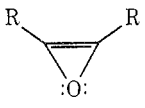

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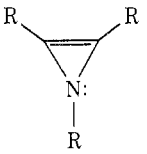

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Major antiaromatic effects have been detected so far principally in the normal 4π conjugated systems: cyclopropenyl anion, cyclobutadiene, and cyclopentadienyl cation. Within these systems, however, the effects are quite substantial, comparable in magnitude of energy to the aromaticity in benzene.

I thank my coworkers, who are named in the references, for their intellectual and experimental contributions to our work. Financial support was furnished by the National Institutes of Health, the National Science Foundation, and Hoffmann-La Roche.

Hydrodynamic Analysis of Human Low Density Lipoproteins†

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Anyone who has faced a clogged kitchen drain will agree that lipids have a very limited solubility in water. Moreover, the consequences of accidental stoppage can be disastrous.

This household problem has a counterpart in the transport of lipids through the blood and lymph of the human body. These fatty substances must be kept in aqueous suspension while they are distributed to the organs and tissues. After a meal very high in lipid, the serum may become a milky white due to light scattered from opalescent particles of fat, which are kept from coalescing by a thin coat of protein, phospholipid, and cholesterol. The particles are large enough to be seen in the dark field microscope, and they are called chylomicra.

Chylomicra are produced by the gut after a meal containing fat.[1] Other lipoproteins are primarily produced by the liver. In fact, on a high carbohydrate diet, lipoprotein levels may rise higher than ever in some people.[2] Fatty acids are synthesized from carbohydrates in the liver or obtained from adipose tissue. In turn, fats and phospholipids are synthesized from the fatty acids. Finally, the newly synthesized fats and phospholipids, together with protein, are packaged and excreted directly into the blood.[3] These particles are usually a little smaller than the chylomicra, but still very large. They are called very low density lipoproteins (conveniently abbreviated VLDL).

Unlike amino acids and carbohydrates, and perhaps unique to lipids, the first step in the metabolism of lipoproteins takes place directly within the blood capillaries. The enzyme, lipoprotein lipase, is attached to sites within the capillary walls, and it can hydrolyze either chylomicra or VLDL, flooding the adjacent tissue with fatty acids. The presence or absence of this lipase is under hormonal control; thus, the fatty acids released from chylomicra may be directed to adipose tissue for storage during feeding, or the VLDL fatty acids, to heart and skeletal muscle for utilization during starvation.[4]

Low density lipoproteins (LDL) are a third class of lipoproteins which are smaller molecules than either the chylomicra or the VLDL. In fact, there is considerable evidence that the LDL constitute the molecular residue left after the hydrolysis of most of the triglyceride from the chylomicra and VLDL by lipoprotein lipase.[5] In addition, some LDL may be produced by the gut or liver.[6] The low density lipoproteins are present in gram quantities in normal human serum, and the average levels depend upon age and sex.[7] The hydrodynamic properties of the low density lipoproteins are the special subject of this Account.

For completeness, we must mention the high density lipoprotein class (HDL). This class is composed of several types of molecules of 200,000 to 400,000 molecular weight, composed of about 50% protein and 50% lipid, mostly phospholipids.[8] All of their functions are not understood, but there is considerable evidence that the HDL plays roles in enzyme activation of lipoprotein lipase and lecithin–cholesterol acyltransferase (LCAT). In Tangier disease, a congenital lack of HDL, massive deposits of cholesteryl ester occur throughout the lymphatic tissues.

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†Contribution No. 3110 from the Department of Chemistry.

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Lending great interest to the study of serum lipoproteins and justifying considerable financial support of lipoprotein research are the distinct possibility of the involvement of these molecules in the development and progress of circulatory disorders.[9] These include both heart and vascular diseases. Victims will eventually include at least half of the American males and a significant fraction of the women.

