# APPLICATION OF PHYSICAL METHODS TO THE STUDY OF SERUM LIPOPROTEINS

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## INTRODUCTION AND SCOPE

During the last several years, our knowledge about the structure and functions of blood serum lipoproteins has increased greatly. Nevertheless, due mainly to the complexity of these particles, many important facets remain to be explored and many questions need to be answered. Since many reviews on the subject have been published,[1-4] our discussion will be limited to an evaluation of some of the methods that have found or should find potential use in the structural analysis of intact lipoproteins of human serum or the serum of other animal species. We shall omit much of the background information on the subject and focus our interest on significant developments in the field. In particular, we will discuss the techniques of analytical ultracentrifugation, electron microscopy, and small angle X-ray scattering as applied to normal serum lipoproteins. A few selected examples of abnormal variants will be chosen to underscore the value of the above methods in approaching the solution of the structure of lipoproteins and of macromolecules in general. A brief introduction on the general properties of serum lipoproteins is helpful for an understanding of the data to be discussed in this review.

The different classes of serum lipoproteins are commonly isolated by ultracentrifugal flotation, following the basic methods of de Lalla and Gofman[5] and Havel et al.,[6] with minor modifications.[7,8] Through the sytematic application of these techniques, various lipoprotein classes have been isolated on the basis of their flotation rate in media of a given density, under constant conditions of speed and temperature. In ultracentrifugal terms, the very low density lipoproteins (VLDL) are those with flotational rates, at $\rho$ = 1.063 g/ml between 20 and 400, whereas the low density lipoproteins (LDL) have flotation rates between 0 and 20. The high density lipoproteins (HDL) are defined against a solvent density of 1.20 g/ml. They are expressed in terms of $S_{f(1.20)}$ values which range between 0 and 12.

The separation of serum lipoproteins into the four classes, chylomicrons, very low, low, and high

*Operated by the University of Chicago for the United States Atomic Energy Commission; formerly the Argonne Cancer Research Hospital

**Abbreviations and Symbols**

| | | |
|---|---|---|
| $S_f$ | = | flotation coefficient |
| $s^0$ | = | sedimentation coefficient at zero concentration |
| $D^0$ | = | diffusion coefficient at zero concentration |
| f | = | frictional coefficient |
| f/fo | = | frictional ratio |
| $v_2$ | = | partial specific volume |
| $M$ | = | molecular weight |
| $[\eta]$ | = | intrinsic viscosity |
| Rg | = | radius of gyration |
| VLDL | = | very low density lipoproteins |
| LDL | = | low density lipoproteins |
| HDL | = | high density lipoproteins |

density lipoproteins, has been found to be an oversimplification, since each class is composed of subclasses, the number and distribution of which may vary among individuals according to their nutritional state, age, and sex. In addition, a number of hyper- or hypolipoproteinemic states have been recognized which may involve one or more of the lipoprotein classes. Significant technical improvements in lipoprotein studies have been achieved during recent years, and computer programs have been developed for the analysis and graphic presentation of lipoprotein distributions from Schlieren optical data.[9] The expected increase in the application of computer technology to the study of serum lipoproteins is likely to lead to a better definition of the various classes and subclasses in normal and disease states. At present, it is not certain whether a given ultracentrifugal class is a homogeneous structural entity or whether, as some believe, it represents a mixture of lipoprotein families. Clearly, an answer to such a question cannot be derived from analytical ultracentrifugation alone, and complementary information must be obtained from the analysis of lipoproteins isolated in the preparative ultracentrifuge or by any other suitable method.

Significant advances have recently been made in the isolation and characterization of the protein moiety of each lipoprotein class. These advances, which are summarized in Tables 1 and 2, indicate that all of the lipoproteins, except perhaps for LDL, contain one or more polypeptides, some of which are shared by very low and high density lipoproteins[3] (Table 2). These observations which apply to man and to other animal species such as *Macacus rhesus*,[10,11] baboon,[10,11] chimpanzee[10,12] and the rat[2] raise important structural questions. For example: (a) Are all of the presently recognized polypeptides constituents of any given ultracentrifugal class or subclass? Or, (b) Do lipoproteins, isolated ultracentrifugally, represent an assembly of species of a similar hydrated density but of a distinct polypeptide composition? (c) Do isolated lipoproteins reflect their natural state in the living organism? Or, (d) Do lipoproteins of a given class when isolated from diseased subjects (i.e., with hyper- or hypolipoproteinemia) have structural properties identical to those of their normal counterparts?

Although studies on the individual components that make up serum lipoproteins have been numerous, with a proportionate proliferation of information on the subject, [1-4,13,14] a cursory examination of the results obtained thus far reveals the intricacy of the system of serum lipoproteins and our relatively modest understanding of their overall structural characteristics. The elucidation of the latter is still the goal of most investigations and justifies the continuing interest in the study of serum lipoproteins as intact particles.

With this preamble, we will now present the information which has been gained by the application of physical methods to the investigation of low and high density lipoproteins.

TABLE 1

**Lipoprotein Classes Found in Human Serum**

| Class | Flotational rate (S) | | Density (g/ml) |
|---|---|---|---|
| Chylomicrons | $S_f$ (1.063) | >400 | 0.95 |
| Very low density (VLDL) | $S_f$ (1.063) | 20–400 | 0.95–1.006 |
| Low density (LDL) | $S_f$ (1.063) | 0–20 | 1.006–1.063 |
| High density (HDL) | $S_f$ (1.20) | 0–9 | 1.063–1.20 |
| Subclass 2 ($HDL_2$) | $S_f$ (1.20) | 3–9 | 1.063–1.125 |
| Subclass 3 ($HDL_3$) | $S_f$ (1.20) | 0.8–3 | 1.125–1.20 |

TABLE 2

**Properties of the Major Classes of Serum Lipoproteins**

| Parameters | Chylomicrons | VLDL | LDL* | HDL |
|---|---|---|---|---|
| Percent composition: | | | | |
| Phospholipids | 7 | 18 | 22 | 24 |
| Unesterified cholesterol | 2 | 7 | 8 | 2 |
| Cholesteryl esters | 5 | 13 | 37 | 20 |
| Triglycerides | 84 | 51 | 10 | 8 |
| Protein | 2 | 9 | 21 | 50 |
| Protein composition:** | | | | |
| B | + | 40 | 100 | – |
| Arg-rich peptide | ? | + | – | + |
| A-I | + | +(?) | – | 65 |
| A-II | + | +(?) | – | 25 |
| C–I | – | – | – | – |
| C-II | – | – | – | – |
| C-III-0 C-peptides | + | 60 | +(?) | 5 |
| C-III-1 | – | – | – | – |
| C-III-2 | – | – | – | – |

*The LDL of $\rho$ 1.006 – 1.019 g/ml, less clearly defined as a distinct class, will not be considered in this review.
**Based on the results of several investigators (see References 1 to 4).
+Present but not quantified.
–Absent.

## ANALYTICAL ULTRACENTRIFUGATION

### 1. General Comments

As is the case with other macromolecules, the application of analytical ultracentrifugation to a given lipoprotein class allows one to obtain several fundamental parameters such as sedimentation and diffusion coefficients, hydrated density, frictional coefficient, and molecular weight.

Both the *sedimentation* and *diffusion* coefficients can be used to determine the *frictional coefficient*. For a two-component system, the equations representing these relationships are

$$s^0 = M(1-\bar{v}_2\,\rho)/N\,f \quad (1)$$

and

$$D^0 = RT/Nf \quad (2)$$

where $s^0$ is the sedimentation coefficient extrapolated to zero concentration; $M$, the molecular weight; $\bar{v}_2$, the partial specific volume of the solute; $\rho$, the density of the solution; N, Avogadro's number; $D^0$, the diffusion coefficient at infinite dilution; R, the gas constant; and T, the absolute temperature.

Comparison of the frictional coefficient of the particle with that of an unhydrated sphere of the same molecular weight, $f_o$, yields the *frictional ratio*, f/fo, as given by

$$f = RT/D^0N = 6\pi\eta\,(f/fo)\,[3\,M\,\bar{v}_2/4\pi\,N]^{1/3} \quad (3)$$

where $\eta$ is the viscosity of the solvent. Deviation of the frictional ratio from unity indicates hydration of the particle and/or a departure from sphericity. If the degree of solvation of the particle is known, a corrected $f/f_o$ value is obtained and may then be used to estimate the axial ratio of the particle under study.

