Early changes in plasma lipoprotein structure and biosynthesis in cholesterol-fed rabbits

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Abstract Plasma lipoproteins of d < 1.063 g/ml from rabbits fed a diet containing 1% cholesterol for 4 days showed changes in concentration and rates of flotation as determined by analytical ultracentrifugation. A marked increase in cholesteryl ester content of lipoprotein with d < 1.019 g/ml was the most prominent change in rabbits fed the diet for 21 days. Gel electrophoresis and immunochemical procedures demonstrated that in control and hypercholesterolemic rabbits there were some common apolipoproteins found in all lipoproteins with density < 1.063 g/ml. In control rabbits, there were also apolipoproteins specific to the lipoprotein fraction with d < 1.019 and to the fraction with d 1.019–1.063 g/ml. However, in rabbits fed the hypercholesterolemic diet for 21 days, the apolipoproteins characteristic of fraction 1.019–1.063 were the most abundant in the fraction with d < 1.019 g/ml. Liver slices from rabbits fed the high cholesterol diet for 7 and 21 days incorporated more L-[^{14}C]leucine into very low density and low density lipoproteins than controls.

The results suggest that cholesterol feeding leads to an increase in biosynthesis of lipoproteins with d < 1.063 g/ml. The newly synthesized lipoprotein contains apolipoproteins similar to those found in controls but with a higher lipid-to-protein ratio. From the apoprotein composition, it is concluded that the very low density fraction present in cholesterol-fed animals is more structurally related to low density lipoproteins than to the very low density lipoproteins isolated from control animals.

Supplementary key words ultracentrifugation · flotation · lipid composition · low density–very low density lipoprotein relationship · apolipoproteins · immunochemical and gel electrophoresis · liver slices

THE STUDY OF hyperlipemic states in laboratory animals could contribute to the understanding of lipoprotein metabolism. Several authors have investigated the plasma lipoproteins of hyperlipemic rabbits that had been fed cholesterol-containing diets for long periods of time (1–4). However, the interpretation of information obtained after prolonged cholesterol administration to rabbits is complicated by the presence of associated disturbances such as hemolytic anemia (5), extensive cholesterol deposition in tissues (6), and liver damage (7). One of the first alterations observed in the cholesterol-fed rabbit is an increased plasma cholesterol concentration, which may reflect a disturbance of plasma lipoprotein metabolism (8). It seems pertinent that before undertaking studies on the mechanisms underlying this hyperlipemia, knowledge on the early qualitative and quantitative changes of plasma lipoprotein should be obtained. The results presented in this paper describe the modifications in lipid composition, apolipoprotein characteristics, flotation rates, and biosynthesis of plasma lipoproteins in rabbits maintained on a hypercholesterolemic diet for periods of up to 3 wk.

MATERIALS AND METHODS

Animal treatment

Commercially purchased rabbits weighing 2–3 kg were fed rabbit chow for 3 wk before placing them on the experimental diet. The cholesterol content of rabbit pellets was increased by the addition of 10 g of cholesterol dissolved in 100 ml of sesame oil to 1 kg of rabbit chow.

Abbreviations: VLDL, lipoprotein with density < 1.019 g/ml; LDL, lipoprotein with density 1.019–1.063 g/ml; HDL, lipoprotein with density 1.063–1.21 g/ml. VLDL-c, LDL-c, and HDL-c denote the respective lipoproteins isolated from control rabbits. VLDL-hc, LDL-hc, and HDL-hc denote the respective lipoproteins from hypercholesterolemic rabbits; the prefix apo- added to the above abbreviations is used to designate the protein portion of the lipoprotein. EDTA, disodium salt of ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

Control animals were given pellets prepared in a similar manner but without the cholesterol. The rabbits were placed in individual cages and allowed to feed ad lib.

