Differential labeling of rat hepatic Golgi and serum very low density lipoprotein apoprotein B variants

Larry L. Swift,* Robert J. Padley,† and Godfrey S. Getz†,**

Department of Pathology,* Vanderbilt University School of Medicine, Nashville, TN 37232, and Departments of Pathology† and Biochemistry and Molecular Biology,** University of Chicago School of Medicine, Chicago, IL 60637

Abstract The synthesis of apoB-100 and apoB-48 by rat liver was investigated by studying the apoB complement of very low density lipoproteins (VLDL) from hepatic perfusates and Golgi fractions. The relative amounts of apoB-100 and apoB-48 in perfusate and Golgi VLDL as determined by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis were similar to those in serum VLDL. To investigate the relative rates of synthesis of the VLDL B proteins, rats were injected intraportally with tritiated amino acid, and hepatic Golgi and serum VLDL were isolated from 7.5 to 120 min later. In hepatic Golgi VLDL, apoB-100 and apoE were maximally labeled at 15 min after the tritiated amino acid pulse. In contrast, VLDL apoB-48 attained maximum radioactivity at 30 min after isotope injection. In serum VLDL, apoB-100 and apoE were maximally labeled at 30 min post-isotope injection, while activity in apoB-48 peaked at 60 min. * The data suggest that the synthesis of the B proteins and incorporation into rat liver nascent VLDL are independently regulated. The differential labeling patterns of the VLDL B proteins may be explained by an intracellular pool of apoB-48 that is larger than that of apoB-100. An alternative explanation of the results is that apoB-100 is a precursor to apoB-48. — **Swift, L. L., R. J. Padley, and G. S. Getz.** Differential labeling of rat hepatic Golgi and serum very low density lipoprotein apoprotein B variants. *J. Lipid Res.* 1987. **28:** 207–215.

Supplementary key words hepatic Golgi apparatus • liver • very low density lipoprotein

The apoprotein B complement found in rat lipoproteins is heterogeneous. Two isomorphs of apoprotein B, apoB-100 and B-48, of apparent molecular weights of 335,000 and 240,000, respectively, have been described (1, 2). A similar heterogeneity has been noted in the apoprotein B of humans (3). Indeed, this heterogeneity has been noted in each species examined thus far except perhaps for the chicken (4). The two apoB proteins are immunologically and structurally distinct as well as metabolically heterogeneous. ApoB-100 and apoB-48 of the rat display partial immunologic identity while apoB-100 also exhibits unique antigenic determinants (1). Both apoB proteins have very similar amino acid compositions, although small but significant differences exist (1, 3). They are structurally distinct with respect to their carbohydrate moieties (Frazier, L., and G. S. Getz, unpublished results) and phosphoserine content (5). Metabolically the apoB-48 of rat serum VLDL exhibits a much shorter plasma residence time than the apoB-100 (2, 6), with only a small proportion of the apoB-48-containing VLDL particles contributing to the rat serum LDL compartment (7).

The representation of the two apoB variants in rat serum lipoprotein fractions has been noted to change upon dietary and physiologic manipulation (1, 8), implying that the intestine contributes a major proportion of the apoB-48 present in serum VLDL. However, serum VLDL from rats fasted for 72 hr still contained a significant quantity of apoB-48, indicating contributions of apoB-48 by sources other than the intestine. The finding that rat liver makes both apoB-100 and apoB-48 (9–11) provided the opportunity to explore not only the hepatic contribution of but also the biosynthetic relationship between these two apoprotein B variants. In this communication we report studies on the role of the liver in the synthesis of the two B apoproteins and on the kinetics of labeling of the apoprotein B complement of VLDL particles isolated from the serum and hepatic Golgi apparatus of rats. A preliminary report of these data has been presented (11).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats were used for all experiments. Rats used for liver perfusion experiments weighed

Abbreviations: VLDL, very low density lipoproteins of d < 1.006 g/ml; apo, apoprotein; B-100, apoprotein B of higher molecular weight; B-48, apoprotein B of lower molecular weight; EDTA, ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulfate.

100–125 g and were purchased from Harlan Industries (Indianapolis, IN). Animals used for hepatic Golgi lipoprotein studies weighed 160–180 g and were purchased from Sasco, Inc. (St. Louis, MO). All rats were fed a standard laboratory diet (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL), and water was available ad libitum.

Materials

L-[4,5-^{3}H]leucine (130–160 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). L-^{3}H-labeled amino acid mixture (163 mCi/mg) was purchased from ICN (Irvine, CA). A kit containing 20 amino acids was obtained from Sigma (St. Louis, MO). Chloramphenicol was purchased from Parke Davis (Morris Plains, NJ), gentamicin sulfate from Schering Pharmaceutical Corp. (Manati, Puerto Rico), and phenylmethylsulfonyl fluoride (PMSF) and aprotinin (Trasylol) from Sigma.

Thoracic duct cannulation

Thoracic duct cannulations were performed as described by Borensztajn et al. (12). Rats were maintained on laboratory diet and water ad libitum. Lymph was collected on ice with preservatives at the concentrations listed below.

Perfusions

Rat liver perfusions were performed by the method outlined by Johns and Miller (13) as modified by Sales (14). The portal vein of a liver was cannulated, and the liver was excised and perfused for 4 hr in a recirculating mode at 37°C with a Krebs-Ringer bicarbonate-based buffer (15) supplemented with 3% (w/v) bovine serum albumin (Sigma), 120 mg/dl glucose, 62.5 mg/dl amino acid mixture (13), 200 μCi of [^{3}H]leucine, 12.5 mU/ml of porcine insulin (Lilly, Indianapolis, IN), and 20% (v/v) of washed out-dated human erythrocytes. The perfusate was oxygenated with a mixture of 95%:5% O_2-CO_2. After the perfusion, erythrocytes were removed by sedimentation at 5000 *g* for 20 min at 4°C. Preservatives were added to the perfusate at the concentrations specified below, and the VLDL were isolated by flotation in the ultracentrifuge as described by Lusk et al. (16).

