# Biosynthesis of High Density Lipoprotein by Chicken Liver: Nature of Nascent Intracellular High Density Lipoprotein

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ABSTRACT Young chickens were administered L-[$^3$H]leucine and after 10 or 30 min the livers were removed and fractionated into rough (RER) and smooth (SER) endoplasmic reticulum fractions and into light, intermediate, and heavy Golgi cell fractions. The labeled high density lipoprotein (HDL), contained within these intracellular organelles was isolated either by immunoprecipitation using rabbit antiserum to rooster HDL, or by ultracentrifugal flotation between densities 1.063 and 1.21 g/ml. The radioactive apoproteins of nascent HDL were analyzed by SDS PAGE and detected by fluorography. Analyses of radioactive apoproteins obtained by immunoprecipitation from the contents of the RER, the SER, and the three Golgi complex fractions revealed only one apoprotein, A1. The C peptide present in serum HDL was not detected intracellularly. The radioactive apoprotein A1 which is present within the cisternae of the RER and the SER fractions failed to float, whereas apoprotein A1, present within the Golgi apparatus, readily floated between densities 1.063 and 1.21 g/ml. The HDL particles, isolated by flotation from the Golgi apparatus content, were further characterized by lipid and protein analyses and by electron microscopy. Golgi HDL particles have the same density as serum HDL. On a percentage basis, Golgi HDL contains less protein and more phospholipids than does serum HDL. Morphologically, Golgi HDL is different in appearance from serum HDL. It is more heterogeneous in size, with most of the particles ranging 8.3–25 nm in diameter. The spherical particles contain small membrane tails. Occasionally, a few disk-shaped bilayer structures are also found within the Golgi apparatus. These studies show that the newly synthesized apoprotein A1, present within the RER and the SER cell fractions, is not fully complexed with lipid and that apoprotein A1 does not acquire sufficient lipid to float at the proper HDL density until it enters the Golgi apparatus. The difference in chemical composition and the heterogeneous size of Golgi HDL may be attributed to the different stages of HDL maturation.

Plasma lipoproteins are lipid-protein complexes responsible for the transport of lipids in the blood. Four major classes of lipoproteins have been isolated from serum in all species tested and are designated according to their particle size and buoyant density as chylomicron (size: >100 nm in diameter; $d$ = <0.95 g/ml), very low density lipoprotein (VLDL: 30–80 nm in diameter, $d$ = 0.95–1.006 g/ml), low density lipoproteins (LDL: 28 nm in diameter; $d$ = 1.006–1.063 g/ml) and high density lipoproteins (HDL: 8–12 nm in diameter; $d$ = 1.063–1.21 g/ml). The distribution, composition, physicochemical properties, and metabolism of HDL have been well studied (7, 13, 19, 28, 48), but very little is known about the mechanisms by which HDL is produced.

In all species that have been studied, the liver and small intestine are found to be the major sites of HDL production (18, 19). HDL is synthesized independently and it is not formed in the blood as a metabolic product of VLDL. In some species, such as the rat, a study of HDL synthesis and secretion is complicated by the fact that both VLDL and HDL are produced simultaneously, that each lipoprotein class is heterogeneous with respect to its apoprotein components, and that several of the apoproteins are present in more than one class of

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lipoprotein (7, 13, 19, 48). However, it has been reported that, in young chicken plasma, HDL is 92% of the total lipoproteins (32) and that 90% of the protein content of HDL is composed of one apoprotein (27, 31). This makes young chickens a useful system with which to study the biosynthesis of HDL.

HDL is known to contain a neutral lipid core that is surrounded by polar lipids, cholesterol, and apoproteins (28). Studies using radioactive precursors in conjunction with cell fractionation procedures have shown that apoprotein synthesis takes place on the rough endoplasmic reticulum (RER) (1, 6, 37) and that lipid synthesis seems to occur on the smooth endoplasmic reticulum (SER) (14, 15, 49, 50, 52). Not known, however, are the intracellular sites or the process by which this complex particle is assembled. In this report we have, therefore, studied the occurrence and nature of nascent HDL at various intracellular locations known to be involved in plasma protein synthesis and secretion.

## MATERIALS AND METHODS

*Animals and Radiochemicals:* Young Leghorn chickens (age 5 to 10 d) were obtained from Spafas Poultry Farms (Norwich, CT.) L-[3,4-$^3$H]leucine (105 mCi/mmol) was purchased from Amersham/Searle Corp. (Arlington Heights, IL).

*Collection of Serum:* Blood was collected from the jugular vein of adult roosters and allowed to clot. The serum was separated from the clot by low speed centrifugation and adjusted to 1 mM EDTA, pH 7.4, and 154 mM NaCl.

*Isolation of HDL and Its Apoproteins:* HDL was purified from rooster serum by sequential flotation as described by Havel et al. (22). HDL was collected at a buoyant density between 1.063 and 1.21 g/ml. The material of $d > 1.21$ g/ml was considered to be nonlipoprotein plasma proteins and that of $d < 1.063$ g/ml to be the VLDL and LDL. The apoproteins were prepared by extracting the lipoproteins with ether:ethanol (2:3) (31). They were analyzed by SDS PAGE.

*Antibodies to Chicken HDL Apoprotein:* Antiserum to rooster HDL apoprotein was prepared by subcutaneous injection of the proteins into a rabbit as described by Hillyard et al. (24). The antiserum was checked by immunoelectrophoresis and by immunodiffusion on agar plates (39) and it was found to react only with HDL apoproteins and not with other plasma proteins which include the apoproteins of other lipoprotein classes. This antiserum was used to identify nascent HDL apoproteins in the liver and in plasma.

*In Vivo Incorporation of L-[$^3$H]Leucine into HDL Apoprotein:* Young chickens were administered L-[$^3$H]leucine intravenously via the wing vein and at various times the livers were removed and serum was collected. To measure the amount of radioactivity incorporated into total intracellular HDL, we rinsed the livers thoroughly, homogenized them in 10 mM sodium phosphate buffer, pH 7.4, containing 154 mM NaCl, 0.5% sodium deoxycholate, and 0.5% Triton X-100, and centrifuged them at 105,000 *g* for 60 min. The detergent soluble fraction was used for immunoprecipitation.

Blood samples were allowed to clot for several hours at room temperature and the serum was separated from the clot by centrifugation. The serum was adjusted to 10 mM sodium phosphate, pH 7.4 containing 154 mM NaCl, 0.5% sodium deoxycholate, and 0.5% Triton X-100 before immunoprecipitation.

Nascent HDL was immunoprecipitated from the detergent-treated samples by the method of Taylor and Schimke (51). The antigen-antibody complex was dissolved in 1 ml of soluene, and the radioactivity incorporated into HDL was measured by counting the solubilized antibody-antigen complex in presence of 0.1 ml of glacial acetic acid and 10 ml of toluene scintillation phosphor in a Packard Scintillation Counter (Packard Instrument Co., Inc., Downers Grove, IL).

