Serum and hepatic nascent lipoproteins in normal and hypercholesterolemic rats[1]

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Abstract The lipids and apoproteins of serum, hepatic Golgi cisternae, and secretory vesicle lipoproteins from hypothyroid, hypercholesterolemic rats were analyzed and compared to homologous lipoprotein fractions from euthyroid rats fed a low fat chow diet in order to determine the nature of the nascent lipoprotein particles and indicate post-secretory modifications. Normal rat hepatic Golgi and secretory vesicles contained predominantly triglyceride-rich very low density lipoprotein (VLDL) which had little associated apoC-II or C-III and was deficient in apoE when compared to serum VLDL. Small quantities of cholesteryl ester-enriched low density lipoprotein (LDL) containing apoB and apoE were also present. Hepatic fractions from hypercholesterolemic rats contained cholesteryl ester- and apoE-rich, triglyceride-depeleted VLDL of similar size, immunodiffusion characteristics, ratio of immunoassayable apoB to apoE, and lipid composition, to hypercholesterolemic serum VLDL. Hepatic levels of LDL in hypercholesterolemic rats were markedly elevated and enriched in cholesteryl esters and apoE when compared to normal hepatic LDL. Cholesteryl ester-rich hepatic VLDL and LDL increased in size and in cholesteryl ester and apoE content during transit from the Golgi cisternae into the secretory vesicles. Triglyceride-rich VLDL only acquired apoE which was further supplemented upon secretion. Nascent VLDL and LDL represented LpB-LpE association complexes and no deficiency in any apoE isoprotein within the cholesteryl ester-rich serum lipoproteins was observed. Thus, dietary-induced hypercholesterolemia in hypothyroid rats results in a fatty liver whose lipoprotein secretory products contribute to the plasma pool of abnormal cholesteryl ester- and apoE-enriched lipoproteins.—**Dolphin, P. J.** Serum and hepatic nascent lipoproteins in normal and hypercholesterolemic rats. *J. Lipid Res.* 1981. **22:** 971–989.

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The feeding of high fat cholesterol-containing diets or saturated fats, with and without added cholesterol, to a variety of primate and non-primate species including monkeys (1–3), swine (4–5), dogs (6), rabbits (7–9), rats (10–13), and guinea pigs (14, 15) results in a marked hypercholesterolemia associated with elevated levels of serum apoE and the presence of two abnormal lipoproteins, β-VLDL and HDL_c. The former, which is rich in cholesteryl ester, contains apoB and apoE with little or no apoC. HDL_c, which migrates as α-2 on electrophoresis, is enriched in cholesteryl ester and contains apoE and apoA-I with variable amounts of apoC but, in most species, no apoB. Both of these cholesteryl ester-rich lipoproteins are believed to be associated with the genesis of premature atherosclerosis (16). HDL_c has been shown to bind to the LDL receptor of human fibroblasts (17) and smooth muscle cells (18, 19). The specificity for binding resides with the apoE component of the HDL_c particle (20, 21). The serum lipoprotein profile seen in these hypercholesterolemic animal model systems shows a marked similarity to that of human familial dysbetalipoproteinemia (type III hyperlipoproteinemia, broad-β disease) which is characterized by an elevation of serum apoE (22) and the presence of β-VLDL (23). Feeding of cholesterol to humans also results in the appearance of β-VLDL (24) and possibly an HDL_c-type particle (25, 26) in the serum. Hypothyroidism will, however, also produce a transient type III pattern in otherwise normal humans (27) and exacerbate the type III lipoprotein profile in familial dysbetalipoproteinemics (28). Care must therefore be taken in correlating the data obtained in some animal species, particularly the dog and rat which generally require reduction in thyroid function by use of propyl-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; β-VLDL, beta-migrating very low density lipoproteins; HDL_c, cholesterol-induced lipoprotein particles that are rich in cholesterol and contain apoE with variable amounts of apoA-I; apo- (as a prefix), defines a lipid-free protein (apolipoprotein) that is normally associated with a lipoprotein; Lp- (as a prefix), defines a particle that contains one or more apoproteins in association with lipids; u.t.s., defines centrifugation time after the rotor has come up to speed; LCAT, lecithin:cholesterol acyltransferase.

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thiouracil to fully express the hypercholesterolemia, with human disorders.

Dory, Delamatre, and Roheim (29) have recently shown that radiothyroidectomized or propylthiouracil-treated (0.3% of diet) rats fed Purina chow without added cholesterol developed a hypercholesterolemia associated with increased plasma apoB, apoE, and apoA–I and decreased plasma triglycerides. Mahley and Holcombe (10), in contrast, showed no increase in serum apoE levels in rats fed a diet containing 0.1% propylthiouracil. It is presently unknown if these observations result from a primary effect of the lack of circulating thyroid hormone upon the liver (30, 31), the production of remnants from chylomicrons and VLDL, or are secondary to the increased intestinal absorption of cholesterol observed in hypothyroid rats (32). Recently Kris-Etherton and Cooper (33) showed that feeding euthyroid rats a high fat, cholesterol-containing diet resulted in hypercholesterolemia and hepatic secretion of a cholesteryl ester-rich VLDL. Livers from propylthiouracil-induced hypothyroid rats fed a normal diet, in contrast, continued to secrete a triglyceride-rich VLDL. Similarly, Swift et al. (13) demonstrated that the hepatic Golgi VLDL of rats fed a diet supplemented with lard, 0.1% propylthiouracil, and taurocholate (LPT-diet) was slightly enriched in total cholesterol, but still contained 66.8% of its mass as triglyceride. A plausible explanation of the effects of hypothyroidism upon serum cholesterol and apoE would therefore be to envisage an increase in the intestinal contribution to the plasma lipoproteins resulting in a cholesteryl ester-enriched fatty liver via increased cholesterol absorption or, alternatively, a decrease in the chylomicron and VLDL remnant removal process by the liver. Previous reports, however, have shown that livers from hypothyroid cholesterol-fed (33) and euthyroid cholesterol-fed rats (34) show no decrease in their ability to rapidly clear remnant-type particles from the circulation despite the fact that the content of hepatic triglyceride lipase is significantly depressed in hypothyroidism (31) and the livers from both groups of rats are considerably enriched in their cholesterol content (12, 35).

Despite the current uncertainties concerning the contribution of hypothyroidism to the observed accumulation of cholesteryl ester-rich particles in the serum of propylthiouracil-treated cholesterol-fed rats, animal model systems are of considerable use in elucidating the fundamental mechanisms that result in hypercholesterolemia and the appearance of abnormal lipoproteins in human serum. Of particular interest to this laboratory is the origin of the β-migrating cholesteryl ester-rich VLDL seen in both human type III hyperlipoproteinemia and dietary-induced hypercholesterolemic rats (12, 23). Earlier studies in humans led to the hypothesis that β-VLDL represented a catabolite or remnant of normal VLDL (23, 36–39) which, due to a metabolic defect in the removal process, accumulates in the serum compartment. As a result of these studies, type III hyperlipoproteinemia has been defined as remnant removal disease (40). There is now an increasing body of evidence from our own (12) and other laboratories (3, 13, 14, 33, 41–43) to suggest that in animal species that develop hypercholesterolemia as a result of feeding a variety of high fat diets, the expansion of the VLDL and LDL plasma cholesteryl ester pools is due, at least in part, to the direct secretion by the liver of cholesteryl ester-rich, triglyceride-depleted lipoproteins. The purpose of this study was to determine if the observed resemblance between cholesteryl ester-rich lipoproteins secreted by the perfused livers from hypothyroid hypercholesterolemic rats and the corresponding serum lipoproteins was a result of postsecretory modification of otherwise normal lipoproteins. To this end, lipoproteins isolated from the hepatic Golgi cisternae and secretory vesicles of normal and hypercholesterolemic rats were compared with those isolated from the serum to determine if postsecretory modification occurred and, if so, the nature and extent of that modification of nascent lipoproteins.

MATERIALS AND METHODS

Experimental animals

Male Long-Evans rats weighing 250–300 g were obtained from Canadian Breeding Farms, St. Constant, Quebec, and were maintained on Purina chow (Ralston Purina Co., St. Louis, MO) or for 42 days on a high fat diet that included 40% butter fat, 5% cholesterol, 0.2% choline chloride, 0.1% sodium cholate, and 0.3% propylthiouracil (thrombogenic diet; ICN Nutritional Biochemicals, Cleveland, OH). Animals fed the thrombogenic diet developed an enlarged (mean weight 17.0 ± 0.5 g, n = 24) and grossly fatty (85 ± 7 mg total lipid/g liver) liver compared to normal animals whose mean liver weight was 10.1 ± 0.2 g (n = 18) which contained 8.5 ± 0.5 mg total lipid/g liver. The serum cholesteryl esters (750 ± 60 mg/dl) and apoE levels (90 ± 15 mg/dl) of rats fed the high fat diet were considerably elevated when compared to those of control animals (serum cholesteryl ester = 45 ± 5 mg/dl and apoE = 24 ± 2 mg/dl). The rats were anesthetized with Nembutal (Abbott Laboratories, North Chicago, IL)

prior to obtaining blood by cardiac puncture and in situ perfusion of the liver.

