Concentration gradient electrophoresis of plasma from patients with hyperbetalipoproteinemia

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Abstract A modification of the Pratt and Dangerfield technique of acrylamide gel concentration gradient electrophoresis has been used to study low density lipoproteins from fasting human beings. Prestained plasma from normal subjects and from patients with Fredrickson's type II hyperbetalipoproteinemia was electrophoresed in continuous gradient gel columns (approximately 3.5–8%). Reproducible low density lipoprotein patterns were obtained, and two major bands of low density lipoproteins as well as trace amounts of two to five other low density lipoproteins were seen in normal plasma. Plasmas from patients with hyperbetalipoproteinemia were more heterogeneous. Abnormal plasmas showed as many as six major low density lipoprotein bands and six minor bands. Patterns were constant for an individual but varied between patients. The major bands frequently had obviously different mobilities from those in normal plasma. Extensive experiments, using a large number and variety of mixed plasmas (normal, type II, and others) or plasmas run separately in divided gel columns, led to a numbering system of the low density lipoproteins comprised of 15 discrete bands. In addition to the changes seen in the low density lipoproteins in patients with hyperbetalipoproteinemia, reduced amounts of high density lipoproteins were consistently found.

Supplementary key words low density lipoproteins · lipoprotein electrophoresis · hypercholesterolemia · high density lipoproteins

PLASMA LIPOPROTEIN ELECTROPHORESIS on a variety of solid media has been accepted as an integral part of the laboratory evaluation of patients with disorders of lipoprotein metabolism (1–3). Electrophoretic supporting materials currently in use include paper, cellulose acetate, agarose gel, and starch. All of these substances have characteristics which limit their resolving power for lipoproteins. Narayan and coworkers have used single density acrylamide gel electrophoresis to study qualitative lipoprotein patterns in man (4, 5) and in experimental animals (6, 7). Recently, Pratt and Dangerfield (8) have used acrylamide gel concentration gradient electrophoresis (CGE) in the study of "hyperlipemic" human plasma. We have applied their basic technique to the study of the plasma low density lipoproteins (LDL) from patients in Fredrickson's category of hyperbetalipoproteinemia (type II), with hypercholesterolemia, normal or slightly elevated plasma triglycerides, and an intensely staining "beta band" on paper strip electrophoresis (2). Plasma from control subjects has been studied by the same technique.

METHODS

Patient material and samples

Normal plasma was obtained after a 12–16 hr fast from ambulatory Caucasian volunteers on a normal diet. Control subjects were nondiabetic and had no history of thyroid, hepatic, or renal disease. They had no clinically evident atherosclerosis and no family history of early atherosclerotic cardiovascular disease. Our selection of younger, normal subjects (mean age 37 yr) was intentional and was justified by our desire to select a population statistically known to have the lowest serum cholesterol, the smallest amount of S_f 0–20 LDL, and the lowest incidence of clinically manifest atherosclerotic vascular disease (9).

Identical studies were performed on plasma from Caucasian patients previously diagnosed by the Fredrickson criteria (2, 3) as having phenotypic type II hyperbetalipoproteinemia. Many had overt atherosclerosis.

Abbreviations: CGE, concentration gradient electrophoresis; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

Mild abnormalities of glucose tolerance were frequent in these subjects, but no patient had glycosuria or symptomatic diabetes mellitus. No other endocrine abnormalities or diseases known to affect lipoprotein metabolism were present. In the majority of patients (except P.L., M.S., and C.B.) the dyslipoproteinemia could not be established definitely as an inherited abnormality despite carefully taken family histories.

All blood samples were collected into a solution of EDTA (1 mg/ml) for lipoprotein electrophoretic analyses and AutoAnalyzer chemical determinations. Samples were immediately centrifuged at 2,000 rpm for 15–20 min. Aliquots were frozen for subsequent chemical determination of cholesterol and triglyceride using the standard AutoAnalyzer procedures suggested by Fredrickson, Levy, and Lindgren (2). Plasma for electrophoresis was kept at 4°C. Paper electrophoresis was performed within 3 days of collection. CGE separations were done for the most part within 1 wk, although no qualitative changes in the acrylamide lipoprotein patterns of control and type II plasma were seen in repetitive runs on random samples for up to 6 wk.

Ultracentrifugation

Selected plasma samples were separated in the Spinco model L preparative ultracentrifuge for the isolation of fractions containing VLDL, LDL, and HDL according to the procedures outlined by Havel, Eder, and Bragdon (10). After dialysis against saline, relative purity of these fractions was studied by immunodiffusion and immunoelectrophoresis (11) in 1.5% agarose gel. Rabbit anti-human-LDL antiserum (Hoechst Pharmaceuticals, Kansas City, Mo.) and rabbit anti-human-serum antiserum (kindly supplied by Dr. John Leddy) were used to determine the relative purity of the fractions.

