Pathways in the formation of human plasma high density lipoprotein subpopulations containing apolipoprotein A-I without apolipoprotein A-II

Alex V. Nichols,[1,*] Elaine L. Gong,[*] Patricia J. Blanche,[*] Trudy M. Forte,[*] and Virgie G. Shore[**]

Donner Laboratory, Lawrence Berkeley Laboratory,[*] University of California, Berkeley, CA 94720, and Lawrence Livermore National Laboratory,[**] P.O. Box 808, Livermore, CA 94550

Abstract The lecithin:cholesterol acyltransferase (LCAT)-induced transformation of two discrete species of model complexes that differ in number of apolipoprotein A-I (apoA-I) molecules per particle was investigated. One complex species (designated 3A-I(UC)-complexes) contained 3 apoA-I per particle, was discoidal (13.5 × 4.4 nm), and had a molar composition of 22:78:1 (unesterified cholesterol (UC):egg yolk phosphatidylcholine (egg yolk PC):apoA-I). The other complex species (designated 2A-I(UC)complexes) containing 2 apoA-I per particle was also discoidal (8.4 × 4.1 nm) and had a molar composition of 6:40:1. Transformation of 3A-I(UC)complexes by partially purified LCAT yielded a product (24 hr, 37°C) with a cholesteryl ester (CE) core, 3 apoA-I, and a mean diameter of 9.2 nm. The 2A-I(UC)complexes were only partially transformed to a core-containing product (24 hr, 37°C) which also had 3 apoA-I; this product, however, was smaller (diameter of 8.5 nm) than the product from 3A-I(UC)complexes. Transformation of 3A-I(UC)complexes appeared to result from build-up of core CE directly within the precursor complex. Transformation of 2A-I(UC)complexes, however, followed a stepwise pathway to the product with 3 apoA-I, apparently involving fusion of transforming precursors and release of one apoA-I from the fusion product. In the presence of low density lipoprotein (LDL), used as a source of additional cholesterol, conversion of 2A-I(UC)complexes to the product with 3 apoA-I was more extensive. The transformation product of 3A-I(UC)complexes in the presence of LDL also had 3 apoA-I but was considerably smaller in size (8.6 vs. 9.2 nm, diameter) and had a twofold lower molar content of PC compared with the product formed without LDL. LDL appeared to act both as a donor of UC and an acceptor of PC. Transformation products with 3 apoA-I obtained under the various experimental conditions in the present studies appear to be constrained in core CE content (between 13 to 22 CE per apoA-I; range of 9 CE molecules) but relatively flexible in content of surface PC molecules they can accommodate (between 24 to 49 PC per apoA-I; range of 25 PC molecules). The properties of the core-containing products with 3 apoA-I compare closely with those of the major subpopulation of human plasma HDL in the size range of 8.2–8.8 nm that contains the molecular weight equivalent of 3 apoA-I molecules.—**Nichols, A. V., E. L. Gong, P. J. Blanche, T. M. Forte, and V. G. Shore.** Pathways in the formation of human plasma high density lipoprotein subpopulations containing apolipoprotein A-I without apolipoprotein A-II. *J. Lipid Res.* 1987. **28:** 719–732.

Supplementary key words model precursors to HDL • discoidal complexes • apolipoprotein A-I (apoA-I) • gradient gel electrophoresis • chemical cross-linking

High density lipoproteins (HDL) are spherical lipid-protein particles, ranging in size from 7 to 12 nm, that contain the major part of apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II) in human plasma. Recent studies (1) utilizing immunoaffinity chromatography and gradient gel electrophoresis have demonstrated that the HDL particle size distribution is made up of two apolipoprotein-specific populations: a population containing apoA-I but no apoA-II (Lp(A-I without A-II)) and a population containing apoA-I with apoA-II (Lp(A-I with A-II)). The Lp(A-I without A-II) population exhibits two major subpopulations: one with mean particle size in the 12–9.8 nm interval (designated the $(HDL_{2b})_{gge}$ interval (2)) and the other with mean size in the 8.8–8.2 nm interval (designated the $(HDL_{3a})_{gge}$ interval). The properties of the subpopulation located within the $(HDL_{2b})_{gge}$ interval correspond closely to those of the ultracentrifugally defined HDL_{2b} subclass (d 1.063–1.100 g/ml (3)). The subpopulation within the $(HDL_{3a})_{gge}$ interval is contained within the ultracentrifugal HDL_3 subclass (d 1.125–1.200 g/ml) together with most of the Lp(A-I with A-II) population (1). More recently, we have shown by chemical (dimethylsuberimidate) cross-linking that the protein moiety of the Lp(A-I without A-II) particles in the $(HDL_{2b})_{gge}$ interval contains the

Abbreviations: apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; UC, unesterified cholesterol; CE, cholesteryl ester; PC, phosphatidylcholine; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

[1]To whom reprint requests should be addressed.

molecular weight equivalent of 4 apoA-I per particle, while that of particles in the $(HDL_{3a})_{gge}$ interval contains the equivalent of 3 apoA-I (4).

Our initial studies (5, 6) on possible pathways in the origin of the two Lp(A-I without A-II) subpopulations focused on lecithin:cholesterol acyltransferase (LCAT)-induced transformation of model precursor complexes comprised of phosphatidylcholine (PC) and apoA-I. The source of substrate unesterified cholesterol (UC) in these experiments was either low density (LDL) or very low density (VLDL) lipoproteins. Particles with properties comparable to those of the $(HDL_{3a})_{gge}$ subpopulation of the Lp(A-I without A-II) population were obtained when we used model precursors containing 2 apoA-I per particle, and comprised of egg yolk PC and apoA-I in approximate molar ratio of 95:1 (with no UC). These precursor complexes were discoidal in shape with long × short dimensions of 10.5 × 4.0 nm, respectively. The core-containing transformation product contained 3 apoA-I per particle and migrated upon electrophoresis either within or proximal to the particle size interval of the native $(HDL_{3a})_{gge}$ subpopulation. These transformation studies suggested that the physiologic origin of the native $(HDL_{3a})_{gge}$ subpopulation may involve fusion of transforming precursor species, initially with 2 apoA-I per particle, leading to a cholesteryl ester (CE)-enriched product with 3 apoA-I per particle. The physiologic relevance of the model precursor studies was further strengthened by the observation of Chen et al. (7) that small spherical HDL (7.6 nm) in plasma of LCAT-deficient patients are also comprised of 2 apoA-I and are transformed by LCAT, in major part, to core-containing product particles with 3 apoA-I per particle.

The present investigation addresses the question of whether model precursor complexes with 3 apoA-I per particle transform via a pathway that conserves the number of apoA-I per particle, at a putatively stable number of 3, or whether a pathway involving fusion, with further increase in number of apoA-I per particle is followed. Model precursor complexes with 3 apoA-I per particle were prepared in substantial amount upon incorporation of UC into recombinant mixtures containing egg yolk PC and apoA-I. In these mixtures, a discrete class of smaller complexes was also formed that contained 2 apoA-I per particle, which migrated electrophoretically in the particle size interval of the small spherical HDL observed in LCAT-deficient plasma. The present report also describes the properties and the LCAT-induced transformation of these small complexes, together with those of the complexes with 3 apoA-I.

MATERIALS AND METHODS

Apolipoprotein preparation

Human high density lipoproteins were prepared and washed by preparative ultracentrifugal methods previously described (3). ApoA-I was isolated according to published methods (8) and subjected to gel filtration chromatography on Sephacryl S-200 (Pharmacia Fine Chemicals, Piscataway, NJ; 2.6 × 100 cm column, 25 ml/hr, 3 M guanidine hydrochloride in 20 mM Tris buffer [11.3 mM Tris-HCl, 8.7 mM Tris base, 150 mM NaCl, 0.27 mM EDTA, and 0.124 mM ethylmercurisalicylic acid, pH 8.0]). Isolated apoA-I gave a single band by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (9); storage and treatment of apoA-I prior to use was previously reported (8).

Plasma lipoprotein analysis

Reference gradient gel electrophoresis patterns demonstrating HDL particle size distributions in normal human plasma and in plasma of a patient deficient in LCAT were obtained on plasma ultracentrifugal d 1.063–1.21 g/ml fractions isolated and analyzed by standard procedures (10). The normal subject was a 39-yr-old female and the LCAT-deficient patient was a 28-yr-old male identified by Dr. John Kane, University of California, San Francisco, who kindly provided the plasma for the present study. Fasting blood was drawn into EDTA-containing tubes (1.5 mg of EDTA/ml blood drawn) and plasma was separated by low-speed centrifugation.

Chemicals

Egg yolk PC (99% pure) was obtained from Grand Island Biological Company (Grand Island, NY). The egg yolk PC produced a single spot by thin-layer chromatography and was used without further purification. Unesterified cholesterol (> 98% pure) was purchased from Supelco, Inc. (Bellefonte, PA) and [4-^{14}C]cholesterol (57.5 mCi/mmol) was from Du Pont NEN Research Products (Boston, MA). Sodium cholate (98% pure) and human serum albumin (fraction V, fatty-acid free) were obtained from Sigma Chemical Company (St. Louis, MO). Dimethylsuberimidate dihydrochloride was obtained from Pierce Chemical Company (Rockford, IL).