Preparation of hepatic subcellular fractions

The livers of exsanguinated rats were perfused with normal saline by the portal vein for at least 10 min to ensure complete removal of serum (12) prior to excision. The livers from nine normal or nine hypercholesterolemic animals were pooled and homogenized in 0.25 M sucrose at 4°C and the Golgi cisternae and secretory vesicles were prepared by a slight modification of the techniques of Redman et al. (44) and Ehrenreich et al. (45) as follows. Ten to twelve ml of the 105,000 *g* microsomal pellet, resuspended and adjusted to 1.17 M sucrose, was placed in the bottom of a 35-ml Beckman SW-27 centrifuge tube and overlayed sequentially with 5 ml of 1.15 M and 13 ml of 0.86 M sucrose. The remaining volume in the tube (approx. 7 ml) was filled with 0.25 M sucrose to form the discontinuous gradient. Following centrifugation at 27,000 rpm in an SW-27 Ti rotor for 3.5 hr (u.t.s.), the *cis* or forming face of the Golgi cisternae (GF_3) banded at the interface between the 1.15 M and 0.86 M sucrose. The secretory vesicles (GF_2 + GF_1) banded between the 0.86 M and 0.25 M sucrose layers. The purity of the Golgi cisternae and secretory vesicle fractions obtained from livers of normal and sucrose-fed rats using the technique of Ehrenreich et al. (45) has previously been demonstrated by our laboratory (46). The modification of Redman et al. (44) facilitates a clearer separation of the Golgi cisternae elements from the more dense endoplasmic reticulum components by interposing a 1.15 M sucrose layer. The modification detailed in this communication ensures maximal recovery of the secretory vesicle fraction by inclusion of the GF_2 fraction of Ehrenreich (45) which contains predominantly small secretory vesicles, with the large secretory vesicles associated with the less dense GF_1 fraction. The technique thus optimizes the recovery of Golgi cisternae and secretory vesicles and results in minimal cross contamination. Small but significant amounts of particulate fat (Figs. 2A and 2C) co-isolate in the sucrose gradient with both the Golgi cisternae and secretory vesicles from the fatty livers of hypercholesterolemic rats. The pooled Golgi cisternae and secretory fractions were diluted 50% by addition of equal volumes of normal saline and each fraction was pelleted by ultracentrifugation at 105,000 *g* for 45 min. The resulting Golgi and vesicle pellets still contained small amounts of particulate fat. Attempts to remove this contaminating lipid by flotation, following addition of higher proportions of saline to the sucrose gradient fractions, were largely unsuccessful and led to substantial lysis of the secretory vesicles with concomitant loss of lipoproteins. The Golgi cisternae and secretory vesicle pellets were lysed by osmotic shock and French pressure cell treatment (47). Prior to isolation of the lipoproteins, the particulate fat and large membrane components were removed by a 60-min centrifugation at 105,000 *g* at d 1.006 g/ml as described by Chapman, Mills, and Taylaur (14). Small secretory vesicle and Golgi membrane fragments pelleted during the subsequent isolation of nascent VLDL and were discarded prior to isolation of the LDL. Due to the high amount of lipid associated with the fatty liver from hypercholesterolemic rats, it was possible that the hepatic Golgi cisternae and secretory vesicles from these animals would not isolate at the same densities in the sucrose gradient as those from normal livers. Accordingly, the Golgi cisternae and secretory vesicles fractions from fatty livers were examined by electron microscopy following negative staining with phosphotungstic acid (13). The results are shown in **Figs. 1** and **2.** The Golgi cisternae fraction (GF_3) was composed almost entirely of Golgi tubules containing encapsulated lipoprotein particles. Secretory vesicles, of any size, were rarely seen in this fraction. However, Fig. 1A shows the presence of one such vesicle that contains small lipoprotein particles. The mean diameter of the entubulated lipoproteins present within this Golgi cisternae fraction was 315 ± 6.8 Å. Figs. 1C and 1D show electronmicrographs of the Golgi cisternae VLDL and LDL lipoprotein fractions after isolation, as described below, and negative staining. The secretory vesicle fraction (GF_1 and GF_2) from the fatty livers contained almost exclusively secretory vesicles of varying sizes when visualized in the electron microscope (Fig. 2). Small Golgi components containing occasional lipoprotein particles were present in low amounts in the secretory vesicle fraction; however, the paucity of their associated lipoproteins would render their presence insignificant with respect to any subsequent characterization of the lipoproteins isolated from this fraction. Figs. 2A–D show electronmicrographs of individual secretory vesicles containing lipoprotein particles ranging in size from 256 Å to 558 Å. The mean particle diameter was 388 ± 9.5 Å. Based on these morphological criteria, the hepatic Golgi cisternae and secretory vesicles, isolated by the above technique from the fatty liver, contain very similar subcellular components to the hepatic Golgi cisternae and secretory vesicle fractions from normal rat livers (44–46). Our Golgi cisternae fraction differs from the Golgi-rich fraction of Swift et al. (13) in that the vast majority of the secretory vesicles have been removed by the sucrose gradient system employed and isolated as a separate fraction (GF_1 and GF_2). Occasionally

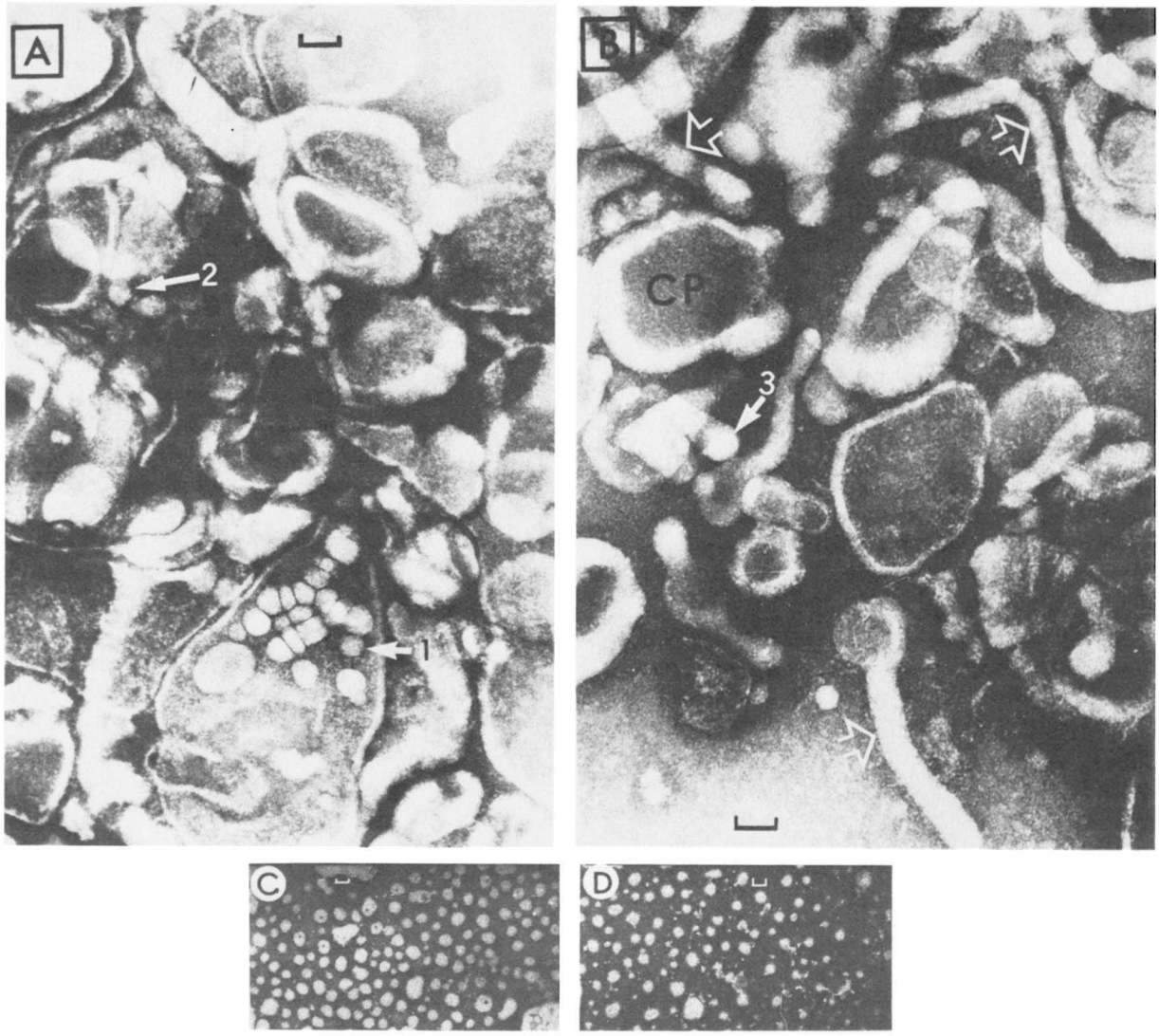

Fig. 1. Electronmicrographs of hepatic Golgi cisternal fractions (GF_3) from hypercholesterolemic rats. Aliquots of the Golgi cisternal fractions isolated at the 1.15 M–0.85 M sucrose interface of the discontinuous sucrose gradient were negatively stained with 2% phosphotungstic acid. A and B: broad arrows indicate Golgi tubules; CP = portion of a cisternal plate; numbered arrows show intubulated or vesiculated lipoproteins of the following diameters: 1, 2 = 300 Å; 3 = 390 Å. C; VLDL (d 1.006 g/ml) and D; LDL (d 1.006–1.063 g/ml) isolated from the lysed Golgi cisternae by ultracentrifugation and visualized after negative staining. Magnification: A = 118,300; B = 120,700; C, D = 45,150. Calibration bars, 500 Å.

large stacked discoidal forms were seen within the isolated hepatic secretory vesicles from the hypercholesterolemic rats. These particles morphologically resemble the discoidal HDL particles seen in the plasma of humans with LCAT deficiency (48) and rat hepatic perfusates in experiments performed with an LCAT inhibitor (49). These particles, however, are considerably larger (60 × 465 Å) than the discoidal HDL particles (45 × 191 Å (49)) and may be formed as a result of particle compaction during isolation by ultracentrifugation or negative staining (Fig. 2D, enclosed area). In this respect, it is noteworthy that

Fig. 2. Electronmicrographs of hepatic secretory vesicles (GF_1 plus GF_2) isolated at the 0.86 M–0.25 M sucrose interface of the discontinuous gradient. Negative staining was as described in Fig. 1. A–D; secretory vesicles containing lipoproteins of the following diameters: 1 = 560 Å; 2, 7 = 260 Å; 3, 6 = 400 Å; 4 = 250 Å; 5 = 380 Å. Note the apparent formation of large discoidal particles in D (enclosed area) which may result from particle packing during isolation. PF, particulate fat. E: Secretory vesicle VLDL (d 1.006 g/ml); G:LDL (d 1.006–1.063 g/ml) isolated by ultracentrifugation from the lysed secretory vesicles. F: cholesteryl ester-rich serum VLDL; H: serum LDL from hypercholesterolemic rats. Particle deformation and rouleaux are seen (F and H) when lipoproteins are present in high concentrations. Magnification: A = 125,550; B = 254,000; C = 175,800; D = 256,000; E–H = 45,150. Calibration bars, 500 Å.