Electrophoretic separation

Paper lipoprotein electrophoresis was performed according to the method of Lees and Hatch (12).

CGE was performed by the method of Pratt and Dangerfield (8), with the following modifications. 0.01 ml of freshly prepared TEMED[1] (10% w/v) and 0.01 ml of ammonium persulfate solution (1.5 g dissolved in 15 ml of water) were added to 7.0 ml of both 2.5% (light) and 8% (heavy) acrylamide solutions. These solutions were degassed by hand pressure for 1 min in corked, 20-ml disposable plastic syringes. Each solution was carefully poured into one chamber of a two-chamber concentration gradient former. The total volume of 14.0 ml from this apparatus was poured, over a 15-min period, into Pyrex glass tubes 12 cm long (I.D. 1.3 cm), which were then plugged with corks covered with Parafilm. The filled tubes were covered with Parafilm and allowed to stand at room temperature overnight. The suggested glycine and Tris buffer solutions were modified by the addition of 350 μmoles of EDTA/liter. 0.4 ml of plasma was prestained for 1 hr with 0.2 ml of ethylene glycol–Sudan black. 0.6 ml of non-cross-linked 4% acrylamide was added to the prestained plasma as an anticonvection medium. Eight concentration gradient tubes were placed in a simple vertical apparatus consisting of upper and lower buffer reservoirs made of plastic dishes, with a ringstand support. Using a tuberculin syringe, 0.1 or 0.2 ml of the prestained plasma–acrylamide solution was carefully layered beneath the glycine buffer of the upper reservoir to cover the top of a concentration gradient column. The electrophoretic runs were conducted at room temperature for 4–5 hr at a constant current of 3 ma/tube (voltage ranged from 25 to 125 v), with insignificant heating of the gels. Progress of the run could be followed by watching the movement of the albumin band, which could be seen without the use of any further marker. At the end of each run, the tubes were photographed with a Polaroid M-3 industrial camera (type 42 roll film), with background illumination provided by an X-ray view box. An orange filter made of cellophane helped reveal lightly staining bands readily visible to the naked eye but occasionally missed by the camera. The gels were then gently removed intact from the Pyrex tube using a no. 22 spinal needle attached to a syringe filled with buffer solution. The gels were allowed to slip into larger, capped glass containers, filled with 10% trichloroacetic acid, for band fixation and for subsequent reference. After band fixation, two nonlipoprotein bands could be clearly seen in the midportion of the gel below the LDL bands which could be used as a check for comparable conditions within a single run.

Numerous controls helped eliminate the possibility of artifacts being responsible for the CGE patterns observed:

1) Duplicate runs in CGE columns were identical; the banding pattern remained similar in the LDL fraction even if there had been slight variation in the gel formation in the upper portion of the solution.

2) Prestained and poststained (8, 13) gel patterns are qualitatively similar, but the latter procedure requires at least 3 days and distorts the gels.

3) Ultracentrifugal separations of a variety of plasma samples at d 1.21 g/ml demonstrated the specificity of Sudan black staining for lipoproteins. Only the supernatant fraction containing the HDL, LDL, and VLDL demonstrated intense blue staining. The infranatant fraction showed a black surface precipitate and a small amount of diffuse gray staining, up to 2 cm beneath the surface, which were not present in the supernatant fraction. This was presumed to be minor, nonspecific banding

[1] *N,N,N′N′′*-Tetramethylethylenediamine, Eastman Kodak Co.

of dye to nonlipoprotein proteins. Small amounts of black-red color moved with the albumin band in the infranatant fraction which we attributed to albumin-binding dye contaminants.

4) The lipoprotein pattern was unaffected by the persulfate catalyst (14), a strong oxidizing agent, as demonstrated by prerunning the gel with thioglycolate solution (15), which inactivates remaining traces of persulfate.

5) 5% sucrose worked as well as 4% acrylamide monomer as an antidiffusion agent, with no difference in the subsequent electrophoretic runs, suggesting no major effect of the antidiffusion agent on the electrophoretic pattern.

6) There was no significant retardation of the lipoprotein bands related to the increased concentration of LDL in hypercholesterolemic patients as tested by serial dilutions of normal and hypercholesterolemic plasma.

