Characterization and quantitation of apolipoproteins A-I and E of normal and cholesterol-fed guinea pigs

Luke S. S. Guo, Robert L. Hamilton, John P. Kane, Christopher J. Fielding, and G. Chi Chen

Cardiovascular Research Institute and the Departments of Anatomy, Physiology, and Medicine, University of California, San Francisco, CA 94143

Abstract We have characterized and quantified the two major plasma apoproteins of high density lipoproteins (HDL), apolipoproteins A-I (apoA-I) and E (apoE), of guinea pigs fed standard chow (normal) or chow supplemented with 1% cholesterol (cholesterol-fed). ApoA-I isolated from plasma HDL of the normal guinea pig exists in six polymorphic forms (pI 5.75–5.40). A similar isoform pattern of this apoprotein was present in nascent HDL isolated from perfused livers of normal and cholesterol-fed animals. This apoprotein contains cysteine and isoleucine and is slightly different in overall amino acid composition from apoA-I of human and rat, but activates lecithin:cholesterol acyltransferase from human plasma with an activation curve almost identical to that obtained with human apoA-I. ApoE present in nascent VLDL and HDL from perfused liver of normal animals contains three isoforms (pI 5.42–5.34). Following cholesterol feeding, the numbers of apoE isoforms from perfused livers were increased from three to five or more by shifting the major component (pI 5.42) to more acidic isoforms (pI 5.28–5.17). This shifting was mostly reversible when apoE was treated with neuraminidase, suggesting that cholesterol feeding leads to a modification of apoE by increasing its content of sialic acid. Similar changes of apoE isoforms were also observed in plasma lipoproteins as early as 10 days after cholesterol feeding. The amino acid compositions of four apoE isoform fractions isolated from plasma HDL of cholesterol-fed guinea pigs were similar to that of parent apoE. The plasma concentrations of apoA-I and apoE, measured by electroimmunoassay, were 6.2 ± 2.0 and 2.2 ± 0.5 mg/dl, respectively, in guinea pigs fed standard chow. In animals that had been fed 1% cholesterol, plasma levels of apoA-I slightly increased in 1 week and showed a twofold increase in 8–10 weeks. Plasma levels of apoE, on the other hand, sharply increased by 10-fold in 1 week and up to 22-fold in 8–10 weeks on the cholesterol diet.—**Guo, L. S. S., R. L. Hamilton, J. P. Kane, C. J. Fielding, and G. C. Chen.** Characterization and quantitation of apolipoproteins A-I and E of normal and cholesterol-fed guinea pigs. *J. Lipid Res.* 1982. **23:** 531–542.

Supplementary key words isoelectric focusing • electroimmunoassay • amino acid composition • LCAT activation

In most animal models, including rats, dogs, swine, monkeys (1), and rabbits (2, 3), cholesterol-induced hyperlipoproteinemia is characterized by significant increases of plasma concentrations of intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and the appearance of β-migrating very low density lipoproteins (VLDL). In addition, an α_2-migrating high density lipoprotein, designated as HDL_c, is induced by cholesterol diet that is accompanied by a markedly reduced α_1-migrating HDL_2 (1). This HDL_c is rich in cholesteryl esters and contains apolipoproteins E and A-I as major apoprotein constituents.

Total plasma cholesterol levels vary widely among mammals (4). In guinea pigs, plasma cholesterol is carried predominantly in LDL; concentrations of plasma HDL are low (5, 6), as in individuals with Tangier disease (7, 8). The HDL-cholesterol in guinea pigs comprises less than 5% of the total plasma cholesterol (80 mg/dl) (5, 9). Feeding cholesterol to this animal rapidly produces hyperlipoproteinemia, associated with increased LDL and IDL levels, and the appearance of β-VLDL; however, in sharp contrast to other animal models, feeding cholesterol to guinea pigs for 8–10 weeks markedly increased plasma HDL levels (5, 9). The HDL is enriched in unesterified cholesterol and apoE, and often contains discoidal particles (9, 10). It is particularly interesting that similar apoE-rich discoidal HDL accumulates in the plasma of humans with genetic lecithin:cholesterol acyltransferase (LCAT) deficiency (11–14), and in perfusates of rat liver when LCAT is inhibited chemically (15).

We have previously isolated and partially characterized two major apolipoproteins, apoA-I and apoE, present in HDL of normal and cholesterol-fed guinea pigs (10). To learn more about relationships between HDL and cholesterol metabolism in this species, we have further characterized these two apolipoproteins with respect to the functional role of the apoA-I as cofactor for lecithin:cholesterol acyltransferase and dietary cholesterol-induced alteration of apoE polymorphisms. Changes of

Abbreviations: apoA-I, apolipoprotein A-I; apoE, apolipoprotein E; HDL, high density lipoprotein(s); LDL, low density lipoprotein(s); VLDL, very low density lipoprotein(s); SDS, sodium dodecyl sulfate; LCAT, lecithin:cholesterol acyltransferase; EGTA, ethylene glycol bis (β-aminoethyl ether) N,N′-tetraacetic acid.

plasma concentrations of apoA-I and apoE in response to cholesterol feeding were also determined by an electroimmunoassay.

EXPERIMENTAL PROCEDURES

Preparation of plasma and perfusate lipoproteins

Adult male guinea pigs (Simonson Lab., Gilroy, CA), were fed Purina chow (normal) or the chow containing 1% cholesterol and 5% cottonseed oil (cholesterol-fed). The cholesterol-fed animals were usually maintained on the diet for 8–12 weeks. Blood was collected in 0.1% Na_2EDTA by heart puncture. Plasma HDL of the normal ($1.10 < d < 1.21$ g/ml) and cholesterol-fed animals ($1.07 < d < 1.21$ g/ml) was isolated by sequential ultracentrifugation using the Beckman L3-50 ultracentrifuge (Beckman Instruments, Palo Alto, CA) and the 40.3 rotor (16). In some experiments, HDL was first isolated with a Ti 50.2 rotor at 48,000 rpm for 18–24 hr and recentrifuged at the same density in the 40.3 rotor. Perfusate VLDL ($d < 1.05$ g/ml) and HDL ($1.10 < d < 1.21$ g/ml) were isolated from liver perfusates of normal and cholesterol-fed guinea pigs as described in the accompanying paper (17). Isolated lipoproteins containing 0.5–2.0 mg protein/ml were delipidated in 20 volumes of ethanol–diethyl ether 3:1 (vol/vol) and diethyl ether at −10°C (18).

Isolation of apoA-I and apoE

ApoA-I and apoE were isolated from plasma apoHDL of normal and cholesterol-fed guinea pigs, respectively, by two column chromatographic systems: a Sephacryl S-200 column (2.5 × 95 cm) equilibrated in 0.015 M Tris–HCl, 6 M urea, pH 8.2 (buffer A) and an anti-apoA-I immunoaffinity column (1.2 × 35 cm) equilibrated in 0.1 M phosphate buffer, containing 0.02% NaN_3, 0.04% EDTA (buffer B).

For isolation of apoA-I, chromatography of the normal apoHDL on a Sephacryl S-200 column yielded two distinct protein peaks. The fractions containing apoA-I were pooled, dialyzed extensively against buffer B, and applied to an anti-apoA-I immunoaffinity column. The unretained fraction was washed through the column with 80–100 ml of buffer B. ApoA-I bound to the antibody column was eluted with 50 ml of 3 M NaSCN.