Complex formation and isolation

Discoidal complexes were prepared by a detergent-dialysis method according to previously published techniques (8). Mixtures of lipid, apoA-I, and sodium cholate were incubated at 4°C for 18 hr in the 20 mM Tris buffer. Following dialysis with at least four 4-liter changes of 20 mM Tris buffer, discoidal complex preparations were passed through a Bio-Gel P-4 column (Bio-Rad Laboratories, Richmond, CA) to ensure removal of sodium cholate. Isolation of specific complex subclasses from multicomponent complex preparations was accomplished by gel filtration chromatography, using Ultragel AcA34 columns (LKB, Bromma, Sweden) in tandem (1.6 × 100 cm each; flow rate, 6.5 ml/hr at 4°C; elution buffer was 20 mM Tris buffer) or a Bio-Gel A5-M column (Bio-Rad, Richmond, CA; 1.6 × 100 cm; flow rate, 6 ml/hr at 4°C; elution buffer, 20 mM Tris buffer).

Preparation of ^{14}C-labeled LDL and partially purified LCAT

Blood from normal healthy volunteers was drawn into EDTA-containing tubes and kept on ice. Plasma was isolated by low-speed centrifugation; penicillin–streptomycin (50 units/ml and 50 μg/ml, respectively) and ethylmercurisalicylic acid (0.124 mM) were added immediately after centrifugation. LDL (d 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation (10) and dialyzed to 20 mM Tris buffer. ^{14}C-labeled LDL were prepared as previously described (11). Partially purified LCAT was prepared from the plasma ultracentrifugal d > 1.21 g/ml fraction according to published methods (12). The purification was 3000-fold; enzyme activity was assayed by the proteoliposome method (13).

Incubation mixtures and conditions

Incubation mixtures consisted of the following components: discoidal complexes (0.20–0.25 mg of apoA-I/ml); partially purified LCAT (at a concentration providing a fractional activity [percentage of UC esterified in 30 min] via the proteoliposome assay (12) in the range of 4–6% as observed for normal plasma); human serum albumin (40 mg/ml); and β-mercaptoethanol (14 mM). All constituents were in 20 mM Tris buffer. Concentrations of egg yolk PC and UC in mixtures depended on composition and concentrations of complexes used. In specific experiments, the available substrate UC in mixture was increased by addition of LDL (d 1.019–1.063 g/ml); the increment of LDL UC to the molar composition of experimental mixtures is indicated in the text.

Interaction mixtures were placed in glass screw-cap vials and flushed with nitrogen prior to incubation. Nonincubated (0 hr) mixtures were immediately placed in an ice-water bath following addition of all components; all other incubations were carried out in a 37°C shaking water bath. Prior to its addition to incubation mixtures, the partially purified LCAT preparation was incubated for 1 hr at 37°C with 14 mM β-mercaptoethanol. At the end of designated incubation periods, aliquots of the incubation mixtures were placed into an ice-water bath with addition of iodoacetic acid to a final concentration of 2 mM.

Fractionation of incubation mixtures

At designated times, aliquots of incubation mixtures were immediately mixed with NaBr–NaCl solution to raise the background density to 1.217 g/ml. These samples were subjected to preparative ultracentrifugation (114,000 *g*, 24 hr, 10°C); the top 1 ml of each tube contained the d ≤ 1.20 g/ml fraction. Isolation of products from LDL-containing d ≤ 1.20 g/ml fractions was accomplished by equilibrium density gradient ultracentrifugation with an SW 41Ti rotor (195,700 *g*, 62.5 hr, 10°C), using discontinuous NaBr gradients. Two density ranges were used: gradient A with density limits of 1.094–1.133 g/ml; and gradient B with limits of 1.114–1.153 g/ml. Gradient A was formed by layering the following NaBr solutions in a 13 ml tube: 4 ml of d 1.133 g/ml; 1 ml of d 1.120 g/ml (containing sample); 4 ml of d 1.107 g/ml; and 3 ml of d 1.094 g/ml. For gradient B, 4 ml of d 1.153 g/ml, 1 ml of d 1.140 g/ml (containing sample), 4 ml of 1.127 g/ml, and 3 ml of d 1.114 g/ml were successively layered.

Gradient gel electrophoresis

Nondenaturing electrophoresis was performed on 4–30% polyacrylamide gradient gels (Pharmacia Fine Chemicals, Piscataway, NJ). Staining, destaining, and analysis followed previously described procedures (2). Peak positions are measured from the top of the gel, and this distance is reported in terms of R_f value (migration distance of the peak relative to the migration distance of the peak of bovine serum albumin in the standard protein mixture run on the same gel). R_f intervals of the major human plasma total HDL subpopulations ($(HDL_{2b})_{gge}$, $(HDL_{2a})_{gge}$, $(HDL_{3a})_{gge}$, $(HDL_{3b})_{gge}$, and $(HDL_{3c})_{gge}$), as defined by gradient gel electrophoresis (2), were used to locate transformation products in the present study. The $(HDL_{2b})_{gge}$ and $(HDL_{2a})_{gge}$ subpopulations fall within the 12.0–9.8 nm and 9.8–8.8 nm size intervals and correspond approximately to the ultracentrifugal HDL_{2b} and HDL_{2a} density subclasses, respectively (3); the $(HDL_{3a})_{gge}$, $(HDL_{3b})_{gge}$, and $(HDL_{3c})_{gge}$ subpopulations fall within size intervals 8.8–8.2 nm, 8.2–7.8 nm, and 7.8–7.2 nm, respectively, and represent three subspecies within the ultracentrifugal HDL_3 density subclass.

Electron microscopy

Aliquots of isolated complexes were dialyzed to 5 mM ammonium bicarbonate and negatively stained with 1% sodium phosphotungstate, pH 7.4. Samples were applied either to Formvar carbon-coated grids or to thin-carbon films mounted on 300-mesh copper grids and examined in a JEOL 100C electron microscope. Particle size was obtained on 100 to 200 particles per sample as previously described (14).

Radial immunodiffusion analysis

Concentration of apoA-I in all preparations was measured by single radial immunodiffusion (15, 16). Wells (2 mm) of immunodiffusion plates containing 1.5% agarose and monospecific polyclonal antisera were filled with 5 μl of sample or standards (0.05–2 μg of apoA-I) and incubated for 48 hr at room temperature in a humid box. Analysis was carried out as previously described (6).

Chemical cross-linking and analysis

Samples for cross-linking analysis were adjusted to 2.8 M NaBr and an apolipoprotein concentration of 0.5–1

mg/ml. A 200-μl aliquot of the sample was cross-linked according to the method of Swaney and O'Brien (17). Cross-linked samples were dialyzed to 0.1 M $NaHPO_4$,1% SDS, pH 7.2, and analyzed by SDS-PAGE on 4–30% gradient gels (9). A standard calibration mixture (SDS-PAGE low molecular weight standards, Bio-Rad Laboratories) was run on each gel for determination of molecular weight.

Chemical analysis

Phospholipids were determined by the method of Bartlett (18) using a standard solution of potassium phosphate in 0.05 N hydrochloric acid (Sigma Chemical Co.). Protein was measured either by the method of Lowry et al. (19) using bovine serum albumin standard or by radial immunodiffusion for apoA-I. Percent esterification was determined by thin-layer chromatography of lipid extracts from incubation mixtures (6). Unesterified and esterified cholesterol concentrations were determined by gas–liquid chromatography (20).

RESULTS

Preparation and characterization of cholesterol-containing model precursor complexes

Formation of cholesterol-containing precursor complexes with 3 and 2 apoA-I per particle was facilitated by addition of sodium cholate (8, 21) to recombinant mixtures of apoA-I, egg yolk PC, and UC. Representative gradient gel electrophoresis patterns of complexes in unfractionated interaction mixtures following cholate removal are shown in **Fig. 1A–C.** To place these patterns into the perspective of patterns of putative physiological precursors of HDL and of mature total HDL particles, Fig. 1D and E show representative patterns of HDL from a subject with LCAT-deficiency and a normal subject, respectively. At initial molar ratio 0:88:1, UC:egg yolk PC:apoA-I, the pattern of the interaction mixture (Fig. 1A) shows a major component with additional minor components at lower and higher R_f values relative to the predominant peak. Based on four separate studies, the mean R_f ($\overline{R}_f$) of the major component is 0.633 ± 0.023 (mean ± standard deviation). We previously described this major component to contain 2 apoA-I and showed that it is comprised of discoidal complexes (10.5 × 4.0 nm, long × short axis, respectively) with a final egg yolk PC:apoA-I molar ratio 95:1. When UC is added to the interaction mixture at a molar ratio of 22:88:1, UC:egg yolk PC:apoA-I, the above component is no longer observed and three new components with R_f values of approximately 0.308, 0.543, and 0.833 appear (Fig. 1B). Interaction mixtures with a higher content of UC (44:88:1) consistently show a bimodal pattern (Fig. 1C). The two major components exhibit $\overline{R}_f$ values of 0.560 ± 0.013 and 0.837 ± 0.008 (n = 4). Incorporation of yet higher amounts of UC into the interaction mixture produces no

