Hepatic Golgi lipoproteins: precursors to plasma lipoproteins in hypercholesterolemic rats[1]

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Abstract Two classes of nascent lipoproteins can be isolated from Golgi apparatus-rich fractions of liver from hypercholesterolemic rats. Golgi very low density lipoproteins (VLDL, d < 1.006 g/ml) are enriched in cholesteryl esters and are similar in many respects to hypercholesterolemic plasma B-VLDL. Golgi low density lipoproteins (LDL, d 1.006–1.04 g/ml) are cholesteryl ester-rich beta-migrating lipoproteins similar to hypercholesterolemic plasma LDL. To determine if this latter lipoprotein is a precursor to plasma LDL, control and hypercholesterolemic rats were injected with Triton WR 1339 (400 mg/kg) to block intravascular lipoprotein catabolism, followed in 30 min with 100 μCi [^{3}H]leucine. At time intervals up to 3 hours after [^{3}H]leucine injection, rats were killed, and plasma lipoproteins and, in some experiments, Golgi lipoproteins were isolated. Three hours after radioisotope injection, 52% of the total lipoprotein radioactivity was found in the plasma VLDL of hypercholesterolemic rats compared to 82% in chow-fed control rats. Twenty-four percent of the total lipoprotein radioactivity appeared in the plasma IDL fraction in hypercholesterolemic rats, while only 3% was found in the same fraction in control rats. After Triton, the time course of specific activities of the Golgi and plasma lipoproteins was consistent with Golgi VLDL and LDL being precursors to plasma VLDL and IDL, respectively. The time course of specific activities of the tetramethylurea-insoluble proteins of plasma and Golgi lipoproteins provided additional evidence in support of this relationship. Furthermore the composition of plasma VLDL and IDL after Triton injection resembled their hepatic Golgi counterparts. We conclude that the liver of the hypercholesterolemic rat synthesizes, assembles, and secretes a cholesteryl ester-enriched VLDL and a cholesteryl ester-rich, beta-migrating LDL. The former is a precursor to plasma VLDL while the latter is a precursor to plasma IDL.—**Swift, L. L., P. D. Soulé, and V. S. LeQuire.** Hepatic Golgi lipoproteins: precursors to plasma lipoproteins in hypercholesterolemic rats. *J. Lipid Res.* 1982. **23:** 962–971.

Supplementary key words Golgi apparatus • Triton WR 1339 • low density lipoproteins

Recently we reported that abnormal hepatic lipoprotein synthesis contributes to diet-induced hypercholesterolemia in the rat (1). Through characterization of nascent lipoproteins retrieved from hepatic Golgi apparatus-rich fractions, we demonstrated that the livers of hypercholesterolemic (HC) rats synthesize and assemble a cholesteryl ester-enriched very low density lipoprotein (Golgi VLDL), similar in many respects to the beta-migrating VLDL (B-VLDL) found in the plasma of these animals. In addition, the HC rat liver synthesizes a cholesteryl ester-rich low density lipoprotein (Golgi LDL, d 1.006–1.04 g/ml) with morphologic, physical, and biochemical properties of cholesterol-induced plasma LDL. This latter finding suggested a de novo synthetic pathway for LDL in the HC rat, a significant finding in light of the traditional concept that LDL arise through the catabolism of VLDL (2, 3). Our studies however, did not permit conclusions regarding the relationship of the nascent Golgi lipoproteins to their plasma counterparts.

The objective of these studies was to determine if hepatic Golgi LDL are precursors to plasma LDL in the hypercholesterolemic rat. Previous studies had established a precursor-product relationship between hepatic Golgi VLDL and serum VLDL in chow-fed rats by comparing the time-dependent specific activities of the apoproteins after injection of radioactive amino acids (4). To study the relationship between hepatic Golgi LDL and plasma LDL in hypercholesterolemic rats through radioisotope incorporation studies, it was necessary to block the intravascular catabolism of VLDL to LDL since this conversion also contributes to the plasma LDL pool. To block this intravascular conversion, rats were injected with Triton WR 1339, a nonionic detergent which has found widespread use in lipid metabolic studies (5–7). Our studies in the Triton-injected HC rat demonstrate that there is secretion of a lipoprotein into the plasma compartment in the IDL fraction that is not observed in chow-fed control animals. In addition, the

Abbreviations: VLDL, very low density lipoproteins of d < 1.006 g/ml; IDL, intermediate density lipoproteins of d 1.006–1.019 g/ml; LDL, low density lipoproteins of d 1.019–1.063 g/ml; HDL, high density lipoproteins of d 1.063–1.210 g/ml; B-VLDL, beta-migrating very low density lipoproteins; HC, hypercholesterolemic; apo, apoprotein.

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time course of radioactivity of hepatic Golgi LDL and the plasma IDL fraction after [^{3}H]leucine injection is consistent with a precursor-product relationship.

MATERIALS AND METHODS

Animals and diet

Male Sprague-Dawley albino rats (Harlan Sprague-Dawley, Indianapolis, IN) were used for all experiments. The rats (80–100 g) were fed a diet of laboratory chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL), (4.1% fat) supplemented with 5% lard, 0.1% 6-N-propyl-2-thiouracil (Sigma Chemical Co., St. Louis, MO), 0.3% sodium taurocholate, and 1% cholesterol (ICN Nutritional Biochemicals, Cleveland, OH), (10.1% fat) for 21 days. At the end of this period serum cholesterol values averaged 430 ± 148 mg/dl and triglycerides 33 ± 12 mg/dl (n = 14), and the animals weighed 151.0 ± 15.9 g (n = 46). Weight-matched groups of rats (140–150 g) maintained on standard laboratory chow served as controls. All animals were housed under constant temperature and humidity conditions, and were permitted access to food and water ad libitum. Animals were fasted for 16 hr prior to the experiments which were usually begun between 8:00 and 9:00 AM.

