FUNCTIONAL ROLES OF PLASMA HIGH DENSITY LIPOPROTEINS

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I. GENERAL OVERVIEW ON HDL STRUCTURE AND METABOLISM

A. Introduction

From the operational viewpoint the high density lipoproteins (HDL) comprise a class of particles that are isolated in the ultracentrifuge between solvent densities of 1.063 to 1.21 g/mℓ. It was long accepted that there are two main subclasses of HDL, namely HDL_2 (d 1.063 to 1.125 g/mℓ) and HDL_3 (d 1.125 to 1.21 g/mℓ) and a third minor one, HDL_1, isolated in the density range of 1.050 to 1.063 g/mℓ only present in detectable amounts in some individuals.[1,2] Recent studies, particularly from the Donner Laboratory, have pointed at a greater complexity of the HDL class. Anderson et al.[3] upon re-evaluation of the Schlieren pattern of HDL in the analytical ultracentrifuge and also from equilibrium density ultracentrifugation analyses, concluded that HDL consists of three different components. One is comparable to the previously described HDL_3 and the other two, HDL_{2a} (d 1.100 to 1.125) and HDL_{2b} (d 1.063 to 1.100), are subcomponents of HDL_2. The authors also found that the plasma levels of HDL_{2a} and HDL_{2b} best correlate with the total HDL levels. More recently, the notion of the homogeneity of the HDL_3 class has been challenged by the rate zonal ultracentrifugal results of Patsch and Gotto[4] and by the studies of Nichols et al.[5] using the technique of gradient gel electrophoresis. It would appear that some subjects have HDL species of density greater than HDL_3, but their actual structural and functional properties are currently not established. Of particular interest would be to establish the relationship between these "heavy" HDL and the early recognized very high density lipoproteins (VHDL) which may be considered to be an ultracentrifugal artifact. Another particle which has entered the HDL nomenclature is HDL_c, where the subscript *c* stands for cholesterol. This is a particle originally described by Mahley et al.[6] in miniature swines during their hyperlipidemic response to diets enriched in saturated fats and cholesterol. Since then, HDL_c also has been reported in other animal species fed cholesterol, namely, man, dog, and rats in the density range between 1.02 and 1.06 g/ml.[7] This particle has a diameter of 150—200 Å, α_2-electrophoretic mobility and is characterized by the presence of apo E, also referred to as arginine-rich peptide.

B. Properties of HDL Particles

The chemical composition of the two major HDL sub-classes HDL_2 and HDL_3 and that of HDL_c is given in Table 1. It may be noted that the apoprotein composition varies. Apo A-I is present in all particles; apo A-II is present in HDL_2 and HDL_3, whereas HDL_c

Table 1
PROPERTIES OF MAIN HUMAN SERUM LIPOPROTEIN SUBCLASSES

Physical properties	HDL_c	HDL_2	HDL_3
Solvent density, g/mℓ	1.04—1.06	1.063—1.125	1.125—1.21
Flotation rate, $S_{f\ 1.21}$	—	3.6—9.0	0—3.5
Hydrated density, g/mℓ	1.050	1.094	1.145
Chemical composition, % weight			
Protein	20.3	41.0	55.0
Phospholipid	29.3	29.5	22.5
Cholesteryl esters	42.5	16.2	11.7
Unesterified cholesterol	7.5	5.4	2.9
Triglyceride	0.5	4.5	4.1
Apoproteins			
A-I	+[a]	75.0	65.0
A-II		15.0	25.0
C-I		1—2	
C-II		1—3	5
C-III (0,1,2)		4—6	
D		—	+
E	+[a]	+	—

[a] Main apoprotein components occurring in varying proportions.

has apo E. The ratio of apo A-I:apo A-II is higher in HDL_2 than in HDL_3. Moreover, HDL_3 contains low concentrations of apo C which are relatively more abudant in HDL_2.

In recent years, important information has accumulated on the amino acid sequence of the various apolipoproteins and on their properties in solution in their lipid-free form.[1,2] Information has also been obtained on their properties at the hydrophilic-hydrophobic interface, largely influenced by the presence of helical segments having amphiphilic characteristics. Moreover, in the case of apo A-I, a high frequency of homology of amino acid segments 22-amino acid long has been observed, each segment considered as having evolved from the duplication of an internal gene coding for an 11-amino acid residue. Taken together these results indicate that plasma apolipoproteins have characteristics in common such as being amphiphilic and, in addition, specific properties related to polypeptide chain length, the presence of structures other than the α-helix, and of other undefined factors. For instance, structural specificity is reflected by the ability of these apoproteins to serve as a co-factor for key lipid modifying enzymes, i.e., apo C-II for lipoprotein lipase and apo A-I for lecithin-cholesterol acyl transferase. Moreover, apo E has been shown to interact specifically with the low density lipoprotein (LDL) receptor even more effectively than apo B. Apo D has been implicated in the process of exchange of cholesteryl esters among lipoproteins although other plasma proteins may participate in this process (see Section II.B).

C. Structural Studies

The early small-angle X-ray scattering studies by Shipley et al.,[8] Atkinson et al.,[9] and Laggner et al.[10] indicated that the HDL particle is spherical or quasi-spherical, exhibiting a diffraction pattern compatible with a model having an electron-deficient core containing the apolar lipids surrounded by an electron-dense shell 10 to 15 Å thick that contains the polar head group of phospholipids and the apoproteins. Later X-ray studies by Tardieu et al.[11] conducted as a function of solvent density have led to the proposal of a

more complex structure where the particle surface is deeply convoluted, allowing for the penetration of solvent to within 25 Å of the center of the particle. The electron microscopic studies of Ohtsuki et al.[12] on negatively stained preparations of human HDL_3 using field emission-conventional transmission electron microscopy and scanning transmission electron microscopy generated images which were interpreted as having a substructure characterized by 12 domains, each 28 Å in diameter, and an inner core, 40 Å in diameter. A subunit structure of HDL was also noted by the electron microscopic studies of Forte and Nichols,[13] but later attributed to artifacts.[14]

The existing physical and chemical data have been used to propose models of HDL structure which are in keeping with the long-range organization suggested by the small-angle X-ray scattering studies. Among the most recent ones are those by Verdery and Nichols[15] and by Shen et al.,[16] later refined by Edelstein et al.[17] All of these models stress the existence of an apolar core and the presence at the lipoprotein surface of apoproteins and polar head group of phospholipids in accord with the results of experiments based on the chemical modification of the lysine groups and particle digestion by phospholipase A_2. However, the detailed structural organization of the various HDL particles is still wanting, and there is no immediate solution on site except for the anticipation that suitable crystalline preparations will become available for X-ray analyses. The solution of the structure of these particles is essential for a thorough understanding of their functional properties and the relationships among HDL subclasses.

D. Modes and Sites of Formation

The spherical structure which appears to characterize the mature HDL particle, i.e., the one which has undergone cholesteryl ester enrichment in the circulation, is thought to be different from the nascent particle newly secreted from the sites of synthesis. The studies by Hamilton et al.[18] with perfused rat livers showed that when the perfusate contained an inhibitor of lecithin:cholesterol acyl transferase (LCAT), the nascent HDL appeared as a disc by electron microscopy with a thickness of 46 Å and a mean diameter of 190 Å. Such disc structures, which contained apo E as the predominant protein, as well as polar lipids and trace amounts of apolar lipids (cholesteryl esters and triglycerides), were found to be a good substrate for LCAT. These studies supported the hepatic derivation of HDL in a form requiring the action of LCAT for the production of mature HDL. In keeping with this conclusion are the studies of patients with familial LCAT deficiency or alcoholic hepatitis showing in their plasma the presence of discoidal HDL particles which acquire a spherical structure upon the action of LCAT.[19]

Besides its hepatic source, HDL appears to be also produced by the intestine also. The mode of formation of HDL as a discoidal structure by the intestine is suggested by the studies of Green et al.,[20] who in studies of the mesenteric lymph collected in the presence of an LCAT inhibitor found these structures by electron microscopy. Moreover, it has been shown that in hepatectomized rats, radioactive amino acids are incorporated into plasma HDL.[21] Currently, the extent of the intestinal contribution to the pool of circulating HDL is not well defined. According to a recent study,[22] the surface polar components of chylomicrons are transferred to HDL upon hydrolysis of the core triglycerides by lipoprotein lipase. This process involves both phospholipids and apoproteins. Evidence for the net transfer of phospholipids from chylomicrons to the HDL surface comes from the work of Minari and Zilversmit,[23] Nestel et al.[24] in the pig, and of Havel et al. in man[25] and rats.[26] Either the incubation of lymph chylomicrons in vitro or their intravenous injection caused enrichment of phospholipids in HDL. According to Havel et al.,[25] the increase in phospholipid involves preferentially HDL_2, which also acquires the C-peptides released from the chylomicron surface. Whether the movement of the phospholipids is synchronous with that of the C-apoproteins is unclear,

but the accumulation of these two surface components appears to be partially responsible for the transformation of HDL_3 into "HDL_2-like" particles. A similar phenomenon has been shown to occur in vitro when VLDL is hydrolyzed by lipoprotein lipase in the presence of HDL_3. The latter acquires phospholipids and C-peptide and flotational properties of HDL_2.[27]

Important evidence has recently accumulated to support the transfer of apo A-I and apo A-II from lymph chylomicrons to HDL. Schaefer et al.[28] have shown that this transfer occurs in human subjects upon intravenous injection into human subjects of radioactively labeled chylomicrons isolated from a patient with chylous pleural effusion. Mass transfer of apoproteins (and phospholipids) from chylomicrons into HDL has also been shown in the rat by Redgrave and Small.[29] Based on these and other observations, Tall and Small[22] have advanced the hypothesis that, following the hydrolysis of the triglycerides of chylomicrons by lipoprotein lipase, the polar surface of these particles becomes redundant and forms bilayer buds which are able to break off in sheets and rapidly fuse into vesicles containing phospholipids, free cholesterol, and possibly small amounts of chylomicron apoproteins. The acquisition of apo A-I will make them a suitable substrate for LCAT and for the generation of mature HDL particles.

Thus LCAT is a key enzyme for the formation of circulating HDL. In principle, any lipid structure present in the plasma, if suited for LCAT action, could become the source of this lipoprotein class.

E. Sites of Removal of HDL

The information available concerning the sites of removal of HDL is still modest and is based on the fate of HDL particles labeled in their apoprotein moiety with radioiodine. This information suffers from the fact that the heterogeneity of HDL class has not always been taken into account.[30] Recent studies by Mahley et al.[31] have shown that apoprotein compositions, i.e., apo E-containing vs. non-apo E-containing particles, have a profound effect on the cellular uptake of HDL. Moreover, sufficient structural differences exist between the rat, often used in catabolic studies, and other animal species, and this prompts a word of caution when extrapolation of the results is attempted.