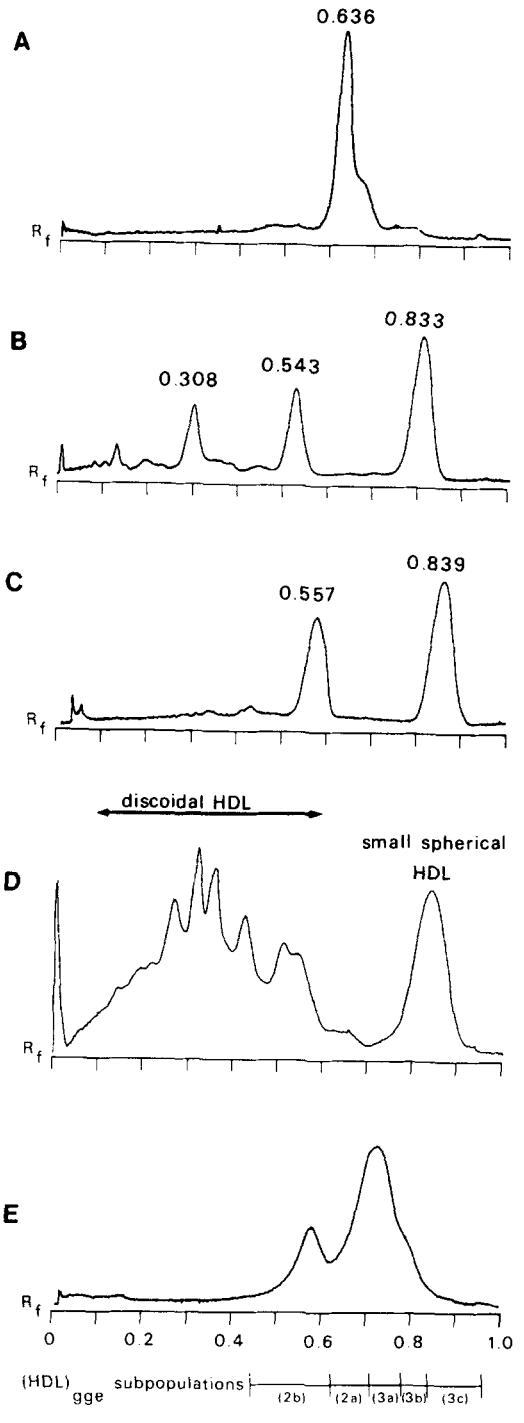

Fig. 1. Gradient gel electrophoresis patterns of complexes formed during cholate-facilitated recombination of apoA-I with egg yolk PC and increasing amounts of UC. Molar compositions (UC:egg yolk PC:apoA-I) of recombination mixtures leading to formation of complexes observed in patterns are: (A) 0:88:1; (B) 22:88:1; (C) 44:88:1. Migration of complexes from top of gel (at left) is given in terms of R_f value relative to migration of bovine serum albumin. For reference, a representative pattern of the HDL fraction (d 1.063–1.20 g/ml) from a subject with LCAT-deficiency is shown (D) to demonstrate migration intervals of native discoidal and small spherical HDL particles. A representative pattern of normal plasma total HDL is shown in (E) together with R_f intervals of total HDL subpopulations and their electrophoretic designation.

further change in the bimodal pattern observed at molar ratio 44:88:1. Peaks with R_f values similar to these two major components are seen in the pattern of the HDL from the LCAT-deficient subject. Particularly close correspondence in major peaks at R_f value of 0.837 can be seen in the pattern of the model complexes and HDL from the LCAT-deficient subject. Most of the HDL forming distinct peaks (R_f values ranging from 0.144 to 0.424) in the pattern from the LCAT-deficient subject are large discoidal particles (22). Unique model discoidal complexes in this size range can be prepared in vitro at higher PC:apoA-I molar ratios and their properties and transformation will be described in a subsequent report.

When interaction mixtures with the 44:88:1 molar ratio (Fig. 1C) were subjected to gel filtration, two elution peaks were observed and fractions under the peaks were separately pooled (**Fig. 2**). The R_f values of the complexes within the two pools were comparable to those of the two complexes in patterns of the total interaction mixture prior to gel filtration. Electron microscopy of the complexes with the lower R_f value (Fig. 2, left) reveals that they are predominantly discoidal particles with dimensions of 13.5 ± 2.1 × 4.4 ± 0.9 nm. En face particles have the same diameter as the long axis of the discs. The higher R_f complexes (Fig. 2, right) present two different images dependent upon the type of grid used. Images on thin-carbon films (Fig. 2A) show primarily discs which form rouleaux and have dimensions of 8.4 ± 0.6 nm × 4.1 ± 0.3 nm. On Formvar surfaces (Fig. 2B), the complexes tend to orient en face and, because they are so uniform, they frequently pack hexagonally; the en face particles have diameters of 7.8 ± 0.5 nm.

Chemical characterization of the two complex classes was performed after isolation from four separate complex preparations (**Table 1**). The lower R_f complexes are characterized by a higher lipid content than the complexes with the higher R_f value (71% vs. 54%, by weight, respectively). The molar ratio of UC:egg yolk PC in the lower R_f complexes is also considerably higher (0.28:1 vs. 0.15:1, respectively); both of these molar ratio values are lower than that of the initial interaction mixture (0.5:1). Chemical crosslinking of the isolated complexes was performed by dimethylsuberimidate, Based on SDS-PAGE of the crosslinked protein moiety, the lower R_f complexes are com-

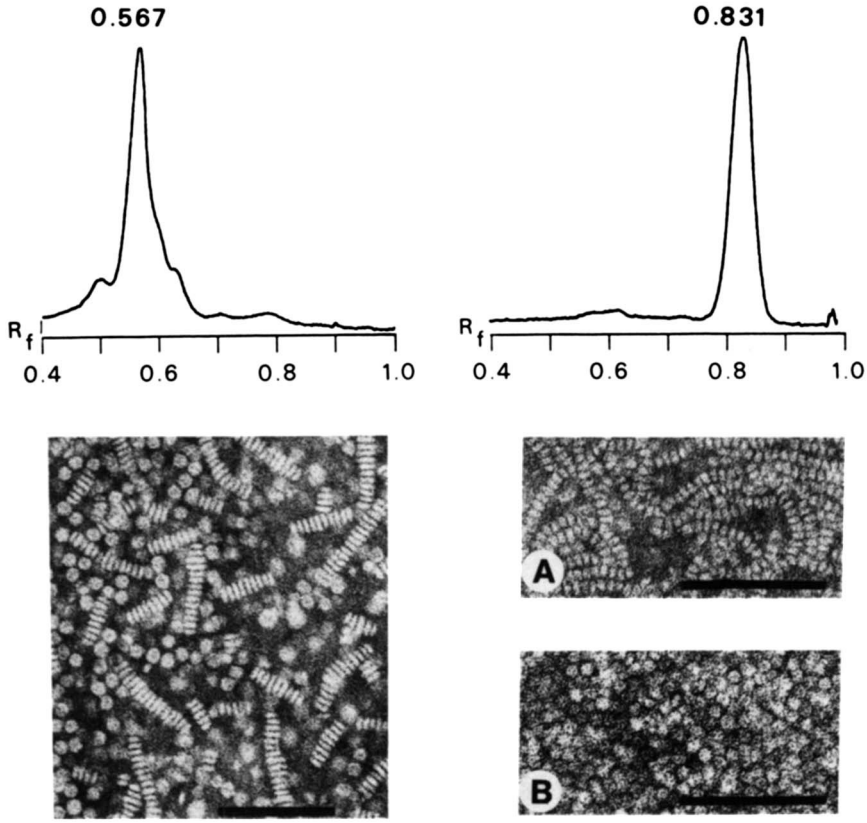

Fig. 2. Gradient gel electrophoresis and electron microscopy of the two major complex species, after isolation by gel filtration, from recombination mixture with molar composition 44:88:1 (UC:egg yolk PC:apoA-I) (see Fig. 1C). Micrograph (lower left) corresponds to complexes in electrophoretic pattern (upper left). Micrographs (lower right (A), thin-carbon film grid; lower right (B), Formvar grid) correspond to complexes in electrophoretic pattern (upper right). Bar marker in these and subsequent micrographs represents 100 nm.

TABLE 1. Composition of cholesterol-containing complexes isolated from recombination mixture (44:88:1, UC:egg yolk PC:apoA-I molar ratio)[a]

	$\bar{R}_f$ 0.560 Complex (13.5 × 4.4 nm)	$\bar{R}_f$ 0.837 Complex (8.4 × 4.1 nm)
	% Composition (by weight)	
UC	8.7 ± 0.6	3.5 ± 0.3
Egg yolk PC	62.3 ± 1.0	50.7 ± 0.5
ApoA-I	29.0 ± 0.8	45.8 ± 0.5
UC:egg yolk PC[b]	0.14:1	0.07:1
	Approximate Molar Composition[c]	
UC	65	12
Egg yolk PC	233	80
ApoA-I	3	2
UC:egg yolk PC[d]	0.28:1	0.15:1

[a]Complexes were prepared and isolated by gel filtration as described in Materials and Methods. Values are means of four preparations.
[b]Weight ratio.
[c]Molar composition calculated using molecular weights: 387 (UC); 775 (egg yolk PC); and 28,000 (apoA-I).
[d]Molar ratio.

prised of particles with predominantly 3 apoA-I per particle (**Fig. 3A**), while the higher R_f complexes are comprised of particles with predominantly 2 apoA-I per particle (Fig. 3B). Combining the chemical data and the results of the cross-linking studies, the molar compositions of the two major components are given in Table 1. The mean molar composition of the lower R_f complexes is 65:233:3 (UC:egg yolk PC:apoA-I) while that of the higher R_f complexes is 12:80:2. These results indicate a 2.9-fold greater number of egg yolk PC molecules and a 5.4-fold greater number of UC molecules in the larger, lower R_f complexes than in the smaller, higher R_f complexes.

In subsequent sections of this report, the above two major classes of cholesterol-containing discoidal complexes are identified in terms of their predominant distinguishing feature, namely the number of apoA-I per particle. Thus, the lower R_f complexes ($\bar{R}_f$ 0.560) are designated 3A-I(UC)-complexes, and the higher R_f complexes ($\bar{R}_f$ 0.837) are designated 2A-I(UC)complexes, indicating cholesterol-containing complexes with 3 and 2 apoA-I per particle, respectively.