Triton injection

Rats were anesthetized with 30 mg/kg body weight Nembutal (50 mg/ml, Abbott Laboratories, North Chicago, IL). A small incision was made in the left supraclavicular fossa, and the jugular bulb was exposed. Triton WR 1339 (Tyloxapol, Sigma Chemical Co., St. Louis, MO) dissolved in normal saline (Abbott Laboratories) to a concentration of 250–300 mg/ml was injected into the jugular bulb at a dose of 400 mg/kg body weight. Thirty minutes after Triton injection, 100 μCi DL-[4,5-^{3}H]leucine (50 Ci/mmol, New England Nuclear, Boston, MA) in 0.01 M phosphate buffered saline, pH 7.4, was injected into the jugular bulb. At selected times after the injection of leucine, rats were exsanguinated from the distal abdominal aorta, under ether as anesthesia, if necessary. Plasma was separated by centrifugation at 2000 *g* for 30 min at 4°C.

In experiments to determine time course of changes in serum triglycerides and cholesterol after Triton injection, blood samples (0.8 ml) were collected from the jugular bulb prior to injection of Triton and placed in siliconized tubes. Rats were killed by exsanguination at 0.5, 1, 2, and 3 hr after Triton injection. Serum was isolated, and triglycerides and cholesterol were analyzed as described below.

Isolation of lipoproteins from hepatic Golgi apparatus and plasma

In studies involving isolation of Golgi lipoproteins, the livers of the animals were removed immediately after exsanguination, and the Golgi apparatus-rich fractions and associated lipoproteins were isolated as has been described previously (1).

Plasma lipoproteins were isolated by the method of Havel, Eder, and Bragdon (8) using a Beckman 40.3 rotor (Beckman Instruments, Palo Alto, CA) at the following densities: <1.006 g/ml, VLDL; 1.006–1.019 g/ml, IDL; 1.019–1.063 g/ml, LDL; and 1.063–1.210, HDL.

In the studies monitoring the appearance of radioactivity in the plasma, the lipoprotein fractions were not washed. However, in the studies comparing specific activities of plasma and Golgi lipoproteins, the lipoprotein fractions were washed one time at the upper density limit of the fraction.

All lipoprotein fractions were exhaustively dialyzed at 4°C against 0.01% EDTA, pH 7.2. Aliquots of the dialyzed lipoprotein fractions were taken for scintillation counting, protein assay, and trichloracetic acid precipitation. Other aliquots were lyophilized for polyacrylamide gel electrophoresis and lipid extraction.

Tetramethylurea precipitation

Plasma and Golgi lipoproteins were dialyzed against 0.154 M NaCl, 1mM EDTA, pH 8.5. Aliquots of the fractions were precipitated with an equal volume of redistilled tetramethylurea (TMU) (Sigma Chemical Co., St. Louis, MO) for 20 hr as described by Kane (9). The insoluble protein pellicles were washed one time with 4.2 M TMU and finally with 0.154 M NaCl, 1 mM EDTA, pH 8.5. Lipids were extracted from the protein pellet with chloroform, methanol, and diethyl ether (10). The insoluble apoB precipitates were dried and solubilized in 0.1 N NaOH. Aliquots were taken for protein estimation and scintillation counting.

Scintillation counting

Aliquots of dialyzed lipoprotein fractions were counted using Aquasol (New England Nuclear) as the fluor. Radioactivity in the total lipoprotein protein was determined after precipitation of lipoprotein fractions with an equal volume of 20% trichloroacetic acid, washing, and delipidation on Whatman glass microfibre filters (Whatman GF/A). Filters were incubated at 60°C with 0.5 ml Protosol (New England Nuclear) for 30 min after which 50 μl of glacial acetic acid and 10 ml of Liquifluor (New England Nuclear) were added. To determine lipoprotein-lipid radioactivity, aliquots of lipoprotein fractions were delipidated with chloroform–

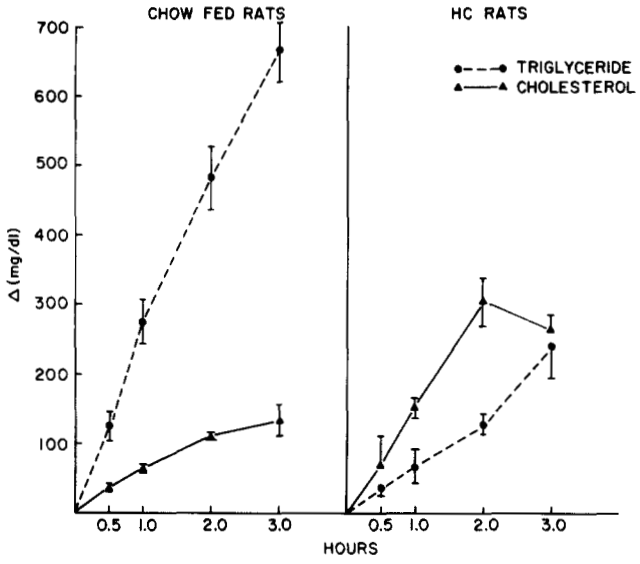

Fig. 1. Changes in serum triglyceride and cholesterol concentrations in chow-fed and hypercholesterolemic (HC) rats following Triton injection. Δ represents difference in serum triglyceride or cholesterol before and after Triton injection. For changes in triglycerides in chow-fed rats and cholesterol in HC rats, each point represents mean ± SEM from four rats. All other data points represent mean ± SEM from three rats.

methanol–ether (10). The lipid extract was dried in a scintillation vial and Liquifluor was added. All samples were counted in a Beckman LS233 scintillation counter using quench standards.

Analytical methods

Total serum cholesterol was determined on ethanol–ethyl acetate extracts as described by Babson, Shapiro, and Phillips (11). Serum triglycerides were determined by the method of Van Handel and Zilversmit (12) on isopropanol-Zeolite extracts. Lipoprotein protein was estimated by the Coomassie microprotein assay using bovine serum albumin as standard (13). Protein in TMU-insoluble proteins was estimated using the method of Lowry et al. (14).

Lyophilized lipoproteins were delipidated by the procedure of Bersot et al. (15). The delipidated apolipoproteins were separated by polyacrylamide gel electrophoresis as described below. The total lipid extract was separated into individual lipid classes on Silica Gel 60 thin-layer plates (EM Laboratories, Elmsford, NY) developed in petroleum ether–diethyl ether–acetic acid 80:20:1. The lipid classes were visualized with iodine vapors, identified by reference to standards (nonpolar lipid mix A, Supelco, Inc., Bellefonte, PA), scraped from the plates, and eluted from the gel (16). Cholesterol (free and esterified) was analyzed by the method of Babson et al. (11); triglyceride according to Van Handel and Zilversmit (12); and phospholipid according to Bartlett (17).

