methods

A rapid calcium precipitation method of recovering large amounts of highly pure hepatocyte rough endoplasmic reticulum

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Abstract **We sought a rapid and non-ultracentrifugal method of recovering large amounts of highly pure rough endoplasmic reticulum (RER) membranes from livers. By substantially modifying a 20-year-old calcium precipitation technique, we obtained a RER fraction from rat liver and established its high degree of purity by quantitating classic membrane markers for different subcellular organelles. This RER fraction is highly enriched in four known proteins (or enzyme activities) required for lipoprotein assembly: apolipoprotein B, microsomal triglyceride transfer protein, acyl CoA:diacylglycerol acyltransferase, and acyl CoA:cholesterol acyltransferase, when compared to two classical RER markers, RNA and glucose-6-phosphatase. From one 10–12 g rat liver, we recover ten to twelve RER pellets of 1.5–1.6 cm in diameter containing ~110–125 mg of total protein, about half of which is sodium carbonate-releasable. By electron microscopy, these large RER pellets from rat livers are homogeneously comprised largely of non-vesiculated short strips of ribosome-rich membranes. This novel technique for isolating RER membranes from liver may provide a useful tool for future studies on the assembly of apolipoprotein B-containing lipoproteins as well as for research focused on mechanisms of secretory and membrane protein translation, translocation, and folding.**—Hamilton, R. L., A. Moorehouse, S. R. Lear, J. S. Wong, and S. K. Erickson. **A rapid calcium precipitation method of recovering large amounts of highly pure hepatocyte rough endoplasmic reticulum.** ***J. Lipid Res.*** **1999.** 40: **1140–1147.**

Supplementary key words apolipoprotein-B • microsomal triglyceride transfer protein • acyl coenzyme A:diacylglycerol acyltransferase • acyl coenzyme A:cholesterol acyltransferase • electron microscopy

The stimulus to develop a new procedure for isolating rough endoplasmic reticulum (RER) membranes from liver arose from ultrastructural observations of rat hepatocytes. Jones, Ruderman, and Hervera (1) reported that very low density lipoprotein (VLDL) precursor particles were formed first within the smooth-surfaced terminal ends of RER, a potentially unique lipoprotein biosynthetic compartment. Claude (2) observed that putative nascent VLDL were never found within the lumenal spaces of flat RER membranes, but were often found within the unique smooth-surfaced terminal ends of RER membranes lacking ribosomes and within the lumenal space of smooth ER (SER) proper. We showed by immunoperoxidase electron microscopy that VLDL-sized lipid-staining particles within the SER proper lacked apolipoprotein(apo) B (3), the large "structural" protein required for circulating VLDL, LDL, and chylomicrons. However, long stretches of RER stained intensely for apoB, as did the unique smooth-surfaced end compartment of the RER. Predictably, clusters of nascent VLDL within forming secretory vesicles of the Golgi apparatus stained most intensely for apoB (3).

These ultrastructural findings evoked a puzzling question: as apoB is synthesized by polysomes of RER whose cisternae lack VLDL-sized particles, and SER cisternae contain lipid-staining particles of VLDL size lacking apoB, where and how does the apoB protein combine with the VLDL-sized lipid particle? We hypothesized that this union between apoB and the VLDL-sized lipid particle occurs in the unique RER–SER junction where both VLDL-sized lipid particles and intense apoB immunostaining were observed (3). However, this did not explain how the large and hydrophobic apoB protein was transferred to this compartment from its site of synthesis on RER polysomes. De-lipidated apoB (and presumably nascent unlipidated apoB) self-aggregates into an insoluble complex in an aqueous milieu in vitro. Therefore, RER membrane-

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; apoB, apolipoprotein B; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; MTP, microsomal triglyceride transfer protein; DGAT, acyl coenzyme A:diacylglycerol acyltranferase; ACAT, acyl coenzyme A:cholesterol acyltransferase; HMG-CoA, 3 hydroxy-3-methylglutaryl coenzyme A.

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associated full-length apoB presumably requires an association with lipids for stability and proper folding to become transport competent within the secretory pathway in vivo (4).

A clue to this mystery emerged when we and others discovered the presence of apoB-containing HDL-sized particles in perfusates of rat livers (5–7), media of isolated primary rat hepatocytes (8), media of isolated primary human hepatocytes (9), media of cultured human and rat hepatoma cells (10–12), media of cultured CaCo-2 (intestinal tumor) cells (13), media of heterologous cells transfected with both the apoB and MTP genes (14), contents of microsomes of rat intestinal cells (15), and in the contents of microsomes from both human and rat hepatoma cells (10–12). This consistent observation from different types of experiments and from different laboratories led us to the hypothesis that apoB might be released first from the RER membrane as a small precursor HDL-sized particle prior to combining with a larger triglyceride-rich particle of VLDL (or chylomicron) size that lacked apoB (4, 16–18). Others have obtained biochemical evidence that is largely consistent with this interpretation (10–12, 19–23). We reasoned that if this were true, both small HDL-sized apoB-containing particles and larger triglyceride-rich particles lacking apoB should be present within the ER lumen, and thus could be recoverable from the ER, provided that adequate amounts could be recovered for biochemical characterization.