For isolation of apoE from the apoHDL of the cholesterol-fed guinea pigs, intact HDL (1–3 mg in 2 ml) was passed through the anti-apoA-I immunoaffinity column to remove particles containing apoA-I. The unretained fraction was delipidated with ethanol–diethyl ether (10). The precipitated apoproteins were recovered in 2 ml of buffer A and applied to a Sephacryl S-200 column. This separated apoE-co-migrating protein (19) and trace amounts of C apoproteins from the major protein peak containing apoE. Isolated apolipoproteins were dialyzed extensively against a 10^{-4} M Tris-buffer and concentrated by partial lyophilization to a volume of 1.0–2.0 ml.

In some experiments, isolated apoA-I and apoE were further fractionated on a DEAE-cellulose column (0.9 × 14 cm) similar to the method of Shore and Shore (20). The column was equilibrated in 0.01 M Tris–HCl, 6 M urea, pH 8.2. Proteins bound to the DEAE-cellulose were eluted with a Tris gradient (0.01 M–0.15 M) formed by 75 ml of the equilibrating buffer and the same volume of 0.15 M Tris, 6 M urea, pH 8.2. All urea solutions were freshly prepared, passed through a Rexyn I-300 (Fisher Scientific Co., Fair Lawn, NJ) column and used immediately.

Desialylation of apoproteins

In lipoprotein solutions containing 0.1 M acetate buffer, pH 5.1, 50 μg (0.6 U/mg) of neuraminidase from *Clostridium perfringens* (Boehringer Mannheim Biochemical, Indianapolis, IN) was added per mg of lipoprotein and the mixture was incubated at 37°C for 1 hr. This mixture was then delipidated with 20 volumes of ethanol–diethyl ether 3:1 (v/v) as described (10).

Preparative isoelectric focusing electrophoresis

For isolation of apoE isoforms, HDL from cholesterol-fed animals was passed through the anti-apoA-I immunoaffinity column and then delipidated. This apoHDL was separated by preparative isoelectric focusing electrophoresis on a flat bed of Ultradex (LKB, Produkter, Bromma, Sweden) with 1.6% Ampholines, pH 4.0–6.0, and 1% 2-mercaptoethanol, as described (21). Isoforms were eluted from the gels with the Tris-buffer containing 0.15% decyl sulfate. For amino acid analysis, Ampholines were removed by precipitation with trichloroacetic acid and followed by three washes with cold acetone (21).

Preparation of anti-apoA-I immunoaffinity column

ApoA-I was isolated from apoHDL by SDS-polyacrylamide gel electrophoresis of multiple samples (66 μg of protein each). One gel was stained to visualize the protein bands, and the corresponding regions from the unstained gels were sliced and each was homogenized with a small volume (~0.2 ml) of saline. Homogenized gel slices, containing 0.3–0.4 mg of protein were mixed with an equal volume of Freund's complete adjuvant and then injected into multiple subcutaneous sites of a rabbit. Two additional injections were made at 14-day intervals with homogenized gel slices containing 0.1 mg protein. Antiserum obtained 10 days after the final injection was fractionated by ammonium sulfate precipitation and column chromatography on DEAE-cellulose (22). The pu-

rified globulin fraction was coupled with CNBr-activated Sepharose (Pharmacia Chemicals, Piscataway, NJ) according to the procedure of Porath, Axen, and Ernback (23).

Analytical procedure

Analytical gel electrophoresis was carried out in the presence of 0.1% SDS and 1% 2-mercaptoethanol at pH 7.2 (24). The gels were stained and destained as previously described (10). For analytical isoelectric focusing electrophoresis (21), the protein was dissolved in sodium decyl sulfate in 0.01 M Tris and 20% sucrose, pH 8.2, and focused between pH 3.5 and 7.0 with or without the addition of 5% 2-mercaptoethanol. For determinations of pI of isoforms of apolipoproteins, unstained gels from the same run were sliced into 5-mm pieces with a gel slicer (Hoefer Scientific, San Francisco, CA). The sliced gels were incubated with 0.5 ml of distilled water at 4°C overnight and the pH of the mixtures was measured at room temperature. The isoelectric points of the stained protein bands were determined from a standard curve established by plots of pH versus length of the unstained gels. Protein was determined by a modified procedure of Lowry et al. (25), with bovine serum albumin as standard. Lipid phosphorus was determined according to Bartlett (26).

Amino acid analysis

Fifty μg of protein was hydrolyzed under vacuum in 6 N HCl for 22 hr at 110°C. Amino acids were quantified on a Beckman model 121 M amino acid analyzer by a two-column technique (27). The contents of methionine and cysteine were determined by the method of Hirs (28). The N-terminal amino acid was identified by a modification of the method of Gros and Labouesse (29). The proteins were dansylated in the presence of 0.1% sodium decyl sulfate, precipitated and washed with 10% trichloracetic acid, and then washed with acetone. After hydrolysis at 110°C for 4 hr, the dansyl amino acids were identified by chromatography on polyamide sheets in two different solvent systems (30).

Assay of apoA-I cofactor activity for lecithin: cholesterol acyltransferase

Lecithin:cholesterol acyltransferase activity was assayed as previously described (31) using purified enzyme (16,000 to 17,000-fold) isolated from human plasma. ApoA-I of guinea pig or human (0 to 10 μg in 0.05 ml) was added to the assay medium (0.2 ml final volume) containing lecithin-cholesterol liposomes prepared with French pressure cell (32), labeled with [^{3}H]cholesterol or [^{3}H]lecithin (0.025 to 0.3 μmol lecithin in 0.05 ml), 0.05 μmol of recrystallized human albumin (Sigma, St. Louis, MO) (0.05 ml, pH 7.5), purified lecithin:cholesterol acyltransferase (0.01 ml containing 0.05 to 0.5 μg of enzyme protein), and EDTA (5 mM in 0.154 ml NaCl). Incubation was for 60 min at 37°C, during which production of cholesteryl esters was linear.

Electroimmunoassay of apoA-I and apoE

Contents of apoA-I and apoE in plasma and in isolated lipoproteins were determined by a modification of the electroimmunoassay of Laurell (33), in which a 1% agarose gel containing 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO) was used. The gel was prepared in equal volumes of 0.0148 M barbital and 0.075 M Tris-glycine buffers (pH 8.8, 0.02 ionic strength), the same buffer used for electrophoresis. We found that in the presence of Triton, a sharp, precipitin-point arch was obtained only by using agarose of low electroendosmosis (Agarose C, Pharmacia Fine Chemicals, Piscataway, NJ).

Immunoassay was performed on glass plates (8.3 × 10.2 cm) coated with 12 ml of 1% agarose containing 100 μl of antiserum and 60 μl of 20% Triton. Monospecific antisera against purified apoA-I or apoE were prepared in rabbits as described above. For electroimmunoassay standards, protein mass of the purified apolipoproteins was determined by amino acid analysis (27) and appropriate dilutions were made with the electrophoresis buffer containing 1% Triton and 0.8% human serum albumin (Cutter Lab., Inc., Berkeley, CA). Samples for assay were preincubated in 1% Triton at room temperature for 1–3 hr before electrophoresis. Five μl of standards and samples was applied on the antibody-containing gel and was subjected to electrophoreses at 3 V/cm, 14-15°C for 18 hr. Nonspecific proteins were removed after the electrophoresis by pressing and rinsing before the gels were stained and destained as described (34). Concentration of apolipoproteins was determined by comparison of the areas enclosed by the precipitates of the samples with those of the standards.

