Plasma Lipoproteins in Familial Lecithin:Cholesterol Acyltransferase Deficiency: Structure of Low and High Density Lipoproteins as Revealed by Electron Microscopy

TRUDY FORTE, KAARE R. NORUM, JOHN A. GLOMSET, and ALEX V. NICHOLS

From the Donner Laboratory, Lawrence Radiation Laboratory, University of California, Berkeley, California 94720, and the Department of Medicine and Regional Primate Research Center, University of Washington, Seattle, Washington 98105

ABSTRACT The low density lipoproteins (LDL) of d 1.019–1.063 g/ml of patients with familial lecithin: cholesterol acyltransferase (LCAT) deficiency show marked heterogeneity when viewed with the electron microscope. At least two types of particles are present, one large and the other small. The large particles predominate in a LDL subfraction of large molecular weight isolated by gel filtration on 2% agarose gel. They appear to be flattened structures with diameters mainly in the range of 900–1200 A. The small particles predominate in a LDL subfraction of smaller molecular weight isolated by filtration on the same type of gel. They are 210–250 A in diameter and are similar to normal LDL in size and shape.

The high density lipoproteins (HDL) also are heterogeneous. The majority of particles are disc-shaped structures 150–200 A in diameter. The discs are mainly present in stacks which have a periodicity of 50–55 A and a variable length. Each disc appears to be made up of a rosette of smaller globular units 50 A in diameter. The appearance of these large molecular weight HDL contrasts with that of normal HDL, which are 70–100 A in diameter and aggregate in monolayers that show hexagonal packing of particles. A small percentage of the patients' HDL consists of structures 45–60 A in diameter. These predominate in a smaller molecular weight HDL subfraction isolated by gel filtration on Sephadex G200. The particles are present in monolayer aggregates but never form stacked structures similar to those seen in the large molecular weight HDL subfraction.

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Dr. Norum's present address is Institute of Nutrition Research, University of Oslo, Oslo, Norway. He was a Visiting Scientist at the Regional Primate Research Center, University of Washington.

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INTRODUCTION

In an accompanying report (1) we show that low and high density lipoprotein fractions and subfractions of patients with familial lecithin: cholesterol acyltransferase (LCAT) [1] deficiency are abnormal by gel filtration, analytical ultracentrifugation, electrophoresis, and chemical analysis. In the present investigation we have studied the morphology of the same fractions and subfractions by electron microscopy. We have endeavored to correlate the structures with the other known properties of the lipoproteins and to compare them with those of normal lipoproteins in order to obtain further insight concerning the nature of protein-lipid interactions in plasma lipoproteins.

METHODS

Materials. Plasma lipoproteins were obtained from the patients and normal individuals described in the accompanying report (1). Although the structure of lipoproteins from both A. R. and A. A. was studied, the micrographs shown here are all of plasma fractions from A. A. However, the structures of the various lipoprotein fractions were comparable in both subjects. The methods employed for isolating the various fractions and subfractions as well as some physical and chemical properties of the fractions are also given in the accompanying article (1).

Electron microscopy. Small aliquots of lipoproteins were mixed with an equal volume of 2% sodium phosphotungstate (PTA), pH 7.4, and a droplet was placed on a Form-

[1] *Abbreviations used in this paper:* HDL, high density lipoprotein(s); LCAT, lecithin: cholesterol acyltransferase; LDL, low density lipoprotein(s); PTA, phosphotungstate.