Chapman (50) has recently reported the presence of discoidal particles in salamander serum HDL preparations and speculated that this phenomenon may be due to a concentration effect. Significantly, deformed or discoidal particles were only observed in this study when the lipoprotein particles were prepared for electron microscopy from solutions of high concentration (Figs. 2F and 2H) or when the lipoproteins were tightly packaged within the secretory vesicles.

Lipoprotein isolation and characterization

Following removal of particulate fat (Golgi and secretory vesicles) or chylomicrons (serum), nascent and serum lipoproteins were isolated by sequential ultracentrifugation at the following densities: VLDL, 1.006 g/ml; LDL, 1.063 g/ml; HDL_1, 1.085 g/ml (51), and HDL_2 + HDL_3 at 1.21 g/ml in a Beckman L5-50B preparative ultracentrifuge (Beckman Industries Inc. Spinco Division, Palo Alto, CA), using an SW-41 rotor by a modification of the method of Havel, Eder, and Bragdon (52) as previously described (12). All serum lipoprotein fractions were washed once by recentrifugation at the appropriate densities prior to analysis. Lipoprotein fractions were delipidated with ethanol–ether 3:1 and ether at −10°C, prior to gel electrophoretic analysis of the soluble apoproteins (12) on 10% polyacrylamide, 7 M urea. Lipoproteins whose apoprotein constituents were analyzed by polyacrylamide gel isoelectric focusing according to the method of Gidez, Swaney, and Murnane (53) were delipidated by sequential extraction with ethanol–acetone 1:1 as described by Utermann et al. (54). The ethanol–ether/ether lipid extracts of lipoproteins were separated by thin-layer chromatography and the various lipid classes were individually quantitated as previously described (12). The recovery of all lipids was >95%. Total hepatic and serum lipids were extracted by the method of Folch, Lees, and Sloane Stanley (55). Total lipoprotein protein was measured by the method of Lowry et al. (56) using bovine serum albumin as standard.

ApoE was determined by the electroimmunoassay procedure of Laurell (57) as described by Wong and Rubinstein (11) with the following modifications. Agarose (1% final concentration) (Seakem LE, Marine Colloids, Rockland, ME) and 5% dextran T-10 (Pharmacia, Upsala, Sweden) were dissolved in 0.06 M sodium veronal buffer, pH 8.5, containing 0.025% Triton X-100 (Sigma Chem. Co., St. Louis, MO) for the assay. The veronal buffer used to dissolve the agarose also served as the electrode buffer and as diluent for the lipoprotein fractions. Plates were run at 2.5 V/cm for 18 hr at 15°C. All other conditions and validation of the assay were as previously described (11). Antibody to rat apoE was raised against apoE purified from rat VLDL by preparative isoelectric focusing (58). ApoB was also determined by electroimmunoassay using antibodies raised in rabbits against native VLDL. All titre against apoE and apoC was removed from this antibody preparation by carefully controlled absorption with limiting quantities of HDL. Antibody specificity was checked by Ouchterlony double diffusion analysis and crossed immunoelectrophoresis as previously described (59). The LpB assay employed 0.7% Seakem LE agarose in 0.06 M sodium veronal buffer, pH 8.4, containing 1% polyethylene glycol (Sigma Chem. Co.), 0.025 M calcium lactate, and 0.016% Triton X-100. Plates were run at 2.2 V/cm for 18 hr at 15°C. The veronal buffer in the agarose was also employed as the electrode buffer and as diluent for the lipoprotein fractions. The assay was validated using rat serum VLDL, whose apoB content was determined after extraction with organic solvents (60) and tetramethylurea (61). The VLDL of known apoB content was then used to calibrate lyophilized rat serum prepared as described by Curry et al. (22), which was later used as the standard in all subsequent assays. The electroimmunoassay was linear for apoB concentrations of 0.08–0.8 μg/10 μl with r^2 values of 0.97 or better. Inter- and intra-assay variations were less than 3%. Lipoprotein particle diameters were determined after negative staining with 2% phosphotungstic acid at pH 7.2, visualization in the electron microscope, and photography (62). Ouchterlony double diffusion analysis was performed as previously described (59).

RESULTS

Analysis and fractional distribution of lipoprotein lipid and protein in hepatic subcellular components and serum

A previous report from our laboratory (12) demonstrated that the perfused fatty liver from hypercholesterolemic rats has the ability to secrete apoE and total lipid at a higher rate than livers from normal animals. There was, however, little difference between the fatty and normal liver secretion rate of total lipoprotein protein when expressed on μg/g liver basis over a 4-hr period. The data in **Table 1** are consistent with this finding and show that there is no significant difference in the total lipoprotein content of the secretory vesicles between normal and hypercholesterolemic rats when the data are expressed in a similar fashion. The Golgi cisternae of hypercholesterolemic rats, however, appeared to contain less lipoprotein protein than normal controls. The secretory

TABLE 1. Lipoprotein lipid and protein content of the secretory vesicles and Golgi cisternae from normal and hypercholesterolemic rat livers

Animals	Lipoprotein			
	Protein		Lipid	
	Secretory Vesicles	Golgi Cisternae	Secretory Vesicles	Golgi Cisternae
	μg/g liver			
Normal	3.5 ± 0.5	1.6 ± 0.4	20.4 ± 1.6	10.1 ± 1.6
Hypercholesterolemic	3.6 ± 0.4	0.8 ± 0.2	29.8 ± 4.2	13.0 ± 2.6

Each value represents the mean ± S.E.M. from five experiments.

vesicles from hypercholesterolemic livers contain significantly higher amounts of total lipoprotein lipid than the control hepatic fraction. **Table 2** shows the data obtained upon chemical analysis of the lipid moieties of the total lipoprotein present in the secretory vesicles and Golgi cisternae of normal controls and hypercholesterolemic rats. In addition to an increment in the total lipoprotein lipid mass in the hypercholesterolemic hepatic fractions (Table 1), the composition of the lipoprotein lipid is markedly different in hypercholesterolemic and control animals. As shown in Table 2 there is a significant decrease in the total triglyceride content of the secretory vesicles and Golgi cisternae of hypercholesterolemic rats and a very marked increment in the lipoprotein cholesteryl ester and cholesterol content of these hepatic fractions when compared to normal controls. **Table 3** shows the percentage distribution of total lipoprotein protein and lipid in the ultracentrifugally isolated lipoprotein fractions of hepatic Golgic cisternae, secretory vesicles, and serum for normal and hypercholesterolemic animals. In normal rat Golgi and secretory vesicles, the majority of the lipoprotein lipid and protein resides within the VLDL density range. A relatively small but significant proportion of the total Golgi and secretory vesicle lipoprotein mass in normal hepatocytes is present within the LDL density class. In the hypercholesterolemic rats, however, there is a very pronounced shift of lipoprotein protein and lipid into the LDL density fraction of the Golgi cisternae and secretory vesicles when compared to normal controls. The data in Table 3 for hypercholesterolemic serum confirm previous reports (10, 11) and show the dramatic elevation in the lipoprotein content of the LDL serum fraction upon induction of hypercholesterolemia.

Lipid analysis of VLDL and LDL from the hepatic fractions compared to serum

The VLDL from hepatic Golgi cisternae, secretory vesicles, and serum was isolated ultracentrifugally and its lipid moiety analyzed. **Table 4** shows the data obtained for nascent VLDL from normal and hypercholesterolemic rats compared to their respective serum counterparts. Nascent VLDL from Golgi and secretory vesicles from normal rats is triglyceride-rich and relatively poor in its cholesterol content. However, it is of interest to note that the normal nascent VLDL is significantly richer in both phospholipids and cholesteryl ester than its serum counterpart. Nascent VLDL from hypercholesterolemic animals, in contrast, is triglyceride-poor and cholesteryl ester-rich. The lipid composition of the nascent vesicle VLDL from the fatty livers has a very similar lipid composition to that of hypercholesterolemic serum VLDL although the former contains somewhat more triglyceride. The lipid moiety of the nascent VLDL from normal livers appears to undergo little or no modification as the particles pass from the Golgi cisternae into the secretory vesicles, there being no significant difference between the relative proportions of the lipid classes analyzed in the two particles. Cisternae VLDL from hypercholesterolemic hepatocytes, however, is richer in triglycerides and phospholipids than the secretory vesicle VLDL from fatty livers; the particles apparently acquire cholesteryl ester relative to triglyceride and phospholipids as they pass from the Golgi into the secretory vesicles. **Table 5**

TABLE 2. Analysis of the total lipoprotein lipid present in the secretory vesicles and Golgi cisternae of normal and hypercholesterolemic rat livers

Animals	Lipoprotein Lipid							
	Triglycerides		Phospholipids		Cholesteryl Esters		Cholesterol	
	Secretory Vesicles	Golgi Cisternae	Secretory Vesicles	Golgi Cisternae	Secretory Vesicles	Golgi Cisternae	Secretory Vesicles	Golgi Cisternae
	μg/g liver							
Normal	8.3 ± 0.4	3.8 ± 0.8	4.5 ± 0.4	2.0 ± 0.1	7.4 ± 2.1	3.6 ± 0.9	2.0 ± 0.2	1.0 ± 0.1
Hypercholesterolemic	3.6 ± 0.6	2.4 ± 0.4	3.5 ± 0.5	1.6 ± 0.5	19.7 ± 3.2	7.2 ± 1.7	3.0 ± 0.4	1.0 ± 0.1

Each value represents the mean ± S.E.M. from five experiments.