To validate the electroimmunoassay of apoA-I, the mass of apoA-I in a sample of HDL was estimated by SDS polyacrylamide gel electrophoresis and by the electroimmunoassay. Isolated apoA-I and HDL of known aminoacyl mass, determined by amino acid analysis, were subjected to electrophoresis. The gels were stained and destained as previously described (10), and then scanned with a Clifford Model 445 densitometer (550 nm, 0.1 mm slit). The mass of apoA-I in HDL was determined by comparison of the area of the densitometric trace of apoA-I with that of the isolated apoA-I. Loads of up to 8 μg of apoA-I yielded a linear densitometric response.

For validation of the electroimmunoassay of apoE, concentration of apoE in plasma VLDL of normal animals and in plasma LDL, HDL, and d < 1.21 g/ml fractions from plasma of cholesterol-fed animals was

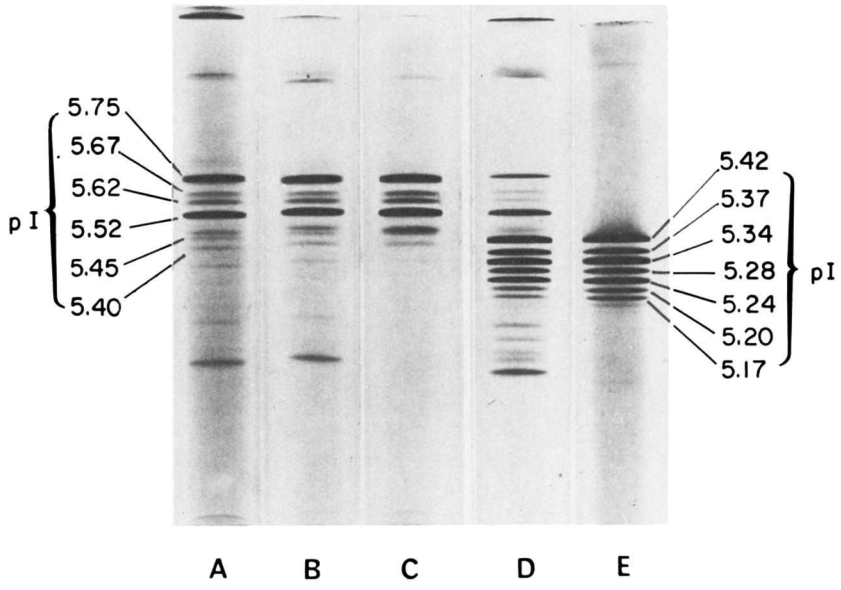

Fig. 1. Isoelectric focusing polyacrylamide gel electrophoretograms (pH 3.5–7.0) of apoHDL, isolated apoA-I, and apoE from the plasma of normal and cholesterol-fed guinea pigs. Gels are apoHDL (A, B) and isolated apoA-I (C) from normal animals, and apoHDL (D) and isolated apoE (E) from the cholesterol-fed animals. Except for gel A, which contained no reducing agent, the remaining apoproteins were incubated with 2-mercaptoethanol and then applied to the gel. Approximately 10 μg of the apolipoprotein was applied in each case.

determined by the electroimmunoassay. These measurements of apoE were compared to those determined by quantitative densitometric analyses of isoelectric focusing gels (21). For the latter technique, apoE comigrating protein called ACP in a previous report (19) is separated from the apoE focusing region (Fig. 3). Between 0.5–3.0 μg of purified apoE applied to the gel yielded linear densitometric response to the Coomassie Blue-stained gels.

RESULTS

Isoforms of apoA-I and apoE

Fig. 1 presents the isoelectric focusing gel electrophoretograms of apoHDL, isolated apoA-I, and apoE from plasmas of normal and cholesterol-fed guinea pigs. Isoform patterns of the isolated apoA-I (Fig. 1C) were closely identical to those of apoA-I present in whole delipidated HDL of the normal guinea pigs, with (Fig. 1B) or without (Fig. 1A) the presence of 2-mercaptoethanol, and showed the presence of six isoforms, two major ones with pI of 5.75 and 5.52 and four minor ones with pI of 5.67, 5.62, 5.45, and 5.40. A protein band present near the top of the gel of the unreduced apoHDL (Fig. 1A) is probably a dimer of apoA-I or a recombinant of apoA-I with other apolipoproteins formed by disulfide linkage, because this component disappeared when 2-mercaptoethanol was present (Fig. 1B).

ApoE isolated from the plasma HDL of the cholesterol-fed animals exhibited five major, with pI ranging between 5.42–5.24, and two minor (pI 5.20 and 5.17) isoforms (Fig. 1E). The same isoforms of apoE were also present in freshly delipidated HDL (Fig. 1D); however, the complete gel pattern was not altered by the presence of 2-mercaptoethanol (not shown).

DEAE-cellulose chromatography

In an attempt to separate the isoforms of the apolipoproteins, isolated apoA-I and apoE were further fractionated by a DEAE-cellulose column. Chromatography of apoA-I, as shown in **Fig. 2** (top), eluted two incompletely resolved peaks between 0.03–0.07 M Tris. Electrophoresis in SDS gels indicated both fractions had the same mobility as the parent apoA-I (insert, left). Some large molecular weight components present on the top part of the gel in fraction 1 may be due to a slight contamination in this preparation. In isoelectric focusing electrophoretograms (insert, right), however, different patterns of apoA-I were evident.

Chromatography of the apoE on DEAE-cellulose yielded broad peaks with major proteins eluted between 0.06–0.10 M Tris (Fig. 2, bottom). Fractions pooled under the marked area showed that the peaks contain some different isoforms (insert, right) and had slightly different electrophoretic mobilities on SDS gels (insert, left). The fraction containing isoforms with the lower pI values had the more retarded mobility. This result suggests that isoforms of apoE may contain different amounts of carbohydrates since glycoproteins migrate

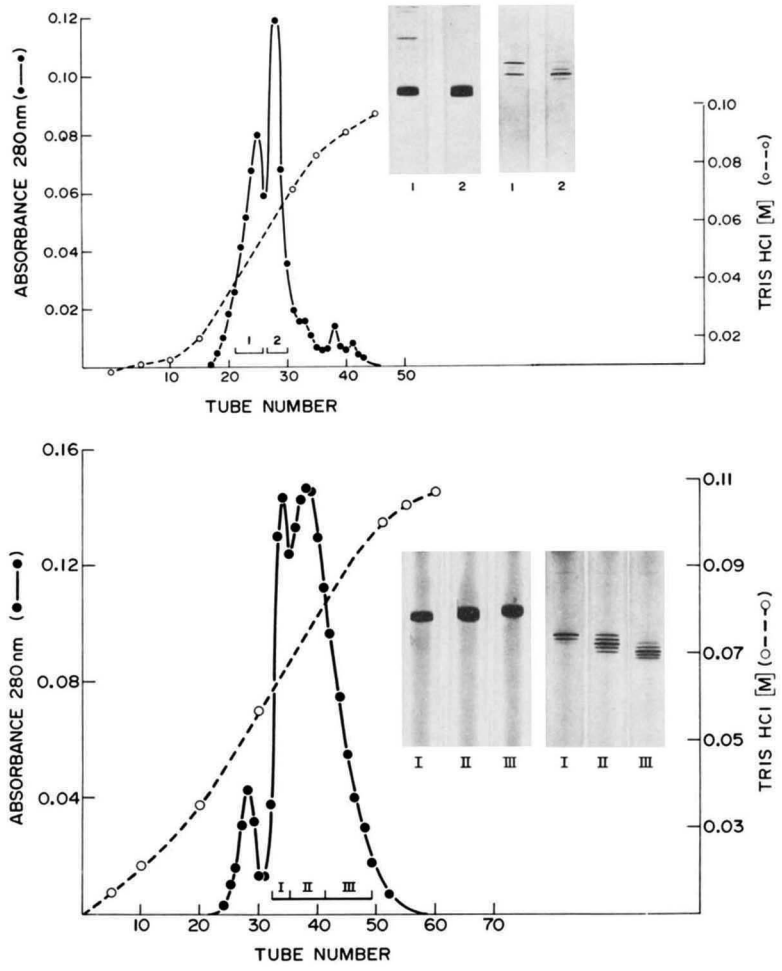

Fig. 2. DEAE-cellulose chromatography of isolated apoA-I (top) and apoE (bottom). Column size was 0.9 × 14 cm and apoproteins were eluted with a Tris gradient, with 75 ml of 0.01 M Tris–HCl, 6 M urea, pH 8.2, as initial buffer and 75 ml of 0.15 M Tris–HCl, 6 M urea, pH 8.2, as limiting buffer. Insert: polyacrylamide gel electrophoretograms of the column fractions pooled under the marked areas. Left: in 0.1% SDS, pH 7.2. Right: in analytical isoelectric focusing system, pH 3.5–7.0.

slower in SDS-polyacrylamide gels than the corresponding protein with the same molecular weight (35).