Therefore, we sought a rapid, non-ultracentrifugal method of preparing large quantities of highly pure ribosome-rich membranes from rat liver that were, in addition, non-vesiculated. We wanted to avoid vesiculation because we were concerned that the rupture and re-sealing of RER membranes during isolation could cause loss of and/or artifactual translocation of apoB from the lumenal contents to the cytosolic side of the RER membranes.

About 20 years ago, Schenkman and Cinti (24) described a non-ultracentrifugal, calcium-precipitation procedure for isolating rat liver microsomes that appeared to fulfill many of our requirements. However, these authors did not establish the purity or homogeneity of their microsome fraction. We modified their method substantially, in order to recover an RER enriched fraction. We established that the RER fraction recovered was virtually free of contaminating membranes from other organelles. In addition, we obtained preliminary evidence that this fraction included some of the smooth-surfaced ends of RER cisternae, the putative lipoprotein assembly compartment. During several years of trial and error, we made a number of substantive changes to their procedure to achieve our goals; These are: *1*) eliminating sucrose (which invariably led to vesiculation); *2*) eliminating buffers (which Claude said were unnecessary (25)); *3*) substituting 140 mm KCl as homogenizing medium; *4*) introducing "gentle" Polytron homogenization (which facilitated the first isolation of "structurally intact" Golgi apparatus (26) instead of the high shear Dounce or Potter-Elvehjem methods); *5*) adding a series of low speed Sorvall spins to eliminate floating fat; and *6*) titration with a very large volume of 8 mm $CaCl_2$ instead of precipitating ribosome-rich membranes with solid salt. Here we describe our modified, rapid, and novel technique of isolating large quantities of highly pure ribosome-rich membranes from rat livers together with biochemical properties of these RER membranes.

MATERIALS AND METHODS

Experimental animals

The vast majority of this research was carried out using male Sprague-Dawley rats (250–350 g), fed ad libitum for several days in the UCSF animal facilities after receipt from B & K Universal (Fremont, CA). We also tested our method on the liver (~60 g) of one 2-kg male New Zealand rabbit for RER pellet size, color, and ultrastructure, but not for biochemistry.

Electron microscopy

To assess homogeneity, the very large RER pellets were sampled by plunging the tip of a Pasteur pipette from the edge to the center of the pellets. Pasteur pipette punches were expressed directly into freshly prepared solutions of ice-cold 2% glutaraldehyde, 1% paraformaldehyde in 0.1 m sodium cacodylate buffer, pH 7.4, and fixed for several hours or longer. This was followed by two 15-min buffer washes before transfer into 2% osmium tetroxide in the cacodylate buffer for post-fixation overnight at 4°C. Samples were block stained in 2% aqueous uranyl acetate overnight at 4°C, rapidly dehydrated in acetones, and embedded in Epon. Ultrathin sections were stained for 5 min with 0.8% lead citrate and photographed in a Siemens 101 electron microscope, Siemens/CTI Corp., Knoxville, TN.

Biochemical organelle markers

Markers for RER membranes and potential contaminating membranes of other organelles were assayed in aliquots of whole liver homogenate and RER fractions to assess purity. Endoplasmic reticulum markers included: RNA, isolated by the TriPure reagent method (Boehringer Mannheim) and estimated by absorbency at 260 nm; glucose-6-phosphatase, a classic ER membrane protein (27); 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme of cholesterol biosynthesis (28) enriched in the smooth ER (29). Markers for potential contaminating membranes included: succinate-INT dehydrogenase localized to the mitochondrial inner membrane (30); 5′ nucleotidase, enriched in plasma membranes and endosomal membranes (31); low density lipoprotein (LDL) receptors (28) enriched in plasma membranes and endosomal membranes (32), and galactosyltransferase, localized to Golgi membranes (33). Protein was estimated by the Biuret method using bovine serum albumin as reference standard (34).