In the rat model, several studies have shown that HDL labeled in the protein moiety by iodine is predominantly removed by the hepatocyte via the lysosomal mechanism.[22,30] The involvement of the liver in the uptake of HDL also has been shown in the dog. However, the liver does not appear to be the exclusive site for HDL removal. Studies by Roheim et al.[32] in the rat have shown that the intestine contributes to HDL uptake, and the studies by Nakai and Whayne[33] have provided evidence for the participation of the kidney. In swine, based on the increased turnover of radioiodine HDL after portacaval shunt, it has been suggested that organs other than the liver participate in the process of HDL uptake and removal.[34] In fact, Snidermann et al.[35] have found that no detectable amount of HDL is extracted by the splanchnic bed of human subjects and Van Tol et al.[36] observed that the catabolism of HDL in the rat is unchanged by the surgical removal of two thirds of the liver. Hence, the studies in the whole animal have not yet provided a quantitative assessment about the contribution of the liver to HDL catabolism. On the other hand, human or rat hepatocytes grown in culture have been shown to take up and degrade radiolabeled HDL. By using the isolated perfused liver system, Nicoll et al.[30] have estimated that the catabolism of radioiodinated HDL in such a system is 10% less than that obtained in the intact rat. On the other hand, one must consider that a quantitative comparison is equivocal in that the nature of the HDL particles seen by the cell is unlikely to be the same in the two experimental conditions. That cells other than hepatocytes are involved in HDL degradation is also indicated by studies on cultured human fibroblasts, rat vascular smooth muscle cells, and rabbit vascular endothelium.[22,30]

However, from the quantitative aspect, the degradation of HDL by these cells appears to be significantly less than that of LDL and to be distinct from the process of receptor-specific endocytosis involving the LDL particles. Thus, these studies indicate that the LDL-receptor pathway is not involved in HDL catabolism, but do not define the nature of the interaction which occurs between HDL and the cell membrane. Although it was initially thought that HDL interferes with the process of specific uptake of cholesterol by the LDL receptor, it now appears that this phenomenon only applies to the fractions of HDL containing apo E, an apoprotein which has been shown to have a great affinity for the LDL receptor.[31]

Taken together, the in vivo and in vitro results indicate that a certain percentage of HDL is taken up by liver and, perhaps, by peripheral cells. However, the mechanism of this uptake remains to be elucidated. Once taken up, the degradation of these particles occurs presumably through the action of lysosomal enzymes (see Section II.B).

F. HDL and Cholesterol Metabolism

Particularly through the seminal studies by Glomset and Norum,[37] the notion has developed that the HDL-LCAT system is involved in the reverse transport of cholesterol as a cholesteryl ester from peripheral tissues to the organs of utilization, namely liver, adrenal cortex, and gonads. Basic to this notion is the concept that HDL and in particular the nascent phospholipid-apoprotein bilayer can acquire unesterified cholesterol from cellular membranes and that this cholesterol initially occupying the particle surface is transformed into cholesteryl esters by the action of LCAT, leading to the formation of mature HDL. The newly formed cholesteryl esters now occupy the particle core and from there are transferred to LDL and VLDL and disposed by the liver, adrenal cortex, or gonads.

According to this notion, HDL plays a key role in tissue cholesterol homeostasis, probably by a process not directly dependent on the LDL-receptor pathway. Thus, the levels of circulating HDL would be crucial for cellular cholesterol egress. Indeed, Miller and Miller[38] have shown that in middle-aged human subjects, there are both rapidly and slowly exchanging pools of tissue cholesterol which are negatively correlated with plasma HDL levels. An extensive literature has since accumulated supporting a negative correlation between levels of plasma HDL and incidence of cardiovascular disease. Such an observation has prompted efforts to raise plasma HDL levels by several means, including physical exercise, diets, and drugs. Other reports invite a more cautious attitude on this subject, particularly since it is difficult to divorce HDL metabolism from that of the serum lipoproteins.

II. FUNCTIONS OF HDL

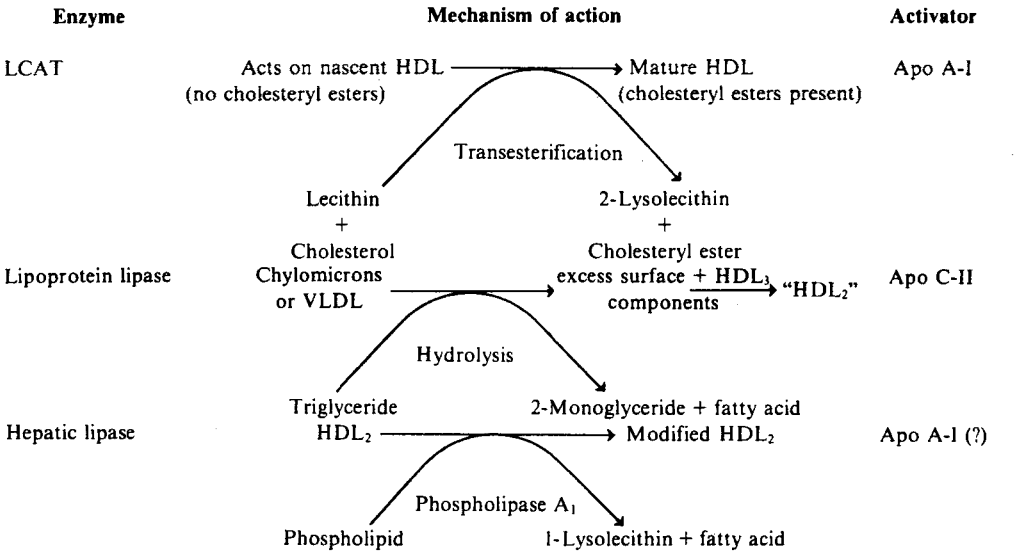
The key role of HDL in lipoprotein metabolism has stimulated increasing interest in the study of this lipoprotein class, in terms of both structure and function. As previously mentioned, several subclasses of HDL are now recognized, and their participation in the overall lipoprotein metabolism is just beginning to emerge. In the following sections, we will examine current concepts on the various functions of HDL as they relate to their interaction with lipid-modifying enzymes, other lipoproteins, and cells.

A. Interaction of HDL With Other Lipoproteins

1. HDL and the LCAT System

The formation of cholesteryl esters in plasma is thought to occur mainly through the transesterification reaction between cholesterol and lecithin catalyzed by the plasma enzyme lecithin-cholesterol acyltransferase (LCAT; EC 2.3.1.43). This reaction

Table 2
PARTICIPATION OF HDL IN LIPOLYTIC REACTIONS



promotes the transfer of an acyl-chain from the 2 position of lecithin to the hydroxyl function of cholesterol, yielding lysolecithin and cholesteryl ester (Table 2). The enzyme is activated by apo A-I[39,40] and apo C-I[40,41] and inhibited by apo A-II.[39,41] Regarding the role played by the A apolipoproteins in LCAT action, Chung et al.[40] have addressed the question by utilizing a highly purified preparation of human LCAT and a substrate composed of single bilayer mixed vesicles with lecithin:cholesterol molar ratios of 4:1.[42] Activation of LCAT was studied as a function of apo A-I concentration and found to obey a hyperbolic saturation curve with maximum activation at approximately a 25-fold molar excess of apo A-I to the enzyme. Apo A-II was found to inhibit the apo A-I-mediated activation of LCAT by displacing apo A-I from the vesicle surface. From these studies it became apparent that the apo A-I:apo A-II ratio in a suitable substrate plays a significant role in the in vitro action of the enzyme, the higher ratios being expected to be attended by a higher degree of enzyme activation. Similar studies were extended to the naturally occurring lipoproteins.[43] The percent cholesteryl ester produced in normal HDL_3 (apo A-I:apo A-II molar ratio; 2.5 to 3:1) was almost eightfold higher than in the HDL_2 (apo A-I:apo A-II molar ratio 8 to 10:1). Yet based on the apo A-I:apo A-II ratios, one would have expected HDL_2 to be a better substrate than HDL_3. This effect may be partially explained by one or more of the following compositional differences between HDL_2 and HDL_3: (1) about 45% less cholesteryl esters in HDL_3, (2) a 25% higher molar ratio of lecithin to unesterified cholesterol in HDL_3 vs. HDL_2, and (3) a 38% lower lecithin:apo A-I ratio in HDL_3 than in HDL_2. With reference to cholesteryl ester content, Chajek et al.[44] have recently found that in lecithin-cholesterol liposomes, these esters inhibit the LCAT action. This inhibition is independent of the amount of enzyme present in the liposome and the proportion of cholesterol relative to lecithin.[44] Another possible explanation for the difference in LCAT action between HDL_2 and HDL_3 may be related to the extent of enzyme binding. This question was recently examined by Chung et al.[45] in the context of their study on the mass:activity distribution of LCAT among lipoproteins isolated by ultracentrifugal and chromatographic procedures. Although they confirmed previous observations on the inability of HDL_2 to be active

either as an enzyme source or as a substrate, they found that this lipoprotein subclass bound LCAT as well as HDL_3 and also provided evidence that the nonfunctional enzyme in HDL_2 can be activated if displaced from the HDL_2 surface. Viewed in the context of lipoprotein metabolism, these findings are compatible with the central role played by HDL_3 both in the plasma synthesis of cholesteryl esters and also as a site from where the newly synthesized cholesteryl esters are delivered to the other plasma lipoproteins. HDL_2 may be considered either as an inert carrier or a reservoir of the enzyme which would become ready for action once displaced from the surface of this lipoprotein. Thus, it is apparent that the HDL subclasses are clearly linked to LCAT action and in consequence with the plasma transport of cholesteryl ester in concert with the cholesteryl ester transport systems to be examined below.

2. Role of HDL in the Transfer of Cholesteryl Esters to the Other Lipoproteins

Several studies have shown that cholesteryl esters undergo exchange or transfer among plasma lipoproteins and that this process is mediated by the cholesteryl ester/triglyceride exchange/transfer protein(s).[46-53] Unfortunately, there is no complete agreement about the nature of this protein or proteins. Pattnaik et al.[47] isolated a glycoprotein of apparent molecular weight 80,000 by a combination of purification steps which included plasma ultracentrifugation at d 1.25 g/mℓ and column chromatography using phenyl-Sepharose, CM-cellulose, and ConA-Sepharose. In contrast, Chajek and Fielding[48] purified the cholesteryl ester transfer protein by a series of procedures which include the isolation of HDL by ultracentrifugal flotation (density 1.063 to 1.21 g/mℓ), the use of anti-apo D affinity chromatography, and Con-A Sepharose chromatography. The resulting product migrated as a single species with an apparent molecular weight of 34,000 by SDS polyacrylamide gel electrophoresis and was considered to be identical to apo D.[48] However, the transport of cholesteryl esters may involve more than one plasma protein, as indicated by the recent report of Morton and Zilversmit.[49] These authors found that apo D and the cholesteryl ester transfer factor behave as two distinct components when fractionated by phenyl-Sepharose and CM-cellulose chromatography, and gel permeation chromatography using Bio-gel A-0.5 *M* concentration or polyacrylamide gel electrophoresis in basic pH. The transfer protein isolated by the procedure of Morton and Zilversmit[49] was also found to be immunologically distinct from apo D, indicating that either apo D is not involved in cholesteryl ester transport or that it contributes to this process in a manner different from the cholesteryl ester transport protein.

The possible existence of multiple forms of cholesteryl ester carrier proteins in the plasma may explain the present uncertainties regarding the issue of undirectional transfer vs. bidirectional exchange of cholesteryl ester between HDL and the triglyceride-rich lipoproteins. Whereas the protein isolated by Chajek and Fielding[48] was shown to accelerate the net transport of cholesteryl ester from HDL to VLDL or to LDL with concomitant equimolar transport of triglyceride from VLDL or LDL to HDL, the protein isolated by Zilversmit et al.[46] and Pattnaik et al.[47] would instead catalyze the bidirectional exchange of cholesteryl ester between HDL and the other plasma lipoproteins. A similar exchange protein for cholesteryl esters has recently been reported by Sniderman et al.[50] in the d 1.25 g/mℓ infranatant of plasma, and Barter et al.[51] have described a serum transfer protein which catalyzes the bidirectional exchange of triglyceride between lipoproteins. Moreover, Ihm et al.[52] recently isolated from plasma a protein(s) of a molecular weight of approximately 150,000 which catalyzes the bidirectional exchange of both phosphatidycholine and cholesteryl esters between HDL and LDL. The measured rates and extents of PC and CE transfer suggest a coupled exchange between core and surface lipids.