Lipoprotein preparation

At the end of the experimental period, animals were anesthetized with 10 ml of aqueous 20% chloral hydrate given by enema. Blood obtained by heart puncture was collected in tubes containing 0.5 ml of a 5% solution of EDTA per 40 ml of blood. Plasma was adjusted to the desired density by the addition of solid KBr. Lipoprotein fractions VLDL (< 1.019 g/ml), LDL (1.019–1.063 g/ml), and HDL (1.063–1.21 g/ml) were obtained sequentially by differential ultracentrifugation at 110,000 g at 20°C in the Beckman rotor 60-Ti. The VLDL was separated after 12 hr of centrifugation; LDL and HDL were obtained after 20 hr. Each fraction collected in the upper 5 ml of the tubes was diluted to 35 ml with KBr solutions of the appropriate density and recentrifuged as mentioned above. To test homogeneity of the isolated VLDL fraction, 2-ml aliquots of VLDL were placed at the bottoms of tubes filled with KBr solution of d 1.019 g/ml. The tubes were ultracentrifuged at 70,000 g in an SW-25-2 rotor for 12 hr. The VLDL was collected in the upper 4 ml of the tube after centrifugation, and no material absorbing at 280 nm could be detected in the rest of the tube contents. When VLDL was isolated by ultracentrifugation of hypercholesterolemic plasma at a density of 1.006 g/ml and examined by analytical ultracentrifugation at a density of 1.21 g/ml, some HDL was found in three cases. This contamination was not detectable when VLDL was isolated by raising the density of plasma to 1.019 g/ml with KBr. The purified LDL and HDL appeared free of contamination with other lipoproteins or plasma proteins when examined by analytical ultracentrifugation and gel electrophoresis.

The purified lipoproteins were dialyzed for 48 hr against 0.15 M NaCl buffered with 5 mM Tris–HCl, pH 7.4, containing 0.5 mM EDTA. The lipoproteins were stored at 2°C until used.

Preparation of lipid-free apolipoproteins

The dialyzed lipoproteins were delipidated with ethanol–acetone as previously described (9). The lipid-free protein residue was dissolved in 8 M urea adjusted to pH 11 with NH_4OH. Protein recoveries of better than 70% were consistently obtained after delipidation.

Analytical ultracentrifugation

Flotation analysis of the total lipoprotein fraction with density less than 1.210 g/ml isolated from plasma of individual animals was carried out using the procedure of Del Gatto, Lindgren, and Nichols (10). Concentrations (%) were calculated with the aid of a calibration cell. Flotation rates are given as F (1.21) values at 20°C.

Gel electrophoresis

Agarose electrophoresis was performed using 1% (w/v) agarose dissolved in 50 mM Tris–HCl, pH 8.4; 4 ml of the melted gel was placed on microscope slides. The samples (2 μl) were placed in 5-mm-long slits. The electrode compartments contained 50 mM sodium barbital buffer (pH 8.6).

When the mobilities of two fractions were compared, both fractions were placed in the same slide and all samples contained bromophenol blue to check the homogeneity of the analyses. Analytical polyacrylamide gel electrophoresis was carried out in a water-cooled vertical cell. The gels were prepared from cyanogum-41 (E-C Apparatus Corp., Philadelphia, Pa.). The conditions and compositions of the gels are given in the figure legends.

Immunological procedure

Antisera were prepared against pooled lipoproteins obtained from five control and five experimental rabbits maintained on their respective diets for 3 wk. White leghorn chickens were given weekly intramuscular injections with 1 ml of lipoprotein containing 2–6 mg of protein over a 4-wk period. After a 2-wk interval the chickens were given one additional intravenous injection. Immunoelectrophoresis and double immunodiffusion were carried out according to the methods described by Ouchterlony (11), using agarose instead of agar. Quantitative immunoelectrophoresis was carried out using the procedure of Clarke and Freeman (12).

Peptide mapping

2 mg of freeze-dried apolipoprotein was taken up in 1 ml of constantly boiling 5.4 N HCl. The sample was then placed in a tube which was sealed and kept for 48 hr at 37°C. At the end of this period the hydrolyzates were lyophilized and the residues were dissolved in water. Aliquots of these hydrolyzates (50 μl) were spotted on silica gel plates 500 μ thick; the peptides were electrophoresed in one dimension and then chromatographed in a second dimension (13). During electrophoresis and chromatography the plates faced each other, and the origin was selected to produce mirror images. This procedure was found to give more reproducible maps.

Methanolysis and gas–liquid chromatography

Triglycerides, phospholipids, and cholesteryl esters were separated by thin-layer chromatography. After the transmethylation of each lipid class according to the method of Kates (14), the fatty acid composition was determined by gas–liquid chromatography.

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Chemical procedures

Analyses of the lipid components of liver and lipoprotein fractions were performed on the chloroform phase of a lipid extract (15). Cholesterol and its esters, after separation by thin-layer chromatography, were measured by the procedure of Bowman and Wolf (16). Phospholipids and glycerides were estimated as previously described (13).

