Changes in the Plasma Lipoprotein-Apoproteins of Guinea Pigs in Response to Dietary Cholesterol†

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ABSTRACT: The major apoproteins from four plasma lipoproteins were isolated from control and cholesterol-fed guinea pigs. Apoproteins were studied by column chromatography, polyacrylamide gel electrophoresis, and amino acid analysis. Dietary cholesterol altered the plasma apolipoproteins mainly by an enrichment in the content of arginine-rich polypeptide (ARP) in all density fractions. This protein had a similar molecular weight (34 000), electrophoretic mobility, amino acid composition, and microheterogeneity as ARP reported in other mammalian species. The estimation of plasma concentration of ARP indicates a higher correlation coefficient with plasma unesterified cholesterol ($r = 0.98$) compared with cholesterol esters ($r = 0.62$).

The response of guinea pigs to dietary cholesterol differs from that of other species by the appearance in plasma of discoidal high density lipoproteins rich in unesterified cholesterol, and by decreased levels of very low density lipoproteins (Sardet et al., 1972). A net accumulation of cholesterol in liver, other tissues, and red cells is associated with pathological changes and with the formation of echinocytes. A fatal hemolytic anemia usually develops after 10-14 weeks on a diet containing 1% cholesterol (Ostwald and Shannon, 1964; Ostwald et al., 1977). We reported earlier on changes occurring in the lipid composition, electrophoretic mobilities, and ultrastructure of guinea pig plasma lipoproteins in response to dietary cholesterol (Puppione et al., 1971; Sardet et al., 1972). The present study presents data on the major apoprotein constituents of the lipoproteins from control and cholesterol-fed guinea pigs. We found that the major apoprotein appearing in the lipoproteins in response to dietary cholesterol is the arginine-rich polypeptide (ARP) and that its concentration may be proportional to that of plasma unesterified cholesterol.

Experimental Procedure

Animals. Male albino guinea pigs weighing 200-250 g (Simonson Lab., Gilroy, Calif.) were fed one of two control diets (Purina chow with or without the addition of 5% cottonseed oil) or the Purina chow with 5% cottonseed oil diet supplemented with 1% cholesterol (Matin and Ostwald, 1975). Cholesterol-fed animals were autopsied when the red cell count dropped below 3.5×10^6 cells/mm^3, usually after 10-12 weeks. Blood was collected in 0.1% Na_2EDTA[1] by heart puncture from animals fasted 15-17 h. Red cells were immediately removed by low speed centrifugation and plasma was recentrifuged at 12 000g for 20 min at 4 °C to remove chylomicrons.

Preparation of Plasma Lipoproteins. Densities of plasma and liporprotein fractions were adjusted by the addition of NaCl-NaBr solutions containing 0.01% Na_2EDTA and these solutions were centrifuged in a 40.3 rotor at 40 000 rpm for 16-24 h at 16 °C (Beckman Model L2-65B ultracentrifuge). Lipoproteins were recovered by standard techniques (Lindgren et al., 1972). VLDL were isolated at 1.006 g/mL and refloated at the same density. LDL_1 (d 1.01-1.05 g/mL) were floated at d 1.07 g/mL, sedimented at d 1.01 g/mL and refloated at d 1.05 g/mL. LDL_2 (d 1.07-1.09 g/mL) and HDL (d 1.09-1.20 g/mL) were floated at d 1.21 g/mL, sedimented at d 1.07 g/mL and refloated at d 1.09 g/mL and d 1.20 g/mL, respectively. Two density fractions of HDL were isolated from cholesterol-fed animals (HDL_1, d 1.07-1.09 g/mL, and HDL_2, d 1.09-1.20 g/mL) because two major HDL components of different flotation rates had been observed by analytical ultracentrifugation (Puppione et al., 1971). We found, as did Chapman et al. (1975), that the lipoproteins of d 1.07-1.09 g/mL of control guinea pigs were typical of low density lipoproteins in their β electrophoretic mobility, content of B-apoprotein, size, shape, and chemical composition. In contrast, this same density fraction from cholesterol-fed guinea pigs contained mainly discoidal high density lipoproteins with α electrophoretic mobility and little or no B-apoprotein. We therefore refer to the density fraction d 1.07-1.09 g/mL as control LDL_2 and cholesterol HDL_1, respectively. Isolated lipoprotein classes were examined by agarose gel electrophoresis (Noble, 1968), electron microscopy using negative staining, and immunological techniques. The results were essentially the same as those we previously reported (Puppione et al., 1971; Sardet et al., 1972).