Lipoprotein assembly proteins and enzymes

Acyl coenzyme A:cholesterol acyltransferase (ACAT) localized largely in the RER (35, 36) and acyl coenzyme A:diacylglycerol acyltransferase (DGAT) localized in both RER and smooth ER (37, 38) were measured by enzyme activity as described previously (39). Albumin, and apolipoproteins A-I, E, A-IV, and C were estimated in aliquots of whole liver homogenate and intact RER by SDS gel electrophoresis and Western blotting using specific antisera provided by Dr. Karl Weisgraber as described previously (28). ApoB was estimated by SDS gel electrophoresis and Western blot as described previously (39). Microsomal triglyceride transfer protein (MTP) was estimated by SDS gel electrophoresis and Western blot using specific antisera for MTP pro-

vided by Dr. David Gordon. Hepatic lipase was estimated by SDS gel electrophoresis and Western blot using a specific antibody provided by Dr. Andre Bensadoun. All determinations were within the linear range of detection as determined in pilot experiments.

Isolation of highly purified RER membranes

Livers were harvested from ether anesthetized rats between 8:00 am and 11:00 am after aortic exsanguination to reduce liver blood content. Livers were minced with double scalpels on an ice-chilled Petri dish using new scalpel blades for each organ. Mincing was carried out until the tissue had the texture akin to a fine paté to preclude excessive Polytron homogenization. Each finely minced liver (10–12 g) was suspended in 20–24 ml of ice-cold 140 mm KCl contained in one 50-ml capacity plastic Sorvall centrifuge tube. Homogenization was carried out by dropping the Polytron generator tip just below the fluid surface, turning the rheostat setting to 0.5, slowly lowering the saw-tooth (ST-10) generator tip into the liquid which mixes the liver into the rotor blades, while continuing a slow up and down motion (~5–10 times) for 25–35 sec, just long enough to eliminate any visible chunks of liver tissue.

Each homogenized liver was then divided equally by decanting back and forth between two 50-ml Sorvall centrifuge tubes, followed by mixing each half with an additional equal volume of ice-cold 140 mm KCl, just to the 40 ml mark. The first pelleting spin in the SS-34 Sorvall rotor was for 10 min at 8,000 rpm at 2°C. Air bubbles and floating fat (which can be quite variable) were water aspirated by carefully sweeping the surface, followed by decanting away from the side of the tube to which additional fat is adherent. The decanting maneuver was designed to separate the supernatant (containing the microsomes) from both the adherent fat on the tube walls and the sedimented intact cells, nuclei, mitochondria, and unwanted cellular debris contained in the first centrifugal pellet. The supernatant was decanted into a Sorvall centrifuge tube, the volume was brought back to the 40-ml line with 140 mm KCl, the tubes centrifuged again for 10 min at 8,000 rpm, and the fat aspiration and decanting (or pipetting) of supernatant was repeated. We did not measure potential losses of RER in these low-speed pelleting spins designed specifically to eliminate virtually all visible floating fat and contaminating organelles before precipitating the RER-rich membranes with $CaCl_2$. This procedure may be repeated one or two more times until there is no visible floating fat. The final supernatant from each rat liver (70–75 ml) was transferred into a 600-ml graduated beaker containing a 2–3 inch magnetic stir bar. The beaker was surrounded by crushed ice and placed atop a magnetic stirrer. Ice-cold 8 mm $CaCl_2$ was slowly titrated from a burette (~20–25 ml/min) into the gently mixing supernatant liquid up to a final volume of ~500 ml. The RER pellet was obtained by centrifugation in the Sorvall SS-34 rotor at 8,000 rpm for 10 min at 2°C. The RER pellets were not resuspended or washed.

Outline of the method

10–12 g liver minced finely with double scalpels
⇓
added to 20–24 ml (1 g/2 ml) of 140 mm KCl
⇓
Polytron at 0.5 rheostat setting for ~25–35 sec
⇓
adjust volume to ~80 ml with 140 mm KCl
⇓
8000 rpm for 10 min in Sorvall SS-34 rotor, 2–4°C
⇓
aspirate floating fat, decant supernatant, adjust volume with 140 mm KCl
⇓
(repeat 1–2 times, depending on the amount of floating fat)
⇓
~75 ml of supernatant titrated slowly to ~500 ml with 8 mm $CaCl_2$
⇓
8000 rpm for 10 min in Sorvall SS-34 rotor, 2–4°C
⇓
ten to twelve pale pinkish RER pellets, each ~1.5–1.6 cm in diameter
⇓
~110–125 mg total RER protein, of which ~50% is sodium carbonate-releasable.

RESULTS

RER mass recovered

When the procedure outlined above is precisely followed, one 10–12 g rat liver yields ten to twelve pinkish RER pellets 1.5–1.6 cm in diameter as illustrated in the life-size color photograph of three such pellets (**Fig. 1**). The total amount of RER mass in these pooled pellets averaged ~110–125 mg protein, and about half of this protein mass was releasable by the high pH(~11.0) sodium carbonate method (40).