7) A normal specimen was run with each series of plasma samples. Each sample was run by itself and, in addition, was mixed with the control sample in a ratio of 1:1 prior to staining in order to more exactly compare the mobilities of the major components of the lipoproteins in each sample. There was no change in the mobility of the major components of each sample when mixed and run together as compared with the electrophoresis of unmixed samples. Similar band comparisons were also made by running control and abnormal samples in one tube. The samples were separated by a paraffin partition that was inserted into the gel just prior to sample application and removed after the samples had entered the gel.

RESULTS

Lipoprotein band identification

As described by Pratt and Dangerfield (8), prestained lipoproteins in whole plasma or after ultracentrifugal separation are easily and rapidly separated on an acrylamide gel concentration gradient. After 4 hr of electrophoresis, the faster, anodal components consist of HDL (d 1.063–1.21), and the slower, mid-gel components consist of LDL (d 1.006–1.063); the larger particles of VLDL (d 1.006) remain near the cathode. After dialysis against saline, supernatant and infranatant fractions of ultracentrifugal separations at densities of 1.006, 1.063, and 1.21 confirmed the above mobility relationships. Minimal LDL contamination of HDL, as obtained in the infranatant fraction of ultracentrifugal separations at d 1.063, was suggested by three pieces of evidence. A minute oil red O-staining arc was seen in agarose immunodiffusion experiments when this HDL-containing infranatant fraction was reacted with rabbit anti-human-betalipoprotein antiserum. This arc showed a band of identity with whole plasma and LDL-containing supernatant fractions. By immunoelectrophoresis, this infranatant fraction also demonstrated a small oil red O-staining arc in the same position as the larger precipitin arc of the supernatant fraction when run against the same antiserum. Lastly, the infranatant fraction when run in CGE did contain one or two slow-moving bands which moved between the LDL and VLDL. These were comparable to the "M band" (similar in mobility to our bands 6–8 as defined below) observed by Pratt and Dangerfield (8) in some plasma samples and attributed by them to the presence of a large amount of HDL. However, when both supernatant and infranatant fractions were run together in a single partitioned CGE gel tube, both demonstrated a band of this mobility, with a majority of the staining in the side containing the supernatant fraction (LDL). This also suggests that the "M band" represents an LDL protein with a density near enough to 1.063 that the relatively inefficient no. 40 rotor we used did not completely separate it from the HDL, even after 22 hr at 100,000 *g*. However, positive identification of this band(s) awaits immunological study on material taken directly from the acrylamide tubes.

As many as 15 different components could be recognized in the composite pattern analysis of the LDL in over 100 normal and abnormal plasmas run in CGE. From this composite picture, and by using mixed plasmas to study comparative lipoprotein mobilities, a tentative numbering system evolved which we found useful in identifying the stained lipoprotein components and in relating the banding in one tube to that in another (Figs. 1 and 2). In the patterns of the subjects comprising this study, only 12 of the 15 bands were seen. Since only five major bands were found in normal plasmas, we have chosen to call these group 4 and have subdivided them with the letters *a*–*e*.

Lipoproteins in normal plasma

Control plasmas demonstrated a normal pattern on paper electrophoresis. The mean total cholesterol concentration was 201 ± 31 mg/100 ml; the mean plasma triglyceride concentration was 104 ± 30 mg/100 ml. Individual plasma cholesterol determinations all fell within the "normal" ranges (age- and sex-matched) suggested by Fredrickson, Levy, and Lees (3). Two plasma triglyceride values (from H.M. and J.K.) were slightly above the upper limit of normal, which is 150 mg/100 ml plasma (3) (Table 1).

Good separation of normal plasma LDL by CGE revealed one heavily stained band in the midportion of the gel (Fig. 3*A*). With close inspection, this band could be