The partial specific volume, $\bar{v}_2$, of the solute can be found either from density measurements or by determination of its sedimentation coefficient in solvents of different density.[15]

If $s^0$, $D^0$, and $\bar{v}_2$ are known, Equations 1 and 2 may be combined to give the molecular weight by the well-known Svedberg equation:

$$M = s^0\,RT/D^0\,(1-\bar{v}_2\,\rho). \quad (4)$$

It should be noted, however, that if $M$ is determined by this method in heterogeneous

TABLE 3

**Properties of Human Low Density Lipoproteins**

| Physical Constants | LDL | Reference |
|---|---|---|
| $s^0_{25,1.20}$ (S) | 38 (18 to 67) | 19 |
| $D^0_{25,w}$ ($cm^2$ $sec^{-1}$) | $2.01 \times 10^{-7}$ | 20 |
| $M$ | $2.73 \times 10^6$ ($2 \times 10^6 - 3 \times 10^6$) | 20 |
| $\rho_h$ (g/ml) | 1.035 (1.004–1.066) | 20 |
| $\bar{v}_2$ (ml/g) | 0.967 | 20 |
| $[\eta]$ (dl/g) | 0.034 | 24 |
| $\beta \times 10^{-5}$ ($atm^{-1}$) (compressibility) | 4.35 | 22 |
| dn/dc ($\lambda$ = 436 nm) (ml/g) (pH 6.7) | 0.162 | 29 |

*Numbers in parentheses are ranges.

systems, the average molecular weight obtained is not necessarily the weight-average molecular weight, even if the weight-average values for $s^0$ and $D^0$ are used.[16]

An evaluation of the molecular weight as well as sample heterogeneity is provided by sedimentation equilibrium measurements employing either conventional or meniscus depletion methods. The technique yields a weight-average or z-average molecular weight depending on the method of analysis used.

For an ideal system, $M$ is obtained by the relation:

$$M = [(2\,RT)/(1-\bar{v}_2\,\rho)\,\omega^2]\,[d\ln c/d(r^2)], \quad (5)$$

where $\omega$ is the angular velocity and c, the equilibrium concentration of the solute at a distance r from the center of rotation.

To correct for concentration dependence, $M$ obtained from Equation 5, should be evaluated at several different concentrations.

## 2. Low-Density Lipoproteins

In spite of their functional importance and their recognized precursor role for LDL, the VLDL particles of $S_{f(1.063)}$ between 20 to 400 have not been clearly defined, partly because of the heterogeneity of the molecular weight, density, and size, and partly because of the problems encountered in assessing the degree of association during analysis. Our discussion will be centered, therefore, on LDL for which a large amount of information is available.

As shown in Table 1, the LDL class floats in the $S_{f(1.063)}$ region between 0 and 12.[7,9] This distribution is clearly observed in flotational profiles of LDL that show a nonsymmetrical pattern which is particularly evident in the case of normal males and some abnormal states.[7]

The value of the hydrated density of human LDL has been obtained from determinations of the variation of the sedimentation coefficient as a function of the density[7,19,20] and from the sedimentation equilibrium in a density gradient[21-23] (Table 3). The relatively large range of values obtained, between 1.004 and 1.066 g/ml, is in keeping with the results of Adams and Schumaker.[22] When these authors studied LDL by sedimentation equilibrium in sucrose-NaBr gradients, they detected at least two components. By further analysis, the apparent molecular weights of these two components were found to be about one fourth those obtained from sedimentation velocity or sedimentation equilibrium. The discrepancy was attributed mainly to the density of heterogeneity of the sample, although the possible role of the preferential hydration of the particles could not be ruled out.

Preferential hydration can influence the hydrodynamic properties of a macromolecule. Fisher et al.,[20] using the procedure suggested by Katz and Schachman,[15] determined the hydrated density of human LDL both in $D_2O$-$H_2O$ mixtures and in aqueous KBr solutions at a density of 1.20 g/ml. The calculated hydrated density of LDL at low ionic strength was essentially equal to that determined from the observed linear relation between sedimentation coefficient and medium density at high salt concentrations. These results and the

TABLE 4

**Properties of Low Density Lipoproteins from Species Other than Man**

| Physical Constants | LDL | $LDL_1$ | $LDL_2$ | Reference |
|---|---|---|---|---|
| *Macacus rhesus* | | | | |
| $S^0_{f(1.063)}$ (S) | 5.80 | – | – | 34 |
| $s^0_{25,1.008}$ (S) | 7.09 | – | – | 34 |
| $D^0_{25,1.008}$ ($cm^2$ $sec^{-1}$) | $2.50 \times 10^{-7}$ | – | – | 34 |
| *M* | $2.26 \times 10^6$ | – | – | 34 |
| $\bar{v}_2$ (ml/g) | 0.960 | – | – | 34 |
| *Porcine* | | | | |
| $S_{f(1.063)}$ | – | 0–13 | 0–4.6 | 35 |
| $s^0_{20,w}$ (S) | – | 8.0 | 9.7 | 35 |
| *M* | – | 2.7–3.0 | 2.0 | 35 |
| $\bar{v}_2$ (ml/g) | – | 0.968 | 0.952 | 35 |
| dn/dc ($\lambda$ = 546 nm) (ml/g) | – | 0.171 | 0.171 | 35 |
| dn/dc ($\lambda$ = 436 nm) (ml/g) | – | 0.180 | 0.180 | 35 |

identical values of the diffusion coefficient, which were found in high and low ionic strength solutions, led to the conclusion that LDL is not preferentially hydrated in KBr solutions. The hydrated density of the particle was 1.035 g/ml, and its partial specific volume, 0.967 ml/g, a value in good agreement with that of 0.970 ml/g determined by pycnometry.[24] According to Toro-Goyco,[24] LDL is preferentially hydrated when dissolved in NaCl solutions. Thus, it appears that LDL may have different properties depending on the nature of the electrolyte used. Further work in this area is needed before definitive conclusions can be drawn.

A hydrodynamic study of macromolecules requires knowledge of their diffusion coefficient, D, which in turn provides a measure of the frictional coefficient. For human LDL, this parameter has been determined by numerous investigators.[19,20,24-28] The following discussion, however, will relate primarily to the recent work of Schumaker and co-workers[19,26] and of Fisher et al.,[20] who reported, for LDL $D^0_{25,w}$ values of $2.17 \times 10^{-7}$ and $2.01 \times 10^{-7}$ $cm^2$ $sec^{-1}$, respectively. By comparison of the frictional coefficient of LDL obtained from $D^0$ with that of an anhydrous sphere of the same molecular weight, the frictional ratio, $f/f_0$ of LDL was found to be 1.11 and 1.10.[19,20,26] It was not possible, however, to determine whether the deviation of $f/f_0$ from unity was due to simple hydration of the particle or to its departure from sphericity.

Interpretation of such experiments depends on the accurate determination of the molecular weight. For LDL, the reported values range between $2 \times 10^6$, and $3 \times 10^6$, The average molecular weight of $2.73 \times 10^6$. reported recently by Fisher et al.,[20] is in good agreement with the values obtained from measurements by light scattering,[29] sedimentation equilibrium,[30,31] and flotation in high-salt media.[32,33] With the assumption of a spherical particle, the radius of LDL was calculated to be 101 Å using the relation $r = (3\bar{v}_2 M/4\pi N)^{1/3}$. This value agrees well with the diameter of 216 to 220 Å obtained from electron microscopic studies (see section C).

Although ultracentrifugal data have provided some insight into the nature of the LDL particle, they have also raised additional questions. For example, it has been suggested that the variation in the molecular weight and sedimentation coefficient found with various preparations of LDL could be explained simply by different complements of lipids (in particular triglycerides), with a constant protein mass.[19] However, proof for this hypothesis will have to come from other approaches, and particularly from a systematic study of the chemical composition of the various LDL samples under consideration.

Preliminary studies of the low density lipoprotein isolated from the serum of the *Macacus rhesus*[34] gave the results summarized in Table 4, which are quite similar to those of human LDL.

The flotation profile of porcine LDL[35] has shown the presence of two distinct fractions ($LDL_1$ and $LDL_2$) with $S_{f(1.063)}$ values of 0 to

TABLE 5

**Properties of Human High Density Lipoproteins**

| Physical Constants | $HDL_2$ | $HDL_3$ | Reference |
|---|---|---|---|
| $s^0_{20,w}$ (S) | 5.45 | 4.65 | 36 |
| $D^0_{20,w}$ ($cm^2$ $sec^{-1}$) | $3.68 \times 10^{-7}$ | $3.93 \times 10^{-7}$ | 38 |
| $M$ | $3.6 \times 10^5$ | $1.75 \times 10^5$ | 36, 37 |
| $\rho_h$ (g/ml) | 1.105 | 1.153 | 36 |
| $\bar{v}_2$ (ml/g) | 0.905 | 0.867 | 36 |
| $[\eta]$ (dl/g) | – | 0.033 | 36 |
| dn/dc ($\lambda$ = 546 nm) (ml/g) | – | 0.166 | 36 |
| dn/dc ($\lambda$ = 436 nm) (ml/g) | – | 0.151 | 36 |

13 and 0 to 4.6. A comparison of the measured parameters, molecular weight, and partial specific volume with those of human LDL showed very similar molecular characteristics for these two animal species (Table 4).

### 3. High Density Lipoproteins

The HDL from human serum are characterized by flotational rates, $S_{f(1.20)}$, between 0 and 12 and can be further subdivided into two subclasses, $HDL_2$ and $HDL_3$, in solutions of densities between 1.063 and 1.125 g/ml and between 1.125 and 1.210 g/ml, respectively.