*Preparation of Endoplasmic Reticulum Fractions and Golgi Complex Fractions:* Livers were homogenized in 0.25 M sucrose, filtered through a layer of cheesecloth, and a postmitochondrial supernatant was obtained by centrifuging the homogenate at 16,000 *g* for 10 min. A total microsomal fraction, i.e., a mixture of microsomes and Golgi complex elements, was obtained by centrifuging the postmitochondrial supernatant at 105,000 *g* for 90 min. The microsomal pellet was suspended in a small volume of 0.25 M sucrose, and sufficient 2.1 M sucrose was added to obtain a refractive index of 1.3920, equivalent to that of 1.22 M sucrose. Three Golgi complex fractions were isolated from this suspension by a modification (42) of the procedures of Ehrenreich et al. (8). 10-ml aliquots of this total microsomal suspension were overlaid with a discontinuous sucrose gradient composed of 8.5 ml of 1.15, 0.86, 0.6, and 0.25 M sucrose and centrifuged for 210 min at 82,500 *g* in a Beckman SW27 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). The materials that floated to the 0.25 M/0.6 M, 0.6 M/0.85 M and 0.85 M/1.15 M sucrose interface were collected and termed light, intermediate and heavy Golgi complex fractions. Details of the isolation procedure and the characterization of the fractions are given elsewhere (3, 8, 25). The remainder of the gradient (below 1.15 M sucrose) contained a mixture of RER and SER. The RER and SER fractions were further separated by the procedure of Ragland et al. (41).

*Isolation of Radioactive Nascent HDL from the RER, the SER, and Golgi Complex Fractions:* Nascent HDL was isolated both by immunoprecipitation and by flotation from the contents of the various intracellular organelles. For immunoprecipitation, the cell fractions were treated with 0.5% sodium deoxycholate and 0.5% Triton X-100 in the presence of phosphate-buffered saline (10 mM phosphate buffer, pH 7.5, 150 mM NaCl), and a soluble fraction was obtained following centrifugation at 105,000 *g* for 60 min. The radioactive HDL apoproteins in the detergent-soluble fraction were then recovered by immunoprecipitation with antiserum to chicken HDL apoproteins following the procedure described by Taylor and Schimke (51).

To float the radio-labeled HDL at the same buoyant density as serum HDL (1.063–1.21 g/ml), we disrupted the RER, SER, and Golgi complex cell fractions by nitrogen cavitation following the procedure of Fleischer (11). The cell fractions were suspended in 2 vol of 2 M NaCl and 7 vol of 0.2 M $NaHCO_3$. The mixture was disrupted in a Cell Disruption Bomb (Parr Instruments Co., Moline, IL) by compressing it at 1,800 psi for 15 min and releasing the mixture dropwise from the chamber. The disrupted mixture was adjusted to 0.25 M sucrose, and the membranes were collected as a pellet by centrifugation at 105,000 *g* for 60 min. The supernate, which contains the intracisternal contents, was dialyzed against 10 mM Tris-HCl, pH 7.4 containing 154 mM NaCl, 1 mM EDTA, and 0.01% $NaN_3$. HDL was obtained from these dialyzed intracisternal contents by sequential flotation as described earlier (22). HDL was collected at a density of 1.063 to 1.21 g/ml.

The efficiency of nitrogen cavitation in releasing nascent HDL apoprotein A1 from RER, SER, and Golgi complex cell fractions was determined by measuring the total amount of radioactive apoprotein A1 in the cell fractions and comparing that value to the amount of radioactive apoprotein A1 released by nitrogen cavitation and that remaining in the membrane subfraction. The total amount of apoprotein A1 in the cell fraction was measured by immunoprecipitation following treatment of the cell fraction with 0.5% sodium deoxycholate and 0.5% Triton X-100. After nitrogen cavitation, the membrane-subfraction and the released materials were separated by centrifugation and the amount of radioactive apoprotein A1 in these subfractions, which were also treated with detergents, was determined. In the RER, 15% of the total protein and 71% of apoprotein A1 were released by nitrogen cavitation. In the SER, 16% of the total protein and 56% of the apoprotein A1 were released and, in combined Golgi complex cell fraction, 29% of the total protein and 76.5% of apoprotein A1 were released. In all three cell fractions, the percent recovery of apoprotein A1 was close to 100%.

*SDS Gel Electrophoresis:* SDS PAGE was carried out following the procedure of Weber and Osborn (53). Lipoproteins were concentrated and their lipid was extracted with ice-cold diethyl ether:ethanol (2:3 vol/vol) followed by three extractions with diethyl ether (31). The lipid-free proteins were collected by centrifugation and prepared for electrophoresis as described (53).

After Coomassie Blue staining, the gels were prepared for fluorography following the procedure of Bonner and Laskey (5) and Laskey and Mills (33). The fluorographs were obtained on Kodak Royal X-omat AR film and exposed at −70° for 14 d.

*Determination of Lipids:* Lipid analysis was performed on HDL isolated by flotation from the Golgi complex fraction and from sera. The lipids were extracted with chloroform-methanol (2:1, vol/vol) by the method of Folch et al. (12). The lipid extracts, dried *in vacuo*, were weighed, redissolved in chloroform, and resolved by thin-layer chromatography on 250-μm layers of silica gel GF Uniplate (Analtech, Inc., Newark, DE) in a solvent system composed of hexane-diethyl ether-acetic acid (80:20:1, vol/vol) (47). This method separates phospholipids, cholesterol, cholesterolesters, and triglycerides.

The phospholipids were further separated by two-dimensional thin-layer chromatography on 250-μm layers of silica gel GF Uniplates (Analtech, Inc., Newark, DE) using the following solvent systems: chloroform-methanol–14.8 N ammonium hydroxide (65:25:5, vol/vol) (43), and chloroform-acetone-methanol-acetic acid-$H_2O$ (50:20:10:10:5, vol/vol) (30). Phospholipids were identified by cochromatography with authentic-reference compounds. The separated phospholipids were recovered from the silica gel plates and analyzed for phosphorus following the procedure of Bartlett (2).

Free cholesterol and cholesterol esters were detected by thin-layer chromatography as described above. The materials were scraped off the plates and quantitated according to the method of Searcy et al. (46). The values for triglycerides were obtained by subtracting the sum of cholesterol, cholesterol esters, and phospholipids from the total lipid values.

