified, i.e., if the enzyme does alter the structure and physical properties of lipoproteins, most of the alterations have already occurred in vivo. Recently, a unique opportunity to circumvent this problem has become available. Six patients who have familial LCAT deficiency (4–6) have been discovered in Scandinavia. The relative content of CE in the plasma of these patients is grossly abnormal, and cannot be explained on the basis of endocrine or nonspecific hepatic dysfunction (7). Consequently, the patients provide a new vantage point from which to study plasma cholesterol metabolism in general and the role of the LCAT reaction in particular. Several investigators, whose work has recently been reviewed (7, 8), have been attempting to characterize the patients' various abnormalities. Our group is currently studying the composition and physical properties of the plasma lipoproteins in order to define the abnormalities and provide a base line for subsequent metabolic studies. Already, several lipoprotein abnormalities have been uncovered. For example, each of the six patients studied has very low density lipoproteins (VLDL) that migrate abnormally on electrophoresis (7); five patients have hyperlipemia (7); the four patients whose low density lipoprotein (LDL) lipids have been measured (9, 10) have increased concentrations of LDL unesterified cholesterol and lecithin in the plasma; and studies (10, 11) of one patient's LDL of *d* 1.019–1.063 g/ml revealed both large and small abnormal lipoproteins. Although each of these abnormalities presumably depends on the LCAT deficiency, none has been adequately explained, and at least one, the hyperlipemia, may be a late indirect effect of renal dysfunction (see Discussion). Furthermore, additional abnormalities may exist since neither the VLDL nor the LDL have been completely characterized. The present investigation was an effort to obtain additional information about the VLDL and about the LDL of *d* 1.006–1.019 g/ml by filtering them through columns of 2% agarose gel and determining the lipid composition of the resulting subfractions. A second aim was to distinguish

TABLE II

Relative Composition of Lipoprotein Fractions of Patients L. G., I. S., and M. R.

Patient and lipoprotein class	CE/UC	CE/TG	CE/protein	UC/protein	TG/protein
	molar ratio			*μmol/mg*	
d < 1.006 g/ml					
Normal*	0.76±0.094	0.34±0.126	—	—	—
L. G.	0.21	0.11	1.20	5.70	11.95
I. S.	0.17	0.12	1.08	6.37	9.18
M. R.	0.44	0.31	1.25	2.86	4.08
d 1.006–1.019 g/ml					
Normal	1.30±0.131	3.09±0.86	—	—	—
L. G.	0.13	0.14	0.29	2.26	2.04
I. S.	0.12	0.13	0.52	4.35	4.02
M. R.	0.37	0.42	0.71	1.96	1.68
d 1.09–1.063 g/ml					
Normal	2.16±0.153	19.21±4.48	1.77±0.359	1.44±0.105	0.11±0.014
L. G.	0.04	0.15	0.15	6.85	1.02
I. S.	0.02	0.15	0.30	20.36	2.00
M. R.	0.09	0.63	0.42	4.42	0.67
d 1.063–1.25 g/ml					
Normal	2.84±0.202	28.92±8.15	0.70±0.102	0.27±0.057	0.03±0.005
L. G.	0.02	0.19	0.04	1.86	0.23
I. S.	0.03	0.18	—	—	—
M. R.	0.07	0.68	0.13	1.82	0.19

* Normal values ±SD for six females aged 22–35. Data from Glomset, Norum, and King, 1970 and Norum, Glomset, Nichols and Forte, 1971.

abnormalities associated with the hyperlipemia from those more directly associated with the enzyme lack by comparing lipoproteins from the nonlipemic plasma of one patient with those from the hyperlipemic patients. A final aim was to determine whether abnormal large and small LDL of *d* 1.019–1.063 g/ml, similar to those described previously (10, 11), are present in the plasma of additional patients.

METHODS

The two patients studied in most of the experiments were M. R., a 23-yr old nonlipemic female, and L. G., a 38-yr old lipemic male. Other patients were I. S., the 34-yr old lipemic sister of M. R., and A. A., the previously studied (10, 11) lipemic sister of L. G. The clinical features, pertinent laboratory findings, and some of the plasma lipoprotein abnormalities of these patients have been described previously (4, 5, 7). None of the patients was obese or showed signs of thyroid, pancreatic, or nonspecific hepatic dysfunction at the time of study. All had slightly reduced concentrations of serum albumin (less than 4, but more than 3 g/ml serum) and moderately increased concentrations of urine protein (0.5–1.5 mg/ml urine). Control subjects were normal females aged 22–35 yr. Neither patients nor control subjects received special diets or medication in connection with the studies, although blood was withdrawn after they had fasted overnight.

Acid citrate dextrose solution (U. S. P. formula A) was used as anticoagulant, blood cells were separated in a refrigerated centrifuge, and *p*-chloromercuriphenylsulfonate (PCMPS) was added to a final concentration of 2 mM to facilitate comparison with previous experiments. Lipoproteins were fractionated by preparative ultracentrifugation, washed twice by recentrifugation using the same conditions, and analyzed for lipid and protein as described previously (9). Tables I and II show the lipid compositions of the major lipoprotein classes of M. R., I. S., and L. G. at the time of study. The concentrations of the lipids of M. R. were very similar to those reported previously (9). Note that the triglyceride (TG) of the lipoproteins of *d* < 1.006 g/ml was elevated in the plasma of I. S. and L. G., but not in that of patient M. R.

The lipoproteins of *d* < 1.006 g/ml were subfractionated on columns of 2% agarose gel (Bio-Gel A 50m, 100–200 mesh, Bio-Rad Laboratories, Richmond, Calif.); equilibrated and eluted with 0.15 N NaCl-1.0 mM EDTA, pH 7.4. Protein in the effluent was measured using a modification (12) of the method of Lowry or using the method of Hirs (13) corrected for the presence of material soluble in chloroform.[2] Human serum albumin was used as a standard. When the lipoproteins of *d* < 1.006 g/ml or *d* 1.006–1.019 g/ml were filtered through fresh beds of gel, recovery based on total cholesterol was usually 70–90%, whereas recoveries of the lipoproteins of *d* 1.019–1.063 g/ml were essentially complete. When identical portions of the lipoproteins of

[2] In no case was the correction more than 10%.

$d < 1.006$ g/ml of patient M. R. were successively filtered through the same column, measurements of effluent absorbance and light scattering and lipid suggested preferential loss of the larger lipoproteins. This loss might have been due to adsorption by agaropectin sulfate present in the "agarose," since commercial preparations of "agarose" usually contain from 0.1 to 0.2% of bound sulfur (14). However, we cannot be sure that breakdown of the lipoproteins did not occur followed by preferential retention of the lipid since we could detect no certain difference in the small amount of protein recovered in association with the larger lipoproteins. In view of the evidence of adsorption or selective retention by the agarose gel, a given column was used for portions of one preparation only, and the gel was subsequently discarded. Subfractionation of the LDL of d 1.019–1.063 g/ml by gel filtration through 2% agarose gel and analytical ultracentrifugation were done as described previously (10). Lipoprotein subfractions obtained in the gel filtration experiments were concentrated by ultrafiltration through Diaflow filters (PM-10; Amicon Corp., Lexington, Mass.).

Electron microscopy was usually performed on samples not more than 1 wk after they had been prepared. Portions were dialyzed against ammonium acetate-ammonium carbonate buffer (15), mixed with an equal volume of 2% sodium phosphotungstate, pH 7.4, and examined on a Formvar/carbon-coated grid as described previously (11).

