SYMPOSIUM ON PLASMA LIPOPROTEINS

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LIPOPROTEINS—A CURRENT PERSPECTIVE OF METHODS AND CONCEPTS*

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Historical Introduction.—About one-half gram of total lipid is normally contained in 100 ml of human plasma. Approximately one third of this lipid is cholesterol. Other major constituents are triglycerides and phospholipids. In the absence of specific interactions, only a fraction of this lipid could be in true molecular solution. A number of early investigators studied the distribution of lipids during precipitation of serum protein fractions. Best known among these investigators were Hardy, Haslam, Chick, Theorell, and Sørensen. The first three of these investigators emphasized the presence of lipids in the serum protein fraction that is insoluble in the absence of salts, i.e., the euglobulin fraction.

The earliest preparation of a lipoprotein isolated from plasma or serum that contained reproducible proportions of lipids and protein was obtained by Macheboeuf.¹ It was obtained from horse serum by precipitation at pH 3.8 of protein soluble at half saturation of ammonium sulfate at neutral pH. Several reprecipitations of this fraction yielded a lipoprotein soluble in water at neutral pH which contained 10 per cent of cholesterol in the esterified form (18% of cholesterol ester) and 23 per cent of phospholipids. It appeared to be free of cholesterol alcohol. This material was called the "coenapse precipitated by acid." Horse serum was estimated to contain about 250 mg of this lipoprotein per 100 ml. Subsequently, this fraction was shown to be an α_1 -globulin in electrophoresis and to have a sedimentation coefficient of 2.5 to 4.0 svedberg units (reduced to water at 20°).²

Blix, Tiselius, and Svensson³ showed that large amounts of lipid were associated with α - and β -globulin fractions electrophoretically separated from normal human plasma. Smaller lipid levels were found in the crude γ -globulin and albumin fractions. Highly purified γ -globulin and albumin subsequently were shown to be almost completely devoid of lipids.^{4, 5} Adair and Adair⁶ described a lipoprotein precipitate from human serum which formed at pH 7 between 50 and 70 per cent saturation with ammonium sulfate. This fraction floated to the surface during centrifugation because of its low density (thought to be about 1.10). Later this fraction was reported⁷ to have a β -globulin electrophoretic mobility.

In 1945, Pedersen⁸ made the discovery that the "X protein" of plasma or serum (as described in ultracentrifuge studies of McFarlane⁹) appeared to be a component which he could concentrate at the top of centrifuged solutions of serum containing 45 per cent saturation of magnesium sulfate, and that this component, β -globulin in nature, appeared to have a density of 1.030 to 1.035 in both purified solutions of the β -globulin and in whole serum or plasma.

Large-scale fractionation of human plasma at low ionic strength in ethanol-water mixtures and temperatures near freezing showed that plasma lipids were precipitated into two readily separated fractions, combined as two distinct types of lipoprotein,^{4, 5} differing in their solubility in water and in ethanol-water mixtures, in their molecular size and shape, in their lipid content, and in their electrical charge. In 1949, Gurd, Oncley, Edsall, and Cohn¹⁰ reported the characterization of these two lipoproteins.

In 1950, Gofman, Lindgren, Elliott, Mantz, Hewitt, Strisower, Herring, and Lyon¹¹ suggested the possible role of the plasma lipoproteins in atherosclerosis. This study was based on a quantitative method for evaluation of plasma lipoproteins by ultracentrifugation studies in solvents with density greater than had normally been employed, in which each of the low-density lipoproteins (LDL) floated with a characteristic rate. This analytical method had been reported by Gofman, Lindgren, and Elliott.¹² This suggestion certainly increased the popularity of lipoprotein studies, and resulted in an almost explosive increase in the number of published papers in this field (Fig. 1).

Methods of Isolation and Characterization.—Since lipids have much lower densities (0.88–1.06) than peptide (1.30–1.35), a characteristic property of all plasma lipoproteins is their low density. Chylomicrons are found to have densities of about 0.93, very-low-density lipoproteins (VLDL) of about 0.93 to 1.01, LDL of 1.01 to 1.05, and high-density lipoproteins (HDL) of 1.08 to 1.16. Most other plasma proteins have hydrated densities above 1.25. In the ultracentrifuge, lipoproteins can be sedimented in solvents having densities lower than those of the lipoproteins, or floated in solvents of higher density.