TABLE 3. Fractional distribution of lipoprotein lipids and proteins in the Golgi cisternae, secretory vesicles, and serum from normal and hypercholesterolemic rats

Fraction	Normal Rats			Hypercholesterolemic Rats		
	Cisternae	Vesicle	Serum	Cisternae	Vesicle	Serum
Lipoprotein Protein						
	% distribution					
VLDL	77.0 ± 9.0	69.0 ± 14.0	6.0 ± 0.6	21.0 ± 6.0	39.0 ± 7.0	6.0 ± 1.0
LDL	22.0 ± 9.0	18.0 ± 3.0	2.0 ± 0.7	55.0 ± 12.0	54.0 ± 7.0	59.0 ± 1.0
HDL_1	1.0 ± 0.1	2.0 ± 0.7	0.5 ± 0.2	15.0 ± 3.0	6.0 ± 4.0	2.0 ± 0.4
HDL_{2+3}	1.0 ± 0.1	13.0 ± 12.0	92.0 ± 0.3	9.0 ± 5.0	3.0 ± 2.0	35.0 ± 2.0
Total protein[a] (mg)	0.24 ± 0.08	0.50 ± 0.14	48.8 ± 3.7[b]	0.24 ± 0.1	0.43 ± 0.1	351.5 ± 29.7[b]
Lipoprotein Lipids						
	% distribution					
VLDL	50.0 ± 7.0	61.4 ± 4.0	28.0 ± 3.0	40.0 ± 1.0	44.0 ± 7.0	18.0 ± 3.0
LDL	25.0 ± 6.0	24.0 ± 5.0	5.0 ± 1.0	34.0 ± 6.0	46.0 ± 8.0	70.0 ± 4.0
HDL_1	14.0 ± 6.0	9.0 ± 2.0	3.0 ± 1.0	13.0 ± 5.0	5.0 ± 1.0	1.0 ± 0.5
HDL_{2+3}	11.0 ± 3.0	7.0 ± 1.0	64.0 ± 4.0	12.0 ± 2.0	4.0 ± 0.6	10.0 ± 1.0
Total lipid[a] (mg)	1.59 ± 0.35	2.67 ± 0.38	75.4 ± 10.8[b]	1.68 ± 0.27	3.94 ± 0.4	1,821 ± 42[b]

[a] These figures represent the quantity of lipoprotein protein or lipid recovered from lysed cisternae or vesicle fractions prepared from a pool of nine normal or hypercholesterolemic livers.

[b] The data for serum are the total lipoprotein protein or lipid expressed in mg/dl. Lipoproteins were isolated sequentially at the following densities: VLDL, 1.006 g/ml; LDL, 1.063 g/ml; HDL_1, 1.085 g/ml; HDL_{2+3}, 1.21 g/ml.

Each value represents the mean % ± S.E.M. from five experiments.

shows a similar lipid analysis for the nascent LDL fraction from normal and hypercholesterolemic rat livers compared to their equivalent serum fractions. It should be noted (Table 3) that the absolute amount of LDL present in normal hepatic fractions and normal rat serum is low in comparison to that of the hypercholesterolemic rat. The LDL from the secretory vesicles of normal rat livers is relatively rich in cholesteryl ester, containing only 26% of its total lipid mass as triglyceride, and has a lipid composition similar to that of the serum LDL. The normal cisternae LDL is poorer in cholesteryl ester and richer in phospholipid and triglycerides than either the vesicle LDL fraction or serum LDL. Thus the normal rat liver would appear to have the capability for synthesis of small quantities of cholesteryl ester-enriched, higher density particles which isolate in the LDL fraction. However, as normal rat serum contains very little true LDL, it is possible that the nascent LDL fraction from normal rat livers contains small VLDL particles which may be modified during the assembly and final packaging process to form the lower density and larger VLDL particles. Similarly, the production of smaller and denser particles from nascent VLDL by the action of triglyceride lipases during isolation cannot at present be ruled out. The LDL isolated from the cisternae and secretory vesicles of hypercholesterolemic rat livers, in addition to representing a much larger proportion of the total lipoprotein present in these fractions, has a strikingly different lipid composition. Table 5 shows that the hypercholesterolemic cisternae and vesicle LDL is extremely rich in total cholesterol, the ester

TABLE 4. Composition of the VLDL lipid from normal and hypercholesterolemic rat liver and serum

	Normal Rats VLDL			Hypercholesterolemic Rats VLDL		
	Cisternae	Vesicle	Serum	Cisternae	Vesicle	Serum
	%			%		
Triglycerides	53.0 ± 1.0	51.1 ± 1.0	69.0 ± 4.0	24.0 ± 2.0	14.0 ± 2.0	9.0 ± 0.2
Phospholipids	18.0 ± 3.0	21.0 ± 2.0	15.0 ± 2.0	15.1 ± 1.0	9.0 ± 1.0	9.0 ± 0.5
Cholesteryl esters	23.0 ± 2.0	21.3 ± 3.0	11.0 ± 1.0	56.0 ± 3.0	71.0 ± 5.0	74.4 ± 0.4
Cholesterol	6.0 ± 1.0	6.0 ± 0.5	2.0 ± 1.0	5.0 ± 0.1	6.0 ± 1.0	8.0 ± 0.4
Total VLDL lipid	0.62 ± 0.10[a]	1.52 ± 0.26[a]	15.1 ± 2.0[b]	0.67 ± 0.10[a]	1.75 ± 0.36[a]	318.8 ± 52.3[b]

[a] These data are the total VLDL lipid (mg) isolated from the lysed Golgi cisternae or secretory vesicles obtained from a pool of nine normal or hypercholesterolemic livers.

[b] Total serum VLDL lipid in mg/dl.

Each value represents the mean % of the individual lipid component ± S.E.M. from five experiments.

TABLE 5. Composition of the LDL lipid from normal and hypercholesterolemic rat liver and serum

	Normal Rats LDL			Hypercholesterolemic Rats LDL		
	Cisternae	Vesicle	Serum	Cisternae	Vesicle	Serum
Triglycerides	29.0 ± 4.0	26.0 ± 1.0	21.0 ± 2.0	12.0 ± 5.0	7.0 ± 1.0	0.8 ± 0.1
Phospholipids	31.0 ± 12.0	22.0 ± 4.0	19.0 ± 4.0	27.0 ± 0.6	17.0 ± 0.7	15.0 ± 3.0
Cholesteryl esters	26.0 ± 6.0	42.0 ± 6.0	47.0 ± 5.0	47.0 ± 7.0	61.0 ± 3.0	69.0 ± 3.0
Cholesterol	18.0 ± 3.0	9.0 ± 2.0	13.0 ± 1.0	14.0 ± 3.0	15.0 ± 1.0	14.0 ± 0.6
Total LDL lipid	0.21 ± 0.04[a]	0.45 ± 0.06[a]	2.4 ± 0.3[b]	0.59 ± 0.14[a]	1.81 ± 0.37[a]	1,284 ± 96[b]

[a,b] These data are expressed as in Table 4.
Each figure represents the mean % value ± S.E.M. for the individual lipid components from five experiments.

form predominating, and contains only small quantities of triglyceride. As seen in the normal rats, the cisternae LDL fraction is relatively richer in triglyceride and phospholipid when compared to either the vesicle or serum LDL fractions. The lipid composition of the secretory vesicle LDL is very similar to that of serum LDL but, as observed with the hypercholesterolemic vesicle nascent VLDL (Table 3), vesicle LDL from the fatty liver is somewhat richer in triglyceride than its serum counterpart. Thus, as noted in Table 4 for VLDL, the transfer of LDL-size particles from the cisternal elements of the Golgi into the secretory vesicles in both normal and hypercholesterolemic rat livers is accompanied by an apparent remodelling of the lipid moiety by enhancement of the cholesteryl ester content and reduction in the proportions of triglycerides and phospholipids.

Hepatic and serum lipoprotein particle sizes

Lipoprotein particles isolated from the hepatic Golgi, secretory vesicles, and serum were sized after negative staining and electromicrographic visualization. The data shown in **Table 6** for the particle sizes of the normal and hypercholesterolemic serum lipoprotein fractions agree well with previous reports (10, 14). In both the normal and hypercholesterolemic rat, the VLDL and LDL particle size increases significantly as the particles pass from the Golgi cisternae into the secretory vesicles. The normal vesicle VLDL is of similar size to normal serum VLDL. The vesicle LDL, however, is somewhat larger than normal serum LDL. There is no significant difference between the hypercholesterolemic secretory vesicle VLDL and that of the serum. The hypercholesterolemic vesicle LDL, in contrast, is significantly larger than the hypercholesterolemic serum LDL.