RESULTS

Gel filtration. Three principal abnormalities became apparent when the patients' lipoproteins of $d < 1.006$ g/ml were separately filtered through the columns of 2% agarose gel. First, in the three lipemic patients L. G., I. S., and A. A., an abnormally large proportion of the lipid associated with these lipoproteins emerged with or soon after the void volume (compare Fig. 1 and Table III with Fig. 2*F*). This observation was not unexpected since both the native plasma and solutions of the lipoproteins of $d < 1.006$ g/ml were very milky and obviously rich in particles large enough to be excluded from the gel. It did not occur in experiments with the lipoproteins of $d < 1.006$ g/ml of M. R., who does not have hyperlipemia. However, the void volume components did show a *second* abnormality common to those of all patients (Figs. 2*A–C*; Table IV). They contained much more UC and PC relative to TG and protein than corresponding normal lipoproteins (compare Figs. 2*B* and 2*C* with Figs. 2*E* and 2*F*). A *third* abnormality of the lipoproteins of $d < 1.006$ g/ml, revealed by the detailed analysis of the lipoproteins of M. R., involved the smaller lipoproteins. In contrast to corresponding normal lipoproteins the content of CE relative to TG or UC remained essentially constant with increasing elution volume, and the content of PC relative to UC consistently increased (compare Figs. 2*B* and 2*E*). Consequently, the smaller VLDL contained considerably less CE and considerably more PC than corresponding normal VLDL. Thus, the VLDL of M. R., eluted in the range of K_d 0.5–0.6 (350–375 ml, Fig. 2) contained an average of 0.68 μmol CE/mg protein, whereas the corresponding value for normal VLDL was 1.30 μmol CE/mg protein. In contrast, the same VLDL of M. R. contained an average of 1.75 μmol PC/mg protein whereas the normal VLDL contained 1.06 μmol PC/mg protein. The VLDL of L. G. and I. S. appeared to share the same abnormality as indicated by the relative compositions of the large and small molecular weight subfractions (Table IV).

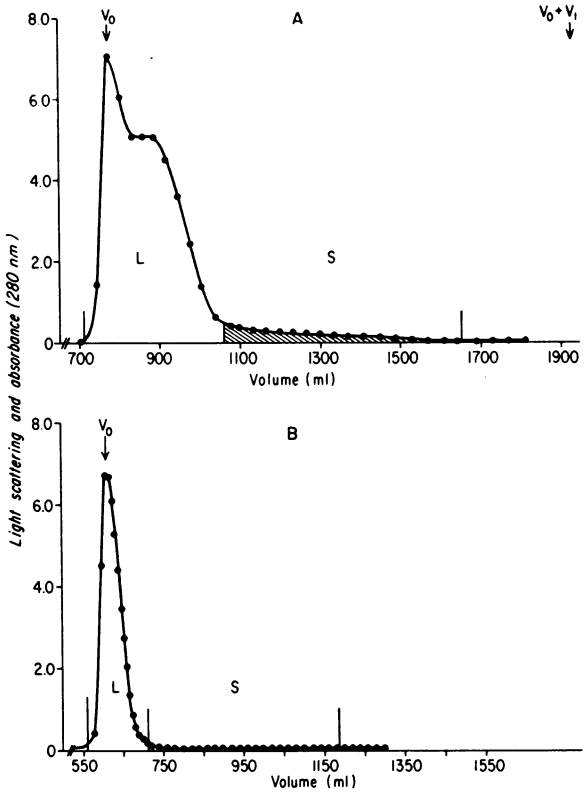

FIGURE 1 Subfractionation of lipoproteins of $d < 1.006$ g/ml from two hyperlipemic patients with familial LCAT deficiency. Section *A* shows lipoproteins of patient L. G. Material corresponding to that present in 73 ml plasma (24.3 mg lipoprotein protein) filtered through a 4.5 × 150 cm column of 2% agarose gel. Note that the "absorbance" of the void volume lipoproteins was mainly light scattering. Effluent corresponding to areas labeled "L" and "S" pooled for analysis (see Table III). Recovery of applied lipoprotein cholesterol was 93%. Section *B* shows lipoproteins of patient I. S. Material corresponding to that present in 25.5 ml plasma (9.5 mg lipoprotein protein) filtered through a 4.5 × 150 cm column of 2% agarose gel. Effluent pooled as in *A*. Note that the void volume peak obtained with the lipoproteins of L. G. has a shoulder whereas the corresponding peak obtained with the lipoproteins of I. S. does not, and that we have no explanation for this difference. Note also that in the experiment shown in *B* the recovery of applied lipoprotein cholesterol was only 62%, and that lipoproteins in concentrations too low to be detected by absorbance measurements may have been eluted after the cut off volume that defines subfraction "S."

TABLE III

Composition of Subfractions of Lipoproteins of d < 1.006 g/ml of Patients L. G. and I. S.

Patient and lipoprotein subfraction	UC	PC	S	CE	TG	Protein
			μmol/ml plasma			*mg/ml plasma*
Patient L. G.						
Large molecular* weight	1.251 (71.1)§	0.631 (64.2)	0.087 (69.0)	0.256 (69.9)	2.780 (71.1)	—
Small molecular‡ weight	0.508 (28.9)	0.354 (35.8)	0.039 (31.0)	0.110 (30.1)	1.128 (28.9)	—
Patient I. S.						
Large molecular* weight	1.176 (78.6)	0.697 (75.3)	0.048 (75.0)	0.244 (79.0)	1.703 (76.9)	0.095 (46.8)
Small molecular‡ weight	0.320 (21.4)	0.229 (24.7)	0.016 (25.0)	0.065 (21.0)	0.511 (23.1)	0.108 (53.2)

* Subfractions labeled "L," Figs. 1*A*, 1*B*.

‡ Subfractions labeled "S," Figs. 1*A*, 1*B*.

§ Number in parentheses refer to percent of recovered component.

Abnormalities also became apparent when the patients' LDL of *d* 1.006–1.019 g/ml were filtered through 2% agarose gel. The lipoproteins of L. G. (Fig. 3*C*) and M. R. (Fig. 3*B*) both yielded abnormal components of large, intermediate, and smaller molecular weight, whereas normal lipoproteins of the same density yielded a single component (Fig. 3*A*) which emerged in a position similar to that of the patients' smaller molecular weight lipoproteins (the K_d for the normal lipoproteins was 0.48 which is comparable to that of the smaller lipoproteins of M. R. [0.50] and L. G. [0.49]). Table V shows that the large and intermediate molecular weight subfractions contained appreciable amounts of cholesterol, particularly in the case of L. G., although most of the cholesterol was associated with the smaller molecular weight subfractions. The tabulated concentration of large molecular weight material is probably lower than the true concentration in the plasma since experiments similar to those mentioned for the lipoproteins of *d* < 1.006 g/ml (see Methods) indicated preferential adsorption of the large LDL of *d* 1.006–1.019 g/ml on the gel.

