

Effect of cholesterol feeding on apo B and apo E concentrations and distributions in euthyroid and hypothyroid rats

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Abstract Lipoprotein profiles in experimental hypercholesterolemia were studied using euthyroid and hypothyroid cholesterol-fed rats. In both groups, serum cholesterol concentration increased, but to a lesser extent in the cholesterol-fed euthyroid rats, with similar changes in distribution among lipoprotein fractions in both groups. Agarose electrophoresis of plasma and individual lipoprotein fractions showed that β -VLDL and HDL_c were present in the hypothyroid, cholesterol-fed rats. In the euthyroid and hypothyroid cholesterol-fed rats, serum apo B concentrations increased three-fold and five-fold, respectively. This reflected increases of apo B in very low density and intermediate density lipoproteins. In the euthyroid cholesterol-fed rat, serum apo E decreased 50%, while the serum apo E concentration was not significantly changed in hypothyroid cholesterol-fed rats. In both the euthyroid and the hypothyroid cholesterol-fed rats, apo E decreased in high density lipoproteins and increased in lower density lipoproteins. We observed qualitative and quantitative differences between hypothyroid and euthyroid cholesterol-fed rats. The major qualitative differences were the appearance of beta migrating very low density lipoproteins (β -VLDL) and HDL_c in the hypothyroid cholesterol-fed rat, and a decrease of serum apo E concentrations in the euthyroid cholesterol-fed rats. Changes in serum cholesterol and apo B concentrations and the distribution of cholesterol, apo B, and apo E among the lipoprotein fractions were similar in direction in both groups, but greater in magnitude in the hypothyroid versus euthyroid cholesterol-fed rats. These data demonstrate that hypothyroidism should be considered when evaluating apolipoprotein changes in hypercholesterolemic animal models. — **DeLamatre, J. G., and P. S. Roheim.** Effect of cholesterol feeding on apo B and apo E concentrations and distributions in euthyroid and hypothyroid rats. *J. Lipid Res.* 1981. **22**: 297–306.

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Hypercholesterolemia is known to be a positive risk factor for the development of atherosclerosis (1). Experimental hypercholesterolemias resemble Type III hyperlipoproteinemia and can serve as models for studying the associated lipoprotein alterations and the

development of atherosclerosis (2). The most common animal models used to study experimental hypercholesterolemia are the monkey, rabbit, swine, dog, and rat (2–4). The dog and rat differ from the others in that attaining a serum cholesterol concentration greater than 300 mg/dl usually requires a hypothyroid state in animals fed a high-cholesterol, high-fat diet; in the other animals a high-cholesterol, high-fat diet alone is sufficient to create hypercholesterolemias capable of inducing atherosclerosis.

We have shown previously that the serum lipoprotein patterns of rats fed a high-cholesterol, high-fat diet differ markedly from those of control rats in that a lipoprotein class of d 1.006–1.03 g/ml (IDL) appears, in addition to an increase in the concentration of VLDL (5). Our studies on the effect of a high-cholesterol, high-fat diet (CF) on the apolipoprotein profiles of rats revealed a decrease in the serum concentrations of apo E (6, 7). This finding was at variance with other reports which showed an increase in apo E with cholesterol feeding (8, 9). Inasmuch as these animals were fed a propylthiouracil- (PTU) containing diet, we postulated that the hypothyroid state might explain the difference. Therefore, we repeated the cholesterol-feeding experiment and included in the study a group of rats that were fed a high-cholesterol, high-fat diet with PTU (CF-PTU). The results reported here show that the difference between our initial results and the results of others was indeed due to the presence of PTU in the diet.

Apo B functions to transport cholesterol and triglyceride in the blood (10) and is recognized in peripheral tissue by an apo B receptor which also recognizes apo E (11, 12). In addition to its possible

Abbreviations: CF, cholesterol-fed; PTU, propylthiouracil; CF-PTU, cholesterol-fed with propylthiouracil; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; EIA, electroimmunoassay; AU, arbitrary units; TMU, tetramethylurea.

role in delivering cholesterol to peripheral tissue, apo E is thought to be important in receptor-mediated chylomicron remnant uptake by the liver (13, 14). In this manuscript we describe changes in lipoproteins and the distribution of apolipoproteins B and E in hypercholesterolemic states, with and without hypothyroidism.

MATERIALS AND METHODS

Animals

For this study male Sprague-Dawley rats, 275–300 g, were used. Control animals were maintained on laboratory rat chow; one experimental group was fed 2% cholesterol and 20% olive oil in laboratory rat chow meal (CF); the other experimental group was fed 2% cholesterol, 20% olive oil, and 0.2% propylthiouracil in laboratory rat chow meal (CF-PTU). Animals fed CF diets gained weight at the same rate as control rats, whereas rats fed CF-PTU diets did not gain weight during the experimental period. After 30 days on the diet, the animals were fasted for 14 hr (midnight to 2:00 PM) and were bled from the abdominal aorta while under light ether anesthesia. Serum was separated and stored in the presence of 0.1% EDTA and 0.1% azide. In order to minimize variations due to differences in rat chow composition and seasonal variations, the reported values for control, CF, and CF-PTU rats were obtained from experiments run simultaneously. For lipid and apolipoprotein analyses, sera from three to four rats were pooled.

Analytical methods

Cholesterol and triglyceride concentrations were determined enzymatically by the methods of Allain et al. (15) and Bucolo and David (16), respectively. Reagents were purchased from Calbiochem-Behring, Dallas, TX. Protein was determined by the method of Lowry et al. (17), as modified by Sata, Havel, and Jones (18), using bovine serum albumin as a standard. Agarose electrophoresis was performed according to the method of Noble (19), using 0.025M Tris-tricine buffer and 1% agarose. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Shapiro, Viñuela and Maizel (20), as described previously (21), using 10% acrylamide gels.