Lipoprotein Delipidation. Lipoprotein fractions at a concentration of 1-5 mg of protein/mL in a solution containing 0.15 M NaCl, 0.02% NaN_3, and 0.04% EDTA, pH 7.6, were delipidated with 20 volumes of ethanol-ether (3:1) at −20 °C

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[1] Abbreviations used: ARP, arginine-rich polypeptide; Na_2EDTA, disodium ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)-aminomethane; DEAE, diethylaminoethyl; VLDL, LDL, and HDL, very low density, low density, and high density lipoproteins; $NaDodSO_4$, sodium dodecyl sulfate; TMU, tetramethylurea; apo-A, apo-B, and apo-C, apolipoproteins A, B, and C, respectively; BSA, bovine serum albumin; LCAT, lecithin-cholesterol acyl transferase.

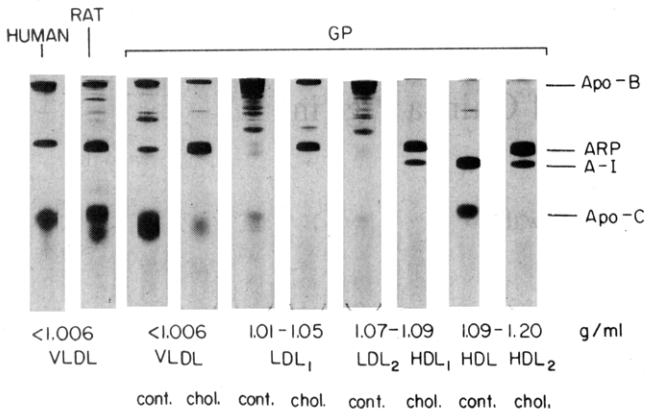

FIGURE 1: Sodium dodecyl sulfate-polyacrylamide gels of human and rat apo-VLDL and apolipoproteins from both control and cholesterol-fed guinea pigs. Approximately 60 μg of apo-VLDL and apo-LDL and 30 μg of apo-HDL were applied. See Experimental Procedure section.

similar to the procedure of Scanu and Edelstein (1971). Recoveries of protein after delipidation were greater than 95% and the apoproteins contained less than 1% residual phospholipids. Apoproteins of control HDL (d 1.09–1.20 g/mL), and of HDL_1 (d 1.07–1.09 g/mL) and HDL_2 of cholesterol-fed animals (d 1.09–1.20 g/mL) were soluble in 0.01 M Tris-HCl buffer (pH 8.2) or the same buffer containing 6 M urea. All apoproteins were soluble in 0.01 M Tris-HCl (pH 8.2) containing 1% sodium dodecyl sulfate (Bio-Rad Lab, Richmond, Calif.) or 1% sodium decyl sulfate (Eastman Kodak, Rochester, N.Y.).

Column Chromatography of Apolipoproteins. Apolipoproteins were fractionated using Sephadex G-200 columns (1.2 × 95 cm) equilibrated with 0.01 M Tris-HCl, 0.1% sodium decyl sulfate, pH 8.2, or Sephadex G-150 columns (1.2 × 95 cm or 0.9 × 27 cm) equilibrated with 0.01 M Tris-HCl, 6 M, urea, pH 8.2 (Ultrapure, Schwarz/Mann, Orangeburg, N.Y.). The effluents were monitored for absorbancy at 280 nm. Some fractions from the decyl sulfate–Sephadex columns were pooled, concentrated by vacuum in dialysis tubing, and further fractionated by column chromatography using urea–Sephadex or DEAE-cellulose (0.9 × 27 cm) similar to the methods of Shore and Shore (1973).

Polyacrylamide Gel Electrophoresis. We used both the 0.1% $NaDodSO_4$–polyacrylamide gel procedure (Weber and Osborn, 1969) and the alkaline polyacrylamide gel–8 M urea system (Kane, 1973). $NaDodSO_4$ gels were stained for 1 h at 70 °C with 0.25% Coomassie brilliant blue (Bio-Rad Lab, Richmond, Calif.) in 50% methanol–10% acetic acid and destained at 70 °C by diffusion in 5% methanol–5% acetic acid. Alkaline urea gels were stained with 1% Amido Black (Matheson Coleman and Bell, Norwood, Ohio) in 50% methanol–10% acetic acid and destained by diffusion in 7.5% acetic acid.

Preparative Gel Electrophoresis. Some apoprotein components of different molecular weights were isolated by electrophoresis of multiple samples (100 μg of protein each) using $NaDodSO_4$–polyacrylamide. One gel was stained to visualize the protein bands, and the corresponding regions from the unstained gels were eluted three times by incubation of the gel slices in 0.1% $NaDodSO_4$ solution at 37 °C for 3 h. The proteins were precipitated from the pooled incubation solutions by the addition of ice-cold acetone (Weber and Osborn, 1969). $NaDodSO_4$ was removed by exhaustive dialysis against a 0.01 M Tris-HCl buffer (pH 8.6) containing 8 M urea prior to reelectrophoresis.