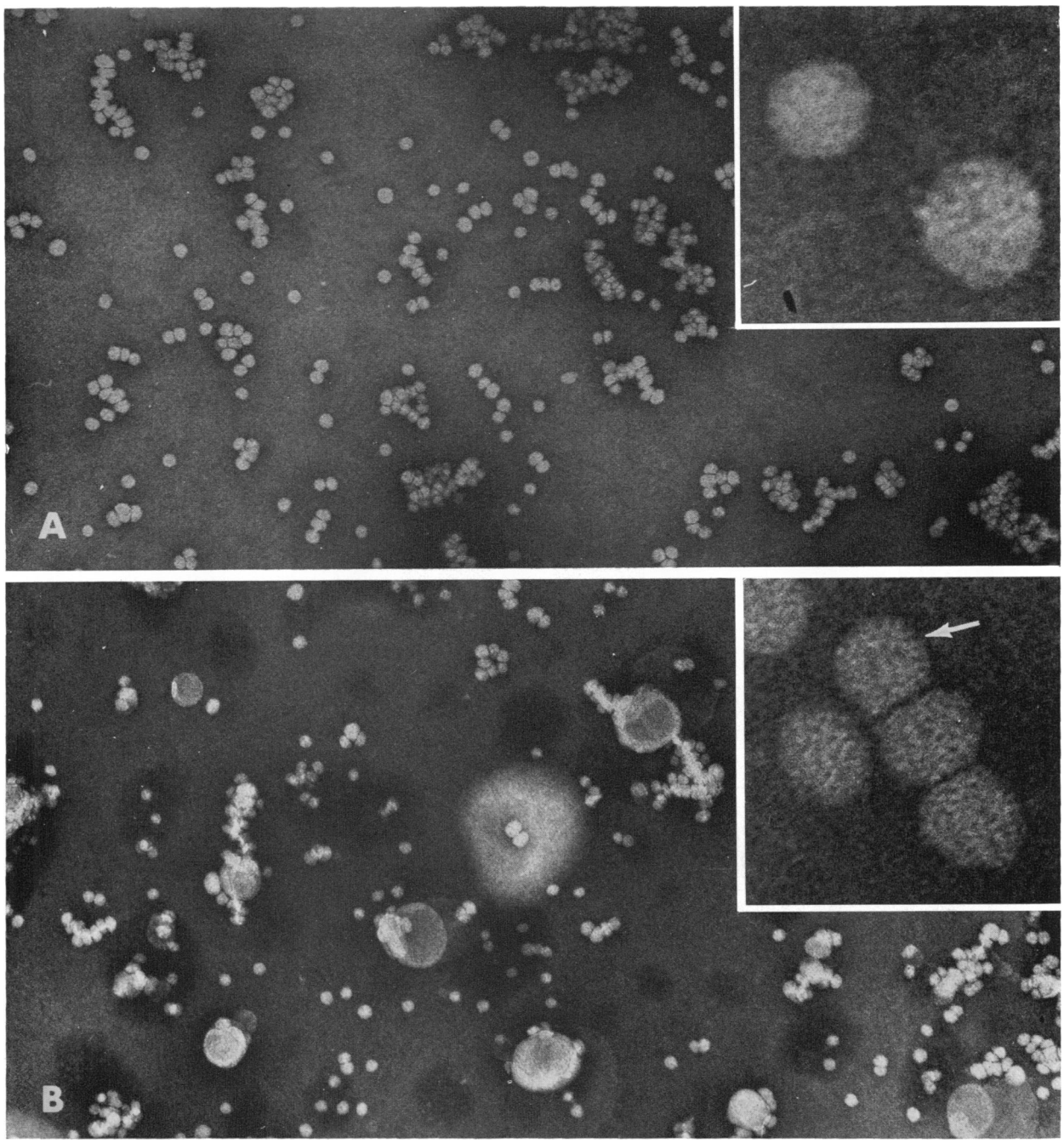

FIGURE 1 Electron micrograph of LDL of d 1.019–1.063 isolated by preparative ultracentrifugation. (A) LDL from a normal subject. Particles are approximately spherical, but become deformed when apposed. × 93,000. Inset shows particle detail at higher magnification. × 705,000. (B) LDL from A. A. Numerous small, approximately spherical particles similar to the particles in normal plasma (cf. 1 A) are visible. Very large particles are also visible in the same field. × 93,000. Inset shows detailed structure of the small particles. The arrow indicates fine structure at the surface. × 705,000.

var/carbon-coated grid by means of a microcapillary. Excess fluid was removed from the grid with filter paper, and then negatively stained preparation was immediately examined in the Hitachi HU 11 microscope.

RESULTS

Low density lipoproteins (LDL). Fig. 1 shows typical structures of LDL fractions isolated by preparative utracentrifugation (d 1.019–1.063 g/ml) from the plasma of a normal subject (Fig. 1 A) and from patient A. A. (Fig. 1 B). Normal LDL particles are quite uniform in size ranging from 200 to 290 A in diameter with 79% of the particles between 210 and 250 A. The inset in Fig. 1 A is a high magnification micrograph of normal LDL; the particles are approximately spherical and show surface irregularities which suggest a fine structure. The LDL from A. A. (Fig. 1 B) are comprised of at least two distinct populations of particles; one consists of large particles which often exceed 1000 A, whereas the other is similar to normal LDL in shape and size (74% between 210 and 250 A). The inset in Fig. 1 B is a high magnification micrograph of a small particle; surface fine structure is apparent (see edge of particle indicated by arrow).