Results obtained when the patients' LDL of *d* 1.019–1.063 g/ml were filtered through 2% agarose are shown in Figs. 4–6. As described previously in the case of patient A. A. (10), the LDL of L. G., I. S., and M. R. all contained abnormally large components in addition to a smaller component that emerged in the same position (K_d 0.56–0.61) as control LDL (10). The LDL of L. G. (Fig. 4*A*), like those of two other hyperlipemic patients, I. S. (Fig. 5) and A. A. (10), yielded a prominent void volume peak of light-scattering material followed by less clearly defined intermediate and smaller molecular weight lipoproteins. In contrast, the LDL of M. R. (Fig. 6*A*) yielded relatively less light scattering material in the void volume and definite peaks of intermediate and smaller molecular weight lipoproteins. The data in Table V substantiate these differences. The large molecular weight subfractions of *d* 1.019–1.063 g/ml of L. G. and I. S. contained 70 and 67%, respectively, of the total cholesterol recovered from the columns whereas the corresponding subfraction of the LDL of M. R. contained only 34% of the total cholesterol

TABLE IV

Relative Composition of Lipoproteins of d < 1.006 g/ml

Patient and lipoprotein subfraction	CE/UC	TG/UC	UC/ protein	PC/ protein	TG/ protein
	molar ratio		*μmol/mg protein*		
Patient L. G.					
Large molecular* weight	0.204	2.22	—	—	—
Small molecular‡ weight	0.216	2.22	—	—	—
Patient I. S.					
Large molecular* weight	0.207	1.45	12.3	7.3	18
Small molecular‡ weight	0.204	1.60	2.96	2.12	4.73

* Subfractions "L," Figs. 1*A*, 1*B*.

‡ Subfractions "S," Figs. 1*A*, 1*B*.

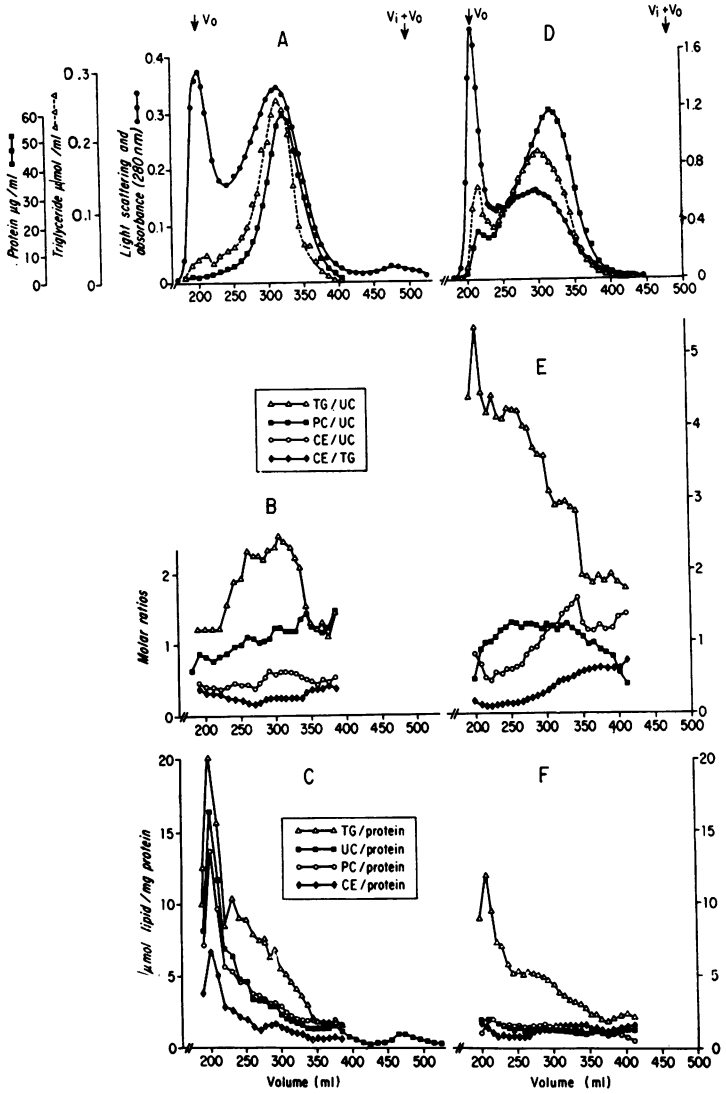

FIGURE 2 Subfractionation of lipoproteins of $d < 1.006$ g/ml from patient M. R. and from normal controls by filtration through 2% agarose. Sections *A-C* show results obtained when lipoproteins corresponding to 74 ml plasma of M. R. (7.2 mg lipoprotein protein) were filtered through a 2.5×90 cm column of the gel. Sections *D-F* show results obtained when the pooled VLDL of two normal females corresponding to 164 ml plasma (15 mg lipoprotein protein) were filtered through a similar column. Note that the ordinate scales for sections *A* and *D* differ, but that those of sections *B* and *C* are identical with those of sections *E* and *F*. As in all other gel filtration experiments, the "absorbance" of the void volume subfractions was mainly light scattering.

recovered. Also, the proportion of the total cholesterol recovered in the intermediate LDL subfractions from M. R. was approximately twice that recovered in the corresponding LDL of L. G. and I. S. The relative differences between the LDL of L. G. and M. R. are of particular interest since the concentrations of cholesterol in the two patients' unsubfractionated lipoproteins of *d* 1.019–1.063 g/ml were almost identical (Table I).

The relative amount of lipoprotein protein in the various subfractions can be approximated from the data in Tables V–VIII. The large LDL of L. G. and M. R. respectively contained 9.5 and 13% of the total LDL protein of *d* 1.019–1.063 g/ml. In contrast, the intermediate LDL of the two patients respectively contained 29 and 36.9% of the total. The remainder, at least half of the total, was in the small molecular weight subfraction,

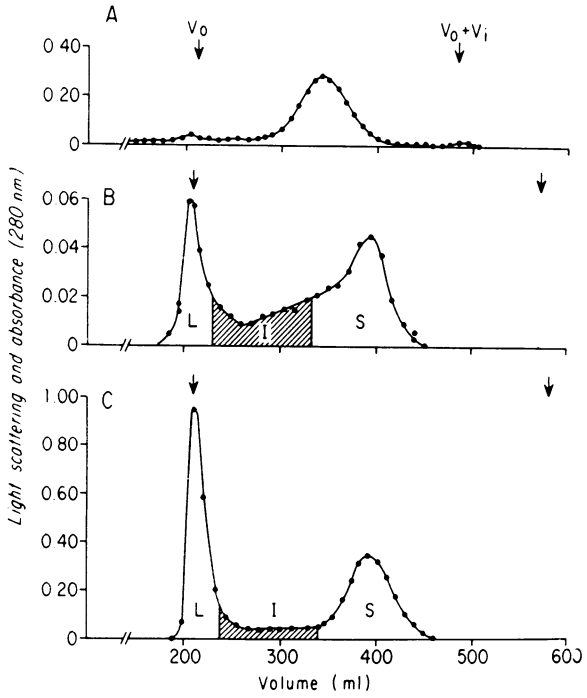

FIGURE 3 Subfractionation of LDL of d 1.006–1.019 g/ml by filtration through 2% agarose. Section A shows results of analysis of LDL from same control plasma used in the experiment shown in Fig. 2. LDL corresponding to that present in 155 ml plasma (8.7 mg lipoprotein protein) were applied to a column sized 2.5 × 90 cm. Section B shows results obtained with the LDL of patient M. R. LDL from 32 ml plasma (1.9 mg lipoprotein protein) were applied to a column sized 2.5 × 112 cm. Recovery of applied lipoprotein cholesterol was 75%. Section C shows results obtained with LDL from patient L. G. LDL from 154 ml plasma (1.2 mg lipoprotein protein) applied to a column sized 2.5 × 112 cm. Recovery of applied lipoprotein cholesterol was 84%.

although in absolute terms, the concentration of protein in this subfraction was only 23% of that of corresponding normal LDL (see Table I).