Recently Fielding and Fielding[53] have reported on the presence of a cholesteryl ester transfer activity in a complex isolated either from plasma or HDL (d 1.063 to 1.21 g/mℓ) by differential immunoadsorption column chromatography. These investigators observed that 99% of plasma LCAT is adsorbed to columns cross-linked to antibodies specific for apo D or apo A-I, and considered these findings as indicative for the presence in plasma of an enzyme complexed with apo A-I and apo D and some lipids. From these results the authors speculated that it is through this complex that transfer of cholesteryl esters occurs and not via HDL_3 as assumed.

In support of this concept, Fielding and Fielding[53a] have recently reported that the cholesteryl ester content in lipoproteins regulates the acceptor capacity of lipoproteins and, thus, LCAT activity in human plasma. These authors found that when the cholesteryl ester content in the HDL was at saturating levels, the rate of cholesteryl ester synthesis, presumably mediated by the cholesteryl ester transfer complex, coincided with the rate of cholesteryl ester transfer in plasma to LDL and VLDL. The reaction proceeded until these acceptor lipoproteins attained saturating levels of cholesteryl esters. It is apparent that the synthesis and transfer of cholesteryl ester in plasma is tightly coupled. However, the mechanism of these processes is not known at this time and requires further study. What appears to be established is that the LCAT-catalyzed cholesteryl esterification in plasma proceeds at a linear rate until the capacity of the acceptor lipoproteins for cholesteryl ester becomes saturated. Thus, rates of cholesteryl ester synthesis and delivery are closely coupled and HDL particles appear to be key in these processes.

3. LCAT and HDL Formation

As mentioned in the Introduction, mature HDL are formed in the plasma from the action of LCAT on nascent discoidal structures containing phospholipids, unesterified cholesterol, and apoproteins. Much knowledge on the relationship between the HDL-LCAT system has derived from the study of patients with LCAT deficiency. In these patients, HDL particles in the circulation[54-56] are very similar in structure to those which have been described in rat liver perfusates containing an inhibitor of LCAT.[18,57] These particles are disc-shaped by electron microscopy (46 Å diameter) and are composed predominantly of polar lipids (lecithin and unesterified cholesterol) and apo E and little cholesterol ester or triglycerides. Purified LCAT converts these nascent particles into mature HDL through a tranesterifying reaction involving both free cholesterol and phospholipids.[18]

The HDL derived from the intestine are also discoidal and consist largely of apo A-I and A-II.[58] "Nascent HDL" particles can be produced in vitro during VLDL lipolysis. They were found to be morphologically indistinguishable from nascent discoidal particles in rat liver perfusates or in rat intestinal lymph.[27] It is interesting that in spite of their morphological similarity these particles have different apoprotein composition: the ones from the liver containing predominantly apo E[18] the ones from the intestine mostly apo A-I and A-II,[20,59,60] and the ones derived from VLDL predominantly apo C.[27] One of the fundamental questions which arises in discussing the in vivo interconversion of nascent HDL pertains to the cofactor requirement for LCAT action. Since detailed in vitro studies with apo E containing LCAT substrates have not been undertaken, little is known about the potential role of apo E in LCAT activation in vivo. Some information can be inferred from the in vitro studies by Glomset et al.[61] These investigators have studied the effect of LCAT on apoprotein distribution in lipoproteins of patients with familial LCAT deficiency. These results show that incubation of LCAT alters the distribution of apo E and apo C in the plasma of these patients. The apo E content in lipoproteins of density greater than 1.019 g/mℓ was found to decrease while those of

density less than 1.019 increased. The opposite trend was true for the C apoproteins. The mechanism of apoprotein transfer was not explained, but evidently the transformation of nascent discoidal structures into mature HDL particles caused differences in the affinity of these apoproteins for the lipoprotein surface. Although the reasons for this difference in affinity remain unexplained, the changes in apoprotein composition will obviously have an important effect on the metabolic fate of the plasma lipoproteins. Thus, HDL particles may be viewed as mediators of apoprotein transfer or exchange in concert with the activity of LCAT.

4. HDL as an Acceptor of Constituents of Triglyceride-Rich Particles

The initial studies by Eisenberg[27] provided evidence for HDL acting as an acceptor for the surface constituents released during the lipolysis of the triglyceride-rich lipoproteins. The in vitro studies by Glangeaud et al.,[62] utilizing radiolabeled apo C-VLDL, later showed that during the lipolysis of VLDL by lipoprotein lipase (LpL), increasing amounts of labeled apo C were transferred to HDL fractions of d 1.04 to 1.21 g/mℓ. In an experimental system containing VLDL, LDL, albumin, and HDL_3, Eisenberg and associates[27,63] showed that the excess surface components of VLDL lipolysis are transferred to HDL_3, resulting in the accumulation of apo C, phospholipid, and unesterified cholesterol in this particle. The modified HDL was "HDL_2-like" (Table 2) in terms of density characteristics, although it retained the A apoprotein composition of HDL_3.[27] When HDL_2 was used as the acceptor, transfer of VLDL components was noted although there were no changes in lipoprotein density.[27]

Recently, Yukawa and Scanu[64] investigated the in vitro effect of adding increasing amounts of VLDL-triglyceride to serum in the presence and absence of milk LpL. In the absence of LpL, addition of exogenous VLDL caused a gradual disappearance of the HDL peak of density 1.14 g/mℓ in the density gradient ultracentrifugal profile and a concomitant increase of the lipoproteins in the region of d 1.038 to 1.054 g/mℓ. When LpL was added to this system, there was a shift of the HDL band to lower densities. The shift was attributed to the net accumulation of lipid, thus producing "HDL_2-like" particles having the same apo A-I:A-II ratio as HDL_3. In general, the "HDL_2" particle produced in vitro differed from HDL_2 found in the normolipidemic sera. The results were interpreted to suggest that in the absence of LpL, addition of an increasing amount of VLDL caused an association of these particles with HDL leading to the disappearance of the HDL peak and the formulation of a complex banding within the density range of d 1.038 to 1.054 g/mℓ. In the presence of LpL, hydrolysis of VLDL triglycerides occurred and this promoted the dissociation from VLDL of the HDL particle which had now acquired a sufficient complement of lipid to become "HDL_2-like."

The relation between these in vitro events and the question of the actual occurrence in the circulation of the two major HDL subfractions, HDL_2 and HDL_3, is unclear. Various metabolic processes apparently affect HDL_2 and HDL_3 to different extents[3,65] although the weight of the evidence is that HDL_2 is a metabolic derivation of HDL_3. On the other hand, in studies of this kind, one must be aware of the possibility that "HDL_2-like" may be generated from HDL_3 in vitro and that this process must be ruled out before attributing changes to events occurring in vivo.

The intravenous injection of heparin releases a lipase of hepatic origin[66,67] in addition to LpL. The functional role of this hepatic lipase in vivo is poorly understood. In contrast to LpL, hepatic lipase activity does not correlate with plasma triglyceride levels.[68-70] In addition, Bengtsson and Olivecrona[71] have recently shown, in vitro, that the activity of the hepatic lipase toward a triglyceride emulsion was strongly inhibited by the presence of HDL. The inhibition was attributed to the higher affinity of the enzyme for HDL than for the emulsion substrate. Kuusi et al.[72] examined the relationship between plasma HDL

levels and hepatic lipase of postheparin plasma in a group of fit young men. A highly significant negative correlation was found between postheparin hepatic lipase activity and HDL_2 cholesterol, phospholipids, and protein levels in plasma. Also, correlation was observed between hepatic lipase activity and HDL_3 lipids. Intravenous injection of hepatic lipase antibody in rats caused a change in the distribution of the HDL subfraction as reported by Jansen and co-workers.[73,74] The chemical composition of the HDL_2 isolated after antibody injection was enriched in phospholipids and relatively poor in protein and cholesteryl ester as compared to control rat HDL_2.[73,74] These investigators also showed that hepatic lipase is capable of HDL_2-phospholipid hydrolysis in vitro, both in control and phospholipid-rich HDL_2.[74] After hydrolysis of the HDL_2 phospholipids, the resulting particle was not converted to HDL_3 but to a product with the characteristics of a cholesteryl ester-rich HDL_2 (Table 2). The notion that HDL_2 and not HDL_3 is affected by the phospholipase action of hepatic lipase[72-74] and the fact that HDL_3 and not HDL_2 is the major acceptor for the excess surface components released during LpL catalyzed VLDL-triglyceride hydrolysis indicate a close interplay between the two heparin-releasable lipases and the metabolism of the HDL subclasses. Clearly, many additional studies both in vitro and in vivo are necessary in order to establish the functional roles of these lipases in HDL metabolism. Conclusive information is likely to be obtained only after properties and mechanisms of action of these enzymes become known. At present we can only conclude that HDL_3 can be viewed as a regulator of the degradation of triglyceride-rich particles. In this process, HDL_3, by acquiring an additional complement of lipids, is transformed into an "HDL_2-like" particle sensitive to the phospholipase action of hepatic lipase (but not to LCAT as seen in the previous section) which may render these particles more suitable to cellular uptake and degradation (see Section II.B). Thus, it is apparent that HDL is closely related to the lipase, and LCAT. The substrate and acceptor roles of HDL in these enzymatic events are depicted in Table 2.

B. Interactions of HDL with Cells

The recognition of the LDL-receptor pathway in the membranes of cells capable of LDL uptake and degradation has stimulated a search for the mode of cell interaction by other serum lipoproteins. In regard to the HDL class, no specific membrane receptors have yet been recognized; however, there is now evidence that HDL can regulate cellular cholesterol metabolism and that the type of effect is dependent upon the nature of the interacting cells. We will examine two aspects of the HDL-cell interactions (1) those leading to entry of cholesterol into cells and (2) those leading to egress of cellular cholesterol.

1. HDL-Mediated Entry of Cholesterol into Cells

Two types of interactions have been recognized. These include HDL with steroidogenic and liver cells.

a. Interactions Between HDL and Steroidogenic Cells

These interactions are of particular interest because of the capacity of these cells to use lipoprotein cholesterol for the synthesis of steroid hormones. Lipoprotein interactions with steroidogenic cells have been investigated in tissues and cells derived from different animal species including man,[76,77] cow,[78] mouse,[79] and rat.[80-82] Of these cellular systems, rat and mouse steroidogenic tissues are highly sensitive to circulating levels of plasma HDL[79-81,83] and have received comparatively more attention than other animal species in relation to HDL recognition and sterol synthesis regulation by the steroidogenic cells. Representative examples are cells from adrenal glands, testis, and ovary.