Large and small molecular weight LDL that correspond to the large and small particles can be isolated by gel filtration on columns of 2% agarose gel (1). The majority of particles in the large molecular weight subfraction (Fig. 2) range in size from 900 to 1200 A. They appear to be flattened as evidenced by the overlapped areas indicated by the arrows. The flattening may be artifactual since negative staining of large particles such as chylomicrons also results in a certain amount of flattening (2). The speckled appearance of the large particles suggests that they are made up of subunits. The diameters of these subunits are difficult to measure but appear to be in the range of 130–170 A. The small molecular weight subfraction mainly contains particles similar in shape and size to the smaller particles in Fig. 1 B, although larger particles also are occasionally present.

High density lipoproteins (HDL). Fig. 3 compares the structures of HDL from plasma of a normal subject (Fig. 3 A) with that from patient A. A. (Fig. 3 B). Normal HDL particles range from 70 to 100 A in diameter and typically form hexagonal arrays in areas of high particle concentration. The inset shows fine structure of these particles at greater magnification and confirms previous observations (2, 3) that the HDL particles appear to be composed of several subunits.

The most striking feature of the HDL of A. A. (Fig. 3 B) is the appearance of stacked structures which display a very regular periodicity of 50–55 A. The width of the units which make up the stacks varies from 100 to 300 A; however, the majority are 150–200 A. The length of the stacks varies from 2 to over 50 distinct units and appears to be concentration dependent since in more dilute preparations the stacks are much shorter. A substantial number of freestanding particles with circular profiles are also present in the micrograph; their diam-

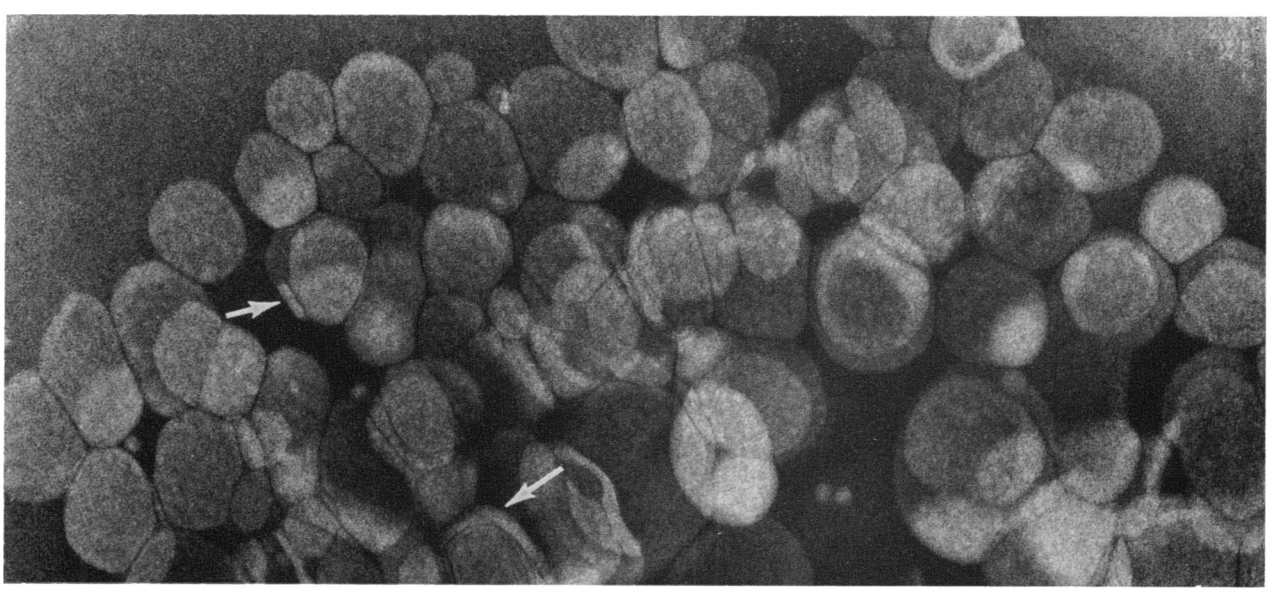

FIGURE 2 Large molecular weight LDL fraction isolated by gel filtration on 2% agarose gel. The structures are extremely large and are apparently flattened as indicated by overlapped structures (arrow). The large structures have a speckled appearance which suggests a fine structure consisting of many small subunits. × 148,000.

