PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 514th Meeting of the Society was held at University College London, Gower Street, London WC1E 6BT, on Thursday and Friday, 22 and 23 April 1971, when the following papers were presented:

SYMPOSIUM ON 'PLASMA LIPOPROTEINS'

Very-Low-Density Lipoproteins and Chylomicrons

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Low-Density Lipoproteins

By BARRY LEWIS. (*Department of Chemical Pathology, Royal Postgraduate Medical School, London W12 0HS, U.K.*)

The low-density class includes the highest concentration of the lipoproteins of human plasma in the fasted state, about 350 mg/dl. Lipid constitutes about three-quarters of the molecule, with cholesterol (47%) as the main component, phospholipid 23% and triglyceride 9% (Bragdon, Havel & Boyle, 1956). Physical features defining the class include a density of 1.006–1.063, S_f (flotation) 0–20 units, β mobility on electrophoresis, molecular weight 2.3×10^6 – 2.8×10^6 and mean diameter 250 Å. Pollard, Scanu & Taylor (1969) suggest that the molecule has 20 subunits forming a dodecahedron, with phospholipid completing the surface and less-polar lipids occupying the core.

As so defined, low-density lipoprotein is heterogeneous, having two ultracentrifugal components, S_f 0–12 and S_f 12–20 units, the latter with higher triglyceride content (Nichols, 1967) and greater therapeutic responsiveness to clofibrate (Strisower, Adamson & Strisower, 1968); there are multiple bands on polyacrylamide-gel gradient electrophoresis (Pratt & Dangerfield, 1969); Oncley (1969) found the sedimentation coefficients of low-density lipoprotein to have a single normal distribution compatible with a molecule of somewhat variable composition. Sodhi (1969) has described an unusual low-density lipoprotein with density 1.040–1.060 but pre- β mobility. Spritz & Mishkel (1969) reported diet-induced changes in cholesterol/phospholipid ratio in low-density lipoprotein; though of smaller magnitude than we find in triglyceride/protein ratios in very-low-density lipoprotein during carbohydrate induction, which rise

by 30–420% (M. Mattock, M. Mancini & B. Lewis, unpublished work), such effects confirm the inconstant composition of lipoproteins. Two forms of genetic polymorphism are known, Lp and Ag (Berg, 1963; Blumberg, Dray & Robinson, 1962).

The lipid-protein bond in low-density lipoprotein probably involves strong electrostatic forces, but interlipid bonds appear to depend on van der Waals forces and hydrophobic interactions, permitting extraction of cholesterol and glycerides by non-polar solvents (Chapman, 1969). Variable composition may result partly from intramolecular changes, but altered proportions of members of the low-density class are also likely. In low-density lipoprotein Shore & Shore (1969) identified two apolipoproteins, and V. Brown, R. I. Levy & D. S. Fredrickson cited by Fredrickson (1970) described C-terminal serine and alanine peptides. Alaupovic, Lee & McConathy (1969) and Lee & Alaupovic (1970) have described the major C-terminal serine apoprotein and also, as minor components, C-terminal alanine peptide, as occurs in very-low-density lipoprotein, and C-terminal threonine peptide, as found in high-density lipoprotein; they introduced a new concept, of lipoprotein families based on a single apoprotein but variable in lipid composition and hence in physical properties.

Low-density lipoprotein, despite its relative abundance in plasma, has a far slower turnover than do triglyceride-rich lipoproteins and albumin-bound free fatty acids. The total turnover of cholesterol through plasma, chiefly transported by low-density lipoprotein, is about 1 g/24 h, and the slowest exponential of [^{14}C]cholesterol has a half-life of some 58 days (Lewis & Myant, 1967). By contrast, apoprotein-labelled low-density lipoprotein has a shorter half-life, 7 days with ^{131}I (Hurley & Scott, 1970) 6.1 days with [^{35}S]cystine (Volwiler *et al.* 1955), presumably because cholesterol recycles between plasma and rapidly exchanging extravascular pools. Low-density lipoprotein is synthesized in the liver, and probably the small intestine, on ribosomes (Bungenberg de Jong & Marsh, 1968). Esterification of its cholesterol, however, occurs chiefly intravenously, by circulating

lecithin (phosphatidylcholine)-cholesterol acyltransferase. Glomset (1968) suggests that this enzyme facilitates the uptake of free cholesterol, released in peripheral tissues in the course of cell turnover, by lipoproteins for transport to the liver. Other functions of low-density lipoprotein or its apoproteins are suggested by the occurrence, in the rare disorder α - β -lipoproteinaemia, of impaired fat absorption, with absence of chylomicron and very-low-density-lipoprotein synthesis (Levy, Fredrickson & Laster, 1966).

Environmental and genetic factors, and several acquired diseases, influence cholesterol and, where studied, low-density-lipoprotein concentrations. There is high concordance between monozygotic twins (Meyer, 1962), and cholesterol concentrations vary with the presence of certain genetic markers (Oliver, Geizerova, Cumming & Heady, 1969). Wide geographical variations are seen, the consequence of environment, not ethnic group (Keys, 1970; Antonis & Bersohn, 1962); even within homogeneous groups, environmental factors contribute. Dietary influences include the type of fat (unsaturation, chain length) and intake of cholesterol and, to a small extent, of sucrose (McGandy, Hegsted, Myers & Stare, 1966). Plasma cholesterol and low-density-lipoprotein concentrations rise during the third to sixth decade of life in industrialized countries; this is not necessarily physiological. It is uncertain to what extent decrease of low-density-lipoprotein concentrations by unsaturated-fat intake results from compartmental shifts of cholesterol (Grundty & Ahrens, 1970) or from increased catabolism of cholesterol to bile acid (Lewis, Pilkington & Hodd, 1961; Wood & Kinsell, 1967; Connor, Witiak, Stone & Armstrong, 1969). Low plasma lipid concentrations occur in the kwashiorkor form of protein-calorie malnutrition (Lewis, Hansen, Wittman, Krut & Stewart, 1964).

Familial hyper- β -lipoproteinaemia shows autosomal dominant inheritance. It may well prove to be heterogeneous, perhaps suggested by therapeutic experience. It may be accompanied by elevated very-low-density-lipoprotein concentrations; sometimes correction of the latter may be accomplished by a prolonged rise in low-density-lipoprotein concentration (B. Lewis, unpublished work).

Low-density-lipoprotein concentrations rise in hypothyroidism, probably owing to impaired excretion and/or catabolism of cholesterol. Concentrations of low-density lipoprotein are lower in females than in males, rising if androgens are administered.

Increased plasma low-density-lipoprotein and cholesterol concentrations are associated with increased incidence of ischaemic heart disease (Kannel, Dawber, Friedman, Glennon & McNamara, 1964). The mechanism is unclear: low-density

lipoprotein is immunologically demonstrable in atheromatous plaques (Woolf & Pilkington, 1965); it may alter platelet function (Bolton, Rampton & Mitchell, 1967) and it can become incorporated into thrombi (Scott & Hurley, 1969).

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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.

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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?