Tetramethylurea Fractionation of Lipoproteins. Some lipoproteins (1 mg of protein/mL) were incubated with an equal volume of 8.4 M tetramethylurea (TMU) (Burdick & Jackson Lab, Inc., Muskegon, Mich., redistilled) in order to isolate apolipoprotein B (Apo-B) as described by Kane (1973). The precipitated proteins were washed twice with 4.2 M TMU solution and were dissolved in 0.01 M Tris buffer (pH 8.6) containing 1% $NaDodSO_4$.

Molecular Weight Estimation. The molecular weights of the apoproteins were estimated from $NaDodSO_4$ gel electrophoresis migration plots constructed from the following standards: bovine serum albumin, fraction V, mol wt 68 000, Armour Pharmaceutical; ovalbumin, mol wt 43 000, Sigma Chem. Co.; bovine heart lactic dehydrogenase, mol wt 36 000, Sigma Chem. Co.; bovine pancreas α-chymotrypsinogen, mol wt 25 700, Sigma Chem. Co.; egg white lysozyme, mol wt 14 300, Mann; bovine pancreas α-chymotrypsin, mol wt 11 000 and 13 000, Worthington.

Estimation of the Arginine-Rich Polypeptides (ARP). To estimate the content of ARP in plasma lipoproteins of guinea pigs with different degrees of hypercholesteremia, we adapted the technique of Hsieh and Anderson (1975) by using myoglobin as an internal standard. Purified myoglobin (mol wt 17 500; Sigma Chem. Co., St. Louis, Mo.) was chosen since the migration of this protein did not overlap the other apoprotein bands. Soluble myoglobin was added to plasma total lipoproteins (d < 1.21 g/mL) prior to dialysis at an approximate protein ratio of 1:10. Lipoproteins containing a known amount of myoglobin (total protein ranged 25–30 μg) were separated by $NaDodSO_4$ gel electrophoresis, and the stained gels were scanned through a 0.1-mm slit at 550 nm using a Clifford Model 445 densitometer. The area of the densitometric trace of ARP on the gels was compared with that of myoglobin. The amounts of ARP were estimated by using a standard curve established by plots of area ratio vs. weight ratio of ARP isolated from cholesterol LDL by column chromatography, and of myoglobin.

Amino Acid Analysis. Apoproteins isolated by the tetramethyl urea procedure, column chromatography, and preparative $NaDodSO_4$ gel electrophoresis were analyzed for their amino acid compositions. The analyses were carried out essentially as described by Moore and Stein (1960) using a Beckman Model 121 amino acid analyzer. Values for threonine and serine were corrected for losses by extrapolating the results from 22, 40, and 70 h hydrolysis to zero time. Neither the tryptophan nor the half-cystine contents of the apoproteins were determined.

Chemical Analyses. Protein was determined by a modified procedure of Lowry et al. (1951), with bovine serum albumin (BSA) (Armour Pharmaceutical; fraction V) as standard. A factor of 0.8 was used to convert BSA protein to lipoprotein protein as previously described (Sardet et al., 1972). Total cholesterol and unesterified cholesterol of plasma and plasma lipoproteins were determined in diethyl ether–ethanol extracts by precipitation of the cholesterol with digitonin followed by the ferric chloride method of Sperry and Webb (1950). Phosphorus was estimated according to Bartlett (1959).

Results

Polyacrylamide Gel Electrophoresis of Apolipoproteins. $NaDodSO_4$ gel electrophoresis patterns of lipoproteins from control and cholesterol-fed animals indicated the presence of four major apoproteins (polypeptides) (Figure 1). The molecular weights, electrophoretic mobilities, and amino acid compositions (Table I) showed the presence of apo-B, arginine-rich polypeptide (ARP), apo-A-I, and the apo-C group corresponding to those of human and rats. An exception was

TABLE I: Amino Acid Composition of Apo-B, ARP, and Apo-A-I of Guinea Pigs, Humans, and Rats.[a]