Several values have been reported for the hydrated density of $HDL_2$ and $HDL_3$. The densities most commonly used, 1.105 g/ml for $HDL_2$, and 1.153 g/ml for $HDL_3$, were determined by Hazelwood[36] from sedimentation coefficients at different densities. From these data, $HDL_2$ and $HDL_3$ will have values of the partial specific volumes of 0.905 and 0.867 mg/g, respectively (Table 5). These results cannot be compared directly with those found by Lindgren et al.,[7,9] who fractionated HDL on the basis of particle size rather than density. Nevertheless, their values for the hydrated densities of $HDL_2$ and $HDL_3$ are quite similar to those reported by Hazelwood.[36] For the "$HDL_2$-like" fraction of $S_{f(1.20)}$ 2.7 to 9.0, Lindgren et al.[7,9] reported values of $\rho_h$ 1.0988 and 1.0950 g/ml for two separate cases. For the same two cases, the "$HDL_3$-like" fraction, having an $S_{f(1.20)}$ of 0.8 to 3.0, gave $\rho_h$ values of 1.1437 and 1.1346 g/ml. However, the density heterogeneity of any of the HDL subclasses should be investigated further before any definitive conclusion as to their hydrodynamic parameters can be reached.[9] Hazelwood[36] has already noted that for $HDL_3$, plots of the sedimentation coefficient versus density of solutions of high and low ionic strength, give slightly different slopes and intercepts at s=0. These results may be attributed to changes of hydration of this subclass as a function of the solvent and also to particle heterogeneity. An equilibrium between $HDL_2$ and $HDL_3$ may occur during ultracentrifugation, and this could give rise to a continuum of HDL particles.[37] The problem of the interrelationship of $HDL_2$ and $HDL_3$ is not yet resolved, thus justifying the continuing study of their individual subclass.

The sedimentation and diffusion coefficients of human $HDL_2$, extrapolated to zero concentration and corrected to standard conditions, are 5.45 S and $3.93 \times 10^{-7}$ $cm^2$ $sec^{-1}$, respectively. For $HDL_3$, $S^0_{20,w}$ is 4.65 S and $D_{020,w}$ is $3.93 \times 10^{-7}$ $cm^2$ $sec^{-1}$.[36,38] The molecular weights of $HDL_2$ and $HCL_3$ found from the approach to equilibrium method are $3.6 \times 10^5$ and $1.75 \times 10^5$, respectively.[36,37] Higher molecular weights for both HDL subclasses were obtained by the method of meniscus depletion.[39]

From the available data on diffusion coefficients and molecular weights, and with the assumption of no hydration, the $f/f_0$ value would be 1.21 for $HDL_2$ 1.40 and for $HDL_3$. The deviation of this ratio from unity is rather large, especially in the case of $HDL_3$. Allowing for an average solvation of 0.2 g $H_2O$ per g lipoprotein, the values reduce to 1.13 and 1.31 for $HDL_2$ and $HDL_3$, respectively. Since the frictional coefficient reflects changes in the preferential hydration of a molecule, and since this value is not known for any other HDL subclasses, it is not yet possible to ascribe the departure of $f/f_0$ from unity to deviations from sphericity alone. Further, it is possible that these particles may have an asym-

TABLE 6

**Properties of High Density Lipoproteins from Species Other than Man**

| Physical constants | HDL | $HDL_2$ | $HDL_3$ | Reference |
|---|---|---|---|---|
| *Macacus rhesus* | | | | |
| $S^0_{20,w}$ (S) | – | 5.59 | 5.33 | 38 |
| $D^0_{20,w}$ ($cm^2$ $sec^{-1}$) | – | $3.60 \times 10^{-7}$ | $3.96 \times 10^{-7}$ | 38 |
| *M* | – | $3.90 \times 10^5$ | $1.97 \times 10^5$ | 38 |
| $\bar{v}_2$ (ml/g) | – | 0.91 | 0.87 | 38 |
| *Porcine* | | | | |
| $S^0_{20,w}$ (S) | 5.0 | – | – | 35 |
| $D_{app,25}$ ($cm^2$ $sec^{-1}$) | $4.9 \times 10^{-7}$ | – | – | 43 |
| *M* | $2.1 \times 10^5$ | – | – | 43 |
| $\bar{v}_2$ (ml/g) | 0.880 | – | – | 43 |
| $[\eta]$ (dl/g) | 0.030 | – | – | 35 |
| dn/dc ($\lambda$ = 546 nm) (ml/g) | 0.174 | – | – | 35 |
| dn/dc ($\lambda$ = 436 nm) (ml/g) | 0.183 | – | – | 35 |
| *Bovine* | | | | |
| $s^0_{20,w}$ (S) | 4.95 | – | – | 41 |
| *M* | $3.76 \times 10^5$ | – | – | 41 |
| $\bar{v}_2$ (ml/g) | 0.910 | – | – | 41 |

metric shape, particularly in the case of $HDL_3$ where the protein subunits make up about 55% of the whole particle.

Frictional ratios may also be obtained from viscosity determinations. Hazelwood[36] reported an intrisinc viscosity $[\eta]$ of 0.033 dl/g for $HDL_3$ in phosphate buffer. Assuming a prolate ellipsoid, this value would correspond to an axial ratio of 3.2 and in turn, to an $f/f_0$ value of 1.126. This author also reported an axial ratio of 4.1 and an $f/f_0$ ratio of 1.187 for $HDL_2$, values that were presumably calculated from the sedimentation coefficient data. Ideally, the frictional ratios obtained from different hydrodynamic techniques should be the same. However, as also noted for other systems, this is not the case for the HDL subclasses.[40] Additional information is obviously needed for a further explanation of the hydrodynamic behavior of $HDL_2$ and $HDL_3$. This information may derive from determinations of the intrinsic viscosity and the diffusion coefficient under well-controlled conditions, particularly in terms of supporting electrolytes.

Recent studies on *Macacus rhesus* $HDL_2$ and $HDL_3$ obtained by ultracentrifugal flotation gave results which were rather similar to those of the corresponding human counterparts (Table 6). For $HDL_2$, $s^0_{20,w}$ was 5.59 S, and $D^0_{20,w}$ was $3.60 \times 10^{-7}$ $cm^2$ $sec^{-1}$. For $HDL_3$ the values were $s^0_{20,w}$, 5.33 S and $D^0_{20,w} = 3.96 \times 10^{-7}$ $cm^2$ $sec^{-1}$. The partial specific volumes calculated from density measurements in a magnetic densitometer were 0.91 ml/g and 0.87 ml/g for $HDL_2$ and $HDL_3$, respectively.[38] However, the hydrodynamic data reported for the *Macacus rhesus* cannot be considered definitive, since the molecular weights reported represent values calculated from $s^0_{20,w}$ assuming unhydrated spheres. From the values of $s^0_{20,w}$ and $D^0_{20,w}$ the authors also reported molecular weights of $4.50 \times 10^5$ and $2.4 \times 10^5$, respectively, for $HDL_2$ and $HDL_3$.

On the other hand, if the particles are assumed to be 20% hydrated, their frictional ratio, obtained from the above data, becomes 1.02 ($HDL_2$) and 1.15 ($HDL_3$). Thus, as already noted for man, rhesus $HDL_3$ has a frictional ratio higher than that of $HDL_2$. Whether this conclusion is valid for other animal species remains to be established. It appears, however, that the pig and the cow, two other animal species reported thus far, have high density lipoproteins that apparently contain a single class of particles with general characteristics comparable, but not identical, to those of human HDL.

Jonas[41] studied bovine serum HDL of $\rho$ = 1.063 to 1.125 g/ml and found that this fraction was identical with that separated at $\rho$ = 1.063 to 1.21 g/ml. The bovine HDL had a flotational rate higher

than that of human HDL and a slightly different sedimentation coefficient: $s^0_{20,w}$ = 4.95 S. From this value and assuming an unhydrated sphere, the molecular weight of bovine in HDL was 2.98 x $10^5$ compared to the value of 3.76 x $10^5$ obtained from sedimentation equilibrium. Again assuming an unhydrated sphere, the $f/f_0$ of bovine HDL is 1.08. The studies on porcine HDL reported by various authors[35,42,43] show some variance in results in terms of molecular weight, partial specific volume, sedimentation coefficients, and chemical composition. However, the isolation procedures vary among investigators. This fact further stresses the need for the introduction of standard methods for obtaining lipoproteins with which comparative studies can be carried out.

The reported values for $s^0_{20,w}$ fell within the range of 4.7 to 5.0 S, with partial specific volumes between 0.880 to 0.882 mg/g. Janado et al.,[35] using a stationary diffusion apparatus, reported a diffusion coefficient, $D^0_{20,w}$ of 2.0 x $10^{-7}$ $cm^2$ $sec^{-1}$. The value is much lower than the apparent $D_{25}$ = 4.9 x $10^{-7}$ $cm^2$ $sec^{-1}$ reported by Cox and Tanford.[43] Several factors may explain the difference in results; first, in the case of the higher D value, only one determination was available, and this when corrected to zero concentration could yield a lower number; second, in the case of the lower D value, as pointed out by the authors,[35] an error could be introduced when the experimental temperature (1°C) is corrected to a standard condition (20°); third, and perhaps most important, there is the potential problem of convection within the stationary diffusion cell which, as noted by Wong[26] in LDL studies, lowers the value of the diffusion coefficient.