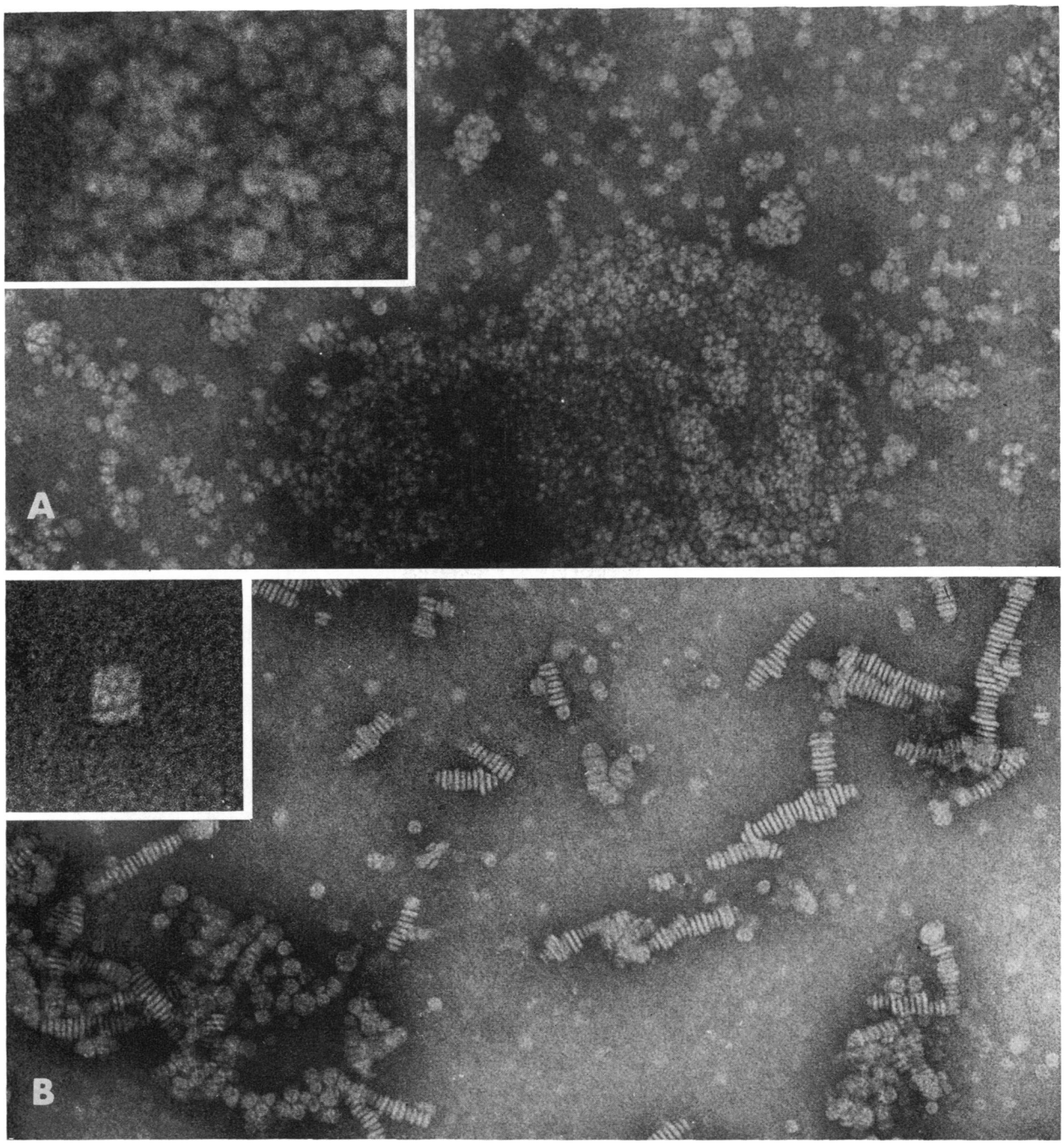

FIGURE 3 Electron micrograph HDL isolated by preparative ultracentrifugation. (A) HDL from normal subject. Particles are very uniform in size and aggregate during drying in a monolayer with a hexagonal packing pattern. × 222,000. Inset shows details of packed particles at high magnification. Note that the particles appear to be made up of several subunits. × 705,000. (B) HDL from A. A. Particles are aggregated into stacked structures with a periodicity of 50–55 A. Free standing particles are also visible. × 182,000. The inset shows two continguous particles one of which is tilted so it is seen face on, revealing that the particles are disclike and composed of several subunits. × 456,000.