Sedimentation rates in the ultracentrifuge are usually measured by a sedimentation coefficient, defined as the sedimentation rate in a unit centrifugal field, $s = (dr/dt)/\omega^2 r$. The sedimentation coefficient in a solvent of density ρ and viscosity η is related to the density x_1 and diameter of d of a spherical molecule by the equation $s = d^2(x_1 - \rho)/18 \eta$. For most proteins, these sedimentation coefficients are of the magnitude of 10^{-13} sec. This unit of 10^{-13} sec has been

FIG. 1.—The number of references to lipoprotein ($\square$), lipid ($\circ$), and total references ($\bullet$) in *Chemical Abstracts* from 1932 to 1967. In order to obtain smooth curves, this data was averaged over five-year periods, and the ordinates on the left-hand side represent these references in the five-year periods. The right-hand ordinate scale represents the average number of references per year.

defined as one svedberg (1S). Sedimentation coefficients depend upon the viscosity and density of the solvent used, and it is usual in protein chemistry to reduce all observed values to some standard density and viscosity, usually that of pure water at 20° or 25°. Such reduced values are commonly written $s_{20,w}$ or $s_{25,w}$. The S_f notation introduced by Gofman and colleagues^{13, 14} expresses the rate of flotation under unit centrifugal field in a solution of sodium chloride of density 1.063 and a temperature of 26°C. The rate is expressed in svedberg units as the usual sedimentation coefficient, except that the data are not reduced to values in water, and the negative sign is omitted. This S_f value is a function of density, size, and shape of the lipoprotein molecule. When solvents other than aqueous sodium chloride of density 1.063 are used, flotation rates can be designated by symbols of the type $S_{f,1.21}$ (1.748 M NaCl; 2.05 M KBr), where the second subscript denotes the density, and the composition of the solvent is then defined in the parenthesis.¹⁵ S_f values for VLDL lie between about 600 and 12, for LDL between 20 and 5. HDL sediments rather than floats, and this notation is not usually used when describing HDL.

As the density of plasma is increased by stepwise addition of sodium chloride, potassium bromide, heavy water, or other reagents, lipoproteins can be floated to the surface during preparative ultracentrifugation in an angle or swinging-bucket head, and thus be concentrated or purified. In ordinary sedimentation or flotation procedures, solvent density is essentially constant. Controlled variation of solvent density permits use of new experimental techniques. Whenever the solvent density is higher than lipoprotein density, molecules float with rates depending upon their densities, sizes, and shapes; whenever the solvent density is lower, molecules sediment at rates depending upon the same parameters. When the two densities are equal, the molecules remain in a condition of isopycnic equilibrium. The solvent density can be increased by addition of a soluble substance of greater density, such as deuterium oxide (D_2O), glycine, sucrose, serum albumin, or an inorganic salt. Such substances do not seem to alter the properties of plasma lipoproteins.

By establishing density gradients over suitable density ranges, it is possible to separate lipoproteins of different densities. If the density gradient is sufficiently stable as compared with lipoprotein sedimentation and flotation rates, one can separate lipoproteins into regions of isopycnic equilibrium. This method, called isopycnic density gradient fractionation can use (1) solvent systems which are slowly changing because of diffusion, but with sufficient stability relative to the sedimentation or flotation rates of the lipoprotein to allow isopycnic equilibrium to become established, or (2) stable density gradients, attained by equilibrium ultracentrifugation of solutions of dense salts, such as cesium chloride, which allow establishment of isopycnic equilibrium even for slowly floating or sedimenting macromolecular components. Gradients of the first kind are especially useful for separation of LDL from plasma;¹⁶ those of the second kind have been used for fractionation of lipoproteins, being of special use in the study of HDL.¹⁷

Solubility methods for isolation and characterization of plasma lipoproteins have been useful. Salting-out methods have not been very helpful, but the low temperature ethanol-water system^{4, 5} and the zinc reagent system^{18, 19} have been extensively used. Certain high molecular weight polyanions precipitate LDL and VLDL rather specifically, and polycations also precipitate lipoproteins. Among the polyanions used are heparin, dextran sulfate, sulfated amylopectin, and other sulfated carbohydrates. The polycation polyvinylpyrrolidone aggregates VLDL.