The molecular weight of 2.1 x $10^5$ for porcine HDL is the average value reported by most workers, based on the techniques of sedimentation equilibrium or on the Svedberg equation. A higher molecular weight (3.1 x $10^5$) has been obtained by light scattering.[35] This may be due to the sensitivity of this method to the presence of small aggregates which, in turn, would be reflected in a higher weight-average molecular weight.

As noted previously in the discussion of the low density lipoproteins, the information obtained on HDL by analytical ultracentrifugation is far from complete. We still need to know more about the effects of various salts on serum lipoproteins, and we must acquire a better knowledge of their particle size distributions. These are only two of the areas that must be explored further if we are to gain a better understanding of the properties of HDL and of the other serum lipoprotein classes.

## ELECTRON MICROSCOPY

Interest in the application of electron microscopic techniques to the study of the morphology and structure of serum lipoproteins has grown during the past few years. Since a comprehensive review on the subject has appeared recently,[44] we will limit our discussion to an analysis of the data pertaining to the structure of LDL and HDL to permit a comparison with the X-ray data presented in the next section.

The usefulness of electron microscopy in resolving the substructure of any of the serum lipoproteins has not been firmly documented. The basic problem lies in the fact that, regardless of the method employed (fixation and shadow-casting, fixation and embedding, freeze-fracturing and etching, negative staining), it is not known whether the particle that is visualized faithfully represents the actual structure of the lipoprotein in solution. By pointing at these limitations, however, we do not intend to discourage the use of electron microscopy for the study of the structure of serum lipoproteins; rather, we wish to stress the need which exists in any complex biological structure for complementary data based on a multidisciplinary approach. The examples that follow should prove this point effectively.

### 1. Studies on Normal Human Serum Lipoproteins

**Low density lipoproteins** – Although there appears to be reasonable agreement on the overall size and shape of LDL, the existence and nature of a substructural organization within this particle is still controversial. By the negative staining technique (Figure 1), the free-standing particles have been described by Forte and Nichols[44] as being spherical or almost spherical with a diameter of 210 to 250 Å, and with a tendency to flatten into edged particles upon packing. Following an analysis at a higher magnification, however, the authors recognized edges and other fine structural details also on the free-standing particles, as is diagrammatically represented in Figure 2. A structural interpretation of such a diagram was not given.

Pollard et al.[30] examined negatively stained preparations of normal human LDL by electron

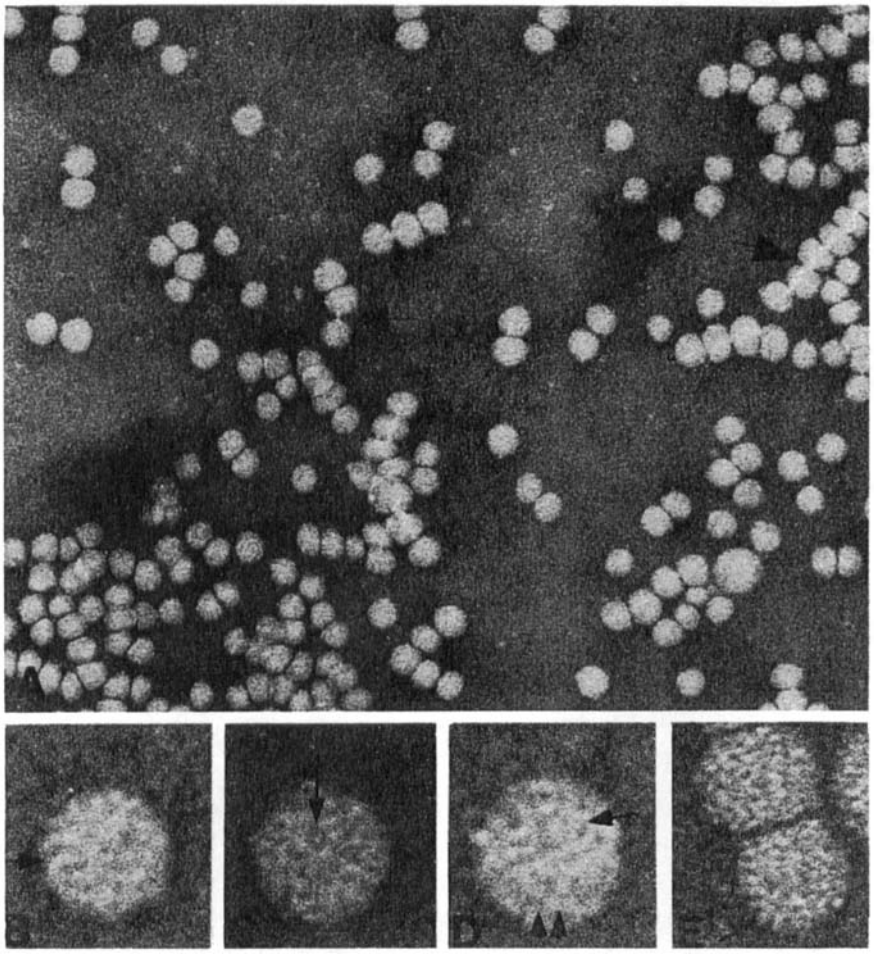

FIGURE 1. Electron microscopy of negatively stained normal human serum LDL. Micrographs B through E (magnification 705,000 x) show surface details. B and E probably represent particles seen in top view. The edges of these particles are indicated by arrows. C and D are tilted particles which show fine structural surface detail (top centers of particles are indicated by single arrows). The image in D suggests that there may be a girdling substructure in the equatorial region. (From Forte, T. and Nichols, A. V., *Adv. Lipid Res.*, 10, 1, 1972. With permission.)

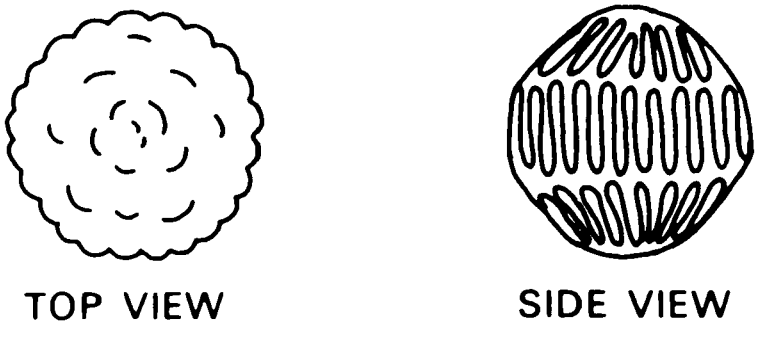


FIGURE 2. Diagrammatic representation of top and side views of LDL from the electron micrographs shown in Figure 1. (From Forte, T. and Nichols, A. V., *Adv. Lipid Res.*, 10, 1, 1972. With permission.)

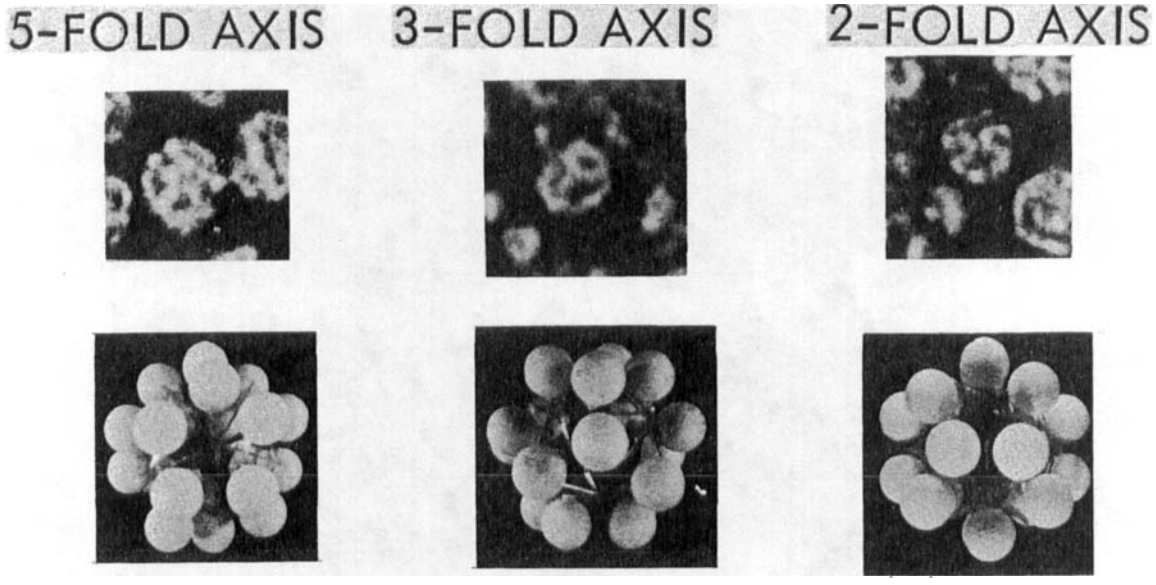


FIGURE 3. Electron micrographs of negatively stained normal human LDL (magnification approximately 260,000 x), compared with proposed model. Down views. (From Pollard, H., Scanu, A., and Taylor, E. W., *Proc. Natl. Acad. Sci. U.S.A.*, 64, 304, 1969. With permission.)