*Electron Microscopy:* Samples of subcellular fractions suspended in 0.25 M sucrose were fixed by mixing an equal volume of sample with a 4%

formaldehyde-glutaraldehyde mixture (the formaldehyde freshly prepared from paraformaldehyde) in 0.2 M Na-cacodylate-HCl buffer, 7.4 (25). After 30 min, the suspensions were centrifuged for 20 min at 37,500 *g* in a Beckman SW 50.1 rotor. The pellets obtained were postfixed with 2% $OsO_4$ for 60 min, stained en bloc with 2% uranyl acetate, dehydrated, and embedded in Epon. Thin sections were cut through the entire depth of each pellet, stained with uranyl acetate and lead citrate, and examined in a Philips 201 microscope.

HDL was isolated by flotation from serum and from the contents of the Golgi complex fractions. They were fixed in suspension by mixing equal volumes of the sample with 4% $OsO_4$ for 24–48 h at 4°C. After fixation, the suspension was centrifuged for 30 min at 84,000 *g* in a Beckman SW 50.1 rotor. The fixed HDL pellet was stained and processed as described above.

Negative staining was done after the floated HDL was extensively dialyzed against 154 mM NaCl and 1 mM EDTA. The standard carbon film technique using 2% phosphotungstic acid (pH 6.4) as described by Hamilton et al. (21) was used.

## RESULTS

### General Morphology of RER, SER, and Golgi Complex Cell Fractions

The RER fraction is composed predominantly of ribosomes bound to membrane vesicles (Fig. 1 *a*) while the SER represents smooth membrane vesicles with a few mitochondria and Golgi complex membrane contaminants (Fig. 1 *b*). The modified procedure of Ehrenreich et al. (42) for the preparation of Golgi complex fractions, when applied to chicken liver, yields three subfractions, light, intermediate, and heavy. The light fraction is composed mostly of vesicles filled with dense particles similar in appearance to very low density lipoproteins (Fig. 2 *a*). The intermediate fraction is composed of some filled vesicles and some Golgi cisternae (Fig. 2 *b*), whereas the heavy Golgi fraction is composed of typical Golgi complex cisternae (Fig. 2 *c*).

### Time Course of Incorporation of [$^3$H]Leucine into Hepatic and Serum HDL

These initial experiments were designed to establish the time course for intracellular synthesis of apoprotein A1 and the relationship of intracellular and secreted apoproteins. Results of such an experiment are shown in Fig. 3. The incorporation of L-[$^3$H]leucine into hepatic HDL was linear for 20 min, and after 30 min it began to decline and dropped to 50% of its maximal level by 60 min. There was a minimal amount of radioactive HDL in the plasma in the first 15 min but it rose linearly, reaching maximal amount by 40 min.

### Nascent HDL in RER, SER, and Golgi Fractions

The chickens were administered L-[$^3$H]leucine and after 10 or 30 min the liver was removed, fractionated into RER, SER, and Golgi cell fractions, and the HDL contained within these cell fractions were isolated by immunoprecipitation or by centrifugal flotation. The radioactive apoproteins were separated by SDS PAGE and detected by fluorography. Most of the protein radioactivity which was immunoprecipitated from the RER and the SER cell fractions after 10 min of in vivo