Incubation procedure to evaluate leucine incorporation into proteins

Rabbits fasted for 18 hr were anesthetized with ether. The livers were removed, rinsed in saline, and lightly blotted, and slices weighing 200–600 mg (± 0.1 mg) were cut with a Stadie-Riggs microtome. The incubation was carried out in 5 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 5 mg of D-glucose and 7.7 mM K^+ according to Judah and Nichols (17).

Prior to the addition of 2–4 μCi of uniformly labeled [^{14}C]leucine (New England Nuclear, sp act 262 mCi/mmole), all the vessels were preincubated on a Dubnoff metabolic shaker at 37°C for 30 min. After the addition of L-[^{14}C]leucine, the incubation was carried out in 95% oxygen for 2 hr. The reaction was terminated by addition of 3 ml of 2.5% unlabeled leucine, and the samples were immediately homogenized in a motor-driven tissue grinder. Unlabeled rabbit plasma (1 ml) was added to the homogenates to act as carrier, and the mixture was transferred to the ultracentrifuge tubes. The density was adjusted to 1.050 g/ml with solid KBr. This density assures that most of the LDL and all of the VLDL is isolated with no detectable contamination with HDL. The ultracentrifugation was carried out twice for 24 hr at 105,536 *g* and 15°C in a 50-Ti Spinco rotor. The lipoprotein contained in the upper 2 ml from the last centrifugation was precipitated with cold 5% trichloroacetic acid. After 10 min the samples were filtered through a GF/A Whatman filter and washed twice with ethanol, ethanol–ether 1:1 (v/v), and finally with ethyl ether. This delipidation procedure is designed to remove the trichloroacetic acid and radioactivity associated with lipids. After drying in the oven at 40°C, the glass fiber filters containing the protein samples were placed into liquid scintillation vials and counted. The samples were counted long enough to secure a standard error of less than 5%. To assay if the radioactivity measured was associated with apolipoproteins of the fraction with d 1.050 g/ml, the lipoproteins from two incubation experiments with liver slices from cholesterol-fed rabbits were isolated in the presence of carrier plasma and delipidated as described above. 1 mg of apoprotein obtained was dissolved in buffer containing 8 M urea and 0.1% sodium dodecyl sulfate and electrophoresed in a preparative polyacrylamide gel slab. After electrophoresis, the gel was stained and scanned at 600 nm. The gel was sliced into 20 pieces, 0.5 cm, which were dissolved in 1 ml of a 1:1 (v/v) solution of 30% H_2O_2 and H_2O. The dissolved fractions were then added to 10 ml of Instagel counting solution (Packard Instrument Co., Downers Grove, Ill.) and counted.

RESULTS

The concentration of plasma lipoproteins from rabbits fed the high cholesterol diet for 3 wk was established by analytical ultracentrifugation. The results are presented in Table 1. It can be observed that a marked increase in the concentration of VLDL plus LDL occurred with no appreciable changes in HDL. In cholesterol-fed animals there was an extension of the upper limit of the flotation range of the VLDL plus LDL fraction. In another experiment, plasma lipoprotein analysis was performed in four individual animals 4 and 8 days after the start of feeding either the control or cholesterol-containing diet. As early as 4 days after the beginning of the experimental diet, the lipoproteins with d < 1.063 g/ml rose from 65 to 229 mg/100 ml of plasma in one rabbit and from 18 to 87 mg/100 ml in the second rabbit. After 8 days these lipoproteins reached values of 711 and 182 mg/100 ml, respectively. In Fig. 1 the results of this typical experiment are illustrated. It can be observed that there is little change in the lipoprotein pattern of a rabbit fed the control diet for 8 days, but sharp changes took place in concentration and flotation ranges of lipoproteins with F

TABLE 1. Ultracentrifugal analysis of plasma lipoproteins from rabbits fed a high cholesterol or a control diet for 21 days

	VLDL + LDL			HDL		
	S_f Range	Maximum Ordinate	mg/100 ml	S_f Range	Maximum Ordinate	mg/100 ml
Controls (8 animals)	28–70	41	23 ± 3[a]	0–12	4.0	134 ± 8
Cholesterol-fed (6 animals)	33–350	46	848 ± 200	0–11	4.5	122 ± 21

[a] Averages ± SD.