microscopy and obtained the type of image shown in Figure 3. The free-standing particles, with an average diameter of 193 Å, were interpreted to have a substructure composed of globular units approximately 40 Å in diameter and with an estimated molecular weight of 30,000 daltons. Similar structures were seen in particles from which the neutral lipids (cholesterol and triglycerides) had been removed by extraction with ethyl ether and in LDL particles which had been cleaved by phospholipase $A_2$ or phospholipase C. Thus, it was concluded that the observed globular units were proteins; this conclusion was reinforced by the fact that the elementary LDL protein units, estimated to have a molecular weight of 28,000 daltons by sedimentation equilibrium ultracentrifugation, appeared on electron microscopy to be globular units, 30 Å in diameter. The analysis of enlarged electron micrographs of LDL suggested the existence of a dodecahedral arrangement of the protein subunits (estimated number of 20 from molecular weight of each subunit and from LDL total protein mass of 500,000 daltons), with characteristic two-, three-, and five-fold axes of rotation (Figure 3). The proteins were assumed to occupy the vertices of the dodecahedron, with phospholipids and neutral lipids occupying the faces and the interior of the geometrical model, respectively. Support for such a model was derived from isodensity maps of electron micrographs of LDL as well as from photographs of a scale model of LDL. Since this model was first proposed in 1969, our understanding of the structure of LDL has not increased substantially except through the small-angle X-ray scattering studies discussed in the next section, which appear to be in general agreement with the model derived from ane analysis of electron micrographs of LDL. Very recently, Piziak et al.[45] isolated the apoprotein subunits of LDL by chromatographic techniques and found them to be identical with a molecular weight of 27,000. This value agrees with that proposed by Pollard et al.[30] but differs from the results of Smith et al.,[46] who arrived at a figure of 265,000 daltons for each of the two subunits which they considered to be the only constituents of LDL. Although the molecular weight determinations were done well technologically, it was not unequivocally proven that the preparations studied were completely dissociated in the solvents employed. Thus, the molecular weights reported by Smith et al.[46] may not necessarily represent those of the elementary protein units.

**High density lipoproteins** – Although visualization of HDL particles by electron microscopy has been reported by various laboratories, extrac-

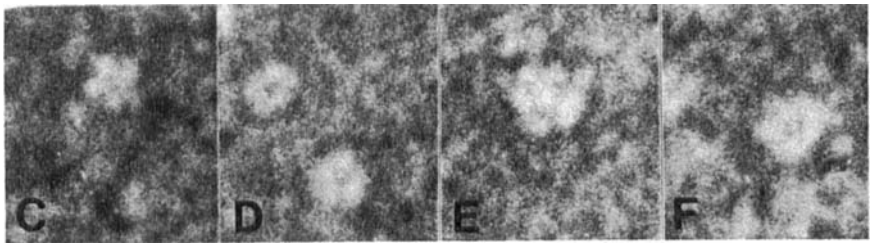


FIGURE 4. Electron micrographs of negatively stained normal HDL. C through F show highly enlarged HDL particles which appear to be composed of several globular subunits.[44] (From Forte, T. and Nichols, A. V., *Adv. Lipid Res.*, 10, 1, 1972. With permission.)

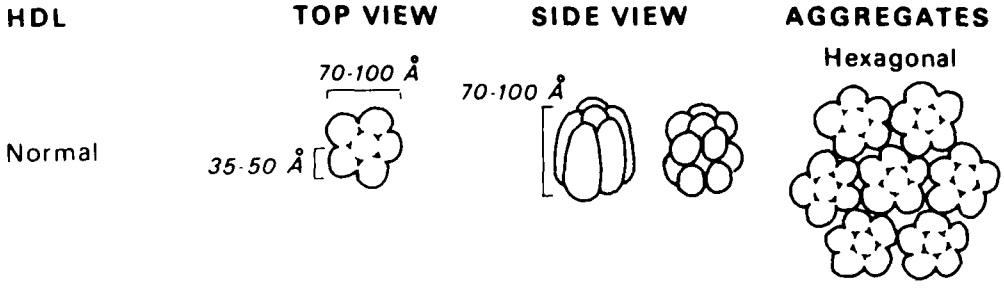


FIGURE 5. Schematic representation of possible structure of HDL. Interpretation derived from electron micrographs in Figure 4. (From Forte, T. and Nichols, A. V., *Adv. Lipid Res.*, 10, 1, 1972. With permission.)

tion of substructural information from negatively stained images has been attempted only by Forte et al.[44] According to these authors, free-standing HDL (diameter: 70 to 100 Å), upon negative staining, appear in the electron microscope to be composed of 5 to 6 subunits which in the $HDL_2$ subclass would have a diameter of 40 to 50 Å and in $HDL_3$ subclass, a diameter of 35 to 40 Å (Figure 4). The structural interpretation provided by the authors is represented schematically in Figure 5 in both top and side views. The figure indicates a set of five subunits surrounding a central core, presumably occupied by an additional subunit. This type of internal structural organization would persist after the particles interact with each other and would explain the hexagonal packing observed when HDL is allowed to dry on a grid.[44] Although the proposed model is of conceptual interest, it has not yet found support by other independent methods. In fact, the small-angle X-ray scattering studies recently reported appear to be at variance with the proposed interpretation derived from the electron microscopic images. The information derived from the analysis of scale models of HDL[47] and the chemical and enzymatic studies on the surface topology of this particle[47] are also incompatible with such an interpretation.

### 2. Lipoproteins from Human Genetic Variants and Subjects with Acquired Dyslipoproteinemia

In Table 7, we have summarized the essential clinical features of some disorders for which serum lipoproteins were analyzed by electron microscopy.

In *abetalipoproteinemia*,[48-50] although normal serum LDL are absent, very small amounts of lipoproteins floating between densities 1.006 and 1.063 g/ml have been recognized. These lipoproteins, which contain apoprotein A-I predominantly, exhibit an unusual morphology by electron microscopy (Figure 6). By negative staining method, the free-standing particles were spherical and had a diameter of 90 to 160 Å, with a mean value of 125 Å ± 15 Å (standard deviation). A large number of these particles exhibited an unusual packing arrangement with a mosaic appearance having a particle length of 102 Å ± 10 Å

TABLE 7

**Basic Clinical Features of Disorders with Dyslipoproteinemia**

| Disease state | Essential clinical features | Serum lipoproteins abnormality |
|---|---|---|
| Abetalipoproteinemia[48-50] | Malabsorption of fat, abnormal red blood cell morphology, involvement of retina, neurological symptoms. | Absence of normal LDL. Presence of abnormal lipoproteins with flotation properties of LDL but polypeptide makeup of HDL. For morphology, see Figure 6.[44,50] |
| Familial lecithin cholesterol acyltransferase deficiency[51] | Opacity of cornea, anemia, presence of protein in the urine, reduced serum levels of cholesterol esters and lysolecithin, deficiency of plasma lecithin: cholesterol acyl transferase. | Presence of lipoproteins abnormal in size, density and composition. For morphology, see Figure 7.[52] |
| Liver disease of the obstructive type.[3,53] | Obstruction to flow of bile. Elevated serum concentrations of cholesterol, mainly unesterified, and phospholipids. | Presence of lipoproteins with abnormal flotation, size, and composition. For morphology of LP-X, see Figure 8.[54,55] |



(standard deviation) and a range of 85 to 120 Å. The structural basis of such an unusual morphology is not apparent; it might be an expression of the easy deformability of these particles which is probably related to their relatively low content of cholesteryl esters.

An unusual morphology of serum lipoproteins has also been observed in patients with lecithin cholesterol acyl transferase (LCAT) deficiency.[51] During drying of phosphotungstic acid (PTA), the HDL fraction from these patients formed disc-like structures with diameters between 150 and 200 Å, which had a tendency to form stacks with a periodicity of 50 to 55 Å (Figure 7). The absence of cholesteryl esters in these particles has been related to the disc formation; this interpretation is supported by the similar morphology exhibited by products reassembled from HDL apoproteins, lecithin, and unesterified cholesterol but in the absence of cholesteryl esters.[4] The inclusion of the latter into the particles caused them to assume a spherical shape with a size approximately that of normal HDL.