The rabbit, a herbivore, will not experience these kinds of circulatory disorders on his normal diet. But if cholesterol is added to rabbit chow, he will rapidly develop a pathological condition bearing a striking resemblance to human arterial atherosclerosis, including thickening of the intima of the aortic walls, lipid accumulation and plaque formation, and deposits of lime granules.[10] Normal rabbit serum usually contains less than 50 mg/100 ml of the low density lipoproteins. After the animal has been on a cholesterol diet for several weeks the concentration of these lipoproteins may increase 10- or 20-fold over the initial level. These lipoproteins contain a high content of cholesterol, and it is likely that they are responsible for the transport of this lipid to the lesions in the vascular walls.[11]

There is a great deal of evidence pointing to diet as an important factor in the rate of lipid deposition in the human as well as the rabbit. High amounts of cholesterol and saturated fats in the diet are associated with increased deposition.[12] Moreover, the lipid composition of the arterial deposits is most similar to the composition found in the low density lipoproteins. Many important factors in addition to diet are involved in the generation of atherosclerosis, among which are blood pressure, turbulence and elasticity within an individual circulatory system, lesions or scarring caused by bacterial or viral infections, and age and genetic differences.

In spite of a large investment of time and money, precise knowledge in many areas is still lacking. Little is known about the details of the molecular architecture of the low density lipoproteins, for example, and even less is known about how they are formed and degraded. For these reasons we have chosen to reexamine some of the fundamental physical parameters of these large, lipid-bearing molecules.

Approaches to the Study of Lipoprotein Structure

Lipoproteins may be isolated, purified, and fractionated according to their densities[13] or selectively precipitated by various sulfonated polysaccharides.[14]

One approach to the study of lipoprotein structure is analytical. Thus, the amounts and kinds of the various lipids and amino acids have been determined,[14] and a sequence has recently been published for one of the HDL peptides.[15]

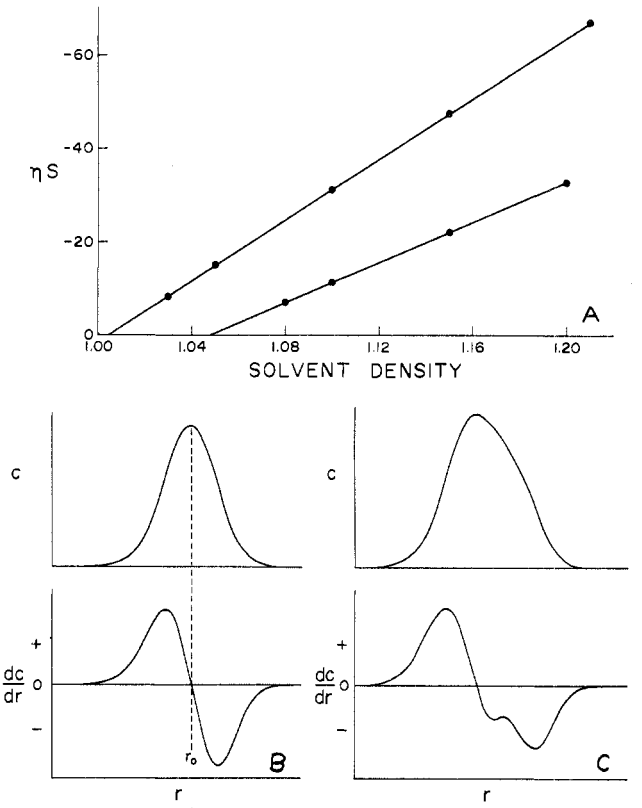

Figure 1. Two methods for the determination of buoyant densities of lipoproteins. (A) A plot of the viscosity-corrected sedimentation coefficient (Svedbergs) against density (g/ml) yields the buoyant density as the horizontal intercept. (B) Sedimentation equilibrium in a buoyant density gradient yields a band of lipoproteins centered at the buoyant density. In the upper curve lipoprotein concentration, c, is plotted against the radial distance, r, in the centrifuge cell. The derivative curve, obtained directly with the schlieren optical system as dc/dr, yields the buoyant density at the crossover point. (C) Lipoprotein samples containing more than one component are frequently clearly resolved and show several maxima and minima in the derivative trace. These figures are meant to be purely schematic, although data points are shown.