Isolation of hepatic Golgi apparatus

At each selected time, a cohort of 8–12 nonfasting rats was used. Rats were anesthetized with ether, and a small midline incision was made. [^{3}H]leucine (100 μCi) in 0.2 ml of phosphate-buffered saline was injected into the portal vein of the rat, and the incision was closed with autoclips. The animals were allowed to recover in a warm box and, at various times after isotope injection, the rats were anesthetized again and exsanguinated from the distal abdominal aorta. The livers were them immediately excised, and the rat liver Golgi apparatus-rich fractions were prepared as described previously (17). These preparations are referred to as Golgi apparatus, Golgi complex, or Golgi. Golgi apparatus subfractions, GF_{1+2} and GF_3, were prepared by modifications of the method of Dolphin (18) and are designated below as Golgi subfractions. Nascent lipoproteins were released from Golgi vesicles and tubules using hypotonic shock (17) and isolated by preparative ultracentrifugation as described below.

Isolation of lipoproteins

Serum and Golgi VLDL were floated at d 1.006 g/ml in a 40.3 rotor (Beckman Instruments, Palo Alto, CA) at 38,000 rpm for 18 hr, recovered by tube slicing, and washed one time under the same conditions. The salt solutions used to isolate and wash the lipoproteins contained the following preservatives to prevent degradation of the proteins: 1.34 mM disodium EDTA, 3.08 mM sodium azide, 0.15 mM thimerosal, 1 mM phenylmethylsulfonyl fluoride, 40 μg/ml chloramphenicol, 25 μg/ml gentamycin sulfate, and 100 units/ml Trasylol.

SDS-polyacrylamide gel electrophoresis and fluorography

Three to 20% SDS-polyacrylamide gradient gels were run as described by Swift et al. (19). Approximately 30 μg of protein of each sample was applied per lane. Gels were stained with 0.01% Coomassie Brilliant Blue in 10:1:10 (v/v/v) solution of methanol–acetic acid–water and then destained. High molecular weight markers (Bio-Rad Laboratories, Richmond, CA) were run in adjacent lanes for reference. Fluorography of SDS gels was accomplished by soaking the gels in five volumes of Enlightening (New England Nuclear, Boston, MA) for 30 min and drying the gels using a Bio-Rad Model 224 gel slab drier (Bio-Rad Laboratories, Richmond, CA). The dried gels were exposed at −70°C for up to 96 hr on Kodak X-OMAT AR Film. Slices of excised gel containing stained protein bands were digested with a solution of 90% NCS tissue solubilizer (Amersham, Arlington Heights, IL) at 50°C for 3 hr (20), cooled, and overlaid with 6 ml of a toluene-based scintillation fluid containing 0.48% (w/v) 2,5-diphenyl oxazole (PPO)/0.05% (w/v) 1,4 bis(2-(4-methyl-5-phenyloxazolyl))-benzene (dimethyl-POPOP) (Research Products International Corp., Elk Grove Village, IL). Samples were counted in a Nuclear Chicago Isocap 300 scintillation counter.

Analytical methods

Agarose electrophoresis was performed using the apparatus and method supplied by Corning ACI (Palo Alto, CA). Protein was estimated according to Lowry et al. (21) as modified by Kashyap, Hynd, and Robinson (22). Lipid analysis was performed as described by Jones et al. (23).

RESULTS

Thoracic duct lymph diversion

To assess the possible contributions by the liver to the pool of apoB-48-containing VLDL particles in the circulation, the contribution of the intestine to the plasma pool was excluded by diversion of lymph from rats whose intestinal lymph ducts were cannulated infradiaphragmatically. Lymph collection was maintained for periods ranging from 24 to 120 hr. Intestinal lymph VLDL collected over 120 hr from such rats contained only apoB-48 (**Fig. 1,** lanes 2). The serum VLDL of this same animal contained both apoB-100 and apoB-48 (Fig. 1, lanes 3) in proportions similar to those found in control rat serum VLDL (Fig. 1, lane 1). This indicated that the apoB-48 of serum VLDL could be derived from tissue sources other than the intestine, probably the liver.

Liver perfusion

Rat livers were perfused in vitro at 37°C in a recirculating mode with a Krebs-Ringer bicarbonate-based medium. VLDL isolated from the perfusate after 4 hr of perfusion contained both forms of apoB (**Fig. 2**) as well as apoE and apoCs. The corresponding fluorogram of this gel (Fig. 2) demonstrated the incorporation of radioactive label into all the apoproteins visualized by staining, and established that both apoB-100 and B-48 recovered from the hepatic perfusate were newly synthesized in the liver.

Characterization of the Golgi apparatus and subfractions

By negative stain electron microscopy, the intact Golgi apparatus exhibited the characteristic features described previously (17, 24). Contaminants such as lysosomes, mitochondria, rough endoplasmic reticulum, and multivesicular bodies were not observed. The features of the GF_{1+2} and GF_3 subfractions were markedly different. The GF_{1+2} subfraction contained predominantly lipoprotein-filled secretory vesicles. Some vesicles appeared to have