Besides their abnormal HDL, patients with LCAT deficiency exhibit both normal LDL and larger particles, 500 to 1,200 Å in diameter, composed almost exclusively of phospholipid, unesterified cholesterol, and approximately 5% protein.[52] The structural significance of these particles has not been established. A special lipoprotein, called LP-X, has proved to be of particular structural interest. This particle is isolated from the plasma of patients with biliary obstruction and is characterized by a high content in phospholipids and unesterified cholesterol and little protein, composed predominantly of C-polypeptides. By electron microscopy (Figure 8), these particles have a diameter of 400 to 600 Å and exhibit vesicular structure, which upon drying in the negative stain, form stacks with a periodicity of approximately 100 Å.[53,54] The possibility that these particles, observed with normal HDL and LP-X may be artifactual, has been recognized by some authors.[53,54]

## SMALL-ANGLE X-RAY SCATTERING

The technique of small-angle X-ray scattering has been highly successful in a number of crystallographic studies, including those on particles in solution or suspension.[56] The combination of sophisticated experimental techniques with elaborate theoretical treatment has made possible the collection and proper interpretation of intensity data, and thus, a precise study of some of the molecular parameters (radius of gyration, molec-

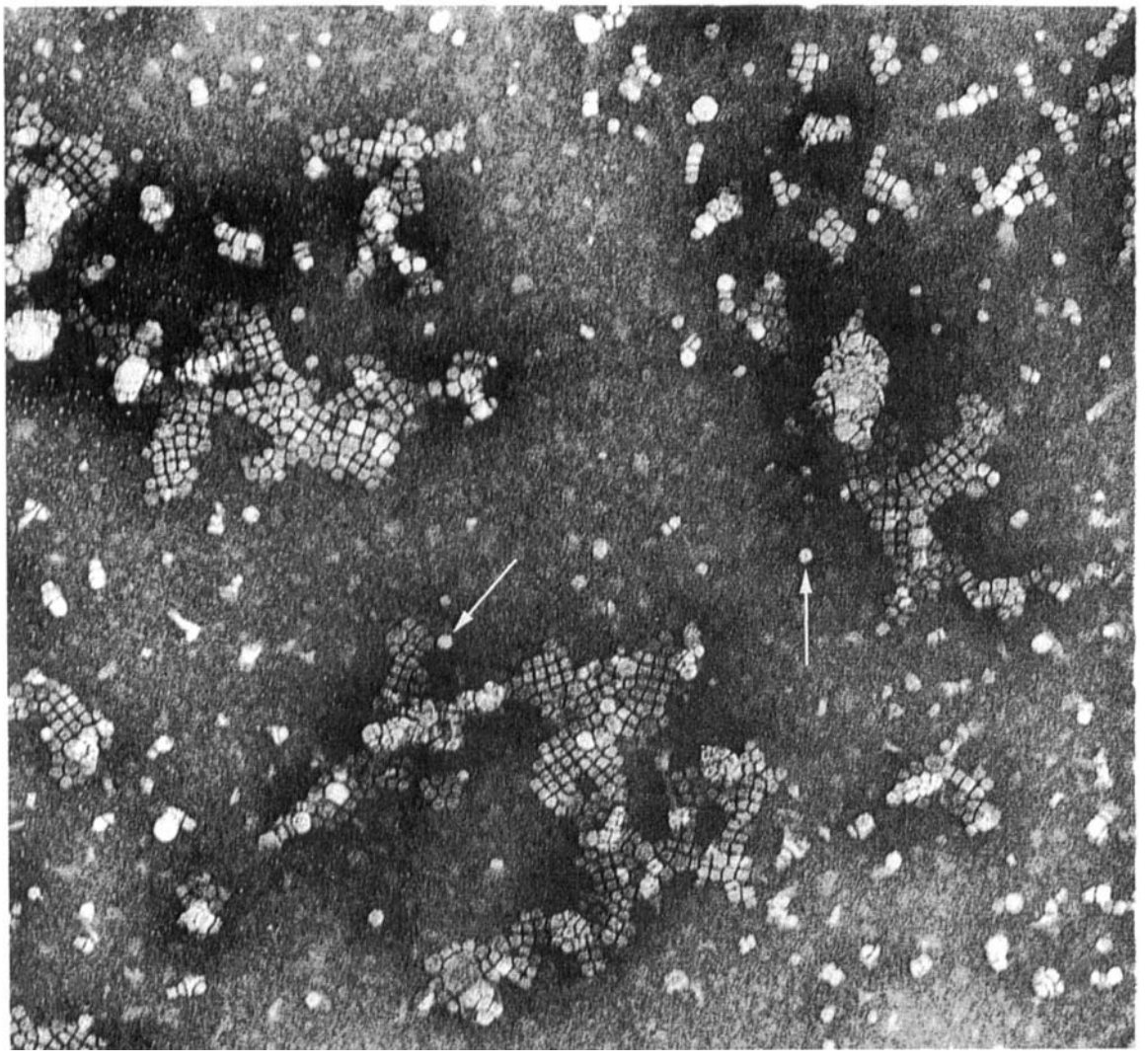

FIGURE 6. Electron micrograph of negatively stained "LDL" in abetalipoproteinemia. Magnification: 150,000 x. Courtesy of Dr. L. Aggerbeck.

ular weight, volume) of the particles under study. Small-angle X-ray scattering is a technique which originated as a specialized tool to complement standard X-ray diffraction procedures. It has become sophisticated enough for a valid application to structural analysis. We will confine our attention to some of the concepts that are applicable to the study of serum lipoproteins by X-ray techniques. No attempt will be made to discuss the physics of X-ray diffraction, since extensive literature on the subject is available.[57-59]

### 1. Sample Size and Ideally Monodisperse Solutions

If one assumes that a beam of X-ray strikes a particle and that the diffracted intensity is isodirectional with respect to the incident beam, all the scattered waves will be in phase. Increasing the glancing angle of the primary beam causes the amplitude of the scattered waves to decrease because of destructive interference and to become zero when

$$2\theta = \lambda/D, \qquad (6)$$

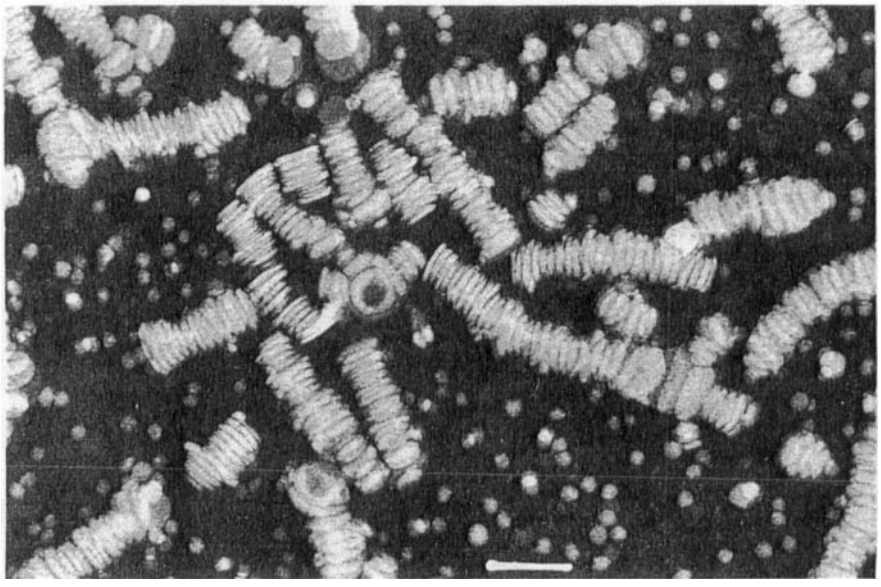

FIGURE 7. Electron micrograph of HDL in a patient with LCAT-deficiency.[52] Magnification 212,000 x. (From Forte, G. M., Norum, K. R., Glomset, G. A., and Nichols, A. V., *J. Clin. Invest.*, 50, 1141, 1971. With permission.)

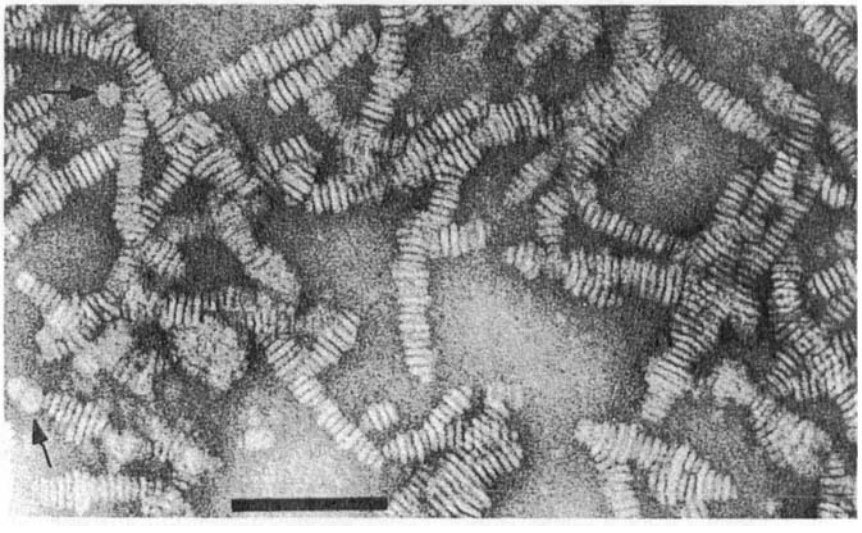

FIGURE 8. Electron micrograph of LP-X from patient with obstructive jaundice.[44] Sample magnification 100,000 x. The bar represents 1,000 A.