The compositions of the subfractions of d 1.006–1.019 g/ml and d 1.019–1.063 g/ml of patients L. G. and M. R. are shown in Tables VI and VII. Corresponding values for the large and small molecular weight subfractions of d 1.019–1.063 g/ml of patient I. S. are shown in Table VIII. In each patient all subfractions contained abnormally small amounts of CE relative to UC or TG and, within each density subclass, showed the same relative changes as a function of molecular size. The large molecular weight subfractions contained the most UC and PC relative to TG, CE, or protein. The smaller molecular weight lipoproteins, which otherwise bore the closest resemblance to corresponding normal lipoproteins, contained unusually large amounts of TG and PC compared with UC. Some differences between the patients' corresponding subfractions also are apparent. Those of L. G. and I. S. contained less CE relative to UC or TG than those of M. R., and the large molecular weight subfractions from the plasma of L. G. and I. S. contained three to five times as much lipid relative to protein as those from the plasma of M. R.

Analytical ultracentrifugation. Several of the lipoprotein fractions and subfractions from the plasma of L. G. (Fig. 7) and M. R. (Fig. 8) were analyzed by analytical ultracentrifugation. Fig. 7 *A* shows the ultracentrifugal distribution obtained for the unsubfractionated lipoproteins of $d < 1.063$ g/ml of L. G. In addition to a

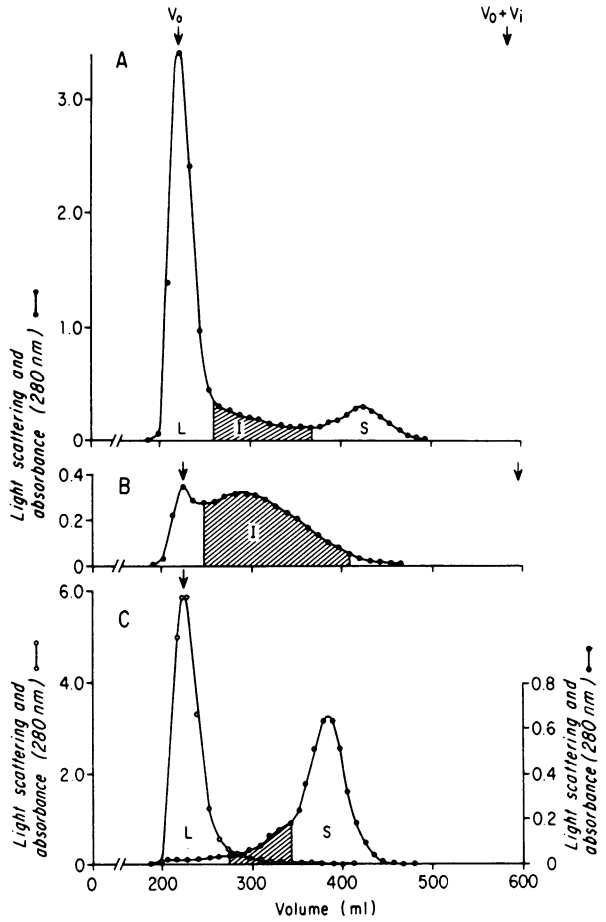

FIGURE 4 Subfractionation of LDL of d 1.019–1.063 g/ml from patient L. G. by filtration through 2% agarose. Section A shows results obtained from filtration of LDL from 85.5 ml plasma (21.7 mg lipoprotein protein) on a column sized 2.5 × 111 cm. Recovery of applied lipoprotein cholesterol was 100%. Sections B and C show experiments in which subfractions combined from three experiments similar to that shown in Fig. 4*A* were refiltered through the same column. Section B shows refiltration of subfractions corresponding to areas labeled "I" in section A. Section C shows two separate refiltration experiments with pooled subfractions corresponding to areas of the filtration diagram in section A labeled "L" and "S," respectively.

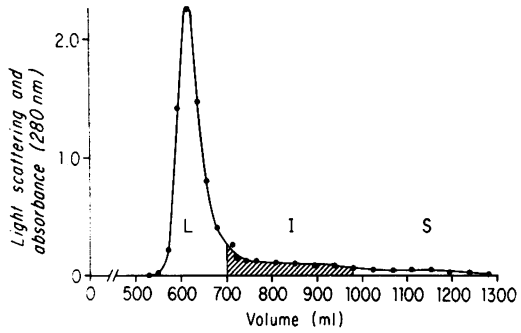

FIGURE 5 Subfractionation of LDL of d 1.019–1.063 g/ml from patient I. S. by filtration through 2% agarose. LDL from 25.4 ml plasma (8.7 mg lipoprotein protein) applied to a column sized 4.5 × 150 cm. Recovery of applied lipoprotein cholesterol was 94%.

broad distribution of rapidly floating material, peaks at S_f 7 and S_f 26 were present. Fig. 7 *B* shows that the lipoproteins of $d < 1.006$ g/ml contained material of $S_f > 20$; however this material contributed little to the peak at S_f 26 in the distribution of lipoproteins of $d <$ 1.063 g/ml. Fig. 7 *C* shows that the LDL of d 1.019–1.063 g/ml accounted for the peaks at S_f 7 and S_f 26 and contributed to material floating in the range of S_f 30–200 (with a peak at S_f 100) in the ultracentrifugal distribution of unsubfractionated lipoproteins of $d < 1.063$ g/ml. Finally, Fig. 7 *D* indicates that the small, intermediate and large molecular weight components, obtained upon refiltration (Figs. 4 *B* and 4 *C*) of the subfractions shown in Fig. 4 *A*, yielded separate peaks which corresponded to those in Fig. 7 *C*. The results of analyses of the lipoprotein fractions of M. R. (Figs. 8 *A–D*) were generally similar to those obtained with the lipoproteins of L. G., but differed in some noteworthy respects. First, the ultracentrifugal distribution of unsubfractionated lipoproteins of $d < 1.063$ g/ml of M. R. contained much less material of $S_f > 100$. This is compatible with the results of the gel filtration experiment shown in Fig. 2 *A*. Second, the lipoproteins of d 1.019–1.063 g/ml yielded a sharper, more slowly floating intermediate peak (S_f 17), compatible with the generally smaller size of the intermediate lipoproteins (the K_d of the intermediate

TABLE V

Distribution of Total Cholesterol among LDL Subfractions from Patients L. G., I. S., and M. R.

Patient and LDL subclass	Large molecular weight subfractions*	Intermediate molecular weight subfractions*	Small molecular weight subfractions*
	μmol total cholesterol/ml plasma		
Patient L. G.			
d 1.006–1.019	0.051 (30.5)‡	0.023 (13.8)	0.093 (55.7)
d 1.019–1.063	1.252 (70.4)	0.370 (20.8)	0.156 (8.8)
Patient I. S.			
d 1.019–1.063	4.35 (67.0)	1.81 (27.8)	0.334 (5.1)
Patient M. R.			
d 1.006–1.019	0.008 (7.3)	0.019 (16.7)	0.087 (76.0)
d 1.019–1.063	0.624 (34.0)	0.942 (51.4)	0.268 (14.6)

* Subfractions labeled "L," "I," and "S" in Figs. 3–6.