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis

The apoproteins were analyzed by a modification of the SDS gel electrophoretic method of Weber and Osborn (18). The apoproteins were solubilized in 0.4% SDS, 0.05 M Tris, 0.38 M glycine, pH 8.2. Approximately 45 μg protein was applied to 11% polyacrylamide gels prepared in the same buffer containing 0.1% SDS. Proteins were electrophoresed at 4 mA/gel for 10 min and 8 mA/gel until the tracking dye (Bromphenol Blue) came to the end of the gel. The gels were stained with 0.1% Coomassie Brilliant Blue in methanol–water–acetic acid 10:10:1 (v/v) and destained.

The apoproteins were identified on the basis of molecular weights, as determined by reference to purified protein standards, and by comparison to published SDS gel electrophoretograms of rat apolipoproteins.

Statistical analyses

Significance of the data was evaluated by a grouped Student's *t* test.

RESULTS

Serum lipid changes after Triton injection

The increases in serum triglycerides and cholesterol in chow-fed and hypercholesterolemic (HC) rats injected with Triton WR 1339 are shown in **Fig. 1.** Three hours after Triton injection, serum triglycerides had increased 670 mg/dl in chow-fed rats but only 245 mg/dl in HC rats. On the other hand, serum cholesterol levels increased 130 mg/dl in chow-fed rats compared to 300 mg/dl in HC rats. The ratios of triglyceride/cholesterol indicated that chow-fed rats secreted predominantly triglyceride-rich lipoproteins while HC rats secreted cholesterol-rich lipoproteins.

Composition of plasma lipoproteins and Golgi lipoproteins before and after Triton injection

The lipid and protein composition of plasma VLDL from HC rats before and after Triton injection is shown in **Table 1.** Also shown is the composition of Golgi VLDL from HC rats reported previously (1). The composition of plasma VLDL changed dramatically after Triton injection, and closely resembled the composition of the Golgi VLDL.

Table 1 also presents the lipid and protein composition of plasma IDL before and after Triton injection and the composition of Golgi LDL from HC rats. The composition of the plasma IDL after Triton injection resembled that of the Golgi LDL.

TABLE 1. Percent composition of plasma and Golgi lipoproteins from hypercholesterolemic rats[a]

	Plasma VLDL				Plasma IDL			
		Triton Injected[b]				Triton Injected[b]		
	Control[c]	1.0 hr	3.5 hr	Golgi VLDL[d]	Control[c]	1.0 hr	3.5 hr	Golgi LDL[d]
Cholesteryl ester	66.3 ± 3.3	32.2	31.1	29.9 ± 1.0	63.7 ± 2.0	52.7	56.6	52.4 ± 1.0
Triglyceride	5.7 ± 1.8	39.8	39.2	40.4 ± 0.7	0.7 ± 0.3	0.9	2.4	4.3 ± 1.0
Unesterified cholesterol	9.3 ± 1.7	7.6	7.5	3.0 ± 0.4	12.8 ± 0.6	10.7	10.4	8.6 ± 0.8
Phospholipid	7.5 ± 1.0	14.0	16.3	10.5 ±0.5	12.8 ± 1.8	16.5	17.1	15.6 ± 2.5
Protein	11.3 ± 3.1	6.4	5.9	16.2 ± 0.9	10.0 ± 1.5	19.2	13.5	19.1 ± 1.2

[a] Values represent mean ± SEM.
[b] Rats were injected with Triton WR 1339 (400 mg/kg) and killed at the indicated times after injection. Values for each time point for Triton-injected rats were obtained from pooled plasma of eight rats each.
[c] Values for control plasma lipoproteins were obtained from four different pooled plasma samples of eight rats each per pool.
[d] Golgi lipoproteins were isolated from control hypercholesterolemic rats. Values were obtained from four preparations involving at least twelve rats per preparation. These compositions are taken from reference 1.

Apoprotein compositions of plasma and Golgi lipoproteins

Fig. 2 presents SDS gel electrophoretograms of apolipoproteins from the hepatic Golgi apparatus and plasma of HC rats. As previously reported, Golgi VLDL contained apoB and apoE as major apoproteins similar to the pattern observed for plasma VLDL from the HC rats. Golgi LDL and plasma IDL from HC rats also contained apoB and apoE as major apoproteins with smaller amounts of 50–75,000 molecular weight proteins being present.

Alterations in plasma lipoprotein apoproteins after Triton injection

The apoprotein patterns of the plasma lipoproteins of chow-fed and HC rats as determined by SDS polyacrylamide gel electrophoresis were altered after Triton injection. **Fig. 3** presents gels of apoproteins of VLDL and IDL from chow-fed and HC rats before Triton (a) and 3 hr after Triton (b). The major changes in all fractions after Triton was a loss of apoE and increasing amounts of high molecular weight proteins not seen in pre-Triton samples. In addition, the HC plasma VLDL contained low molecular weight proteins that were not observed prior to Triton injection.

In the LDL fractions from chow-fed and HC rats (not shown) only apoB was observed after Triton.

Lipoprotein synthesis after Triton injection

To determine the lipoprotein fractions that were being secreted into the plasma compartment, chow-fed and HC rats were injected with Triton WR 1339 followed in 30 min with injection of [^{3}H]leucine. The incorporation of [^{3}H]leucine into the plasma lipoproteins of Triton-injected chow-fed and HC rats is shown in **Fig. 4.** Incorporation of [^{3}H]leucine into VLDL of Triton-injected chow-fed rats increased constantly over the 3-hr period (Fig. 4A). At 3 hr, 82% of the total lipoprotein-associated radioactivity was found in the VLDL fraction in chow-fed rats. In HC rats there was a delay in the appearance of activity in the VLDL fraction, after which the incorporation of [^{3}H]leucine increased steadily over the time period studied. Total activity incorporated into plasma VLDL from HC rats after 3 hr was approximately 55% of that incorporated into VLDL from chow-fed rats, and represented 52% of the total lipoprotein associated radioactivity found in HC rats.