Electrophoresis alone has not yielded purified lipoprotein fractions, but when zonal electrophoresis is applied to subfractions of plasma and used as an analytical method for study of lipoprotein homogeneity, very useful results are obtained. Starch granules or gel, paper, cellulose acetate, acrylamide gel, agar, and agarose gels have all been used as supporting materials. Chromatography and gel filtration have also been applied to lipoprotein purification with considerable success.²⁰

General Properties of Plasma Lipoproteins.—Nearly all workers in the human plasma lipoprotein field have obtained four principal lipoprotein fractions designated here as chylomicrons, VLDL, LDL, and HDL. Although lipoprotein fractions are often further subfractionated, and other minor lipoprotein fractions

have been reported, we will consider only these four major fractions. Oncley²¹ reviewed the chemical composition of these fractions as measured by a half-dozen workers. Hatch and Lees²⁰ have recently made a similar review. These sets of data are presented in Table 1.

A considerable number of amino acid analyses have now been reported for HDL, LDL, and VLDL, and there is generally good agreement in all recent work. Table 2 records averages for VLDL, LDL, and HDL (columns 1-3), and contrasts these values with average values for other human plasma proteins (albumin, transferrin, fibrinogen, and γ -globulin). Although there are obvious differences between these values, there does not seem to be any easy explanation of the fact that lipoproteins have the property of binding from 1 to 12 times their weight of lipid, while other plasma proteins bind little or no lipid (except for non-esterified fatty acid, NEFA). We have looked for similarities or differences in these seven proteins by means of a parameter Z representing the sum of the absolute values of the differences between any two proteins: $Z_{jk} = \sum_{i=1}^{20} |x_{ij} - x_{ik}|$,

where x_{ij} represents the mole fraction of amino acid i in protein j , and x_{ik} a similar value in protein k . Identical protein fractions give values for this parameter Z_{jk} very near zero (an error of 3 per cent in all values would yield $Z = 0.03$). Table 3A records these comparisons and Table 3B records values calculated from the complete sequence analysis of a few mammalian insulins, albumins, and hemoglobins, in order to give an indication of the significance of the different Z values. The lowest Z value was obtained by comparison of VLDL and LDL ($Z = 0.06$), a value near that for the horse and human insulin comparison ($Z = 0.08$). Many of the values for supposedly unrelated proteins lie between 0.25 and 0.50. The Z value for the LDL-HDL comparison, 0.30, would appear to indicate little similarity between these lipoproteins.

The similarity between VLDL and LDL on the basis of gross amino acid analysis is especially interesting since native VLDL and LDL are also very similar immunochemically (to be discussed later), and metabolic studies using ¹³¹I-labeled VLDL show transfer of the label from VLDL peptide to LDL peptide, but no transfer to HDL peptide.²²

The preparation of apolipoproteins from purified lipoproteins is not a simple procedure. Unless rather drastic conditions are used, all of the lipid cannot be removed. Such drastic conditions usually yield an insoluble or partially soluble apoprotein. The use of detergents seems to help in the lipid extraction, although the complete removal of the detergent is then difficult or impossible. Chemical modification of the lipoprotein before lipid extraction can often yield a more soluble product. Succinylation is often used as a modification method. Here the amino groups (normally positive at pH 7) are modified into negative residues to yield a modified protein of considerably enhanced negative charge at neutral pH.