A second approach involves a detailed examination of hydrodynamic properties,[16,17] and is the subject of this Account.

A third approach involves optical probes of macromolecular structure.[8] Reassembly of lipoprotein structure holds promise of yielding valuable information.[8] Also, a fascinating series of genetic diseases provides an elegant tool for the study of these important macromolecules.[5,8,18]

Hydrodynamic Studies on Low Density Lipoproteins

The Buoyant Densities of Low Density Lipoproteins. One of the molecular parameters readily determined for serum lipoproteins is their buoyant density. The buoyant density is obtained by plotting the velocities of sedimentation and/or flotation as a function of density of the solution and extrapolating to zero velocity. NaCl, NaBr, and KBr have been extensively used as density-increasing salts. Addition of these salts will change both the viscosity, η, and density, ρ, of the solution; the product of the viscosity and the sedimentation coefficient, s, will be a function of the solution density, according to eq 1.

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Table I
Physical Parameters of Low Density Lipoproteins

Sample[a]	Ref	$s^0_{26,1.063}$, S	$s^0_{25,1.481}$, S	$s^*_{25,1.20}$,[b] S	Buoyant density, ρ_b, g/ml	Mol wt, g/mol × 10^{-6} [c]	Mol vol, ml/mol × 10^{-6} [d]	Lipid density g/ml[e]
876	23	−13.5		−54.3	1.0079	3.55	3.52	0.9648
877	23	−13.5		−56.6	1.0110	3.89	3.84	0.9723
876	23	−6.89		−38.4	1.0272	2.52	2.46	0.9654
877	23	−6.87		−39.1	1.0281	2.62	2.54	0.9692
876	23	−4.96		−33.5	1.0348	2.22	2.14	0.9639
877	23	−4.13		−32.3	1.0393	2.20	2.11	0.9682
ML	24		−45.07	−38.4	1.0290	2.57	2.50	0.9688
BT	24		−47.29	−40.4	1.0278	2.74	2.66	0.9718
EP	24		−40.33	−33.5	1.0359	2.24	2.16	0.9659
WP	24		−45.32	−38.8	1.0276	2.57	2.50	0.9673
FK	24		−45.50	−38.0	1.0344	2.67	2.58	0.9774
WE	24		−41.88	−34.3	1.0390	2.40	2.31	0.9749
VLD-PRE	24		−59.45	−54.0	1.010	3.59	3.55	0.9676
S_f20	25			−67	1.004	4.70	4.69	0.9722
S_f17	25			−61	1.008	4.23	4.20	0.9725
S_f10	25			−45	1.025	3.13	3.06	0.9768
S_f4	25			−32	1.048	2.37	2.26	0.9842
S_f0	25			−18	1.066	1.23	1.15	1.007[f]
WF	25			−38.5	1.034	2.71	2.62	0.9775
JM	25			−38.0	1.034	2.66	2.57	0.9767
							Average (excluding S_f0)	0.9715

[a] Samples S_f0 through S_f20 are from hyper-pre-β-lipoproteinemic patients. The other samples are from "normal" donors. [b] $s^*_{25,1.20} = (\eta_{25,1.20}/\eta_{25,w})s^0_{25,1.20}$; $s^0_{25,1.20} = \{s^0_{x,y}(1 - 1.20/\rho_b)\eta_{x,y}\}/\{(1 - y/\rho_b)\eta_{25,1.20}\}$; $\eta_{25,w}$ = 0.8904 cP (H_2O), $\eta_{25,1.063}$ = 1.0260 cP (NaCl), $\eta_{25,1.20}$ = 0.8534 cP (KBr), $\eta_{25,1.481}$ = 2.000 cP (NaBr). [c] $M = (6.63\eta_{25,w}s^*_{25,1.20}/(1 - 1.20/\rho_b))^{3/2}(3/4\rho_b)^{1/2}\pi_N$. [d] Molecular volume = M/ρ_b. [e] Apparent lipid density = $(M - 510{,}000)/((M/\rho_b) - 510{,}000 \times 0.728)$. [f] For this sample only, we assumed the protein molecular weight was 255,000 for the lipid density calculation. This lipoprotein behaves like a "half-molecule" of the S_f4 lipoprotein.