Only small amounts of radioactivity were found in the plasma IDL fraction in chow-fed rats (Fig. 4B). The amount of activity found in this fraction did not increase from 1 to 3 hr, and at 3 hr the activity represented only

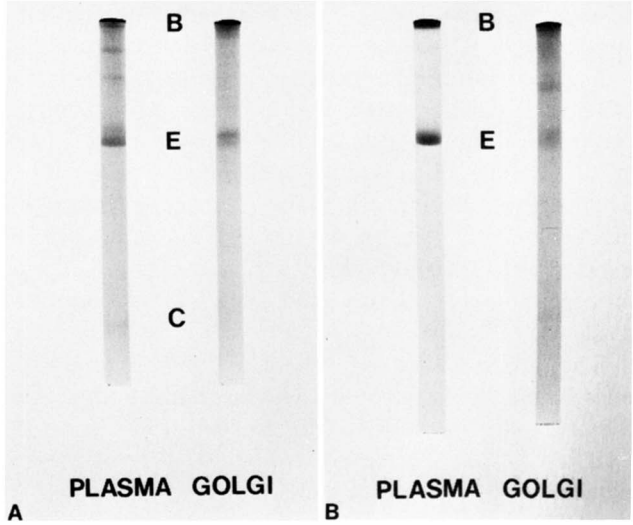

Fig. 2. SDS polyacrylamide gel electrophoretograms of (A) hepatic Golgi VLDL and plasma VLDL from HC rats; and (B) hepatic Golgi LDL and plasma IDL from HC rats. Approximately 30 μg protein was electrophoresed on each gel.

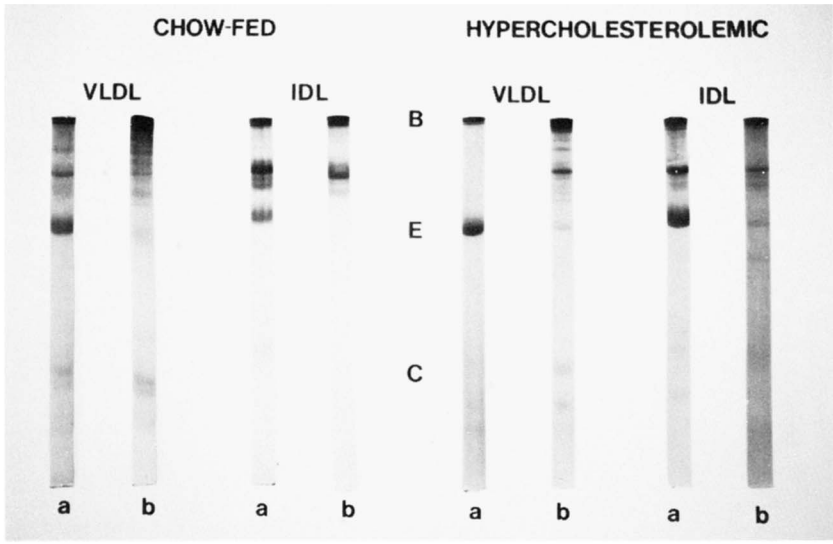

Fig. 3. SDS polyacrylamide gel electrophoresis of apoproteins of VLDL and IDL from chow-fed and HC rats before Triton (a) and 3 hr after Triton injection (b). Equal amounts of apoprotein from each lipoprotein class were applied to each gel pair.

3% of total plasma lipoprotein-associated radioactivity. In contrast, incorporation of [^{3}H]leucine into the IDL fraction in HC rats increased steadily over the 3-hr period. Three hours after leucine injection, the total activity (dpm/ml plasma) was over seven times that found in IDL fraction from chow-fed rats. Furthermore, the activity in the IDL fraction at 3 hr represented 24.1% of the total plasma lipoprotein associated radioactivity in HC rats.

Less than 5% of the total plasma lipoprotein-associated radioactivity was found in the LDL fractions of chow-fed and HC rats (Fig. 4C). Little or no increase in activity was noted in this fraction after the 1-hr sample.

The incorporation of activity into HDL fractions was quite variable (Fig. 4D). The irregular pattern of incorporation may be related to the dissociating effects of Triton on high density lipoproteins (19). Total activity incorporated into the d > 1.210 g/ml fraction 3 hr after leucine injection was nearly identical in Triton-injected HC and control rats ($4.27 \pm 0.17 \times 10^5$ dpm/ml plasma in control and $4.80 \pm 0.77 \times 10^5$ dpm/ml plasma in HC rats).

The incorporation of [^{3}H]leucine into the lipid moieties of plasma lipoproteins was negligible. Less than 2% of total lipoprotein activity could be extracted with $CHCl_3$–CH_3OH 2:1 (v/v). More than 95% of the radioactivity in VLDL from HC or chow-fed rats or in the IDL fraction from HC rats was precipitable with 20% trichloroacetic acid. It is interesting to point out that lipoproteins from Triton-injected rats could not be completely precipitated using 10% trichloroacetic acid. This is presumably due to residual Triton associated with the lipoproteins which acted as solubilizing agent for the lipoproteins.

Time course relationship of specific activity of plasma and Golgi lipoproteins after Triton injection

The metabolic relationship between the protein moieties of hepatic Golgi and plasma lipoproteins was studied in HC rats following injection of [^{3}H]leucine. In these studies rats were injected with Triton (400 mg/kg) 30 min before [^{3}H]leucine injection. At various intervals after [^{3}H]leucine injection, the rats were killed and the specific activities of lipoprotein protein isolated from Golgi apparatus and plasma were determined. Each point in **Fig. 5** represents the average of two experiments. Specific activity of Golgi VLDL decreased rapidly from the 0.5-hr sample over the time period studied. Plasma VLDL specific activity increased up to 2 hr and decreased slightly at the 3 hr time point. The specific activity curves of plasma and Golgi VLDL intersected between 0.5 and 1.0 hr after radioisotope injection.