Lipoproteins were separated according to the method of Havel, Eder and Bragdon (22), using an SW-41 rotor in a Beckman L5-50 preparative ultracentrifuge. Very low density lipoproteins (VLDL) of $d < 1.006$ g/ml, intermediate density lipoproteins

(IDL) of $d 1.006$ – 1.03 g/ml, and low density lipoproteins (LDL) of $d 1.03$ – 1.063 g/ml were separated by a 24-hr ultracentrifugation at 265,000 *g*. High density lipoproteins (HDL) of $d 1.063$ – 1.21 g/ml were separated by a 40-hr ultracentrifugation at 265,000 *g*. VLDL and IDL were resuspended in saline of the appropriate density and washed twice by ultracentrifugation. LDL and HDL were washed once by increasing the supernate density slightly (1.063 to 1.08 and 1.21 to 1.24 g/ml, respectively), placing a layer of saline of the original density over this solution (1.063 for LDL and 1.21 g/ml for HDL), and recentrifuging. The fractions were dialyzed against 2–4 liters of saline at a density of 1.006 g/ml which contained 0.1% EDTA and 0.1% azide. The dialysis solution was changed at least four times in a 24-hr period.

For determination of cholesterol, triglyceride, and apolipoprotein distributions in VLDL, IDL, and LDL, one-ml aliquots of serum were adjusted to the following densities: 1.006 g/ml; 1.03 g/ml; 1.063 g/ml. HDL was further subdivided to HDL₁, HDL₂, and HDL₃ using the densities: 1.08 g/ml; 1.125 g/ml; and 1.21 g/ml. Samples were subjected to a single spin in a Beckman L3-40 ultracentrifuge using a 40.3 rotor with 2-ml adaptors. The tubes were cut by a Spinco tube cutter (Spinco Division Beckman Instruments, Inc., Palo Alto, CA). The infranates were brought up to the same volume and used for the analysis. Apolipoprotein concentrations were then determined in serum and in each infranate fraction. The quantity of apolipoprotein in each lipoprotein fraction was calculated by subtracting one infranate value from the preceding lower density infranate value (e.g., IDL = $d > 1.006$ – $d > 1.03$). Since the infranate albumin concentration should equal the serum albumin concentration, it is possible to correct for non-specific losses of apoproteins. The albumin concentrations of serum and the infranate were determined and a correction factor was calculated by dividing serum albumin concentration by the infranate albumin concentration. The infranate apoprotein concentration was multiplied by the correction factor in order to correct for non-specific losses of apoprotein concentration. The differences between serum and infranate concentration were never greater than $\pm 5\%$.

Apolipoprotein concentrations were determined by the electroimmunoassay (EIA) technique of Laurell (23), as modified by Bar-On, Roheim, and Eder (21). Antisera used in this study were prepared as previously described (21). Nonidet 40 (NP-40), a non-ionic detergent, was added to a final concentration of 1% in the samples and standards and 0.05% in the agarose (24). Standard curves were obtained by dilu-

tion of pooled rat serum and were run simultaneously with the samples. The arbitrary units (AU) used to express the data are a percentage of standard pool serum.

Apolipoprotein concentrations were also estimated using SDS-PAGE of $d < 1.21$ g/ml lipoproteins. These lipoproteins were subjected to a single spin in a Beckman L5-50 preparative ultracentrifuge using an SW-60 rotor. The serum density was adjusted to 1.24 g/ml by the addition of KBr, and the sample was placed in the ultracentrifuge tube. Saline of $d 1.21$ g/ml was then layered over this solution, and the lipoproteins were spun for 60 hr at 210,000 g . Total apo B content of the $d < 1.21$ g/ml serum fraction was determined by the tetramethylurea (TMU) precipitation technique of Kane (25). Apolipoprotein E concentration of the $d < 1.21$ g/ml serum fraction was estimated by scanning stained SDS polyacrylamide gels of the apolipoproteins using an EC densitometer (Model No. B5216-II, EC Apparatus Corp., St. Petersburg, FL) and measuring the area under the peaks by planimetry using an A. Ott compensating planimeter (Epic, Inc., New York, NY). The fraction of the total area that the apo E peak represented was multiplied by the TMU-soluble protein concentration to estimate apo E concentration in $d < 1.21$ g/ml serum. Although variations in apolipoprotein chromogenicity may limit this technique of protein quantification, comparing only the same protein (apo E) between groups minimizes this source of error.

For statistical analysis, one-way analysis of variance was used followed by the Student-Neuman Kuels multiple range test (26). A P value < 0.05 was considered significant.

RESULTS

Lipoprotein electrophoresis

Marked changes in the relative concentrations and mobilities of both serum and isolated fractions were found when serum and individual lipoprotein fractions were analyzed by 1% agarose electrophoresis (Fig. 1). The sera of both CF and CF-PTU showed broad β patterns. However, the broad β patterns were not identical. The CF serum stained for more lipid in the pre- β region than the CF-PTU sera; the CF-PTU sera showed a much stronger staining in the β region and had slower mobility than CF sera (Fig. 1A). VLDL of CF-PTU rats contained a component which migrated in the β region and may be similar to β -VLDL, which has been found in other studies involving hypothyroid cholesterol-fed animals (Fig. 1C) (2, 4, 8).

VLDL of CF rats did not show a band in the β region (Fig. 1C). The IDL fractions of both CF and CF-PTU rats exhibited electrophoretic patterns similar to their respective VLDL patterns. LDL of CF-PTU rats also exhibited a strong band of α_2 mobility (Fig. 1C). This most likely corresponds to HDL_c, a particle previously described by Mahley (2, 4) and Mahley and Holcombe (8). In both CF and CF-PTU sera there was a marked reduction in α lipoproteins (Fig. 1A).

Serum lipid and lipoprotein concentrations

Serum cholesterol concentration tripled in the CF rats and increased six-fold in the CF-PTU rats (Table 1). The cholesterol distributions among the lipoprotein fractions are seen in Fig. 2. VLDL and IDL cholesterol concentrations increased above control in CF rat sera and to a greater extent in the CF-PTU rat sera. No significant changes occurred in LDL. The amount of cholesterol present in HDL₁ and HDL₂ decreased to the same extent in CF rat sera and CF-PTU rat sera. HDL₃ and $d > 1.21$ g/ml serum cholesterol concentrations were similar to those in control rats for both experimental groups.

Triglyceride concentration was unchanged in the CF rats but decreased significantly in the CF-PTU rats. Fig. 3 shows the triglyceride distribution in the control and experimental rats. Control VLDL contains 63% of total triglyceride with the rest distributed in descending order in IDL, LDL, and HDL. In CF rats there was an increase in IDL triglyceride, although this was not statistically significant, probably due to the small sample size. Triglyceride concentrations in other lipoprotein classes remained unchanged. The decreased serum triglyceride concentration in the CF-PTU rats reflected a decrease in VLDL triglyceride.