	Apo-B		ARP			Apo-A-I		
	GP[b]	GP[c]	GP[b]	Humans[d]	Rats[e]	GP[b]	Humans[f]	Rats[e]
Lys	75 ± 1.2	79	44 ± 3.5	48	54	65 ± 1.0	80	98
His	22 ± 0.4	23	11 ± 1.2	13	8	18 ± 0.9	23	24
Arg	37 ± 0.7	37	104 ± 5.0	106	130	39 ± 1.1	69	70
Asp	109 ± 2.9	109	62 ± 1.5	48	91	108 ± 2.5	79	134
Thr	61 ± 0.2	64	36 ± 3.6	38	44	34 ± 1.3	44	42
Ser	65 ± 1.1	77	57 ± 4.5	54	35	139 ± 4.3	57	33
Glu	121 ± 3.1	117	248 ± 4.1	233	257	131 ± 3.5	160	200
Pro	42 ± 1.0	40	26 ± 2.1	27	18	34 ± 2.2	42	24
Gly	52 ± 0.1	50	54 ± 1.6	58	28	198 ± 9.4	50	24
Ala	73 ± 1.6	70	101 ± 1.5	108	57	70 ± 2.8	80	48
Val	60 ± 1.4	55	68 ± 4.0	68	51	43 ± 3.2	56	46
Met	12 ± 0.1	9	22 ± 2.3	24	45	Trace	9	28
Ile	58 ± 1.6	57	20 ± 2.0	13	23	12 ± 1.3	2	18
Leu	133 ± 2.1	136	113 ± 1.6	109	112	89 ± 3.4	166	126
Tyr	29 ± 1.2	30	20 ± 1.3	14	18	8 ± 1.4	26	29
Phe	49 ± 2.3	47	16 ± 1.0	14	19	11 ± 3.1	26	37
Tryp	ND[g]	ND[g]	ND[g]	28	10	ND[g]	30	15

[a] Moles/10^3 moles of amino acid. [b] Values obtained in the present study are means ± SEM from single analyses of three separate preparations of apo-B, and four preparations of ARP, and from duplicate analyses of two preparations of apo-A-I. Apo-B was isolated from control LDL by Sephadex column chromatography or tetramethylurea precipitation (Kane, 1973). ARP was isolated from cholesterol apo-LDL and cholesterol apo-HDL by column chromatography. Values from preparations of ARP isolated by preparative $NaDodSO_4$ gel electrophoresis were very similar but are not included in these data. Apo-A-I was isolated from control apo-HDL and cholesterol apo-HDL_2 by preparative $NaDodSO_4$ gel electrophoresis. [c] Recalculated from Chapman et al. (1975). [d] From Shore and Shore (1973). [e] From Swaney et al. (1977). [f] Recalculated from Edelstein et al. (1972). [g] Not determined.

the amino acid composition of apo-A-I (see below).

Apo-B was present in VLDL and LDL of both control and cholesterol-fed animals as indicated by a stained band on top of the polyacrylamide gels, whereas this material was not detected in HDL.

The major difference between lipoproteins of control and cholesterol-fed guinea pigs was a substantial increase in a protein corresponding in molecular weight to that of ARP in all density fractions of cholesterol-fed guinea pigs. It constituted the predominant apoprotein component of both HDL_1 and HDL_2, whereas in controls it represented only a minor band in VLDL and was apparently absent in HDL.

Apo-A-I was present in all HDL fractions and was the major protein of control HDL. The apo-C group of bands was prominent in control VLDL and control HDL, but was present in only trace amounts in the two control LDL fractions. This group of proteins was greatly decreased in all lipoproteins of cholesterol-fed animals as compared with their corresponding fractions from control animals.

In addition to the four major apoproteins described, a group of slowly migrating proteins was observed, predominantly in LDL, but they were not further studied.

The apolipoprotein patterns of the two control groups (with or without addition of 5% cottonseed oil) were similar except that the control LDL from animals fed cottonseed oil showed a faint band corresponding to apo-A-I, and the proportion of the apo-C group appeared to be increased (not shown).

Chromatographic Separation of Apolipoproteins. Figure 2 shows the apolipoprotein elution profiles of LDL from control and cholesterol-fed animals separated on Sephadex G-200 using 0.1% sodium decyl sulfate. Apo-LDL of controls yielded three peaks. As shown by $NaDodSO_4$ gel electrophoresis, the void volume peak (N I) contained apo-B and constituted approximately 75% of the recovered control LDL protein. The second peak (N II) contained the group of slowly migrating proteins (not characterized) as well as a band corresponding in molecular weight to ARP. The apo-C group was eluted in the third peak (N III).

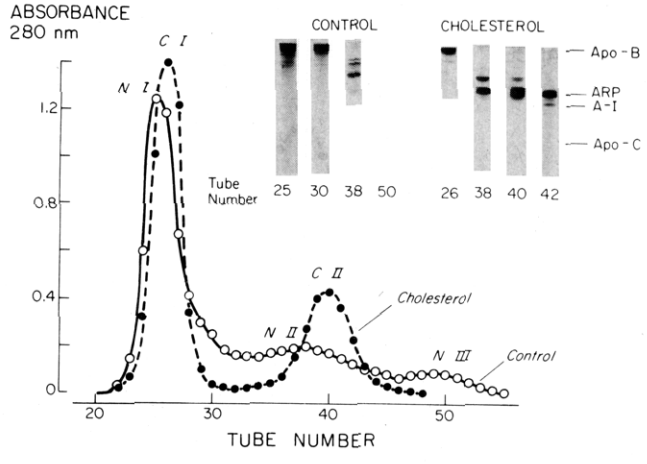

FIGURE 2: Sephadex gel filtration chromatography of control and cholesterol-fed apo-LDL on a 1.2 × 95 cm Sephadex G-200 column in 0.01 M Tris buffer containing 0.1% sodium decyl sulfate (pH 8.2). Approximately 15 mg of control apo-LDL and 17 mg of cholesterol-fed apo-LDL were applied. Upper right: sodium dodecyl sulfate-polyacrylamide gels of selected fractions. Fifty microliters of each fraction was applied.