The specific activity of Golgi LDL decreased steadily from the 0.5 hr time point, similar to Golgi VLDL. The specific activity of the plasma IDL fraction increased over the 3-hr time period after a delay in the appearance of radioactivity in this fraction. The specific activity curves of the plasma IDL fraction and the Golgi LDL intersected approximately 1.7 hr after [^{3}H]leucine injection. The specific activity of HC plasma LDL increased to 14 dpm/μg protein at the 3-hr time period,

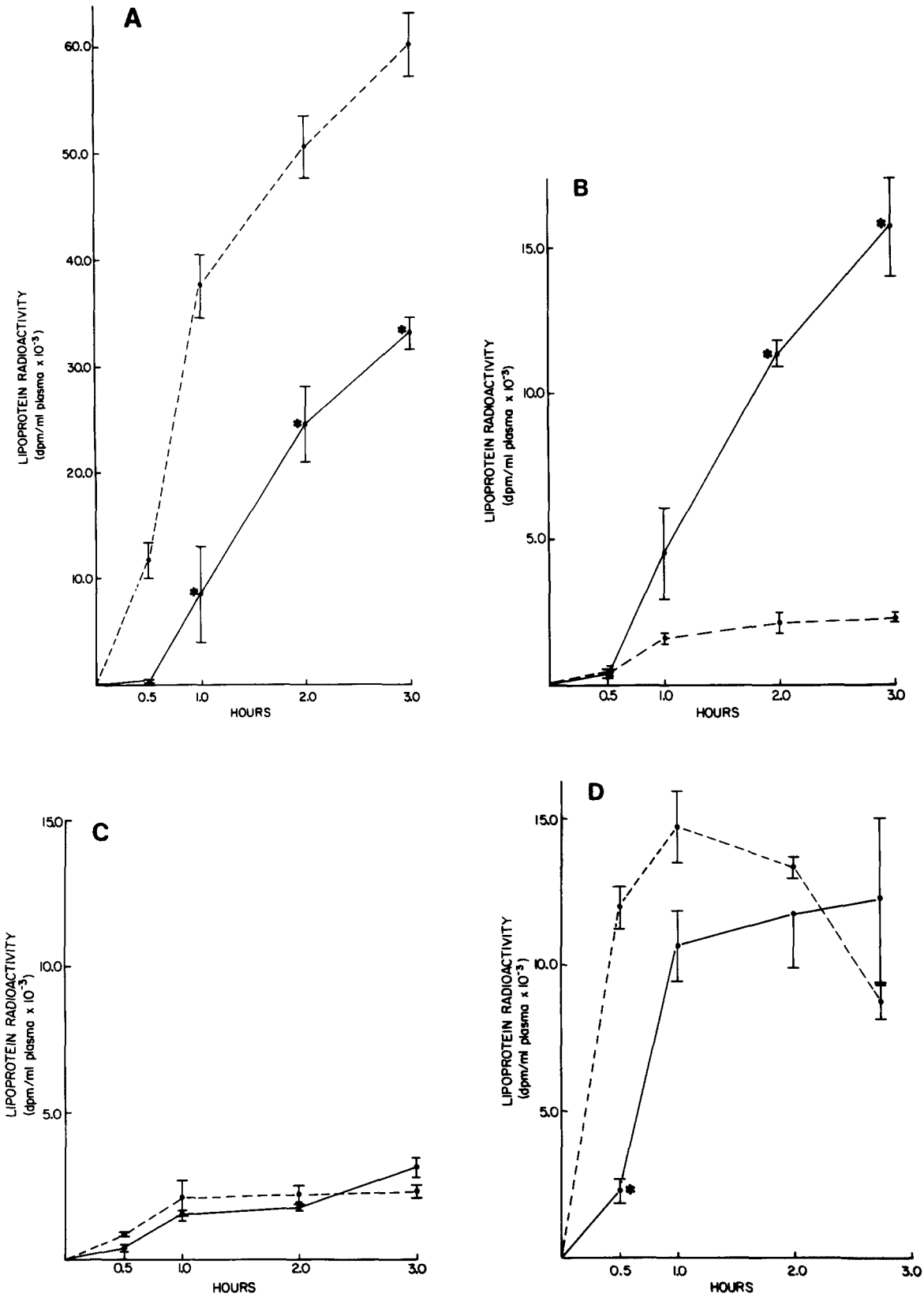

Fig. 4. Time course of incorporation of [^{3}H]leucine into plasma lipoproteins of chow-fed (– – –) and hypercholesterolemic (——) rats. Each point is the mean of three rats ± standard error of mean. Statistical significance ($P < 0.05$) between HC and chow-fed control is indicated by an asterisk. (A) VLDL, (B) IDL, (C) LDL, (D) HDL.

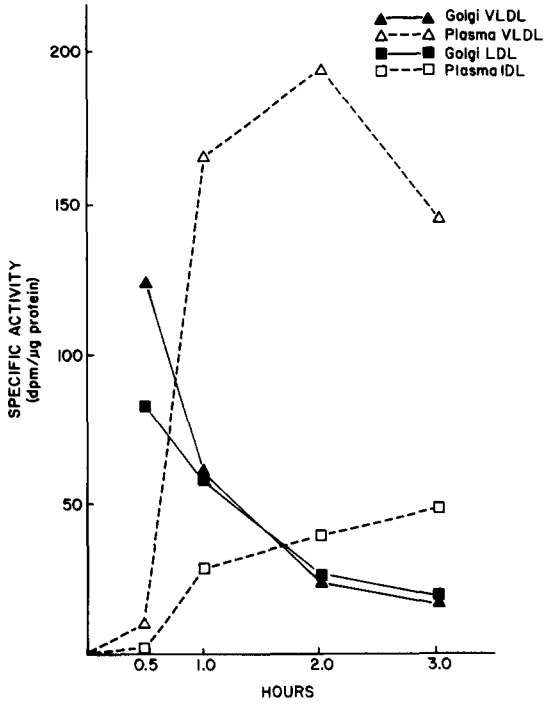

Fig. 5. Specific activity (dpm/μg protein) of lipoproteins from hepatic Golgi apparatus and plasma of HC rats. The values for each time point represent the mean of two experiments involving 9–12 animals each. Golgi VLDL ▲——▲; plasma VLDL △- - -△; Golgi LDL ■——■; plasma IDL □- - -□

but did not intersect the Golgi VLDL or LDL curve over the time period studied.

To characterize the time-dependent specific activity changes in the major apoproteins of the Golgi and plasma lipoproteins, the activity in TMU-insoluble proteins after Triton and [^{3}H]leucine injection was monitored (**Fig. 6**). The specific activity of the TMU-insoluble proteins in the Golgi VLDL decreased from the 0.5-hr time point and intersected the specific activity curve for TMU-insoluble proteins of plasma VLDL between 0.5 and 1.0 hr, as observed with the intact lipoproteins (Fig. 5).