‡ Numbers in parentheses refer to percent distribution of recovered cholesterol.

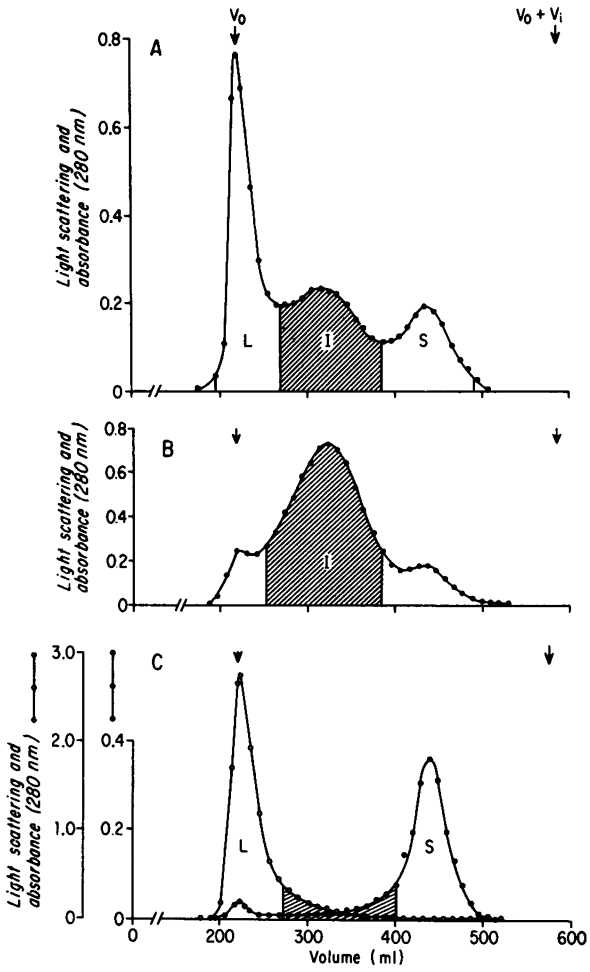

FIGURE 6 Subfractionation of LDL of d 1.019–1.063 g/ml from patient M. R. by filtration through 2% agarose. Section *A* shows results obtained upon filtration of LDL from 46.2 ml plasma (18.1 mg lipoprotein protein) on a column sized 2.5 × 111 cm. Recovery of applied lipoprotein cholesterol was 97%. Sections *B* and *C* show experiments in which subfractions from five experiments similar to that shown in Fig. 5*A* were pooled and refiltered through the same column. Section *B* shows refiltration of subfractions corresponding to the area labeled "I" in section *A*. Section *C* shows two separate refiltration experiments with pooled subfractions corresponding to areas of the filtration diagram in section *A* labeled "L" and "S," respectively.

TABLE VI

Relative Composition of LDL Subfractions of Patient L. G.

LDL subclass and/or subfraction	TG/UC	PC/UC	CE/UC	CE/TG	PC/S	CE/protein	UC/protein	PC/protein	TG/protein
			molar ratios				*μmol/mg*		
Large molecular weight									
d 1.006–1.019*	0.34	0.58	0.038	0.110	8.93	0.60	15.9	9.15	5.45
d 1.019–1.063*	0.05	0.57	0.008	0.176	7.60	0.43	54.7	31.0	2.45
d 1.019–1.063‡	0.04	0.54	0.010	0.235	7.64	0.40	39.8	21.5	1.72
Intermediate molecular weight									
d 1.006–1.019*	0.59	0.61	0.082	0.139	9.07	0.46	5.64	3.41	33.31
d 1.019–1.063*	0.13	0.65	0.021	0.161	7.19	0.11	5.13	3.34	0.65
d 1.019–1.063‡	0.11	0.58	0.022	0.204	8.22	0.19	8.64	5.05	0.95
Small molecular weight									
d 1.006–1.019*	1.68	0.86	0.165	0.098	9.05	0.36	2.20	1.89	3.71
d 1.019–1.063*	1.34	0.96	0.171	0.127	8.57	0.15	0.89	0.85	1.21
d 1.019–1.063‡	1.52	1.00	0.192	0.126	9.46	0.19	1.00	1.00	1.53

* Lipoproteins of *d* 1.006–1.019 g/ml and 1.019–1.063 g/ml subfractionated by gel filtration on 2% agarose gel; not rechromatographed.

‡ Rechromatographed subfraction of *d* 1.019–1.063 g/ml.

lipoproteins of L. G. shown in Fig. 4 *B* was 0.18 whereas that of the intermediate lipoproteins of M. R. shown in Fig. 6 *B* was 0.29) and compatible with their lower TG content (compare the compositions of the refiltered intermediate lipoproteins of *d* 1.019–1.063 g/ml shown in Tables VI and VII). A third difference between the lipo-

TABLE VII

Comparison of Normal LDL with LDL subfractions of Patient M. R.

LDL subclass and/or subfraction	TG/UC	PC/UC	CE/UC	CE/TG	PC/S	CE/protein	UC/protein	PC/protein	TG/protein
			molar ratios				*μmol/mg*		
Normal lipoproteins									
d 1.006–1.019§	0.45	0.455	1.30	3.00	5.89	—	—	—	—
d 1.019–1.063	0.11	0.519	2.16	19.4	2.21	1.77	1.44	0.482	0.114
Large molecular weight									
d 1.006–1.019*	0.34	0.52	0.222	0.65	2.69	1.08	4.85	2.50	1.65
d 1.019–1.063*	0.06	0.59	0.041	0.68	5.62	0.61	14.8	8.70	0.90
d 1.019–1.063‡	0.06	0.58	0.024	0.41	7.27	0.50	20.9	12.0	1.23
Intermediate molecular weight									
d 1.006–1.019*	0.67	0.72	0.269	0.40	6.48	1.32	4.89	3.50	3.28
d 1.019–1.063*	0.12	0.62	0.062	0.53	6.56	0.48	7.84	4.83	0.91
d 1.019–1.063‡	0.07	0.68	0.039	0.55	7.68	0.32	8.24	5.54	0.58
Small molecular weight									
d 1.006–1.019*	1.26	0.87	0.440	0.35	8.48	1.72	3.92	3.43	4.95
d 1.019–1.063*	1.16	0.94	0.531	0.46	8.24	0.61	1.14	1.07	1.33
d 1.019–1.063§	1.14	0.92	0.564	0.49	16.6	0.47	0.83	0.78	0.95

* Lipoproteins of *d* 1.006–1.019 g/ml and 1.019–1.063 g/ml subfractionated by gel filtration on 2% agarose gel; not rechromatographed.

‡ Rechromatographed subfraction of *d* 1.019–1.063 g/ml.

§ From references 9 and 10.

TABLE VIII

Comparison of Normal LDL with LDL Subfractions of Patient I. S.