Polyacrylamide gel electrophoretic analysis of nascent and serum lipoprotein apoproteins

Fig. 3 shows the typical 7 M urea polyacrylamide gel electrophoretic profile for normal rat serum lipoprotein fractions and apoVLDL and LDL from the normal rat liver secretory vesicles and Golgi cisternae. As previously reported (46), both the secretory vesicles and cisternae VLDL are virtually deficient in apoC-II and C-III when compared to serum VLDL, and contain apoB and apoE almost exclusively. Rat serum LDL contains predominantly apoB with small quantities of apoE, apoC-II, and apoC-III. The secretory vesicles and Golgi cisternae LDL fractions have apoprotein patterns similar to the nascent VLDL and contain predominantly apoB and apoE with trace amounts of apoC-II and C-III. The serum HDL_1 fraction of normal rats contains apoB and apoE with small amounts of apoC-II, apoC-III, and apoA-I. In Fig. 3, gel 8 shows the typical rat HDL_{2+3} apoprotein profile; apoA-I is the major apoprotein. Insufficient protein was obtained in the HDL_1 and HDL_{2+3} density fractions of lysed secretory vesicles and Golgi cisternae to permit analysis of the constituent apoproteins by polyacrylamide gel electrophoresis. **Fig. 4** shows the apoprotein profiles obtained upon polyacrylamide gel

TABLE 6. Lipoprotein particle sizes[a]

	Normal Rats			Hypercholesterolemic Rats		
Fraction	Serum	Secretory Vesicles	Golgi Cisternae	Serum	Secretory Vesicles	Golgi Cisternae
VLDL	431 ± 9	408 ± 11	227 ± 6	405 ± 15	413 ± 20	360 ± 6
LDL	222 ± 6	271 ± 13	195 ± 4	227 ± 5	346 ± 6	313 ± 7
HDL_1	171 ± 3			150 ± 3		
HDL_{2+3}	120 ± 2			141 ± 3		

[a] Mean diameter, Å ± S.E.M. The diameters of at least 200 particles for each lipoprotein class were measured.

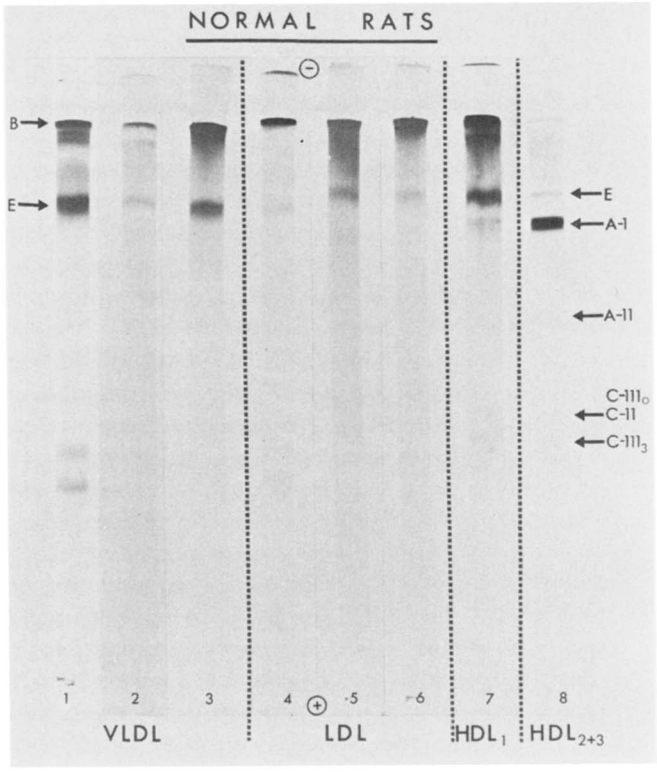

Fig. 3. 7 M urea, 10% polyacrylamide gel electrophoretic analysis of apolipoproteins from normal rat serum, hepatic secretory vesicles, and Golgi cisternae. Apoproteins were designated according to their relative migrations when compared to known purified apoprotein standards. Gel 1, serum apoVLDL (d 1.006 g/ml); 2, apoVLDL isolated from the lysed hepatic secretory vesicles; 3, apoVLDL from the hepatic Golgi cisternae; 4, serum apoLDL (d 1.006–1.063 g/ml); 5, hepatic secretory vesicle apoLDL; 6, hepatic Golgi cisternae apoLDL; 7, serum apoHDL_1 (d 1.063–1.085 g/ml); 8, serum apoHDL_{2+3} (d 1.085–1.21 g/ml). Fifty to 150 μg of apoprotein were applied to each gel.

electrophoresis of the serum and hepatic lipoprotein apoproteins from hypercholesterolemic rats. The serum VLDL from these animals is characterized by the presence of apoB and apoE with very little or no apoC-II and C-III. The secretory vesicle and Golgi cisternae VLDL from hypercholesterolemic rats, like the normal controls, contained only apoB and apoE. The hypercholesterolemic serum LDL fraction, as the VLDL, contained almost exclusively apoB and apoE. Similarly, the nascent LDL from the vesicle and cisternae contained apoB and apoE. The serum HDL_1 fraction, as in normal rats, contained apoB, apoE, and apoA-I, but, unlike normal serum HDL_1, little or no apoC-II and C-III was present. Gel 8 in Fig. 4 shows that the secretory vesicle HDL_1 fraction contains apoB and apoE with no apoC-II or C-III. The pattern obtained for the cisternae HDL_1 is rather more diffuse, however apoB and apoE (**Table 7**) are present, together with a faint band which co-migrates with apoA-I. The hypercholesterolemic serum HDL_{2+3} fraction contains all of the known HDL apoproteins with the exception of apoE, the virtual absence of which in HDL_{2+3} from hypercholesterolemic rat serum has previously been observed by Mahley and Holcombe (10).

It is also of interest to note that both of the closely migrating apoE bands previously observed in hepatic perfusate apo VLDL from fatty livers (12) are also seen in the nascent apoVLDL and LDL from the secretory vesicles and Golgi cisternae (Fig. 2, gels 3 and 5) of fatty livers.

Human type III dysbetalipoproteinemia is characterized by the apparent absence of one of the apoE isomorphic forms (apoE-III) in the serum VLDL (54). This may result in impaired clearance of the VLDL metabolites by the liver (63) and accumulation of cholesteryl ester-rich particles in the serum. ApoVLDL and LDL from hypercholesterolemic rat serum (**Fig. 5**) shows a high degree of similarity of the apoE isomorphic patterns when compared to normal serum apoVLDL. Thus, no deficiency of a specific isomorphic form of apoE is evident upon induction of hypercholesterolemia. The apoE isomorphic pattern shown in Fig. 5 is significantly different from that reported for rat serum VLDL by Gidez et al. (53) and has been attributed to the different delipidation method employed (58).

Immunodiffusion analysis of serum and nascent lipoproteins

Previous studies in my laboratory (59) have shown that on analysis of VLDL isolated ultracentrifugally from normal rat plasma by Ouchterlony double diffusion, two types of lipoprotein particle are observed. **Fig. 6A** demonstrates the diffusion pattern obtained. Reaction 1 is due to an association complex of apolipoproteins B, E, and C on the same lipoprotein particle. Reaction 2 was shown to be due to a discrete LpE particle that contains no apoB or apoC. Reaction 3 was similarly shown to represent a discrete LpC particle that contains no apoB or apoE. Double diffusion analysis of the cholesteryl ester-rich VLDL from hypercholesterolemic rat serum and VLDL from both normal and hypercholesterolemic rat liver perfusates, in contrast, showed only LpB-LpE-LpC (normal) or LpB-LpE (hypercholesterolemic) association complexes to be present within the ultracentrifugally isolated VLDL fraction and no discrete LpE or LpC were observed.

In order to compare further the intracellular nascent lipoproteins from normal and fatty livers with those present in hepatic perfusates and serum, and to establish that nascent very low and low density