The specific activity of the TMU-insoluble proteins of Golgi LDL was higher than that observed for Golgi VLDL at all time points. The time-dependent curves for Golgi LDL and plasma IDL intersected at approximately 1.7 hr, similar to that observed with the intact lipoproteins (Fig. 5).

DISCUSSION

Our previous studies of hepatic lipoprotein synthesis in the hypercholesterolemic (HC) rat have characterized the nascent lipoproteins isolated from the Golgi apparatus (1). It was demonstrated that the livers of rats made hypercholesterolemic by feeding diets containing cholesterol, lard, taurocholate, and propylthiouracil synthesized *a*) a cholesteryl ester-enriched VLDL and *b*) a cholesteryl ester-rich, beta-migrating lipoprotein containing apoE and apoB as the major apoproteins. Since this latter lipoprotein was similar to serum LDL from HC rats, we referred to it as Golgi LDL. Characterization of lipoproteins isolated from the hepatic Golgi apparatus affords the opportunity to assess morphologic, biochemical, and biophysical properties of newly synthesized lipoproteins prior to enzymatic alterations and apoprotein transfer which occur after secretion into the plasma compartment. However, such characterization studies are not sufficient to establish the precursor-product relationship between the Golgi and plasma lipoproteins.

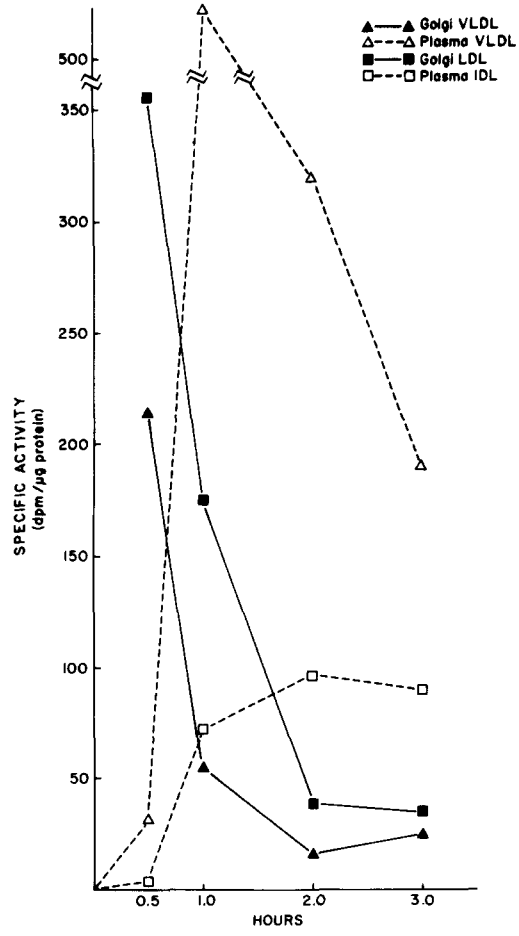

Fig. 6. Specific activity (dpm/μg) of TMU-insoluble apoproteins from hepatic Golgi apparatus and plasma of HC rats. Groups of ten rats were injected with Triton WR-1339 and 100 μC [4,5-^{3}H]leucine as described in the text. Hepatic Golgi and plasma lipoproteins were isolated, and TMU-insoluble apoproteins from these lipoprotein fractions were prepared. The values for each time point represent the mean of at least three determinations on lipoprotein fractions from one group. Golgi VLDL ▲——▲; plasma VLDL △- - -△; Golgi LDL ■——■; plasma IDL □- - -□

Previous studies by Nestruck and Rubinstein (4) have defined the relationship between VLDL isolated from the serum and hepatic Golgi apparatus of chow-fed rats. In these studies rats were injected with [^{3}H]leucine, killed at various time intervals, and the specific activities of VLDL from hepatic Golgi apparatus and serum were determined. The time-dependent specific activity curves provided kinetic evidence that the nascent VLDL retrieved from the Golgi apparatus are precursors of serum VLDL.

The application of radioisotope kinetic studies in HC rats presents a problem. To investigate if Golgi LDL were a precursor to plasma LDL through radioisotope incorporation studies, it was necessary to block the intravascular catabolism of VLDL to LDL. This was necessary because radioactivity appearing in plasma LDL fractions could arise through catabolism of previously secreted ^{3}H-labeled VLDL as well as through the direct secretion of ^{3}H-labeled LDL.

To block the intravascular catabolism of lipoproteins, the nonionic detergent Triton WR 1339 was used. Triton has had widespread use for the study of lipid metabolism (19, 20). Its capacity to block lipid clearance has been utilized to measure the rates of triglyceride and cholesterol synthesis under a variety of experimental conditions (5, 21–23). It has also been used successfully to study intestinal lipoprotein production (24, 25) and the interrelationships between the plasma lipoproteins (6, 7, 26, 27). Although it is effective in blocking intravascular lipoprotein catabolism and removal, it is not without disadvantages. Triton injection of rats has been shown to result in the dissociation of apoE and apoA-I from the serum VLDL and HDL, as well as in the appearance of proteins not normally present in these lipoprotein fractions (27). Our studies confirm that apoE is dissociated from the lipoproteins of control and HC rats after Triton injection (Fig. 3), and we also observed unidentified proteins in the SDS gels of lipoproteins from these animals. Scanu and Oriente (28) established that the detergent, in vitro, produced a dissociation of HDL lipid and protein, while Scanu et al. (29) found that HDL were markedly reduced in Triton-injected dogs.

As has been demonstrated previously (5, 24), injection of chow-fed rats with Triton WR 1339 led to a sharp rise in serum triglyceride levels and more modest increases in serum cholesterol over the 3-hr period studied (Fig. 1). These serum lipid increases after Triton injection suggested the secretion of triglyceride-rich lipoproteins. Furthermore the relative increases of triglyceride and cholesterol were similar to the concentrations of triglyceride and cholesterol within newly synthesized VLDL (1). In contrast, administration of Triton WR 1339 to HC rats led to a marked increase in serum cholesterol levels and smaller increases in serum triglyceride concentrations (Fig. 1). This suggested that one or more classes of cholesterol-rich lipoproteins were being synthesized and secreted in HC rats. Kris-Etherton and Cooper (30) reported that perfused livers of cholesterol- and fat-fed, hypothyroid rats secreted significantly less triglyceride and five times more cholesterol than livers of control rats. This increased secretion of cholesterol was associated in part with the secretion of cholesterol-rich VLDL. Noel et al. (31) have also demonstrated that the perfused livers of HC rats secrete abnormal cholesterol-carrying lipoproteins found in both the very low and low density lipoprotein ranges.