Time course of transformation of 3A-I(UC)complexes upon interaction with LCAT

The time course of transformation of 3A-I(UC)complexes was investigated in incubation mixtures containing partially purified LCAT, human serum albumin, and β-mercaptoethanol. No additional UC was introduced into the incubation mixtures beyond that present in the discoidal complexes themselves. **Fig. 4** shows gradient gel electrophoresis patterns of d ≤ 1.20 g/ml fractions isolated by ultracentrifugation from incubation mixtures in a representative time course experiment (0 hr, 2.5 hr, 6 hr, 24 hr; 2.5 hr not shown). Similar data were obtained from three separate incubation experiments. The patterns consist of one predominant peak whose R_f value progressively increases with time (from R_f 0.544 at 0 hr to R_f 0.665 at 24 hr). At 0 hr incubation the R_f value of the peak is comparable to that of the stock nonincubated and nonultracentrifuged 3A-I(UC)complexes. By electron microscopy, particles isolated in the d ≤ 1.20 g/ml fraction from the 0 hr incubation mixture (Fig. 4A) are discoidal and of a size similar to that of the stock untreated preparation. Particles in fractions isolated from mixtures incubated for 2.5 hr, 6 hr, and 24 hr all appear round and no stacking struc-

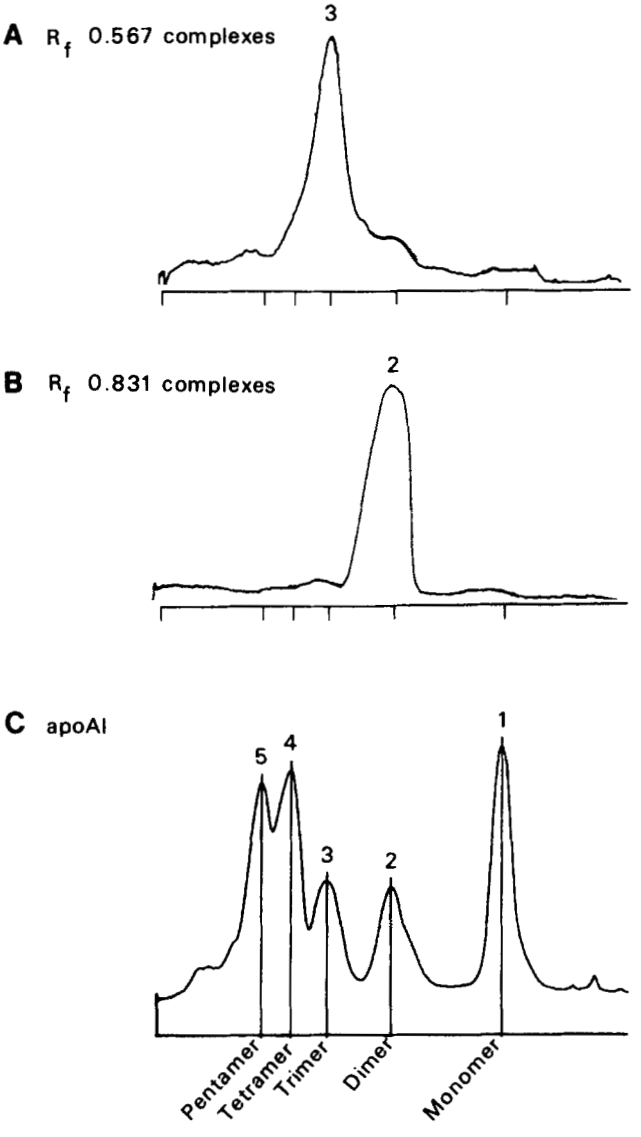

Fig. 3. SDS-PAGE (4-30% gradient gel) of cross-linked (dimethylsuberimidate) protein moieties of isolated complexes described in Fig. 2. Patterns correspond to following: (A) complexes with R_f 0.567; (B) complexes with R_f 0.831; and (C) isolated apoA-I preparation. Migration positions of peaks of monomeric and cross-linked oligomeric forms of apoA-I are indicated. Conditions of cross-linking are described in Materials and Methods.

tures are observed (see Fig. 4C for micrograph of 24-hr sample). Since considerable esterification had occurred in the incubation mixtures at these times (2.5 hr, 73%; 6 hr, 84%; and 24 hr, 97%), the round shapes most likely represent spheroidal particles with CE core. From peak positions on electrophoresis patterns, the particle diameters are: 10.0 nm (2.5 hr); 9.7 nm (6 hr); and 9.2 nm (24 hr). Based on three incubation experiments, the mean particle size of the final 24-hr product is 9.2 ± 0.04 nm and falls close to the upper bound of the size interval (8.8–8.2 nm) containing the peak of the native plasma Lp(A-I without A-II) subpopulation with protein molecular weight equivalent to 3 apoA-I per particle.

The mean chemical composition of the particles isolated in the d ≤ 1.20 g/ml fractions for the three experiments as described in Fig. 4 are given in **Table 2.** Consistent with the cholesterol esterification data for the total incubation mixture prior to ultracentrifugal processing, the particles in the d ≤ 1.20 g/ml fractions show compositional changes characterized by build-up of CE and reduction of UC and egg yolk PC. The build-up of core CE in product particles is rapid and is already 13% of the overall chemical composition at 2.5 hr and ultimately 17% at 24 hr, when near-complete esterification of cholesterol in the incubation medium is observed. Recovery of apoA-I in the d ≤ 1.20 g/ml fraction during the course of the incubation period reproducibly ranges from 85% (0 hr) to 81% (2.5 hr) to 77% (24 hr). In our experiments with 3A-I(UC)complexes, the recovery of apoA-I in d ≤ 1.20 g/ml fractions from control samples is 85–90% of the apoA-I incorporated into the incubation mixture. Assuming comparable recovery for incubation products, the decrease in apoA-I content in the d ≤ 1.20 g/ml fraction at 24 hr, relative to the apoA-I content at 0 hr, is about 10%. Thus, the bulk of the transformation appears to proceed by core build-up in the 3A-I(UC)complexes.

Chemical cross-linking was performed on the protein moiety of the products in the d ≤ 1.20 g/ml fraction to determine any changes in the number of apoA-I molecules per particle. At 0 hr and all subsequent incubation times, the product particles consisted predominantly of species with 3 apoA-I molecules per particle (**Fig. 5A,** 24-hr product). Thus, LCAT-induced transformation of cholesterol-containing discoidal complexes with 3 apoA-I molecules per particle does not involve fusion but follows a pathway in which the number of apoA-I molecules is conserved.