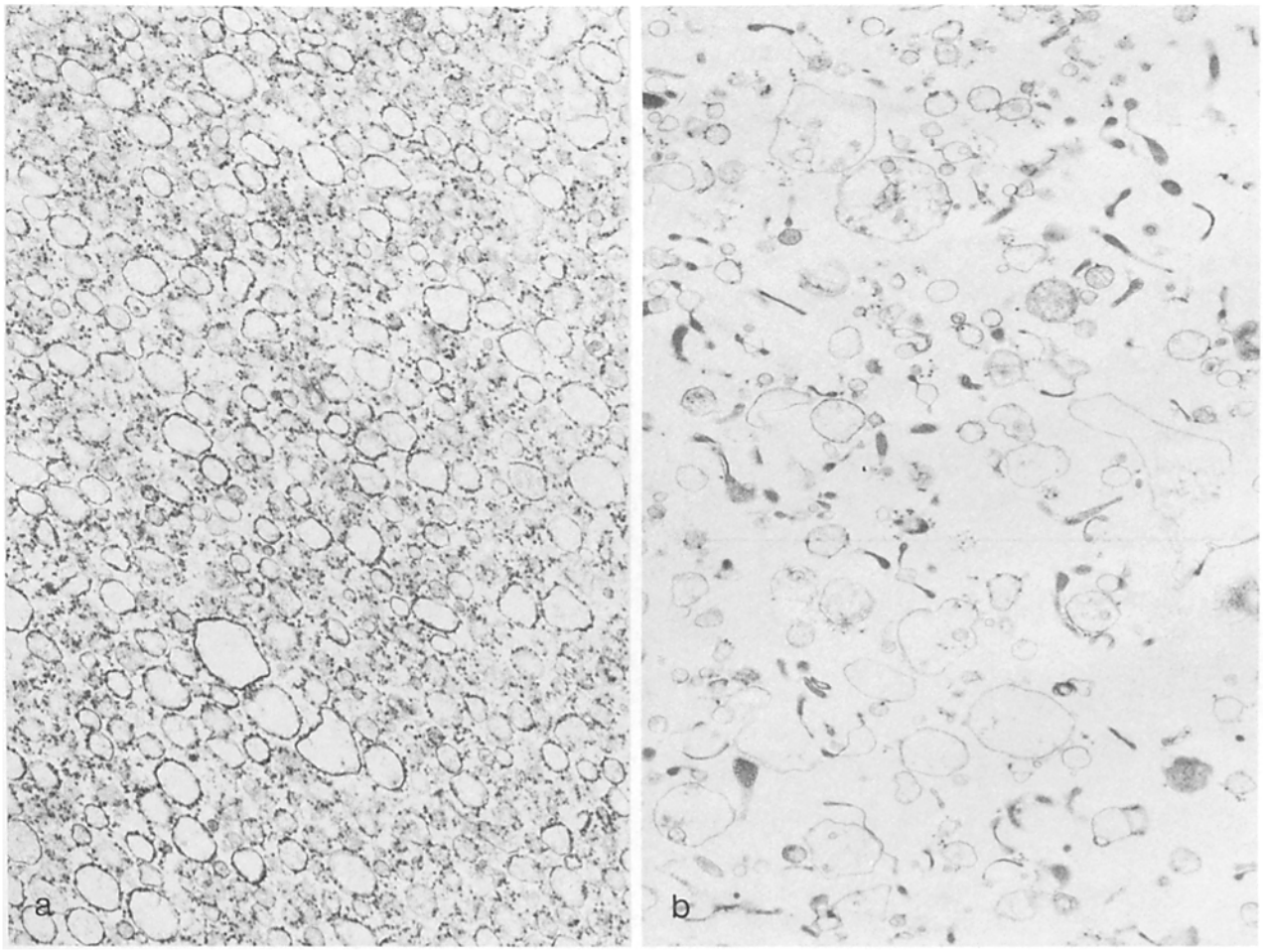
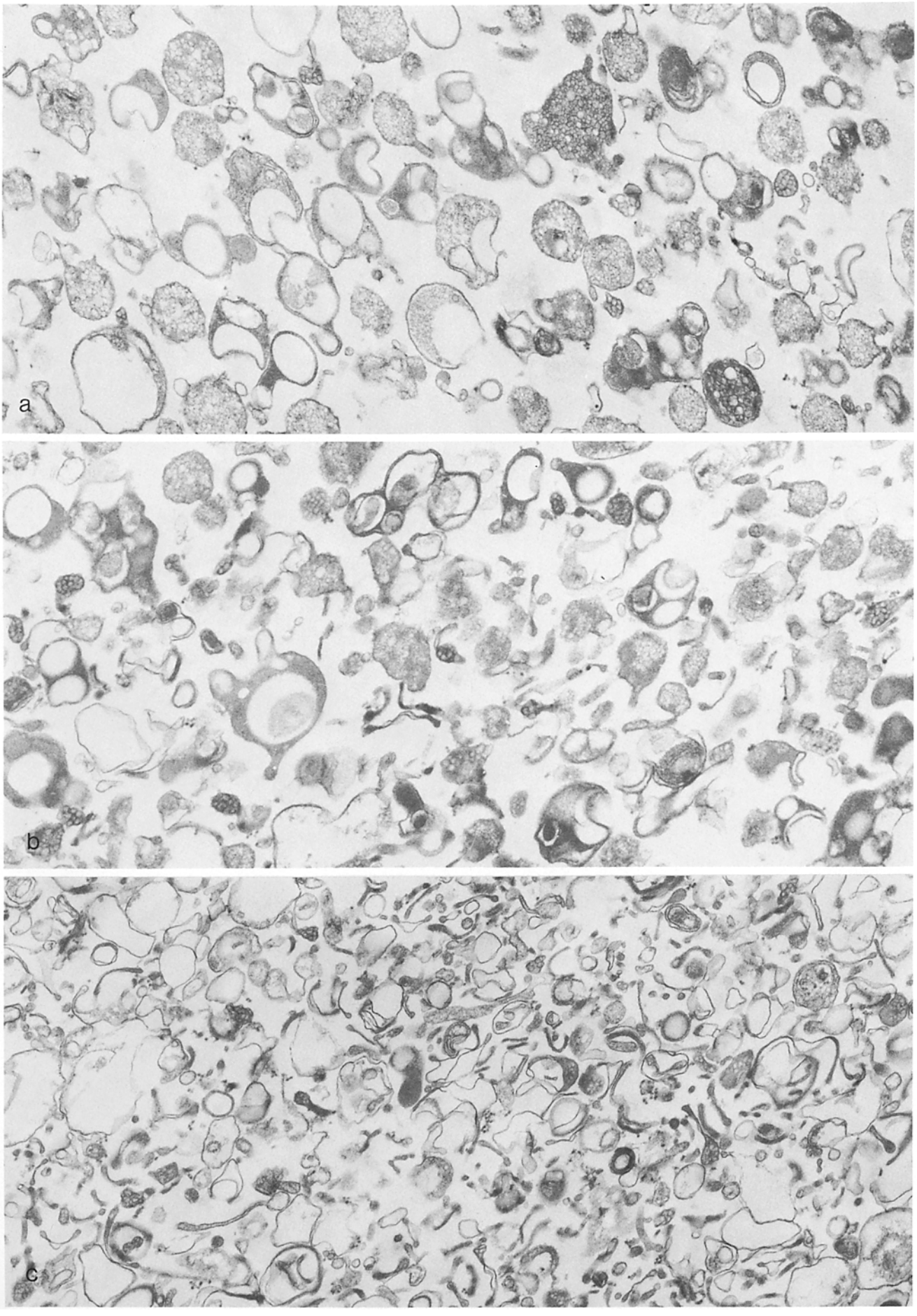



FIGURE 1 RER and SER cell fractions were prepared from livers of young chickens and fixed with glutaraldehyde-formaldehyde and postfixed in $OsO_4$ as indicated in Materials and Methods. (*a*) RER fraction. It consists mostly of membrane vesicles bound with ribosomes. (*b*) SER consisting mostly of smooth membrane vesicles with a few mitochondria and some Golgi elements. × 28,000.


a
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c

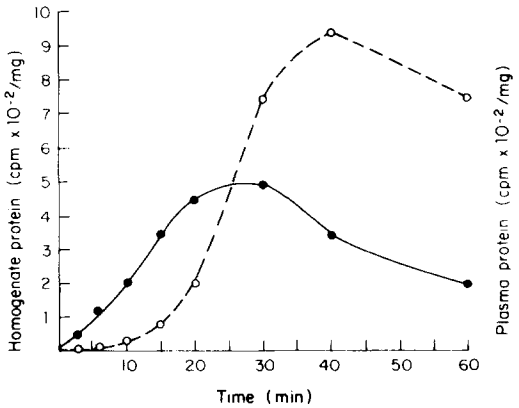


FIGURE 3 Time course of HDL secretion by chicken liver. Young chickens were injected intravenously with L-[$^3$H]leucine and at indicated times the blood and livers were collected. Radioactive HDL was isolated by immunoprecipitation from a detergent-treated liver homogenate and from serum. Details are given in Materials and Methods. (●) Liver. (○) Serum.

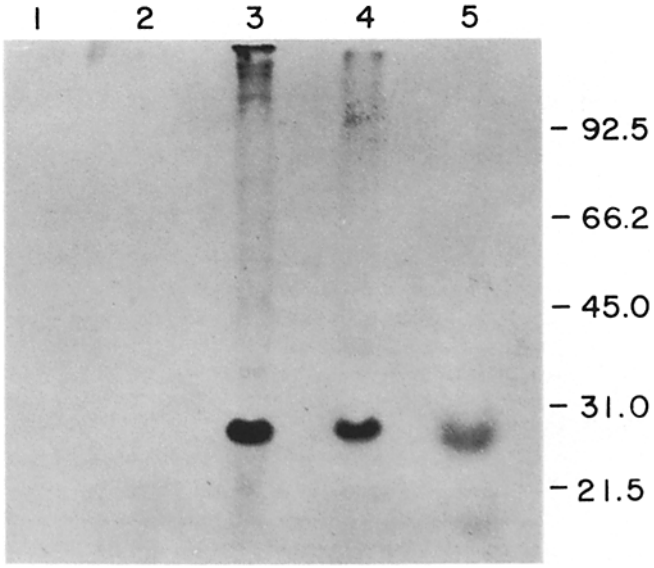


FIGURE 4 Fluorogram of HDL apoproteins isolated from rough- and smooth-endoplasmic reticulum fractions. Young chickens were administered L-[$^3$H]leucine and, after 10 min, the livers were removed, fractionated into RER and SER fractions, and the HDL contained within these fractions was isolated by centrifugal flotation and immunoprecipitation. The apoproteins were analyzed by SDS PAGE and identified by fluorography. The details of the procedures are given in Materials and Methods. Lanes *3* and *4* represent nascent HDL apoproteins obtained by immunoprecipitation from the contents of the RER and the SER fractions from 2 g of liver. Lanes *1* and *2* represent nascent HDL obtained by flotation from the contents of the RER and SER fractions from 26 g of liver. Lane *5* shows the apoprotein pattern of serum HDL obtained by flotation. Measurements, × $10^{-3}$.