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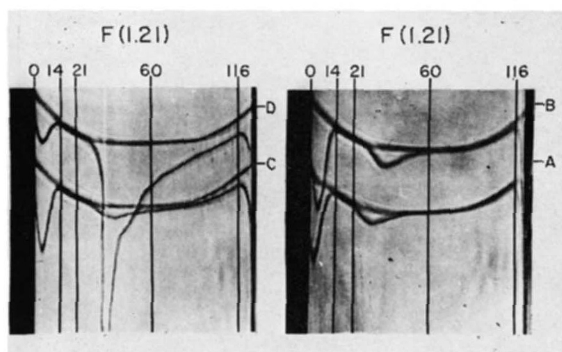

FIG. 1. Ultracentrifugal pattern of rabbit plasma lipoproteins. Flotation analysis of lipoproteins with density less than 1.21 g/ml from a control rabbit: *A*, at the initiation of the control diet; *B*, after 8 days on diet. Lipoproteins from a rabbit fed a high cholesterol diet: *C*, at the beginning of the diet; *D*, after 8 days on the diet. This last pattern was obtained from the fraction of d < 1.210 g/ml diluted twofold in order to visualize the large low density components; for this reason the HDL peak appears smaller than in pattern *C*.

(1.21) values above 14 in a rabbit fed the hypercholesterolemic diet for 8 days.

Results of chemical analysis of plasma lipoproteins from rabbits fed the cholesterol diet for 21 days are presented in Table 2. The most noticeable changes were a marked increase in the content of cholesterol, both free and esterified, of the d < 1.019 lipoprotein fraction and a decrease in the content of glycerides in this fraction. It is shown that the proportion of protein in VLDL of the cholesterol-fed group is lower than that of the controls. Apart from the increase in percentage of esterified cholesterol, no other differences were demonstrated between the LDL composition of control and treated animals.

Liver lipids of rabbits fed cholesterol for 25 days exhibited a large increase in concentration of cholesteryl esters, as indicated in Table 3. In Table 4 is presented the fatty acid composition of cholesteryl esters from plasma and liver. In plasma the relative content of cholesteryl oleate was higher and that of cholesteryl linoleate was lower in cholesterol-fed animals than in controls. However, in absolute terms all cholesteryl esters, except perhaps cholesteryl arachidonate, were elevated in plasma of hypercholesterolemic rabbits. In liver the major change induced by cholesterol feeding in the composition of cholesteryl esters was an increment in cholesteryl oleate.

The study of lipoprotein biosynthesis by liver in the presence of an altered lipid composition is of importance. Results of the incorporation of L-[^{14}C]leucine into lipoprotein fractions by incubated liver slices are presented in Table 5. Two of four rabbits fed the cholesterol diet for 1 wk showed an increased rate of incorporation of [^{14}C]-leucine into the lipoproteins. By the end of the second week this increase was very clear in three out of five animals. The close correspondence between the densitometric tracing of the polyacrylamide gels of apolipoproteins from the d < 1.050 g/ml lipoproteins and the distribution of radioactivity (Fig. 2) confirm that the radioactivity measured is that incorporated by the slices into the protein moiety of the lipoproteins. No differences were detected in the incorporation of [^{14}C]leucine into plasma proteins in the fraction with d < 1.21 g/ml from control and cholesterol-fed animals.

TABLE 2. Percentage composition of plasma lipoproteins isolated from control rabbits and rabbits fed the cholesterol-containing diet for 21 days

	Protein	Phospholipids	Glycerides	Free Cholesterol	Esterified Cholesterol
Lipoproteins with density < 1.019 g/ml					
Control (5)[a]	19.5 ± 3.2[b]	17.2 ± 2.5	54.1 ± 4.7	8.9 ± 0.9	4.3 ± 0.9
Cholesterol-fed (5)	9.9 ± 1.0	22.1 ± 3.7	15.1 ± 2.5	12.4 ± 2.7	40.3 ± 1.8
Lipoproteins with density 1.019–1.063 g/ml					
Control (5)	28.1 ± 2.2	26.5 ± 0.8	11.9 ± 1.8	9.1 ± 1.0	24.4 ± 2.0
Cholesterol-fed (5)	26.7 ± 4.7	23.7 ± 2.2	8.7 ± 2.8	12.9 ± 1.2	33.5 ± 2.9

[a] The number of rabbits is indicated in parentheses.
[b] Means ± SD. All values are percentages.