Lipoprotein Homogeneity.—Proof of lipoprotein homogeneity is a difficult question to treat briefly. Immunochemically, the HDL and LDL classes appear to meet this test, but because of multiple antigenic determinants eliciting heterogeneous antibodies, the test is probably not sufficiently stringent. We are at-

TABLE 1. *Composition and normal concentration in plasma of the four major lipoprotein families.*

	Chylomicrons		VLDL		LDL		HDL	
Apoprotein	2	2.0	8	5-12	21	22	50	50
Phospholipid	7	4.3	19	13-20	22	20	22	24
Cholesterol alcohol	2	2.1	7	3-5	8	10	3	2
Cholesterol ester	5	3.9	13	10-13	37	36	14	20
Triglyceride	84	87.0	51	50-60	11	12	8	4
NEFA†	‡	‡	2	‡	1	‡	3	‡
Approx. conc., mg/dl								
Male	30	—	150	159	360	369	260	275
Female	30	—	80	56	330	303	330	436

* First value is average value from Oncley,²¹ second value is from Hatch and Lees.²⁰ All composition figures are weight per cent.

† NEFA = nonesterified fatty acid.

‡ Value not recorded.

TABLE 2. *Amino acid composition of some human plasma proteins.**

Amino acid	VLDL	LDL	HDL	Albumin	Transferrin	Fibrinogen	γ-Globulin
Asx	110	108	<u>78</u>	89	<u>118</u>	<u>117</u>	<u>78</u>
Glx	122	121	<u>180</u>	137	<u>89</u>	111	100
Thr	65	65	48	46	<u>44</u>	63	<u>81</u>
Ser	84	86	64	<u>38</u>	59	101	<u>122</u>
Pro	<u>32</u>	38	49	46	50	57	<u>76</u>
Gly	49	47	43	<u>22</u>	72	<u>86</u>	68
Ala	63	62	77	<u>108</u>	84	<u>48</u>	53
Cys/2	<u>4</u>	<u>6</u>	<u>6</u>	<u>60</u>	<u>56</u>	25	23
Val	57	55	57	73	67	<u>44</u>	<u>92</u>
Met	15	15	10	10	13	<u>20</u>	<u>8</u>
Ile	45	<u>58</u>	<u>8</u>	14	22	43	22
Leu	111	115	<u>130</u>	104	86	62	75
Tyr	32	32	34	29	35	37	<u>40</u>
Trp	15	9	<u>20</u>	1	12	18	16
Phe	<u>52</u>	<u>51</u>	34	<u>53</u>	40	29	<u>32</u>
Lys	89	77	98	<u>101</u>	87	<u>66</u>	<u>63</u>
His	19	24	<u>17</u>	<u>27</u>	<u>28</u>	21	21
Arg	37	<u>32</u>	<u>48</u>	40	39	<u>52</u>	<u>30</u>

* Mole fraction in parts per thousand.

The amino acid values have been calculated from the following sources:

VLDL: Granda, J. L., and A. Scanu, *Biochemistry*, 5, 3301 (1966); Levy, R. S., A. C. Lynch, E. D. McGee, and J. W. Mehl, *J. Lipid Res.*, 8, 463 (1967).

LDL: Margolis, S., and R. G. Langdon, *J. Biol. Chem.*, 241, 469 (1966); Granda, J. L., and A. Scanu, *Biochemistry*, 5, 3301 (1966); Levy, R. S., A. C. Lynch, E. D. McGee, and J. W. Mehl, *J. Lipid Res.*, 8, 463 (1967).

HDL: Shore, V., and B. Shore, *Biochem. Biophys. Res. Commun.*, 9, 455 (1962); Levy, R. I., and D. S. Fredrickson, *J. Clin. Invest.*, 44, 426 (1965); Scanu, A., and J. L. Granda, *Biochemistry*, 5, 446 (1966); Levy, R. S., A. C. Lynch, E. D. McGee, and J. W. Mehl, *J. Lipid Res.*, 8, 463 (1967).

Other proteins from Schultze, H. E., and J. F. Heremans, *Molecular Biology of Human Proteins* (Amsterdam: Elsevier Publishing Co., 1966).

Underlined values represent values more than one standard deviation above the mean, and overlined values represent values less than one standard deviation below the mean, considering these seven human proteins to make up a set.