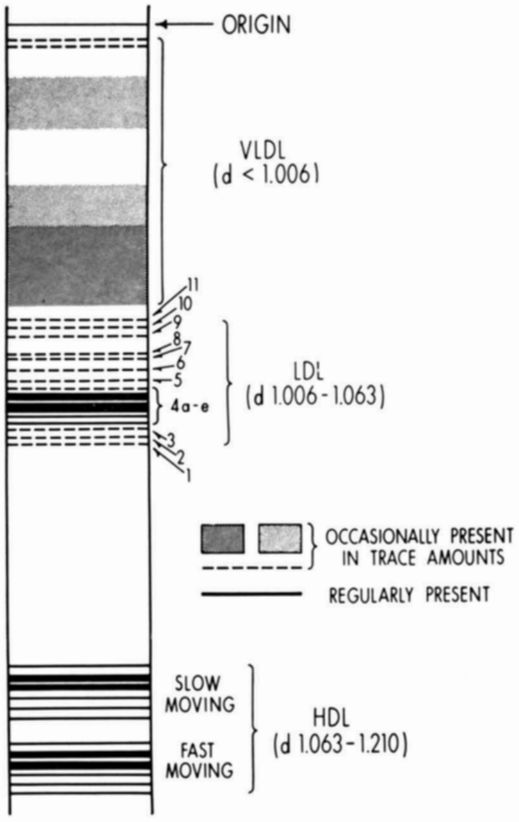

FIG. 1. Lipoprotein bands seen in CGE of normal plasma. Composite graphic representation of the different lipoprotein bands that have been seen in plasma samples studied by CGE. Numbering system of LDL bands was derived from results of experiments with mixed normal and abnormal plasmas, comparing the different mobilities of LDL bands. Variations in HDL are seen in normals, but the distribution of HDL into fast- and slow-moving components and the increased number and staining intensity of HDL bands are regular features of the normal pattern.

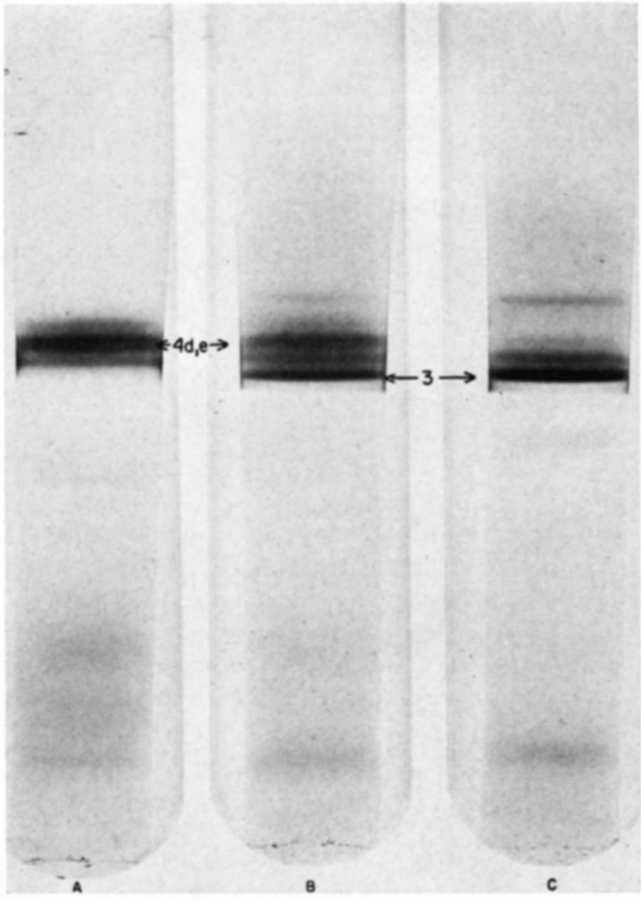

FIG. 2. Example of mixing experiments applied to all samples to study lipoprotein mobility and heterogeneity. *A*, plasma from a normal subject with major bands 4d and 4e. In the illustration these appear almost blended together into a single heavy band, preceded by the lighter-staining band 4b and followed by band 5. Other lighter-staining bands (e.g., 4a and 4c) are present but lost in photographing because of contrast differences emphasizing separation of heavily staining bands. *B*, mixed sample (1:1) of plasmas *A* and *C* shows the differences in mobility of major bands from each sample, with diminished staining of 4d and 4e, primarily derived from *A*, as well as diminished staining of 3, primarily derived from *C*. Despite mixing, LDL bands appear to retain the mobilities they exhibited in unmixed plasma. These same mobility differences are maintained when individual samples are run side by side in partitioned gel tubes. *C*, plasma from a subject with the greatest banding at 3 and 4b.

seen to be comprised of two major[2] bands (4c and 4d), and these were preceded by two minor components (4a and 4b) (Fig. 4). The above major bands were seen in 13 of the 16 normal subjects (81%). The three exceptions were C.V., R.F., and our oldest control subject, B.A. (Table 1). Occasionally, the major bands were followed by one or more extremely lightly staining bands (4e and 5–11). Increasing sample size made such bands more clearly visible and occasionally revealed others previously unseen. In addition, there was usually a small amount of diffusely staining VLDL (slightly more prominent in H.M., J.K., and B.A.). Furthermore, the HDL staining in normal plasma was marked and always consisted of two band groups termed here "fast" and "slow" HDL, each composed of up to six components. We emphasize that there was variation among normal plasmas in the amount of each LDL and HDL component by qualitative visual examination, although in general, this banding pattern remained surprisingly uniform.