RER ultrastructure

During the several years when we were modifying different aspects of the Schenkman and Cinti microsomal method for isolation of RER membranes, we relied solely on a thorough ultrastructural analysis of the final RER pellets after each procedural change. Because of the unusually large size of the RER pellets, two to three punches from one representative pellet from each experiment

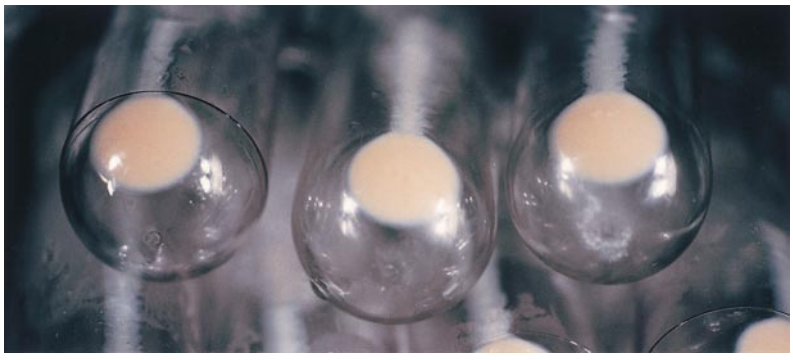

Fig. 1. Life-size color photograph of three (of twelve) 1.5 cm diameter pinkish RER pellets isolated from one rat liver.

were examined by standard transmission electron microscopy to assess the purity and homogeneity of the fraction. As each punch may be 3–4 mm long, these were subsequently cut into sections of ~1 mm in length and thoroughly examined. Although tedious in approach, these analyses guided our efforts in assuring that the subcellular