Changes of apoE isoforms induced by cholesterol-feeding

To determine the differences of apoA-I or apoE isoforms present in normal and cholesterol-fed guinea pigs, nascent VLDL and HDL isolated from the perfused livers were analyzed by isoelectric focusing polyacrylamide gel electrophoresis as shown in **Fig. 3.** Similar isoforms of apoA-I were found in the perfusate HDL of normal and cholesterol-fed animals (Figs. 3C and D). ApoE isoforms, on the other hand, consisted of one major (pI of 5.42) and two minor bands (pI of 5.37 and 5.34) in both perfusate VLDL and HDL of the normal animals (Figs. 3A and C), but the number increased to five or more isoforms with lowered pI values when the animals were fed cholesterol (Figs. 3B and D). This change in apoE isoforms was observed in plasma VLDL as early as 10 days after the cholesterol feeding. Differences of the apoE isoforms were also evidenced by quantitative densitometric analyses of these isoelectric focusing gel electrophoretograms (**Table 1**). Cholesterol feeding caused a shift of apoE isoforms from the higher pI of 5.42 to two lower pI of 5.28 and 5.24, which were absent in the normal lipoproteins (Table 1). Closely similar isoform patterns of apoE were found in all density fractions of liver perfusates of cholesterol-fed guinea pigs. When more apoproteins were loaded to the gels, more isomers with an even lower pI (5.20 and 5.17) were detected, as shown in Fig. 1.

Desialylation of apoE

Because sialic acid represents one of the major sugar residues in apoE from humans (36), apoE from the perfusate HDL of the cholesterol-fed animals was treated

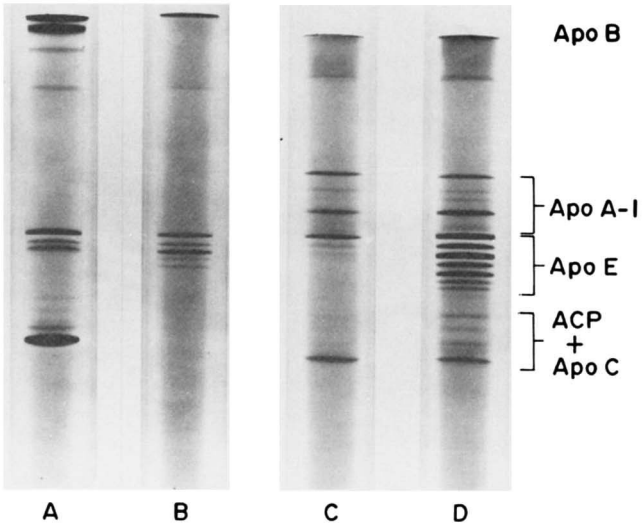

Fig. 3. Isoelectric focusing polyacrylamide gel electrophoretograms of nascent VLDL and HDL from perfused livers of guinea pigs. Gels are VLDL (A), HDL (C) from normal and VLDL (B), HDL (D) from the cholesterol-fed animals. Approximately 2 μg (A, B, C) and 10 μg(D) of the apoE determined by the electroimmunoassay were applied.

with neuraminidase. As shown in **Fig. 4,** desialylation reduced the number of isomers of apoE. The more acidic isoforms, induced by cholesterol feeding, were almost completely transformed to the three major isoforms of apoE that occurred in normal guinea pig perfusate VLDL and HDL. This same conversion of apoE isoforms was produced by neuraminidase treatment of liver perfusate VLDL, plasma HDL, or isolated apoE obtained from cholesterol-fed guinea pigs.

Amino acid composition

The overall amino acid composition of the isolated apoA-I from three different preparations was slightly different from that of apoA-I of humans (37, 38) and rat (39) (**Table 2**). Compared to human apoA-I, which lacks isoleucine and cysteine, apoA-I of guinea pigs contains 20 and 8 moles/10^3 mol of isoleucine and cysteine, respectively (Table 2). The N-terminal amino acid of the apoA-I of guinea pigs, determined by the dansylation, was Asp X (Table 2), as reported for human (37, 38) and rat (40).

To determine the amino acid compositions of the apoE isoforms, fractions containing one or two major isoforms were isolated by preparative isoelectric focusing electrophoresis as shown in **Fig. 5.** These fractions had the same amino acid composition as that of the parent apoE. The N-terminal amino acid of the apoE was lysine (**Table 3**). Attempts to isolate apoE from normal guinea pigs for amino acid analysis were unsuccessful because of the very low plasma concentrations of apoE in this species (**Table 4**).

Cofactor activity of apoA-I of guinea pigs for lecithin:cholesterol acyltransferase

The cofactor activity of apoA-I of guinea pigs was tested with lecithin:cholesterol acyltransferase from human plasma. As shown in **Fig. 6,** activation of LCAT with guinea pig apoA-I isolated from peak 2 of the DEAE-cellulose column (Fig. 2, top) was almost the same as that obtained with purified human apoA-I. The slightly lower activation obtained with apoA-I from peak 1 may be due to the contaminating apoproteins in this fraction (Fig. 2, top).

Plasma concentrations of apoA-I and apoE

Fig. 7 shows a typical electroimmunoassay pattern of apoA-I standard in the presence of 0.1% Triton. The area enclosed by the precipitates was linear between 25 and 150 ng of apoA-I applied. The inter-assay and intra-assay variations from three different apoA-I preparations were less than 3%. Immunoassayable apoA-I in a pooled

TABLE 1. Distribution of apoE isoforms in nascent VLDL and HDL from perfused livers of normal and cholesterol-fed guinea pigs

Apo E Isoform (pI)	Normal		Cholesterol-fed	
	VLDL	HDL	VLDL	HDL
		%		
5.42	60.8 ± 14.9	66.9 (64.4–69.5)	31.8 ± 2.1	28.5 ± 4.3
5.37	18.9 ± 6.0	20.4 (19.3–21.5)	18.0 ± 1.7	19.1 ± 0.6
5.34	20.1 ± 8.0	12.5 (11.0–14.0)	25.0 ± 1.4	27.1 ± 1.1
5.28	0	0	9.9 ± 0.3	11.9 ± 1.5
5.24	0	0	15.0 ± 1.4	13.3 ± 2.4

Distribution of apoE isoforms was determined by densitometric analysis of isoelectric focusing polyacrylamide gel electrophoresis (21). About 2 μg of the apoE measured by the electroimmunoassay was applied in each case. Numbers represent mean ± SD from four samples, or mean and range (in parentheses) of two samples.