Lipoproteins in hyperbetalipoproteinemia

Paper electrophoretic patterns and chemical lipid determinations clearly separated Fredrickson type II patients (Table 2) from control subjects. Paper electrophoresis uniformly showed a beta band more heavily stained with oil red O than found in normal subjects. A variable, but usually small, degree of pre-β staining was noted, which roughly correlated with the amount of triglyceride present. In addition, α-lipoprotein staining

[2] A major band is defined as one which is at least 0.5 mm in width.

TABLE 1. Control subjects: lipid fractionation and concentration gradient electrophoresis

Subject	Age	Sex	Weight	Diagnosis	Total Cholesterol[a]	Triglycerides[a]	Concentration Gradient Electrophoresis Bands: Major	Minor	Alpha
	yr		*kg*		*mg/100 ml plasma*				
S.L.	30	M	67.0	Dyspepsia	223 (210 ± 33)	120 (78 ± 39)	4c, 4d	4a, 4b, 5	normal
S.W.	45	F	63.4	Mild asthma	200 (217 ± 35)	104 (80 ± 42)	4c, 4d	4a, 4b	normal
J.R.	32	M	72.6	Asymptomatic	190 (210 ± 33)	130 (78 ± 39)	4c, 4d	4a, 4b, 5	normal
K.H.	24	F	45.1	Anxiety state	208 (179 ± 35)	70 (62 ± 29)	4c, 4d	4a, 4b	normal
P.F.	28	M	58.0	Asymptomatic	167 (183 ± 37)	104 (73 ± 32)	4c, 4d	4b, 4e, 5, 6	normal
J.K.	29	M	92.9	On allopurinol	195 (183 ± 37)	166 (73 ± 32)	4c, 4d	4a, 4b, VLDL	normal
C.V.	29	F	46.0	Ptotic kidney	183 (179 ± 35)	42 (62 ± 29)	4d, 4e	4a, 4b, 5	normal
J.M.	30	M	77.0	Asymptomatic	200 (210 ± 33)	112 (78 ± 39)	4c, 4d	3, 4a, 4b	normal
R.G.	27	M	67.0	Asymptomatic	195 (183 ± 37)	80 (73 ± 32)	4c, 4d	4a, 4b, 7, 8	normal
A.W.	32	M	69.2	Asymptomatic	190 (210 ± 33)	112 (78 ± 39)	4c, 4d	4a, 4b	normal
D.H.	28	M	75.9	Asymptomatic	200 (183 ± 37)	64 (73 ± 32)	4c, 4d	4b, 4e, 5, 7, 8	normal
R.A.	45	F	53.6	Renal cyst	280 (217 ± 35)	88 (80 ± 42)	4c, 4d	4a, 4b, 5	normal
R.F.	57	M	60.0	Asymptomatic	170 (240 ± 48)	76 (104 ± 45)	4b, 4c	4a, 5, 6, 7, 8	normal
H.M.	56	M	75.0	Asymptomatic	235 (240 ± 48)	184 (104 ± 45)	4c, 4d	4a, 4b, VLDL	normal
B.A.	76	F	65.4	Osteoporosis	230	142	4a, 4c	3, 9, 10, VLDL	normal
D.D.	30	M	76.1	Asymptomatic	145 (210 ± 33)	76 (78 ± 39)	4c, 4d	4a, 4b	normal
Mean	37				201 ± 31	104 ± 30			

[a] Normal values from sex- and age-matched subjects are shown in parentheses (page 151, Ref. 3).

TABLE 2. Phenotypic type II hyperbetalipoproteinemia: lipid fractionation and concentration gradient electrophoresis