labeling electrophoresed the same as HDL apoprotein A1 obtained from the serum. There was a small amount of radioactivity in the high molecular weight range, which could be due to aggregation of protein, but there was no evidence of the presence of radioactive proteins of lower molecular size than apoprotein A1 (Fig. 4, lanes *3* and *4*). By contrast, the contents of the RER and the SER cell fractions did not yield radioactive proteins in complexes that could be floated to a buoyant density (1.063–1.21 g/ml) similar to that of serum HDL (Fig. 4, lanes *1* and *2*). This indicates that apoprotein A1 is present in these cell fractions but has not been sufficiently complexed with lipids to allow it to gain its appropriate buoyant density.

Nascent intracellular HDL from the contents of the hepatic Golgi complex cell fractions was also isolated by immunoprecipitation and flotation. In these experiments, the chickens were labeled in vivo for 30 min. Immunoprecipitation of the radioactive protein from the three Golgi cell fractions again yielded one major radioactive protein with electrophoretic properties similar to those of serum apoprotein A1. There was no evidence of other radioactive proteins, except for lanes *1* and *2* (Fig. 4), in which some of the radioactivity remained at the top of the running gel as an aggregate (Fig. 5, lanes *1*, *2*, and *3*).

To examine the ability of nascent HDL from the Golgi cell fractions to float at its appropriate density (1.063–1.21 g/ml),

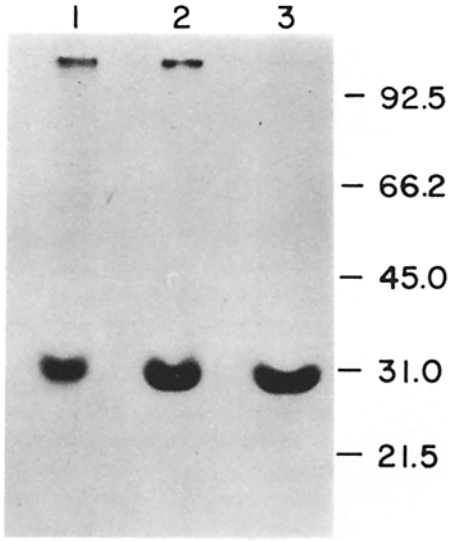


FIGURE 5 Fluorogram of HDL apoproteins isolated by immunoprecipitation from three Golgi cell fractions. Young chickens were administered L-[$^3$H]leucine and, after 30 min, the livers (6 g) were removed and fractionated into light, intermediate, and heavy Golgi complex cell fractions (42). The HDL apoproteins present within these fractions were obtained by immunoprecipitation and analyzed by SDS PAGE and fluorography. For details see Materials and Methods. Lane *1* contains the apoproteins isolated from light Golgi complex cell fraction, lane *2* that from the intermediate fraction, and lane *3* that from the heavy cell fraction.

FIGURE 2 The light, intermediate and heavy Golgi complex cell fractions were prepared from the livers of young chickens as described in Materials and Methods. (*a*) Golgi complex light fraction. The preparation is homogeneous, consisting of rounded secretion vacuoles, some of which are filled and marked by lipoprotein particles. There are relatively few Golgi cisternae. (*b*) Intermediate Golgi complex fraction. The predominant elements are secretion vacuoles and Golgi cisternae. A few broken membranes and a few lysosome-like vesicles are also evident. (*c*) Heavy Golgi complex fraction. This fraction is composed mostly of flattened Golgi cisternae, some with distended rims. Occasionally, some of these distended rims are marked by lipoprotein particles. A few smooth membrane vesicles and a few broken membranes are also present. × 28,000.

we prepared $^{3}$H-labeled HDL from both the Golgi complex content, obtained by disruption of the total Golgi complex cell fraction, and from sera by centrifugal flotation between densities of 1.063 and 1.21 g/ml. About 50% of the radioactive apoprotein A1 released by nitrogen cavitation was recovered at this density. Fig. 6 shows the fluorogram of the SDS PAGE analysis of HDL apoprotein isolated by flotation from the Golgi complex content and from sera. The results show that the newly synthesized HDL obtained from the Golgi complex fraction, unlike nascent HDL obtained from RER and SER fractions, is capable of floating at the appropriate density (1.063–1.21 g/ml) and consists mainly of apoprotein A1. A few minor radioactive protein bands which migrate more slowly on SDS PAGE are also evident (Fig. 6, lane *1*). These may represent contaminating membrane proteins. The serum HDL consists mainly of radioactive apoprotein A1, but a low molecular weight protein which migrates like the C peptides is also evident (Fig. 6, lane *2*). This low molecular weight radioactive protein is not seen in the Golgi complex fraction. These experiments indicate that similar apoprotein patterns are obtained whether nascent HDL is isolated from the liver by immunoprecipitation or by flotation, that the lipid and protein moieties are assembled by the time the nascent HDL reaches the Golgi vesicles, and that the low molecular weight apoprotein component (C peptide) is probably added to HDL outside of the liver.

## Composition of Golgi HDL

HDL, obtained from the combined Golgi complex cell fractions by flotation, was analyzed for proteins and lipids and a comparison was made with HDL obtained by flotation from serum. HDL from the Golgi complex cell fractions is composed of 42% protein, 40% phospholipids, 11% cholesterol esters, 5% cholesterol, and 2% triglycerides. Serum HDL is 48% protein, 26% phospholipids, 12% cholesterol esters, 6% free cholesterol, and 8% triglycerides. Thus, the Golgi complex HDL has a larger percentage of phospholipids and a lower content of triglycerides than serum HDL (Table I). The phospholipid compositions of serum and Golgi complex HDL were further analyzed by two-dimensional thin layer chromatography. The phospholipids of Golgi complex HDL contained a greater percentage of lysolecithin, sphingomyelin, phosphatidylethanolamine, and phosphatidylserine than those of serum HDL. Serum HDL contained a larger percentage of phosphatidylcholine and phosphatidylinositol (Table II).