TABLE 3A. *Comparison of the amino acid composition of various human proteins.*

VLDL	LDL	HDL	Albumin	Transferrin	Fibrinogen	γ -Globulin	Insulin	
0	0.06	0.27	0.35	0.29	0.27	0.36	0.52	VLDL
	0	0.30	0.36	0.31	0.28	0.36	0.51	LDL
		0	0.30	0.34	0.43	0.44	0.60	HDL
			0	0.26	0.52	0.50	0.52	Albumin
				0	0.31	0.34	0.53	Transferrin
					0	0.26	0.60	Fibrinogen
						0	0.57	γ -Globulin

TABLE 3B. *Comparison of the amino acid composition of certain proteins.*

		Insulin		Human				
Human	Sperm whale	Horse	Sei Whale	Beef	Sheep	Albumin	LDL	
0	0.04	0.08	0.08	0.12	0.16	0.51	0.50	Human insulin
	0	0.04	0.04	0.08	0.12	0.49	0.50	Sperm whale insulin
		0	0.08	0.12	0.08	0.49	0.54	Horse insulin
			0	0.04	0.08	0.45	0.50	Sei whale insulin
				0	0.04	0.46	0.53	Beef insulin
					0	0.46	0.57	Sheep insulin
			Human hemoglobin		Human albumin			
α	β	γ						
0	0.27	0.29	α -chain				0.12	Bovine albumin
	0	0.23	β -chain					
		0	γ -chain					

tempting to apply a rigorous ultracentrifuge test for homogeneity by determining the distribution of sedimentation coefficients in a given sample, after correction for effects of diffusion and concentration dependence of the sedimentation coefficient.²³ The method was introduced by Baldwin and Williams,²⁴ and applied to a number of protein and polymer systems. Considerable heterogeneity is found in this test, but when sedimentation distribution functions are interpreted in terms of variations in size and density, a single bivariate normal distribution is found in the case of LDL. This behavior would be expected of an apoprotein which bound lipids, but not in exactly specific locations or of exactly specific structures. A strong negative correlation (-0.472) between the size parameter and the density parameter was observed, as would be expected if a specific apoprotein bound more or less lipid, being less dense and larger when binding more lipid and more dense and smaller when binding less lipid.

When the same method is applied to VLDL fractions, it is necessary to introduce two bimodal normal distributions, one around a diameter of 269 Å and density 0.992, and the other around diameter 4.02 Å and density 0.965. Here, too, each of these distributions shows a strong negative correlation between size and density. The simplest model which would explain this sort of distribution would seem to involve two apoproteins, one lying in the range S_r from 60 to 12S, and the other S_r from 600 to 30S. In our preparations, about 36 per cent weight concentration of lipoprotein was in the low S_r range, and 64 per cent in the high S_r range.

Figure 2 shows these distributions for VLDL and LDL, and some approximate distributions for chylomicrons and HDL. The HDL distribution is not based

FIG. 2.—Distribution of the principal lipoprotein families in normal human plasma, shown as a three-dimensional plot with lipoprotein concentration as the vertical coordinate recorded as a function of lipoprotein density and lipoprotein size. Lipoprotein size is plotted on a logarithmic scale, with diameter indicated on the left, and molecular weight on the right. The families recorded here are, from right to left, HDL, LDL, VLDL, chylomicrons. (From Oncley²¹.)

on this sort of measurement, but rather depends largely upon a study of the distribution of a broad preparation of HDL in stable cesium chloride gradients reported by Oncley and Allerton,¹⁷ where there appeared to be a single distribution of density 1.12.

Numerous experiments have indicated that most, if not all, of the lipid components of the plasma lipoproteins are in rapid equilibrium with one another, and with other tissues. The work of Hagerman and Gould^{25, 26} showed clearly that the cholesterol alcohol component of plasma is in rapid equilibrium with the cholesterol alcohol component of the red blood cell. This exchange is a rapid reaction, with half time of about one hour with dog blood at 37°. Gurd²⁷ reviews this and other similar studies, pointing out that "since the phospholipids, triglycerides, cholesterol, and cholesterol esters are very insoluble in water, it is likely that exchanges and transfers are brought about by actual collision between the lipoprotein complexes themselves." This situation would appear to indicate that lipoprotein structure was one which could provide reasonably close contact between the lipid moieties of various lipoproteins. Gurd suggests that "the rate of diffusion is determined by energy barriers within the collision complex itself, and does not give a direct indication of how deeply a given type of lipid molecule is normally buried within an individual complex."