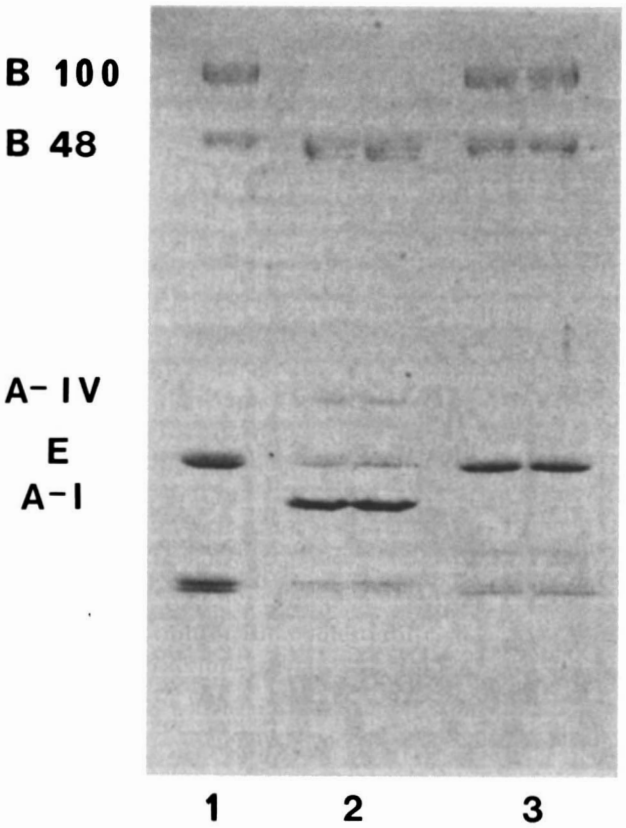

Fig. 1. SDS polyacrylamide gel electrophoretograms of serum VLDL from control rats and serum and lymph VLDL from rats in which mesenteric lymph had been diverted for 120 hr. Approximately 20 μg of protein was applied to each lane. 1, Serum VLDL from control rats; 2, lymph VLDL from 120-hr lymph-diverted rats; 3, serum VLDL from 120-hr lymph-diverted rats.

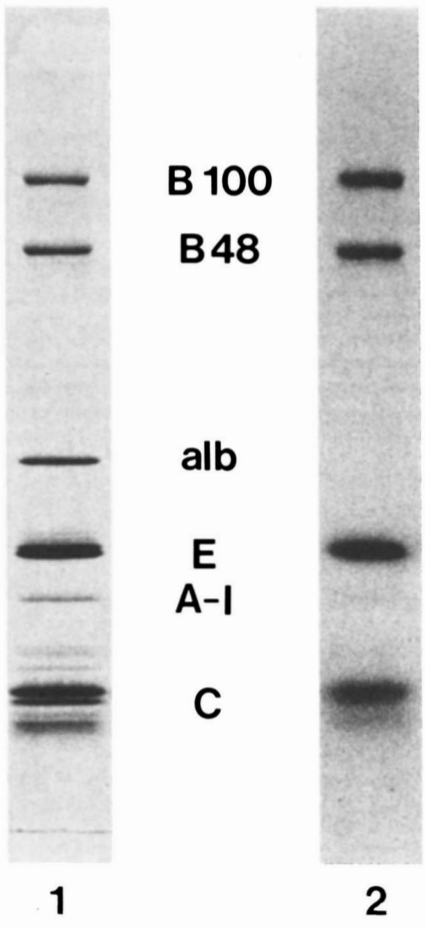

Fig. 2. SDS polyacrylamide gel electrophoretogram and corresponding fluorogram of rat liver perfusate VLDL. Livers were perfused for 4 hr as described in Methods. Electrophoresis and fluorography were performed as described in Methods. Approximately 30 μg of protein was applied to each lane. Lane 1, Coomassie Blue-stained gel; lane 2, fluorogram developed for 45 hr; alb, albumin.

been ruptured, allowing content lipoproteins to be released. The GF_3 subfraction was enriched in cisternal plates, some with anastomotic tubular network intact.

Characterization of VLDL isolated from intact Golgi apparatus

The lipid compositions of the VLDL obtained from hepatic Golgi apparatus and serum were very similar (**Table 1**). Furthermore, the lipid composition of the nascent hepatic Golgi VLDL was nearly identical to that reported for rat hepatic perfusate VLDL (25).

Fig. 3 presents the agarose electrophoretogram of the VLDL from hepatic Golgi apparatus and serum. Golgi VLDL migrated somewhat slower than serum VLDL. Using the Corning system, isolated rat serum VLDL migrated faster than the VLDL in serum.

Fig. 4 illustrates the apoprotein profile of serum VLDL (lane 1) and nascent VLDL of hepatic Golgi apparatus (lane 2) from the same group of rats. The protein profiles of the two fractions were qualitatively the same comprising the apoprotein B, E, and C classes. Golgi VLDL contained more apoA-I than serum VLDL.

Isotopic labeling of VLDL of intact Golgi apparatus

To further substantiate that both apoB isoforms were synthesized in the liver, [^{3}H]leucine was injected into the portal veins of rats and 15 min later the VLDL of the intact Golgi apparatus and serum were isolated. It has been shown previously that hepatic Golgi lipoproteins are optimally labeled at 15 min post-isotope injection (26). The stained protein profile and the corresponding fluorographic pattern of Golgi VLDL apoproteins separated by SDS gel electrophoresis are illustrated in **Fig. 5.** The stained gel pattern of the VLDL of hepatic Golgi apparatus (lane 2) clearly exhibited both apoB isomorphs. However, the fluorogram revealed very little isotope incorporated into the apoB-48 protein (lane 1). This same pattern was obtained in three experiments with different lots of [^{3}H]leucine and in one experiment with a ^{3}H-labeled amino acid mixture.

TABLE 1. Lipid composition of VLDL from serum and intact Golgi apparatus

	Serum VLDL (n = 18)	Golgi VLDL (n = 12)
	% distribution	
Triglycerides	74.3 (3.4)	73.2 (3.2)
Phospholipids	16.9 (3.3)	21.7 (3.9)
Cholesteryl esters	5.7 (2.7)	4.1 (1.6)
Free cholesterol	3.1 (0.8)	1.0 (0.5)

Each value represents the mean ± (standard deviation).