Subject	Age	Sex	Weight	Diagnosis	Total Cholesterol[a]	Triglycerides[a]	Concentration Gradient Electrophoresis Bands: Major	Minor	Alpha
	yr		*kg*		*mg/100 ml plasma*				
E.R.	52	F	63.3	Angina pectoris; abnormal GTT[b]	375 (251 ± 49)	108 (83 ± 46)	4c, 4e	4b, 5, 8	sl. dec.
L.G.	37	M	75.0	Asymptomatic; normal GTT	306 (210 ± 33)	195 (78 ± 39)	3, 4a, 4c	2, 5, 6, 8, VLDL	decreased
G.N.	42	M	86.4	Angina pectoris; abnormal GTT	400 (230 ± 55)	252 (90 ± 41)	3, 4a, 4c	2, 4e, 6, 8, VLDL	decreased
L.B.	18	M		Son of C.B.; asymptomatic	341 (172 ± 34)	110 (61 ± 34)	4c, 4d	3, 4a, 4b, 8, 10, VLDL	decreased
N.A.	28	M	83.6	Hypertension; on allopurinol	320 (183 ± 37)	236 (73 ± 32)	4c, 4d, 5	4a, 6, 8, VLDL	decreased
T.C.	47	M	66.8	Asymptomatic; abnormal GTT	400 (230 ± 55)	258 (90 ± 41)	4a, 4c, 5	4b, 6, 8, 10, VLDL	decreased
B.C.	35	F	40.9	Schizophrenia	317 (204 ± 37)	88 (67 ± 48)	4b, 4c, 4d	3, 4a, 4e, 5, 8	decreased
G.R.	50	M	84.8	Asymptomatic	423 (240 ± 48)	184 (104 ± 45)	3, 4a, 4c, 4d	3, 5, 6, 7, 8	decreased
C.B.	45	M	60.3	Myocardial infarct; tendinous xanthomata	590 (230 ± 55)	300 (90 ± 41)	4c, 4d, 5, 6	3, 4a, 4b, 7, 8, 10, VLDL	decreased
P.L.	26	F	52.2	Tendinous xanthomata; normal GTT	455 (179 ± 35)	172 (62 ± 29)	4b, 4c, 4d, 6, 7, 8	3, 4a, 5, 10	decreased
M.S.	52	F	56.0	Angina pectoris; abnormal GTT	490 (251 ± 49)	170 (83 ± 46)	4a, 4c, 4d	3, 5, 6, 7, 8	decreased
Mean	39				401 ± 86	188 ± 69			

[a] Normal values from sex- and age-matched subjects are shown in parentheses (page 151, Ref. 3).
[b] GTT, glucose tolerance test.

was usually diminished compared with normals. Individual plasma cholesterol determinations were clearly above the "normal" ranges suggested by Fredrickson et al. (3). Mean total cholesterol was 401 mg/100 ml. Triglycerides were normal to moderately elevated (range 88–300 mg/100 ml), with a mean value of 188 mg/100 ml. The cholesterol value was always the larger of the two.

CGE in all hyperbetalipoproteinemic patients demonstrated an increased staining intensity of several components of band 4 (100%) and band 3 (27%), and others less often (Figs. 3*B* and *C* and 5*B*, *C*, and *D*, and Tables 2 and 3). Both type II and control subjects had major bands 4c and 4d in comparable frequency, but in general there were more major components present in type II patterns (Table 2). The components of band 4 were at times so intensely stained that they blended together to form a single band which often later resolved into its several parts with fading of the lipid stain in trichloroacetic acid. One or more of the slower-moving bands in

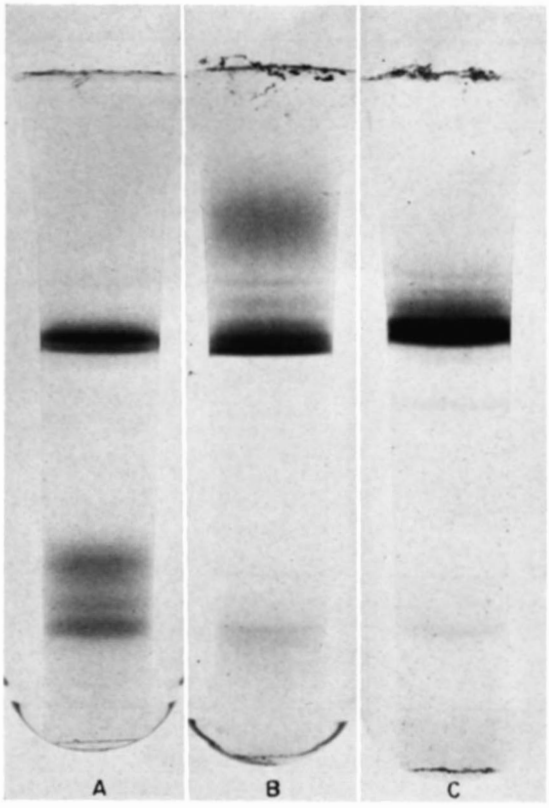

FIG. 3. Comparison of normal plasma with type II plasma. *A*, normal subject R.A.; *B*, phenotypic type II, T.C.; *C*, familial type II, C.B. Type II plasma patterns demonstrate increase in number, width, and staining intensity of major LDL bands compared with the normal subject. Note the increased amount of HDL staining and banding in the normal pattern, although this is less clearly defined than suggested by Fig. 1 because of rapid fading and diffusion of HDL stain in trichloroacetic acid before photography of the gel. Below the stained LDL in the midportion of the gels can be seen the heaviest of two nonlipoprotein bands which are seen in all separations after fixation in trichloroacetic acid. These bands can serve as markers to compare one banding pattern with another.