i. Adrenal Glands

The early reports on mouse adrenal tumor cells (y-1 clone)[84] and cells from adult bovine adrenal glands[78] have shown that these cells take up LDL cholesterol via the LDL-receptor pathway, namely by adsorptive endocytosis and hydrolysis of the protein and cholesteryl esters of the lipoproteins in the lysosomes. The cholesterol so released serves either as a substrate for steroid synthesis or, if in excess, is re-esterified and stored as cholesteryl esters.[82] It has also been observed that the LDL-receptor activity is enhanced by the adrenocorticotropic hormone (ACTH).[82,85] More recent studies have provided evidence that rat and mouse adrenal glands may also contain specific sites for HDL uptake and that these are under the control of the pituitary hormone ACTH.[79,86] These observations have been made possible in vivo by the use of intact animal models where the secretion of plasma lipoprotein from the liver was blocked by 4-amino pyrazolo pyrimidine (4APP), a purine analogue.[87] Under these experimental conditions, the plasma cholesterol of these animals falls dramatically, inducing the adrenal gland to become depleted of cholesteryl esters and to increase cholesterol synthesis through an increase of the HMGCoA reductase activity.[83] In the case of the intact mouse[79] and rat,[80–83] the effects of 4APP on the adrenal glands were found to be reversed by the infusion of lipoproteins. Since both LDL and HDL were effective in this regard,[79,83] the data were taken to indicate that the mouse and rat adrenal glands have two independent specific uptake systems, one for LDL and the other for HDL. Kovanen et al.[79] have shown that in the mouse adrenal gland, both uptake mechanisms were saturable. In each case the uptake of the radiolabeled lipoprotein exhibited a preferential competition towards its homologous lipoprotein (i.e., ^{125}I-LDL vs. LDL; ^{125}I-HDL vs. HDL). Further proof of a specific mechanism of HDL adrenal uptake came from the recent report of Kita et al.,[88] who, in 4APP-ACTH-treated mice, showed that the intravenous injection of rabbit antibodies directed against the LDL receptor, purified from bovine adrenal cortex, blocked the in vivo adrenal uptake of ^{125}I-LDL while the ^{125}I-HDL uptake was not affected. The lipoprotein uptake was under ACTH control (i.e., it was suppressed by dexamethasone which inhibits the secretion of ACTH) and caused cholesteryl ester content in the adrenal gland to rise while a suppression of the activity of microsomal HMGCoA reductase took place.[79] It should be noted that HDL uptake and the presence in the adrenal gland of a specific receptor for HDL was only noted in the in vivo studies. Kovanen et al.,[79,89] who carried out both in vivo and in vitro experiments, suggested several possible mechanisms to account for these apparent discrepancies. One of these possibilities is that the adrenal gland may express in vivo an HDL receptor which is not present in the isolated adrenal cells or tissue culture. A second possibility is that, in vivo, this putative HDL receptor is not needed, in that neighboring cells (i.e., endothelial or sinusoidal lining cells) may be able to supply cholesterol to the adrenal gland. An alternative possibility, perhaps more realistic than the others, is that upon intravenous infusion HDL is modified into an active form recognized by the adrenal receptor. Also left unresolved was the issue of whether the process of HDL uptake involved internalization of the intact lipoprotein or just its binding to surface structures. Such an issue was addressed more directly by Gwynne and Hess[90] in rat adrenal cells grown in vitro. Uptake of HDL was demonstrated in primary cell cultures either previously stimulated by ACTH or in freshly isolated cells obtained from rats in which their cholesterol stores were depleted by the injection of 4APP. The presence of HDL in the medium stimulated steroid production and an increase in cell cholesterol content, without stimulation of cholesterol synthesis. Both the increase in cellular cholesterol content and steroid production were saturable with respect to the HDL particles present in the medium; moreover, steroid production was inhibited by the presence of protein

inhibitors such as aminoglutethimide or cycloheximide. These results were in agreement with previous work by Gwynne et al.[86] in adrenal glands derived from hypophysectomized rats. The results are supportive of the presence in the rat adrenocortical cells of a specific saturable, ACTH-dependent mechanism for uptake of HDL cholesterol. Gwynne and Hess[90] also showed that in ACTH-treated adrenocortical cells derived from 4APP-treated rats, the amount of fluorogenic steroids produced exceeded at least four times the amount of iodinated apoprotein degraded from ^{125}I-HDL. That is, the uptake of HDL cholesterol exceeded, by at least fourfold, the amount of cholesterol associated with the HDL apoprotein degraded by the cell, suggesting that utilization of HDL cholesterol does not require endocytosis and lysosomal degradation of the entire HDL particle. Even at low HDL concentrations the rate of cholesterol uptake was found to be far in excess to the amount which was converted into steroid hormones, indicating that the newly accumulated cholesterol is partitioned between at least two pools: a storage pool and a steroidogenic substrate pool.

How HDL uptake regulates the distribution of the entering cholesterol into these two pools remains unclear. However, what appears to be established is that, in the absence of endogenous cholesterol stores, the entering cholesterol from HDL is responsible for the increase in steroid production and cellular cholesterol content and that ACTH participates in this regulation by stimulating HDL cholesterol[81,86,90] or apoprotein[79] uptake by the adrenal cells. Evidence for a high affinity binding site for rat HDL in rat adrenal glands has been obtained by Chen et al.[91] However, the specificity of such a "site" has not been documented and could as well represent binding of apo E-containing rat HDL to the LDL adrenal receptor. Gwynne and Hess[92] have reported binding and degradation of human ^{125}I-HDL by suspensions of intact rat adrenal cortical cells. The binding sites were not highly specific and LDL (in molar terms) was a good competitor for ^{125}I-HDL binding. This lack of high specificity towards other lipoprotein fractions is well documented for HDL binding to liver cells (Table 4), rat testis membranes,[91] virus-transformed human lung fibroblasts,[93] and human skin fibroblasts.[94]

ii. Testis

The studies of Anderson and Dietschy[81] indicated that 4APP treatment of rats did not deplete the testis of its cholesteryl ester stores, nor did it stimulate sterol synthesis. However, administration of chorionic gonadotropin to 4APP-treated animals increased the rate of synthesis without a decrease in cholesteryl ester content. The HDL infusions to 4APP-chorionic gonadotropin-treated animals resulted in a decrease of sterol synthesis and an increase in cholesteryl ester content of the testis and in plasma testosterone levels. LDL had only a limited effect on these parameters. These results suggest that cholesterol derived from HDL is responsible for steroid hormone production in this organ.

Recent studies have provided evidence for high affinity saturable binding sites for rat HDL in rat testis.[91] Freshly prepared rat testicular membranes were found to bind iodinated rat HDL with high affinity ($Kd_{37°C} = 32\ \mu g$ HDL protein/mℓ). The binding was found not to be affected by Ca^{+2}, EDTA, LDL, pronase, or trypsin and to be primarily located in the interstitial tissue containing Leydig cells. Administration of chorionic gonadotropin to rats resulted in testicular fragments that bound twice as much rat ^{125}I-HDL with no change in binding affinity, indicating that for the rat testis-rat HDL system, hormonal control is operational over the expressed number of lipoprotein binding sites. In this respect, LDL receptor expression is subjected to hormonal control.[85] The binding characteristics of HDL to the Leydig cell membrane fragments were similar to those reported for liver membrane fragments[95] (Table 3), liver cells (Table 4), and fibroblasts (Table 5), but distinct from those of the LDL receptor. No high affinity binding of ^{125}I-LDL could be detected in the testicular membranes,[91] indicating that

Table 3
BINDING CHARACTERISTICS OF ^{125}I-HDL TO CELLULAR MEMBRANE FRAGMENTS

System	Affinity	Divalent cation requirement	Sensitivity to proteases	Specificity	Ref.
Rat testis membranes	32 μg HDL protein/mℓ t°37°C	No	No	Competed by rat VLDL, but not by rat LDL	91
Rat ^{125}I-HDL	70 μg HDL protein/mℓ t°4°C (dissociation constant)				
Rat liver membranes	85 μg HDL protein/mℓ t°37°C				
Human HDL_3	75 μg HDL protein/mℓ t°0°C (half maximal saturation)	No	No		95

steroidogenesis in the rat testis is dependent upon the entry of HDL cholesterol. These studies suggest that HDL and LDL receptors are distinct, a knowledge which should facilitate future studies on these receptors.

iii. Ovary

Treatment of rats with 4APP resulted in a decrease in cholesteryl ester content in the ovary and stimulation of sterol synthesis.[80,81] HDL infusion in such animals restored the cholesteryl ester content of the ovary[81] to normal levels and reduced the sterol synthesis,[80,81] while human LDL infusions caused only a slight increase in cholesteryl ester content[81] and a modest suppression of the HMGCoA reductase activity.[80,81] HDL also increased the plasma progesterone levels.[81] Taken together, these results suggest that HDL cholesterol and not the newly synthesized sterol in the ovaries is the major substrate for the steroid hormone production in rat ovary. Rat HDL showed a higher efficiency than human HDL in the suppression of sterol synthesis,[81] and this observation may be attributable to a difference in apoprotein makeup of both lipoproteins.

b. Interactions of HDL with Liver Cells

The studies on the interaction of HDL with liver cells have been carried out in a number of systems, including the total animal, the perfused organ, freshly isolated parenchymal (hepatocytes) and nonparenchymal liver cells (mainly Kupffer cells), primary hepatocyte cultures, and hepatic membrane fragments. The rat has been the main source of liver tissue or cells, whereas the lipoproteins more commonly used have been derived from rat or human plasma.

Hepatocytes constitute about 60 to 70% of the liver cells, the remainder being nonparenchymal cells.[96] Separation of these two types of cells is achieved by collagenase perfusion of liver, followed by mild mechanical disruptive methods and differential centrifugation.[97-109] In some cases, purification of nonparenchymal cells by centrifugation is preceded by a pronase digestion,[98,99,102] which selectively destroys the hepatocytes. These methods insure cellular fractionations in which contamination of one cell type by the other does not exceed 5%. Because hepatocytes and nonparenchymal cells have different metabolic roles, these cells will be dealt with separately.

i. Hepatocytes

Binding of rat ^{125}I-HDL to freshly isolated rat hepatocytes has been reported by several investigators[97,104-106] to occur at 0°C[105] and 4°C.[97,104,106] Binding was assessed either after

Table 4
CHARACTERISTICS OF RAT ^{125}I-HDL BINDING TO FRESHLY ISOLATED RAT LIVER CELLS AND LIVER CELL MEMBRANES

System	Affinity	Number of high affinity binding sites	Divalent cation requirement	Pronase sensitivity	Concanavalin A sensitivity	Others	Ref.
Freshly isolated hepatocytes in suspension: rat ^{125}I-HDL t° 4°C	$1.219 \times 10^{-7} M$[a] (dissociation constant)	2.2×10^{6}/cell[a]	No	Yes (partial)	Yes (partial)	Low specificity towards other lipoproteins; binding not affected by neuraminidase treatment of HDL or hepatocytes and not affected by 1,2-cyclohexanedione treatment of HDL	106
Freshly isolated hepatocytes in suspension: rat ^{125}I-HDL; t° 37°C	$1.75 \times 10^{-7} M$[b] (dissociation constant)	2.4×10^{5}/cell[b]	ND[c]	ND[c]	ND[c]	Low specificity; competed by rat LDL and VLDL; bound HDL is trypsin releasable	97
Freshly isolated hepatocytes in suspension; rat [^{3}H]-cholesteryl ester-HDL; t° 37°C	ND[c]	ND[c]	ND[c]	ND[c]	Yes (partial)		108
Freshly isolated nonparenchymal cells; rat ^{125}I-HDL; t° 4°C	$4.54 \times 10^{-7} M$[a] (half maximal saturation)	5×10^{4}—34×10^{4}/cell[a]	ND[c]	Yes (partial)	ND[c]	Low specificity; competed by rat LDL and VLDL; binding not affected by acetylation of HDL	107
Freshly isolated nonparenchymal cells; rat [^{3}H]-cholesteryl ester HDL; t° 37°C	ND[c]	ND[c]	ND[c]	ND[c]	No		108
Liver cell membrane fraction; human ^{125}I-HDL$_3$; t° 0°C	$7.8 \times 10^{-7} M$[b] (half maximal saturation)	1300 ng HDL/mg of membrane protein	No	No	ND[c]	Binding not affected by 17-α ethinyl estradiol pretreatment of the animals $Kd_{37°C} = 8.8 \times 10^{-7} M$[b]	95

[a] Based on mol wt = 110,000 for HDL protein.[91,92]
[b] Based on mol wt = 96,000 for HDL protein.
[c] ND = not determined.