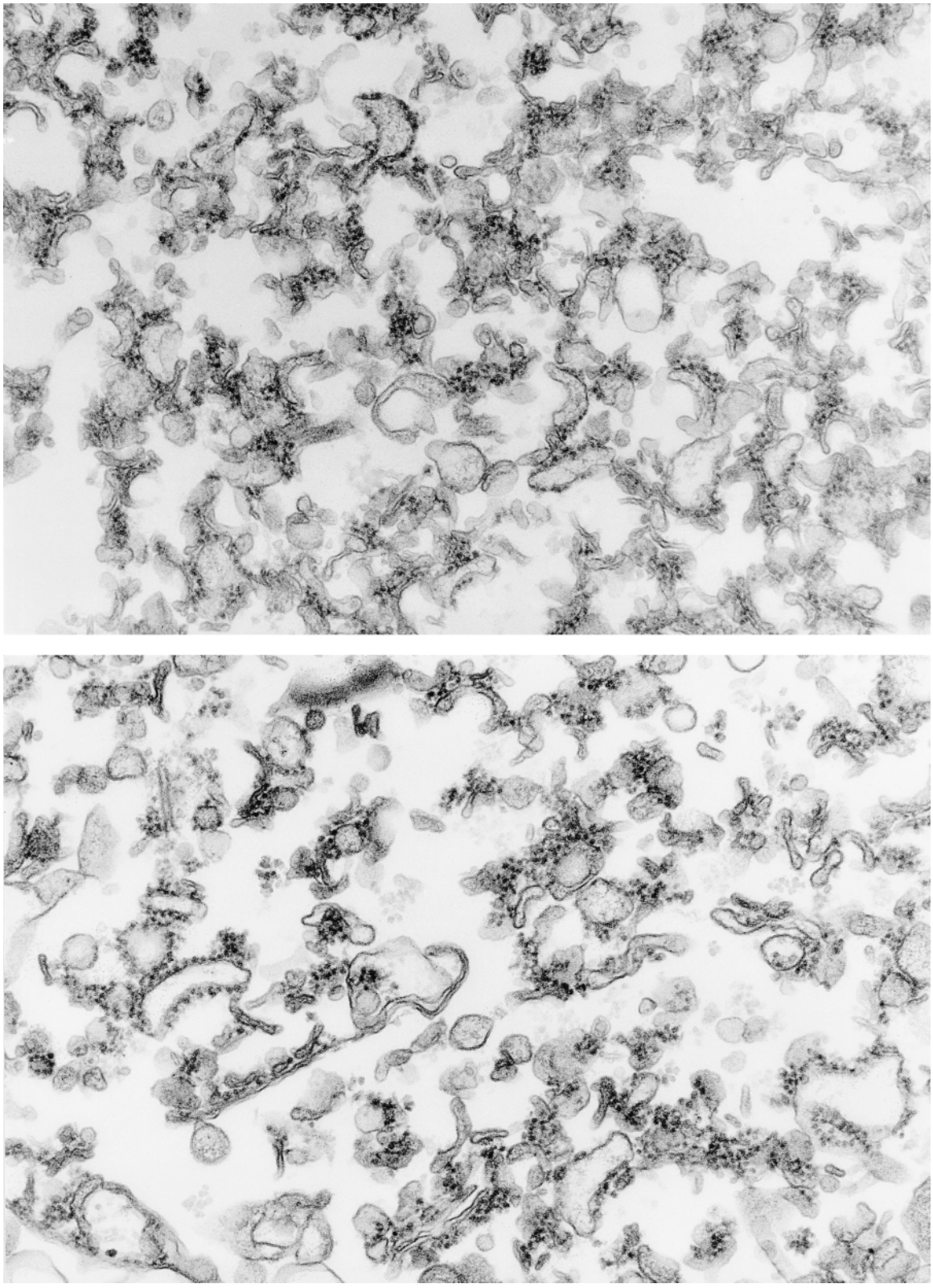

Fig. 2. Representative electron micrograph of thin sections of the RER pellets isolated from rat livers (top) are compared to that from one rabbit liver (Bottom). Note that the predominant structures are polyribosomes attached to elongate and often curved, but non-vesiculated (i.e., not spherical) membranes. Some smaller, smooth-surfaced spherical vesicles lacking ribosomes are also present (×40,000).

fraction recovered after each modification was comprised largely of non-vesiculated fragments of ribosome-rich membranes (**Fig. 2**). When other organelles such as Golgi apparatus membranes, endosomes, mitochondria etc. were observed in the pellet, we abandoned the modification and pursued a different course. We emphasize that the ultrastructural appearance of our final RER fractions is remarkably consistent throughout the body of individual rat RER pellets. We also found this to be true in the RER pellets from the liver of one rabbit (Fig. 2). Our RER pellets differ morphologically from most others in the literature in that our fraction is comprised mostly of short strips of curved and parallel membrane fragments covered on one or both sides by polyribosomes, and sometimes containing intralumenal secretory proteins. Smaller and many fewer smooth-surfaced vesicles are also present. These RER fragments appeared non-vesicular, suggesting that they may be open-ended, in contrast to classic RER preparations that consist of rounded vesicles considered to be fused membranes enclosing a sealed "latent" space containing RER secretory and resident lumenal proteins.

RER enrichment of classic RER markers

The purity of our RER fraction is demonstrated in **Fig. 3** in which the two best known markers for this organelle, ribosomal RNA and the membrane-bound enzyme, glucose-6-phosphatase, are enriched 3- and 4-fold over homogenate, whereas membrane markers for five potentially contaminating subcellular structures were always less than one. The rate-limiting enzyme for cholesterol biosynthesis, a smooth ER membrane protein, HMG-CoA reductase was barely detectable, and the mitochondrial membrane marker succinate-INT dehydrogenase was virtually absent. Small and variable amounts of plasma membranes and endosomes were detected by the presence of 5′ nucleotidase and LDL receptors, but these markers were never enriched over homogenate. Similarly, Golgi apparatus membranes assessed by galactosyltransferase activity were also very low, comparable to those markers for plasma membranes and endosomes. The presence of some portion of these three markers likely represents proteins newly synthesized by the RER that are destined for the Golgi apparatus, plasma membranes, and endosomes, as opposed to simple contamination. These data taken together indicate that our fraction is a highly enriched RER membrane preparation with an estimated purity of ~90% or greater.

RER enrichment of lipoprotein assembly proteins

As a major objective of this research was to prepare a highly pure RER fraction in order to study lipoprotein assembly intermediates from within the ER lumen, we compared the enrichment of the two major proteins that are known to be essential for plasma lipoprotein assembly/secretion, apoB, the structural protein of circulating chylomicrons, VLDL, LDL, and microsomal triglyceride transfer protein (MTP), the "core-lipidating" RER lumenal residence protein (14). The "core" lipids, triacylglycerols and cholesteryl esters, are synthesized by two ER membrane-bound enzymes DGAT and ACAT that have only re-