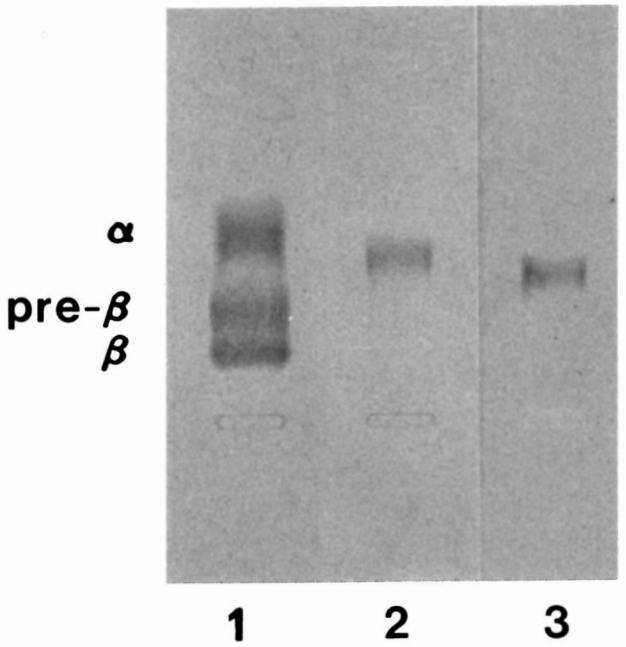

Fig. 3. Agarose gel electrophoretograms of serum (lane 1), serum VLDL (lane 2), and VLDL from hepatic Golgi apparatus (lane 3) from nonfasting rats. Samples were electrophoresed using a Corning electrophoresis system and stained with Fat Red 7B stain.

Kinetic labeling of VLDL apoproteins from intact Golgi apparatus

In order to probe the basis for the differences in the apparent labeling rates of the two apoB variants, serum VLDL and VLDL from hepatic Golgi apparatus were isolated from rats at various times after the intraportal injection of tritiated amino acid. Equal quantities of protein from each VLDL fraction were analyzed by SDS-gel electrophoresis at each time point, and the isotopic incorporation into each protein was determined by scintillation counting. In the absence of a suitable method for the determination of the specific radioactivity of each of the apoB species, the data for each protein were expressed as percent of the radioactivity incorporated at the peak labeling time and are illustrated in **Fig. 6.** At their respective peaks, maximal label incorporation into each of the apoB isomorphs was equivalent at approximately 1000 cpm. These labeling curves are an average of three separate time course experiments, two generated with [^{3}H]leucine and one with a ^{3}H-labeled amino acid mixture.

In the Golgi VLDL, label appeared most rapidly in the apoE moiety, attaining 80% of its maximum activity 7.5 min post-injection, peaking at 15 min, and quickly declining thereafter. The apoB-100 radioactivity was also maximal at 15 min in Golgi VLDL, after which it declined. ApoB-48 attained its maximum activity more slowly,

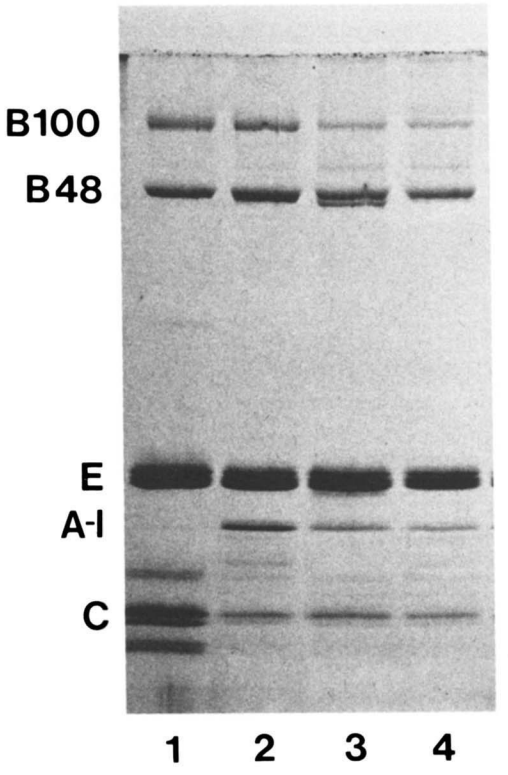

Fig. 4. SDS polyacrylamide gel electrophoretograms of VLDL from serum and hepatic Golgi apparatus and Golgi subfractions of nonfasting rats. Approximately 30 μg of protein was applied to each lane. 1, Serum VLDL; 2, VLDL from Golgi apparatus; 3, VLDL from Golgi subfraction GF_{1+2}; 4, VLDL from Golgi subfraction GF_3. Golgi preparations were isolated as described in Methods and VLDL was isolated by ultracentrifugation.

reaching its zenith at 30 min post-injection, 15 min later than the apoB-100.

The serum VLDL apoprotein labeling pattern closely paralleled that found in the Golgi VLDL but with an intervening delay. At 30 min post-injection, the peaks of apoE and apoB-100 activity in the serum VLDL were attained, after which the amount of label in the apoB-100 declined while that in the apoE reached a plateau. The appearance of radioactivity in apoB-48 again lagged after that of apoB-100 and apoE, attaining maximum label incorporation at 60 min. Decay of label in the apoB-48 then occurred in a fashion roughly parallel to that in apoB-100.

Subfractionation of the hepatic Golgi complex

To define the relationship of the two apoB proteins within Golgi subfractions (GF_{1+2}, GF_3) and to examine whether selective partitioning of the apoB moieties could account for the differential labeling pattern found in the Golgi complex, hepatic Golgi subfractions were prepared from animals injected with [^{3}H]leucine 15 or 30 min earlier. The VLDL retrieved from each of these Golgi subfractions revealed an apoprotein distribution similar to that observed in the VLDL of intact Golgi apparatus (Fig. 4, lanes 3 and 4). The distribution of radioactive label in the apoproteins of each Golgi subfraction at the time points examined (**Table 2**) indicated the progression of the newly synthesized apoproteins through the Golgi complex and were consistent with the previous data demonstrating the sequence of apoprotein labeling and secretion.