$\theta$ is the diffracting angle, $\lambda$ the wavelength of the radiation used, and D the average dimension of the particle. In a system of identical particles widely separated from one another, the intensity of the scattered radiation is identical, if properly scaled to the average intensity scattered by a single particle. Such a dependence of the physical dimension of the sample on the scattered intensity and the need for a system of identical particles in a state of large separation imposes serious difficulties on the study of nonperiodic arrays such as solutions. The latter represent a statistical distribution of particles which cannot be properly handled from a mathematical standpoint to define interparticle distances or an ordering parameter. Thus, the verification of a large separation depends, in practice, on a number of physical factors, such as dilution, thermal vibration, statistical probability that all particles are moving in equally probable directions, and degree of close packing. These factors may be difficult to assess in an experimental diffraction pattern. Even assuming that the particles in solution have the same volume, they may distribute themselves as in a Newtonian fluid in statistically equivalent although different groups. Such a distribution, which is reminiscent of the types of problems encountered in the study of periodic arrays, when domain structures are studied, can result in wavefronts of scattered intensity that are rather different from the actual electron distribution of the particle. In order to minimize these difficulties, it is customary to study solutions at different particle concentrations by selecting a solvent the electronic density of which differs as much as possible from that of the particle under study. While the analysis of different solutions allows for the verification of the experimental scattering curves as a function of concentration, the appropriate choice of solvent ensures discrimination between the scattering contribution by solvent and solute, respectively.

## 2. Radius of Gyration

From Equation 6, it is deduced that $D = \frac{\lambda}{2\theta}$, and this immediately indicates that experiments of small-angle X-ray scattering can be used profitably for measurements of particle size. The problem has been given theoretical and practical emphasis by Guinier,[57,60] who introduced the concept of radius of gyration. This parameter, obtained by extrapolating to zero the slope of the logarithmic curve obtained from the plot of scattered intensity versus angular position, provides a sensitive mathematical characterization of a particle. It should be clear, however, that no projection of particle shape can be derived from measurements of the radius of gyration since, in practice, different particle shapes can yield very similar scattering curves.

## 3. Fourier Synthesis

It is a standard routine in studies of crystal structure analysis to collect intensity data, I, from atomic planes (hkl) and to express them in terms of a so-called structure factor (F), as shown in Equation 7. After proper allowance has been made for a number of experimental parameters;

$$\sqrt{I(hkl)} = |F(hkl)|, \tag{7}$$

it is possible to map out all the scattering properties of a periodic array. It can also be shown that

$$F_{hkl} = A + iB, \tag{8}$$

where A and B are the real and imaginary components, respectively.

Thus, an unambiguous reconstruction of such a scattering distribution coupled with a knowledge of the scattering characteristics of the constituent atoms makes possible a precise reconvolution of any structural framework. This is commonly done by use of the properties of Fourier analysis, which requires a knowledge of the so-called phase angle in exponentials of the type

$$e^{i\alpha_{hkl}}. \tag{9}$$

The value of $\alpha$ is generally not available from experiments; this represents a formidable limitation that constitutes the so-called phase problem in X-ray crystallography. In principle, since small-angle scattering techniques yield intensity data in terms of angular position, it would appear feasible to apply standard Fourier techniques to the analysis of a small-angle diffraction pattern; some clarification is needed, however.

## 4. Effect of a Center of Symmetry

In particles having a center of symmetry, Equation 8 reduces to:

$$F_{hkl} = \pm |F_{hkl}|, \tag{10}$$

since the imaginary component, iB, is zero. The

transform of a centrosymmetric distribution, which is itself centrosymmetric and has no imaginary counterpart, is represented by lines having phase angle values of 0 or $\pi$. These lines are usually referred to as "nodal lines." In the case of a particle having spherical symmetry, the nodes establish sign relationships for all the fringes of the transform. Thus, an ideal transform of a sphere should show intensity maxima represented by regions of zero density. Similar arguments may be carried out for other centrosymmetrical loci (ellipsoids, cylinders, etc.), the scattering properties of which can be predicted with precision. In practice, this knowledge of the theoretical scattering curves for a given geometrical shape permits the preliminary interpretation of a small-angle scattering pattern. The presence of a center of symmetry causes the intensities in between the maxima to fall to zero, and this should be observable experimentally. Lack of such evidence, although partially justifiable in terms of technical considerations, i.e., a distribution of particle sizes, appears to support the view that deviation from centrosymmetry may indeed occur. This may apply to serum lipoproteins. (In this context, it is worth mentioning that the chain folding in proteins usually prevents crystallization in centric space groups.) The absence of a center of symmetry causes phasing according to criteria incompatible with a simple + or - relationship since, in transforms where B differs from zero, the phase angle can have any value around the nodal points, which are specific points characterized by F = 0 in the general transform F = A+iB. . . . This ought to be considered during examination of a diffraction pattern that is characterized by well-resolved side maxima. This symmetric distribution could be due to several causes, such as a symmetrical arrangement of some subunits in an otherwise asymmetric distribution.

## 5. Truncation of the Fourier Series

Since a Fourier series is truly representative of a structural continuum only when convergence is reached at infinity, the higher the number of intensity data, the more precise is the representation of an array. Truncation of data introduces an error in the reconvolution of a pattern, the importance of which has been the subject of extensive theoretical work, especially in connection to two- and three-dimensional arrays.[61,62] No work of this kind has been reported with reference to patterns reconvoluted from small-angle scattering experiments, but the problem is presently being investigated (A. Tardieu, personal communication). However, the extension of an approach proposed by Cruickshank[63] should allow for some qualitative considerations. Assuming that a particle has a uniform, close packed array of identical atoms, it can be shown that, in the truncated series, the intensity of each of the n atoms will be displaced in position by the intensities of all the other atoms. For two atoms separated by a distance d, such a displacement can be expressed, based upon a point atom approximation, as

$$(d)_{max} = \frac{2S_0^2 f_{01}}{\tau^2 C_2}, \qquad C_2 = \frac{\partial^2 \rho_2}{\partial \tau^2}, \tag{11}$$

where $f_{01}$ is the scattering factor of the first atom at a point S, $\rho_2$ the density of the second atom and $\tau$ the interatomic distance. From Equation 11, one can deduce that the effect of truncation of a series decreases with increasing distance that the error is particularly serious in the case of scatterers with a low atomic number. (These scatterers exhibit high values of f for small values of sine $\theta/\lambda$.) Thus, we would intuitively infer that, for a region extending at least up to 20 to 25 Å from the origin of reciprocal space, errors in series termination should preclude any reliable use of the Fourier transform in small-angle scattering experiments. This kind of error, for instance, could be encountered in the analysis of serum lipoproteins. The transform, however, should be representative at greater distances from the origin. Short of a solid theoretical framework, the introduction of an artificial Gaussian temperature factor during the computation of the series could help in checking the sensitivity and thus the validity of the results of the transform from a diffraction pattern. It would be interesting, furthermore, to assess the magnitude of the displacement, if any, induced to an adjacent peak from an intensity maximum.

## 6. Studies on LDL

Mateu et al.[64] monitored the small-angle scattering of LDL particles suspended in NaBr at different concentrations. The presence of bands separated by low minima was considered to represent a high-particle symmetry, which is

probably a centric one, within the limits of resolution (33 Å) of the technique. The intensity data in terms of standard Fourier techniques, when appropriately corrected for background and computed according to phasing criteria which are only compatible with a + or − correlation (centric distribution), indicated the presence of a minimum centered at 65 Å from the origin of reciprocal space. The low density of such a shell and the presence of subsidiary maxima at each side of the minimum were taken to indicate that LDL is a lipid bilayer coated by an outer shell of protein molecules. These proteins would have a regular distribution and would be separated by a center-to-center distance of ≅ 45 Å, as indicated by a band exhibiting such a spacing. The proposed symmetry was considered compatible with an icosahedron, although the existence of 60 subunits was not documented experimentally. The proposed model, which is still under investigation, is clearly consistent although it does not prove the correctness of the model derived from electron microscopic data. Furthermore, due to the low resolution of the experimental technique, the structural role for $H_2O$ was not defined. It is worth noting that the measure of indeterminacy in this method, 33 Å, could be larger than the magnitude of any of the crystallographic translations of a crystalline array.