LDL proteins from cholesterol-fed guinea pigs yielded two clearly separated peaks. The apo-B (C I) constituted approximately 67% of the recovered apo-LDL. The second peak (C II) had a slightly greater elution volume than that of the N II fraction and contained mainly ARP. The apo-C group seemed to be absent from LDL of cholesterol-fed animals.

In order to isolate ARP for further characterization, the peak fractions of C II were pooled, concentrated, and fractionated on urea-Sephadex, yielding three peaks: C I, C II, C III (Figure 3). C II contained apparently pure ARP as shown by $NaDodSO_4$ gel electrophoresis. C III was shown to be a previously unidentified apoprotein that will be described in a separate report.

Elution patterns of HDL proteins from control and cholesterol-fed guinea pigs are shown in Figures 4 and 5, respectively.

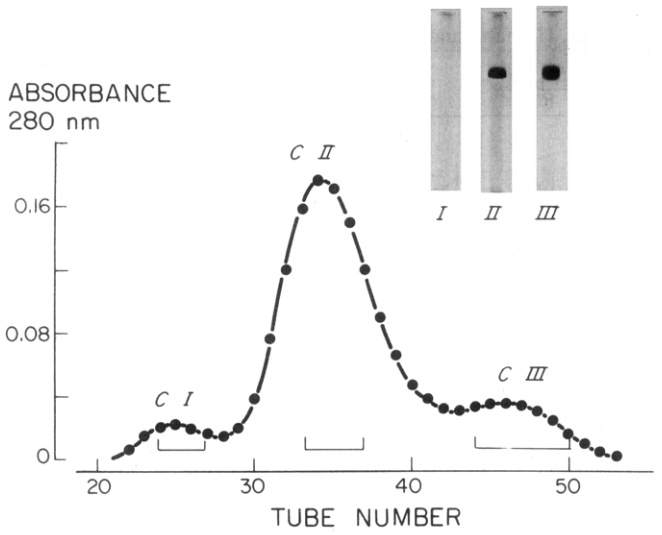

FIGURE 3: Sephadex gel filtration chromatography of C II (tubes 39–41, Figure 2) proteins on a 1.2 × 95 cm column of Sephadex G-150 in 0.01 M Tris buffer containing 6 M urea (pH 8.2). Upper right: sodium dodecyl sulfate–polyacrylamide gels of eluted peaks. Approximately 15 μg of protein was applied.

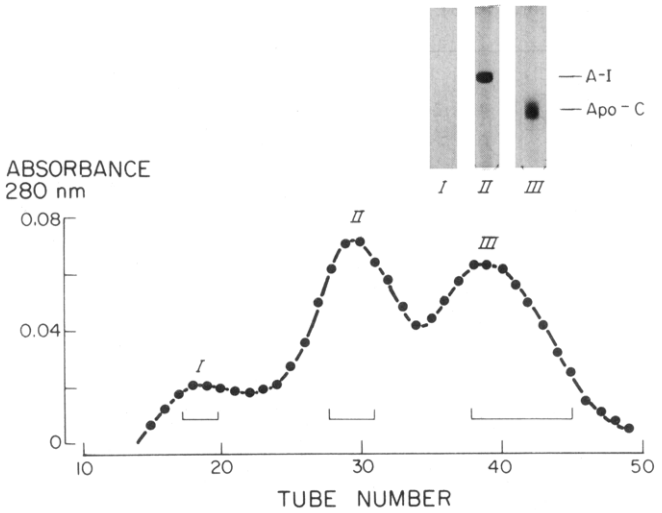

FIGURE 4: Sephadex gel filtration chromatography of control apo-HDL on a 0.9 × 27 cm column of Sephadex G-150 in 0.01 M Tris buffer containing 6 M urea (pH 8.2). Approximately 0.8 mg of protein was applied. Upper right: sodium dodecyl sulfate–polyacrylamide gels of eluted peaks. Approximately 10 μg protein was applied.