LDL subclass and/or subfraction	TG/UC	PC/UC	CE/UC	CE/TG	PC/S	CE/protein	UC/protein	PC/protein	TG/protein
			molar ratios				*μmol/mg*		
Normal lipoproteins									
d 1.019–1.063‡	0.11	0.52	2.16	19.4	2.21	1.77	1.44	0.48	0.11
Large molecular weight									
d 1.019–1.063*	0.05	0.63	0.01	0.24	7.72	0.74	74.6	46.3	3.57
Small molecular weight									
d 1.019–1.063*	0.45	0.76	0.07	0.16	8.85	0.14	2.22	1.70	1.00

* Lipoproteins of *d* 1.019–1.063 g/ml subfractionated by gel filtration on 2% agarose gel; not rechromatographed.
‡ From references 9 and 10.

protein distributions of L. G. and M. R. was that the LDL of M. R. contained much less material of $S_f > 30$. This difference is compatible with the differences in large molecular weight material shown by the gel filtration experiments in Figs. 4 and 6.

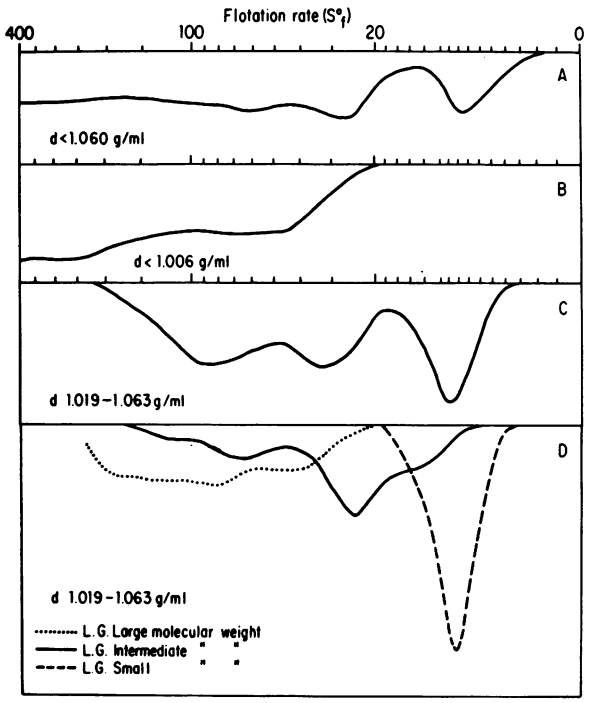

FIGURE 7 Analytical ultracentrifugation of the VLDL and LDL of patient L. G. Section *A* shows results of analysis of the combined lipoproteins of $d < 1.063$ g/ml. Section *B* shows results of analysis of the lipoproteins of $d < 1.006$ g/ml. Section D shows results of analysis of the large (L), intermediate (I) and smaller (S) molecular weight subfractions obtained in the gel filtration experiments shown in Figs. 4*B* and 4*C*. Note that the results of the analyses have been normalized so that the total peak areas are identical.

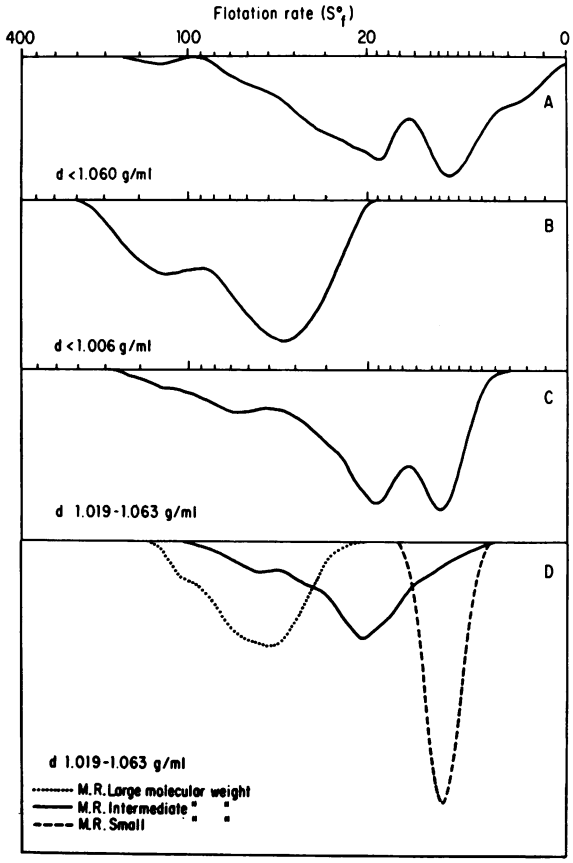

FIGURE 8 Analytical ultracentrifugation of the VLDL and LDL of patient M. R. Section *A* shows results of analysis of the combined lipoproteins of $d < 1.063$ g/ml. Section *B* shows results of analysis of the lipoproteins of $d < 1.006$ g/ml. Section *C* shows results of analysis of the lipoproteins of *d* 1.019–1.063 g/ml. Section *D* shows results of analysis of the large (L), intermediate (I) and smaller (S) molecular weight subfraction obtained in the gel filtration experiments shown in Figs. 6*B* and 6*C*. The results were normalized as in the experiments shown in Fig. 7.

