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Human Plasma High-Density Lipoproteins: Past, Present and Future

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High-density lipoproteins represent a class of plasma lipoproteins of high hydrated density commonly separated by ultracentrifugal flotation in media of density 1.063–1.21 g/ml. Two major subclasses have been recognized, with densities of 1.063–1.125 g/ml (HDL₂) and 1.125–1.21 g/ml (HDL₃). Both fraction HDL₂ and fraction HDL₃ have been intensively characterized by many chemical and physical techniques, although their relationship, whether physiological or merely an artifact, has not been established.

The demonstration that the apolipoprotein (referred to earlier as α P) of high-density lipoprotein can be isolated in an essentially lipid-free form and solubilized in aqueous media in the absence of surface-active agents or dissociating agents like urea or guanidine hydrochloride was a significant development in the understanding of the chemistry and the structure of high-density lipoprotein. Such an observation made possible the application to the apolipoprotein of the many techniques available in protein chemistry. It is now established that apo-(fraction HDL₂) and apo-(fraction HDL₃) each contain two major polypeptide chains, referred to in our laboratory as fractions III and IV, which are chemically and immunologically distinct from each other. Another minor class of polypeptides, fraction V, has been identified, and there is evidence for its derivation from the very-low-density-lipoprotein peptides as one of the consequences of the very-low-density lipoprotein-high-density lipoprotein exchange process.

From the structural viewpoint, the studies directed at defining the lipid-binding properties of fractions III and IV with the ultimate goal of reassembling *in vitro* high-density lipoprotein from its isolated constituents have been particularly fruitful. Methods have been described for the

reassembly of a high-density-lipoprotein-like complex with properties strikingly similar to those of native high-density lipoprotein. This has led to a detailed analysis of the mode of solubilization of cholesteryl esters in high-density lipoprotein, particular attention being paid to the role played by the apolipoprotein and phospholipids. Of no less significance have been the studies demonstrating that some of the polypeptide chains of the apolipoprotein centre in important enzymic reactions. For example, one of the polypeptides of fraction V has been shown to act as a cofactor in the activity of the enzyme lipoprotein lipase (clearing-factor lipase).

With regard to future trends, it is expected that important progress will be made in defining the structure and function of the various high-density-lipoprotein polypeptides. Reassembly methods, making use of ultracentrifugal, spectroscopic (n.m.r., spin-label methods, fluorescence etc.) and X-ray techniques for the analysis of the products obtained, are expected to provide some clarification of the molecular details of the protein-lipid interactions in high-density lipoprotein. Many of these studies are now under way.

The Argonne Cancer Research Hospital is operated by the University of Chicago for the U.S. Atomic Energy Commission.

Some Physical Approaches to the Structure of Serum High-Density Lipoproteins

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The serum lipoproteins have been subjected to an increasing range of physical techniques and physico-chemical approaches in recent years. These studies have had a dual motivation. On the one hand it was hoped that these approaches might lead to a detailed elucidation of the molecular structures of these systems, which in turn would lead to an understanding of their role in lipid metabolism. On the other hand the serum lipoproteins appear to be receiving increasing attention as models of lipid-protein interaction in general. This paper will be limited to the high-density lipoproteins of human serum, specifically the HDL₂ fraction, the major molecular components of which are protein, phospholipids (mainly phosphatidylcholine) and neutral lipids (mainly cholesteryl esters). The major questions to which the studies to be described refer are simply stated: what are the gross structural features and underlying molecular interaction in fraction HDL₂ that lead to a mutual solubilization of the otherwise water-insoluble phospholipids and cholesteryl esters?

Fraction HDL₂ has a molecular weight of 386 000 and has been observed directly in the electron microscope; the mean diameter was about 95 Å, in agreement with the size determined by low-angle-X-ray-scattering techniques. The water-solubility of fraction HDL₂ and the high phospholipid content suggest a structure in which many of the charged groups, such as the lysine residues of the protein moieties, and the choline head group of the phospholipids are on the molecular surface. This suggestion receives support from chemical and enzymic accessibility data and also n.m.r. data.