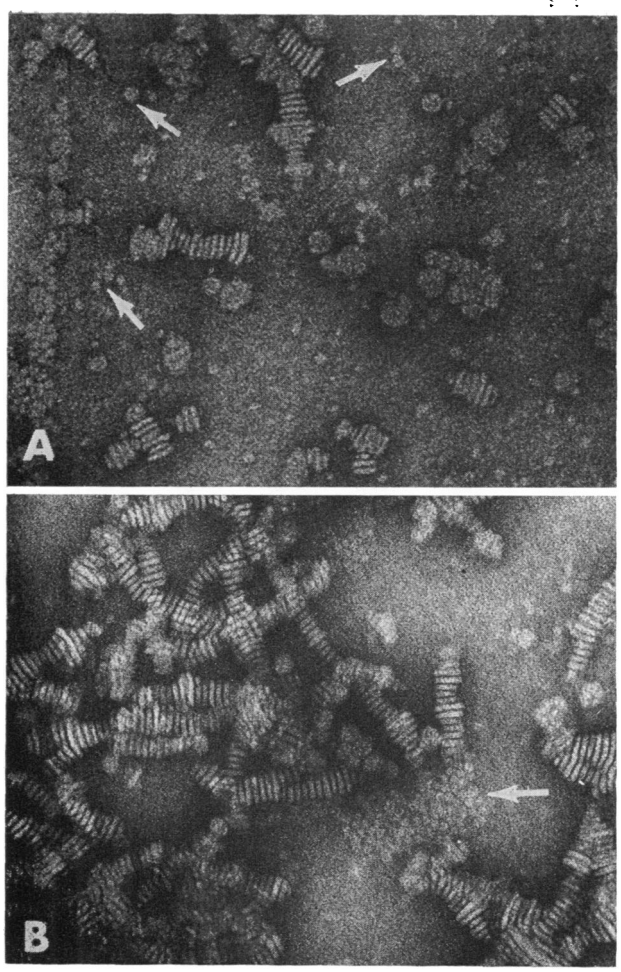

FIGURE 4 Demonstration of small structures in the HDL of A. A. (A) Scattered among the larger disc-shaped particles are numerous small (45–100 A) particles (arrows). × 182,000. (B) Small particles similar in dimension to those in 4A are aggregated in a monolayer (arrow). × 182,000.

eters range between 100 and 250 A, but the majority are between 150 and 200 A. Occasionally these particles show a suggestion of substructure consisting of several subunits in the form of a rosette. Such fine structural detail is revealed in the inset (Fig. 3 B) which shows two contiguous particles. One of the particles is tilted so that its flat end is visible showing what appears to be a rosette of globular subunits of approximately 50 A diameter. Viewed in this light, the HDL structures isolated from the patient consist of discs 50 A high by 150–200 A across, each disc being made up of several globular subunits. During drying the disclike particles presumably aggregate into stacks with a fairly regular periodicity (see Discussion).

Although large disclike particles 150–200 A in diameter predominate in the HDL fraction, occasional small structures 45–100 A in diameter are also visible. The fields in Fig. 4 A and 4 B show both freestanding and concentrated particles of these smaller dimensions. Such particles when concentrated appear to pack in a monolayer (Fig. 4 B) and in this respect more nearly resemble normal HDL. However, these small particles are not found in a hexagonal array when concentrated during drying.

As in the case of the LDL, large and small molecular weight HDL that correspond to the large and small particles can be isolated by gel filtration (1). An electron micrograph of the large molecular weight HDL obtained by filtration through a column of Sephadex G200 is shown in Fig. 5. Particles 150–200 A in diameter are present which often form stacked structures identical with those seen in the electron micrographs of the unfractionated HDL. The small molecular weight subfraction (Fig. 6) contains numerous particles which range in diameter from 45 to 100 A, at least 50% of the structures being between 45 and 60 A. Particles 70–100 A in diameter appear to have a definite substructure consisting of four to five subunits and correspond well with normal HDL.