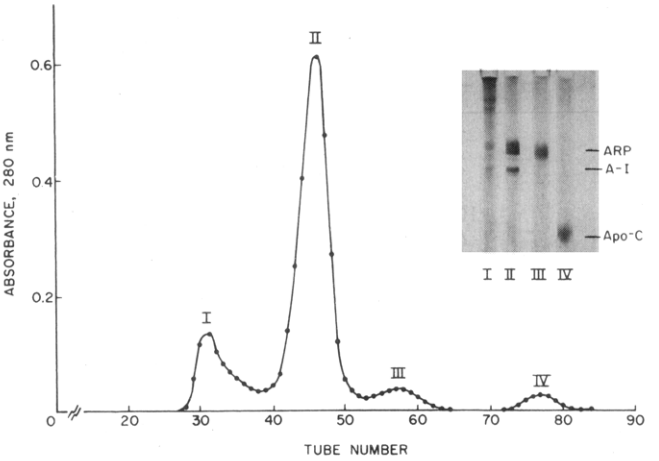

FIGURE 5: Sephadex gel filtration chromatography of cholesterol-fed apo-HDL (1.07–1.20 g/mL) on a 2.5 × 95 cm column of Sephadex G-150 in 0.01 M Tris-HCl containing 6 M urea (pH 8.2). Approximately 12 mg of protein was applied. Upper right: sodium dodecyl sulfate–polyacrylamide gels of eluted peaks. Approximately 15 μg of protein was applied.

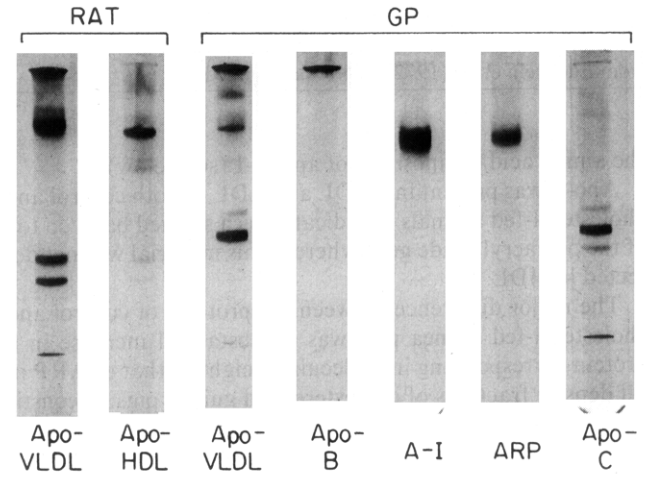

FIGURE 6: Urea–polyacrylamide gels of rat apo-VLDL and apo-HDL, guinea pig apo-VLDL, and guinea pig polypeptides isolated by Sephadex gel filtrations. Apo-B and apo-C were isolated from control LDL, ARP was isolated from cholesterol-fed LDL, and apo-A-I was isolated from control apo-HDL. Approximately 60 μg of apo-VLDL and 30 μg of apo-HDL and isolated proteins were applied.

Control apo-HDL exhibited two major peaks that were identified from their $NaDodSO_4$ gel electrophoresis patterns as apo-A-I and apo-C (Figure 4). Apo-HDL from cholesterol-fed animals was eluted in four peaks. $NaDodSO_4$ gel electrophoresis patterns indicated that peak I contained mainly aggregated proteins, peak II contained both ARP and apo-A-I, peak III was the unidentified apoprotein, and peak IV contained apo-C. DEAE-cellulose chromatography of peak II did not adequately separate ARP and apo-A-I.

Molecular Weight of Apolipoproteins. ARP and apo-A-I had molecular weights of 34 000 and 25 000, respectively, as estimated by $NaDodSO_4$ gel electrophoresis, whereas the apo-C group generally contained two components with molecular weights of 8100 and 11 000. Apoproteins isolated by Sephadex gel chromatography or by preparative $NaDodSO_4$ gel electrophoresis had identical molecular weights as determined by $NaDodSO_4$ gel electrophoresis using known standards.

Alkaline Urea Gel Electrophoresis of Apolipoprotein Components. Apo-B from LDL of both control and cholesterol-fed guinea pigs isolated by Sephadex gel chromatography (Figure 2) did not enter the separating gel (Figure 6). ARP separated into three components of very similar R_f values (0.15–0.21) that resembled the mobilities of ARP from rat apo-VLDL. Apo-A-I exhibited three closely spaced major and several minor bands with R_f values of 0.17–0.25 (Figure 6) similar to the mobilities of apo-A-I of rat HDL. ARP and apo-A-I isolated from HDL_2 of cholesterol-fed animals by preparative $NaDodSO_4$ gel electrophoresis were not cross-contaminated, and had essentially identical patterns in alkaline urea gels to those separated by Sephadex gel columns. The apo-C group separated into three distinct bands in the alkaline urea gel system with R_f values of 0.50–0.65 (Figure 6) that are lower than those of the apo-C components of rats. The mobilities of these bands were very similar to those of bands II, III, and IV of normal guinea pig serum lipoproteins reported by Chapman et al. (1975).