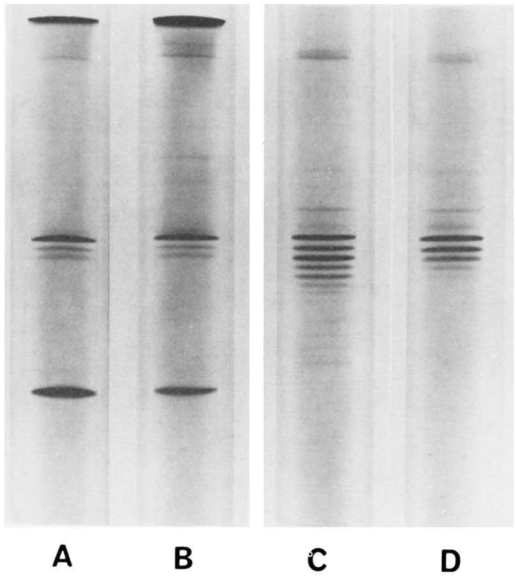

Fig. 4. Effect of neuraminidase treatment on apoE isoforms as analyzed by isoelectric focusing polyacrylamide gel electrophoresis (pH 3.5–7.0). Gels are liver perfusate VLDL (A, B) from the normal and liver perfusate HDL (C, D) from the cholesterol-fed animals. A and C were control, B and D were neuraminidase-treated.

sample of HDL accounted for 67.7% of the total aminoacyl mass. From quantitative polyacrylamide gel electrophoresis, a value of 64.0% was obtained. With the same electroimmunoassay there was no significant difference in the levels of immunoassayable A-I present in plasma (from six animals) when antiserum was prepared from apoA-I isolated by column chromatography or from SDS gels, or when the assay was performed with intact or delipidated plasma.

The electroimmunoassay standards for the apoE were between 50 to 200 ng. The inter-assay and intra-assay variations were less than 5%. Immunoreactivity of apoE in lipoproteins was evaluated by comparison of immunoassayable apoE with estimation of apoE by quantitative polyacrylamide gel electrophoresis in samples of plasma VLDL from normal guinea pigs and from samples of LDL, HDL, and the d < 1.21 g/ml fractions from the plasma of cholesterol-fed animals. As shown in **Fig. 8,** there was good agreement ($r = 0.989$) between apoE concentrations determined by these two methods.

The plasma concentrations of immunoassayable apoA-I and apoE from male guinea pigs that had been on a regular chow diet was 6.2 ± 2.0 and 2.2 ± 0.5 mg/dl, respectively (Table 4). Guinea pigs that had been fed 1% cholesterol for 1 week had slightly increased plasma levels of apoA-I, but showed a 10-fold increase of apoE. After a longer period of the cholesterol feeding, up to 8–10 weeks, both apoA-I and apoE were significantly increased by 2- and 22-fold, respectively, above the plasma levels of guinea pigs fed control diet.

TABLE 2. Amino acid composition of apoA-I of guinea pig, human and rat

	Guinea Pig[a]	Human[b]	Rat[c]
	mol/10³ mol		
Lys	96 ± 8	86	98
His	21 ± 2	20	24
Arg	51 ± 2	65	70
Asp	110 ± 4	86	134
Thr	46 ± 2	41	42
Ser	61 ± 3	57	33
Glu	185 ± 5	192	200
Pro	33 ± 3	41	24
Gly	43 ± 5	41	24
Ala	79 ± 2	78	48
½Cys	8	0	4
Val	50 ± 3	53	46
Met	5	12	28
Ile	20 ± 3	0	18
Leu	147 ± 12	159	126
Tyr	20 ± 1	29	29
Phe	24 ± 1	24	37
Tryp	N.D.	16	15
N-Terminal	Asp X	Asp X	Asp X

[a] Values are means ± SD of three different apoA-I preparations. The contents of methionine and cysteine were the mean of two analyses from different preparations of apoA-I.
[b] Calculated from Delahunty et al. (37) and Baker et al. (38).
[c] Obtained from Swaney et al. (39).

DISCUSSION

ApoA-I, isolated from plasma apoHDL of normal guinea pigs, exists in six polymorphic forms. Because the same isoform pattern was observed in freshly delipidated

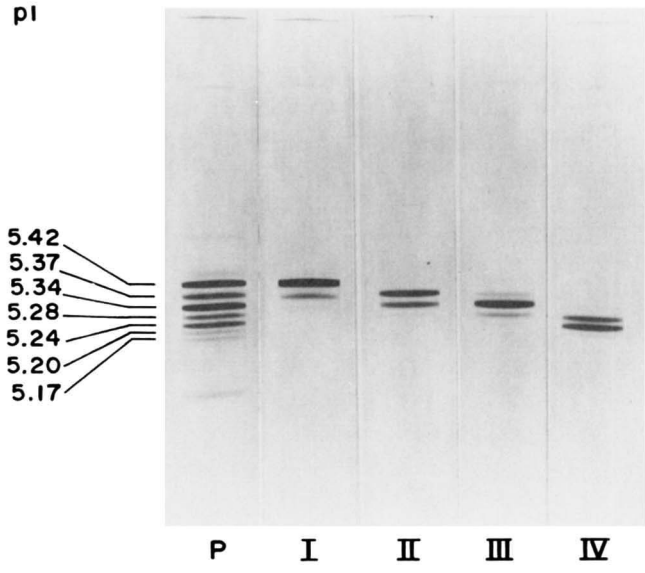

Fig. 5. Isoelectric focusing polyacrylamide gel electrophoretograms of apoHDL from cholesterol-fed guinea pigs (P) and its isoform fractions (I–IV). Isoforms were separated by preparative isoelectric focusing gel electrophoresis. ApoA-I present in the HDL fraction was removed by passing this lipoprotein through an anti-apoA-I immunoaffinity column.

TABLE 3. Amino acid composition of apoE and its isoform fractions

	Isoform Fraction				
	Parent	I	II	III	IV
	mol/10³ mol				
Lys	53 ± 0.6	52 ± 0.6	51 ± 1.0	54 ± 3.0	52 ± 6.0
His	5 ± 1.5	5 ± 0.7	5 ± 1.0	6 ± 0.7	6 ± 1.0
Arg	95 ± 2.7	108 ± 10	103 ± 7.0	104 ± 7.0	104 ± 9.0
Asp	47 ± 6.0	45 ± 5.0	50 ± 1.0	45 ± 6.0	47 ± 4.0
Thr	31 ± 1.7	28 ± 0.5	29 ± 1.0	29 ± 0.2	31 ± 0.5
Ser	62 ± 6.6	59 ± 4.0	60 ± 2.0	59 ± 5.0	60 ± 6.0
Glu	247 ± 7.0	248 ± 4.0	242 ± 6.0	243 ± 11	240 ± 13
Pro	32 ± 0.8	30 ± 2.0	32 ± 1.0	33 ± 1.0	32 ± 3.0
Gly	58 ± 7.0	48 ± 2.0	52 ± 3.0	50 ± 0.8	50 ± 5.0
Ala	105 ± 3.9	105 ± 1.0	104 ± 3.0	104 ± 1.0	104 ± 5.0
Val	74 ± 3.0	76 ± 2.0	75 ± 1.0	74 ± 5.0	75 ± 5.0
Ile	17 ± 1.5	18 ± 0.1	19 ± 2.0	19 ± 2.0	20 ± 0.8
Leu	107 ± 5.4	113 ± 3.0	111 ± 4.0	113 ± 5.0	112 ± 2.0
Tyr	14 ± 1.3	15 ± 2.0	16 ± 1.0	16 ± 0.5	16 ± 1.0
N-Terminal	Lysine	N.D.	N.D.	N.D.	N.D.