TABLE 3. Liver lipid composition of rabbits fed a high cholesterol or a control diet for 15 days

	Glycerides	Cholesterol		Phospholipids
		Total	Esters	
	mg/g tissue wet wt (mean ± SE)			
Control (5)[a]	3.5 ± 0.9	2.6 ± 0.4	0.8 ± 0.2	16.4 ± 0.7
Cholesterol-fed (7)[a]	8.7 ± 1.9	11.1 ± 1.2	7.5 ± 0.9	16.5 ± 0.8
P[b]	0.01	< 0.001	< 0.001	0.1

[a] Number of animals.
[b] Student's t test.

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TABLE 4. Composition of fatty acids in cholesteryl esters of liver and plasma from rabbits fed a control or a cholesterol-containing diet for 15 days

	16:0[a]	16:1	18:0	18:1	18:2	18:3	20:4
				% of total fatty acids			
Plasma							
Control (5)[b]	18.1[c] ± 1.1	3.8 ± 1.0	3.6 ± 0.2	22.2 ± 0.6	49.7 ± 2.5	1.6 ± 0.3	2.1 ± 0.3
Cholesterol-fed (7)	16.1 ± 1.5	3.2 ± 0.3	5.3 ± 0.4	40.6 ± 1.8	32.1 ± 1.1	2.1 ± 0.3	0.3 ± 0.1
P[d]				0.001	0.001		0.001
Liver							
Control (4)	20.5 ± 0.9	3.7 ± 0.7	8.6 ± 1.2	36.3 ± 1.4	25.9 ± 1.6	3.1 ± 0.7	2.1 ± 0.3
Cholesterol-fed (7)	15.7 ± 1.9	3.7 ± 0.3	4.2 ± 0.4	48.5 ± 4.1	25.2 ± 1.4	1.6 ± 0.2	0.6 ± 0.2
P[d]				0.02			0.01

[a] Number of carbon atoms: number of double bonds.
[b] The number of animals is in parentheses.
[c] Means ± SE.
[d] Significance of difference, Student's *t* test.

TABLE 5. Incorporation of [^{14}C]leucine into d < 1.050 lipoproteins by liver slices from control and cholesterol-fed rabbits

Days on Diet	Control	Cholesterol-fed
	cpm/g[a]	
7	7.112	11.303
	9.856	10.519
	5.396	5.907
	4.690	5.534
21	3.300	13.940
	3.210	13.000
	1.350	10.000
	1.210	2.590
	830	1.060

[a] The figures represent cpm/g of wet tissue for a 1-hr incubation.

Electrophoresis of purified lipoproteins

Fig. 3 presents the results of agarose electrophoresis of purified VLDL and LDL from control and experimental animals fed the cholesterol diet for 3 wk. Freshly prepared VLDL-c shows a pre-β mobility and LDL-c a β mobility. On the other hand, VLDL-hc has mobility similar to LDL-c. No differences in the electrophoretic behavior of LDL from control or cholesterol-fed rabbits could be detected.

Immunological properties of purified lipoproteins

Anti-VLDL-hc sera gave common precipitin lines with VLDL-hc, VLDL-c, LDL-hc, and LDL-c (Fig. 4). Also in Fig. 4 are shown the precipitin arcs given by anti-LDL-c sera reacting against VLDL-hc, VLDL-c, LDL-hc, and LDL-c. No reaction was observed between the above antisera and HDL-c or HDL-hc. When lipoprotein-specific antisera were reacted with total rabbit plasma, only the arcs corresponding to LDL or VLDL were present in immunoelectrophoretic experiments. γ-Globulin isolated from anti-VLDL-hc serum (18) was adsorbed with LDL-hc. After separation of the immunoprecipitate, the supernatant γ-globulin failed to react against LDL-c, LDL-hc, VLDL-c, and VLDL-hc. These results indicate that the particles present in LDL-hc and VLDL-hc contain common antigenic determinants, and the anti-VLDL-hc γ-globulin used in this study cannot detect VLDL lipoproteins with segregated antigenic characteristics.