DISCUSSION

These data confirm previous observations that both B proteins are synthesized by the rat liver and secreted on VLDL particles (9–11). This conclusion is supported by three different findings. *1*) The relative amount of apoB-48 in serum VLDL was unchanged despite removal of most or all of the intestinal contribution by lymph diversion, indicating there are sources of this apoprotein other than the intestine (Fig. 1). *2*) When tritiated amino acid was added to rat liver perfusates, both B proteins of perfusate VLDL were labeled providing direct evidence

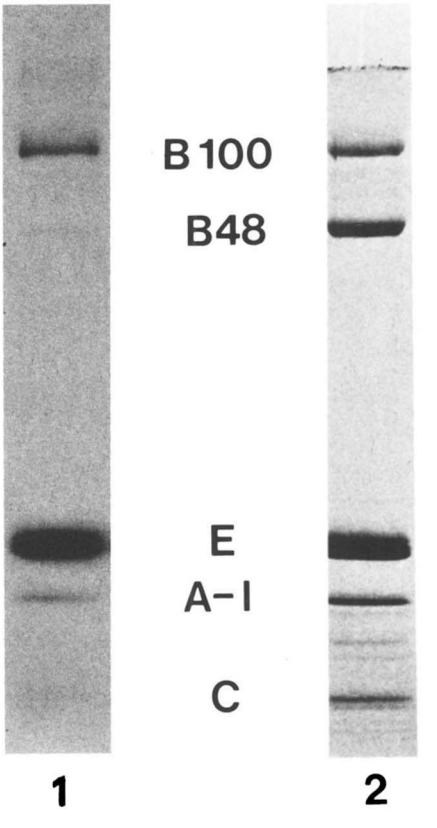

Fig. 5. SDS polyacrylamide gel electrophoretogram and corresponding fluorogram of VLDL of intact Golgi apparatus isolated from rats 15 min after tritiated amino acid injection. Electrophoresis and fluorography were performed as described in Methods. Approximately 30 μg of protein was applied to each lane. Lane 1, fluorogram developed for 96 hr; lane 2, Coomassie Blue-stained gel.

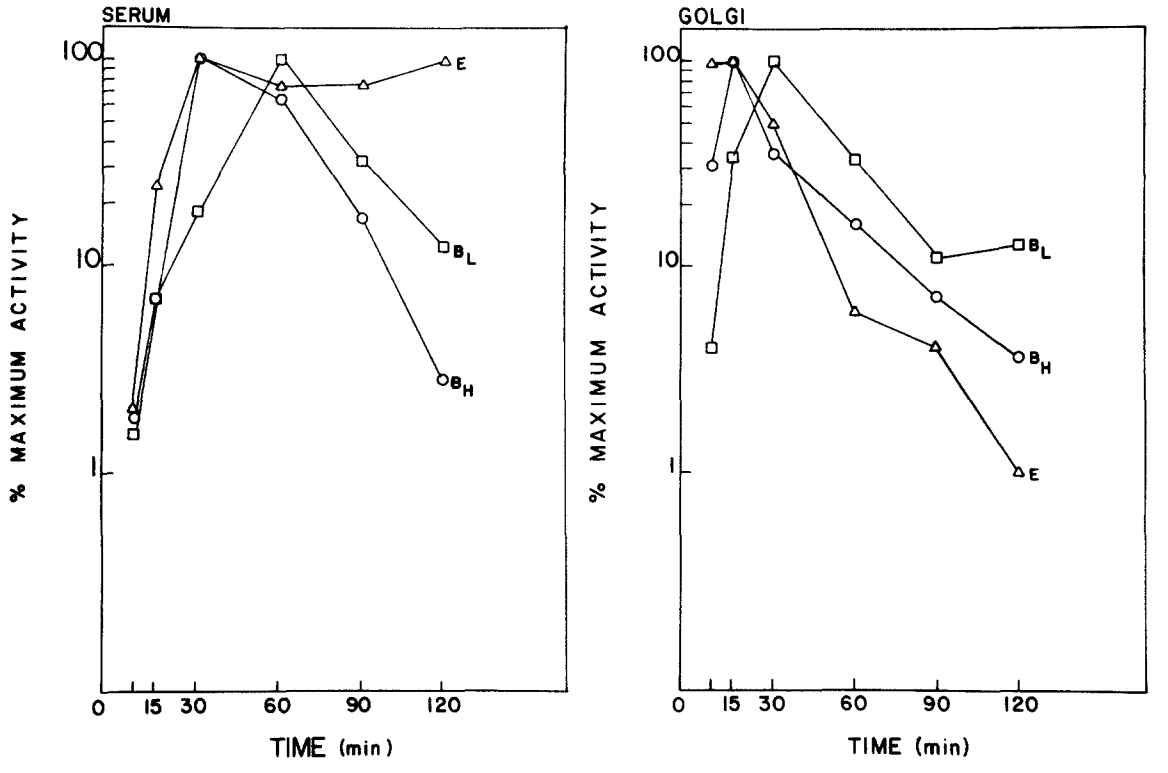

Fig. 6. Time course isotope incorporation into apoB-100 (B_H), apoB-48 (B_L), and apoE of VLDL of intact Golgi apparatus and serum VLDL. Rats were injected intraportally with 100 μCi tritiated leucine and killed at various times after injection. Serum and hepatic Golgi VLDL were isolated, apoproteins were separated by SDS polyacrylamide gel electrophoresis, and activity in individual apoproteins was determined by liquid scintillation techniques. Each point represents the average of three separate experiments.

for the hepatic synthesis of these proteins (Fig. 2). *3*) Both apoB-100 and apoB-48 of intracellular hepatic Golgi VLDL were labeled after intraportal infusion of isotope (Fig. 6).