Values are mean ± SD of three different preparations. Contents of methionine and cysteine are assumed to be 28 and 5 mol/10^3, respectively, based upon mean of two analyses of parent apoE from different preparations.

HDL (Fig. 1), it is not likely that the heterogeneity of the isolated apolipoprotein was generated by carbamylation. The isoform pattern of this apolipoprotein was not changed by cholesterol feeding, although a twofold increase of the plasma apoA-I was noted in animals fed cholesterol for 8 to 10 weeks (Table 4). The pI range of guinea pig apoA-I was 5.75–5.40, which was between that of human (5.62 to 5.32 (41)) and rat (5.82 to 5.55 (42)).

Guinea pig A-I is slightly different in amino acid composition from apoA-I in humans (37, 38) and rats (39), but was the same N-terminal amino acid, Asp X. Like the apoA-I of rat, guinea pig apoA-I contains cysteine and isoleucine (Table 2), whereas these two amino acids are absent in apoA-I from the normal human subjects. Recent studies by Franceschini et al. (43) and Weisgraber et al. (44) have shown that the apoA-I_{Milano}, isolated from individuals with a new type of familial lipoprotein disorder, contains both isoleucine and cysteine. It is interesting that these individuals have reduced plasma levels of high density lipoproteins and apoA-I. We previously reported a somewhat different amino acid composition for this apolipoprotein, isolated from SDS-polyacrylamide gels (10). The high content of glycine and serine in that preparation probably indicates contamination with some non-A-I peptides. Despite a slightly different amino acid composition, isolated guinea pig apoA-I was as active as human A-I as a cofactor for purified LCAT from human plasma.

ApoE in liver perfusate VLDL and HDL of the normal guinea pigs contains one major and two minor isoforms (Fig. 3) similar to the common polymorphism of apoE present in humans (45), but had lower pI (5.42–5.34) than that of human apoE. Upon cholesterol feeding, plasma concentration of apoE was sharply increased (Table 4), accompanied by a dramatic change in apoE polymorphism (Fig. 3). Numbers of isoforms were increased from three to five or more by shifting the major components (pI 5.42) to more acidic isoforms (Table 1). This alteration of apoE isoforms was almost reversible

TABLE 4. Immunoassayable apoA-I and apoE in plasma of normal and cholesterol-fed guinea pigs

	Weeks Fed Cholesterol		
	0	1	8–10
ApoA-I (mg/dl)	6.2 ± 2.0 (18)	8.5 ± 3.7 (6)	13.8 ± 1.9 (10)
ApoE (mg/dl)	2.2 ± 0.5 (18)	24.2 ± 6.6 (10)	49.3 ± 10.8 (10)

Values represent mean ± SD from numbers of animals indicated in parentheses. The normal animals were fed regular Purina chow and the cholesterol-fed animals were fed the same chow with an addition of 1% cholesterol and 5% cottonseed oil. Animals were fasted for 16–18 hr.

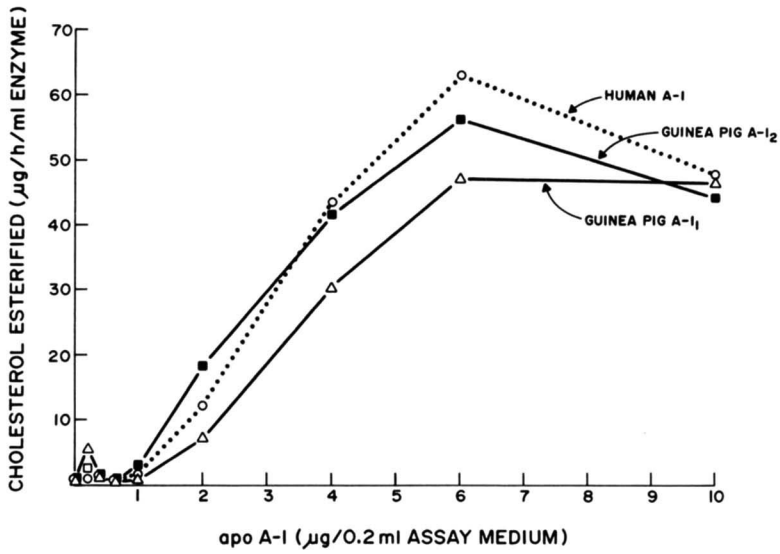

Fig. 6. Cofactor activities of apoA-I of guinea pig and human for purified lecithin:cholesterol acyltransferase (16,000–17,000-fold) isolated from human plasma. ■ —— ■, guinea pig apoA-I (peak 2 of DEAE-cellulose column, Fig. 2); Δ —— Δ, guinea pig apoA-I (peak 1 of DEAE-cellulose column, Fig. 2); O-----O, human apoA-I.

with treatment of neuraminidase, suggesting that apoE from cholesterol-fed guinea pigs contains more sialic acid. Increasing content of this sugar residue in apoE can also explain the existence of different electrophoretic mobilities on SDS polyacrylamide gels from DEAE-chromatographic fractions of this apolipoprotein (Fig. 2). This is consistent with the observations that rat apoE fractions from DEAE-cellulose columns contain different

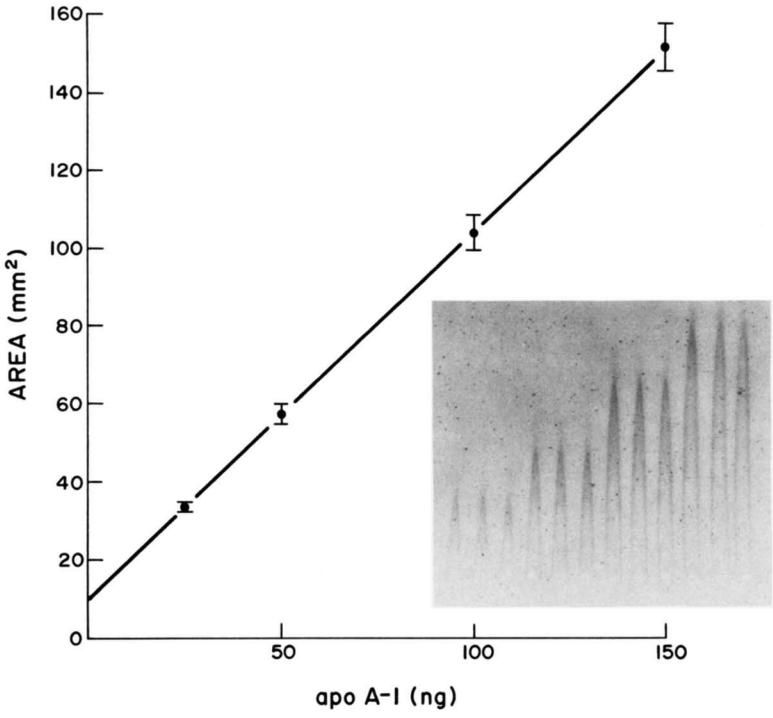

Fig. 7. Standard curve for electroimmunoassay analysis of apoA-I, plotted by measuring the area enclosed by precipitates against concentrations of apoA-I. Each point represents mean and standard deviation of a total of nine experiments from three different plasma apoA-I preparations. Insert: typical patterns for increasing concentrations of apoA-I standard.