Quantitative immunoelectrophoresis

Quantitative immunoelectrophoresis was used to follow in four rabbits the changes in the immunoelectro-

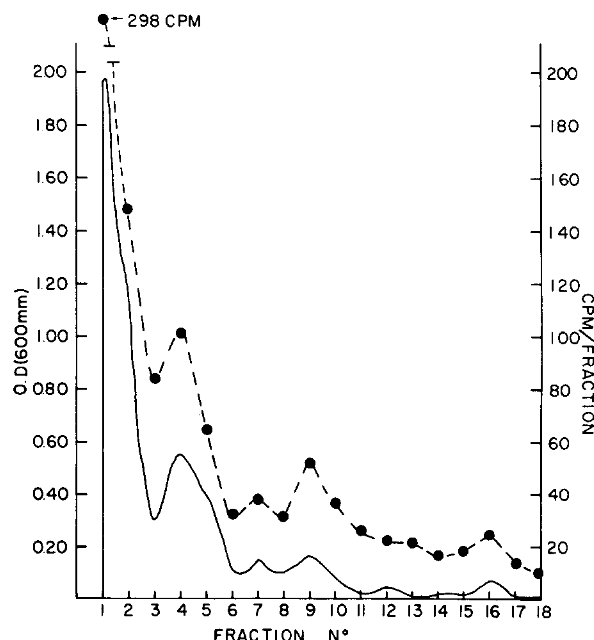

FIG. 2. Densitometric tracing and radioactivity after preparative polyacrylamide gel electrophoresis of apolipoproteins obtained from carrier plasma combined with the fraction of d < 1.050 g/ml from liver slices incubated with [^{14}C]leucine. The continuous line represents the absorbance of the stained gel, and the broken line is the radioactivity present in 0.5-cm-long slices obtained from the same gel.

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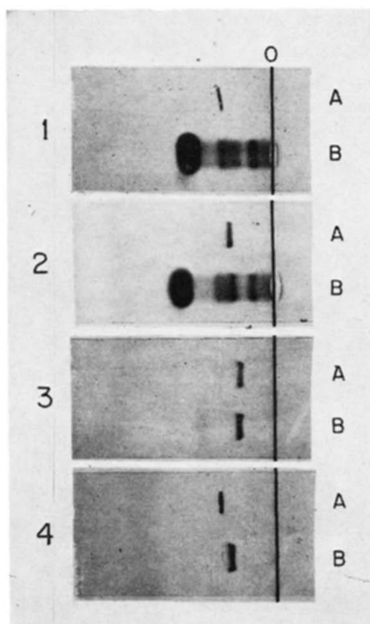

FIG. 3. Agarose electrophoresis of rabbit plasma and purified lipoproteins. Slide *1*: sample *A*, VLDL-c; sample *B*, plasma from a control rabbit. Slide *2*: sample *A*, LDL-c; sample *B*, plasma from a hypercholesterolemic rabbit. Slide *3*: sample *A*, VLDL-hc; sample *B*, LDL-hc. Slide *4*: sample *A*, VLDL-c; sample *B*, VLDL-hc.

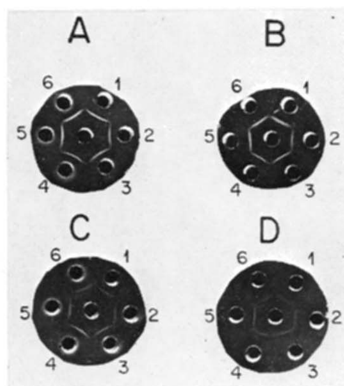

FIG. 4. Double immunodiffusion in agarose gels. The center wells contained: *A*, anti-VLDL-hc serum; *B*, anti-VLDL-hc serum; *C*, anti-VLDL-c serum; and *D*, anti-VLDL-c serum. The outer wells contained: *A-1*, *A-2*, and *A-3*, VLDL-c; *A-4*, *A-5*, and *A-6*, VLDL-hc; *B-1*, *B-2*, and *B-3*, LDL-c; *B-4*, *B-5*, and *B-6*, LDL-hc; *C-1*, *C-2*, and *C-3*, VLDL-c; *C-4*, *C-5*, and *C-6*, VLDL-hc; *D-1*, *D-2*, and *D-3*, LDL-c; and *D-4*, *D-5*, and *D-6*, LDL-hc.

phoretic properties of their lipoproteins. Fig. 5 presents the results obtained for two rabbits at 0, 4, and 8 days after the beginning of feeding the hypercholesterolemic diet. These animals had levels of plasma cholesterol of approximately 100, 500, and 850 mg/100 ml at the time of bleeding. The antiserum used in all cases was anti-VLDL-hc. The change in the immunoelectrophoretic properties of the reacting lipoproteins can be observed as well as the rapid increase in their concentration after 4 days (pattern 2). Measurements of the peak areas (pat-