Incorporation of [^{3}H]leucine into the plasma lipoproteins of Triton-injected HC and chow-fed rats revealed differences in the relative amounts and types of lipoproteins being secreted (Fig. 4). More activity was incorporated into VLDL of chow-fed rats over the 3-hr period than in HC rats, suggesting decreased synthesis of VLDL protein in Triton-injected HC rats (Fig. 4A). Noel et al. (31) reported secretion of VLDL protein by perfused livers of HC rats was 53% of that secreted by livers of control rats (51 μg/g liver per 4 hr vs. 95 μg/g liver per 4 hr). However, Kris-Etherton and Cooper (30) reported that the rate of protein secretion into the d 1.006 g/ml fraction by the perfused livers of control and hyperlipidemic rats was comparable. Marsh reported similar findings (32), while, Frnka and Reiser (33) reported increased VLDL protein synthesis in rats fed diets containing 1% cholesterol and 10% safflower oil. Some of these differences may be explained by the different diets and varying degrees of hypercholesterolemia in the rats.

The incorporation of [^{3}H]leucine into IDL fractions of Triton-injected HC and chow-fed rats was markedly different (Fig. 4B). Little radioactivity was found in this fraction in chow-fed rats, while significant amounts of radioactivity appeared in this fraction in HC rats. This finding suggests a direct de novo synthesis of a lipoprotein into this fraction, since the intravascular catabolism of VLDL to lower density lipoproteins is blocked by Triton as evidenced by the linear appearance of activity in both the VLDL and the IDL fractions. Mahley and Holcombe (34) reported the appearance of a lipoprotein in the d 1.006–1.020 g/ml range in HC rats that was rich in cholesteryl esters and apoE. Lasser et al. (35) also reported the appearance of a lipoprotein in the d 1.006–1.030 g/ml range of rats fed diets containing 1% cholesterol and 10% olive oil. These researchers did not speculate as to the origin of this lipoprotein. However, Roheim et al. (36) demonstrated that the perfused livers of cholesterol-fed rats secreted 50% more lipoprotein cholesterol than control livers, and a fraction

of this was found in the d 1.006–1.019 g/ml range. From our radioisotope incorporation studies we concluded that the HC rat synthesizes and secretes a very low density lipoprotein and a lipoprotein found in the IDL fraction. Little activity appeared in the LDL fraction of chow-fed or HC rats (Fig. 4C).

A precursor-product relationship between the hepatic Golgi lipoproteins and plasma lipoproteins could not be established directly through radioisotope kinetic data because of the presence of Triton. Thus, to determine if hepatic Golgi VLDL were precursors to plasma VLDL and Golgi LDL precursors to plasma IDL, several pieces of evidence were required. First, the apoprotein compositions of plasma and hepatic Golgi VLDL were identical as were the apoprotein compositions of plasma IDL and Golgi LDL (Fig. 2). Second, the time-dependent specific activity curves for Golgi and plasma VLDL intersected as did the curves for Golgi LDL and plasma IDL (Fig. 5). The intersection of the curves is necessary but not sufficient evidence for a precursor-product relationship. Third, the time-dependent specific activity curves of the TMU-insoluble proteins (apoB) follow patterns similar to that observed for the holo-lipoprotein classes. Thus the time-dependent specific activity changes in Golgi and plasma lipoproteins and their major apoproteins are consistent with Golgi VLDL being precursors to plasma VLDL and Golgi LDL being precursors to plasma IDL. Finally, after Triton, the compositions of the plasma VLDL and IDL are strikingly similar to Golgi VLDL and LDL, respectively. Compositional changes in plasma VLDL of Triton-treated animals have been noted previously (7, 23, 27) with the suggestion that plasma VLDL after Triton resemble nascent VLDL (27). Our studies confirm that when intravascular lipoprotein catabolism is blocked by Triton, the compositions of the plasma VLDL and IDL became very similar to those of Golgi VLDL and LDL, respectively. This is strong evidence for precursor-product relationships between these lipoproteins.

It is interesting to note the differences in specific activities of apoB of Golgi VLDL and LDL at the 0.5 and 1-hr time periods. These differences may be related to the B-variant composition of the two Golgi lipoprotein classes and the rate of synthesis of the two variants. Krishnaiah et al. (37) have identified two B variants, B-335 and B-240, in rat serum lipoproteins. We have shown that the livers of chow-fed rats synthesize both variants (38). In addition, we have shown that the liver synthesizes B-335 more rapidly than B-240.[2] Studies are in progress to determine the variants secreted with hepatic Golgi VLDL and LDL in HC rats and the rates at which they are synthesized.

These studies coupled with our previous studies (1) indicate that the livers of HC rats synthesize and secrete a cholesteryl ester-enriched VLDL and a cholesteryl ester-rich beta-migrating low density lipoprotein. The former lipoprotein is secreted in the VLDL fraction, and the latter is secreted in the IDL fraction. Our data do not eliminate the possibility that accumulation of cholesteryl ester-rich remnants contribute to the elevated lipoprotein levels in HC rats as suggested by Ross and Zilversmit (39). However our data provide conclusive evidence that abnormal hepatic lipoprotein synthesis and secretion contribute to the development of dietary-induced hypercholesterolemia. Of major importance is the elucidation of the mechanisms by which the hepatic synthesis of low density lipoproteins is triggered. Several recent studies have suggested the presence of a de novo synthetic pathway for LDL (or IDL) in patients with various hyperlipoproteinemias (40, 41). Understanding the factors that control this pathway could be of clinical relevance in view of this evidence that increased dietary intake of cholesterol appears to be one of these factors.