Based on the results of the chemical analyses and the

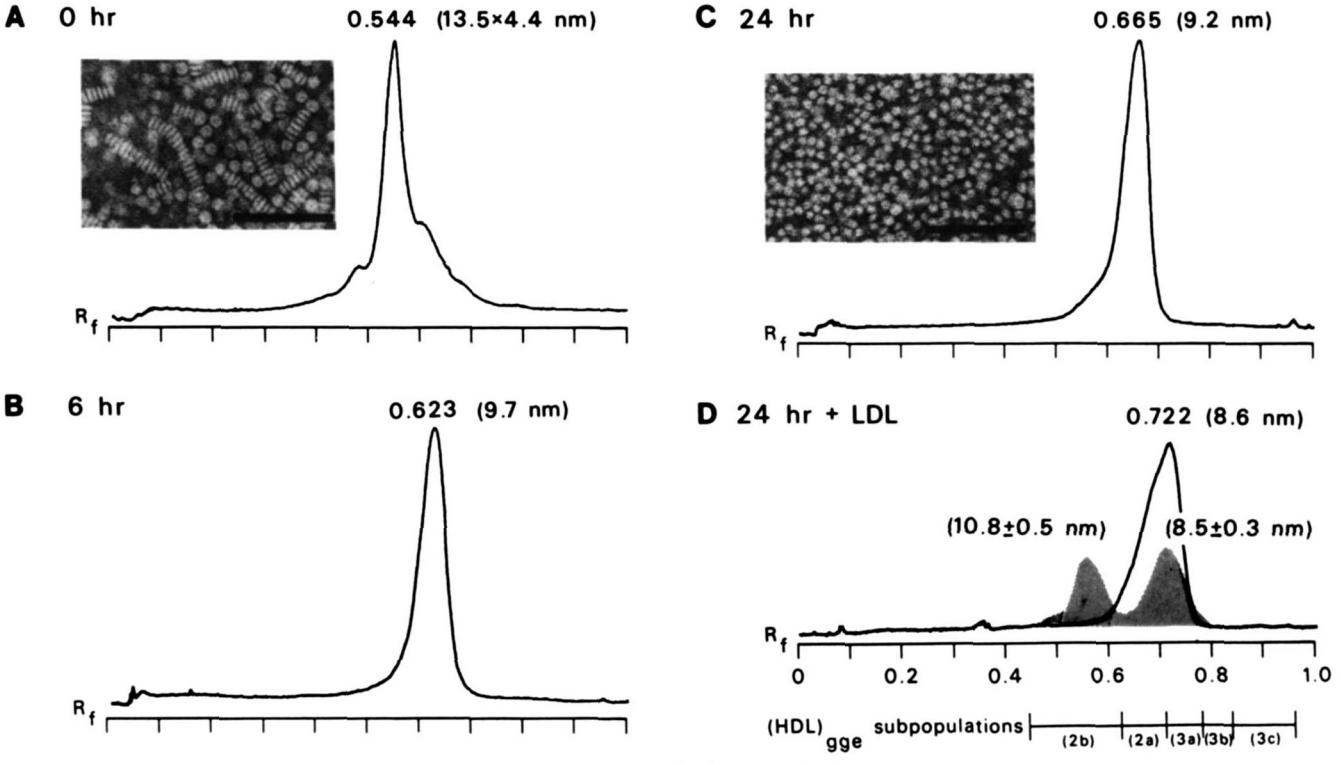

Fig. 4. Gradient gel electrophoresis patterns of transformation products in d ≤ 1.20 g/ml fractions isolated from incubation mixtures comprised of 3A-I(UC)complexes, partially purified LCAT, human serum albumin, and 14 mM β-mercaptoethanol. LCAT inhibitor (2 mM iodoacetic acid) was added at the end of incubation prior to processing. Patterns correspond to the following incubation (37°C) periods: (A) 0 hr; (B) 6 hr; and (C) 24 hr. Electron micrographs (Formvar grids) of material in fractions from 0-hr and 24-hr mixtures are shown with corresponding electrophoretic patterns. Pattern (D) is of 24-hr transformation product isolated from mixtures with same composition as in (A)–(C) above, but containing LDL as source of additional UC (see text for details). Product in (D) was isolated by equilibrium density gradient ultracentrifugation (gradient A) at banding density 1.114 g/ml. The shaded pattern in (D) is a schematic representation of the R_f distribution of the two major subpopulations observed in the human plasma HDL population containing apoA-I without apoA-II (Lp(A-I without A-II)). For reference, the particle size intervals of the major subpopulations of total HDL, as previously defined (13), are indicated below pattern (D).

TABLE 2. Composition of products from transformation of 3A-I(UC)complexes by lecithin:cholesterol acyltransferase[a]

	0 hr	2.5 hr	6 hr	24 hr	24 hr (+ LDL)[b]
			% Composition (by weight)		
UC	8.9 ± 0.6	3.4 ± 0.4	1.7 ± 0.2	0.9 ± 0.5	1.5 ± 0.3
CE		12.8 ± 1.3	15.6 ± 0.3	17.1 ± 0.1	23.2 ± 0.4
Egg yolk PC	63.4 ± 1.1	53.0 ± 1.3	49.5 ± 1.2	47.1 ± 0.6	30.3 ± 0.6
ApoA-I	27.7 ± 0.6	30.8 ± 2.2	33.2 ± 1.8	34.9 ± 0.4	45.0 ± 2.9
			Approximate Molar Composition		
UC	70	24	11	6	7
CE		54	61	63	67
Egg yolk PC	248	187	162	146	73
ApoA-I	3	3	3	3	3
Δ Egg yolk PC:Δ CE[c]		1.13:1	1.41:1	1.60:1	2.61:1

[a]Components of incubation mixture, incubation conditions, and product isolation are described in Materials and Methods and text. Values are means of three experiments.
[b]Product composition when incubation mixture contains LDL; details are given in text.
[c]Ratio of change in moles egg yolk PC to change in moles CE; changes are calculated relative to 0-hr time point. Molecular weights used in calculation are as in Table 1; the molecular weight for CE is 650.

cross-linking data, the approximate molar compositions of the transformation products were calculated and are listed in Table 2. The molar content of total cholesterol remains essentially constant during the overall transformation of precursor complexes to products. The molar content of CE in the product approaches that of the UC in the initial precursor complex, approximately 63 CE per particle at 24 hr. The molar content of CE at 2.5 hr is already 77% of the initial UC content and at 6 hr it is 87% of that value. The mean molar composition of the final (24 hr) core-containing product with 3 apoA-I is 6:63:146:3, UC:CE:PC:apoA-I. With incubation, the ratio of the molar change in PC relative to that in CE increases (1.13 [2.5 hr], 1.41 [6 hr], and 1.60 [24 hr]), indicating loss of PC from the d $\leq$ 1.20 g/ml fraction beyond that due to transesterification via LCAT activity. The additional decrease of PC suggests the presence of phospholipase activity in the incubation medium. Corresponding increases in PC phosphorus in the d $>$ 1.21 g/ml fraction are observed.

In previous studies (5), we demonstrated that LDL can serve as a source of UC for LCAT-induced transformation and CE core formation in complexes of apoA-I and egg yolk PC. In the present studies, we investigated whether the presence of LDL in the incubation mixture, by making available additional UC, would result in additional transesterification and further build-up of core in the product particles. The amount of LDL increased the initial molar composition of the incubation mixture from 22:78:1 (UC:egg yolk PC:apoA-I) to 60:78:1, increasing the available UC content by 2.7-fold. The 24-hr transformation product was isolated by equilibrium density gradient ultracentrifugation (gradient A, Materials and Methods) and banded at a mean density of 1.114 g/ml. The electrophoretic pattern (Fig. 4D) of an isolated 24-hr product shows a major component and, based on two studies, the mean particle diameter is 8.6 ± 0.08. This component is smaller than the 24-hr product obtained in the absence of LDL (9.2 nm). Chemical cross-linking of the product fraction shows almost exclusively the presence of particles with 3 apoA-I (data not shown), just as observed for products obtained in the absence of LDL. By chemical analysis (Table 2), the molar content of CE in these particles is comparable to that in products formed without LDL (67 vs. 63 CE per particle). A major chemical diffference, however, which is most likely associated with the smaller size of the products, is an almost twofold lower molar content of PC compared with that in products formed in the absence of LDL. Thus, the molar composition of the 24-hr product fractions formed in the presence of LDL is 7:67:73:3 (UC:CE:egg yolk PC:apoA-I) while that formed in the absence of LDL is 6:63:146:3. This comparison indicates that 3 apoA-I-containing transformation products are relatively tightly constrained in the number of CE molecules they can accomodate, but are considerably less constrained in the range of PC molecules they can incorporate into their surface.

Time course of transformation of 2A-I(UC)complexes upon interaction with LCAT

The time course of transformation of 2A-I(UC)complexes was evaluated in incubation mixtures containing partially purified LCAT, human serum albumin, and β-mercaptoethanol. As with the 3A-I(UC)complexes, no additional UC was introduced into the incubation mixtures. **Fig. 6** shows the time course of change in electrophoresis patterns of ultracentrifugal d $\leq$ 1.20 g/ml fractions isolated from the incubation mixtures. Similar results were obtained from three separate studies. At 0 hr incubation the R_f value of the peak (R_f 0.820) is comparable to that of the stock nonincubated and nonultracentrifuged 2A-I(UC)complexes ($\bar{R}_f$ 0.837). Incubation results in build-up of a major

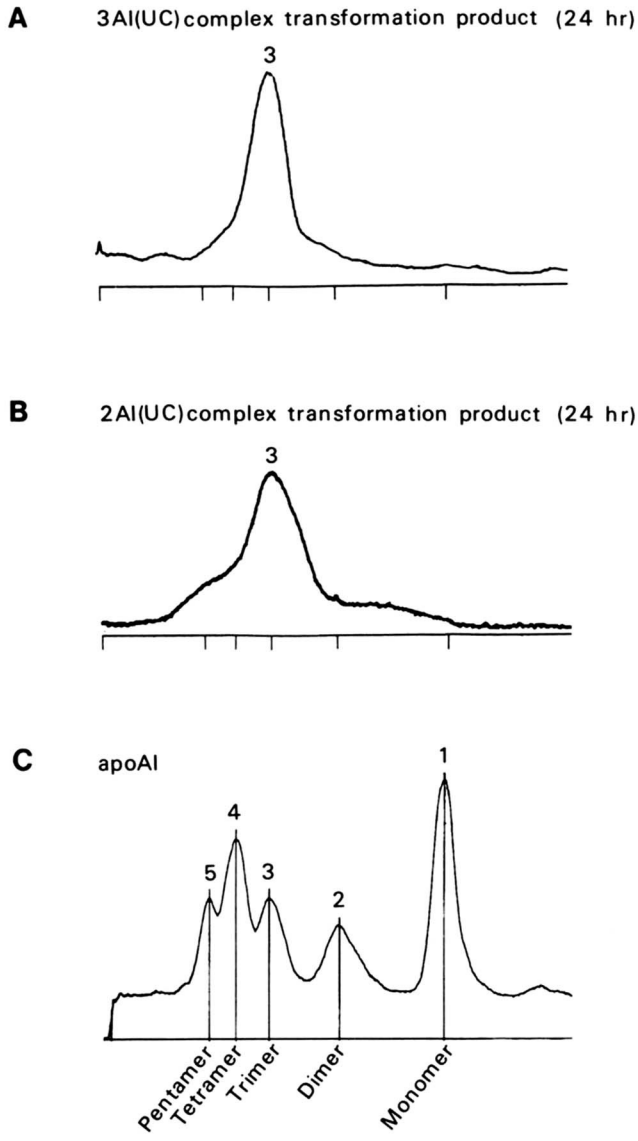

Fig. 5. SDS-PAGE (4–30% gradient gel) of cross-linked (dimethylsuberimidate) protein moieties of 24-hr transformation products isolated from incubation mixtures with precursors of (A) 3A-I(UC)complexes; and (B) 2A-I(UC)complexes. Cross-linked preparation of isolated apoA-I is shown in (C). Migration positions of peaks of monomeric and cross-linked oligomeric forms of apoA-I are indicated. Conditions of cross-linking are described in Materials and Methods.