$$\eta s = k(1 - \rho/\rho_b) \quad (1)$$

where k is a function of molecular weight, size, and shape. The quantity ρ_b is called the buoyant density of the lipoprotein. Typical ηs *vs.* ρ experiments are illustrated in Figure 1A. ρ_b is the density intercept where ηs equals zero. Equation 1 is linear when ρ_b is constant.

Buoyant densities may also be determined by sedimentation equilibrium in a buoyant density gradient.[19-22] This is illustrated in Figure 1B. The equilibrium banding technique is particularly useful for determining density heterogeneity, as shown in Figure 1C.

Buoyant densities for the low density lipoproteins are shown in column 6 of Table I. These samples have been selected from the published data of three different groups of workers.[23-25] Buoyant densities range from 1.004 to 1.066. These represent the extrema of the density range occupied by the low density lipoproteins.

Partial Specific Volumes of the Low Density Lipoproteins. Katz and Schachman[26] had suggested that buoyant densities determined in D_2O–H_2O mixtures might yield partial specific volumes, and later we showed this was the case for the enzyme ribonuclease.[27] Fisher, *et al.*,[28] have applied this technique to the low density lipoproteins, and they have found the same values for the buoyant densities of low density lipoproteins in D_2O–H_2O mixtures and in KBr solutions. These agree with direct measurements of partial specific volume.[29]

Therefore, the buoyant densities listed in column 6 of Table I are expected to approximate the reciprocal partial specific volumes of these macromolecules.

Sedimentation Coefficients of the Low Density Lipoproteins. Lipoproteins are usually studied in salt solutions which are more dense than the lipoproteins. The sedimentation coefficients listed in Table I, columns 3–5, are negative numbers, which indicates that the lipoproteins float instead of sediment under the action of the centrifugal field. The interpretation of the subscripts of the symbol $s^0_{26,1.063}$ indicates that the sedimentation measurements were carried out at 26° in a solvent of density 1.063 g/ml. The superscript 0 indicates a correction for concentration dependence. When the superscript * is used, it indicates that a solvent viscosity correction has also been applied.

As can be seen by an inspection of eq 1, sedimentation coefficients measured in solutions of very high density ρ are much less sensitive to small variations in the buoyant density ρ_b. From such sedimentation coefficients, accurate determinations of molecular weight are possible.[30]

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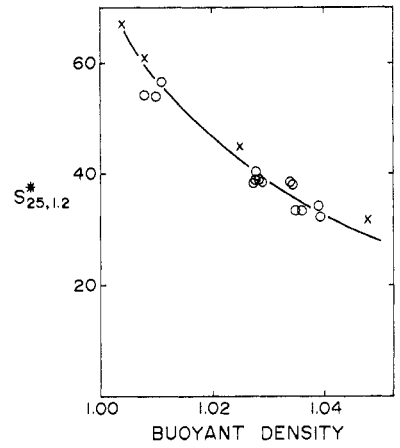

Figure 2. Plot of sedimentation coefficient (Svedbergs) against buoyant density (g/ml) for low density lipoproteins. These are the data presented in columns 5 and 6 of Table I. (○) Lipoproteins from "normal" donors; (×) lipoproteins from hyper-pre-β-lipoproteinemia patients. The line drawn through the points is for hypothetical lipoproteins containing 510,000 daltons of protein, $\bar{v}$ = 0.72 ml/g, various amounts of lipid of density of 0.97 g/ml, and $f/f_0 = 1.105$.

