Composition and structure of soluble lipoproteins*

Two models of lipoprotein structure have been proposed or implied by different investigators[1-6]. One is a molecular model, as the protein is believed to interact with lipid without losing its molecular integrity. The other, termed a micellar model, is described as a lipid core surrounded by a film of protein, or protein and phospholipid. The lipoproteins vary so widely in their lipid content that both types may exist. Experimentally two main classes of lipoprotein have been recognized on the bases of certain properties, *i. e.*, α- and β-lipoproteins distinguished by their electrophoretic mobility[7], and "high"- and "low"-density lipoproteins separated by centrifugation in solvents of known density[5], but these classes cannot be rigorously identified with the proposed models. For example, numerous lipoprotein fractions have been isolated from blood serum and they do not show a sharp change in properties that could be interpreted as a change in structure. We have therefore examined the reported composition of lipoproteins for evidence that two distinguishable types, indicative of the proposed structures, actually exist.

Natural lipoproteins contain proteins, phospholipids, and neutral lipids, combined in reasonably constant, but non-stoichiometric proportions through forces weaker than the covalent bond. Each of these components has characteristic properties that could affect lipoprotein structure. Proteins can solubilize lipid and, being large molecules, confer structural integrity. Phospholipid can form micelles and also solubilize neutral lipid but, being small molecules, would contribute less structural integrity. Likewise, neutral lipids are small molecules that must also be solubilized by the other components and might therefore be considered "dependent" components.

The reported[5,8-21] protein, phospholipid, and neutral lipid contents, on a weight basis, of some 40 lipoproteins obtained from blood serum, chyle, and egg yolk, are shown in Fig. 1. Some contain 80% protein, others 90% neutral lipid, but none has over 35% phospholipid. The points show a sharp change in direction at a protein content of about 33%. At higher protein contents the phospholipid/neutral lipid

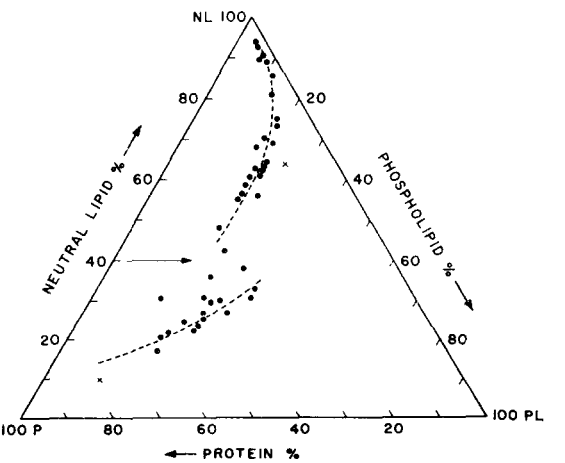

Fig. 1. Protein, phospholipid and neutral lipid contents of lipoproteins. Curves derived from linear equations in Fig. 2.

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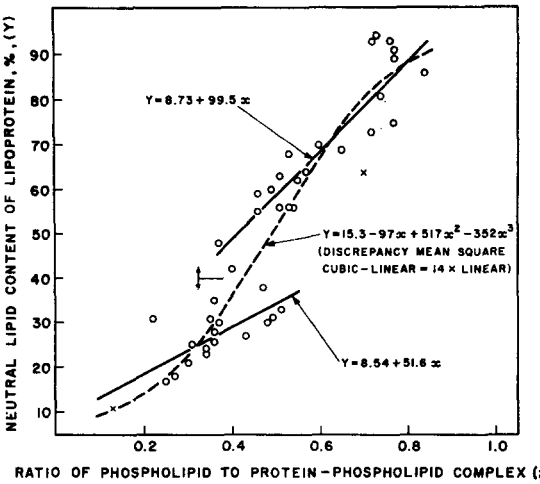

Fig. 2. Neutral lipid content of lipoproteins in relation to the composition of their protein–phospholipid complexes.

ratio is about 1:1 but at lower protein contents it increases to 1:10. The two broken-line curves represent the two linear equations derived from Fig. 2, and the crosses indicate the composition of the hen's egg yolk lipoproteins referred to later.

As the three quantities in Fig. 1 must total 100%, there are only two independent variables available for analysis. Neutral lipid content and the proportion of phospholipid in the phospholipid–protein complex were chosen as the two variables in Fig. 2, since they represent the "insoluble" and "solubilizer", respectively. While the neutral lipid increases with the phospholipid content of the complex, there is clearly a transition zone where the neutral lipid increases sharply from about 35 to over 50% in the region where phospholipid forms about half the complex.