The schematic diagram in Fig. 7 summarizes the salient features of the various HDL particles seen in the electron micrographs. Possible shapes of the various particles as well as their patterns of aggregation under conditions of negative staining are represented.

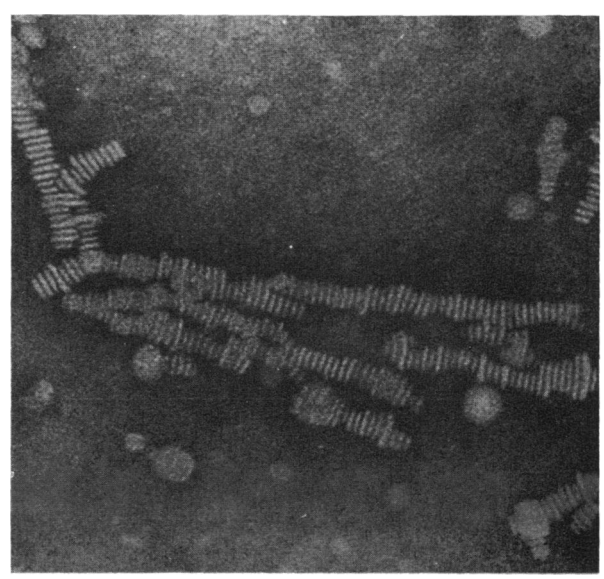

FIGURE 5 Large molecular weight subfraction of the HDL of A. A. isolated by gel filtration on Sephadex G200. Only larger particles are visible and most of these are aggregated into stacks. × 182,000.

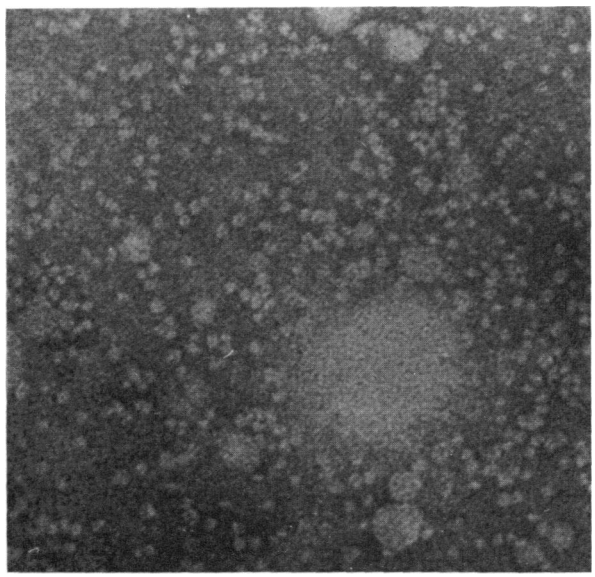

FIGURE 6 Small molecular weight component of the HDL of A. A. isolated by gel filtration on Sephadex G200. Numerous small particles are present; the large round structures presumably represent clumped material. × 222,000.

DISCUSSION

In the accompanying paper (1) we show that many of the physical and chemical properties of the LDL and HDL of patients with familial LCAT deficiency are abnormal. Consistent with these observations, the present study shows that the LDL and HDL are also abnormal when viewed in the electron microscope. The presence of a wide range of particles in the LDL fraction is consistent with the heterogeneous flotation pattern (1). The large, flattened LDL particles are highly abnormal, although similar structures have been observed in the LDL of two patients with biliary obstruction.[2] Because many of the particles are about 1000 A in diameter, they could account for the light scattering properties of the infranatant after preparative ultracentrifugation (1). The fine structural details of the large particles suggest that the latter may be composed of smaller subunits. However, the nature of the subunits is unknown. The smaller LDL, 210–250 A in diameter, are similar in appearance to normal LDL, which is interesting in view of the fact that their lipid composition is so abnormal (1).

[2] Forte, T. Unpublished observations.