Total $d < 1.21$ g/ml protein was decreased in CF rat serum and increased in CF-PTU rat serum (Table 2). In CF rats, lipoprotein protein concentration in VLDL, IDL, and LDL increased greatly over control (Table 3). IDL showed the largest increase. The protein concentration in HDL of CF rats decreased 44% from control values. As in the CF animals, CF-PTU treatment increased VLDL, IDL, and LDL protein concentrations. However, the increase for CF-PTU was less in VLDL but greater in IDL and LDL than in the CF animals. The protein concentration of HDL was similarly decreased in CF and CF-PTU rats.

Apolipoprotein concentration and distribution

Serum apolipoprotein B concentrations increased 3-fold over control values due to the CF diet and 6-fold due to the CF-PTU regimen, as determined by EIA (Table 1). These findings were confirmed with

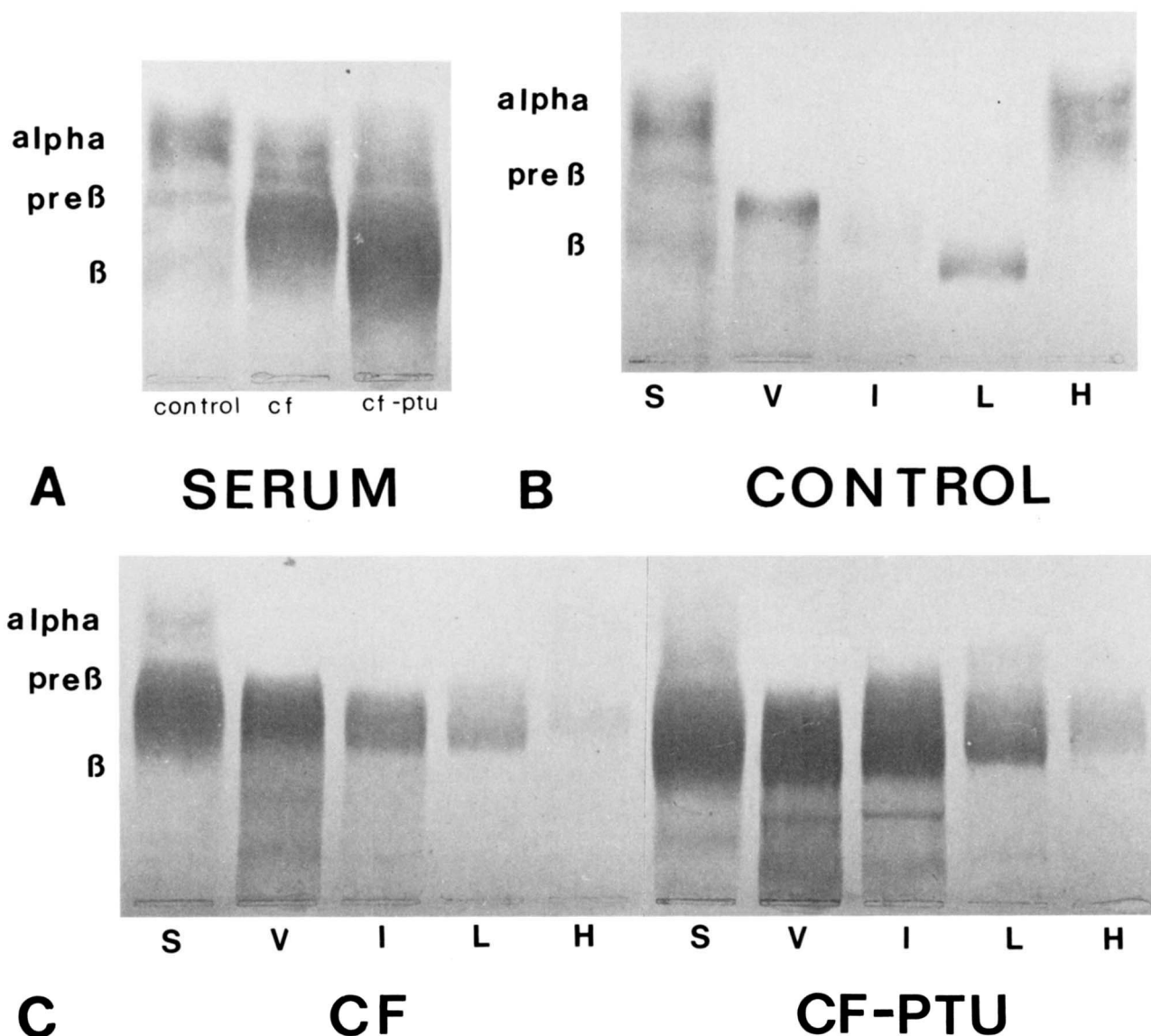


Fig. 1. Agarose electrophoresis of lipoproteins. Panel A, comparison of serum samples of control, CF, and CF-PTU rats. Panel B, lipoprotein fractions from control rats. Panel C, lipoprotein fractions from CF and CF-PTU rats. Serum samples were run simultaneously as were CF and CF-PTU lipoprotein fractions in order to allow direct comparison. Abbreviations: S, serum; V, very low density lipoproteins; I, intermediate density lipoproteins; L, low density lipoproteins; H, high density lipoproteins.

TMU-precipitable protein determinations of the lipoprotein fractions, which indicate apo B concentration (25). Table 2 shows that the concentration of TMU-precipitable protein in $d < 1.21$ g/ml lipoprotein fractions is 3-fold greater than control in CF rats and 6-fold in the CF-PTU rats. **Fig. 4** depicts the distribution of apolipoprotein B in control and experimental groups. The CF diet resulted in a 12-fold increase of apo B in VLDL and IDL, while no changes occurred in LDL of CF rats. The CF-PTU diet led to a 15-fold increase in VLDL apo B and a 33-fold increase in IDL apo B. No significant differences in LDL apo B concentrations were present between any of the

groups. Only traces of apo B were present in the $d 1.063-1.08$ g/ml region in all groups. No apo B was detected in $d > 1.08$ g/ml fractions.