Amino Acid Composition of Apolipoproteins. The amino

TABLE II: Amino Acid Composition of Apo-A-I Isolated by Preparative Gel Electrophoresis from HDL of Control and Cholesterol-Fed Guinea Pigs.[a]

	Control	Cholesterol fed
Lys	64	67
His	18	18
Arg	40	38
Asp	105	112
Thr	35	33
Ser	132	145
Glu	132	131
Pro	37	32
Gly	183	213
Ala	70	70
Val	48	38
Met	Trace	Trace
Ile	14	10
Leu	94	83
Tyr	10	6
Phe	17	6

[a] Apo-A-I polypeptides were isolated from one sample each of control and cholesterol apo-HDL by preparative gel electrophoresis using 0.1% $NaDodSO_4$-polyacrylamide gels (see Experimental Procedure). Values (moles/10^3 moles of amino acids) are means of duplicate assays.

acid composition of apo-B (Table I) isolated from control LDL by tetramethylurea and by column chromatography were identical and were closely similar to that reported earlier for normal guinea pigs (Chapman et al., 1975). ARP isolated from either HDL or LDL of cholesterol-fed guinea pigs showed an identical amino acid composition (Table I) that was very close to that of ARP previously identified in humans (Shore and Shore, 1973) and rats (Swaney et al., 1977). Apo-A-I from HDL of both control and cholesterol-fed guinea pigs was distinguished by a high proportion of glycine and serine and the absence of methionine. Table II shows that the amino acid compositions of apo-A-I isolated by preparative $NaDodSO_4$ gel electrophoresis from control and cholesterol apo-HDL were very similar. Since the parent apo-HDL of control and cholesterol-fed guinea pigs differed by the absence and presence of ARP, respectively (Figure 1), these results indicate that this method is useful for the isolation of polypeptides for amino acid analyses.

Correlation of Arginine-Rich Polypeptide and Plasma Cholesterol Levels. Table III presents data on the levels of plasma unesterified cholesterol, cholesterol esters, and estimated amounts of ARP in plasma lipoproteins from 11 cholesterol-fed guinea pigs with different degrees of hypercholesterolemia. There was a high correlation between the plasma levels of ARP and unesterified cholesterol (r = 0.98). The correlation between cholesterol esters and ARP was much less pronounced (r = 0.62).

Discussion

Dietary cholesterol produced changes in guinea pigs not only in the absolute amounts of plasma lipoproteins, their density distributions, and lipid composition (Sardet et al., 1972) but significantly altered their apoprotein composition. The major change was a greatly increased amount of the arginine-rich polypeptide in all density fractions. It represented approximately half of HDL proteins in cholesterol-fed animals, whereas it was not detected in HDL fractions of control animals. The amino acid composition and molecular weight of this protein isolated from both LDL and HDL of cholesterol-fed guinea pigs was very similar to ARP in healthy humans (Shore and Shore, 1973; Shelburne and Quarfordt, 1975), in humans with dys-β-lipoproteinemia (Havel and Kane, 1973; Shore et al., 1974a), in humans with LCAT deficiency (Utermann et al., 1974), in normal rats (Swaney et al., 1977), and in cholesterol-fed rats (Mahley and Holcombe, 1977), rabbits (Shore et al., 1974b), and swine (Mahley et al., 1975). ARP contained at least three protein staining components separable on alkaline urea–polyacrylamide gels. A similar microheterogeneity has been demonstrated for ARP in lipoproteins of humans (Shore and Shore, 1973; Shore et al., 1974a; Havel and Kane, 1973; Utermann et al., 1975) and rats (Gidez et al., 1977). The molecular basis for this phenomenon remains to be elucidated. Chapman et al. (1975) reported the presence of small amounts of an arginine-rich polypeptide in VLDL and LDL of normal guinea pigs. The amino acid composition of their ARP was rather different from that isolated from LDL and HDL of cholesterol-fed animals in this study (Table I) except for an identical arginine content. Chapman et al. also reported that normal guinea pig HDL contain as much as 50% apo-B as a major protein. We found no evidence of apo-B in HDL of either control or cholesterol-fed animals as shown by $NaDodSO_4$ gel electrophoresis, by full solubilization of apo-HDL in Tris buffer and by the absence of immunoprecipitin reaction with antisera against LDL.