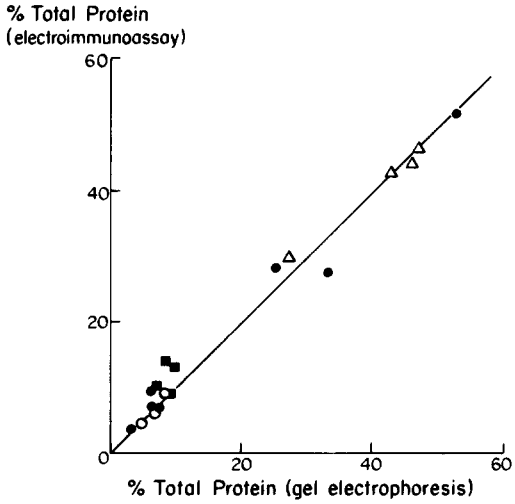

Fig. 8. Comparison of measurements of apoE in VLDL from the plasma of normal (■) and in LDL (○), HDL (△) and d < 1.21 fractions (●) from the plasma of cholesterol-fed guinea pigs by quantitative isoelectric focusing gel electrophoresis and by electroimmunoassay. The d < 1.21 g/ml fractions were isolated from animals with 1 to 10 weeks on cholesterol feeding; $r = 0.989$.

proportions of sialic acid (46) and that isolated rat apoE also exists in several (four to six) isoforms (42). Treatment of human VLDL with *Clostridium perfringens* neuraminidase also caused the disappearance of the minor acidic isoproteins from the apoE pattern, as determined by two-dimensional polyacrylamide gel electrophoresis (47).[1]

The isolated fractions of apoE had amino acid compositions similar to their parent apoE isolated from the cholesterol-fed guinea pigs (Table 3). The higher content of glutamic acid in the apoE of guinea pig than that of man (21) reflects the finding that guinea pig apoE is more acidic, as indicated by the lower pI values (Fig. 1). In addition to the previously reported amino acid composition for this apolipoprotein (10), the current studies added further information on the contents of cysteine and methionine, and identified lysine as the N-terminal amino acid.

To determine the plasma concentrations of apoA-I and apoE and the secretion rates of these apolipoproteins by perfused livers of normal and cholesterol-fed guinea pigs (described in the accompanying paper (17)), we have developed an electroimmunoassay for these two apolipoproteins. In a pooled sample of HDL from normal guinea pig plasma, a similar content of apoA-I was found by our electroimmunoassay and by quantitative polyacrylamide gel electrophoresis. Because the immunoassayable apoA-I content in plasma is unaffected by delipidation when Triton was present, it suggests that the current electroimmunoassay is capable of detecting all apoA-I present in lipoproteins. The immunoreactivity of apoE in isolated VLDL from plasma of normal guinea pigs and isolated LDL, HDL, and d < 1.21 g/ml fraction from plasma of the cholesterol-fed animals was identical to that of isolated apoE standards (Fig. 8), as judged by comparison with quantitative gel electrophoresis. This result indicates that the electroimmunoassay measures apoE equally in its isoforms in different lipoprotein particles in both normal and cholesterol-fed animals.

Among known mammals, guinea pigs have the lowest reported plasma concentration of apoA-I (6.2 ± 2.0 mg/dl) and apoE (2.2 ± 0.5 mg/dl) as measured by electroimmunoassay (Table 4). Compared to the serum concentrations of these apolipoproteins in human (21, 48, 49) and rat (50–52), apoA-I of guinea pigs represents only about one-twentieth and one-sixth of the values, respectively, whereas apoE of guinea pigs represents about one-half and one-eighth of the values from human and rat, respectively. Following the cholesterol-feeding, levels of apoA-I were slightly increased without alteration of isoform patterns. Levels of apoE, on the other hand, were sharply increased by cholesterol diet, and were accompanied by remarkable changes of apoE isoforms. These changes in apoE appeared to be due to a higher content of sialic acid, as discussed above, and may be in some way related to the unusually high content of unesterified cholesterol in the lipoproteins. Previously, we reported that a very high correlation exists between plasma unesterified cholesterol and apoE concentrations (10) in these animals on cholesterol diet. The molecular basis of the apoE polymorphism or an association between these lipids and the metabolic fate of lipoproteins containing altered apoE isoforms from cholesterol-fed animals remains to be elucidated.

We are grateful to Carlene Chang, Stephen Jones, and Ksenia Tomitch for their expert technical assistance. The authors also wish to thank Dr. Richard Havel and Leila Kotite for their advice on desialylation of apoproteins. This work was supported by Arteriosclerosis SCOR Grant HL-14237 from the United States Public Health Service and was presented in part at the Fifth International Symposium on Atherosclerosis in Houston, Texas, November 1979.

Manuscript received 15 September 1981 and in revised form 18 December 1981.

REFERENCES

1. Mahley, R. W. 1978. Alterations in plasma lipoproteins induced by cholesterol feeding in animals including man. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, Jr., and J. A. Ontko, editors. American Physiological Society, Bethesda, MD. 181–197.

[1] Human apoE isomers, analyzed by one-dimensional isoelectric focusing electrophoresis, are also reduced in number to higher pI by treatment with neuraminidase from *Clostridium perfringens* (Havel, R. J. Unpublished observations).