Serum apolipoprotein E concentrations decreased from control values by more than 50% with the CF diet, but did not differ from control with the CF-PTU regimen, as determined by EIA (Table 1). In addition, concentrations of apo E were estimated by densitometric scanning of SDS gels. This technique showed that apo E concentrations in $d < 1.21$ g/ml fractions of sera from rats fed CF diets were approximately half the control, while sera of animals fed CF-PTU diets had slightly greater apo E concentrations than control

(Table 2). In the control animals, apo E is found mainly in HDL₂ (42%), HDL₁ (23%), d > 1.21 g/ml (14%), and LDL (12%). While the CF diet lowers serum apolipoprotein E concentrations, it also alters its normal distribution (Fig. 5). In CF rats, apo E concentrations were not significantly changed in VLDL and increased 10-fold in IDL. HDL₂, the major apo E-containing fraction in the control, showed a 95% decrease in CF rats. Other lipoprotein fractions that showed significant decreases included: LDL, HDL₁, HDL₃, and d > 1.21 g/ml. Serum apolipoprotein E concentrations of CF-PTU rats remained unchanged. Nevertheless, a redistribution of apo E similar to that seen with the CF rats is present. In VLDL of CF-PTU rats, apo E concentrations increased from 1 AU to 15 AU, which is a value 7-fold higher than the CF apo E concentration. The apo E concentrations in IDL increased from 1 AU to 38 AU, which is 3-fold higher than the CF apo E concentration. Apo E concentrations in LDL, HDL₁, HDL₂, HDL₃, and d > 1.21 g/ml were all decreased to the same extent as in CF rats.

We have observed these changes resulting from CF diets in other experiments, in addition to the data

TABLE 1. Plasma concentrations of cholesterol, triglyceride, and apolipoproteins B and E of control and experimental rats^a

Diet	Control (6) ^b	Cholesterol-fed (6)	Cholesterol-Propylthiouracil (6)
Cholesterol, mg/dl	55 ± 3 ^c	165 ± 23 ^{d,e}	291 ± 22 ^{d,e}
Triglyceride, mg/dl	73 ± 5	85 ± 7 ^e	34 ± 7 ^{d,e}
Apo B, arbitrary units	60 ± 3	182 ± 33 ^{d,e}	327 ± 20 ^{d,e}
Apo E, arbitrary units	49 ± 4	20 ± 3 ^{d,e}	51 ± 5 ^c

^a Length of treatment period was 4 weeks.

^b Number of samples: six pools of three animals for all determinations.

^c Values ± S.E.M.

^d Significantly different than control, $P < 0.05$.

^e Significantly different than other experimental group, $P < 0.05$.

presented above. The comparison between these experiments is shown in Table 4. Changes in cholesterol concentrations varied within the three experiments from an increase of 83% to 300%; apo B from an increase of 111% to 303%; apo E from a decrease of 59% to 45%. The direction of change was very consistent with respect to cholesterol apo B and apo E;

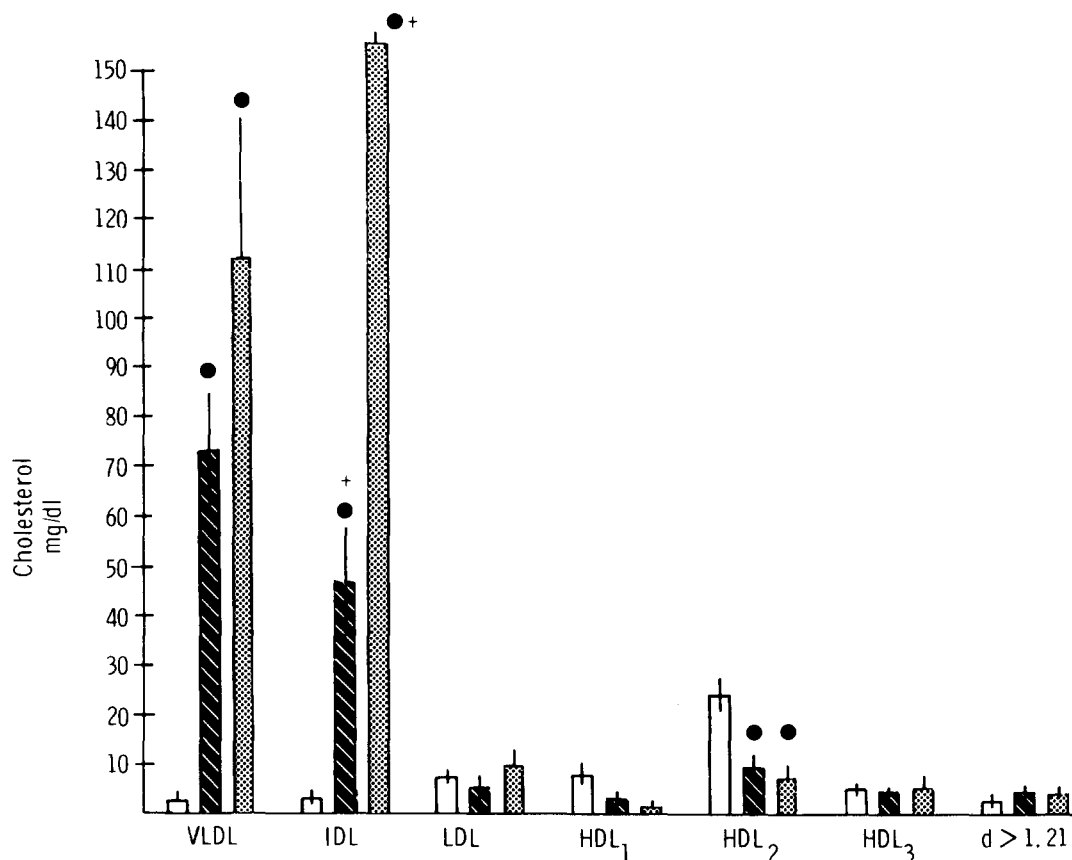


Fig. 2. The effect of cholesterol feeding and cholesterol feeding with propylthiouracil on the distribution of plasma cholesterol. □, Control; ▨ cholesterol-fed; ▩ cholesterol-fed with propylthiouracil. Values based on three pools of six rats per pool. (●) Indicates significant difference from control, $P < 0.05$. (+) Indicates significant difference from other experimental group, $P < 0.05$.