Apo-A-I was present mainly in the HDL of both control and cholesterol-fed guinea pigs. It had a molecular weight of 25 000 and formed multiple bands on alkaline urea gels, similar to apo-A-I of man (Edelstein et al., 1972) and of rats (Swaney et al., 1977). Its amino acid composition, however, was strikingly different from that of the other species. This difference may reflect functional differences of this protein in guinea pigs compared with apo-A-I of other species, and may be related to the very low concentrations of HDL found in control guinea pig plasma.

Our work and that of others has shown that many animal species respond similarly to the need to transport and metabolize increased amounts of dietary cholesterol. The chemical composition, electrophoretic mobility (Puppione et al., 1971; Sardet et al., 1972), and the apoprotein content of VLDL in cholesterol-fed guinea pigs (Figure 1) are similar to those of

TABLE III: Unesterified Cholesterol, Cholesterol Ester, and Arginine-Rich Polypeptide in Plasma of Cholesterol-Fed Guinea Pigs with Different Degrees of Hypercholesteremia.[a]

		Guinea pig no.										
		1	2	3	4	5	6	7	8	9	10	11
C,	mg/dL	26.6	45.0	41.3	65.0	77.1	95.1	101.3	113.0	141.8	155.4	157.8
CE,	mg/dL	73.4	91.4	78.7	119.6	104.6	137.6	173.7	185.7	93.5	172.6	157.5
ARP,	mg/dL	4.0	4.7	17.6	16.6	25.3	34.5	29.6	40.2	55.2	65.7	66.0

[a] Unesterified cholesterol (C) and cholesterol ester (CE) levels were determined in plasma of 11 guinea pigs fed a cholesterol-containing diet for different periods of time (see Experimental Procedure). Concentration of arginine-rich polypeptide (ARP) was estimated in delipidated total lipoproteins (d < 1.21 g/mL) by densitometry after $NaDodSO_4$ gel electrophoresis, using myoglobin as internal standard. The values were calculated from a standard curve of ARP/myoglobin weight ratio versus ARP/myoglobin peak area ratio.

β-VLDL reported in type III hyperlipoproteinemic subjects (Havel and Kane, 1973), cholesterol-fed rabbits (Shore et al., 1974b), and dogs, swine, and rats (Mahley et al., 1974, 1975; Mahley and Holcombe, 1977). The LDL of cholesterol-fed guinea pigs, on the other hand, apparently are different from that of many other cholesterol-fed species that respond to excess dietary cholesterol with the accumulation of an unusual lipoprotein designated HDL_c (Mahley et al., 1974, 1975; Mahley and Holcombe, 1977). This lipoprotein floats in the normal LDL density range but differs from LDL by electrophoretic mobility on paper or agarose gel, while LDL fractions of cholesterol-fed guinea pigs have normal LDL electrophoretic mobility (Puppione et al., 1971; Sardet et al., 1972). Electron-microscopy of LDL of cholesterol-fed guinea pigs (Sardet et al., 1972) showed large translucent vesicles (800–1100 Å) in addition to normal LDL size particles in contrast to the 100–450 Å particles of HDL_c (Mahley et al., 1975; Mahley and Holcombe, 1977).

Much of the circulating HDL of cholesterol-fed guinea pigs are discoidal particles that form stacks or rouleaux when negatively stained and are remarkably enriched in unesterified cholesterol (FC/CE ratio approximately 2) (Sardet et al., 1972). The discoidal characteristics of the HDL present in cholesterol-fed guinea pigs resemble those observed in humans with LCAT deficiency (Glomset and Norum, 1973; Utermann et al., 1974, 1975) and cholestasis (Blomhoff, 1974; Kostner et al., 1976) and those found in perfusates of rat liver when LCAT is inhibited (Hamilton et al., 1976). Discoidal HDL usually occur in association with reduced or absent LCAT. Their presence in cholesterol-fed guinea pig plasma may reflect a functional deficiency of this enzyme caused by the excess free cholesterol. The cholesterol-to-phospholipid molar ratio in the discoidal HDL of cholesterol-fed guinea pigs is about 2:1 (Sardet et al., 1972) whereas even a 1:1 molar ratio has been reported to almost completely inhibit LCAT activity in vitro (Fielding et al., 1972).

The very large increase of an arginine-rich polypeptide also appears to be a response to dietary cholesterol common to many species. This protein, which is virtually absent from normal HDL, represents half of the HDL in cholesterol-fed guinea pigs. It may therefore be specifically involved in the formation of cholesterol-rich lipoprotein particles and/or the metabolism of cholesterol as has been previously suggested (Shore et al., 1974b; Mahley et al., 1975; Mahley and Holcombe, 1977). The high correlation between the amounts of ARP and of unesterified cholesterol in plasma lipoproteins of cholesterol-fed guinea pigs suggests a specific function of this polypeptide for the transport and/or metabolism of cholesterol. The molecular basis for this high correlation remains to be elucidated.

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