Table 5
CHARACTERISTICS OF HUMAN HDL BINDING TO FIBROBLASTS AS COMPARED TO LDL BINDING

Cell	HDL density range	Observation	Ref.
Human fibroblasts	1.09<d<1.21	^{125}I-HDL binding to fibroblast derived from individuals with homozygous familial hypercholesterolemia (HFH) is slightly greater than for normal cells; while under similar circumstances ^{125}I-LDL binding to HFH cells is reduced by 70%	127, 129
		Incubation of normal cells in the presence of sterols caused no change in ^{125}I-HDL binding in contrast with the drastic decrease in ^{125}I-LDL binding (49 to 80% decrease depending on the conditions)	129
		Preincubation of cells with pronase does not affect ^{125}I-HDL binding to the cells, as opposed to an 82% reduction in ^{125}I-LDL binding	129
		On a molar basis (0.1—0.2 nmol of lipoprotein/mℓ), surface binding of ^{125}I-HDL is slightly less than that of ^{125}I-LDL (approx. 1 nmol/g cell); however, rates of internalization and degradation of ^{125}I-HDL are very low compared to those of ^{125}I-LDL and are accounted for by bulk endocytosis and adsorptive endocytosis occurring randomly at the fibroblast surface; as opposed to LDL, HDL (approx. 2 nmol/mℓ) does not increase cellular cholesterol content or inhibit sterol biosynthesis	128
Virus transformed human lung fibroblasts	1.063<d<1.21	Pronase pretreatment of cells does not affect ^{125}I-HDL binding but decreases ^{125}I-LDL binding by 50%	93
		Incubation of doubly labeled LDL (^{3}H-cholesterol/^{125}I-apoprotein) with cells, up to 1 hr, results in LDL internalization "in toto"; the cholesterol/apoprotein ratio of internalized material increases to a maximum of 2 with longer incubations; in contrast, HDL cholesterol is internalized 2- to 10-fold more effectively than HDL apoproteins as the incubation time elapses.	93
		Chloroquine stimulates ^{125}I-LDL (but not ^{125}I-HDL) cellular uptake	93
Mouse transformed fibroblasts	1.063<d<1.21	Transfer of HDL cholesterol to cells is not accompanied by significant hydrolysis of the HDL apoproteins	93

three successive washings of the cells in isotonic buffers[97,105] or after separation of the cells from the incubation media by pelleting them through an oily phase.[104,106] The binding was found to be maximal after about 1 hr of incubation[104,106] and to be reversible based on the release of radioactivity either upon transfer of the cells to a lipoprotein-free medium[105] or by addition of unlabeled rat HDL to the medium.[106] Two types of binding sites, one with higher affinity and saturable (see Table 4) and the other with lower affinity and nonsaturable,[106] were observed. However, the high affinity site was limited in its specificity due to the fact that human LDL and HDL and rat VLDL and LDL[104] were partial competitors for rat ^{125}I-HDL.[106] In the absence of detailed information on the apoprotein composition of these lipoproteins, the results of the competition experiments cannot be readily interpreted since the presence of apo E was not ruled out.[110,111] However, Ose et al.[106] showed that rat HDL treated with 1,2 cyclohexanedione, which is known to prevent apo E-containing lipoproteins from binding to peripheral cells,[112] competed as effectively as unmodified HDL for the ^{125}I-HDL binding and that this binding did not require divalent cations. These results, although not defining the characteristics of the HDL binding sites, do indicate however that these sites are different from the LDL receptor found in extrahepatic cells.[85,113] Kovanen et al.[95] followed a different approach to determine the binding of HDL to liver cells by employing rat liver membranes prepared from normal and 17α-ethinyl estradiol-treated rats. These rats are known to develop a profound hypolipidemia due to an increased hepatic catabolism of LDL and of apo E-containing particles[114] with no significant alteration of HDL_3 hepatic catabolism.[95,114] Though this model was primarily used to study the mechanism by which the liver recognizes LDL and apo E-containing lipoproteins, it did establish that the binding of human HDL_3 to rat liver membranes was the same in both normal and estradiol-treated animals. Under those experimental conditions, HDL_3 binding was specific (in terms of competition with unlabeled human HDL_3) and saturable with half-maximum binding attained at about 80 μg/mℓ of HDL_3 at both 0 and 37°C. Unlike LDL binding, HDL_3 binding was insensitive to pronase, Ca^{+2}, or EDTA. The characteristics of the human HDL_3 binding sites were grossly compatible with those described by Ose et al.[106] in the rat (see Table 4), although some differences were observed. Overall, the binding characteristics of HDL to the hepatic cells appear to be distinct from those of the LDL receptor in peripheral cells, a receptor which is dependent on Ca^{+2} and EDTA and exhibits a high affinity and specificity, and also sensitivity to proteases.[85,113] However, Kita et al.,[115] using a rabbit liver membrane system, and Bachorik et al.,[116] using a porcine liver membrane system, have reported the existence of a low specificity LDL binding site able to recognize HDL. This binding site was not affected by pronase or trypsin treatment[116] and was either dependent[116] or independent[115] of divalent cations. At 37°C, there was an increase in the amount of rat ^{125}I-HDL bound to the rat hepatocytes,[104-106] as compared to the binding at low temperature. Moreover, also at 37°C, the bound ^{125}I-HDL became progressively less prone to dissociation from the cells in the presence of excess unlabeled HDL in the medium.[103,106] After 10 min of incubation at 37°C essentially all of the ^{125}I-HDL was displaced by unlabeled HDL, whereas after 1 hr incubation (under conditions in which binding of HDL, at 4°C, to the cell surface was maximal) about 70% of the radioiodinated HDL was displaced.[103] The kinetics of binding of rat ^{125}I-HDL at 37°C exhibited a fast component (up to 1 hr of incubation), followed by a slower component.[102,104] The fast component was saturable with respect to the HDL concentration in the medium, suggesting the existence of a finite number of binding sites for this lipoprotein[102,105] and also that the internalization of the ^{125}I-HDL is a process that takes several minutes to occur. Both HDL apoproteins and cholesteryl ester were taken up by the hepatocytes.[102,108] The uptake of cholesteryl ester was time dependent and reached saturation, with respect to the HDL cholesterol concentration in

the medium, after a 30-min incubation.[108] More important however, this uptake was partially inhibited by the presence in the medium of Concanavalin A,[108] suggesting that it was mediated via the binding of rat HDL to the hepatocyte cell membrane (Table 4).[106] The mode of binding of HDL to liver cells is poorly understood, although it appears to involve absorptive endocytosis.[102,104,109] However, the mechanism differs from other endocytotic processes which involve participation of cellular microtubules and microfilaments, because neither colchicine (a depolymerizer of microtubules) nor cytochalasin B (a destabilizer of microfilaments) inhibits ^{125}I-HDL uptake.[117] The studies of Quarfordt et al.[118] suggest that the apoprotein composition of rat HDL may play a role in lipoprotein uptake. These authors measured the uptake of (4-^{14}C) cholesteryl ester-labeled rat HDL or rat ^{125}I-HDL by rat livers using a nonrecycling perfusion system and reported that, with apo A-I-enriched rat HDL, the recovery of (4-^{14}C) cholesteryl ester in the liver paralleled that of the ^{125}I apoproteins. On the other hand, with apo E-enriched rat HDL, the cholesteryl ester uptake was twofold higher than the apoprotein uptake. Moreover, a tenfold higher cholesteryl ester uptake was measured for apo E-enriched HDL than for apo A-I-enriched HDL. The internalization of rat ^{125}I-HDL by hepatocytes in vitro led to the accumulation of radioactivity in a membrane fraction enriched with the plasma membrane marker 5′ nucleotidase,[102,103,117] while in vivo, the injected ^{125}I-HDL accumulated in a membrane fraction enriched with the lysosomal marker acid phosphatase.[117] This discrepancy in subcellular distribution between in vitro and in vivo studies could be the result of the in vivo modification of the injected ^{125}I-HDL and/or a modification occurring in the hepatocytes upon purification. However, in both instances, the internalized iodinated HDL was partially degraded in the lysosomes regardless of the cell system used since leupeptin[102,117] and chloroquine,[97,102,117] which are both inhibitors of lysosomal hydrolases, reduced the degradation at 37°C of the apoproteins[97,102,117] and cholesteryl esters[102] of HDL. About 5 to 10% of the cell-associated ^{125}I-HDL was degraded per hour,[102,117] while, under similar conditions, about 6% of the ^{3}H-cholesteryl ester-labeled HDL was degraded.[102] Taken together, the data in the literature indicate that the rat liver cell is able to bind rat HDL particles by a high-affinity, low-specificity process and that the binding is followed by a slow uptake of the lipoprotein through adsorptive endocytosis. However, it is unclear whether the entire HDL particle is internalized into the liver cell or whether there is a preferential internalization of the cholesteryl esters. Nevertheless, the internalized components undergo lysosomal hydrolysis to a large extent.

In relation to cholesterol metabolism Breslow et al.,[100] in short-term monolayer cultures of primary rat hepatocytes, and Edwards,[101] in freshly isolated rat hepatocytes, observed that the HMGCoA reductase activity after 3 hr[101] or 48 hr[100] of incubation was stimulated by both human or rat HDL, whereas under comparable medium conditions human LDL inhibited the enzyme.[101] Further studies by Jakoi and Quarfordt[119] showed that a 4-hr infusion of human HDL into rats led to a stimulation of both hepatic HMGCoA reductase activity and cholesterol synthesis, with a concomitant decrease in total hepatic microsomal cholesterol content. In addition, apo HDL was able to stimulate HMGCoA reductase activity, whereas the infusion of LDL, VLDL, and apo VLDL had the opposite effect.[119] As to the mechanism of stimulation of HMGCoA reductase activity, the authors[101,119] attributed this process to an HDL-mediated increased cholesterol efflux from the hepatic cells. However, the experimental evidence indicates that, overall, HDL cholesterol is taken up by the liver.[120,121] Rothblat et al.[122] showed that cholesterol-enriched human HDL induces the accumulation of sterol in a rat hepatoma cell line under conditions in which the apoprotein moiety of HDL was poorly internalized and degraded. These data indicate that the lipid composition of HDL is able to regulate free cellular cholesterol uptake and, in turn, regulate cellular cholestero-

genesis: as postulated by Jakoi and Quarfordt[119] an increased uptake of free cholesterol by HDL at the peripheral sites would decrease the cholesterogenic potential in the liver, whereas an enhanced removal of HDL cholesterol by the LCAT reaction would enhance the ability of this lipoprotein to stimulate hepatic cholesterogenesis. Since it is known that cholesterol serves as a precursor for the synthesis of bile acids, a link between this synthesis and HDL uptake may be postulated. In fact, Schwartz et al.[120,121] have shown that injection of human HDL or LDL labeled with ^{3}H- or ^{14}C-cholesterol into a patient with a bile fistula resulted in preferential recovery of the HDL-free cholesterol in the bile. Thus, the binding of HDL to liver cells may be viewed as a regulator of the rate of cholesterol incorporation into bile products and cholesterol homeostasis in general.