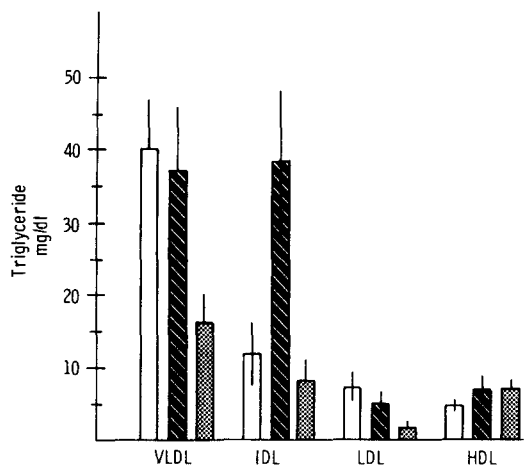


Fig. 3. The effect of cholesterol feeding and cholesterol feeding with propylthiouracil on the distribution of plasma triglyceride. □ Control; ■ cholesterol-fed; ▨ cholesterol-fed with propylthiouracil. Values based on three pools of six rats per pool.

however, there was some variation in the magnitude of the response. The distribution of apo B and apo E showed similar patterns in an additional experiment (data not shown). Animals with the largest increase in cholesterol had the highest increase in IDL apo E and the largest decrease in HDL apo E.

SDS gel electrophoresis

Fig. 6 shows the SDS gel electrophoresis pattern of the total lipoprotein fractions ($d < 1.21$ g/ml), and **Fig. 7** shows VLDL of control, CF, and CF-PTU rats. The pictures of the densitometric scans of the $d < 1.21$ g/ml gels demonstrate that apo E is lower in the CF rats than in the control. In the control, the major apoprotein is A-I, while in CF-PTU rats, apo E becomes the predominant apoprotein. VLDL gels of CF rats show a stronger apo B band than control, with no apparent differences from control in the apo E or apo

TABLE 2. Concentrations of total protein and apolipoproteins B and E in $d < 1.21$ g/ml serum of control and experimental rats^a

	Control (n = 3) ^b	Cholesterol-fed (n = 3) ^b	Cholesterol- Propylthiouracil (n = 1) ^c
Total protein ^d , mg/dl	121 ± 3 ^e	102 ± 3	150
Apo B ^f , mg/dl	10 ± 3	23 ± 4	55
Apo E ^g , mg/dl	24 ± 5	13 ± 2	33

^a Length of treatment period was 6 weeks.

^b Number of samples: three pools of six animals.

^c Number of samples: one pool of nine animals.

^d Serum was from an unwashed single spin.

^e Values ± S.E.M.

^f TMU-precipitable protein in $d < 1.21$ g/ml serum.

^g Proportion of TMU soluble protein as determined by densitometric scan of SDS gels of $d < 1.21$ g/ml serum.

TABLE 3. Distribution of protein in lipoprotein fractions of control and experimental rats

	Control	Cholesterol-fed	Cholesterol-PTU
	<i>mg/dl^a</i>		
VLDL ^b	3.2 (5.6) ^c	6.1 (11.7)	4.7 (7.2)
IDL	1.5 (2.7)	11.1 (21.6)	22.0 (33.5)
LDL	4.9 (8.8)	8.2 (16.0)	13.5 (20.6)
HDL	46.4 (82.9)	25.9 (50.4)	25.5 (38.8)

^a All values were obtained from duplicate determinations on two pools of nine animals.

^b Densities used: very low density lipoproteins (VLDL), $d < 1.006$ g/ml; intermediate density lipoproteins (IDL), 1.006–1.03 g/ml; low density lipoproteins (LDL), 1.03–1.063 g/ml; high density lipoproteins (HDL), 1.063–1.21 g/ml.

^c Numbers in parentheses refer to percent of total lipoprotein protein.

C regions. VLDL gels of CF-PTU rats differed considerably from both control and CF rats; they consisted almost entirely of apo B and apo E, while C apolipoproteins were greatly lowered.

DISCUSSION

The present study describes apolipoprotein profiles in control and cholesterol-fed rats and further compares cholesterol-fed to hypothyroid cholesterol-fed

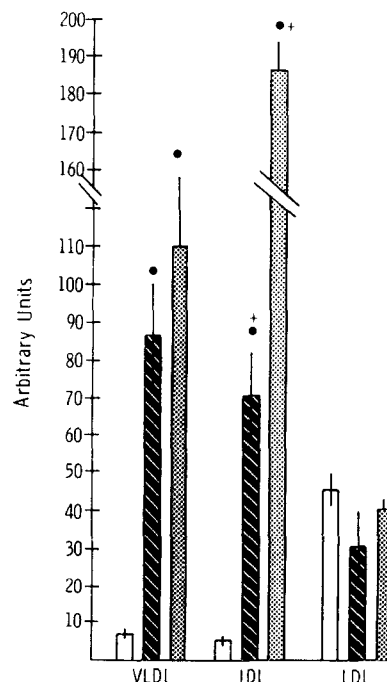


Fig. 4. The effect of cholesterol feeding and cholesterol feeding with propylthiouracil on the distribution of apolipoprotein B. □ Control; ■ cholesterol-fed; ▨ cholesterol-fed with propylthiouracil. Values based on six pools of three rats per pool. (●) Indicates significant difference from control, $P < 0.05$. (†) Indicates significant difference from other experimental group, $P < 0.05$.

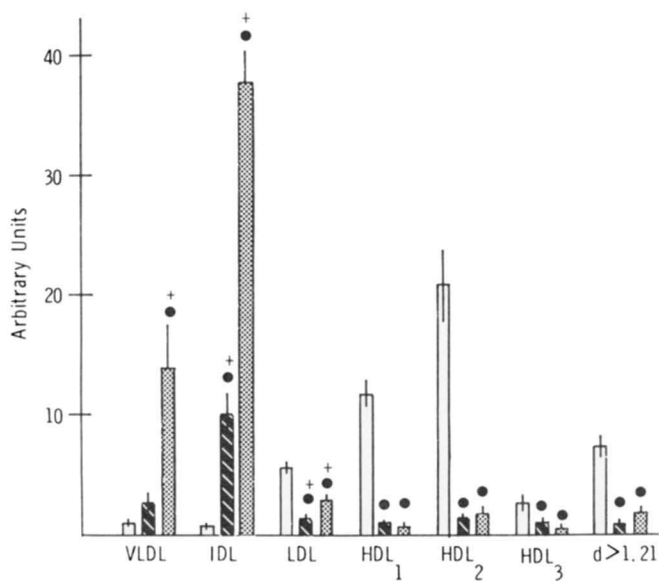


Fig. 5. The effect of cholesterol feeding and cholesterol feeding with propylthiouracil on the distribution of apolipoprotein E. □ Control; ■ cholesterol-fed; ▨ cholesterol-fed with propylthiouracil. Values based on six pools of three rats per pool. (●) Indicates significant difference from control, $P < 0.05$. (+) Indicates significant difference from other experimental group, $P < 0.05$.

rats. It should be mentioned that this study cannot differentiate between the effects of olive oil and cholesterol. Compared to controls, the hypothyroid rats weighed less and consumed less food, reflecting their metabolic state. We elected not to use pair-fed controls for the hypothyroid animals, since this would involve comparing a starved rat with one whose caloric intake was in line with its metabolic requirements.