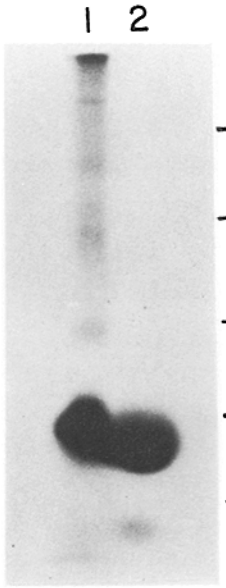


FIGURE 6 Fluorogram of HDL apoproteins isolated by flotation from Golgi complex cell fractions and from serum. The apoproteins of HDL contained within the Golgi cell fractions were labeled in vivo by administering L-[$^{3}$H]leucine to chickens as described in Fig. 5. The Golgi fractions from 28 g of liver were pooled and ruptured to release their contents as described in Materials and Methods. Radioactive nascent HDL was prepared both from the Golgi contents and from sera (18 ml) by centrifugal flotation. The apoproteins were analyzed by SDS PAGE and detected by fluorography. For details see Materials and Methods. In lane *1*, 4,500 cpm of radioactive HDL from the Golgi cell fraction was electrophoresed, and in lane *2*, 3,800 cpm of serum HDL was analyzed. Measurements, $\times 10^{-3}$.

## Morphology of HDL Particles Obtained from Chicken Serum and Golgi Complex Cell Fractions

An examination of the Golgi complex cell fractions prepared from chicken livers showed many vesicles containing lipoprotein particles. Often, these lipoprotein particles were irregularly shaped and sometimes appeared as a unique bilayer structure with a major axis measuring 44 to 66 nm and a minor axis measuring ~9–13 nm (Fig. 7). The occurrence of this bilayer structure within the secretory vesicles has not heretofore been described. However, it has been postulated (21) that the liver secretes HDL in the form of bilayer disks composed of phospholipids and cholesterol. The presence of bilayer sheets within the Golgi complex vesicles is therefore interesting and may support the hypothesis mentioned above. With this in mind, serum HDL and Golgi complex HDL were prepared and processed for electron microscopy as described in Materials and Methods.

In thin sections, serum HDL and Golgi complex HDL did not yield clear profiles and thus are not illustrated. However, there were some apparent differences. The serum HDL were mostly spherical particles and Golgi complex HDL were elongated particles reminiscent of the structures described by Hamilton et al. (21) in rat liver perfusate.

In negatively stained preparations, serum HDL appeared as dispersed spherical particles, with a mean diameter of 9.5 nm. Most of the particles were of a similar size, but a few particles

TABLE I

*Protein and Lipid Composition of Serum and Golgi Complex HDL*

| Component | Serum HDL | Golgi complex HDL |
|---|---|---|
| Protein | 48.0 ± 0.50 | 42.0 ± 1.86 |
| Lipids | 52.0 ± 1.0 | 58.0 ± 1.86 |
| Phospholipids | 26.5 ± 1.5 | 40.0 ± 4.41 |
| Cholesterol esters | 12.0 ± 0.5 | 11.0 ± 3.8 |
| Cholesterol | 6.0 ± 0.5 | 5.0 ± 2.08 |
| Triglycerides | 8.0 ± 0.5 | 2.0 ± 0.67 |

The results are expressed as percent by weight ± standard error of three determinations. Individual lipids are expressed as percentage of total lipid fraction.

TABLE II

*Percent Phospholipid Distribution in HDL Floated from Serum and from the Contents of the Golgi Cell Fraction*

| Phospholipids | Serum HDL | Golgi complex content HDL |
|---|---|---|
| Lysolecithin | — | 6.9 ± 3.68 |
| Sphingomyelin | 2.5 ± 1.15 | 5.8 ± 2.93 |
| Phosphatidylcholine | 54.0 ± 1.0 | 39.0 ± 3.06 |
| Phosphatidylethanolamine | 15.0 ± 1.53 | 18.0 ± 3.07 |
| Phosphatidylinositol | 12.0 ± 2.73 | 8.9 ± 2.53 |
| Phosphatidylserine | 16.0 ± 1.86 | 21.3 ± 2.77 |

Values are mean plus and minus standard error of three determinations. Phospholipid = lipid phosphorus × 25.