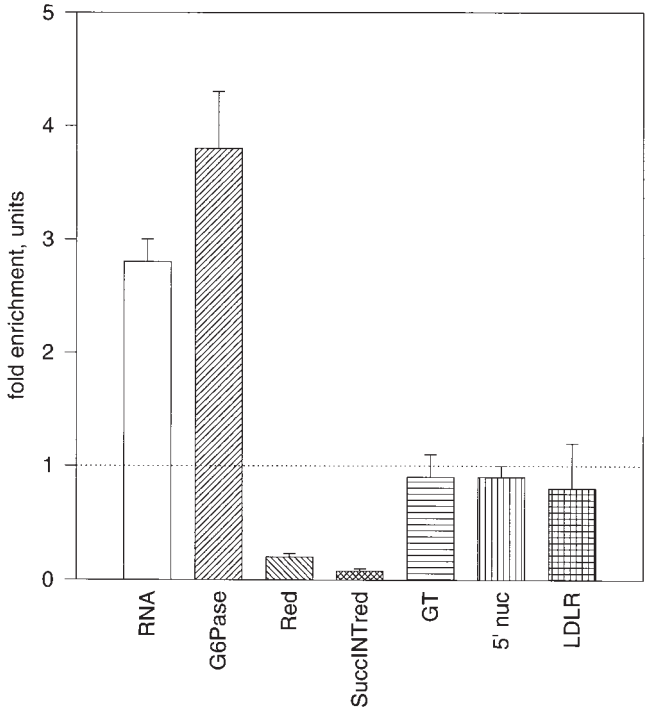

Fig. 3. Purity of the RER membranes from rat livers estimated by organelle markers. RER markers, RNA, and glucose-6-phosphatase (G-6-Pase); SER marker, HMG CoA reductase (Red); mitochondria marker, succinate INT reductase (Succ INT red); Golgi apparatus marker, galactosyltransferase, (GT); and endosome and plasma membrane markers, low density lipoprotein receptors (LDL R) and 5′ nucleotidase (5′ nuc). The data are expressed as fold enrichment over whole rat liver homogenate set as 1.0. The data are the means ± SD of 17 independent experiments. Values for the homogenates were: (a) for RNA, 25.5 ± 6.8 μg/mg protein; (b) for G-6-Pase, 0.93 ± 0.27 μg Pi/mg protein per min; (c) for Red., 139.7 ± 71.6 pmol MVA/mg protein per min; (d) for succ. INT red., 36.3 ± 22.7 pmol/mg protein per min.; (e) for GT, 6.5 ± 3.4 pmol/mg protein per min.; (f) for LDL-R, 2.13 ± 0.71 units/mg protein; and (g) for 5′ nuc., 0.47 ± 0.12 μg Pi/mg protein/min.

cently been cloned (41–45), but have not been isolated or purified to homogeneity. In this experiment, we contrasted the enrichment of these four functional proteins in plasma lipoprotein assembly to the RER standard membrane marker glucose-6-phosphatase, and to our plasma membrane and endosomal markers. As shown in **Fig. 4A**, all four of these lipoprotein assembly macromolecules were enriched 4.5- to 7-fold over homogenate, equal to or even greater than standard markers for RER membranes. In these experiments, we achieved even less contamination than before (Fig. 3) for other cell organelles, in particular plasma membranes and endosomes which might have been a rich source of apoB (Fig. 4A).

Recoveries of organelle markers and lipoprotein assembly proteins in RER fractions

We also measured the percent recovery of microsomal membrane markers together with the four lipoprotein assembly proteins that were recovered in our RER membrane fractions and compared these to markers of poten-