ii. Nonparenchymal Cells

The participation of the nonparenchymal cells of the liver in HDL uptake has been shown in several reports. Freshly isolated nonparenchymal cells are able to bind rat ^{125}I-HDL[104,105,107] at 0°C[105] and 4°C.[104,107] In these studies binding was measured after separation of the cells from the incubation medium by pelleting through an oily phase[104,107] or after successive washes with isotonic buffers.[105] The binding reached a maximum after 30 min[104] to 1 hr of incubation,[107] was reversible in that excess unlabeled HDL displaced a significant amount of radioactivity from the cells,[107] and was partially abolished by pretreatment of the cells with pronase.[107] Wandel et al.[107] also showed that HDL binding was the result of a high affinity (saturation at about 100 $\mu g/m\ell$ of the ligand) and a low affinity process; the number of "high affinity" sites varied from experiment to experiment, between 5×10^4 to 34×10^4 sites per cell, considering a molecular weight of 110,000 for the HDL protein[107] and the binding was rather aspecific, since both rat VLDL and LDL were able to partially compete with unlabeled HDL for the binding of ^{125}I-HDL. Since denatured albumin did not compete with unlabeled rat HDL for binding, and "screening" of ^{125}I-HDL through a previous exposure to nonparenchymal cells did not affect the binding results,[107] the authors conclude that binding was not due to the presence of denatured proteins, known to be taken up by nonparenchymal cells.[123] At 37°C, there was an increase in the amount of labeled lipoprotein bound to the nonparenchymal cells as compared to the binding at 4°C[104,107] or 0°C.[105] With longer times of incubation at 37°C less labeled material was displaced by the addition of a 50-fold excess of unlabeled rat HDL.[107] After 15 min and 2 hr of incubation at 37°C, the addition of HDL caused the displacement of 60 and 20% of the cell-bound radioactivity, respectively. Both the apoproteins and cholesteryl esters of the HDL particles were taken up by the nonparenchymal cells. The uptake of the ^{3}H-cholesteryl ester was time dependent and differed from the uptake by the hepatocytes in that it was not saturable with respect to the HDL concentration in the medium and was not affected by Concanavalin A.[108] Moreover, it was noted that the acyl-CoA cholesterol acyl transferase (ACAT) activity was only present in the hepatocytes.[108] The mechanism of uptake of ^{125}I-HDL appears to involve adsorptive endocytosis;[104,107,109] however, in view of the difference in the kinetics of uptake between the apoproteins[107] and cholesteryl esters[108] it is likely that both of these components of HDL are not internalized at the same time. Regardless of the rates of uptake, both apoproteins[104,107] and cholesteryl esters[108] were hydrolyzed at least partially in the lysosomes since chloroquine[107] decreased the extent of hydrolysis. As is the case with hepatocytes, the enzymatic degradation was slow; about 10% of the cell associated ^{125}I-HDL was degraded per hour.[107]

Taken together the studies indicate that nonparenchymal cells bind, internalize, and degrade HDL by a low specificity process and by a still undefined mechanism of internalization. It is also apparent that this binding may be independent from that observed with denatured proteins such as formaldehyde and heat-treated albumin, since

these proteins do not affect the binding of ^{125}I-HDL. In keeping with this notion, heat denaturation or acetoacetylation of HDL resulted in products which exhibited two- to threefold increase in uptake by the cells[107] with no change in the binding mechanism, since the acetylated HDL was a good competitor for the ^{125}I-HDL binding. These modified HDL exhibited a higher rate of hydrolysis which Wandel et al.[107] found to be compatible with the observations of Goldstein and Brown[124] and Mahley et al.[125] in macrophages.

Nonparenchymal cells appear to be proportionally more efficient in their uptake of HDL than the hepatocytes.[107] The relative rates of uptake of HDL at 37° C between these two cell types indicate that the nonparenchymal cell is about five times more efficient than the hepatocyte in HDL uptake.[107] In terms of cellular protein content Van Berkel et al.[98,99,105] reported that nonparenchymal cells bind four to five times more HDL than hepatocytes[105] and that nonparenchymal cell homogenates are ten times more efficient in HDL hydrolysis than homogenates derived from hepatocytes.[98,99] Moreover, Wandel et al.[107] observed a sixfold higher HDL uptake by the nonparenchymal cells and Ose et al.[102] showed that the in vivo uptake of ^{125}I-HDL by the nonparenchymal cells is 15 times higher than that of hepatocytes. These figures indicate that about 40% of the hepatic catabolism of HDL is due to nonparenchymal cells; this implies that within the liver, there is an important mechanism of HDL degradation which may not be involved in the process of bile acid formation. Other cells involved in HDL degradation are the endothelial cells[126] and cells from the spleen, lungs, and kidney.[36,102]

C. HDL and Cellular Cholesterol Egress

Several reports have provided evidence that HDL binds no cultured fibroblasts[93,94,127-129] and other nonendocrine peripheral cells, including porcine[130] and rat[131,132] smooth muscle cells, as well as endothelial cells.[126] Table 5 summarizes the characteristics of HDL binding to fibroblasts as compared to those of LDL.[93,94,127-129] Commonly, the HDL particles were obtained in the density range between 1.090 to 1.21 g/mℓ and their binding to the cells was shown to be independent of apo B. However, not all of the studies made in these cellular systems have ruled out the presence of apo E in the HDL fractions tested, and the possibility remains that this apoprotein was responsible for the observation that at high molar ratios HDL competed with LDL binding.[93,94] In this respect, Drevon et al.[133] have reported that human HDL stimulates HMGCoA reductase activity and inhibits ACAT activity in both human and rat fibroblasts independent of apo E, an apoprotein which is known to inhibit HMGCoA reductase activity in human fibroblasts;[134] moreover, no differences were observed in HDL binding between normal cells or cells derived from individuals homozygous for familial hypercholesterolemia (HFH) (Table 5). Similar behavior of normal and HFH human fibroblasts towards HDL binding has been reported by Emeis et al.[135] In the same vein, work in this laboratory has shown that canine HDL, which contains essentially only apo A-I, competes with human HDL_3 for binding to lymphoblastoid cells.

A major function attributed to HDL is to regulate cholesterol efflux from cells in culture; namely aortic rat muscle cells,[136,137] mouse peritoneal macrophages,[138,139] human fibroblasts,[140] normal and HFH lymphoblasts,[182] L-mouse fibroblasts,[141] and ascite cells.[137,142,143] To measure cholesterol efflux, the cells were prelabeled by several different procedures, including loading with radioactive cholesterol[126,137,140,142-144] or a cholesterol precursor,[141,145,146] loading with radiolabeled cholesteryl esters by exposing the cells to LDL in the presence of chloroquine[126,136,144] or acetylated LDL,[138,139] and loading with both free and esterified cholesterol by preincubation in the presence of LDL.[147] Measurement of net efflux was based on the quantitative assessment of cholesterol and cholesteryl ester in the medium, cell, or both.[126,136,138,139,141,144,145,147] Irrespective of the

system under study, HDL stimulated the removal of cholesterol from the cells. However, cholesterol efflux was also promoted by lipoprotein-deficient serum,[136,145] abetalipoproteinemic serum,[145] lipid-free serum,[145,146] cholesterol-depleted HDL,[147] and by all fractions that contained HDL apoproteins.[136,137] However, the HDL apoproteins were not as effective as HDL in cholesterol removal, but if combined with lecithin[137,142,143] and/or sphingomyelin[137,142] their capacity of cholesterol removal was enhanced, the total capacity far exceeding that of lecithin and/or sphingomyelin dispersions alone. Apo A-I was particularly influenced by the addition of lecithin and sphingomyelin.[137] The release of cholesterol from cultured cells was found to occur predominantly as free cholesterol;[147-149] subsequent studies by Ho et al.[138] using cholesteryl ester-loaded macrophages not only indicated that cholesterol efflux requires the presence of an acceptor in the medium, but also that such an acceptor is essential for the net hydrolysis of cytoplasmatic cholesteryl esters to occur. Thus, the excreted cellular sterol paralleled the amount of cellular cholesteryl ester hydrolyzed.[138] Brown et al.[139] used a double-labeling technique in which macrophages loaded with cholesteryl (^{3}H)-oleate were transferred to a fresh medium containing (^{14}C)-oleate in the presence or absence of HDL. The presence of HDL in the medium had no effect on the hydrolysis of cholesteryl esters (measured as decline of cholesteryl (^{3}H)-oleate cellular content), but inhibited the re-esterification of cellular cholesterol (determined as an increase of cholesteryl (^{14}C)-oleate cellular content). Cell-free extracts from systems containing HDL exhibited a reduction in ACAT activity, a phenomenon which was observed in cholesteryl ester-laden rat and human fibroblasts[133] and also would account for the predominant decrease in cholesteryl ester content observed in human skin fibroblast and rat aortic smooth muscle cells[132] upon incubation with an apo HDL-sphingomyelin mixture. In this same context, Daniels et al.[147] have suggested that the rate-limiting step for cholesterol efflux in cholesterol and cholesteryl ester-laden fibroblasts could be the net hydrolysis of cellular cholesteryl esters when cholesterol-depleted HDL is used as the acceptor. Thus, in peripheral cells loaded with cholesterol, HDL causes the net hydrolysis of cholesteryl esters, removal of free cholesterol from the cell, and suppression of ACAT activity. The mechanism of this HDL effect is unknown. A participation by apo A-I is suggested[138] by the observation that lipoprotein-depleted or deficient serum is effective in cholesterol removal from cholesteryl ester-loaded macrophages, while albumin and globulins were not effective cholesterol acceptors. The involvement of Apo A-I in the stimulation of cellular cholesterol efflux has also been observed in rat smooth muscle cells,[137] ascites cells,[137,143] and lymphoblastoid cell lines.[182]

Glomset[150] has proposed that esterification of HDL cholesterol by LCAT might facilitate the uptake of additional cholesterol molecules by the lipoproteins and has shown that LCAT promotes transfer of cholesterol from the erythrocyte ghosts to HDL. However, at present, there is no firm evidence that LCAT facilitates cholesterol efflux from cultured cells. In fact, heat[126,141,144,147] or *N*-ethyl maleimide inactivation[126,138] of LCAT failed to reduce the ability of serum[138,141] or the d >1.21 g/mℓ fraction of serum[126,144,147] to promote cellular cholesterol efflux even though HDL binding to peripheral cells, namely porcine[130] and rat[131,132] smooth muscle cells, skin fibroblasts,[128] endothelial cells,[126] and normal and HFH lymphoblast,[182] has been documented to occur with a limited internalization and degradation of the apoprotein component. Taken together, these observations suggest that HDL (or HDL subsets), by interacting with the peripheral cells, "load" their surface with free cholesterol molecules which are then esterified by the action of LCAT for subsequent delivery to the liver[120,121] and steroidogenic tissues.[80,81] In turn, the reduction of cellular cholesterol secondary to an increased cholesterol efflux could stimulate the activity of HMGCoA reductase[145,146] and the synthesis of LDL receptors.[151]

The idea that HDL binding is the initial signal which leads to the increase in HMGCoA reductase activity and sterol synthesis has not yet been proven, although it remains as a possibility. Interestingly, we have observed that either canine HDL, which contains essentially only apo A-I, or human lipid-free apo A-I are able to stimulate HMGCoA reductase activity. From these observations one is tempted to conclude that apo A-I is the modulator of the cellular effects by HDL. However, apo A-II has also been reported to promote cholesterol efflux.[137,143] Thus, this process is either of low specificity or apo A-I and apo A-II may have common functional segments.