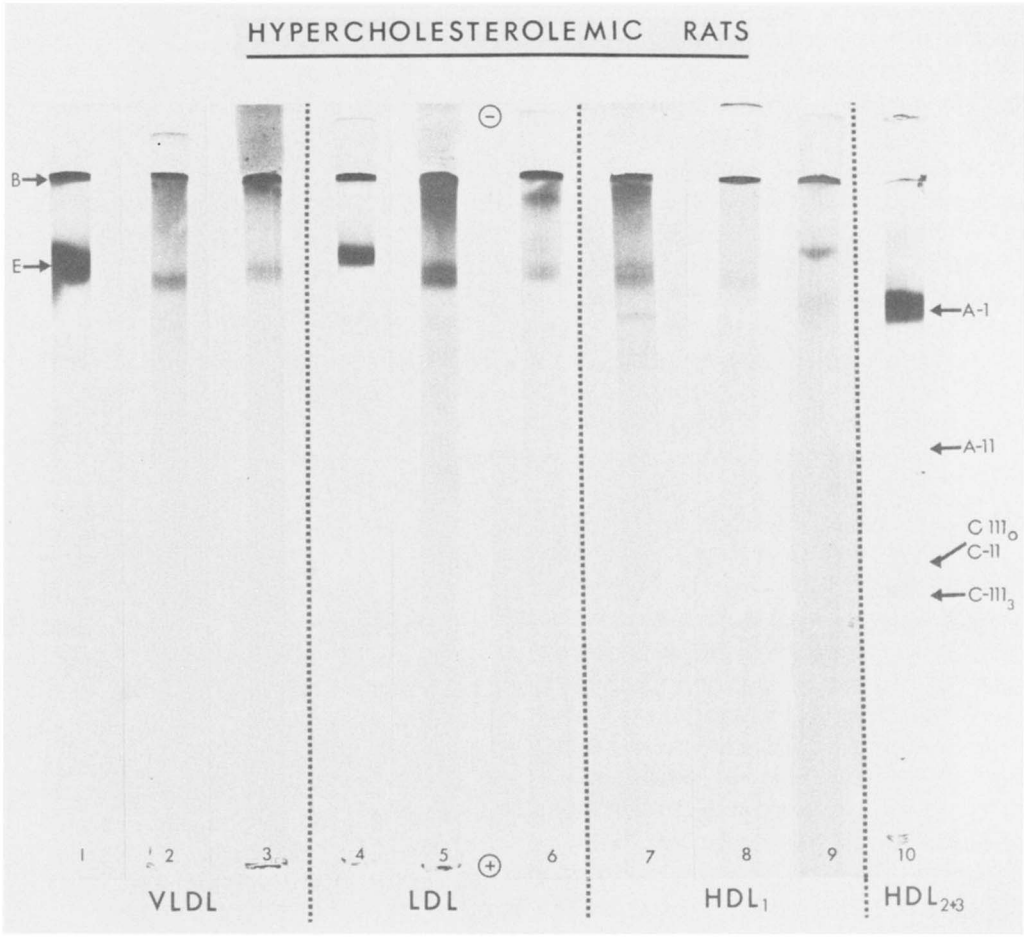

Fig. 4. 7 M urea, 10% polyacrylamide gel electrophoretic analysis of apolipoproteins from hypercholesterolemic rat serum, hepatic secretory vesicles, and Colgi cisternae. Apoprotein designations correspond to those in Fig. 1. Gels 1, 4, 7, and 10 represent serum apoVLDL, apoLDL, apoHDL$_1$, and apoHDL$_{2+3}$, respectively; gels 2, 5, and 8 represent hepatic secretory vesicles apoVLDL, LDL, and HDL$_1$, respectively; gels 3, 6, and 9 represent Golgi cisternae to apoVLDL, apoLDL, and apoHDL$_1$, respectively. Each lipoprotein species was isolated by ultracentrifugation at the hydrated densities quoted for Fig. 3. Fifty to 150 μg of apoprotein was applied to each gel. The two bands seen within the apoE region of the gels of lipoproteins secreted by perfused livers (12) are also seen in the Golgi lipoproteins (gels 3 and 5). Gels 4 and 9 were not run at the same time as the remaining gels.

lipoproteins are indeed secreted as association complexes of apoproteins B and E, nascent hepatic VLDL and LDL were analyzed immunochemically in a similar fashion to that previously reported (59). Fig. 6B shows an Ouchterlony double diffusion analysis of ultracentrifugally isolated VLDL from normal rat hepatic secretory vesicles. In contrast to Fig. 6A, no discrete LpE particles are seen in the normal vesicle VLDL which appears to represent an association complex of LpB and LpE. Fig. 3 shows the almost complete lack of apoC-II and C-III in nascent vesicle VLDL and thus very little if any reaction to anti-apoC-II or C-III whether in discrete or associated form would be expected in this preparation. Fig. 6C shows a similar double diffusion analysis of nascent vesicle VLDL from the livers of hypercholesterolemic rats. A reaction of complete identity is seen between the VLDL and anti-apoB and anti-apoE and no reaction is apparent to anti-apoC-III. This confirms the visual absence of this apoprotein on polyacrylamide gels (Fig. 4). The nascent LDL fraction from the secretory vesicles of hypercholesterolemic rat livers similarly contained an LpB-LpE association complex which lacked apoC and, as in Fig. 6B and 6C, no discrete LpE could be detected. Analysis of the hepatic Golgi cisternae VLDL and LDL from both normal and hypercholesterolemic rats revealed only LpB-LpE association complexes lacking apoC and a complete absence of any discrete LpE (results not shown). The ultracentrifugally isolated serum LDL fraction from hypercholesterolemic rats (Fig. 6E) contained an LpB-LpE association complex devoid of apoC as did the

TABLE 7. The distribution of apoE between ultracentrifugally isolated lipoprotein fractions from hepatic Golgi cisternae and secretory vesicles

Fraction	Secretory Vesicles		Golgi Cisternae	
	Normal	Hypercholesterolemic	Normal	Hypercholesterolemic
	% distribution of apoE			
VLDL	38.8 ± 10.5	29.2 ± 3.9	50.8 ± 17.6	31.7 ± 4.2
LDL	37.5 ± 6.8	58.9 ± 4.3	27.7 ± 13.7	52.5 ± 8.9
HDL_1	5.6 ± 1.6	6.8 ± 3.6	2.4 ± 1.4	2.2 ± 0.5
d > 1.085 g/ml	13.4 ± 4.3	6.7 ± 2.2	15.0 ± 5.9	5.7 ± 2.9
Total apoE (μg)[a]	48.9 ± 13.3	54.6 ± 8.1	9.0 ± 1.7	13.8 ± 1.1
Total apoB (μg)[a]	307.0 ± 69.0	118.0 ± 31.0	88.0 ± 22.0	65.0 ± 11.0
ApoB/apoE (mass ratio)	6.5 ± 0.8	3.4 ± 0.25	11.0 ± 1.8	4.6 ± 0.5

[a] These figures represent the quantity of immunoassayable apoE and apoB recovered from within the Golgi cisternae and secretory vesicles isolated from a pool of nine normal or nine fatty livers.

All data are expressed as mean ± S.E.M. for three experiments.

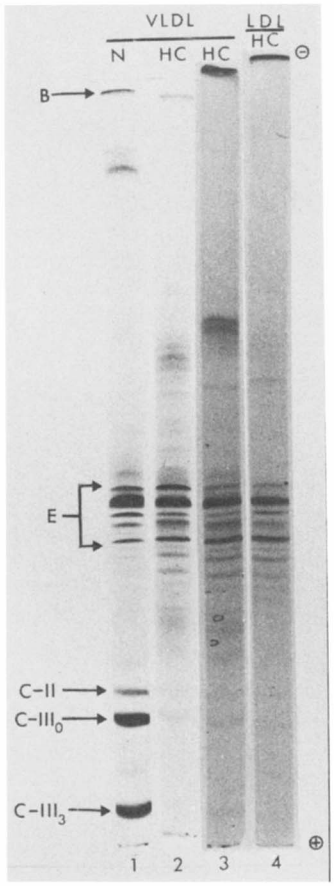

Fig. 5. Analysis of apoVLDL from normal and apoVLDL and LDL from hypercholesterolemic rat serum by polyacrylamide gel isoelectric focusing (pH 4–6.5) in the presence of 7 M urea. Gel1, apoVLDL from normal rat serum; gels 2 and 3, apoVLDL; gel 4, apoLDL from hypercholesterolemic rat serum. The apoproteins and their isoforms were designated using purified standard apolipoproteins. The apoE region corresponded to pI = 5.3–5.4 in all gels. The more acidic pH is at the anodic end of each gel. No deficiency or modification of the apoE isoprotein pattern is evident upon induction of hypercholesterolemia.

secretory vesicle LDL fraction isolated from the fatty liver. However, unlike the vesicle LDL fraction, discrete LpE (reaction 2) was also detected in the serum LDL. Thus the ultracentrifugally isolated serum LDL fraction from hypercholesterolemic animals contains at least two lipoprotein species. Conceivably, the discrete LpE could have arisen from the LDL LpB-LpE association complex during ultracentrifugal isolation as in Fig. 6A or it may represent the apoE antigenic component of HDL_c that would be present in this serum fraction.

Quantitation of apoB and apoE present within isolated nascent and serum lipoprotein fractions

Polyacrylamide gel electrophoretic analysis of the isolated lipoproteins from the Golgi cisternae, secretory vesicles, and serum of normal and hypercholesterolemic rats (Figs. 3 and 4) showed that apoB and apoE were the predominant apoproteins in all the ultracentrifugal fractions from both groups of rats, with the exception of normal serum VLDL where apoC-II and apoC-III were present, and serum HDL_{2+3} which contains predominantly apoA-I. In order to obtain quantitative data on the relative proportions of apoB and apoE associated with each of the isolated lipoprotein classes, specific immunoassays were employed. The data in Table 7 show the distribution of apoE among the ultracentrifugally isolated lipoprotein fractions of the lysed secretory vesicles and Golgi cisternae from normal and hypercholesterolemic rat livers. It is of some interest to note that a significant proportion of the total apoE present within the secretory vesicles and Golgi cisternae of normal rat livers is associated with the LDL fraction, although only 18 and 22%, respectively, of the total lipoprotein protein is present in this fraction