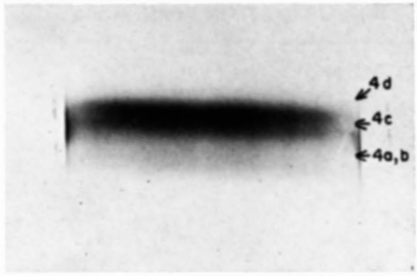

FIG. 4. Close-up view of the CGE pattern of the darker-staining LDL major bands (4c and 4d) and the lighter-staining LDL minor bands (4a and 4b) isolated in the ultracentrifuge (d 1.006–1.063) from the plasma of a fasting normal subject. The minor bands are immediately below the major bands and, in the photograph, appear to blend together.

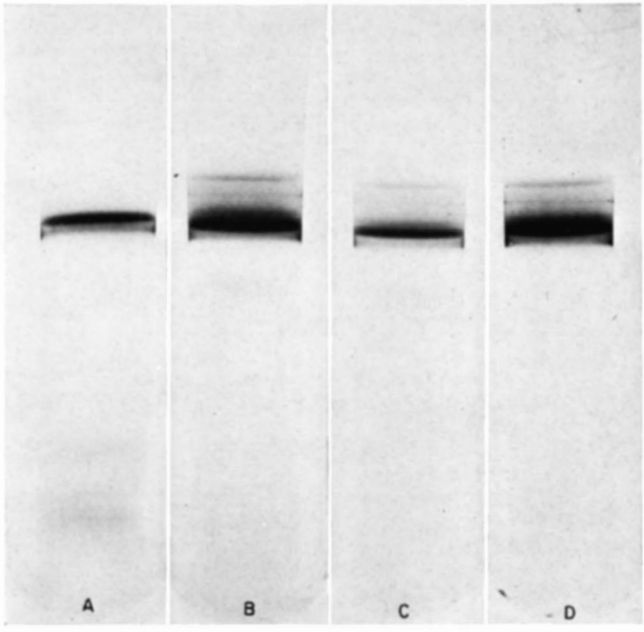

FIG. 5. Dilution and ultracentrifugation of type II plasma subsequently run in CGE. *A*, normal subject, J.M., whole plasma; *B*, familial Type II, M.S., whole plasma; *C*, familial type II, M.S., plasma diluted with saline (1:1); *D*, familial type II, M.S., infranatant fraction of plasma sample centrifuged at 100,000 *g* for 22 hr in a Spinco model L ultracentrifuge (no. 40 rotor) at native density of plasma (1.006). CGE reveals that LDL bands retain their banding pattern and mobility despite dilution and ultracentrifugation. Dilution is often necessary to demonstrate separation of heavily stained adjacent bands which tend to blend together. Tube *D* demonstrates that the mid-gel bands do not contain VLDL (d < 1.006). Other ultracentrifugal separation experiments have revealed these mid-gel bands to be LDL (d 1.006–1.063).

the range of 4e–11 was also seen in all specimens. Some increased VLDL staining occurred (54%) in six patients. In all cases, the HDL bands appeared relatively decreased in staining intensity compared with normal subjects. This decreased HDL staining occurred even in the absence of increased VLDL. Prominence of several bands in the number range 4e–11 was associated with slight elevations of triglyceride levels above 150 mg/100 ml (e.g., in patient M.S.) (Table 2 and Fig. 5), even when only a normal amount of VLDL was present. It is important to stress that there was considerable variation among patterns obtained from different type II patients, but repeated fasting samples from individual patients did not show variations. We note the absence of band 1 in all subjects reported here and the relative infrequency of band 2 (two of the type II patients), yet these faster bands were seen as major components with great frequency in the CGE patterns of patients with other hyperlipemic states.

DISCUSSION

This study demonstrates the remarkable complexity of the lipoproteins as seen with CGE. The gel medium separates these substances on the basis of both size and

TABLE 3. Band patterns in concentration gradient electrophoresis

A. Normal Subjects (n = 16)[a]

	LDL Major Bands (5)					LDL Minor Bands (10)									
Band number	4a	4b	4c	4d	4e	3	4a	4b	4e	5	6	7	8	9	10
Number of subjects with each band	1	1	15	14	1	2	13	14	2	7	2	3	3	1	1
Percentage with each band	6	6	94	88	6	13	81	88	13	44	13	19	19	6	6

B. Phenotypic Type II (n = 11)[b]

	LDL Major Bands (10)										LDL Minor Bands (10)									
Band number	3	4a	4b	4c	4d	4e	5	6	7	8	2	3	4a	4b	4e	5	6	7	8	10
Number of subjects with each band	3	5	2	11	7	1	3	2	1	1	2	6	5	4	2	6	6	3	10	4
Percentage with each band	27	45	18	100	64	9	27	18	9	9	18	55	45	36	18	55	55	27	91	36

[a] Total number of different major and minor LDL bands observed: 12. Average number of LDL major bands per subject: 2 (100%). Average number of LDL minor bands per subject: 3 (range 2–5).