In order to investigate further the hepatic synthesis of B proteins, the labeling kinetics of the apoB-100 and apoB-48 of VLDL contained within the hepatic Golgi complex were studied. VLDL from intact Golgi apparatus were similar to serum VLDL. However, since Golgi VLDL represent uncirculated lipoproteins that have not interacted with serum enzymes and/or other lipoprotein classes, they contained less apoC (Fig. 4) and proportionately more phospholipid (Table 1) than rat serum VLDL (27). The relative deficiency of apoC probably resulted in a slower mobility on agarose electrophoresis as compared with serum VLDL (Fig. 3).

Both the serum and Golgi VLDL fractions exhibited essentially the same distribution of apoB proteins. The apoB profile of the VLDL from intact Golgi apparatus did not vary when isolated from either the cisternal or vesicular subfractions of the Golgi complex (Fig. 4). These subfractions represent the *cis* and *trans* faces of the Golgi, respectively, the *cis* face receiving products from the endoplasmic reticulum and the *trans* face exporting the processed material (28, 29). The uniformity in the apoB profile across the Golgi apparatus showed that there is no preferential processing or isolation of the apoB isomorphs and suggested that there are no alternate pathways that contribute apoprotein B to the Golgi apparatus as the VLDL traverses this compartment, unlike that implied for the addition of apoC-II and apoC-III (26, 30).

The pulse labeling of the B apoproteins of Golgi VLDL produced unexpected results. Previous work on the more rapid clearance of apoB-48 compared to that of apoB-100 from the rat serum VLDL compartment (2, 6) led to the expectation that, in the steady state, apoB-48 would be more rapidly labeled. Quite the contrary was observed. Of the major VLDL protein constituents secreted by the rat liver, apoB-48 was the most slowly labeled whether examined in the Golgi or the serum compartments (Fig. 6). Before attempting to interpret the differential labeling of apoB-100 and B-48, it should be emphasized that this study reports the incorporation of radioactive apoB-100 and B-48 into nascent hepatic VLDL rather than the rate of isotopic precursor into the respective B apoproteins themselves.

A number of hypotheses could be proposed to explain the differential labeling of the two rat liver apoB variants. *1*) Contamination of Golgi preparations with endocytic vesicles containing lipoproteins rich in apoB-48 could account for the slower labeling of apoB-48. Recent studies

TABLE 2. Labeling of hepatic Golgi subfraction VLDL apoproteins

	15 Minutes		30 Minutes	
	Cisternae	Vesicles	Cisternae	Vesicles
	counts per minute			
ApoB-100	327	220	201	326
ApoB-48	73	74	182	383
ApoE	1770	1695	242	344

Twenty μg of VLDL protein from each fraction was separated by SDS gel electrophoresis; the gel containing each of the apoproteins was excised, processed, and counted as described in Materials and Methods. Each value represents the average of duplicate determinations.

by Hornick et al. (31) have shown that multivesicular body-like organelles containing endocytosed lipoproteins contaminate some Golgi preparations. It should be noted that the Golgi isolation procedure used in these studies was the same as that described by Hornick and coworkers (31). In addition, the lipid composition of the Golgi VLDL was very similar to the composition of rat hepatic perfusate VLDL (25), indicating little contamination of the isolated lipoproteins by cholesteryl ester-enriched remnants. Finally, we do not feel that multivesicular body-like organelle contamination can explain our results since the sequence of labeling of apoB isomorphs in serum VLDL closely paralleled that of the intracellular VLDL apoB proteins. A variant of the contamination hypothesis is that plasma VLDL recycles through the Golgi compartment, perhaps with VLDL containing apoB-48 being more likely to recycle. In one experiment, radioiodinated rat plasma VLDL, containing labeled apoB-100 and apoB-48, was injected into the jugular bulbs of rats 15 min prior to killing and preparation of hepatic Golgi apparatus. Less than 0.5% of the injected ^{125}I was found associated with the Golgi complex, and the material so associated was not enriched in radioactive apoB-48.

2) A large intracellular pool of apoB-48 in the hepatocyte, which a newly synthesized apoB-48 moiety would traverse before being assembled onto a VLDL particle, could result in a delay in the appearance of label in apoVLDL B-48 as seen here. The pool could be a reservoir of apoB-48 stored within the cell, or the pool of apoB-48 could take the form of a metabolic pool. The latter could represent the obligatory posttranslational modification of apoB-48, such as phosphorylation (5), or it could reflect the preferential association of apoB-48 with large triglyceride-rich nascent VLDL whose transit time through the hepatocyte may be slow. However, there is no concrete evidence for such a slow transit time of large VLDL. The presence of intracellular apoprotein pools in the liver and intestine has been suggested by a number of studies (32). Nestruck and Rubinstein (26) and Dolphin and Rubinstein (30) proposed the existence of hepatic apoprotein pools to account for the slow appearance of labeled apoC-II and apoC-III in the hepatic Golgi lipoproteins as well as the labeling characteristics of apoB in the Golgi VLDL (30). Bisgaier and Glickman (33), examining the lipoprotein secretory capacity of rat intestinal mucosa after the administration of cycloheximide, found that apoB-48 continued to be secreted on lipoproteins up to 20 min after the cessation of protein synthesis. A pool of apoB-48 of this size could account for the labeling pattern found in this study.