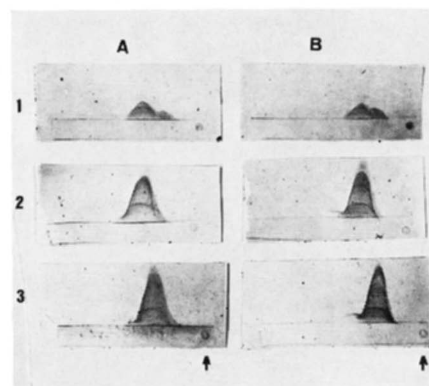

FIG. 5. Quantitative immunoelectrophoresis of rabbit plasma lipoproteins. Patterns are from plasma of two rabbits (*A* and *B*) fed the hypercholesterolemic diets for 0 days (*1*), 4 days (*2*), and 8 days (*3*). The cholesterol levels were 100, 500, and 850 mg/100 ml, respectively. The gel used for the electrophoresis in the second dimension contained anti-VLDL-hc chicken antiserum.

terns 2 and 3) suggest that although the plasma cholesterol concentration increased from 500 to 850 mg/100 ml there was not a proportional increase of the protein portion of the lipoprotein. These results indicate that the main antigenic lipoprotein protein increases rapidly in the first days of the diet, reaching a plateau at plasma cholesterol levels of approximately 500 mg/100 ml. The data suggest, therefore, that an increment of plasma cholesterol above this value is produced by the presence of lipoproteins with lipid-to-protein ratios higher than those of the VLDL present at the beginning of the diet. These results are in agreement with those obtained by analytical ultracentrifugation and analysis of the isolated fractions.

Comparison of the apolipoproteins

The electrophoretic behavior of totally delipidated VLDL, LDL, and HDL isolated from rabbits maintained on the experimental or control diets for 3 wk is compared in Fig. 6.

In gels containing 8 M urea (Fig. 6*A*), apoVLDL-c and apoVLDL-hc had a fast component with similar mobility, although a slower protein component present in apoVLDL-hc was not observed in apoVLDL-c. The electrophoretic patterns of apoHDL-c and HDL-hc were indistinguishable. The incorporation of sodium dodecyl sulfate into the running gels resulted in better resolution. Fig. 6*B* demonstrates that apoLDL-c, apoVLDL-hc, and apoVLDL-c had a protein that barely entered the gel. LDL-c and VLDL-hc had two other bands with similar mobilities not visible in VLDL-c. However, VLDL-c and VLDL-hc both contained a fast-moving protein not present in LDL. In 8% gels containing 6 M urea and 0.2% sodium dodecyl sulfate (Fig. 6*C*), the apolipoproteins were further resolved. In this system apoLDL-c and apoVLDL-hc had several bands in common. On the

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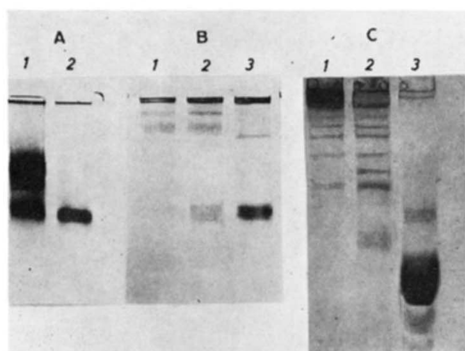

FIG. 6. Polyacrylamide gel electrophoresis of apolipoproteins. *A:* gel 4% cyanogum-41 in 0.37 M Tris-HCl, pH 9.0, 8 M urea, stained with amido black; *1*, apoVLDL-hc; *2*, apoVLDL-c. *B:* gel 6% cyanogum-41, 0.37 M Tris-HCl, pH 9.0, 8 M urea, and 0.2% sodium dodecyl sulfate, stained with amido black; *1*, apoLDL-c; 2, apoVLDL-hc; *3*, apoVLDL-c. In *A* and *B* the electrode buffer was 40 mM Tris-glycine, pH 8.4. *C:* gel 8% cyanogum-41 in 0.1 M phosphate buffer, pH 7.4, containing 6 M urea and 0.2% sodium dodecyl sulfate, stained with Coomassie brilliant blue; *1*, apoLDL-c; *2*, apoVLDL-hc; *3*, apoHDL-c.