### 7. Studies on HDL

Shipley et al.[65] studied human serum $HDL_2$ at different solvent (NaCl) concentrations and determined a conventional value of the radius of gyration, Rg = 46 Å. Desmearing the pattern to approximate the finite cross section of the experimental beam to an infinitely fine one[66-68] gave a value of Rg = 44 Å. Under the assumption that $HDL_2$ particles are spherical, as suggested by the presence of resolved subsidiary maxima, and using the carefully determined value of the radius of gyration, the authors calculated a particle diameter of ≅ 119 Å. (Using a molecular weight of 386,000 daltons, the resultant molecular diameter is 104 Å.) Both these values were consistent with observations by electron microscopy which gives particle diameters of the order of 90 to 100 Å. By the assumption of a spherical model with a nonuniform, electron density, distribution, considerable agreement was obtained between observed and computed diffraction profiles. This was done by systematically arranging for variations in the values of the radii of the particle (external and internal) and of the electron densities of both core and shell. Thus, it was concluded that $HDL_2$ particles consist of a relatively electron deficient core (≅ 86 Å in diameter), surrounded by an electron-rich outer shell (11 Å). As stated by the authors, further study should be devoted to a positive verification of the spherical shape of $HDL_2$. For instance, it would be of interest to interpret the fitted experimental and theoretical curves in terms of a Fourier transform, taking into account the limited intensity data and the lack of knowledge of the phase angle. Such studies are in progress.

### 8. Studies on $HDL_3$

*Human* – Laggner et al.[69] recently reported on small-angle scattering experiments on human $HDL_3$ which was assumed to represent a highly homogenous material. After proper corrections for several technical factors (influence of the $K_\beta$ emission line, correction for interparticle interference, collimation errors), small-angle diffraction profiles obtained at 4° and 23°C yielded an estimated Rg - 50.5 ± 0.15 Å. On the grounds that the observed value of Rg was inconsistent with a closely packed spherical or cylindrical particle shape, the authors suggested that the pronounced side maxima, exhibited in the pattern, ought to be attributed to internal correlation of subunits. Therefore, they proposed a model consisting of a "lipid core surrounded in a more or less symmetrical way by subunits." Additional data are obviously needed for further support of this model.

**Porcine** – Atkinson et al.[70] carefully studied the small-angle scattering of $HDL_3$ from porcine plasma. They measured a conventional Rg of 54 Å from diffraction profiles which were characterized by "high homogeneity or particle symmetry" as evidenced by two well-resolved subsidiary maxima. Since fitting of the experimental data on the basis of a model exhibiting a uniform, close packed particle shape was not possible, reproducibility of the theoretical and experimental pattern was achieved through a careful assessment of the values of the radii (internal and external) of the inner and outer portions of the particle. Computation of the Fourier transform from the diffraction profile, corrected for termination errors through the introduction of a Gaussian temperature factor and phased according to a centrosymmetric distri-

bution indicated the presence of a low-density core which was reported to have a nominal radius of 42 Å and to be surrounded by an annulus of proteins of 12 Å thickness. The transform was reproducible in three different patterns obtained at different concentrations of solvent.

### 9. General Remarks

As anticipated by Guinier et al.,[57] studies of small-angle scattering of X-rays are particularly useful for a quantitative characterization of the size (radius of gyration) of particles in solution. Such information, if properly analyzed in terms of other physicochemical data, permits a good determination of parameters such as molecular weight and particle volume. As shown from the examples listed above, a careful extrapolation of the information obtained through small-angle X-ray scattering experiments can give a broad insight into the structural features of serum lipoproteins. However, no information on the internal structural arrangement of the constituent peptides nor any quantitative characterization of their crystallographic properties (space group, lattice parameters) can be obtained by this method. This would require the solution of details on a scale smaller than that of the resolution offered by the techniques of small-angle X-ray scattering. Nonetheless, this technique at present offers the simplest and most direct approach to the study of solutions of serum lipoproteins or of other macromolecules in general. As stated in section C, radiation damage and drying of the specimen under vacuum are still serious drawbacks in the various techniques of direct observation of high resolution microscopy. Methods of optical rotatory dispersion, circular dichroism, nuclear magnetic resonance, and electron spin resonance used individually are not likely to be more efficient probers of the internal structure of serum lipoproteins, but the information that can be gathered by their simultaneous application can prove extremely useful and rewarding. Thus, until some crystalline material is available and therefore a three-dimensional (or at least two-dimensional) study is made possible, our knowledge of serum lipoproteins must rely on the intelligent evaluation of data from the physicochemical approaches that are available. Apart from the expected differences in the magnitude of the radii of gyration, the reported studies on serum HDL have given very similar macroscopic structural features. This is gratifying in view of the number of different observers involved. The area of research on LDL is comparatively less settled at this moment, and the structural and physiological implications regarding this particle must await further experimentation. It should be stressed that nothing is known on the role of structural water, both in the HDL and in LDL; yet such an information should prove essential to an understanding of some of the physicochemical properties of serum lipoproteins. Studies now in progress combining the small-angle X-ray scattering technique and neutron diffraction analysis should allow for a more sophisticated evaluation of the diffraction patterns.

## OTHER PHYSICAL METHODS EMPLOYED IN THE STUDY OF INTACT SERUM LIPOPROTEINS

Since the subject has been reviewed recently,[4,47] the most relevant information has been summarized in Table 8. This includes the results obtained by the techniques of optical rotatory dispersion, circular dichroism, infrared and fluorescence spectroscopy, proton and $^{13}C$ nuclear magnetic resonance, and electron spin resonance.

## CONCLUDING REMARKS AND PERSPECTIVES

In this article, we have attempted to provide a panoramic view of the results which have been obtained by the application of a number of physical methods to the study of the structure of serum lipoproteins, with particular reference to the low and high density classes. It is clear from the preceding discussion that no one method is likely to resolve the structure of any lipoprotein, rather it appears that a combination of methods is necessary. It was shown recently that X-ray techniques can be applied to the study of lipoproteins in solution, and certainly more advances are expected in this area of investigation. Similarly, contributions by electron microscopy have centered primarily around the technique of negative staining. Promising developments may be expected through the application of freeze-etching or scanning electron microscopy, provided that methods are devised to prevent particle disruption

| | |
|---|---|
| Presence of α-helix, antiparallel β-sheet, and unordered structure. Proportions dependent upon temperature and lipid content of the particle. The β-structure appears to predominate at high temperatures (37°–50°C), and the α-helix at temperatures around 4°C. | Predominance of α-helical conformation; the remainder represented by unordered structure. A relatively high α-helical structure was also observed in the two major HDL apoproteins after delipidation. Lipids may play a stabilizing role in the conformation of the HDL protein. |
| Data corroborate the structural information derived from the above studies. | |
| | Limited information available. Studies indicate that there are three apolar binding sites in HDL. Also, the aromatic amino acids are located in a relatively polar environment. |
| Spectra indicate that the polar head groups of phospholipids are quite free and are probably in an aqueous environment. Lipid hydrocarbon chains are also highly mobile. The nonpolar aromatic amino acid residues of the protein are immobilized somehow, probably by apolar interactions with lipids. | No evidence for apolar interactions by line-broadening criteria. Data suggest a "micellar" model for HDL structure. The data did not rule out the existence of a certain percentage of protein and the lipid protons constrained in their motion. |
| Only preliminary data available. Proton-decoupled spectra showed most intense peaks deriving from fatty acid acyl moieties of cholesterol esters, phospholipids, and triglycerides, and of choline methyl derivatives. There was little contribution by protein. | |
| Two types of signals observed: narrow and broad, the latter indicative of areas in the particle with constrained motion and possibly relating to protein-lipid interactions. | Spectra showing a narrow and a broad component, the latter attributable to protein-lipid interactions. The narrow component probably indicates lipids with unrestricted motion. |

om References 72 and 73.

under the electron beam. This optimistic outlook may be extended to the various spectroscopic techniques, particularly to nuclear magnetic resonance using $^{13}C$, $^{31}P$, or other suitable nuclides, electron spin resonance, and fluorescence techniques. Important information on intact lipoproteins is also likely to be gained if more effort is directed toward the elucidation of the structure of the abnormal lipoproteins present in genetic or acquired human variants which are already known or are still to be discovered. Studies of various animal species should prove valuable, particularly of those species that have a close phylogenetic relation to man. In view of the structural complexity of serum lipoproteins and of their relatively large dimensions, however, analyses which are limited to the intact particle are not likely to provide information on the molecular structure of these particles. Complementary studies are clearly needed, and these should include detailed investigation of each of the lipoprotein constituents either alone or after recombination. The work on the structural characterization of each of the lipoprotein polypeptides is well under way, as are studies aimed at explaining the great avidity of these polypeptides for lipids. Looking at the rapidly growing literature on serum lipoproteins, the increasing number of investigators entering the field, and the technological developments in the areas of both lipid and protein research, one is led to suspect that many of the present structural uncertainties are likely to become clear in the not too distant future. It is also expected that the information acquired will help to define the function of these lipoproteins which are still largely unknown.

## ACKNOWLEDGMENTS

The senior author is recipient of USPHS Career Development Award HL #24867 and Dr. Lidia Vitello of USPHS Postdoctoral Fellowship #HL52752. The work by the authors cited in this version was supported by USPHS grants HL #08727 and HL #06481 and from grants from the Illinois and Chicago Heart Association (A 72-6) and the Atomic Energy Commission.

The authors wish to thank Drs. Phillip Keim and Lon Aggerbeck for valuable comments and Mrs. E. Lanzl for the final editing of the manuscript, and Ms. F. Skozen for the typing of the manuscript.

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