In order to compare the sedimentation properties of the various lipoprotein samples studied by three groups of workers, we have converted all values to $s^*_{25,1.2}$, using the conversion formulas shown in the footnote to Table I. A wide range of sedimentation values are found, extending from −18 to −67 S (1 S = 10^{-13} sec).

It is of immediate interest to see if there exists any correlation between these sedimentation values and the buoyant densities. Therefore, these quantities are cross-plotted in Figure 2. Although there is some scatter to the points, an obvious reciprocal relationship is apparent; those molecules with the higher densities have smaller negative sedimentation coefficients.

One sample tabulated in Table I has not been included in Figure 2. This is the S_f0 lipoprotein. As will be discussed later, we believe this is a half-molecule.

Lipoprotein Diffusion Coefficients. Lipoprotein diffusion coefficients have been difficult to measure by conventional techniques because the low densities of the lipoproteins provide little density increment to stablize the boundaries. Recently it has been found that the additional field strength provided by a low centrifugal field can magnify the density increment sufficiently to permit satisfactory measurements of diffusion coefficients. Thus, Fisher, Granade, and Mauldin have shown that diffusion coefficients measured either in low salt or 1.20 g of KBr/ml yield $D^0_{25,w}$ = 2.17 F (25° in water; 1 Fick = 10^{-7} cm²/sec). We find that measurements in 1.400 g of NaBr/ml yield the similar value of 2.25 F.[31,32]

The diffusion coefficient is a particularly important measurement because it may be combined with sedimentation coefficients to yield hydrated molecular weights (eq 2, where R and T are the gas constant and absolute temperature). Once M is known, the translational frictional ratio may be determined (eq 3, where N is Avogadro's number). The frictional

$$M = \frac{s}{D}\frac{RT}{(1 - \rho/\rho_b)} \quad (2)$$

$$\frac{f}{f_0} = \frac{M^{2/3}(1 - \rho/\rho_b)}{Ns6\pi\eta}\left(\frac{4\pi N\rho_b}{3}\right)^{1/3} \quad (3)$$

ratio should be a function of shape and hydration only. For this quantity Fisher, *et al.*,[28] find a value of 1.11. We find 1.10. For subsequent computations, therefore, we will assume an average value of 1.105.

If we now make the assumption that the shape and hydration of the low density lipoproteins are independent of their sizes, then we can use this value of 1.105 for all low density lipoproteins.

Equation 3 may then be rewritten as eq 4. A similar expression has been developed by Fisher, *et al.*[28]

$$M = (6.63\eta s/(1 - \rho/\rho))^{2/3}(3/4\rho_b)^{1/2}\pi N \quad (4)$$

Equation 4 has been employed to determine all of the molecular weight values listed in the seventh column of Table I.

Molecular Weight–Molecular Volume Relationship. If two lipoproteins differ in molecular weight by ΔM, their molecular volumes, V_m, will differ by the sum of the differences in the volumes of the constituent lipids and proteins. Thus

$$\Delta V_m = \sum \Delta M_i/\rho_i \quad (5)$$

where ρ_i is the density of an individual component and ΔM_i is the difference in amounts of that component found in the two lipoproteins. Let ρ_{av} be the average density of the differences. Then

$$\rho_{av} = \frac{\Delta M}{\sum \Delta M_i/\rho_i} \quad (6)$$

Equations 5 and 6 are combined to yield

$$\frac{\Delta V_m}{\Delta M} = \frac{1}{\rho_{av}} \quad (7)$$

Thus, if the molecular volume is plotted as a function of the molecular weight, the reciprocal of the slope will yield the average value for the density of the mass that must be added to convert the first lipoprotein into a particle of the same mass and density as the second.[33]

Listed in the penultimate column in Table I are the values of the molecular volume. These values are plotted as a function of the molecular weight in Figure 3. The points fall on a straight line with a slope of 1.03 ml/g. The reciprocal of this quantity yields the density of the mass by which these lipoproteins differ. This is 0.97 g/ml, a low value, which indicates that the substance must be primarily lipid. These data are consistent, therefore, with a model for the low density lipoproteins in which the amount of protein is constant while the amount of lipid varies from one molecule to the next.