2. Shore, V. G., B. Shore, and R. G. Hart. 1974. Changes in apolipoproteins and properties of rabbit very low density lipoproteins on induction of cholesteremia. *Biochemistry.* **13:** 1579–1584.
3. Ross, A. C., and D. B. Zilversmit. 1977. Chylomicron remnant cholesteryl esters as the major constituent of very low density lipoproteins in plasma of cholesterol-fed rabbits. *J. Lipid Res.* **18:** 169–181.
4. Alexander, C., and C. E. Day. 1973. Distribution of serum lipoproteins of selected vertebrates. *Comp. Biochem. Physiol.* **46B:** 295–312.
5. Puppione, D. L., C. Sardet, W. Yamanaka, R. Ostwald, and A. V. Nichols. 1971. Plasma lipoproteins of cholesterol-fed guinea pigs. *Biochim. Biophys. Acta.* **231:** 295–301.
6. Bøhmer, T., R. J. Havel, and J. A. Long. 1972. Physiological fatty liver and hyperlipemia in the fetal guinea pig: chemical and ultrastructural characterization. *J. Lipid Res.* **13:** 371–382.
7. Fredrickson, D. S., A. M. Gotto, and R. I. Levy. 1972. Familial lipoprotein deficiency (abetalipoproteinemia, hypobetalipoproteinemia and Tangier disease). *In* The Metabolic Basis of Inherited Disease. 3rd ed. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill, Inc., New York. 493–530.
8. Assmann, G., E. Smootz, K. Adler, A. Capurso, and K. Oette. 1977. The lipoprotein abnormality in Tangier disease. *J. Clin. Invest.* **59:** 565–575.
9. Sardet, C., H. Hansma, and R. Ostwald. 1972. Characterization of guinea pig plasma lipoproteins: the appearance of new lipoproteins in response to dietary cholesterol. *J. Lipid Res.* **13:** 624–639.
10. Guo, L. S. S., M. Meng, R. L. Hamilton, and R. Ostwald. 1977. Changes in plasma lipoprotein-apoproteins of guinea pigs in response to dietary cholesterol. *Biochemistry.* **16:** 5807–5812.
11. Norum, K. R., J. A. Glomset, A. V. Nichols, and T. Forte. 1971. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: physical and chemical studies of low and high density lipoproteins. *J. Clin. Invest.* **50:** 1131–1140.
12. Glomset, J. A., and K. R. Norum. 1973. The metabolic role of lecithin:cholesterol acyltransferase: perspectives from pathology. *In* Advances in Lipid Research. Vol. II. R. Paoletti and D. Kritchevsky, editors. Academic Press, New York. 1–65.
13. Utermann, G., and H. J. Menzel. 1974. On the polypeptide composition of an abnormal high density lipoprotein (LP-E) occurring in LCAT-deficient plasma. *FEBS Lett.* **45:** 29–32.
14. Mitchell, C. D., W. C. King, K. R. Applegate, T. Forte, J. A. Glomset, K. R. Norum, and E. Gjone. 1980. Characterization of apolipoprotein acyltransferase deficiency. *J. Lipid Res.* **21:** 625–634.
15. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
16. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
17. Guo, L. S. S., R. L. Hamilton, R. Ostwald, and R. J. Havel. 1982. Secretion of nascent lipoproteins and apolipoproteins by perfused livers of normal and cholesterol-fed guinea pigs. *J. Lipid Res.* **23:** 543–555.
18. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solutions of ethanol of the small molecular weight peptides of the serum very low density and high density lipoproteins: relevance to the recovery problem during delipidation of serum lipoproteins. *Anal. Biochem.* **44:** 576–588.
19. Meng, M., L. Guo, and R. Ostwald. 1979. Isolation and partial characterization of a guinea pig serum apolipoprotein comigrating with apoE on sodium dodecyl sulfate-polyacrylamide electrophoretograms. *Biochim. Biophys. Acta.* **576:** 134–140.
20. Shore, V. G., and B. Shore. 1973. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry.* **12:** 502–507.
21. Havel, R. J., L. Kotite, J-L. Vigne, J. P. Kane, P. Tun, N. Phillips, and G. C. Chen. 1980. Radioimmunoassay of human arginine-rich apolipoprotein, apolipoprotein E: concentration in blood plasma and lipoproteins as affected by apoprotein E-3 deficiency. *J. Clin. Invest.* **77:** 1351–1362.
22. Levy, H. G., and H. A. Sober. 1960. A simple chromatographic method for preparation of gamma globulin. *Proc. Soc. Exp. Biol. Med.* **103:** 250–252.
23. Porath, J. R., R. Axen, and S. Ernback. 1967. Chemical coupling of proteins to agarose. *Nature.* **215:** 1491–1492.
24. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
25. 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.
26. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
27. Spackman, D. H. 1967. Accelerated methods. *Methods Enzymol.* **2:** 3–15.
28. Hirs, C. H. W. 1967. Determination of cystine as cysteic acid. *Methods Enzymol.* **2:** 59–62.
29. Gros, C., and B. Labouesse. 1969. Study of the dansylation reaction of amino acids, peptides and proteins. *Eur. J. Biochem.* **7:** 463–470.
30. Woods, K. R., and K. T. Wang. 1967. Separation of dansyl-amino acids by polyamide layer chromatography. *Biochim. Biophys. Acta.* **133:** 369–370.
31. Aron, L., S. Jones, and C. J. Fielding. 1978. Human plasma lecithin:cholesterol acyltransferase. Characterization of cofactor-dependent phospholipase activity. *J. Biol. Chem.* **253:** 7220–7226.
32. Hamilton, R. L., Jr., J. Goerke, L. S. S. Guo, M. C. Williams, and R. J. Havel. 1980. Unilamellar liposomes made with the French pressure cell: a simple preparative and semiquantitative technique. *J. Lipid Res.* **21:** 981–992.
33. Laurell, C. B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15:** 45–52.
34. Weeke, B. 1973. Rocket immunoelectrophoresis. *In* A Manual of Quantitative Immunoelectrophoresis. N. H. Axelson, J. Krøll, and B. Weeke, editors. Universitetsforlaget, Oslo. 16–35.
35. Segrest, J. P., R. L. Jackson, E. P. Andrews, and V. T. Marchesi. 1971. Human erythrocyte membrane glycoprotein: a re-evaluation of the molecular weight as determined by SDS polyacrylamide gel electrophoresis. *Biochem. Biophys. Res. Commun.* **44:** 390–395.

36. Jain, R. S., and S. H. Quarfordt. 1979. The carbohydrate content of apolipoprotein E from human very low density lipoproteins. *Life Sci.* **25:** 1315–1324.
37. Delahunty, T., H. N. Baker, A. M. Gotto, Jr., and R. L. Jackson. 1975. The primary structure of human plasma high density apolipoprotein glutamine 1 (apoA-I). I. The amino acid sequence of cyanogen bromide fragment II. *J. Biol. Chem.* **250:** 2718–2724.
38. Baker, H. N., A. M. Gotto, Jr., and R. L. Jackson. 1975. The primary structure of human plasma high density apolipoproteins glutamine I (apoA-I). II. The amino acid sequence and alignment of cyanogen bromide fragments IV, III, and I. *J. Biol. Chem.* **250:** 2725–2738.
39. Swaney, J. B., F. Braithwaite, and H. A. Eder. 1977. Characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry.* **16:** 271–278.
40. Edelstein, C., C. T. Lim, and A. M. Scanu. 1972. On the subunit structure of the protein of human serum high density lipoprotein. I. A study of its major polypeptide component (Sephadex, Fraction III). *J. Biol. Chem.* **247:** 5842–5849.
41. Albers, J. J., L. V. Albers, and F. Aladjem. 1971. Isoelectric heterogeneity of the major polypeptide of human serum high density lipoproteins. *Biochem. Med.* **5:** 48–55.
42. Gidez, L. I., J. B. Swaney, and S. Murnane. 1977. Analysis of rat serum apolipoproteins by isoelectric focusing: I. Studies on the middle molecular weight subunits. *J. Lipid Res.* **18:** 59–68.
43. Franceschini, G., C. R. Sirtori, A. Capurso, K. H. Weisgraber, and R. W. Mahley. 1980. A-I_{Milano} apoprotein: Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *J. Clin. Invest.* **66:** 892–900.
44. Weisgraber, K. H., T. P. Bersot, R. W. Mahley, G. Franceschini, and C. R. Sirtori. 1980. A-I_{Milano} apoprotein: Isolation and characterization of a cysteine-containing variant of the A-I apoprotein from human high density lipoproteins. *J. Clin. Invest.* **66:** 901–907.
45. Utermann, G., M. Mees, and A. Steinmetz. 1977. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. *Nature.* **269:** 604–607.
46. Weisgraber, K. H., R. W. Mahley, and G. Assmann. 1977. The rat arginine-rich apoprotein and its redistribution following injection of iodinated lipoproteins into normal and hypercholesterolemic rats. *Atherosclerosis.* **28:** 121–140.
47. Zannis, V. I., and J. L. Breslow. 1981. Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and posttranslational modification. *Biochemistry.* **20:** 1033–1041.
48. Albers, J. J., P. W. Wahl, V. G. Cabana, W. R. Hazzard, and J. J. Hoover. 1976. Quantitation of apolipoprotein A-I of human plasma high density lipoprotein. *Metabolism.* **25:** 633–644.
49. Karlin, J. B., D. J. Jahn, J. I. Starr, A. M. Scanu, and A. H. Rubenstein. 1976. Measurement of human high density lipoprotein apolipoprotein A-I in serum by radioimmunoassay. *J. Lipid Res.* **17:** 30–37.
50. Schonfeld, G., M. S. Frick, and A. P. Bailey. 1976. Measurement of apolipoprotein A-I in rat high density lipoprotein and in rat plasma by radioimmunoassay. *J. Lipid Res.* **17:** 25–29.
51. Fainaru, M., R. J. Havel, and T. E. Felker. 1976. Radioimmunoassay of apliproprotein A-I of rat serum. *Biochim. Biophys. Acta.* **446:** 56–68.
52. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Radioimmunoassay of arginine-rich apolipoprotein of rat serum. *Biochim. Biophys. Acta.* **490:** 144–155.