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REFERENCES

1. Swift, L. L., N. R. Manowitz, G. D. Dunn, and V. S. LeQuire. 1980. Isolation and characterization of hepatic Golgi lipoproteins from hypercholesterolemic rats. *J. Clin. Invest.* **66:** 415–425.
2. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. I. Fate in circulation of the whole lipoprotein. *Biochim. Biophys. Acta.* **326:** 378–390.
3. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. II. Fate in circulation of apoprotein subunits. *Biochim. Biophys. Acta.* **326:** 391–405.
4. Nestruck, A. C., and D. Rubinstein. 1976. The synthesis of apoproteins of very low density lipoproteins isolated from the Golgi apparatus of rat liver. *Can. J. Biochem.* **54:** 617–628.
5. Otway, S., and D. S. Robinson. 1967. The use of a non-ionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J. Physiol.* **190:** 321–332.
6. Illingworth, D. R. 1975. Metabolism of lipoproteins in nonhuman primates. Studies on the origin of low density lipoprotein apoproteins in the plasma of the squirrel monkey. *Biochim. Biophys. Acta.* **388:** 38–51.
7. Portman, O. W., M. Alexander, N. Tanaka, and D. R. Illingworth. 1977. Triacylglycerol and very low density

[2] Swift, L. L., R. J. Padley, V. S. LeQuire, and G. S. Getz. Unpublished results.

lipoprotein secretion into plasma of squirrel monkeys. *Biochim. Biophys. Acta.* **486:** 470–482.
8. 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.
9. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
10. Weisgraber, K. H., R. W. Mahley, and G. Assmann. 1977. The rat arginine-rich apoprotein and its redistribution following injection of iodinated lipoproteins into normal and hypercholesterolemic rats. *Atherosclerosis.* **28:** 121–140.
11. Babson, A. L., P. O. Shapiro, and G. E. Phillips. 1962. A new assay for cholesterol and cholesterol esters in serum which is not affected by bilirubin. *Clin. Chim. Acta.* **7:** 800–804.
12. Van Handel, E., and D. B. Zilversmit. 1957. Micromethod for the direct determination of serum triglycerides. *J. Lab. Clin. Med.* **50:** 152–157.
13. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
15. Bersot, T. P., W. V. Brown, R. I. Levy, H. G. Windmueller, D. S. Fredrickson, and V. S. LeQuire. 1970. Further characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry.* **9:** 3427–3433.
16. Skipski, V. P., and M. Barclay. 1969. Thin-layer chromatography of lipids. *Methods Enzymol.* **14:** 530–598.
17. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
18. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
19. Scanu, A. M. 1965. Factors affecting lipoprotein metabolism. *Adv. Lipid Res.* **3:** 63–138.
20. Recknagel, R. O. 1967. Carbon tetrachloride hepatotoxicity. *Pharm. Rev.* **19:** 145–208.
21. Schurr, P. E., J. R. Schultz, and T. M. Parkinson. 1972. Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs. *Lipids.* **7:** 68–74.
22. Klauda, H. C., and D. B. Zilversmit. 1974. Influx of cholesterol into plasma in rabbits with fasting hyperbetalipoproteinemia. *J. Lipid Res.* **15:** 593–601.
23. Merrill, J. M. 1967. The effect of nicotinic acid on lipoprotein synthesis and triglyceride secretion by the liver. *In* Progress in Biochemical Pharmacology. Vol. 2. D. Kritchevsky, R. Paoletti, and D. Steinberg, editors. S. Karger-Basel, New York. 412–418.
24. Risser, T. R., G. M. Reaven, and E. P. Reaven. 1978. Intestinal contribution to secretion of very low density lipoproteins into plasma. *Am. J. Physiol.* **234:** E277–E281.
25. Holt, P. R., and A. A. Dominguez. 1980. Triton-induced hyperlipidemia: a model for studies of intestinal lipoprotein production. *Am. J. Physiol.* **238:** G453–G457.
26. Illingworth, D. R., L. E. Whipple, and O. W. Portman. 1975. Metabolism of lipoproteins in nonhuman primates. Reduced secretion of very low density lipoproteins in squirrel monkeys with diet-induced hypercholesterolemia. *Atherosclerosis.* **22:** 325–334.
27. Ishikawa, T., and N. Fidge. 1979. Changes in the concentration of plasma lipoproteins and apoproteins following the administration of Triton WR 1339 to rats. *J. Lipid Res.* **20:** 254–264.
28. Scanu, A., and P. Oriente. 1961. Triton hyperlipemia in dogs. I. In vitro effects of the detergent on serum lipoproteins and chylomicrons. *J. Exp. Med.* **113:** 735–757.
29. Scanu, A., P. Oriente, J. M. Szajewski, L. J. McCormack, and I. H. Page. 1961. Triton hyperlipemia in dogs. II. Atherosclerosis, diffuse lipidosis, and depletion of fat stores produced by prolonged administration of the nonionic surface-active agent. *J. Exp. Med.* **114:** 279–294.
30. Kris-Etherton, P. M., and A. D. Cooper. 1980. Studies on the etiology of the hyperlipemia in rats fed an atherogenic diet. *J. Lipid Res.* **21:** 435–442.
31. 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.
32. Marsh, J. B., and F. Sherry. 1962. Effects of a high cholesterol diet on synthesis of plasma low density lipoprotein. *Proc. Soc. Exptl. Biol. Med.* **109:** 14–15.
33. Frnka, J., and R. Reiser. 1974. The effects of diet cholesterol on the synthesis of rat serum apolipoproteins. *Biochim. Biophys. Acta.* **360:** 322–338.
34. Mahley, R. W., and K. S. Holcombe. 1977. Alterations of the plasma lipoproteins and apoproteins following cholesterol feeding in the rat. *J. Lipid Res.* **18:** 314–324.
35. 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.
36. 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.
37. Krishnaiah, K. V., L. F. Walker, J. Borensztajn, G. Schonfeld, and G. S. Getz. 1980. Apolipoprotein B variant derived from rat intestine. *Proc. Natl. Acad. Sci. USA.* **77:** 3806–3810.
38. Swift, L. L., R. J. Padley, V. S. LeQuire, and G. S. Getz. 1981. Presence of apoprotein B 240 within the Golgi apparatus of rat liver. *Circulation.* **64(4):** IV–101.
39. 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.
40. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1977. Simultaneous measurement of apolipoprotein B turnover in very-low and low-density lipoproteins in familial hypercholesterolaemia. *Atherosclerosis.* **28:** 247–256.
41. Berman, M., M. Hall III, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apoB and apoC lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **19:** 38–56.