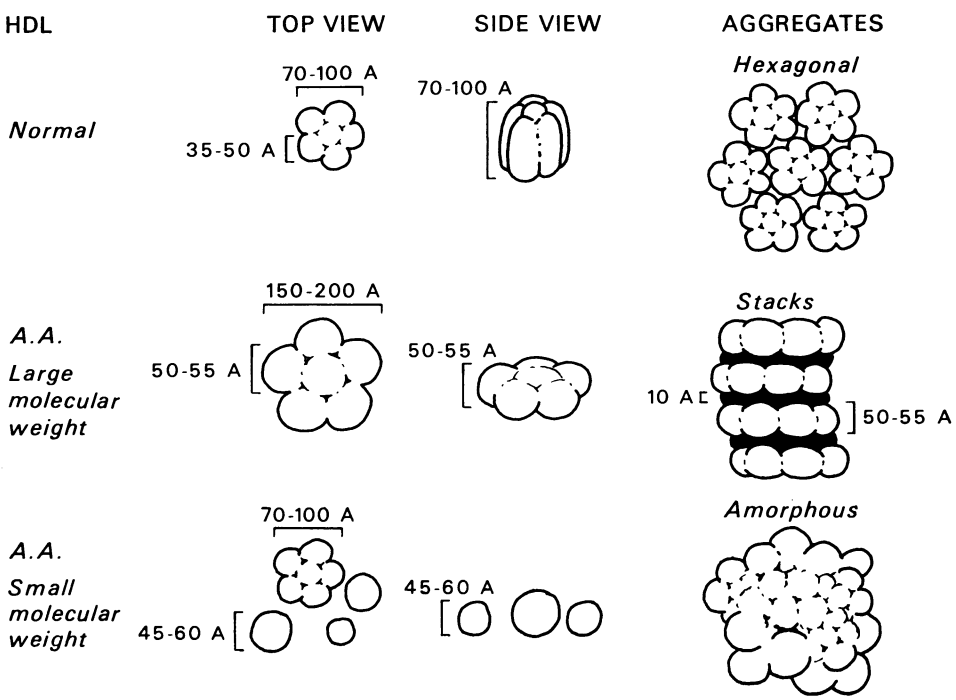

FIGURE 7 Schematic comparison of the HDL of normal subjects and of a patient with familial LCAT deficiency. The representation of a side view of normal HDL is based on previously reported data (2, 4). Note that the normal HDL are shown to be composed of six subunits, but that this is only an average value. In the representation of the aggregated large molecular weight HDL of the patient (A. A.), the light bands indicate disclike particles, and the dark bands indicate the hydrophilic, PTA-containing space. In the side view of the small molecular weight HDL, no structures similar to those shown for normal HDL are shown. They may exist, but they have not as yet been seen.

The great diversity of particle sizes seen in the HDL fraction is compatible with the heterogeneity indicated on analytical ultracentrifugation and gel filtration (1). However, aggregation of the disc-shaped HDL into stacks is probably an artifact caused by concentration during drying. The fact that shorter stacks are observed in preparations made from more dilute HDL solutions has already been mentioned. Also, the behavior of the large molecular weight HDL on gel filtration and analytical ultracentrifugation (1) would suggest separate discs and not large aggregates. Presumably, the geometry of the discs favors the formation of stacks as the lipoprotein solution becomes concentrated during drying; a similar phenomenon has been shown for ringlike erythrocyte membrane subunits during negative staining (5). Normal HDL, on the other hand, never form stacked structures during drying, but rather pack in a hexagonal array. Such a packing arrangement may result from their more nearly spherical geometry, as suggested in the diagram in Fig. 7 and their uniformity of size.

The 50–55 A periodicity of the stacked discs is very similar to the periodicity of the lamellar, bilayer structures seen in negatively stained phospholipid or phospholipid-cholesterol dispersions (6, 7). Very recently, structures resembling the HDL stacked discs have been described by Howell, Lucy, Pirola, and Bouchler (8) for macromolecular assemblies of phospholipid and cholesterol with bile salts. Like these structures, the abnormal large molecular weight HDL particles may represent a mixed aggregate of phospholipid and cholesterol, which in this case is stabilized by the HDL protein. The protein is presumably responsible for the subunit character of the entire structure. The formation of disclike particles instead of more nearly spherical ones is probably promoted by the near absence of nonpolar "core" lipids. Indeed, studies being carried out at the present time[3] indicate that stacked discs can be formed "in vitro" with mixtures of HDL peptides plus phospholipid or phospholipid and unesterified cholesterol. With the addition of cholesteryl ester to the peptide-phospholipid-unesterified cholesterol mixture, structures approaching those of normal HDL are primarily formed, and stacked discs are rarely seen. Thus cholesteryl esters may prevent disc formation by favoring a more spherical configuration. Further evidence for the importance of nonpolar lipids in maintaining HDL structure has been suggested by the reconstitution work of Scanu et al. (9). These investigators showed that only upon the addition of both polar and nonpolar lipids to apo-HDL was it possible to assemble HDL macromolecules with properties similar to native HDL.