product peak with R_f value of 0.671 at 6 hr and 0.725 ± 0.007 (n = 3) at 24 hr. The transformation appears as a single-step process proceeding from the discoidal complexes to particles with electrophoretic migration at 24 hr corresponding to round particles with mean diameter of 8.5 ± 0.04 nm (n = 3). This particle diameter falls within the size interval (8.8–8.2 nm) of the human plasma Lp(A-I without A-II) subpopulation with protein molecular weight equivalent to 3 apoA-I per particle. A shift to higher R_f values with incubation is also observed for the peak of the residual precursor complex (0.905 ± 0.011 [n = 3] at 24 hr). Assuming spherical shape, the mean particle size of the 24-hr residual complex is 7.4 ± 0.07 nm. Average recovery of apoA-I in the ultracentrifugal d ≤ 1.20 g/ml fractions isolated from the incubation mixtures is as follows: 83% (0 hr); 56% (2.5 hr); 41% (6 hr); and 26% (24 hr). By measurement, the reduction reflects a shift of the apoA-I into the d > 1.21 g/ml fraction. The relative contribution of residual precursor 2A-I(UC)complexes to the overall pattern area decreases with incubation but is still approximately 45% at 24 hr even though esterification of cholesterol is essentially complete (in two experiments, 98%

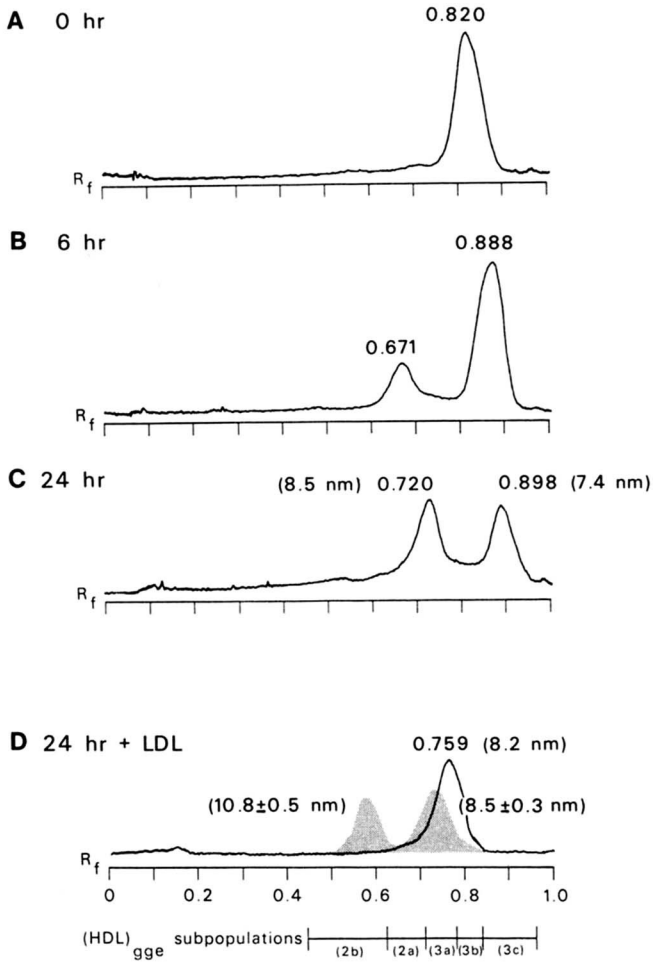

Fig. 6. Gradient gel electrophoresis of transformation products of d ≤ 1.20 g/ml fractions isolated from incubation mixtures comprised of cholesterol-containing 2A-I(UC)complexes, partially purified LCAT, human serum albumin, and 14 mM β-mercaptoethanol. LCAT inhibitor (2 mM iodoacetic acid) was added at end of incubation period prior to processing. Patterns correspond to following incubation (37°C) periods: (A) 0 hr; (B) 6 hr; and (C) 24 hr. Pattern (D) is 24-hr transformation product isolated from mixtures with same composition as in (A)–(C) above, but containing LDL as source of additional UC (see text for details). Product in (D) was isolated by equilibrium density gradient ultracentrifugation (gradient B) at mean banding density of 1.133 g/ml. See legend, Fig. 4, for explanation of shaded area.

TABLE 3. Composition of products (24 hr incubation) from transformation of 2A-I(UC)complexes by lecithin:cholesterol acyltransferase[a]

	% Composition		Approximate Molar Composition	
	24 hr	24 hr (+LDL)	24 hr	24 hr (+LDL)[b]
UC	1.0 ± 0.4	2.2 ± 0.6	4	10
CE	14.6 ± 0.1	15.0 ± 0.2	47	39
Egg yolk PC	44.6 ± 1.5	33.5 ± 1.2	121	74
ApoA-I	39.8 ± 2.5	49.3 ± 3.0	3	3

[a]Components of incubation mixtures, incubation conditions, and product isolation are described in Materials and Methods and text. Values are means of two experiments. Molar components were calculated using molecular weights listed in Tables 1 and 2.

[b]Product composition when incubation mixture contains LDL; details are given in text.

and 97%). This suggests that the net amount of CE formed may not be sufficient to transform the precursor complexes completely to core-containing product species.

Chemical cross-linking of the protein moiety of the core-containing product after isolation by gel filtration shows almost exclusively a product with 3 apoA-I per particle (Fig. 5B). **Table 3** presents the mean chemical composition data for the 24-hr transformation product of 2A-I(UC)complexes from two separate experiments. Comparison with the 24-hr product (also with 3 apoA-I) from transformation of 3A-I(UC)complexes (Table 2) shows that these products exhibit roughly comparable percent composition. Approximate molar contents calculated for CE and PC of the product from 2A-I(UC)complexes are 25% and 17%, respectively, lower than those of the 3A-I(UC)complex product. This is consistent with the smaller size of the product of 2A-I(UC)complexes relative to that of the 3A-I(UC)complexes (8.5 vs. 9.2 nm).

When LDL was added to the incubation mixture, resulting in a change of initial molar composition of the mixture from 6:40:1 (UC:egg yolk PC:apoA-I) to 22:40:1, a more extensive transformation (Fig. 6D) of the precursor complexes is observed. The 24-hr transformation product was isolated by equilibrium density gradient ultracentrifugation (gradient B, Materials and Methods) and banded at mean density of 1.133 g/ml. The major transformation product has a mean particle size of 8.2 ± 0.07 nm (n = 2) at 24 hr, which is smaller than the product obtained in the absence of LDL, but is still within the size range (8.8–8.2 nm) of the human plasma Lp(A-I without A-II) subpopulation with protein molecular weight equivalent to 3 apoA-I. In the presence of LDL, the total amounts of CE and apoA-I isolated within the product fraction are approximately twofold greater than those in the product fraction formed in the absence of LDL. Chemical cross-linking of the protein moieties in association with the lipoprotein species contained in the d ≤ 1.20 g/ml fraction, isolated from the incubation mixture, shows a predominance of species with 3 apoA-I molecules per particle (not shown). The mean molar composition of the isolated 24-hr product is 10:39:74:3 (UC:CE:PC:apoA-I) (Table 3). As was the case with transformation of 3A-I(UC)complexes, the smaller size of the product from 2A-I(UC)complexes, obtained in the presence of LDL, also appears associated with a lower PC:apoA-I molar ratio than observed in the absence of LDL (4:47:121:3). Recovery of apoA-I in the d ≤ 1.20 g/ml fraction when the incubation mixture (n = 3) contains LDL is approximately 83% (0 hr) and 59% (24 hr). Assuming recovery of product is comparable to that of control, the mean reduction of apoA-I in the d ≤ 1.20 g/ml fraction, associated with the transformation at 24 hr, is 28%.

DISCUSSION

We previously demonstrated that a series of discoidal complexes of unique size and composition is formed when apoA-I and egg yolk PC are recombined using a detergent (sodium cholate)-dialysis method (8). At least eight classes of discoidal complexes of discrete size and composition can be identified by gradient gel electrophoresis. With increasing molar ratio of egg yolk PC:apoA-I in the recombination mixture, these components are assembled into larger discoidal complexes with corresponding reduction in formation of the smaller discoidal complex classes. Thus, at the lowest molar ratio investigated (25:1, egg yolk PC:apoA-I), the predominant component is a small discoidal complex, approximately 8.7 × 4.0 nm, long × short dimension with $\bar{R}_f$ of 0.819. At molar ratio 88:1 in the recombination mixture, formation of $\bar{R}_f$ 0.819 complexes is negligible and the predominant class is now a larger discoidal complex, 10.5 × 4.0 nm, with $\bar{R}_f$ of 0.633. A further shift in assembly products to larger discoidal complexes is observed upon additional increase in molar ratio.

In the present investigation, addition of UC to the 88:1 recombination mixture redirected the recombination away from assembly of $\bar{R}_f$ 0.633 complexes, containing 2 apoA-I per particle (6). At molar ratio 44:88:1 (UC:egg yolk PC:apoA-I) two major cholesterol-containing, discoidal complex species are formed, one at $\bar{R}_f$ 0.560 with 3 apoA-I per particle and dimensions of 13.5 × 4.4 nm (3A-

I(UC)complexes) and the other at $\overline{R}_f$ 0.837 with 2 apoA-I per particle and dimensions of 8.4 × 4.1 nm (2A-I(UC)complexes). Addition of UC to recombination mixtures sufficient to raise the UC:egg yolk PC molar ratio above 0.5:1, when the egg yolk PC:apoA-I ratio is 88:1, results in the same bimodal electrophoretic pattern of complexes observed at molar ratio 44:88:1.