3) The differential rates of label incorporation into the Golgi VLDL apoB complement suggest a precursor–product relationship between apoB-100 and apoB-48. Such a relationship could account for the observed pattern of apoB-48 labeling in the Golgi VLDL. Radioactivity peaked in apoB-100 of Golgi VLDL 15 min post-injection of [^{3}H]leucine, at which time the label first appeared in apoB-48, our data suggest that this interconversion must take place prior to the entry into the *cis* Golgi subfraction. the apoB-48 radioactivity declined in a fashion parallel to that of apoB-100. If apoB-100 serves as a precursor of apoB-48, our data suggest that this interconversion must take place prior to the entry into the *cis* Golgi subfraction.

A number of structural and immunologic studies on rat apoprotein B provide data consistent with the precursor–product relationship. The two forms of apoprotein B are closely similar but not identical in amino acid composition both in humans (3) and in the rat (1). Peptide maps obtained by chymotryptic cleavage of the rat apoproteins (2) or staphylococcal V8 proteolysis of human B apoproteins (34) reveal that all the peptides of apoB-48 are included in apoB-100, but not all peptides of apoB-100 are present in apoB-48. Double immunodiffusion with polyclonal antibodies raised against apoB-100 produced a spur pattern when reacting with lipoproteins containing predominantly apoB-100 and apoB-48 in adjacent wells, suggesting that apoB-100 contains epitopes not present on apoB-48 (1). On the other hand, in a similar diffusion of the two apoprotein B-containing lipoproteins against anti-apoB-48 polyclonal antibodies, a precipitin line of identity with no spur was produced suggesting that all the epitopes of apoB-48 were included within those of apoB-100 (1). Three laboratories, each with their own battery of monoclonal antibodies raised against the human equivalents of the rat apoB isomorphs, have mapped a number of epitopes on the human apoB species (35–38). The only unique epitopes found thus far have been on the apoB-100 equivalent, while all of the epitopes detected on the apoB-48 equivalent are also found on the apoB-100 protein. In addition to these structural and immunologic studies, the recent description of the selective phosphorylation of apoB-48 (5) also relates to the possibility of a precursor–product relationship between apoB-100 and apoB-48. Phosphorylation of apoB-100 could provide the signal for conversion of the larger apoB-100 into apoB-48,

or phosphorylation could occur after apoB-48 was formed from apoB-100, providing a basis for distinguishing the variants in subsequent intracellular pathways.

The above structural and immunological characterizations of the rat apoprotein B variants, though very limited in extent, can be readily accommodated to the precursor–product relationship between apoB-100 and apoB-48 suggested by the kinetic labeling data. On the other hand, alternative relationships between these two cognate apoproteins must certainly be entertained.

Lee and coworkers (39, 40) have suggested that apoB-100 may be cleaved to form apoB-48. However, they postulate that this scission occurs in the plasma compartment and not within the cell. Under stringent anaerobic isolation conditions in the presence of antioxidants, these workers found only apoB-100 in serum lipoproteins. According to these studies oxidation or proteolysis of apoB-100 produces the smaller apoB-48. However, efforts in our laboratory to reproduce these findings have been unsuccessful (Strauss, P. S., R. J. Padley, and G. S. Getz, unpublished observations).

The full biological meaning of the kinetics of labeling of apoB-100 and apoB-48 awaits further work on the structure and biosynthesis of these apoproteins. This study indicates that apoB-100 and apoB-48, though closely related in structure, may be synthesized by separate pathways that bear a complex relationship to one another.

This work was supported by Grants HL-23525 and HL-15062 from the National Heart, Lung, and Blood Institute, U.S. Public Health Service, and a grant from the University Research Council, Vanderbilt University. L. Swift is an Established Investigator of the American Heart Association. R. J. Padley was supported by the United States Public Health Medical Scientist Training Program 5 T32 GM 07281 and by the Standard Brands Lipid Fellow Grant 2-3300-42-5818. The excellent technical assistance of Patricia Soule and Robin Goble is gratefully acknowledged.

Manuscript received 21 May 1986 and in revised form 14 October 1986.