D. Modification of HDL After Exposure to Cells

Work reported from our laboratory has indicated that structural changes occur when HDL_3 is exposed to leukocytes[152] and fibroblasts.[153] The reisolated HDL_3 after exposure to cells retained its hydrodynamic and spectral properties of mature HDL_3; however, intact apo A-II was absent and replaced by hydrolytic fragments.[152,153] The particles exhibited an increased content in lysophosphatidyl choline[152] and glycolipids due to the transfer of newly synthesized glycolipid as a response of the cells to exogenous HDL_3.[153] The changes in the HDL_3 particles attending their exposure to cells were prevented by the addition of lipoprotein-depleted serum (LPDS) to the medium. Several lines of evidence[152] indicate that the proteolysis of apo A-II occurred at the cell-lipoprotein interface: (1) lysosomes did not participate in the hydrolysis since chloroquine, which is known to inhibit the lysosomal function, did not inhibit the apoprotein hydrolysis, (2) the apo A-II fragments produced during proteolysis remained attached to the lipoprotein particle, (3) there was minimal binding and internalization of HDL_3 under the experimental conditions used, and (4) the medium pre-exposed to cells did not promote apo A-II hydrolysis. The increase in lysophosphatides noted in HDL_3 exposed to cells[152] may also reflect an interaction occurring at the cell-lipoprotein interface, since in human granulocytes, phospholipases have been shown to be membrane-bound.[154] Of interest to both leukocytes and fibroblasts is that HDL_3 promoted the synthesis of cellular glycolipid and also served as an acceptor of the glycolipids transferred from the cell.[153] Little is known regarding the mechanisms leading to the structural modifications of HDL_3 after exposure to cells. Although these changes have been noted in vitro and in the absence of LPDS, they may have metabolic relevance in vivo. We may speculate that particularly the formation of lysophospholipids may be associated with the early events in HDL-cell interactions and serve as an initiator of the metabolic processes following these interactions. More work in this direction is, obviously, needed.

E. Miscellaneous Effects

Although much attention has been placed on the role of HDL in lipid metabolism, this lipoprotein class has now been found to function in a number of other biological systems. Representative examples are given in Table 6. Ulevitch et al.[155] have recently demonstrated that the bacterial lipopolysaccharide (LPS) from salmonella minnesota R595, when injected intravenously into the rabbit, forms a complex which can be isolated from the other plasma constituents by affinity chromatography using a column containing Sepharose-4B-anti-LPS antibodies. The major protein component of the potassium thiocyanate eluate was identical with rabbit apo A-I in electrophoretic migration behavior and amino acid composition. The buoyant density of the complex was similar to rabbit HDL but differed from this lipoprotein in cholesteryl ester:cholesterol and phosphatidylcholine:phosphatidylethanolamine ratios. In spite of this complexation with HDL, LPS retained its property of producing shock, disseminated intravascular coagulation, and even death of the rabbit.[156,157] Thus, the functional groups, determinant of the toxic action of LPS, were not neutralized by HDL.

Table 6
MISCELLANEOUS ROLES OF HDL

Observation	Comment	Ref.
Bacterial lipopolysaccharide (LPS) from salmonella minnesota R595 binds to rabbit HDL	Interaction of LPS with HDL results in loss of some endotoxic activity	155—157
Human HDL has cytotoxic activity against *T. brucei*	Trypanocidal activity is the result of HDL interaction with the plasma membrane of the parasite	158
Human HDL inhibits LDL-induced cytotoxicity in smooth muscle cells and endothelial cells	Inhibition of LDL-induced cytotoxicity by HDL at 100 μg LDL cholesterol/mℓ of medium; no inhibition by HDL at a 2.5-fold higher concentration of LDL in the medium	165, 166
Human HDL stimulates prostaglandin I_2 synthetase in pig aorta microsomes	Platelet hyperfunction in patients with ischemic heart disease may be explained by the regulation of PGI_2 concentration; LDL inhibits the production of this platelet antiaggregating agent	167
HDL inhibits the in vitro binding of LDL-lipids to arterial elastin	HDL may play a role, here, as an anti-atherogenic agent	169
Serum amyloid protein (SAA) associates with HDL	A correlation exists between high levels of serum glucose and SAA and decreased levels of HDL	159—164

An interesting functional role of HDL has been reported by Rifkin[158] who found that HDL is the serum factor responsible for the lysis of *Trypanosoma brucei.* The cytotoxic action of HDL toward this parasite may explain why human subjects are not infested by the *T. brucei* subgroup. In contrast, Trypanosomes *rhodesience* and *gambience,* which cause African sleeping sickness in man, are resistant to the action of normal human HDL or serum. The lysis of *T. brucei* by human HDL may be the result of alterations in lipid composition of the trypanosomal plasma membrane induced by interactions with the lipoprotein.[158] An intriguing question which remains unexplained is why *T. brucei* is resistant to rabbit or rat HDL. This suggests interesting structural differences between these lipoproteins which require further investigation. An additional role of HDL has been recognized through the work of Benditt and Eriksen,[159] who showed that serum amyloid protein AA (SAA) is associated with HDL in plasma. The proteins designated SAA_1 and SAA_2 are homologous to the 9000 molecular weight AA[160] found in tissues of patients having a variety of ailments including tuberculosis and rheumatoid arthritis. The recent studies on the characterization of SSA_1 and SAA_2[160] indicate that in some human subjects HDL contains potential precursors of tissue amyloid AA. The significance of the finding cannot be appreciated at this time since a functional correlation of AA in plasma and tissues is not known. However, a recent report by Hoffman et al.[161] indicates that mouse HDL containing SAA is cleared from the circulation at a higher rate than control HDL. Of related interest is the observation that intravenous glucose infusions apparently increase the levels of SAA in HDL.[162,163] Also, the plasma HDL levels in patients with diabetes mellitus and nephrotic syndrome are low and, moreover, proteins with similar properties as SAA are present in their HDL.[164]

Taken together, the data suggest that serum glucose levels may effect the metabolism of circulating HDL constituents by increasing the production of AA which appears to have an affinity for this lipoprotein in vivo. Further studies on the physicochemical properties and function of the AA-HDL complex appear necessary.

In addition to the direct roles outlined above, HDL may have an indirect functional role by competing with LDL.[165,169] Hessler et al.[165] reported that HDL inhibits the LDL-

induced cytotoxicity in human vascular smooth muscle cells and umbilical vein endothelial cells in culture. Henricksen et al.[166] confirmed these results with studies using umbilical cord endothelial cells. No cytotoxic effects by HDL were noted at any concentration tested. In another study, Beitz and Forster[167] reported opposite effects between HDL and LDL on the in vitro prostaglandin I_2 synthetase activity of pig aorta microsomes. A 21% stimulation of prostaglandin I_2 synthesis was found for HDL (1 mg cholesterol per milliliter), while LDL (2 mg cholesterol per milliliter) inhibited the enzyme activity by 64%. Since prostaglandin I_2 inhibits platelet aggregation, a relatively high plasma HDL:LDL ratio may be antithrombotic with regard to platelet function.

Studies have shown that LDL associates with arterial wall connective tissue constituents.[170-173] The consequence of this interaction is the accumulation of cholesterol and other lipids in the arterial wall and, thus, initiation of atherosclerotic lesions.[170,171,174] Recently, Noma et al.[169] investigated the mechanism of lipoprotein binding to arterial elastin in vitro. When a constant amount of LDL was incubated with elastin, in the presence of increasing concentrations of HDL, the binding of lipids to elastin progressively decreased. From these studies, indicating an opposing role of HDL against LDL, it can be speculated that other biological events may be regulated by HDL:LDL molar ratios. For instance, the bioregulation of lymphocyte function by LDL subsets[175-177] has been inferred from studies of inhibition of T-lymphocyte rosette formation with erythrocytes[178,179] and suppression of lymphocyte stimulation by mitogens and allogeneic cells[180,181] The mechanism of LDL action in these lymphocyte functions remains unclear, but it is anticipated that HDL may also play a role in these and other cell-surface interactions.

III. CONCLUSIONS

When viewed in the overall context of lipoprotein metabolism (Figure 1), several important functional roles for HDL and its HDL subsets emerge. Of particular importance among them are those relating to cholesteryl ester synthesis and transport; modulation of steroidogenesis in adrenal glands, testis, and ovary; likely participation in the process of bile acid formation; and involvement in cellular cholesterol egress. This plurifunctional role is afforded by the structural heterogeneity and flexibility of this lipoprotein class to an important extent due to its apoprotein composition. The ratio of the two main apoproteins, apo A-I and apo A-II, varies among subclasses by processes which are still poorly understood but likely dependent on their rates of synthesis and degradation and on their relative affinity for the HDL surface. It is reasonable to postulate that the proportion of these two apoproteins is an important determinant in defining lipoprotein size, curvature, and surface structure, parameters which must be key factors in HDL function. If we think of HDL as the prime lipoprotein in reverse cholesterol transport, i.e., movement of cholesterol from the peripheral site to the liver, it is not surprising that the various HDL subsets are directly or indirectly related to the action of three of the recognized plasma lipid-modifying enzymes: lipoprotein lipase, hepatic lipase, and LCAT, as well as involved in the action of the cholesteryl ester/triglyceride exchange/transfer proteins. The HDL subclasses appear to be eminently suited for insuring a continuous flow of cholesterol from the peripheral cells to tissues either producing steroid hormone or bile or destined for storage. In all of these processes a very fundamental role appears to be played by apo A-I. This apoprotein is the cofactor of LCAT. Any abnormality affecting the LCAT-apo A-I system will be reflected in alterations in HDL structure and, in consequence, of all HDL-dependent functions. We have also evidence that apo A-I binds to cells, and although an apo A-I "receptor" has not been clearly documented, it is tempting to speculate that this apoprotein is capable of

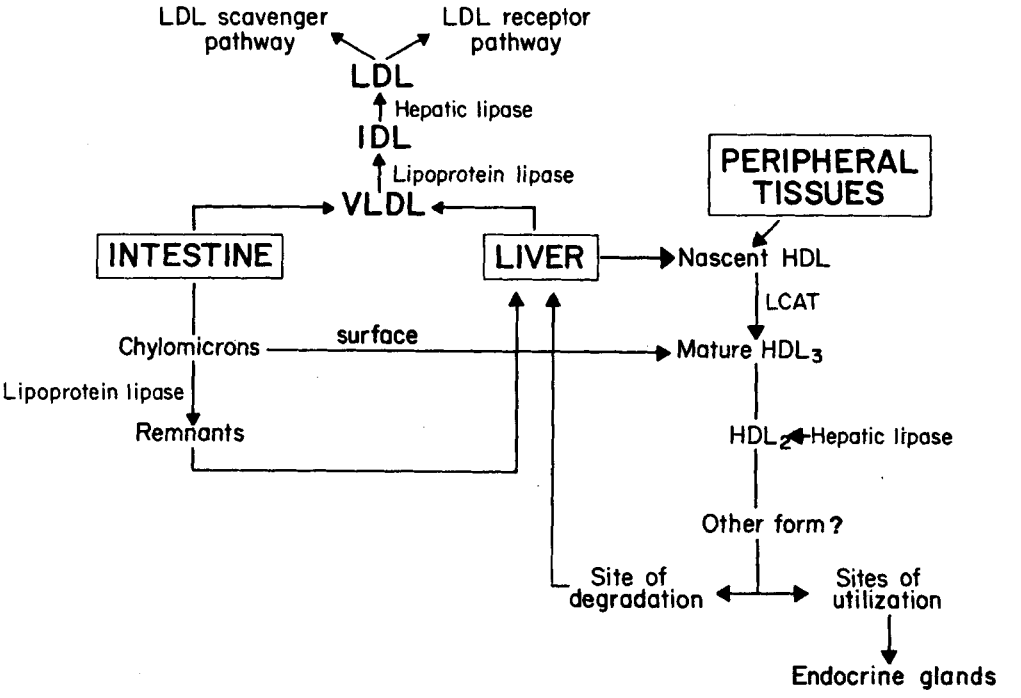


FIGURE 1. Scheme of lipoprotein metabolism. The scheme illustrates that all lipoproteins once secreted into circulation undergo structural modifications through the action of lipid-modifying enzymes in concert with physicochemical processes. The end products are then taken up by cells through either specific or aspecific pathways. The cholesterol entering the cells can regulate endogenous production of sterol and in given cells serves as a precursor of steroid hormones (adrenal glands) or bile acids (liver).

modulating cholesterol homeostasis in the cell independently from the LDL-receptor pathway. According to current views, the structural maturation of HDL, that is, the stage when cholesteryl ester-rich spherical structures of defined size, density, and composition are present in plasma, is only reached upon secretion into the circulation. Before this time, the nascent particles are thought to be rather specific discoidal structures, although with the necessary chemical makeup to serve as a substrate for LCAT. Thus, mature HDL is the consequence of an enzymatic process which requires the main apoprotein constituent of this apoprotein. Once a mature HDL_3-like structure is formed, its surface is now capable of accepting new polar components from the hydrolysis of TG-rich particles while its core is active delivering cholesteryl esters to the other lipoproteins and, in concert, LCAT is making new cholesteryl ester molecules from the free cholesterol leaving the cells.