(Table 3). The distribution of apoE among the lipoprotein fractions from hepatic Golgi and secretory vesicles of hypercholesterolemic animals showed a marked accumulation of apoE in the LDL fraction and a concomitant decrease in the apoE associated with the $d > 1.085$ g/ml fraction. This latter decrease of apoE in HDL and $d > 1.21$ g/ml fractions upon induction of hypercholesterolemia has previously been noted for rat serum (11) and liver perfusate of hypercholesterolemic rat livers (12). The fractional distribution analysis for apoB showed a similar elevation in the LDL vesicle, cisternae, and serum fractions upon induction of hypercholesterolemia (results not shown). The values for the total immunoassayable apoB and apoE present within the lysed Golgi cisternae and secretory vesicles, isolated from a pool of nine rat livers, is also given in Table 7. These data were used to calculate the mass ratios for total apoB to apoE in the last line of Table 7. The numerical values of these ratios of both the secretory vesicles and Golgi cisternae lysates from hypercholesterolemic rat livers are significantly lower than those of the corresponding fractions from normal livers. These data indicate a relative enrichment in apoE versus apoB within the nascent lipoproteins present in the secretory vesicles and Golgi cisternae of the livers from hypercholesterolemic animals when compared to nascent lipoproteins from normal livers. This observation is confirmed and extended by the data shown in **Table 8**, in which the apoE and apoB content of individual serum and nascent lipoprotein fractions were determined and expressed as apoB to apoE ratios. These results demonstrate that as both cholesteryl ester-rich (hypercholesterolemic) and triglyceride-rich (normal) lipoproteins pass from the *cis* or forming face of the Golgi into the secretory vesicles, they appear to acquire more apoE, the mass ratio of apoB to apoE being numerically higher in the cisternae than in the vesicle fraction in all cases. Values for HDL_{2+3} are not reported, as virtually no apoB was present in that fraction. Of considerable interest was the observation that normal serum VLDL was significantly richer in apoE than the nascent VLDL from the secretory vesicles of normal rat livers, indicating a postsecretory addition of apoE. This does not appear to be the case with the nascent cholesteryl ester-rich VLDL of hypercholesterolemic hepatic secretory vesicles, whose apoB to apoE ratio is identical to that of the hypercholesterolemic serum VLDL fraction. The possibility that the apoB to apoE ratio for normal serum VLDL is adversely affected by the presence of chylomicron and VLDL remnant particles is unlikely as chylomicron remnants are cleared very rapidly from the serum compartment (64) and VLDL remnants tend to be apoE-depleted as a result of triglyceride lipolysis (65). Similarly, preliminary experiments have indicated that incubation of nascent triglyceride-rich hepatic VLDL with the $d > 1.063$ g/ml fraction normal rat serum results in the acquisition of apoE by the ultracentrifugally re-isolated nascent VLDL fraction. Thus, loss of apoE from the nascent VLDL during ultracentrifugal isolation would not appear to account for the observed differences in the apoB to apoE mass ratios. The relative enrichment in apoE of the nascent VLDL and LDL fractions from hypercholesterolemic rat livers is exemplified by the much lower numerical apoB to apoE ratios obtained for these lipoprotein fractions when compared to nascent lipoproteins from normal rat livers. Also of note are the data for the serum LDL fraction from hypercholesterolemic animals. In contrast to the VLDL, the apoB to apoE ratio for serum LDL from hypercholesterolemic rats is almost twice that of the cholesteryl ester-rich LDL from secretory vesicles of the fatty livers, which indicates a loss of apoE from this fraction after entry into the serum compartment. The very high apoB to apoE ratios obtained for normal rat serum LDL and HDL_1 indicate the low amounts of apoE isolated within these density ranges.

DISCUSSION

This study clearly demonstrates that the feeding of a high fat, cholesterol-containing diet to rats with

TABLE 8. Ratio of apoB to apoE in lipoproteins from serum, hepatic Golgi cisternae, and secretory vesicles of normal and hypercholesterolemic rats

	VLDL			LDL			HDL_1		
Rats	Serum	Secretory Vesicles	Golgi Cisternae	Serum	Secretory Vesicles	Golgi Cisternae	Serum	Secretory Vesicles	Golgi Cisternae
Normal	4.5 ± 0.1	5.8 ± 0.4	9.3 ± 1.2	34.3 ± 4.6	7.6 ± 1.1	13.4 ± 1.9	54.8 ± 6.8	7.2 ± 0.1	18.1 ± 3.9
Hypercholesterolemic	3.6 ± 0.2	3.6 ± 0.2	5.4 ± 0.2	8.0 ± 0.5	4.1 ± 0.5	5.4 ± 0.5	2.8 ± 0.05	2.0 ± 0.4	2.1 ± 0.5

Each value is the mean ratio of immunoassayable apoB to apoE ± S.E.M. for three experiments.

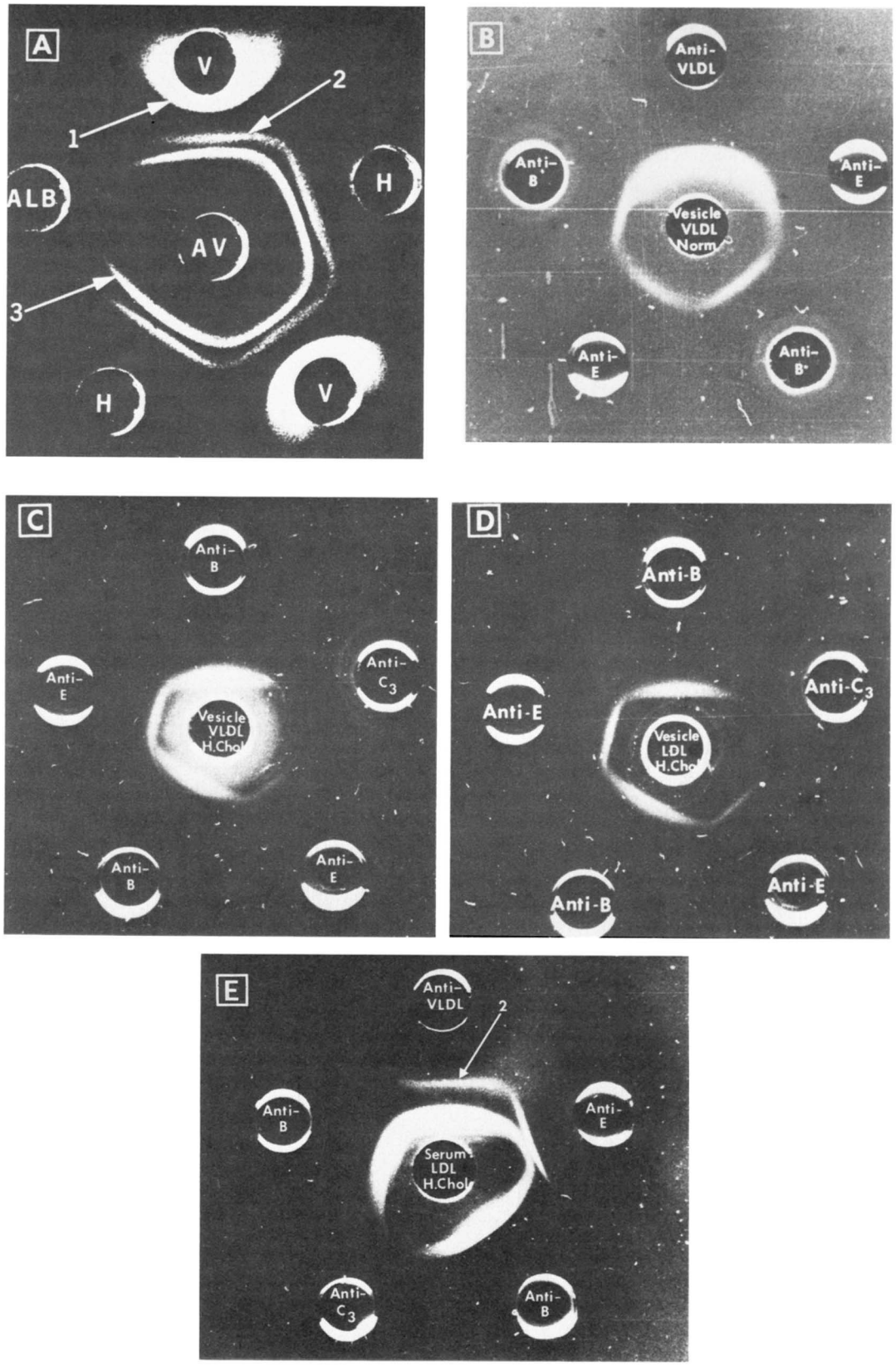

Fig. 6. Ouchterlony double diffusion analysis of nascent and serum lipoproteins from normal and hypercholesterolemic rats. A, immunodiffusion of ultracentrifugally isolated normal rat serum VLDL (V); HDL_{2+3} (d 1.09–1.21 g/ml (H), and rat serum albumin (ALB) against anti-rat VLDL (AV). Reaction 1 represents an LpB-LpC-LpE association complex, reaction 2 a discrete LpE particle, and reaction 3 a

the concomitant induction of hypothyroidism results in marked changes in the lipid and apolipoprotein composition of the nascent lipoproteins isolated from the hepatic Golgi cisternae and secretory vesicles of the fatty livers. The hepatic VLDL contains cholesteryl ester as its major lipid class, unlike the triglyceride-rich VLDL from normal livers. As both triglycerides and cholesteryl esters are hydrophobic core components of lipoprotein particles, it is probable that cholesteryl ester molecules of intestinal origin are progressively substituted for core triglycerides in the nascent VLDL as the hypercholesterolemia develops and the liver becomes progressively cholesteryl ester-enriched (12) as a result of chylomicron remnant uptake. Significantly, Swift et al. (13) demonstrated that upon induction of a milder hypercholesterolemia (432 mg/dl) by feeding 5% lard and 1% cholesterol to hypothyroid rats, the Golgi VLDL, although cholesteryl ester-enriched, only contained 30% of its total mass as this lipid. In the present study, the serum cholesteryl ester level was 750 mg/dl and the hepatic secretory vesicle VLDL contained 70% of its lipid mass as cholesteryl esters (Table 4). The lipid composition of the hepatic secretory vesicle VLDL from hypercholesterolemic rats closely approximates that of the cholesteryl ester-rich serum VLDL. Similarly, the apolipoprotein composition of the nascent VLDL, which contains almost exclusively apoB and apoE, is very similar to that of the serum VLDL and the mass ratios of apoB to apoE are identical for the two lipoprotein particles. Furthermore, the secretory vesicle and serum VLDL from the hypercholesterolemic rats are of very similar size (Table 6) and both represent stable association complexes of apoB with apoE. These data together with those from our previous study (12) and that of Swift et al. (13) strongly support the hypothesis that the fatty liver from hypercholesterolemic animals is capable of synthesizing and secreting cholesteryl ester- and apoE-enriched VLDL particles that contribute to the hypercholesterolemia observed in these animals. Although a significant proportion of the lipoproteins present within the subcellular fractions of fatty livers possesses the flotation characteristics of VLDL in the ultracentrifuge, the major lipoprotein class is within the LDL or IDL (13) density range. The fatty liver would appear to secrete at least half of its total lipoprotein within this density class (12). Clearly, therefore, the fatty liver has the ability to synthesize and secrete smaller, higher density lipoproteins that are rich in cholesteryl esters and apoE (Tables 5 and 8). The lipid composition of these particles bears a striking resemblance to that of the serum LDL fraction which, as in the Golgi cisternae and secretory vesicles, is markedly elevated in hypercholesterolemia. Although ultracentrifugal isolation and/or negative staining procedures may result in lipoprotein particle deformation and the appearance of large discoidal particles within the hepatic secretory vesicles (Fig. 2), it is evident from this study that small, LDL-size particles are present in the secretory vesicles of the fatty livers from hypercholesterolemic animals. Furthermore, these LDL-size lipoproteins are secreted into the serum compartment (12) and contribute significantly to the observed increments in the serum cholesteryl esters and apoE of these cholesterol-fed animals. Inspection of the data in Tables 6, 8, and Fig. 6E shows, however, that the serum LDL fraction is immunochemically heterogeneous, containing a discrete LpE particle in addition to the LpB-LpE association complex. The serum LDL also contains less apoE relative to apoB and has particles of significantly smaller mean diameter than the LDL fraction from the lysed secretory vesicles of the fatty liver. Thus the nascent cholesteryl ester-rich LDL particles, inasmuch as they contain apoB and apoE, may be considered as small VLDL particles. They probably undergo post-secretory modification within the serum and are metabolized to smaller particles containing less apoE than their nascent precursors.

