Serum Lipid Transport Systems: Recent Advances

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ABSTRACT

Lipids circulating in the plasma are transported in water soluble form as lipid-protein complexes. Lipoproteins can be classified according to size, density, electrophoretic mobility and protein composition. The ability of low density lipoproteins and very low density lipoproteins (VLDL) to form complexes with different polyanions has been also used as a method for separation and study of serum lipoproteins. Even within classes of lipoproteins closely related otherwise, the amount of different lipids and their ratios to each other and to protein are variable. Two enzymatic systems seem to be at least partly responsible for the different lipid compositions of serum lipoproteins: lipoprotein lipase and lecithin-cholesterol acyltransferase (LCAT). LCAT, which seems to be associated with α-lipoproteins, is responsible for the formation of the bulk of cholesteryl-esters in human serum. Changes in activity of this enzyme may explain the observed changes with age and disease of serum cholesteryl-ester fatty acids (CEFA). Differences in CEFA pattern are found between newborn and adult animals, including man. The activity of serum LCAT was observed to increase with age in animals and to decrease markedly in patients with liver cirrhosis. These patients show abnormal serum CEFA patterns and abnormally low proportions of pre-β- (VLDL) and α- (high density) lipoproteins.

INTRODUCTION

At the International Symposium on Lipid Transport celebrated in Nashville in 1963 it was predicted that the “finding of specific proteins as lipid acceptors was very exciting and that further study of these apoproteins will bring about a better understanding of how lipids are transported in the blood” (1). This prediction proved to be true although a great deal still remains to be learned.

In this paper no attempt will be made to present a comprehensive review of all the advances in the field of lipoprotein and lipid transport that have occurred since the 1963 meeting. We will discuss selected aspects of recent advances in methodology which have led to a better understanding of the structure and function of lipoproteins. We will discuss new advances in our knowledge of enzymes which are known to participate in lipoprotein metabolism and function and, finally we will discuss the transport of a specific serum lipid fraction which is suspected of having an important physiological role, the cholesteryl-esters.

ADVANCES IN LIPID METHODOLOGY

In the past few years important advances have been made in this area. A summary of the techniques currently used in the study of lipoproteins follows.

1. differential salt precipitation
Macheboeuf (1929), Adair and Adair (1944)

2. Cohn cold ethanol fractionation
Gurda, Oncley, Edsel and Cohn (1949), Cohn (1950)

3. analytical ultracentrifugal flotation
Pederson (1945), Gofman et al. (1951)

4. preparative ultracentrifugation, followed by chemical analysis
Lindgren et al. (1951), Hillyard et al. (1955), Havel et al. (1955), Bragdon et al. (1956)

5. complexing with polyanions
Walton (1952), Oncley and Mannick (1954), Burstein and Samaille (1955), Bernfeld et al. (1957)

6. electrophoresis
a. moving boundary electrophoresis
Blix, Tiselius and Swensson (1941)
b. zone electrophoresis
i. starch block, Kunkel and Slater (1952)
ii. paper, Swahn (1953), Durrum et al. (1952), Strauss and Wurm (1958), 369

1One of five papers to be published from the Symposium "Lipid Transport" presented at the AACS Meeting, New Orleans, April 1970.
iii. starch gel, Smithies (1955)  
iv. cellulose acetate  
v. acrylamide, Narayan et al. (1965)  
vi. agar and agarose gel, Graber and Williams (1955) Ressler et al. (1961), Nobel (1968)  
c. immunoelectrophoresis, Graber and Burtin (1964)  

7. immunodiffusion  
Ouchterlony (1953), Mancini (1965)  
8. membrane filtration  
Stone and Thorp (1966)  
9. others  
a. nuclear magnetic resonance, Chapman (1968)  
b. refractometry, Lindgren et al. (1960)  
c. electron microscopy, Hayes and Hewitt (1957)  
d. x-ray  
e. spectroscopy  
i. infrared, Scanu and Granda (1968)  
ii. circular dichroic, Scanu and Hirz (1968)  

The two techniques most widely used have been ultracentrifugation, either ultracentrifugal flotation or ultracentrifugation followed by chemical and physiochemical analysis, and different types of electrophoresis. In general, good correlation has been found between electrophoretic and ultracentrifugally isolated lipoproteins. These correlations have been extremely useful in establishing bridges of communication between clinicians and biochemists and have permitted a better understanding of the physiological role of lipoproteins.

On the basis of density, flotation, electrophoretic behavior and amino acid residues, four main groups of lipoproteins have been characterized (Table I): 1) chylomicrons, 2) very low density lipoproteins (VLDL) or pre-β-lipoprotein by electrophoresis, 3) low density lipoproteins (LDL) or β-lipoproteins by electrophoresis and 4) high density lipoproteins (HDL) or α-lipoproteins by electrophoresis. The presence of four major residues, aspartic acid, glutamic acid, serine and threonine as N-terminal amino acids, in these lipoproteins suggests that there must be at least four different apoproteins forming part of the lipoproteins. We will discuss this point later.

There are techniques used in the study of lipoproteins based on the ability of LDL and VLDL to form complexes with different polyanions (2,3). We have studied the formation of these complexes using different mucopolysaccharides (MPS), cations, etc. (4) and found that at physiological pH, maximum complex formation takes place at concentration of Ca ++ of 20 mg/ml of serum and at concentrations of heparin below 2 mg/ml serum (5). At higher concentration of heparin, other serum proteins in addition to β-lipoproteins are coprecipitated. The presence of N-sulfated groups in the hexosamine residue of MPS increased their complexing ability with the β-lipoproteins. We have concluded that in the formation of these complexes between LDL, VLDL and MPS, Ca ++ seems to interact first with phosphate groups of phospholipids of lipoproteins, thereby providing catonic sites to the reacting group of MPS. However, other types of interaction such as electrostatic forces, hydrogen bonds or hydrophobic bonds cannot be excluded as participating in the formation of the com-

### TABLE I

<table>
<thead>
<tr>
<th>Lipoprotein class</th>
<th>Flotation Sf.</th>
<th>Density range (g/ml)</th>
<th>Paper electrophoresis</th>
<th>N terminal amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>&gt;400</td>
<td>&lt;0.95</td>
<td>Origin</td>
<td>Ser, Thr</td>
</tr>
<tr>
<td>VLDL a</td>
<td>20-400</td>
<td>0.95 - 1.006</td>
<td>pre-β</td>
<td>Ser, Thr, Glu, Asp</td>
</tr>
<tr>
<td>LDL</td>
<td>0-20</td>
<td>1.006-1.063</td>
<td>β</td>
<td>Glu, Ser</td>
</tr>
<tr>
<td>HDL</td>
<td>--</td>
<td>1.063-1.21</td>
<td>α</td>
<td>Asp</td>
</tr>
</tbody>
</table>

aAbbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; Ser, serine; Thr, threonine; Glu, glutamic acid; and Asp, aspartic acid.