[b] Total number of different major and minor LDL bands observed: 12. Average number of LDL major bands per subject: 3 (range 2–6). Average number of LDL minor bands per subject: 4 (range 3–6).

charge. Large lipoprotein molecules such as VLDL that normally do not enter even dilute gels (e.g., the 3.75% gels studied by Narayan [4]) move easily into the CGE medium of approximately 3.5% acrylamide and are subsequently separable into several fractions. More important than the separation of LDL and VLDL (which also may be done with agarose gel), CGE reveals differences in the LDL that are only suggested by ultracentrifugation and chemical lipid fractionation as well as paper electrophoresis of plasma samples.

Many authors have found multiple bands in acrylamide gel lipoprotein separations which they have attributed to heterogeneity among LDL. Pratt and Dangerfield (8) found numerous LDL bands similar to those we have observed. Borrie (16) noted many lipid-staining bands suggestive of LDL in type III patients. Frings, Foster, and Cohen (17) used single density gels and described several bands, possibly LDL, which were not further characterized. Similarly, Raymond, Miles, and Lee (18) found "one or more" LDL bands in 3.0% acrylamide gel slabs. In contrast to data from CGE, however, those bands appeared to show mobility differences from sample to sample, even within the same patient.

Our work extends the above observations and suggests that there is a general, reproducible lipoprotein pattern in CGE. The unifying concept of a general LDL pattern is suggested by the observation that the different lipoprotein bands of mixed plasma retain the mobilities they exhibited when run individually. Nevertheless, a key assumption is that lipoprotein bands with the same mobilities, bands that are superimposed in mixed plasma runs, are in fact identical LDL. If we accept this premise, our evidence suggests that major bands may vary from patient to patient; for example, band 4a may dominate in one patient and 4c in another. Technical problems such as small sample size or imperfect LDL separations might explain the single bands noted occasionally by Raymond et al. (18) and by Pratt and Dangerfield (8). Our numbering system is tentative but it is operationally useful, though it may require revision with further study, especially in patients with other well-defined lipoprotein disorders. More important than numbering bands will be techniques for isolation of individual bands from the gel columns so that possible immunological and chemical differences between the LDL may be investigated.

A "normal" LDL pattern consists of two prominent but separable bands, usually 4c, 4d, which were observed in 13 of 16 subjects. Variations from this pattern of major bands was seen in three subjects, also considered normal. Other LDL bands are represented in usually insignificant amounts. We also note the presence in normal subjects of two major components of HDL, with twelve minor components variably represented. In contrast, CGE patterns in patients with phenotypic hyperbetalipoproteinemia reveal variable elevations in one or more LDL bands that correspond to major or minor bands in normal plasma. Also, HDL appeared relatively and absolutely reduced in the type II group. The variability of the pattern in type II also suggests the possibility of subgroups within the presently defined Fredrickson classification.

Other data related to the physical chemistry of LDL suggest that they are a heterogeneous group of proteins; for instance, the variations in peak S_f 0–12 lipoproteins in the analytical ultracentrifuge most recently discussed by Lindgren and coworkers (19). These variations may reflect the weighted contribution of the numerous LDL noted in CGE, rather than differences in the lipid or protein content of a single, homogeneous "betalipoprotein." In addition, Lees (20) noted that in the immunodiffusion measurements for the quantitation of ultracentrifugally isolated LDL there were marked differences between the LDL cholesterol and the LDL protein ratios in normal subjects, in type II patients, and in patients with other lipoprotein disorders. We suggest that the predominance

of one or more LDL as seen in CGE, each with its unique cholesterol to protein ratio but with its common LDL protein antigen, may explain the wide range of cholesterol/protein ratios determined by Lees (20).

Finally, the physiological and pathophysiological significance of these multiple LDL bands remains to be determined. Family studies may reveal that the LDL species are inherited in a way similar to the variety of haptoglobins that are genetically transmitted in known combinations. Also, CGE pattern variations may well show new relationships between LDL and early arteriosclerosis. For example, six patients who did not fit our clinical definition of normal subjects (many had severe atherosclerotic vascular disease) revealed differences from the normal CGE pattern as described in this paper, showing banding patterns similar to those with hyperbetalipoproteinemia. These individuals had statistically normal plasma lipids and normal paper electrophoretic patterns. A possibility exists that certain types of betalipoprotein as well as an increase in amount of lipoprotein are associated with the process of atherosclerosis.

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