Since all plasma lipoproteins have high solubility in aqueous salt solutions and show the normal solubility characteristics of proteins, it has generally been supposed that the protein moiety occupies a considerable part of their surface. McFarlane⁷ pointed out in 1949 that the LDL molecule contained so little apoprotein that there is insufficient protein to cover even a spherical model with a monolayer of peptide (assuming about 8.5 Å as a minimum thickness). This first estimate probably involved too low a molecular weight estimate and too large a hydration factor, but even with better data there still appears to be a real

difficulty in this regard in the LDL class, and certainly the VLDL class cannot be covered more than partially with peptide. A number of recent papers have discussed this problem.²⁷⁻³⁰

Immunochemical Reactivity.—Immunochemical characterization of antigens depends on specific reactions with antibodies produced in vertebrates in response to injection of foreign antigenic materials. Although completely homogeneous antibody protein is seldom available, antisera of defined specificity is obtained by appropriate absorption techniques. Immunochemical techniques are exquisitely sensitive in detecting similarities and differences among protein molecules. Antisera specific for lipoproteins can be produced using whole serum or plasma for immunization. The antisera is made specific by absorption with serum or plasma from which lipoproteins have been removed by ultracentrifugal flotation. High titer antisera specific for each major class of lipoproteins can also be obtained using purified lipoproteins as antigen.

There are a variety of methods of varying sensitivity used to study antigen-antibody reactions.³¹ In lipoprotein work, immunodiffusion and immunoelectrophoresis are most common, although quantitative precipitin and hemagglutination techniques have been applied.

The results of these studies can be divided into three categories: (1) reaction of purified but otherwise nontreated lipoproteins, (2) reactions of modified purified lipoproteins, and (3) reactions of apolipoproteins produced by delipidation of naturally occurring or modified lipoprotein molecules. Detection of genetic polymorphic forms of β -lipoproteins is also included in this discussion of immunochemical characterization of lipoproteins as these forms are detected by virtue of their possession of specific antigenic determinants.

When dealing with purified unmodified lipoproteins, the results indicate that HDL and LDL have antigenic specificities which are not shared.^{32, 33} Whether they also possess common determinants has not been investigated and certainly is of interest. On the other hand, VLDL, unmodified and unextracted, is immunochemically identical to the LDL.^{32, 34, 35} Reports of differences in reactivity have appeared^{36, 37} but in the experience of most laboratories, including ours, antibodies formed against either class are completely absorbed by the other.

Physicochemical heterogeneity within the HDL class has been studied extensively.^{37, 38} HDL₂ and HDL₃ are distinguishable, if overlapping, subgroups of the HDL characterized by differences in lipid content and thus density, but which probably contain qualitatively, if not quantitatively, identical subunits. It has been suggested that HDL₃ is an artifact of ultracentrifugation resulting from lipid loss by HDL₂.³⁹ Antisera against either of these HDL are completely absorbed using either HDL₂ or HDL₃ as antigens.³⁷

The LDL class of lipoproteins is relatively homogeneous although it is this class that contains genetically determined polymorphic forms. Reports of immunochemical heterogeneity may be due to polymorphisms or more likely to technical problems in gel diffusion and, in certain cases, to the use of preparations made from abnormal plasma.⁴⁰ Lipoproteins isolated from abnormal plasma often differ from normal lipoproteins.

Immunochemically detectable subgroups of VLDL have not been reported

although the very large size and high lipid content of these molecules, in addition to their broad density and size range, makes characterization by immunodiffusion and immunoelectrophoresis very difficult.

Both HDL and LDL have been modified by various methods, most commonly succinylation. In both cases, antigenic specificity is markedly altered by these procedures.

Immunochemical specificity of apolipoproteins depends both on their original class (VLDL, LDL, or HDL) and whether they were prepared from modified or unmodified lipoproteins. Apoproteins A(HDL₂) and A(HDL₃) appeared immunochemically⁴¹ identical and had the same N-terminal⁴¹ and C-terminal^{38, 41} amino acids. Recently, apoprotein B(LDL) has been obtained in completely soluble form^{42, 43} using dodecyl- or decylsulfate. Soluble apoproteins from both modified and unmodified LDL seemed homogeneous. As with HDL, modification significantly changed the immunochemical specificity.