Agarose electrophoresis provided a means of assessing changes in lipoproteins due to the CF and CF-PTU regimens. The plasma of CF-PTU animals contained VLDL and IDL that had a slower mobility than the VLDL and IDL of CF rats and probably represents β -VLDL (8). The SDS-PAGE gels of the CF-PTU rats showed that the VLDL and IDL fractions contained a smaller quantity of C apoproteins than VLDL and IDL of CF rats, which may explain their slower mobility

TABLE 4. Comparison of cholesterol-fed and control plasma apolipoproteins B and E concentrations

Experiment	Cholesterol	Apo B ^a		Apo E ^a
		(percent of control)		
A (n = 6 pools) ^b	183	211	48	
B (n = 6 pools) ^b	400	403	41	
C (n = 3 pools) ^c	366	325	51	

^a Determined by electroimmunoassay.

^b Each pool contained equal volumes of plasma from three to four animals.

^c Each pool contained equal volumes of plasma from six animals.

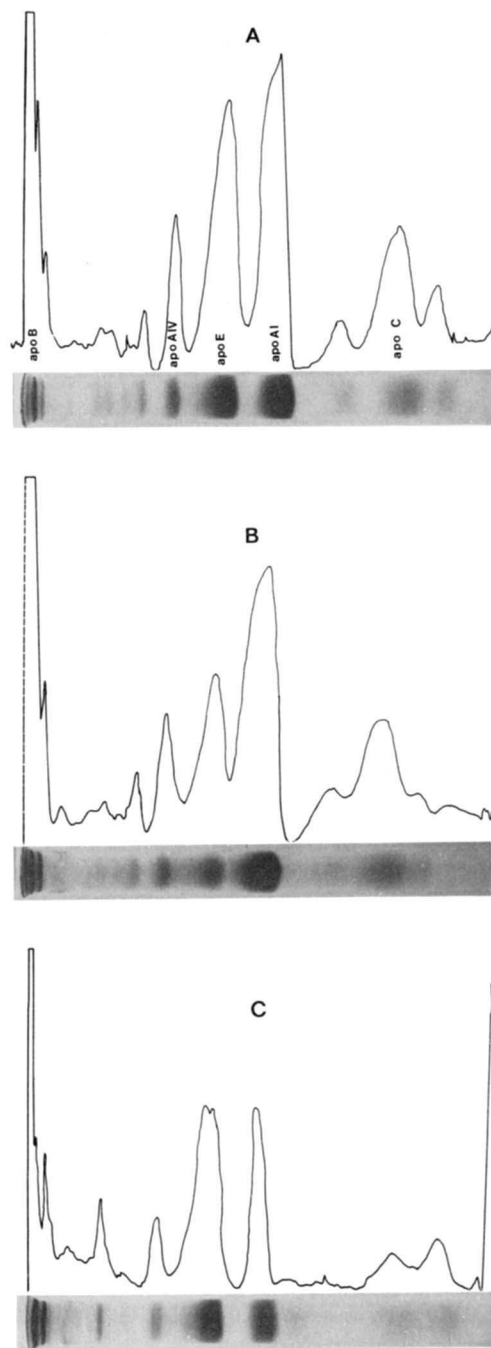


Fig. 6. SDS-PAGE and densitometric scanning of $d < 1.21$ serum from rats: (A) Control; (B) cholesterol-fed; (C) cholesterol-fed with propylthiouracil.

(Fig. 7). Plasma of rats, dogs, and swine fed atherogenic diets show HDL_e, an α_2 migrating particle that appears primarily in the 1.03–1.08 g/ml density range (4). A strong α_2 migrating band, which is probably HDL_e, was detected in the LDL fraction (d 1.03–1.063 g/ml) of the CF-PTU rats (Fig. 1C). A lighter band was sometimes visible in LDL of the CF rats. These data suggest that hypothyroidism plays an important role

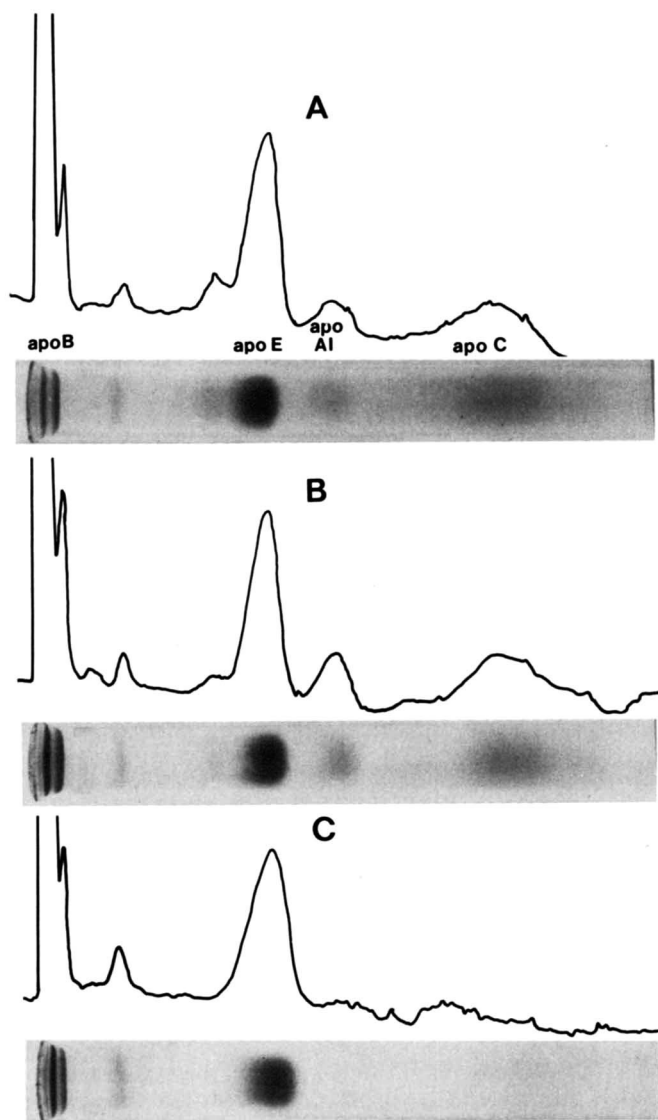


Fig. 7. SDS-PAGE and densitometric scanning of very low density lipoproteins from rats: (A) Control; (B) cholesterol-fed; (C) cholesterol-fed with propylthiouracil.

in the development of β -VLDL and HDL_c in the rat. However, the appearance of β -VLDL and HDL_c may also be related to attainment of high serum cholesterol concentrations (2).