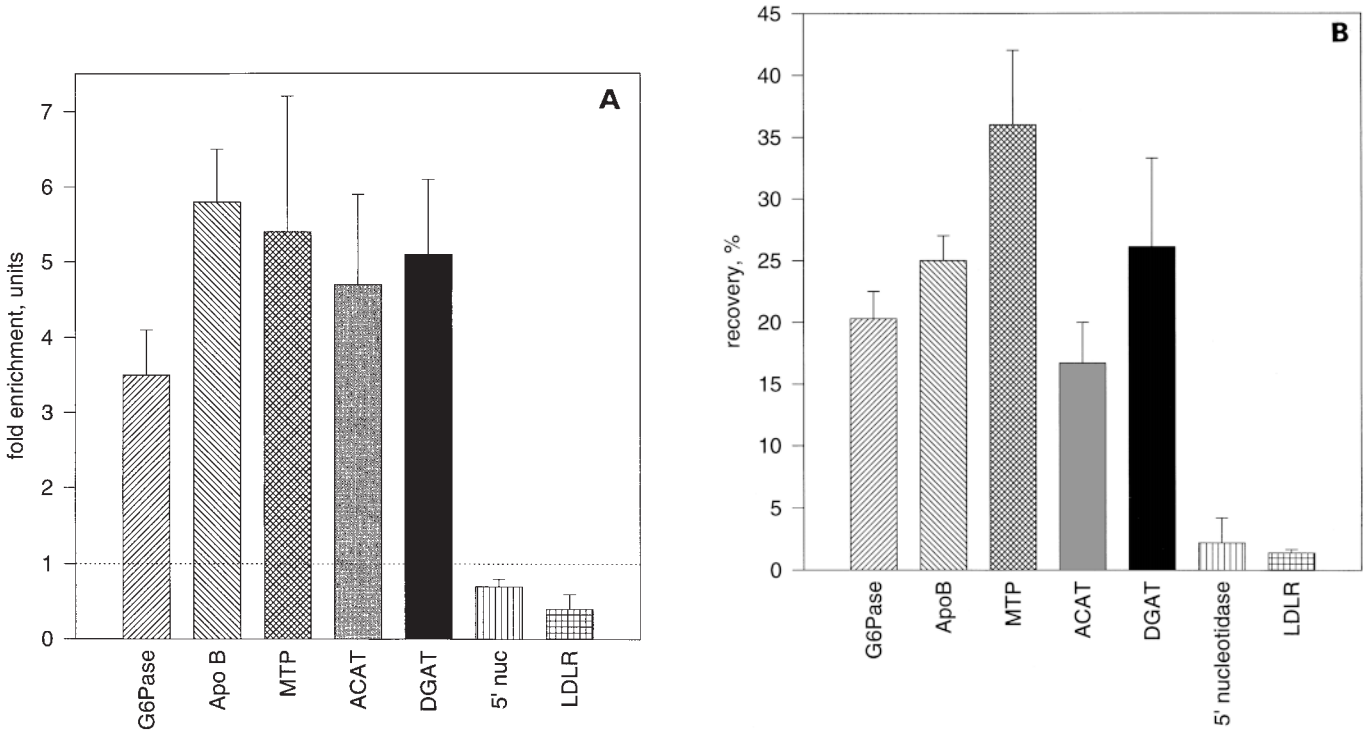

Fig. 4. Recoveries and enrichment of proteins and enzyme activities in the RER fraction that are implicated in lipoprotein assembly. G-6-Pase was used as RER reference whereas 5′ nuc and LDL R were measured to assess purity. ApoB, MTP, ACAT, and DGAT were measured as described in Materials and Methods. Their recoveries were equal to or greater than G-6-Pase in the RER membranes. Panel A: fold enrichment over homogenate set as 1.0. Panel B: % Recovery with the homogenate set as 100%. The data are the means ± SD of 6 independent experiments.

tially contaminating organelles (Fig. 4B). Whereas contaminating membrane protein recoveries were always less than 2–3%, RER markers averaged greater than 15% and reached as high as >40% for MTP, a known RER resident protein (14). Interestingly, about 25% of both apoB and DGAT activity in the whole homogenate was recovered in our RER fraction, indicating a comparatively high yield of these proteins involved in plasma lipoprotein biogenesis. Indeed, compared to the classic RER markers, RNA and glucose-6-phosphatase, the recoveries of apoB, MTP, and DGAT were greater in our RER fraction, and the recovery of ACAT was only slightly less at >15% (Fig. 4B).

RER enrichment of secretory proteins

We also examined the enrichment of known hepatocytic secretory proteins that were putatively contained within the lumenal space of our RER membrane fraction. As shown in **Fig. 5**, some of these proteins were barely detectable, such as hepatic lipase, or were even undetectable, such as apoC-III. Serum albumin and apoE were enriched about 2-fold over homogenate, whereas apoA-I was not. Surprisingly, apoA-IV was enriched an average of 5-fold with a wide range of deviation among experiments.

DISCUSSION

Compared to other methods of isolating ribosome-rich membranes from liver, the procedure described herein has several advantages. First, it is comparatively simple in that it does not require an ultracentrifuge, only a refrigerated Sorvall-type centrifuge with a large volume (e.g., SS-34 comparable) rotor. A Polytron with an ST-10 generator is, however, required for "gentle" homogenization if our method is to be authentically reproduced. Although it is possible that similar results might be achieved by different techniques of liver homogenization, other investigators will have to establish whether an equally large amount of pure and homogeneous RER fraction could be recovered after high-shear stress methods of homogenizing livers (e.g., Dounce or Potter/Elvhjem), a result that we think is unlikely. It should be emphasized that this is the first description, to our knowledge, of a non-ultracentrifugal RER isolation procedure in which the purity was established by quantitating membrane markers for potentially contaminating organelles. We established these measurements rigorously because we required the absence of other plasma lipoprotein-containing organelles (such as endosomes and Golgi apparatus) in order to isolate and biochemically characterize truly nascent plasma lipoprotein precursors assembled within the RER lumen.