The Weight and Density of Protein and Lipid in Low Density Lipoproteins. Recently Smith, Dawson, and Tanford[34] have measured the molecular weight of the apoprotein of low density lipoproteins and suggest that it consists of two polypeptide

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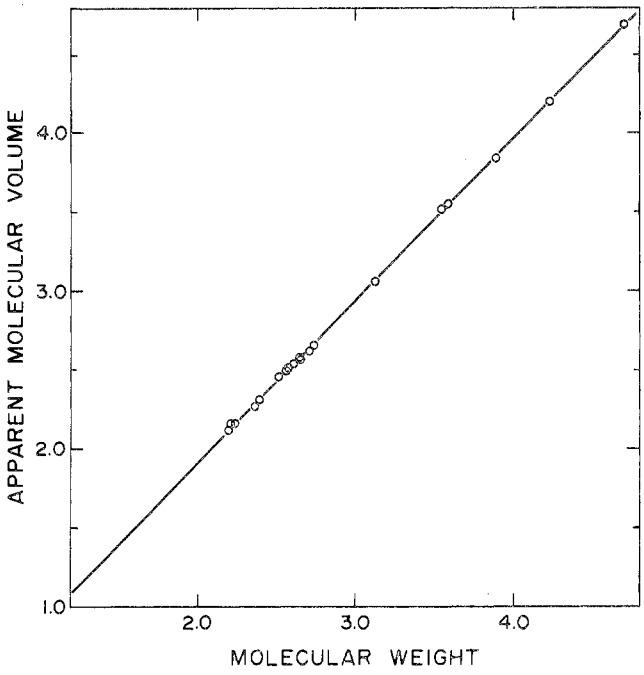

Figure 3. A plot of the molecular volume, M/ρ_b ($\times 10^{-6}$ ml/g), as a function of the molecular weight, M ($\times 10^{-6}$ g/mol), for all of the serum lipoproteins listed in Table I. The slope of the line is 1.03 ml/g. The reciprocal of the value, 0.97 g/ml, is the density of the material which would have to be added or subtracted to move a molecule along this line.

chains of 255,000 +5% daltons. Other investigators prefer a model in which a number of smaller chains are held together by very strong noncovalent binding to form an aggregate of about this size.[8] Chemical tests also show that the amount of apoprotein in each lipoprotein molecule is constant throughout the low density lipoprotein range from S_f4 to S_f20.[25]

The partial specific volume of the succinylated apoprotein has been measured as 0.716 ml/g.[35] From amino acid composition we calculate a value of 0.738 ml/g. However, the apoprotein also contains about 7% carbohydrate.[14] Inclusion of the carbohydrate reduces the calculated partial specific volume to 0.728 ml/g, which is in reasonable agreement with the experimental value.

The quantity of lipid associated with a low density lipoprotein is now readily determined as the difference in molecular weight between the lipoprotein and apoprotein, $M - 510{,}000$. The volume occupied by this lipid is determined as the difference in molecular volumes between the lipoprotein and its apoprotein, $M/\rho_b - 510{,}000 \times 0.728$. Finally, the ratio of these two quantities yields the density of the lipid moiety. The apparent lipid densities are listed in the last column of Table I. Excluding the S_f0 sample they are seen to range between 0.965 to 0.985 g/ml. The average value is 0.9715 g/ml. There is no correlation between these lipid densities and the lipoprotein molecular weights. On the other hand, the lipid density should be related to the lipid composition. In the next section a possible relationship is explored.