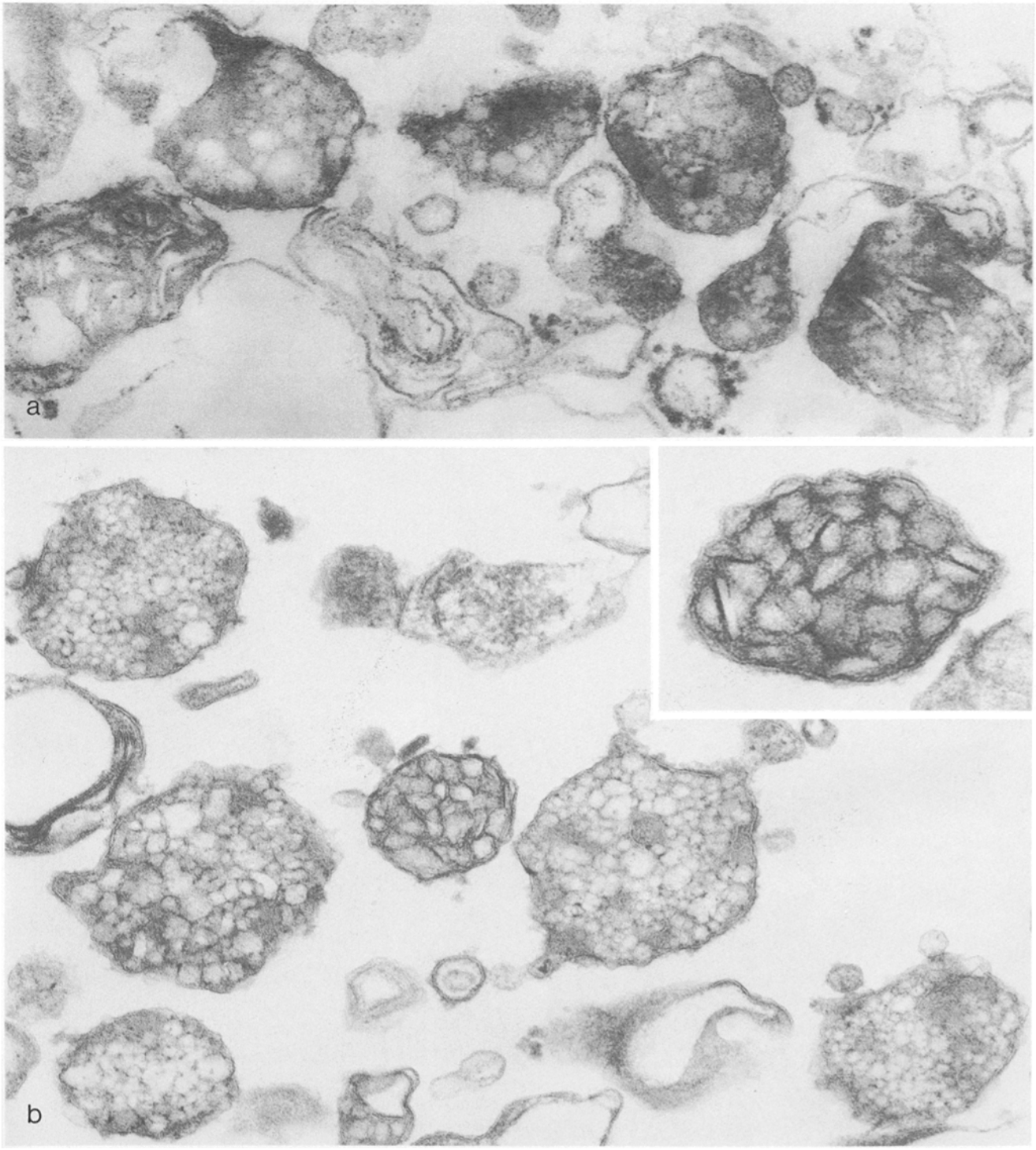


FIGURE 7 A section through a pellet of isolated Golgi complex cell fractions showing the presence of membranelike structures within the secretory vesicle. The light and intermediate Golgi complex cell fractions were isolated from livers of young chicken as described in Materials and Methods. The pooled Golgi complex fractions were fixed with glutaraldehyde-formaldehyde and postfixed in $OsO_4$ as indicated in Materials and Methods. (*a*) Represents a field towards the bottom of the pellet of the Golgi cell fractions exhibiting Golgi vesicles containing either spherical or membranous particles of varied sizes. A few broken membranes, rough microsomes and lysosomes appear as contaminants. × 90,000. (*b*) Represents a field from the top layer of the same preparation. The secretory vesicles are filled with clusters of lipoprotein particles, and the contents of some of these secretory vesicles again include membranous elements. × 90,000. The *inset* illustrates one secretory vesicle at higher magnification. The membranous nature of the content is apparent. × 180,000.

with larger diameters were also present in this preparation (Fig. 8 *a*).

Golgi complex HDL are quite different in appearance from serum HDL. They are more heterogeneous in size, appearing as spherical structures with diameters ranging from 8.3 to 25 nm. Most of the Golgi complex HDL particles contain small membrane tails. The origin of these tails is not clear. In addition to these particles some disk-shaped structures are also present.

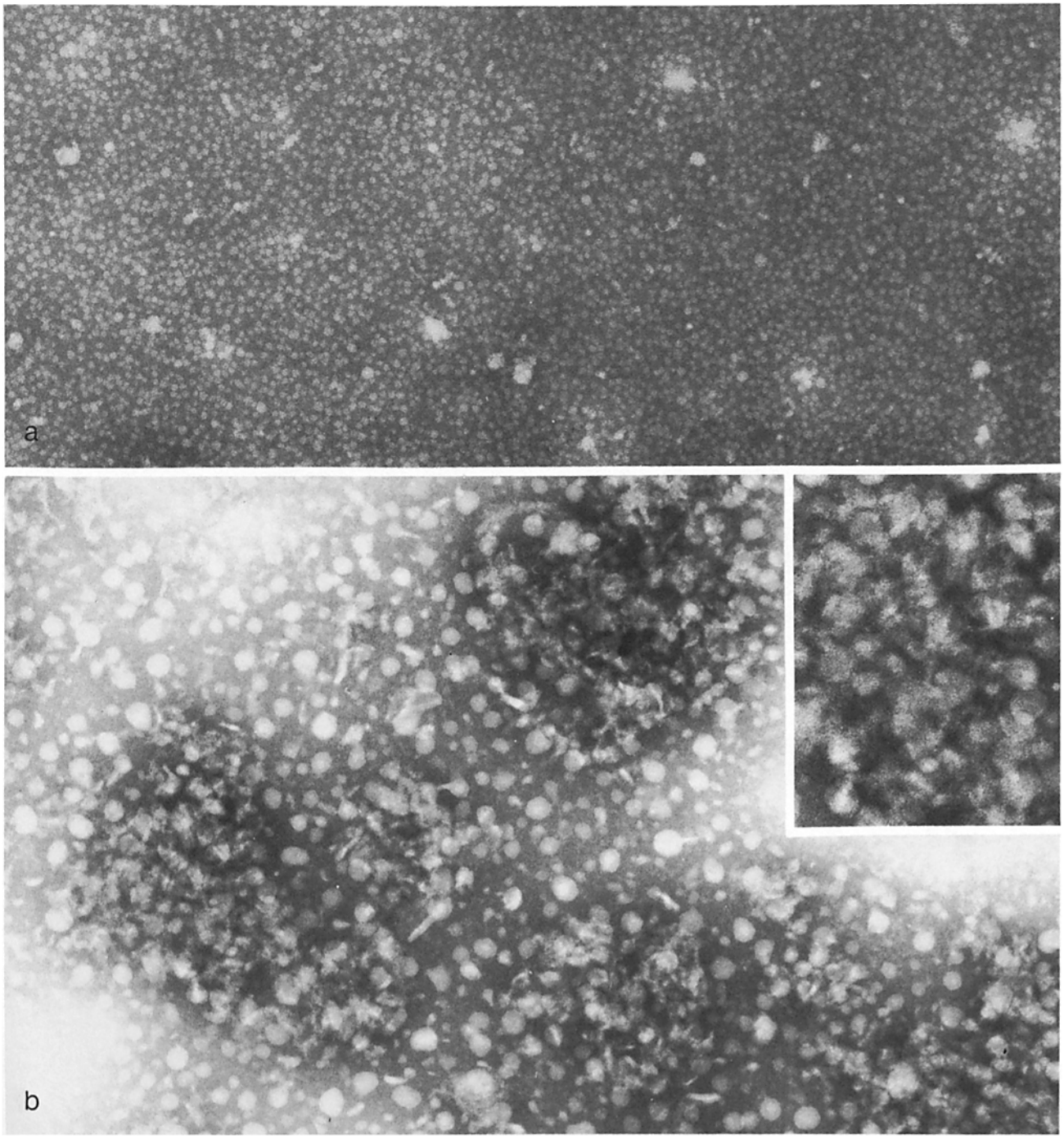


FIGURE 8 Negative-stained preparations of unfixed HDL obtained from chicken serum and from the contents of Golgi complex cell fractions. (*a*) Serum HDL appears homogeneous in size (9.5 nm diameter). A few larger particles are usually present which may represent an aggregate of smaller particles. × 141,000 (*b*) HDL particles isolated from Golgi complex cell fractions appear to have two populations of particles. One group is spherical and smaller in size. The diameter of this smaller particle ranges between 8.3–25 nm, and they possess a short membrane tail. A second group of larger particles, ranging from 12.5 to 21 nm, is also present. These larger particles appear to be short membrane stacks. All specimens were prepared, as described in Materials and Methods, from particles which floated at a *d* of 1.063–1.21 g/ml. × 144,000. The *inset* illustrates a higher magnification of the larger particle showing the membrane stacks. × 240,000.