[3] Forte, T., S. Lux, E. Gong, R. I. Levy, and A. V. Nichols. Unpublished observations.

The small molecular weight HDL subfraction obtained by gel filtration is not comprised simply of dissociated subunits of the larger, disc-shaped HDL, although the apparent size (50–55 A) of the subunits of the larger HDL (inset, Fig. 3 B) might be consistent with an aggregation-disaggregation relationship. Neither discs nor stacks of discs are formed upon electron microscopy of the small molecular weight HDL (Fig. 6). Also, the two HDL subfractions differ greatly in lipid composition, electrophoretic mobility (1), and reactivity toward LCAT (10). Although the small molecular weight HDL constitute only a small fraction of the HDL of LCAT-deficient plasma, they are highly interesting. Many of the particles seen in the small molecular weight subfraction are smaller than the particles normally observed in preparations of HDL_3. Comparable particles may be present in small concentrations in normal plasma, since particles with similar flotation properties are observed upon analytical ultracentrifugation of normal HDL (1), and since observations by Glomset, Janssen, Kennedy, and Dobbins (10) of HDL subfractions obtained by gel filtration suggest that HDL of smaller dimensions than HDL_3 probably exist. These small molecular weight HDL may be functionally significant. Glomset, Norum, and King (11) have shown that the small molecular weight HDL of the patients serve as effective substrates for LCAT and have postulated that they may constitute recently secreted HDL. This is a possibility that clearly warrants further investigation.

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REFERENCES

1. Norum, K. R., J. A. Glomset, A. V. Nichols, and T. Forte. 1971. Plasma lipoproteins in familial lecithin: cholesterol acyltransferase deficiency: physical and chemical studies of low and high density lipoproteins. *J. Clin. Invest.* **50**: 1131.
2. Forte, G. M., A. V. Nichols, and R. M. Glaeser. 1968. Electron microscopy of human serum lipoproteins using negative staining. *Chem. Phys. Lipids.* **2**: 396.
3. Puppione, D. L., G. M. Forte, A. V. Nichols, and E. H. Strisower. 1970. Partial characterization of serum lipo-

proteins in the density interval 1.04—1.06 g/ml. *Biochim. Biophys. Acta.* **202**: 392.
4. Puppione, D. L., T. Forte, and A. V. Nichols. Serum lipoproteins of killer whales. *Comp. Biochem. Physiol.* In press.
5. Harris, J. R. 1968. Release of a macromolecular protein component from human erythrocyte ghosts. *Biochim. Biophys. Acta.* **150**: 534.
6. Bangham, A. D., and R. W. Horne. 1964. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. *J. Mol. Biol.* **8**: 660.
7. Lucy, J. A.. and A. M. Glauert. 1964. Structure and assembly of macromolecular lipid complexes composed of globular micelles. *J. Mol. Biol.* **8**: 727.
8. Howell, J. I., J. A. Lucy, R. C. Pirola, and I. A. D. Bouchier. 1970. Macromolecular assemblies of lipid in bile. *Biochim. Biophys. Acta.* **210**: 1.
9. Scanu, A., E. Cump, J. Toth, S. Koga, E. Stiller, and L. Albers. 1970. Degradation and reassembly of a human serum high-density lipoprotein. Evidence for differences in lipid affinity among three classes of polypeptide chains. *Biochemistry.* **9**: 1327.
10. Glomset, J. A., E. T. Janssen, R. Kennedy, and J. Dobbins. 1966. Role of plasma lecithin: cholesterol acyltransferase in the metabolism of high density lipoproteins. *J. Lipid Res.* **7**: 639.
11. Glomset, J. A., K. R. Norum, and W. King. 1970. Plasma lipoproteins in familial lecithin: cholesterol acyltransferase deficiency: lipid composition and reactivity in vitro. *J. Clin. Invest.* **49**: 1827.