It is interesting to note that if we calculate a lipid density for the S_f0 lipoprotein of Table I, assuming 510,000 daltons of protein, we obtain a lipid density of 0.92 g/ml. This is obviously low. If we assume there is only one-half of the protein, mol wt 255,000,

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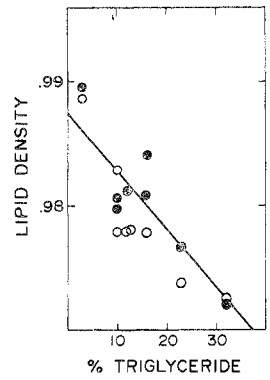

Figure 4. A plot of lipid density (g/ml) against triglyceride content for the low density lipoproteins listed in Table II. (●) Calculated from molecular weights and densities and (○) calculated from chemical composition, as tabulated in columns 9 and 10, Table II, respectively. The line drawn through the points is for a hypothetical lipoprotein containing 510,000 daltons of protein, $\bar{v}$ = 0.728 ml/g, 14% free cholesterol (1.033 g/ml), 28% phospholipid (1.031 g/ml), the indicated percentage of triglyceride (0.915 g/ml) and the remainder as cholesteryl ester (0.958 g/ml).

however, we obtain a more reasonable lipid density of 1.007 g/ml.

Lipid Composition and Density. Lipid composition is usually reported in terms of percentages of the four major classes of lipids found in the serum lipoproteins: cholesteryl esters, free cholesterol, triglycerides, and phospholipids. In Table II are listed compositional data for those samples for which sedimentation coefficients and buoyant densities are also available.[25,36]

Inspection of the data in Table II discloses no trends in composition with either the molecular weight or the buoyant density of the lipoproteins. The percentages of phospholipid and free cholesterol remain essentially constant across the entire range of buoyant densities. The weight ratio of phospholipid to free cholesterol is approximately 2:1, corresponding to a molar ratio of roughly 1:1. The quantity of hydrophobic lipids, cholesteryl ester, and triglyceride, added together, comprise approximately 60% of the lipids in the low density lipoprotein.

Careful inspection of the data in Table II reveals that the cholesteryl ester and the triglycerides do vary, in a reciprocal, compensatory fashion, over a considerable range of composition. Thus, the triglycerides vary from 3 to 32%, while the cholesteryl ester changes from 55 to 26%. Again there is no correlation with molecular weight or density of the whole lipoprotein. Thus, samples A, C and "S_f10" have 3, 13, and 26% triglycerides, but they have sedimentation coefficients of −45.5, −42, and −45 S, respectively.

A relation does appear to exist, however, between the density of the lipid moiety of the low density lipoprotein and the content of triglycerides. When this lipid density is plotted as a function of triglyceride content, a rough correlation is seen to exist, as shown by the solid circles in Figure 4.

The correlation shown in Figure 4 may be obtained by an independent method of approximating the lipid density. Thus, Sata, *et al.*,[37] use values of 1.033, 0.958, 1.031, and 0.915 g/ml for the average densities of free cholesterol, cholesteryl ester, phos-

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Table II
Lipid Composition and Buoyant Densities of Low Density Lipoproteins

Sample[a]	Ref	% of Total Lipid: Cholesteryl ester	Cholesterol	Triglyceride	Phospholipid	$s^0_{25,1.2}$, S	ρ_b, g/ml	Lipid density: From density mol wt,[b] g/ml	From composition,[c] g/ml
A	36	55	14	3	28	−45.5	1.0325	0.9896	0.9862
B	36	48	14	10	28	−41.5	1.0315	0.9807	0.9829
C	36	48	16	13	24	−42	1.031	0.9809	0.9798
E	36	53	11	10	27	−40	1.033	0.9798	0.9797
G	36	50	12	12	27	−38.5	1.036	0.9813	0.9794
S_f20	25	26	18	32	24	−67	1.004	0.9721	0.9725
S_f10	25	39	14	23	24	−45	1.025	0.9767	0.9738
S_f4	25	44	14	16	26	−32	1.048	0.9841	0.9785