The conformation of the protein subunits within fraction HDL₂ have been extensively studied under a range of conditions by optical rotatory dispersion and circular dichroism, and these studies have suggested that a relatively high degree of helical structure may be present. N.m.r. and fluorescence studies indicated that any proposed structure must account for a high degree of molecular mobility of the lipid hydrocarbon chains. Surface-balance and electron-spin-resonance (spin-labelling) techniques have both been applied recently to this problem, with encouraging results. It seems that these two techniques in particular may be able in due course to provide considerable insight into some of the molecular details of the underlying interactions.

We have to admit, however, that in spite of a considerable amount of effort with whole fraction HDL₂ the extent of our information on the gross structure and, more specifically, the underlying molecular interactions remains extremely meagre. The delipidation and the partial and total reconstitution techniques that have been introduced by Scanu, Cump, Toth, Koga, Stiller & Albers (1970) have led to a feeling of greater optimism that the structural information in the required degree of depth may be obtainable by presently available methodology. However, this feeling still seems far removed from reality, and the experimental results to be described can at this stage be presented as showing only possible approaches, rather than in any sense a final answer, to this important problem.

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Biosynthesis of Plasma Lipoproteins

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The biosynthesis of plasma lipoproteins, which can be carried out by the cells of the liver (Marsh & Whereat, 1959) and the intestinal mucosa (Kessler, Stein, Dannacker & Narcessian, 1970), proceeds by assembly of the polypeptide chains on the mem-

brane-associated polyribosomes. At this stage labelled peptides can be isolated that are capable of binding lipids (Bungenberg de Jong & Marsh, 1968). The subcellular site of attachment of lipid is not known, but in liver the molecules reach the Golgi apparatus, where carbohydrate is covalently attached (Lo & Marsh, 1970) and very-low-density lipoproteins can be seen in the electron microscope (Hamilton, Regen, Gray & LeQuire, 1967). Details of the secretory mechanism are not known at present. Although net synthesis of the protein moieties of both low-density lipoprotein and high-density lipoprotein has been demonstrated in slices of rat liver, the very-low-density lipoproteins have been found to contain the same peptides, though not in the same proportions, as do low-density lipoproteins and high-density lipoproteins (Fredrickson, 1959). Consequently it seems possible that very-low-density lipoproteins may be the only form in which lipoproteins are secreted by the liver. With regard to the lipids, the newly secreted lipoproteins are not identical with those found in the circulation, at least in the case of cholesteryl esters, which are mainly synthesized by the plasma lecithin (phosphatidylcholine)-cholesterol acyltransferase (Glomset, 1968). There is evidence that this enzyme is secreted by the liver, thereby accounting for the low concentration of plasma cholesteryl esters in liver disease (Simon & Scheig, 1970). The availability of free fatty acids to the liver from the plasma or the endogenous synthesis of fatty acids from carbohydrate (Windmueller & Spaeth, 1967) appears to be one of the most important factors regulating hepatic lipoprotein synthesis. This concept is supported by recent work on lipoprotein synthesis after ethanol administration (Baraona & Lieber, 1970) or partial hepatectomy (Infante *et al.* 1969). When the liver is stimulated to release more lipid after perfusion of high concentrations of free fatty acid, an increased incorporation of labelled amino acids into very-low-density lipoprotein has been demonstrated (Ruderman, Richards, Valles de Bourges & Jones, 1968), indicating that lipid release under these conditions is accompanied by the synthesis of the protein of the very-low-density lipoproteins. The mechanism of this effect has not yet been established.

This work was supported by U.S. Public Health Service Grant HE-05285 from the National Institutes of Health and by the Center for Oral Health Research, University of Pennsylvania.

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