When the molar ratio of the recombination mixture is 44:88:1 (UC:egg yolk PC:apoA-I), the molar compositions of the 3A-I(UC)complexes and 2A-I(UC)complexes are 22:78:1 and 6:40:1, respectively. The molar composition of the 3A-I(UC)complex closely approximates that (20:80:1) of the cholesterol-containing micellar complex described by Matz and Jonas (21). The mean molar ratio of UC:egg yolk PC obtained for each of the complex species, when isolated from the 44:88:1 recombination mixture, is 0.28:1 for 3A-I(UC)complexes and 0.15:1 for 2A-I(UC)complexes. These molar ratios are considerably lower than that of the initial recombination mixture (0.5:1). Furthermore, there is a direct correlation between the UC:egg yolk PC molar ratio of the complexes and the complexes' particle size. These observations are consistent with the results of Tall and Lange (23) who proposed that apoA-I-PC complexes containing cholesterol are organized into two domains, a boundary region in proximity to apoA-I from which cholesterol is excluded and a nonboundary region that contains cholesterol in equilibrium with cholesterol in its milieu. The net effect of this organization is to lower the molar ratio of the resultant complexes relative to the initial recombinant mixture ratio. Our observation indicating that the complex molar ratio value approaches that of the recombinant mixture with increasing particle size is also consistent with the proposed two-region organization.

In our previous transformation studies (6), we showed that $\overline{R}_f$ 0.633 discoidal complexes, containing 2 apoA-I and comprised of only egg yolk PC and apoA-I, are initially transformed by LCAT, in the presence of LDL as a source of cholesterol, into small (approximate diameter 7.7 nm), apparently round particles also containing 2 apoA-I. These particles contain a small amount of CE in the approximate molar ratio of 2–3:1, CE:apoA-I. With longer incubation, there is further build-up of a core CE, resulting in production of larger round particles (approximate diameter 8.8 nm) now with 3 apoA-I per particle. The increase in number of apoA-I molecules appears to involve fusion of intermediate structures as they become enriched in CE. The availability of 3A-I(UC)complexes afforded us an opportunity to explore transformation of complexes initially with 3 apoA-I per particle and to evaluate whether a fusion pathway or some other pathway leads to a core-containing product. Our experiments indicate that transformation of 3A-I(UC)complexes leads directly to formation of a CE core within the precursor particle, without occurrence of intermediate steps involving either production of smaller particles or fusion. The mean molar composition of the 24-hr transformation product of 3A-I(UC)-complexes is 2:21:48:1 (UC:CE:PC:apoA-I). Using a precursor complex of comparable molar composition (20:80:1) but with different enzyme preparation and incubation conditions, Matz and Jonas (21) determined a molar ratio of 4:16:65:1 for a 24-hr conversion product. During transformation of 3A-I(UC)complexes the number of apoA-I molecules per particle remains at 3. Reduction of PC in the product exceeds the amount required for the transesterification reaction, suggesting removal either by phospholipase activity or transfer. We have previously noted (6) phospholipase A_2 activity upon interaction of cholesterol-poor discoidal complexes with LCAT. Reduction of PC, beyond transesterification requirements, in transformation products has also been observed during LCAT-induced conversion of nascent HDL in perfusate of liver of the African green monkey (24). Associated with the transformation of the 3A-I(UC)complexes, we also observed some decrease of apoA-I in the d ≤ 1.20 g/ml fraction at 24 hr. The basis for this decrease in apoA-I is not clear, but may indicate some surface destabilization from phospholipase activity and lysophosphatidylcholine accumulation.

The present observations suggest that stabilization of a CE core, comprised of 13–22 CE molecules per apoA-I and resulting from LCAT action on discoidal complexes, requires at least 3 apoA-I molecules. CE-containing products with 2 apoA-I molecules appear to be stable only at very low values of CE:apoA-I molar ratio (2–3:1 (6)). Availability of additional substrate cholesterol (from LDL) during 3A-I(UC)complex transformation does not significantly increase the core CE content per particle, nor lead to fusion with production of still larger particles containing a higher number of apoA-I molecules per particle. Interestingly, the product formed in the presence of this source of additional substrate cholesterol is actually smaller and markedly lower in PC content than that formed in the absence of LDL. Transfer of PC from model discoidal complexes to LDL, in the absence of LCAT activity, has recently been described (25). Conditions facilitating further transformation of products with 3 apoA-I to larger species, with properties of the larger human plasma Lp(A-I without A-II) subpopulation with an apparent protein moiety equivalent to 4 apoA-I, are under investigation. The possibility that PC enrichment of the 3 apoA-I product may facilitate incorporation of additional apoA-I allowing further core build-up is under investigation. Likewise, the potential transformation–enhancing effects of fusogenic components, such as unesterified fatty acids and lysoPC, appearing during lipolytic degradation of triglyceride-rich particles, are also under investigation. It is also possible, however, that the origin of the larger Lp(A-I without A-II) subpopulation may involve precursor species that either initially start with a higher number of apoA-I per particle (e.g., 4 apoA-I per

precursor particle) or contain other apolipoproteins (e.g., apoE) that may facilitate incorporation of a higher number of apoA-I into the core-containing product.

LCAT-induced transformation of 2A-I(UC)complexes without additional cholesterol in the incubation medium results only in their partial transformation (at 24 hr) to a core-containing product with 3 apoA-I per particle. The incomplete conversion appears due to an insufficient amount of substrate cholesterol available for build-up of core in most of the precursor complexes. Our current hypothesis for the mechanism involved in formation of the 3A-I-products (with 47 moles of CE and 4 moles of UC; at 24 hr) from 2A-I(UC)complexes (each initially with only 12 moles of UC) proposes that fusion of 2A-I-containing intermediates is the predominant process. The cholesterol required for build-up of core in the intermediates that leads to fusion is derived, in part, by transfer of UC contained within untransformed 2A-I(UC)complexes present in the incubation medium. This mechanism is essentially the same one we have proposed for fusion transformation of cholesterol-free 2A-I-precursors that proceeds by transfer of UC from LDL in the medium. The residual precursor species in the present studies probably represent cholesterol-depleted particles comprised primarily of apoA-I and PC. If these residual particles contain a small number of CE molecules (2–3 per apoA-I) then their overall physical-chemical properties would closely approximate those of the small transformation products obtained when discoidal complexes (95:1 egg yolk PC:apoA-I; 2 apoA-I per particle) interact with LCAT-active plasma d $>$ 1.21 g/ml fraction (6). Two additional features of this transformation system should be noted: *1)* the electrophoretic migration of the residual 2A-I(UC)complexes is greater than that of the starting 2A-I(UC)complexes and corresponds to that of the small round particles of mean size 7.4 nm and *2)* a large proportion (approximately 74%) of apoA-I, initially associated with the 2A-I(UC)complexes of d $\leq$ 1.20 g/ml, shifts into the d $>$ 1.21 g/ml fraction. The electrophoretic migration of residual 2A-I(UC)complexes is similar to that of human plasma HDL species which appear to be the smallest HDL species (apparent diameter 7.8–7.2 nm) particles that float into the d $\leq$ 1.20 g/ml fraction. ApoA-I-enriched small particles with apparently round shape and of size comparable to that of such residual 2A-I(UC)complexes are observed in HDL of LCAT-deficient subjects (7.6 nm and 2 apoA-I per particle; (7)), in HDL isolated from Hep G2 medium (7.4 nm; (26)), and in HDL secreted by rat intestine (7.8 nm; (27)). In the latter three situations, the small particles are considered to be spherical since stacking images are rarely encountered and round profiles predominate. Our present results suggest that some caution must be exercised in interpreting the images since the electron image of small lipid-protein complexes, as shown for the 2A-I(UC)complexes in the present study, is somewhat dependent upon staining conditions such as surface properties of the grid and particle concentration. Thus, it is altogether possible that the small HDL particles obtained from plasma, mesenteric lymph, and Hep G2-conditioned media may actually include discoidal particles as well as small HDL in early stages of core formation. The migration properties of such diverse particles on gradient gel electrophoresis are quite comparable (R_f values in the range of 0.825–0.905).

The basis for the shift of apoA-I from the d $\leq$ 1.20 g/ml fraction into the d $>$ 1.21 g/ml fraction during transformation of 2A-I(UC)complexes is not clear, but appears to involve further degradation (e.g., lipid depletion) of the residual 2A-I(UC)complexes. Control studies on 2A-I(UC)complexes in incubation mixtures containing human serum albumin but without LCAT activity do not show this shift. It is of interest to note that sedimenting apoA-I is observed in substantial amounts in plasma of LCAT-deficient subjects (28) and in Hep G2 medium which also contain the small apoA-I-enriched HDL particles (26).

Transformation of the 2A-I(UC)complexes in the presence of LDL, which provides additional UC to the incubation mixture, results in formation of a core-containing product with 3 apoA-I per particle and leaves no residual 2A-I(UC)complexes. The particle size of this product is smaller than that of the product formed in the absence of LDL. As was the case in the transformation of the 3A-I(UC)complexes, the conversion product of 2A-I(UC)complexes is significantly lower in PC content compared with that when LDL is absent; likewise, the CE content remains essentially the same as that of the product formed in the absence of LDL. In general, the quantum-like transformation of the 2A-I(UC)complexes (in the presence or absence of LDL) is similar to that observed for small core-poor particles (derived from LCAT-induced transformation (6) of discoidal complexes) that contain 2 apoA-I per particle and transform to products with 3 apoA-I per particle in the presence of a source of cholesterol. Transformation of the small, core-poor particles is associated with a reduction at 24 hr of approximately 25% in apoA-I content of the d $\leq$ 1.20 g/ml fraction, isolated from the incubation mixture. This is consistent with the hypothesis that fusion of 2A-I-intermediates occurs and yields stable 3A-I-products upon release of one apoA-I molecule from the product surface. In the present studies, transformation of the 2A-I(UC)complexes (in the presence of a source of cholesterol) is associated (at 24 hr) with a mean reduction of apoA-I of 28% in the d $\leq$ 1.20 g/ml fraction and thus appears to follow a comparable pathway.