Apo B increased 2- to 3-fold in the serum of CF rats. This increase occurred as a result of a greatly increased amount of apo B in VLDL and IDL. In the CF rats, apo E decreased overall by about 50%. The apo E distribution changed, with more appearing in IDL and much less in HDL and the $d > 1.21$ g/ml fraction. All HDL subfractions showed a marked decrease of apo E, and there were no apparent differences between the subfractions.

A large percentage of VLDL cholesterol in the cholesterol-fed rabbit originates from intestinally absorbed exogenous cholesterol and is carried in remnant particles formed from chylomicrons (27, 28). The large quantity of dietary fat presented to the CF rats increases the intestinal chylomicron production many-fold (29, 30). Our data show that in the CF rat, apo E is greatly diminished in HDL (by 92%). Since apo E transfers from HDL to chylomicrons upon exposure of chylomicrons to serum (31, 32), the large increase of chylomicron production that occurs in CF rats may be of sufficient magnitude to reduce drastically the apo E concentration in HDL. Subsequently, the apo E becomes a component of a chylomicron remnant, which the liver removes from the plasma at a very fast rate (33). It has been suggested that apo E provides chylomicron remnants with an hepatic recognition signal, allowing the remnant particles to be removed by a high-affinity, receptor-mediated process (13, 14). Therefore, we postulate that when the liver removes a large mass of chylomicron remnants from the serum of CF rats, a concomitantly large mass of serum apo E is removed by the liver of these rats. This mechanism may be responsible for the lower apo E in the serum and HDL of CF rats. However, it cannot be ruled out at the present time that a decreased HDL apo E secretion by the liver may account for the lowered concentration of apo E in the serum and HDL of CF rats, since apo E is thought to be primarily synthesized and secreted by the liver as HDL (34).

Perfused livers of cholesterol-olive oil-fed rats secrete a greater quantity of d 1.006–1.019 g/ml cholesterol into liver perfusates than control livers (35). Thus, the liver probably contributes to the IDL found in the serum of CF rats, either directly or by catabolism of VLDL. In the present study, the greatest increase of apo E and apo B was found in the IDL fraction. Since apo E and apo B are normally components of VLDL derived from the liver (36, 37), the IDL apolipoproteins E and B may be at least partially derived from these lipoproteins produced by the liver.

The serum concentration of apo B in CF-PTU rats increased from 60 AU for control to 327 AU. This increase exceeds the 182 AU observed in the CF rats. No change from control occurred in serum concentrations of apo E in the CF-PTU rats, which contrasts with a 50% decrease of apo E concentrations in CF rats. The VLDL and IDL of CF-PTU rats contained much greater concentrations of apo B and apo E than were observed in the CF rats. As with the CF rats, HDL apo E decreased to 8% of control concentrations in the serum of CP-PTU rats. The general shifting

of apo E from high-density lipoproteins to lower-density lipoproteins in hypothyroid cholesterol-fed rats agrees with the observations of others (8, 9).

We have shown that in hypothyroid rats the concentrations of both apo B and apo E increase in the serum (38). Thus, hypothyroidism potentiates the apo B increase induced by the CF diets. The influence of hypothyroidism alone on apo E concentrations could explain why apo E is lower in CF but remained unchanged in CF-PTU animals from our experiments. Mahley and Holcombe (8) and Wong and Rubinstein (9) observed an increase in apo E in hypothyroid cholesterol-fed rats. However, there were differences in dietary composition used in this study versus the above-quoted reports; both studies (8, 9) used diets containing fat of animal origin (5% lard or 40% butterfat), and both used bile acids. In the present study, no bile acids were included in the diet, and olive oil was the source of fat. This probably explains why apo E concentrations were unchanged in the serum of our hypothyroid-hypercholesterolemic animals.

There is evidence that the livers of CF-PTU rats contribute directly to the increased apo E, VLDL, and IDL concentrations. Studies using perfused livers of rats fed 5% cholesterol, 40% butterfat, 0.1% sodium chloride, and 0.3% PTU reveal that apo E secretion by these livers is greater than controls (39). Further, the livers secrete a greater quantity of VLDL and IDL. Considering all the information available from other reports, at least five possibilities exist to explain why VLDL and IDL of CF-PTU rats are greater than in control and CF rats: increased hepatic production (39), decreased hepatic uptake (40), decreased peripheral uptake (41), increased intestinal absorption (42), or most likely some combination of the above.

These studies demonstrate that many specific differences in the lipoprotein profiles exist between the euthyroid and hypothyroid cholesterol-fed rats; however, there are also some fundamental similarities between these two hypercholesterolemic models. The major differences are the appearance of β -VLDL and HDL_c in the hypothyroid cholesterol-fed rats and the decrease of serum apo E concentrations in euthyroid cholesterol-fed rats. The distribution of serum cholesterol and apolipoproteins B and E among the lipoprotein fractions was drastically altered in both groups: apoproteins and cholesterol were increased in VLDL and IDL and reduced in HDL. The decrease in HDL apo E and the increase in IDL apo E in both hypothyroid and euthyroid cholesterol-fed rats was of special interest. From these data it is apparent that these similarities are the result of cholesterol feeding,