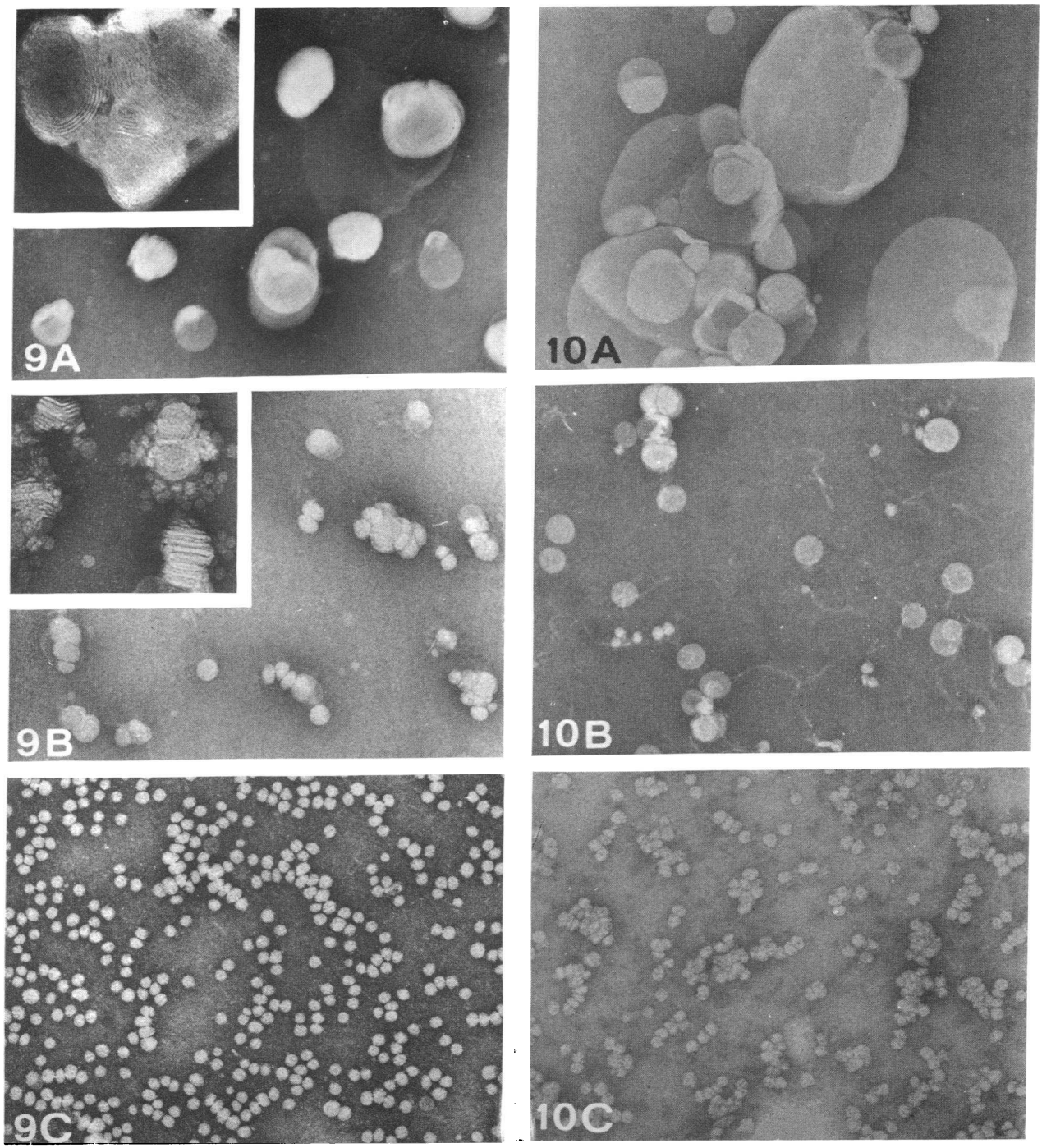

FIGURE 9 Electron micrographs of negatively stained lipoproteins of *d* 1.019–1.063 g/ml of patient L. G. (*A*) Large molecular weight subfraction obtained in the experiment shown in Fig. 4*C*. Large, nearly round structures predominate; occasionally slumped regions which contain myelin figures are apparent (insert). (*B*) Intermediate molecular weight subfraction obtained in experiment shown in Fig. 4*B*. Particles in this fraction are heterogeneous in size and occasionally form short stacks or rouleaux when aggregated (insert). (*C*) Small molecular weight subfraction obtained in experiment shown in Fig. 4*C*. Particles are round and homogeneous in size. Micrographs *A*, *B*, and *C* are magnified × 89,000; inserts, × 118,000.

FIGURE 10 Electron micrographs of negatively stained lipoproteins of *d* 1.019–1.063 g/ml of patient M. R. showing large (*A*), intermediate (*B*) and smaller (*C*) molecular weight subfractions obtained in experiments shown in Figs. 6*B* and 6*C*. Magnification × 89,000.

Electron microscopy. Figs. 9 and 10 show electron micrographs of the large, intermediate, and small molecular weight LDL subfractions from patients L. G. and M. R. The large molecular weight LDL from both patients consist of particles which, for the most part, are greater than 1000 Å in size. Occasionally the negative stain can be seen penetrating into the very large particles so that myelin figures are produced (Fig. 9 *A*). Flattening and aggregation of the particles makes it difficult to determine whether there is a difference in size distribution between the large molecular weight LDL in the two patients.

The intermediate molecular weight subfractions of both subjects (Figs. 9 *B* and 10 *B*) show two distinct populations of particles. In each case the smaller of these consists of round particles 170–340 Å in diameter which is consistent with the dimensions of the small molecular weight LDL described previously (11). The small particles thus presumably represent contaminating small molecular weight components. The larger particles of intermediate molecular weight LDL consist of electron transparent structures which appear to be flattened and occasionally form arrays of stacked particles (Fig. 9 *B*) of variable thickness (50–110 Å). This subfraction from both patients displays a wide range, 340–1000 Å, of particle diameter. Within this range the intermediate molecular weight LDL from M. R. and L. G. show distinct differences in size distribution. 68% of the particles from M. R. are between 450 and 600 Å in diameter whereas only 48% of the particles from L. G. fall into this range. On the other hand, 38% of the particles from L. G. are greater than 600 Å compared with 20% from M. R. These results are consistent with the analytical ultracentrifugal data (Figs. 7 *D* and 8 *D*) which indicate that the lipoproteins of the intermediate molecular weight subfraction from M. R. exhibit a sharper and more slowly floating peak than the intermediate lipoproteins of L. G.

The low molecular weight LDL subfractions from both L. G. (Fig. 9 *C*) and M. R. (Fig. 10 *C*) are very similar with diameter ranges of 170–310 and 170–280 Å, respectively. In both cases more than 50% of the particles were between 220 and 250 Å in diameter; thus, in size and appearance these lipoprotein subfractions are indistinguishable from normal LDL (11).

DISCUSSION

This investigation of the lipoproteins of patients with familial LCAT deficiency has provided new information concerning several abnormalities. First, abnormal lipoproteins, large enough to be excluded from 2% agarose gels and unusually rich in UC and PC, are present throughout the range of $d < 1.063$ g/ml. In the case of the large lipoproteins of $d < 1.006$ g/ml components of this size are normally present. Therefore, we cannot be certain whether the increased UC and PC associated with large molecular weight material is due to the presence of a unique type of abnormal lipoprotein or whether these lipids simply "overload" large, relatively normal VLDL and/or chylomicrons. In addition, the possibility exists that these lipoproteins may be aggregates of smaller lipoproteins. Information about lipoprotein aggregation phenomena is scanty, and we do not know whether the unusual composition of the patients' lipoproteins would promote or retard aggregation. In the case of the LDL, however, less ambiguity exists. The large molecular weight material is clearly separate from the normal-sized lipoproteins which emerge much later from columns of 2% agarose gel (Fig. 3; reference 10). On the other hand, the question of artifact must be considered. We have discussed previously (10) our reasons for believing that the large LDL are not preparative artifacts. One of these is the fact that particles large enough to scatter light form a well-defined band just above the sedimenting proteins when the patients' native plasma is ultracentrifuged overnight and the fact that the bulk of this light-scattering material appears in the large molecular weight subfraction of *d* 1.019–1.063 g/ml. Another reason is that material of $S_f > 20$ is present in the lipoproteins of *d* 1.019–1.063 g/ml before gel filtration as well as in the large molecular weight subfraction after gel filtration.[3] This indicates that the large molecular weight material is not an artifact caused by the gel filtration procedure. One possibility that cannot be excluded is that the abnormal lipoproteins of patients with familial LCAT deficiency are more readily disrupted than normal lipoproteins by procedures such as ultracentrifugation. However, if the lipoproteins are this labile, spontaneous disruption might also occur in vivo, creating "biological" as opposed to preparative artifacts. A final possibility is that the large molecular weight material of *d* 1.019–1.063 g/ml is formed by aggregation of the intermediate molecular weight material. This type of phenomenon seems to account for the large molecular weight LDL of cholesterol-fed guinea pigs (16) since the compositions of the large and intermediate molecular weight LDL of these animals are very similar and since intermediate molecular weight material is generated upon rechromatography of the large molecular weight material. However, simple aggregation cannot account for the large LDL of familial LCAT deficiency since the composition of these

[3] The rate of flotation of lipoproteins in a centrifugal field is not only influenced by density, but also by molecular size. The fact that some of the patients' LDL of densities within the relatively narrow range of 1.019–1.063 g/ml float at rates in excess of S_f 12 indicates therefore that very large particles are present.