These disk structures have 4.1-nm thick membranes of varying lengths ranging from 12.5 to 21 nm. The majority of Golgi complex HDL is of the spherical type with membrane tails (Fig. 8*b*).

## DISCUSSION

These studies indicate that in young chickens the synthesis of the major apoprotein of HDL in the liver precedes its appearance in the plasma and that the intracellular transit time for radioactive HDL is ~20–30 min which is in agreement with the transit time of other plasma proteins (40). Apoprotein A1 is present in the RER, SER, and Golgi complex cell fractions but it only achieves the buoyant density of HDL when it reaches the Golgi apparatus. It appears, however, that nascent HDL may not be fully assembled in the Golgi apparatus since there are biochemical and morphological differences between Golgi complex and serum HDL.

HDL apoprotein has been detected in rat hepatocytes and, using radioactive precursors, it has been determined that in the perfusate there is an accumulation of both lipids and protein at the expected buoyant density of HDL (36, 38). However, none of the previous studies have elucidated the exact cellular sites at which the attachment of the various lipid components to the apoprotein occurs or the mechanisms governing this assembly process. Using radioactive precursors, in conjunction with cell fractionation procedures, investigators have shown that VLDL apoprotein synthesis takes place on the RER (1, 6, 37) and, on the basis of morphological and autoradiographic studies, it has been suggested that triglyceride and phospholipid assembly into VLDL takes place in the SER (14, 15, 49, 50, 52). Nascent VLDL, obtained from a Golgi cell fraction from rat liver, has been analyzed biochemically and morphologically and was found to be similar to serum VLDL (15, 20, 34, 35, 37).

Our work demonstrates that the newly synthesized apoprotein A1 fails to float between densities 1.063 and 1.21 g/ml, even though it is clearly present within the RER and SER fractions. On the other hand, in the Golgi complex, the same apoprotein A1 is capable of floating at a density of 1.063–1.21 g/ml. This indicates that while apoprotein A1 is within the cisternae of the RER and SER it has not yet acquired its full complement of lipids and that most of the assembly of lipids and protein occurs in the Golgi apparatus.

Fluorographic analysis of the newly synthesized radioactive HDL obtained by ultracentrifugal flotation from the contents of the Golgi apparatus demonstrated that apoprotein A1 is the predominant major protein and that the low molecular weight C peptide present in plasma HDL is missing. The absence of this low molecular weight C peptide in nascent HDL suggests either that the techniques used to isolate nascent HDL fail to detect this protein or that this protein is added to plasma HDL outside of the liver. The C peptides are known to recycle in the plasma between the VLDL and HDL classes of lipoprotein (9, 10, 23, 44). A few minor high molecular weight protein bands were also found to be associated with the HDL isolated from the Golgi complex cell fractions. These high molecular weight protein bands may represent contaminating membrane proteins which floated with HDL since they were not present in the immunoprecipitate (Fig. 5).

A comparison of chemical composition of Golgi complex and serum HDL showed that serum HDL is slightly higher in protein content than Golgi complex HDL. Analysis of the lipid of Golgi complex HDL indicates that it may be different from that of serum HDL since Golgi complex HDL contains proportionally less triglyceride and more phospholipids than does serum HDL. The distribution of phospholipids in Golgi complex HDL appears to bear an overall resemblance to that of its serum counterpart, with a few exceptions. The Golgi complex HDL is characterized by a greater percentage of lysolecithin and sphingomyelin than occurs in serum HDL. We do not know whether the greater percent of lysolecithin and the decrease in lecithin in Golgi complex HDL (Table II) can be attributed to the esterification of cholesterol by lecithin cholesterol acyltransferase (LCAT). This reaction, which is known to occur during the formation of HDL, produces lysolecithin from lecithin (16, 17). Another possibility is that, since lysolecithin and sphingomyelin are known to be major components of Golgi membrane lipids (29, 54), they are contributed by broken pieces of Golgi complex membrane which may float at a density similar to that of HDL. The presence of a few minor high molecular weight protein bands (Fig. 6) in this fraction may also indicate the presence of some membrane components.

Comparison of the morphology of Golgi complex HDL to that of serum HDL suggests that the Golgi complex HDL is not yet fully complexed since the Golgi complex HDL is more heterogeneous and larger in size. HDL particles obtained from the Golgi complex content appear to be a mixture of disk and spherical forms (Fig. 8*b*). Most of the spherical particles are associated with short membrane tails. Similar association between membranous fragments and all classes of lipoproteins isolated from the rat liver Golgi apparatus has been reported (26). The origin of these membrane tails is not known. They may be a part of the Golgi complex membrane which is associated as a contaminant with HDL particles or they may reflect an intermediate stage in particle formation.

In thin sections, HDL fractions isolated from the Golgi apparatus appear to be membranelike structures, while in negative stain they appear as discoidal or spherical forms. The occurrence of trilaminal disks within the secretory vesicles is, however, rare. Only 10% of the Golgi complex preparations exhibit such a membranous structure within the secretory vesicle. Hamilton et al. (21) have reported that HDL originates as bilayer disks when rat livers are perfused with an inhibitor of LCAT. Discoidal HDL has also been reported in patients suffering either from LCAT deficiency (17) or from cholestasis (4), and in cholesterol-fed guinea pigs (45). At present it is not clear whether the membranous nature of some of the Golgi HDL represents a precursor of serum HDL or whether it is an aberrant lipoprotein influenced by an unusual physiological state of the liver. The infrequent occurrence of the trilaminar structure within the secretory vesicles may also be due to a rapid conversion of HDL particles from discoidal to spherical forms within the Golgi complex.

Only two classes of lipoproteins, HDL and VLDL, are secreted by the hepatocytes (1, 7, 13, 19, 28, 48). Available evidence indicates that most of the LDL present in plasma is generated by the catabolism of VLDL (10) and is not synthesized by the liver. In young chickens, 90% of the total plasma lipoproteins is HDL (32). Thus, unlike the situation in other species, the majority of the lipoproteins produced by young chickens is HDL. Yet, our observations, as well as those of Howell and Palade (26) with rat liver, indicate extensive heterogeneity in the size of lipoprotein particles isolated from the Golgi apparatus. The similarity between rat and chicken lipoprotein particles indicates that the heterogeneity in size is not solely due to different lipoprotein classes within the Golgi complex vesicles, since young chickens primarily produce HDL, but that it may reflect different stages of lipoprotein maturation.

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