[a] Samples A through G are from hyper-β-lipoproteinemic patients. Samples $S_f4,10,20$ are from hyper-pre-β-lipoproteinemic patients. [b] Lipid density = $(M - 510{,}000)/((M/\rho_b) - 510{,}000 \times 0.728)$. [c] Lipid density = $(\Sigma\%)/(\%\ CE/0.958 + \%C/1.033 + \%\ TG/0.915 + \%\ PL/1.031)$. Note that samples C, E, and G sum to 101%.

pholipid, and triglycerides, respectively. On this basis we have computed the densities recorded in the final column of Table II. These are also plotted in Figure 4 as open circles. Reasonable agreement between the two sets of data is obtained. Thus, we may conclude that the density of the lipid moiety of these low density lipoproteins is primarily a reflection of the triglyceride content. This variation in lipid density with triglyceride content could be the explanation for the microheterogeneity in density that is found among the low density lipoproteins in buoyant density experiments.[22]

Some Thoughts on the Origin of Low Density Lipoproteins

We may now attempt to draw some conclusions concerning the origin of the low density lipoproteins. First, one hypothesis could be that the low density lipoproteins represent a series of macromolecules undergoing progressive digestion by lipoprotein lipase. This enzyme hydrolyzes triglycerides, and is certainly involved in the metabolism of chylomicra and the very low density lipoproteins. But if this hypothesis were correct, we would expect to find the slope of the M–V plot, Figure 3, to be about 0.92 g/ml, instead of the observed value of 0.97 g/ml. Moreover, none of the lipid composition or density data supports this hypothesis.

We suggest an alternate possibility for the formation of LDL: that the first step is the attachment of the VLDL to sites of lipoprotein lipase. This enzyme will hydrolyze and cause the loss of triglycerides. We suggest the process continues until the cholesteryl ester/triglyceride ratio reaches about 0.5. Above this value, release of the degraded VLDL becomes increasingly favorable, and when the ratio reaches 0.9, all of the lipoprotein has been freed. Thus, the molecular weights of the released lipoprotein would depend upon the absolute amount of cholesteryl ester originally present in a particular VLDL. After release, further metabolism of surface by LCAT would still be possible. Additional cholesteryl ester also might be acquired through interaction with HDL. These latter processes would stop when the surface became taut,[38] since surface tension forces would then contribute a strongly unfavorable free energy term to the reaction. The final particle resulting from these multiple processes is the LDL according to this hypothesis.

Concluding Remarks

In this Account we have examined sedimentation and buoyant density data from a number of laboratories. Consistent results were obtained, yielding some definite conclusions. Thus, we found a reciprocal s–ρ_b relationship for the low density lipoproteins. Diffusion data allowed the development of an expression relating s to M. The series of low density lipoproteins was found to be generated by the addition of a substance of average density 0.97 g/ml. The average density of the lipoprotein lipids is 0.97 g/ml. These data for the low density lipoproteins are consistent with a model in which the amount of protein is constant with a molecular weight of about 510,000 daltons. Variation in the density of the low density lipoprotein lipids is principally due to the triglyceride content of the lipoproteins examined.

Consideration of these conclusions has allowed us to speculate upon the origin of the low density lipoproteins. We advance a new hypothesis for the formation of LDL based upon the cholesteryl ester/triglyceride ratio being the factor which determines the release of the pubescent LDL from the site of attachment of VLDL to lipoprotein lipase.

Our knowledge of the serum lipoproteins is still rudimentary. A detailed understanding of their molecular architecture is hampered by the lack of X-ray information, although some data from low-angle scattering are beginning to appear.[39] The isolation, purification, and sequencing of the polypeptide chains are just beginning. The origin and catabolism of the low density lipoproteins are still unknown. Thus, we may predict with confidence that many significant discoveries are to be made in this very important field during the next decade. We encourage young investigators to join the search.

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