Riley et al. (66) have recently reported that the intestine of chronically cholesterol-fed euthyroid rats synthesizes elevated quantities of IDL type lipoproteins (d 1.006–1.030 g/ml) which contain 42% of their lipid mass as cholesteryl esters and have apoB and apoA-I as their major apoprotein species. These particles may well contribute to the plasma LDL pool after exchange of their apoA-I for apoE (67). Such an exchange would result in a decrease in the apoE and an increase in the apoA-I content of the serum higher density lipoprotein fractions, which would be consistent with the observations that total HDL_{2+3} protein is markedly elevated (Table 3) and contains predominantly apoA-I with little or no apoE (Fig. 4), as re-

discrete LpC particle (59). B, immunodiffusion of nascent VLDL isolated from the secretory vesicles of normal rat livers against the indicated monospecific antibodies and anti-VLDL. C, diffusion of the cholesteryl ester-rich VLDL from the secretory vesicles of hypercholesterolemic rat livers against the designated monospecific antibodies. D, the center well contained the LDL fraction isolated from the secretory vesicles of hypercholesterolemic rat livers. The peripheral wells containing antibodies were as designated in the figure. E, analysis of the serum LDL fraction from hypercholesterolemic rats by diffusion against the specified antibodies. Note the presence of a discrete LpE (labeled as 2), equivalent to reaction 2 in A. Each antigen well contained 5–8 μg of total protein; each antibody well contained 0.5–1.0 mg of gammaglobulin. Antibodies against rat serum VLDL and apoB were prepared as in (59) and against apoE and apoC-III as in (58).

ported in this communication. There is presently little quantitative data available that delineates the relative contributions made by the intestine and the liver to the cholesteryl ester- and apoE-rich serum lipoprotein pools responsible for the hypercholesterolemia induced by cholesterol feeding of hypothyroid rats. Indeed the relative contribution made by each organ may change as the hypercholesterolemia develops and the liver becomes progressively enriched with cholesteryl esters as a result of uptake of cholesteryl ester-rich chylomicron remnant-type particles of intestinal origin. Critical assessment of the role of the liver and intestine in the biosynthesis of serum-destined cholesteryl ester-rich lipoproteins during the development of hypercholesterolemia will certainly be facilitated when quantitative techniques for the assay of intestinal and hepatic apoB (68) are developed.

Analysis of the serum VLDL from normal rats and the serum VLDL and LDL from hypercholesterolemic rats by analytical gel isoelectric focusing (Fig. 5) showed that there was no obvious deficiency in, or difference between, the isoprotein patterns observed for the apoE component of each lipoprotein species. Thus, while the cholesterol-fed hypothyroid rat shows many of the serum lipoprotein changes seen in human type III hyperlipoproteinemia, it does not exhibit the VLDL apoE-III and E-IV deficiency (54) characteristic of, and diagnostic for, the human disorder. This observation is of interest in light of consistent findings that there appears to be no defect or decrease in the rate of removal of rat cholesteryl ester-rich lipoprotein particles from the serum compartment (69) or by perfused rat liver preparations (33, 34). Lipid-apoE complexes lacking apoE-III and E-IV, in contrast, are not cleared very rapidly by perfused rat livers (63). Thus the accumulation of cholesteryl ester- and apoE-rich particles in the serum of cholesterol-fed rats may be entirely due to increased synthesis of these particles by the liver and intestine and a saturation of the hepatic removal mechanism. The accumulation of β-VLDL in human Type III hyperlipoproteinemia may, in contrast, result from both an increased synthesis of hepatic apoE-III- and E-IV-deficient cholesteryl ester-rich particles and decreased clearance of the hepatic lipoprotein metabolites and serum chylomicron remnants due to lack of the required isoproteins in their endogenous or acquired apoE components.

Previous reports from our laboratory (46, 47) demonstrated that the small proportion of apoC-II and apoC-III seen in association with nascent hepatic triglyceride-rich VLDL is added at a late stage in VLDL biosynthesis as the lipoprotein particles pass from the Golgi cisternae into the secretory vesicles. The major proportion of the apoC-II and C-III are added after secretion of the nascent VLDL into the space of Disse and serum compartments (46). This study demonstrates (Table 8) that the relative proportion of apoE which, unlike apoC-II and apoC-III, is present in significant quantities in both triglyceride-rich (normal) and cholesteryl ester-rich (hypercholesterolemic) nascent (intracellular) VLDL and LDL, is increased during passage of the nascent lipoproteins through the Golgi apparatus and into the secretory vesicles. The apoE content of the triglyceride-rich secretory vesicle VLDL from normal livers, unlike the cholesteryl ester-rich VLDL, is further supplemented upon passage of the nascent VLDL into the space of Disse or serum compartment (Table 8). The concept that LpE, like LpC, can be acquired by, or exchanged with, nascent VLDL is substantiated by previous data from Nestruck and Rubinstein (46). These authors incubated nascent Golgi VLDL from normal rats with serum HDL radioactively labeled in the protein moiety. Isolation of the VLDL after incubation and analysis of the apoproteins by polyacrylamide gel electrophoresis showed radioactivity associated with the apoC-II and C-III and also the apoE area of the gel. Similarly, preliminary experiments in this laboratory have demonstrated the ability of triglyceride-rich, but not cholesteryl ester-rich, nascent (intracellular) VLDL to acquire LpE upon incubation with VLDL-free ($d > 1.063$ g/ml) normal rat serum. Thus nascent hepatic triglyceride-rich VLDL acquires both LpE and LpC upon entry into the serum, as do chylomicrons (67). The source of these acquired lipoproteins would appear to be the serum HDL fraction, although the possible involvement of the low molecular weight, virtually lipid-free, LpE secreted by normal perfused rat livers (12) in this process remains to be determined.

Apolipoproteins E and C bind fairly rapidly to, and remain firmly associated with, phospholipids (70, 71). It is therefore improbable that apoproteins exist in a totally delipidated state within the serum compartment (71). The amount of their associated lipid may however be small. Thus the exchange or acquisition of apolipoproteins between or by lipoprotein fractions is most probably accompanied by a transfer of lipid. As apoE is strongly implicated in cholesterol transport and forms an integral part of cholesteryl ester-rich lipoproteins, it is plausible to suggest that the acquisition of LpE by nascent triglyceride-rich hepatic VLDL may be accompanied by a mass transfer of phospholipid and cholesterol to the nascent lipoprotein. Previously published data have indicated, however, that a significant proportion of

the apoC-II and C-III (46, 72) and possibly all of the apoE (73) components that are acquired by nascent hepatic VLDL are added between the time of fusion of the secretory vesicle with the liver plasma membrane and the appearance of the VLDL in the hepatic perfusate. Furthermore, incubation of hepatic perfusate VLDL with VLDL-free rat serum resulted in no significant change in the lipid composition or apoE/triglyceride ratio of the VLDL, although the apoC-II and C-III components were enhanced (73). These cumulative data suggest that the acquisition of apoproteins by nascent VLDL upon secretion is accompanied by the net transfer of only small amounts of lipid which only slightly influences the overall lipid composition of the VLDL particle. The role this acquisition of apoproteins plays in the subsequent metabolism of hepatic VLDL in the rat is thus most probably confined to stimulation of triglyceride lipolysis by addition of apoC-II (74), inhibition of premature hepatic clearance of undegraded VLDL by addition of apoC-III (75), and hepatic clearance of triglyceride and apoC-depleted VLDL remnants by hepatic receptor recognition of apoE (76).

In view of the observations that cholesteryl ester-rich serum VLDL and LDL contain little or no apoC-III (Fig. 5) and their nascent counterparts, unlike triglyceride-rich nascent VLDL, will not acquire this apoprotein upon incubation with serum,[2] the question why these lipoproteins are not cleared by the liver (75) but accumulate in the serum compartment is raised. A plausible explanation consistent with the currently available data would be that the apoE-mediated hepatic receptor, which does not appear to be depressed or malfunctional in hypercholesterolemia (33, 34), becomes saturated due to the 1.5-fold increase in hepatic secretion of apoE with its attendant cholesteryl ester-rich lipoproteins (12). Saturation of the hepatic clearance mechanism for the apoE-rich lipoproteins would naturally lead to the accumulation of these abnormal lipoproteins in the serum.

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