A second and more important advantage is the demonstrated purity (assessed by membrane markers) and homogeneity (assessed by thorough ultrastructural analyses) of our RER fractions. The latter is remarkable because despite the unusually large size of the pellets, they were uniformly comprised largely of ribosome-rich membranes with no evidence of stratification of other organelles, a

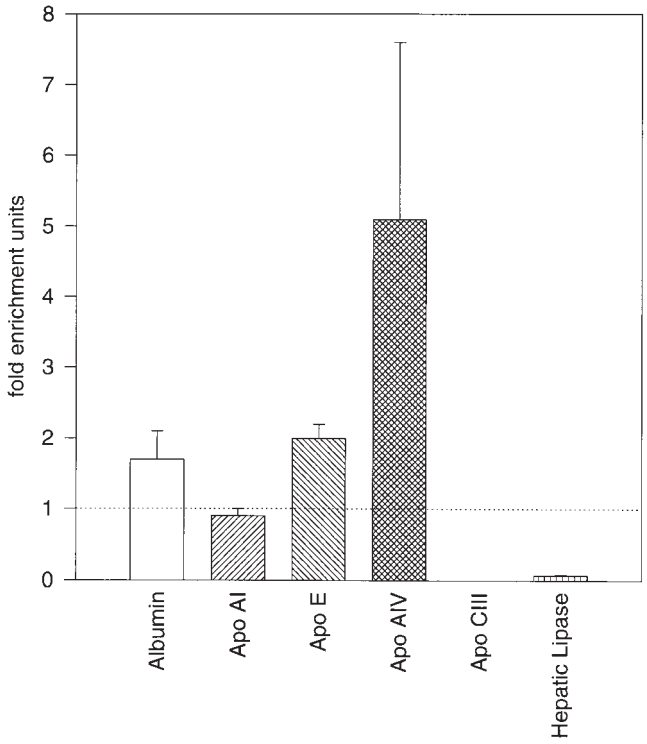

Fig. 5. Enrichment of hepatocyte secretory proteins in the RER fraction. Serum albumin, apoA-I, apoE, apoA-IV, apoC-III, and hepatic lipase content in the RER were measured as described in Materials and Methods and were expressed as fold enrichment over whole homogenate set as 1.0 The data are the means ± SD of 3 independent experiments.

flaw that often characterizes subcellular fractions obtained by ultracentrifugal methods. We estimate from our analyses of classical organelle membrane markers that our RER fraction is 90% or greater pure ribosome-rich membranes. In addition, the 'gentle" Polytron homogenization technique, together with a "gentle" calcium precipitation method followed by low speed centrifugal isolation, appeared to facilitate the recovery of a substantial proportion of the unique smooth-surfaced terminal ends of RER seen in intact hepatocytes by transmission electron microscopy and to which has been ascribed the function of VLDL and chylomicron assembly (1–4). Two observations contribute to this conclusion. First was the unexpectedly high recovery and enrichment of DGAT, an enzyme usually assigned to the SER compartment, whereas a classic marker for this latter organelle, HMG-CoA reductase, was virtually absent from our RER fraction. Second, our unpublished studies have shown that this RER fraction contains lipoprotein precursor particles of VLDL size, indicating that some of the unique smooth-surfaced terminal ends of hepatocyte RER cisternae (a compartment that has been implicated in VLDL assembly (1–3)) are also present. The very high levels of recovery and enrichment in our RER fraction of all four of those proteins (apoB, MTP, DGAT, and ACAT) that are known to be required for VLDL and chylomicron assembly also support the hypothesis that substantial amounts of the smooth-surfaced terminal ends of RER, the putative compartment of nascent VLDL assembly (1–4), were also recovered in our RER pellets.

A third attribute is the large quantity of RER membranes recovered per gram of liver. We are unaware of any previously described RER isolation procedure that recovers ~110–125 mg RER protein from 10–12 g of rat liver. This represents a yield of between 30–50% of that expected, based on the observation that one gram wet weight of rat liver contains about 40–50 mg of ER protein, about 60% of which is RER (46). Crude preparations of classic rat liver microsomes may yield equal masses of protein, but these fractions also contain substantial amounts of other subcellular organelles, in particular, sinusoidal plasma membranes (35) and thus are not comparable. A final attribute is the rapidity (~2 h) with which one can obtain this RER fraction from a liver. For our purposes, eliminating all visible floating fat from the supernatant was necessary, requiring an additional two to three low speed spins that other researchers may prefer to omit in order to further shorten the method. However, we emphasize that we did not analyze the purity or homogeneity of RER pellets under those abbreviated conditions, and consequently we do not know whether the low speed spins also reduced the amount of contaminating organelles, decreased the RER yield, or changed the results in some other way, although we suspect that it would.

The ultrastructural architecture, rich source of lipoprotein assembly-related proteins, and presence of VLDL precursor particles suggest that our RER fraction reflects a more structurally and functionally intact organelle as compared to classical microsomal vesicles. Therefore, in addition to the increased purity and mass of our RER fraction, we predict that other researchers may discover important new and unexpected biological molecules and other advantages to our RER fraction that are absent from typical whole microsomes or from ER subfractions prepared by density gradient ultracentrifugation.

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