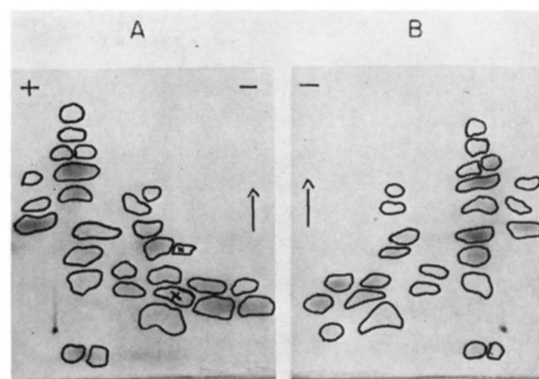

FIG. 7. Peptide maps of partial hydrolyzates of apolipoproteins. Silica gel G plates, 500 μ, air-dried. Electrophoresis buffer, pyridine–acetic acid–water 2:20:1000 (v/v/v). Chromatography solvent, *n*-butanol–acetic acid–water 60:20:20 (v/v/v). *A*, apoVLDL-hc; *B*, apoLDL-c.

other hand, apoHDL-c showed no major components with mobilities similar to those of apoLDL-c and apoVLDL-hc.

To explore further the similarities of the major protein constituents of VLDL-hc and LDL, suggested by immunological studies and gel electrophoresis, peptide maps of the apolipoproteins were obtained. Gel filtration analysis indicated that the peptides resulting after controlled partial acid hydrolysis contained two to eight amino acids. The peptide maps of apoLDL-c and apoVLDL-hc appear very similar except for those spots marked *X* on the map corresponding to apoVLDL-hc (Fig. 7).

DISCUSSION

The results presented indicate that cholesterol feeding produces in rabbits a rapid modification of lipoprotein concentrations. Huang and Kako (8) have observed an early increase in plasma cholesterol concentrations in rabbits after initiation of a hypercholesterolemic diet. As suggested by Gofman et al. (1), such an increase in plasma cholesterol could be the reflection of either an elevation in the concentration of normally occurring lipoproteins or the assembly of new lipid-carrying macromolecules structurally different from those found in normal animals. The change in lipid composition, as pointed out previously by Schumaker (2), is most marked in the VLDL fraction. Analysis of the apolipoproteins of control and experimental rabbits indicated that in normal rabbits VLDL and LDL have a major protein component in common and some proteins which are characteristic of each fraction. In the animals fed the high cholesterol diet for 3 wk, the protein components typical of LDL were also found in the lipoprotein with density less than 1.019. These results suggest that in hypercholesterolemic animals, the VLDL fraction is made up of lipoproteins with the apoproteins of normal LDL but with a higher lipid-to-protein ratio which caused them to be collected in the usual density range of a normal VLDL. Schumaker (2) and Huang and Kako (8) have found that VLDL from hypercholesterolemic rabbits is a poor substrate for lipoprotein lipase, and it has been suggested (8) that perhaps this VLDL was structurally more related to LDL. Our results give experimental basis to this suggestion. Studying the *N*-terminal amino acids of lipoproteins from long-term cholesterol-fed rabbits, Shore and Shore (19) found no large qualitative or quantitative changes. Our results explain these findings; high cholesterol diets lead to an increased concentration of lipoproteins with density less than 1.063 with changes in their lipid composition, but with the major apoproteins normally present in this fraction. It is of interest that in the control rabbits some protein components of VLDL and LDL appear to be similar, but no major apoprotein from HDL could be detected in VLDL. This situation contrasts with that found in rat and man, where VLDL contains substantial amounts of the apoprotein present in HDL (20, 21).

The increased incorporation of L-[^{14}C]leucine into lipoproteins with density less than 1.050 in cholesterol-fed rabbits suggests that one of the factors leading to hypercholesterolemia is an enhancement of the lipoprotein biosynthetic mechanism in response to an increased intracellular cholesterol concentration.

Several investigators have shown that long-term cholesterol feeding in rabbits (22), dogs (23), and rats (24) causes an alteration of the fatty acid composition of liver and plasma cholesteryl esters, characterized by an increased 18:1/18:2 ratio. We observed a similar alteration in rabbits fed a high cholesterol diet for 2 wk. The

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significance of this rapid change should be investigated further.

As a whole, our results stress the necessity for careful studies of the early effects of cholesterol feeding in cell metabolism to advance our knowledge about the influence of dietary modification on lipoprotein structure and metabolism.

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