In summary, for complexes containing 2 apoA-I, the pathway appears to involve fusion with formation of core-containing products having 3 apoA-I per particle. For complexes containing 3 apoA-I, the pathway proceeds directly to a core-containing product with 3 apoA-I per particle without formation of smaller intermediate species or fusion. Additional important properties of our transforma-

tion products include the following: *1)* the range of the number of CE molecules per apoA-I from the lowest (13:1) to the highest (22:1) CE:apoA-I molar ratio observed in products is 9, but *2)* the range of the number of PC molecules per apoA-I from the lowest (24:1) to the highest (49:1) PC:apoA-I molar ratio observed in products is 25. These observations indicate that the CE content of the product is relatively tightly controlled by the number of apoA-I per particle while the PC content is less restricted by the number of apoA-I. It is well established that native HDL are efficient recipients and carriers of additional PC and that this capability plays an important role in the metabolism of triglyceride-rich lipoproteins (29). Since only a partially purified LCAT preparation was used in the present experiments, the possible contribution of factors, such as lipid transfer protein and HDL-converting protein, cannot be excluded and requires evaluation using purified components.

Our studies suggest that the origin of the human plasma Lp(A-I without A-II) subpopulation, with protein molecular weight equivalent to 3 apoA-I per particle, may involve either or both of the two pathways described in this report. The fusion pathway may use as precursors the frequently observed small HDL particles enriched in apoA-I but low in UC. As shown in our present studies, transformation of these small precursor species to larger lipoproteins is more extensive in the presence of additional sources of UC and hence these particles appear to be excellent candidates for participation in reverse cholesterol transport (30). The direct core build-up pathway may utilize larger apoA-I-enriched discoidal complexes, containing at least 3 apoA-I per particle, such as have been observed in HDL of LCAT-deficient subjects and which may derive in part from lipolytic degradation of triglyceride-rich particles. The above two pathways could provide core-containing particles with 3 apoA-I per particle which, upon encountering lipoprotein remodeling factors in human plasma (e.g., lipid transfer proteins and lipases), would be ultimately transformed to the physiologically observed HDL product.

The authors are indebted to Ms. Mary Lou Olbrich for excellent assistance in the preparation of this manuscript. We are grateful to Robert W. Nordhausen and Marie Laskaris for their technical assistance. This work was supported by NIH Program Project Grant HL-18574 from the National Heart, Lung, and Blood Institute of the National Institutes of Health.

Manuscript received 1 July 1986 and in revised form 13 January 1987.

REFERENCES

1. Cheung, M. C., and J. J. Albers. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. *J. Biol. Chem.* **259:** 12201–12209.
2. Nichols, A. V., P. J. Blanche, and E. L. Gong. 1983. Gradient gel electrophoresis of human plasma high density lipoproteins. *In* CRC Handbook of Electrophoresis. Vol. III. L. Lewis, editor. CRC Press, Boca Raton, FL. 29–47.
3. Anderson, D. W., A. V. Nichols, T. M. Forte, and F. T. Lindgren. 1977. Particle distribution of human serum high density lipoproteins. *Biochim. Biophys. Acta.* **493:** 55–68.
4. Nichols, A. V., M. Cheung, P. Blanche, E. Gong, and J. Albers. 1985. Relevance of model transformation studies to origins of HDL subpopulations. *Circulation.* **72, Supp. III:** III–364.
5. Nichols, A. V., E. L. Gong, P. J. Blanche, T. M. Forte, and V. G. Shore. 1984. Interaction of model discoidal complexes of phosphatidylcholine and apolipoprotein A-I with plasma components: physical and chemical properties of the transformed complexes. *Biochim. Biophys. Acta.* **793:** 325–337.
6. Nichols, A. V., P. J. Blanche, E. L. Gong, V. G. Shore, and T. M. Forte. 1985. Molecular pathways in the transformation of model discoidal lipoprotein complexes induced by lecithin:cholesterol acyltransferase. *Biochim. Biophys. Acta.* **834:** 285–300.
7. Chen, C., K. Applegate, W. C. King, J. A. Glomset, K. R. Norum, and E. Gjone. 1984. A study of the small spherical high density lipoproteins of patients afflicted with familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* **25:** 269–282.
8. Nichols, A. V., E. L. Gong, P. J. Blanche, and T. M. Forte. 1983. Characterization of discoidal complexes of phosphatidylcholine, apolipoprotein A-I, and cholesterol by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **750:** 353–364.
9. Weber, K., and M. Osborne. 1970. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
10. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins. G. J. Nelson, editor. John Wiley-Interscience, New York, NY. 181–274.
11. Barter, P. J., G. J. Hopkins, and L. Gorjatschko. 1984. Comparison of human plasma low- and high-density lipoproteins as substrates for lecithin:cholesterol acyltransferase. *Biochim. Biophys. Acta.* **792:** 1–5.
12. Chen, C-H., and J. J. Albers. 1981. Further characterization of purified lecithin:cholesterol acyltransferase from human plasma. *Biochem. Med.* **25:** 215–226.
13. Chen, C-H., and J. J. Albers. 1982. Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin:cholesterol acyltransferase activity. *J. Lipid Res.* **23:** 680–691.
14. Forte, T. M., P. A. Davis, R. W. Nordhausen, and C. J. Glueck. 1982. The electron microscopic structure of human umbilical cord blood lipoproteins. *Artery.* **10:** 223–236.
15. Ouchterlony, O., and L-A. Nilsson. 1978. Immunodiffusion and immunoelectrophoresis. *In* Handbook of Experimental Immunology. 3rd ed. D. M. Weir, editor. J. B. Lippencott Co., Philadelphia, PA. 19.10–19.44.
16. Albers, J. J., and M. C. Cheung. 1979. Radial immunodiffusion assay of lipoproteins and apoproteins: application to high density lipoproteins. *In* Report of the HDL Methodology Workshop, San Francisco, March 1979. K. Lippel, editor. NIH Publication #79-1661. 251–264.
17. Swaney, J. B., and K. O'Brien. 1978. Cross-linking studies of the self-association properties of apoA-I and apoA-II from human high density lipoprotein. *J. Biol. Chem.* **253:** 7069–7077.
18. Bartlett, G. F. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.

19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
20. Hindriks, F. R., B. G. Wolthers, and A. Groen. 1977. The determination of total cholesterol in serum by gas-liquid chromatography compared with two other methods. *Clin. Chim. Acta.* **74:** 207–215.
21. Matz, C. E., and A. Jonas. 1982. Reaction of human lecithin:cholesterol acyltransferase with synthetic micellar complexes of apolipoprotein A-I, phosphatidylcholine, and cholesterol. *J. Biol. Chem.* **257:** 4541–4546.
22. Soutar, A. K., B. L. Knight, and N. B. Myant. 1982. The characterization of lipoproteins in the high density fraction obtained from patients with familial lecithin:cholesterol acyltransferase deficiency and their interaction with cultured human fibroblasts. *J. Lipid Res.* **23:** 380–390.
23. Tall, A. R., and Y. Lange. 1978. Interaction of cholesterol, phospholipid, and apolipoprotein in high density lipoprotein recombinants. *Biochim. Biophys. Acta.* **513:** 185–197.
24. Babiak, J., H. Tamachi, F. L. Johnson, J. S. Parks, and L. L. Rudel. 1986. Lecithin:cholesterol acyltransferase-induced modifications of liver perfusate discoidal high density lipoproteins from African green monkeys. *J. Lipid Res.* **27:** 1304–1317.
25. Shahrokh, Z., and A. V. Nichols. 1985. Interaction of human plasma low density lipoproteins with discoidal complexes of apolipoprotein A-I and phosphatidylcholine: characterization of the interaction products. *Biochim. Biophys. Acta.* **837:** 296–304.
26. Thrift, R. N., T. M. Forte, B. E. Cahoon, and V. G. Shore. 1986. Characterization of lipoproteins produced by the human liver cell line, Hep G2, under defined conditions. *J. Lipid Res.* **27:** 236–250.
27. Forester, G. P., A. R. Tall, C. L. Bisgaier, and R. M. Glickman. 1983. Rat intestine secretes spherical high density lipoproteins. *J. Biol. Chem.* **258:** 5938–5943.
28. Norum, K. R., J. A. Glomset, A. V. Nichols, T. Forte, J. J. Albers, W. C. King, C. D. Mitchell, K. R. Applegate, E. L. Gong, V. Cabana, and E. Gjone. 1975. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: effects of incubation with lecithin:cholesterol acyltransferase in vitro. *Scand. J. Clin. Lab. Invest.* **35, Suppl. 142:** 31–55.
29. Eisenberg, S. 1984. High density lipoprotein metabolism. *J. Lipid Res.* **25:** 1017–1058.
30. J. F. Oram, J. J. Albers, M. C. Cheung, and E. L. Bierman. 1981. The effects of subfractions of high density lipoprotein on cholesterol efflux from cultured fibroblasts: regulation of low density lipoprotein receptor activity. *J. Biol. Chem.* **256:** 8348–8356.