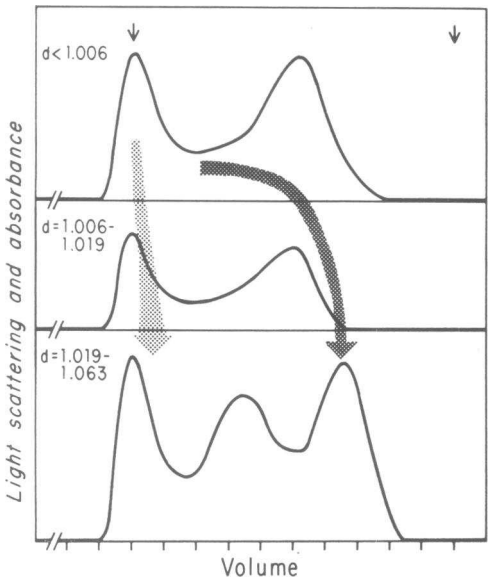

FIGURE 11 Schematic diagram showing postulated relationships among the plasma lipoproteins of patients with familial LCAT deficiency.

LDL differs from that of the intermediate LDL, since intermediate LDL are not generated upon rechromatography of the large LDL, and since only small amounts of large LDL are apparent upon rechromatography of intermediate LDL (Figs. 4 and 6).

If the large lipoproteins are not artifacts, how are they formed and why are they particularly abundant in the plasma of the hyperlipemic patients? Neither question can be answered definitively. However, dietary experiments (Glomset, Norum, Nichols, King, Mitchell, Applegate, and Gjone, to be published) have shown that the concentrations of the large lipoproteins all diminish markedly and disproportionately when the patients consume fat-free diets. This is compatible with the postulate of Schumaker and Adams (3) that a normal function of the LCAT reaction is to dispose of the surface UC and PC of large, TG-rich lipoproteins, once the TG has been hydrolyzed by lipoprotein lipase. Thus, the large lipoproteins might be formed from surface "remnant" UC, PC, and protein in the absence of the normal disposal mechanism. If this occurs, increased turnover of plasma TG might promote accumulation of the large lipoproteins in the patients' plasma and thus account for the increased concentrations of these UC- and PC-rich components in the plasma of L. G. and I. S. However, substantiation of this possibility will have to await further studies. At present our only reason for believing that the turnover of plasma TG may be increased in the hyperlipemic patients is the still tenuous association between the renal component of familial LCAT deficiency and the hyperlipemia. Although none of the patients studied here has the nephrotic syndrome, all have moderate proteinuria, and A. R., the sister of M. R., did develop the nephrotic syndrome. Of most significance, however, is the fact that the plasma TG of A. R. increased as her renal function deteriorated, then decreased to almost normal as all renal function ceased, and has since remained low during treatment by dialysis (E. Gjone, unpublished observations).

In our previous study (10) of the abnormal LDL of patient A. A. we focused attention on the largest and smallest components of d 1.019–1.063 g/ml obtained upon gel filtration. In the present study we have in addition analyzed the LDL of intermediate molecular weight. These LDL are clearly present in the plasma of all the patients and are readily recognized upon analytical ultracentrifugation as material of S_f 20–30. They are very similar to abnormal LDL observed in the plasma of cholesterol-fed guinea pigs (16, 17) and of patients with cholestasis (18, 19). They emerge from columns of 2% agarose gel in the same general position (16, 20), have similar flotation properties (17, 19), and negatively stained preparations have the same general appearance upon electron microscopy (16, 18). Furthermore, the LDL of intermediate molecular weight from patients with familial LCAT deficiency have recently been shown (21, 22) to be immunologically similar to the abnormal LDL of cholestasis. One difference is that the intermediate LDL of familial LCAT deficiency, in contrast to the abnormal LDL of cholestasis, show very little tendency to form extensive rouleaux. This might reflect differences in specimen preparation before electron microscopy. On the other hand the compositions of the abnormal LDL differ somewhat, and one or more of the differences might be critical. Reported values (18) for the refiltered abnormal LDL of cholestasis were: 0.3% CE, 0.9% TG, 31% UC, 65% PL, and 2.5% protein, whereas the composition of refiltered intermediate LDL from M. R., expressed in the same terms, was 2.1% CE, 5.1% TG, 32.7% UC, 49.8% PC + S (sphingomyelin), and 10.2% protein.

An important question that has not yet been resolved concerns the possible metabolic relation between the intermediate and larger sized LDL. The dietary experiments cited above have shown that the intermediate LDL also decrease when the patients consume fat-free diets although not as markedly as the large LDL. Consequently, the postulated relation between chylomicron and VLDL surface UC and PC and the large LDL may also apply to the intermediate LDL.

Finally, this investigation has provided information concerning the lipid composition of the patients' smaller lipoproteins of $d < 1.006$ g/ml and d 1.006–1.063 g/ml. Normal lipoproteins of $d < 1.006$ g/ml comprise a spectrum of molecules of different sizes and compositions

(23–26). Therefore, the abnormalities of the patients' lipoproteins of this density are best shown by our analyses of the successive subfractions obtained upon gel filtration of the lipoproteins of M. R. (Fig. 2). Comparison of the spectrum of her lipoproteins of $d < 1.006$ g/ml with that of corresponding normal lipoproteins showed two abnormalities: (*a*) the relative content of CE was essentially constant, and (*b*) the relative content of PC increased with increasing elution volume. The most likely explanation of these abnormalities is that normal VLDL are progressively converted into smaller lipoproteins by the simultaneous action of several enzymes, one of which is LCAT. Evidence has already been reported (27, 28, 9) that LCAT indirectly increases the CE and decreases the TG, UC, and PC of VLDL in vitro. Also, we have found (29) that LCAT increases the mobility of the patients' VLDL, and that this is associated with in vitro fragmentation of the VLDL into LDL (Norum, Glomset, Nichols, Forte, King, Applegate, Mitchell, and Albers, to be published). In the absence of LCAT, one might therefore expect abnormalities with regard to the lipid composition of VLDL. Similarly, since LCAT increases the CE and decreases the TG, UC, and PC of the patients' unsubfractionated LDL (9), one would expect that any normal-sized LDL generated during the catabolism of VLDL (30, 31), in the absence of LCAT, would contain abnormally high amounts of TG, UC, and PC, and abnormally low amounts of CE.

Two postulates concerning the patients' abnormal lipoproteins of $d < 1.063$ g/ml are schematically summarized by Fig. 11. The heavily shaded arrow represents a metabolic pathway, similar to one that appears to exist in normal plasma (30, 31), by which VLDL are progressively converted into smaller VLDL and LDL of *d* 1.006–1.019 g/ml and *d* 1.019–1.063 g/ml. We suggest that the patients' smaller VLDL and LDL are formed by this pathway since they are essentially normal in size and in relative protein content, and since the ratio of "surface" lipid to "core" lipid (PC + S + UC/TG + CE) is nearly normal. However, since the concentration of the small LDL is low and the CE content abnormal, fragmentation of the VLDL and replacement of the "core" TG of the VLDL and LDL by exchange with HDL CE are probably reduced. In contrast to the heavily shaded arrow, the lightly stippled arrow represents an abnormal pathway caused by deficient removal of the surface UC and PC of chylomicrons and large VLDL, once the triglyceride of these lipoproteins has been hydrolyzed by lipoprotein lipase. In the absence of LCAT this excess lipid spontaneously dissociates from the surfaces of these lipoproteins and forms bilayers which form variously sized, large aggregates depending on the amount of apoprotein present.

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