while the above-mentioned differences are most likely due to hypothyroidism. ■

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REFERENCES

1. Kannel, W. B., W. P. Castelli, T. Gordon, and P. M. McNamara. 1971. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham Study. *Ann. Int. Med.* **74**: 1-12.
2. Mahley, R. W. 1979. Dietary fat, cholesterol, and accelerated atherosclerosis. In *Atherosclerosis Reviews*. R. Paoletti and A. M. Gotto, Jr., editors. Raven Press, New York, NY. **5**: 1-34.
3. Kritchevsky, D. 1963. Experimental atherosclerosis. In *Lipid Pharmacology*. R. Paoletti, editor. Academic Press, New York, NY. 63-130.
4. 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. Dietchy, A. M. Gotto, Jr., and J. A. Ontko, editors. Waverly Press, Inc., Baltimore, MD. 181-197.
5. Lasser, N. L., P. S. Roheim, D. Edelstein, and H. A. Eder. 1973. Serum lipoproteins of normal and cholesterol-fed rats. *J. Lipid Res.* **14**: 1-8.
6. Kuehl, K., P. S. Roheim, and H. A. Eder. 1974. Alterations in subunit composition of serum lipoprotein of cholesterol-fed rats. *Federation Proc.* **33**: 351.
7. DeLamatre, J. G., and P. S. Roheim. 1979. Effect of feeding a high cholesterol-high fat diet on the apolipoprotein profiles of rats. *Federation Proc.* **38**: 896.
8. Mahley, R. W., and K. S. Holcombe. 1977. Alterations of the plasma lipoproteins and apolipoproteins following cholesterol feeding in the rat. *J. Lipid Res.* **18**: 314-324.
9. Wong, L., and D. Rubinstein. 1978. The levels of apolipoprotein-E in hypercholesterolemic rat serum. *Can. J. Biochem.* **56**: 161-166.
10. Schaefer, E. J., S. Eisenberg, and R. I. Levy. 1978. Lipoprotein apoprotein metabolism. *J. Lipid Res.* **19**: 667-687.
11. Innerarity, T. L., R. E. Pitas, and R. W. Mahley. 1979. Binding of arginine-rich (E) apoprotein after recombination with phospholipid vesicles to the low-density lipoprotein receptors of fibroblasts. *J. Biol. Chem.* **254**: 4186-4190.
12. Innerarity, T. L., and R. W. Mahley. 1978. Enhanced binding by cultured human fibroblasts of apo E containing lipoproteins compared with low-density lipoproteins. *Biochemistry* **17**: 1440-1447.
13. Sherrill, B. C., T. L. Innerarity, and R. W. Mahley. 1980. Rapid hepatic clearance of the canine lipoproteins containing only the E apoprotein by a high affinity receptor. *J. Biol. Chem.* **255**: 1804-1809.
14. Shelburne, F., J. Hanks, W. Meyers, and S. Quarfordt.

1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65**: 652-658.
15. Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20**: 470-475.
16. Bucolo, G., and H. David. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* **19**: 476-482.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
18. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13**: 757-768.
19. Noble, R. R. 1968. Electrophoretic separation of plasma lipoproteins in agarose gel. *J. Lipid Res.* **9**: 693-700.
20. Shapiro, A. L., E. Viñuela, and J. V. Maizel, Jr. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. *Biochem. Biophys. Res. Commun.* **28**: 815-820.
21. Bar-On, H., P. S. Roheim, and H. A. Eder. 1976. Serum lipoproteins and apolipoproteins in rats with streptozotocin-induced diabetes. *J. Clin. Invest.* **57**: 714-721.
22. 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.
23. Laurell, C. B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15**: 45-52.
24. Roheim, P. S., and G. Vega. 1979. Electroimmunoassay of apolipoprotein A-I. In Report of the High Density Lipoprotein Methodology Workshop, San Francisco, CA. K. Lippel, editor. NIH Publication No. 79-1661. 241-251.
25. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53**: 350-364.
26. Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ. 133-139, 151-154.
27. 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.
28. Redgrave, T. G., K. B. Dunne, D. C. K. Roberts, and C. B. West. 1976. Chylomicron metabolism in rabbits fed diets with or without added cholesterol. *Atherosclerosis.* **24**: 501-508.
29. Zilversmit, D. B. 1978. Assembly of chylomicrons in the intestinal cell. In Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, Jr., and J. A. Ontko, editors. Waverly Press, Inc., Baltimore, MD. 69-81.
30. Windmueller, H. G., and A. E. Spaeth. 1972. Fat transport and lymph and plasma lipoprotein biosynthesis by isolated intestine. *J. Lipid Res.* **13**: 92-105.
31. Robinson, S. F., and S. H. Quarfordt. 1978. Chylomicron apoprotein alteration after plasma exposure. *Biochim. Biophys. Acta.* **541**: 492-503.
32. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum proteins. *J. Lipid Res.* **19**: 712-722.
33. Redgrave, T. G. 1970. Formation of cholesterol ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49**: 465-471.
34. Hamilton, R. L. 1978. Hepatic secretion and metabolism of high-density lipoproteins. In Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, Jr., and J. A. Ontko, editors. Waverly Press, Inc. Baltimore, MD. 155-171.
35. Roheim, P. S., D. E. Haft, L. I. Gidez, A. White, and H. A. Eder. 1963. Plasma lipoprotein metabolism in perfused rat livers. II. Transfer of free and esterified cholesterol into the plasma. *J. Clin. Invest.* **42**: 1277-1285.
36. Marsh, J. B. 1976. Apoproteins of the lipoproteins in a nonrecirculating perfusate of rat liver. *J. Lipid Res.* **17**: 85-90.
37. Felker, T. E., M. Fainaru, R. L. Hamilton, and R. J. Havel. 1977. Secretion of the arginine-rich and A-I apolipoproteins by the isolated perfused rat liver. *J. Lipid Res.* **18**: 465-473.
38. Dory, L., and P. S. Roheim. 1981. Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism. *J. Lipid Res.* **22**: 287-296.
39. Noel, S. P., L. Wong, P. J. Dolphin, L. Dory, and D. Rubinstein. 1979. Secretion of cholesterol rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64**: 674-683.
40. Redgrave, T. G., and D. A. Snibson. 1977. Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. *Metabolism.* **26**, 493-503.
41. Chait, S., E. L. Bierman, and J. J. Albers. 1979. Regulatory role of triiodothyronine in the degradation of low density lipoprotein by cultured human skin fibroblasts. *J. Clin. Endocrinol. Metab.* **48**: 887-889.
42. Mathe, D., and F. Chevallier. 1976. Effects of the thyroid state on cholesterol metabolism in the rat. *Biochim. Biophys. Acta* **441**: 155-164.