REFERENCES

1. 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.
2. Elovson, J., Y. O. Huang, N. Baker, and R. Kannan. 1981. Apolipoprotein B is structurally and metabolically heterogeneous in the rat. *Proc. Natl. Acad. Sci. USA.* **78:** 157–161.
3. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
4. Blue, M-L., A. A. Protter, and D. L. Williams. 1980. Biosynthesis of apolipoprotein B in rooster kidney, intestine, and liver. *J. Biol. Chem.* **225:** 10048–10051.
5. Davis, R. A., G. M. Clinton, R. A. Borchardt, M. Malone-McNeal, T. Tan, and G. R. Lattier. 1984. Intrahepatic assembly of very low density lipoproteins. Phosphorylation of small molecular weight apolipoprotein B. *J. Biol. Chem.* **259:** 3383–3386.
6. Sparks, C. E., and J. B. Marsh. 1981. Metabolic heterogeneity of apolipoprotein B in the rat. *J. Lipid Res.* **22:** 519–527.
7. Van't Hooft, F. M., D. A. Hardman, J. P. Kane, and R. J. Havel. 1982. Apolipoprotein B (B-48) of rat chylomicrons is not a precursor of the apolipoprotein of low density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **79:** 179–182.
8. Swift, L. L., R. J. Padley, V. S. LeQuire, and G. S. Getz. 1981. Presence of apoprotein B240 within the Golgi apparatus of rat liver. *Circulation.* **64:** IV–101.
9. Sparks, C. E., O. Hnatiuk, and J. B. Marsh. 1981. Hepatic and intestinal contribution of two forms of apolipoprotein B to plasma lipoprotein fractions in the rat. *Can. J. Biochem.* **59:** 693–699.
10. Wu, A-L., and H. G. Windmueller. 1981. Variant forms of plasma apolipoprotein B. *J. Biol. Chem.* **256:** 3615–3618.
11. Padley, R. J., L. L. Swift, and G. S. Getz. 1982. Differential synthesis of apoprotein B variants in rat liver. *Circulation.* **66:** II–101.
12. Borensztajn, J., G. S. Getz, R. J. Padley, and T. J. Kotlar. 1982. The apoprotein B-independent hepatic uptake of chylomicron remnants. *Biochem. J.* **204:** 609–612.
13. John, D. W., and L. L. Miller. 1969. Regulation of net biosynthesis of serum albumin and acute phase plasma proteins. *J. Biol. Chem.* **244:** 6134–6142.
14. Sales, D. J. 1976. Thesis. University of Chicago. Chicago, IL.
15. Dawson, R. M. C., D. C. Elliott, W. H. Elliott, and K. M. Jones, editors. 1969. Data for Biochemical Research. Clarendon Press, Oxford. 507–508.
16. Lusk, L. T., L. F. Walker, L. H. DuBien, and G. S. Getz. 1979. Isolation and partial characterization of high-density lipoprotein HDL_1 from rat plasma by gradient centrifugation. *Biochem. J.* **183:** 83–90.
17. 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.
18. Dolphin, P. J. 1981. Serum and hepatic nascent lipoproteins in normal and hypercholesterolemic rats. *J. Lipid Res.* **22:** 971–989.
19. Swift, L. L., P. D. Soulé, M. E. Gray, and V. S. LeQuire. 1984. Intestinal lipoprotein synthesis. Comparison of nascent Golgi lipoproteins from chow-fed and hypercholesterolemic rats. *J. Lipid Res.* **25:** 1–13.
20. Product information bulletin: NCS Solubilizer for liquid scintillation counting. Amersham Corporation. Arlington Heights, IL.
21. 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.
22. Kashyap, M. L., B. A. Hynd, and K. Robinson. 1980. A rapid and simple method for measurement of total protein in very low density lipoproteins by the Lowry assay. *J. Lipid Res.* **21:** 491–495.
23. Jones, L. A., T. Teramoto, D. J. Juhn, R. B. Goldberg, A. H. Rubenstein, and G. S. Getz. 1984. Characterization of lipoprotein produced by the perfused rhesus monkey liver. *J. Lipid Res.* **25:** 319–335.

24. Morre, D. J., R. L. Hamilton, H. H. Mollenhauer, R. W. Mahley, W. P. Cunningham, R. D. Cheetham, and V. S. LeQuire. 1970. Isolation of a Golgi apparatus-rich fraction from rat liver. I. Method and morphology. *J. Cell Biol.* **44:** 484-491.
25. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667-680.
26. 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.
27. Hamilton, R. L. 1983. Hepatic secretion of nascent plasma lipoproteins. *In* Plasma Protein Secretion by the Liver. H. Glaumann, T. Peters, Jr., and C. Redman, editors. Academic Press, Inc., New York. 357-374.
28. Bergeron, J. J. M., D. Borts, and J. Cruz. 1978. Passage of serum-destined proteins through the Golgi apparatus of rat liver. *J. Cell. Biol.* **76:** 87-97.
29. Rothman, J. E. 1981. The Golgi apparatus: two organelles in tandem. *Science.* **213:** 1212-1219.
30. Dolphin, P. J., and D. Rubinstein. 1976. Glycosylation of apoproteins of rat very low density lipoproteins during transit through the hepatic Golgi apparatus. *Can. J. Biochem.* **55:** 83-90.
31. Hornick, C. A., R. L. Hamilton, E. Spaziani, G. H. Enders, and R. J. Havel. 1985. Isolation and characterization of multivesicular bodies from rat hepatocytes: an organelle distinct from secretory vesicles of the Golgi apparatus. *J. Cell Biol.* **100:** 1558-1569.
32. Getz, G. S., and R. V. Hay. 1979. The formation and metabolism of plasma lipoproteins. *In* The Biochemistry of Atherosclerosis. A. M. Scanu, R. W. Wissler, and G. S. Getz, editors. Marcel Dekker, New York. 151-188.
33. Bisgaier, C. L., and R. M. Glickman. 1983. Intestinal synthesis, secretion, and transport of lipoproteins. *Annu. Rev. Physiol.* **45:** 625-636.
34. Le Boeuf, R. C., J. E. Shively, M. A. Balla, and V. N. Schumaker. 1983. Human apolipoprotein-B: partial amino acid sequence. *Circulation.* **68:** III-217.
35. Marcel, Y. L., M. Hogue, R. Theolis, Jr., and R. W. Milne. 1982. Mapping of antigenic determinants of human apolipoprotein B using monoclonal antibodies against low density lipoprotein. *J. Biol. Chem.* **257:** 13165-13168.
36. Hahm, K-S., M. J. Tikkanen, R. Dargar, and G. Schonfeld. 1982. Antigenic mapping of human apolipoprotein B subspecies with monoclonal antibodies. *Circulation.* **66:** II-240.
37. Curtiss, L. K., and T. S. Edgington. 1982. Immunochemical heterogeneity of human plasma apolipoprotein B. I. Apolipoprotein B binding of mouse hybridoma antibodies. *J. Biol. Chem.* **257:** 15213-15221.
38. Tsao, B. P., L. K. Curtiss, and T. S. Edgington. 1982. Immunochemical heterogeneity of human plasma apolipoprotein B. II. Expression of apolipoprotein B epitopes on native lipoproteins. *J. Biol. Chem.* **257:** 15222-15228.
39. Lee, D. M., and S. Singh. 1982. Studies of apolipoprotein B in chylomicrons of human plasma and serum. *Circulation.* **66:** II-239.
40. Lee, D. M., E. Koren, S. Singh, and T. Mok. 1984. Presence of B-100 in rat mesenteric chyle. *Biochem. Biophys. Res. Commun.* **123:** 1149-1156.