This transition could be either a discontinuity between two linear relations, or a sigmoid distribution of the points in Fig. 2. Separate linear equations were therefore fitted to data above and below 40% neutral lipid and a cubic equation (sigmoid) to all the points. The cubic equation did not fit the data adequately since its discrepancy mean square was 14 times greater than the experimental error indicated by the linear equations. Evidently the results are best represented by two linear equations, of significantly different slope, separated by a discontinuity where the lipoprotein contains about 40% neutral lipid and 33% protein (density about 1.09). As this transition distinguishes two classes of lipoprotein on the basis of composition rather than density, they have been termed high- and low-protein lipoproteins (HPL and LPL).

Experimental evidence relating lipid content and particle size is shown in Fig. 3[7,20,22–29]. The results show considerable variability: some of the reported estimates of size assume a spherical molecule, and the lipid content of others was estimated from their density. If BULL's[30] data at an air–water interface are applicable, curve P shows the minimum amount of protein required to cover lipid spheres of various sizes[4] converted to the parameters of Fig. 3. Curve PP shows the same relation assuming that the surface contains equal weights of protein and phospholipid.

Clearly, the size of the LPL type increases with lipid content and, as most of the

points lie to the right of curve P, the protein either provides more coverage at an oil–water than at an air–water interface or phospholipid also participates at the surface. Particles with a lipid core or a phospholipid surface clearly resemble microemulsions or micelles rather than molecules.

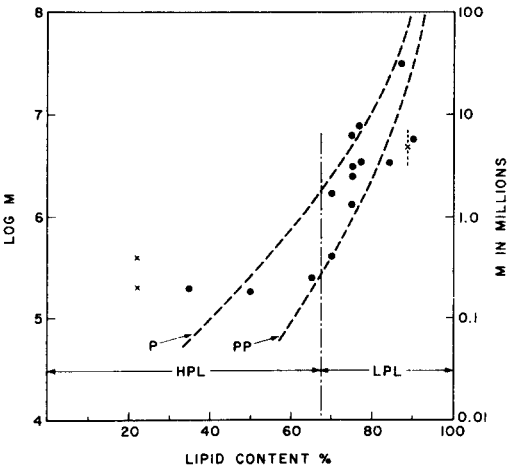

Fig. 3. Relation between lipoprotein size and total lipid content (experimental points). Curve P calculated for lipid sphere with protein coverage only, and curve PP with coverage by equal amounts of protein and phospholipid (see text).

The few experimental points representing the HPL type show no relation between size and lipid content, and none would be expected if they have a molecular structure. Strong evidence for a molecular structure in this type of lipoprotein is obtained from those in egg yolk[31] that have been shown to form pH-dependent reversible dissociation systems[20,21] similar to those exhibited by certain proteins[32,33].

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Intestinal phosphatidate phosphatase*

Recent evidence has accumulated that the mechanism for fatty acid absorption and its conversion into triglycerides in the intestinal mucosa is quite similar to the system proposed for liver triglycerides as suggested by KENNEDY[1]. Labeled phosphatidic acids have been isolated from the intestinal mucosa following the incubation of segments of this tissue with labeled inorganic phosphate or ^{14}C-labeled fatty acids[2]. The enzymes involved in this process and in this tissue have not been fully investigated with the exceptions of the demonstrated presence of thiokinase by SENIOR AND ISSELBACHER[3] and the failure to find the enzyme glycerolkinase as reported by BRUELL AND RISER[4]. L-α-Phosphatidate phosphohydrolase (phosphatidate phosphatase, EC 3.1.3.4), an enzyme involved in these reactions, was first described by SMITH *et al.*[5] and its distribution in a variety of tissues, as well as the specificity, was examined. These investigators did not report on the occurrence of this enzyme in the intestinal mucosa. In addition, when the specificity of this enzyme was examined, utilizing such substrates as phosphatidyl choline, only the liberation of inorganic phosphate was determined. If the enzyme displays a similar action on this substrate as on phosphatidic acids, the expected product would be phosphorylcholine which would not be determined in the analysis for inorganic phosphate. It is the purpose of this paper to describe the presence of this enzyme and its intracellular distribution in intestinal tissues. In addition, the specificity of this enzyme has been examined not only from the aspect of inorganic phosphate liberation but also with regard to the production of certain phosphorylated bases.

Phosphatidic acids were isolated by the method of CHIBNALL AND CHANNON[6]. Phosphatidic acids were synthesized according to the procedure of WAGNER-JAUREGG AND ARNOLD[7]. The lysophosphatidic acid was prepared by the method of KABASHIMA[8]. Phosphatidyl choline was isolated by the method of HANNAHAN, TURNER AND JAKO[9]. Phosphatidyl ethanolamine, phosphorylethanolamine, phosphorylcholine, and α-glycerophosphate were obtained from commercial sources and their purity checked by chromatography.

* Previously referred to as phosphatidic acid phosphatase.