In contrast to HDL and LDL, the VLDL class was reported to yield apoproteins of different specificities.⁴⁴⁻⁴⁷ Studies on VLDL from normal individuals have shown two apoproteins to be present, one which seems identical with the apoprotein of LDL and the other which reacts with antisera against HDL. Unextracted VLDL do not react with anti-HDL but do react completely with anti-LDL. A third unrelated apoprotein has been described from VLDL isolated from hyperlipemic plasma. There is some discussion as to whether this component is actually a distinct peptide of VLDL or whether the results are due to occluded proteins in preparations from abnormal plasma. Apoproteins extracted from normal LDL and VLDL exhibited identical immunochemical reactivity with either anti-LDL or anti-VLDL.

Heterogeneity in the form of genetic polymorphisms occurs in plasma lipoproteins just as it does in other plasma proteins. Polymorphic forms of LDL were first described by Blumberg and collaborators and were designated Ag.⁴⁸ A number of different forms of Ag have been described and the genetics of the system is being studied. Antibodies against Ag are found in multiply transfused humans, particularly those suffering from thalassemia. Many of these individuals have had over 200 blood transfusions, providing ample opportunity for immunization against foreign polymorphic proteins. Attempts to produce rabbit antibodies specific for Ag have been unsuccessful, probably due to the low antigenicity of the Ag determinant as compared to other LDL determinants. So far there have been no attempts to study the structural basis of the polymorphism but existence of these forms will be helpful when amino acid sequencing of apoprotein B is attempted.

A second LDL polymorphic system, Lp, has been described by Berg⁴⁹ and the genetics worked out by a number of different laboratories. In this case Lp(a) lipoproteins are quite antigenic in rabbits. In addition to genetic studies, at least two groups of investigators^{50, 51} are studying physicochemical properties of Lp(a) lipoproteins. Partial purification has been accomplished. Lp(a) molecules have an average density somewhat greater than LDL and may be the previously described HDL₁ class which forms an upper density tail on LDL ultracentrifugal patterns from the sera of some individuals. It has been reported

that Lp(a) molecules are completely absorbed by anti-Lp(a) negative LDL and also that similar absorption occurs using anti-Lp(a) negative HDL serum. These results suggest that the Lp(a+) determinant marks a minor subclass of lipoproteins sharing antigenic properties with both HDL and LDL.

No normal polymorphic forms of either HDL or VLDL have been reported. However, in Tangier disease, which is characterized by a genetically determined lack of HDL, there is an altered apoprotein A(HDL) present in the plasma. Heterozygous individuals make a reduced amount of HDL and also make some of this altered apoprotein.⁵²

It is likely that there are a number of distinct lipoprotein classes present in minor amounts in normal plasma which may be present in much larger amounts in abnormal plasma. The importance of these lipoproteins cannot be estimated solely in terms of their concentration in plasma.

There have been reports of native apolipoproteins in normal plasma. A number of investigators have shown that apolipoproteins can combine, either *in vitro* or *in vivo*, with lipid to become normal, or nearly normal lipoproteins. Roheim, Miller, and Eder⁵³ conclude that "rat plasma contains an apoprotein which combines with lipid in the liver and is released into the plasma as lipoprotein." More recently, the presence of B protein in normal and abetalipoproteinemic plasma has been suggested.⁵⁴ A plasma protein (not a lipoprotein with density less than 1.21) capable of specific interaction with cholesterol has been reported to be present in normal plasma.⁵⁵ Fractionation of this protein, called "transcholesterin," has shown that the activity resides in several α_1 - and α_2 -globulin fractions separable by DEAE-cellulose chromatography.⁵⁶

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² Macheboeuf, M., and P. Rebeyrotte, *Discussions Faraday Soc.*, **6**, 62 (1949).

³ Blix, G., A. Tiselius, and H. Svensson, *J. Biol. Chem.*, **137**, 485 (1941).

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⁵ Oncley, J. L., M. Melin, D. A. Richert, J. W. Cameron, and P. M. Gross, Jr., *J. Am. Chem. Soc.*, **71**, 541 (1949).

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