The overall picture of the various HDL functions which is now emerging is likely to be incomplete, and those which are now recognized still require definition in molecular terms. Of the many questions still unanswered, these are just a few outstanding ones: (1) preference of LCAT for HDL_3 and not HDL_2, (2) preference of hepatic lipase for HDL_2 and not HDL_3, (3) preference of the cholesteryl ester/triglyceride exchange/transfer protein systems for HDL_3, (4) the molecular specificity of these reactions and their rate-limiting factors, and (5) the molecular basis for the interactions between HDL and cells. Although emphasis has been placed on apo A-I, we need also to gain a better understanding on the role played by apo A-II and the other apoproteins associated with HDL. Apo A-II presents a rather fascinating challenge in that this apoprotein is present in man as two identical chains linked together by a disulfide bridge. In turn it is a single

chain in new and old world monkeys and is absent in lower animal species. The function of this apoprotein is largely unknown, although it is apparent that its degree of surface occupancy, probably by competing with apo A-I, influences the capacity of HDL to interact with LCAT, to accept the surface components originating from the enzymatic hydrolysis of the triglyceride-rich particles, and to probably participate in HDL-cell interactions. In the latter regard, the studies thus far conducted in vitro indicate that this apoprotein is exquisitely sensitive to the action of the proteolytic enzymes present in cells grown in culture, although the physiological significance of this observation is still undetermined. It is evident that in order to gain a thorough understanding of the dynamic aspects of the structure-function relationships of the HDL subclasses there is a need of further studies. Those based on coordinated multidisciplinary approaches should prove to be particularly rewarding.

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REFERENCES

1. **Osborne, J. C., Jr. and Brewer, H. B., Jr.,** The plasma lipoproteins, *Adv. Prot. Chem.* 31, 253,1977.
2. **Scanu, A. M., Edelstein, C., and Keim, P.,** Serum lipoproteins, in *The Plasma Proteins,* Vol. I, 2nd ed., Putnam, F., Ed., Academic Press, New York, 1974, 317.
3. **Anderson, D. W., Nichols, A. V., Pan, S. S., and Lindgren, F. T.,** High density lipoprotein distribution: resolution and determination of three major components in a normal population sample, *Atherosclerosis* 29, 161, 1978.
4. **Patsch, R. J. and Gotto, A. M., Jr.,** Separation and analysis of HDL subclasses by zonal ultracentrifugation, in *Report of the High Density Lipoprotein Workshop,* Lippel, K., Ed., NIH Publ. 79-1661, National Institutes of Health, Bethesda, Md., 1979, 310.
5. **Nichols, A. V., Blanche, P. J., Krauss, R. M., and Gong, E. L.,** Gradient gel electrophoresis of HDL within the ultracentrifugal d 1.20 fraction from human serum, in *Report of the High Density Lipoprotein Workshop,* Lippel, Kenneth, Ed., NIH Publ. 79-1661, National Institutes of Health, Bethesda, Md., 1979, 303.
6. **Mahley, R. W., Weisgraber, K. H., Innerarity, T., Brewer, H. B., and Assman, G.,** Swine lipoproteins and atherosclerosis. Changes in the plasma lipoproteins and apoproteins induced by cholesterol feeding, *Biochemistry,* 14, 2817, 1975.
7. **Mahley, R. W.,** Alterations in plasma lipoproteins induced by cholesterol feeding in animals including man, in *Disturbances in Lipid and Lipoprotein Metabolism,* Dietchy, J. M., Gotto, A. M., and Ontko, J. A., Eds., Waverly Press, Baltimore, 1978, 181.
8. **Shipley, G. G., Atkinson, D., and Scanu, A. M.,** Small angle X-ray scattering of human serum high-density lipoproteins, *J. Supramolec. Structure,* 1, 98, 1972.
9. **Atkinson, D., Smith, H. M., Dickson, J., and Austin, J. P.,** Interaction of apoprotein from porcine high density lipoprotein with dimyristoyl lecithin. I. Structure of the complexes, *Eur. J. Biochem.,* 64, 541, 1976.
10. **Laggner, P., Muller, H., Kratky, O., Kostner, G., and Holasek, A.,** Studies on the structure of lipoprotein A of human high density lipoprotein HDL_3 — the spherically averaged electron density distribution, *FEBS Lett.,* 33, 77, 1973.
11. **Tardieu, A., Mateu, L., Sardet, C., Weiss, B., Luzzati, V., Aggerbeck, L., and Scanu, A. M.,** The structure of human serum lipoproteins in solution. II. A small-angle X-ray scattering study of HDL_3 and LDL, *J. Mol. Biol.* 101, 129, 1976.
12. **Ohtsuki, M., Edelstein, C., Sogard, M., and Scanu, A. M.,** Electron microscopy of negatively stained and freeze-etched human serum high density lipoprotein-3, *Proc. Natl. Acad. Sci. U.S.A.,* 74, 5001, 1977.
13. **Forte, T. and Nichols, A. V.,** Application of electron microscopy to the study of plasma lipoprotein structure, *Adv. Lip. Res.,* 10, 1, 1972.
14. **Forte, N. and Nordhausen, R.,** Structural analysis of plasma lipoproteins by novel electron microscopic techniques, *Circulation,* 54(Suppl. II, Abstr. 368), 94, 1976.

15. **Verdery, R. B., III and Nichols, A. P.,** Arrangement of lipid and protein in human serum high density lipoproteins: a proposed model, *Chem. Phys. Lipids,* 14, 123, 1975.
16. **Shen, B., Scanu, A. M., and Kezdy, F.,** The structure of human serum lipoproteins inferred from compositional analysis, *Proc. Natl. Acad. Sci. U.S.A.,* 74, 837, 1977.
17. **Edelstein, C., Kezdy, F. J., Scanu, A. M., and Shen, B. W.,** Apolipoproteins and structural organization of plasma lipoproteins: human plasmic high density lipoprotein-3, *J. Lipid Res.,* 20, 143, 1979.
18. **Hamilton, R. L., Williams, M. C., Fielding, C. J., and Havel, R. J.,** Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver, *J. Clin. Invest.,* 58, 667, 1976.
19. **Glomset, J. A.,** Plasma lecithin: cholesterol acyl transferase, in *Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism,* Nelson, G. J., Ed., John Wiley & Sons, New York, 1972, 745.
20. **Green, P. H. R., Tall, A. R., and Glickman, R. M.,** Rat intestine secretes discoid high density lipoprotein, *J. Clin. Invest.,* 61, 528, 1978.
21. **Roheim, P. S., Gidez, L. I., and Eder, H. A.,** Extrahepatic synthesis of lipoproteins of plasma and chyle: role of the intestine, *J. Clin. Invest.,* 45, 297, 1966.
22. **Tall, A. R. and Small, D. M.,** Body cholesterol removal: role of plasma high density lipoproteins, *Adv. Lipid Res.,* 17, 1, 1980.
23. **Minari, D. and Zilversmit, D.,** Behavior of dog lymph chylomicron lipid constituents during incubation with serum, *J. Lipid Res.* 4, 424, 1963.
24. **Nestel, P. J., Havel, R. J., and Bezman, A.,** Sites of initial removal of chylomicron triglyceride fatty acids from the blood, *J. Clin. Invest.,* 41, 1915, 1962.
25. **Havel, R. J., Kane, J. P., and Kashyap, M. L.,** Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man, *J. Clin. Invest.* 52, 32, 1973.
26. **Mis, O. D., Faegermean, O., Hamilton, R. L., and Havel, R. J.,** Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat, *J. Clin. Invest.,* 56, 603, 1975.
27. **Eisenberg, S.,** Plasma lipoprotein conversion: the origin of low-density and high-density lipoprotein, *Ann. N.Y. Acad. Sci.,* 348, 30, 1980.
28. **Schaefer, E. J., Jenkins, L. L., and Brewer, H. B.,** Human chylomicron apolipoprotein metabolism, *Biochem. Biophys. Res. Commun.,* 80, 405, 1978.
29. **Redgrave, T. G. and Small, D. M.,** Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat, *J. Clin. Invest.* 64, 162, 1979.
30. **Nicoll, A., Miller, N. E., and Lewis, B.,** High-density lipoprotein metabolism, *Adv. Lipid Res.,* 17, 53, 1980.
31. **Mahley, R. W., Innerarity, T. L., and Weisgraber, K. H.,** Alterations in metabolic activity of plasma lipoproteins following selective chemical modification of the apoproteins, *Ann. N.Y. Acad. Sci.,* 348, 265, 1980.
32. **Roheim, P. S., Rachmilewitz, D., Stein, O., and Stein, Y.,** Metabolism of iodinated high density lipoproteins in the rat, *Biochim. Biophys. Acta,* 248, 315, 1971.
33. **Nakai, T. and Whayne, T. F.,** Catabolism of canine apolipoprotein A-1: purification, catabolic rate, organs of catabolism, and the liver subcellular catabolic site, *J. Lab. Clin. Med.,* 88, 63, 1976.
34. **Carew, T. E., Sark, R. P., Johansen, K. H., Dennis, C. A., and Steinberg D.,** Low density and high density lipoprotein turnover following portacaval shunt in swine, *J. Lipid Res.,* 17, 441, 1976.
35. **Snidermann, A., Thomas, D., Marpole, D., and Teng, B.,** Low density lipoproteins: a metabolic pathway for return of cholesterol to the splanchnic bed, *J. Clin. Invest.,* 61, 867, 1978.
36. **Van Tol, A., Van Gent, T., Van 'T Hooft, F. M., and Vlaspolder, F.,** High density lipoprotein catabolism before and after partial hepatectomy, *Atherosclerosis,* 29, 439, 1978.
37. **Glomset, J. A. and Norum, K. R.,** The metabolic role of lecithin: cholesterol acyl transferase: perspective from pathology, *Adv. Lipid Res.,* 11, 1, 1973.
38. **Miller, G. J. and Miller, N. E.,** Plasma high density lipoprotein concentration and development of ischemic heart disease, *Lancet,* 1, 16, 1975.
39. **Fielding, C. J., Shore, V. G., and Fielding, P. E.,** A protein cofactor of lecithin:cholesterol acyltransferase, *Biochem. Biophys. Res. Commun.,* 46, 1493, 1972.
40. **Chung, J., Abano, D. A., Fless, G. M., and Scanu, A. M.,** Isolation, properties, and mechanism of in vitro action of lecithin:cholesterol of acyltransferase from human, plasma, *J. Biol. Chem.,* 254, 7456, 1979.
41. **Soutar, A. K., Garner, C. N., Baker, H. N., Sparrow, J. T., Jackson, R. L., Gotto, A. M., and Smith, L. C.,** The effects of plasma apolipoproteins on lecithin:cholesterol acyltransferase, *Biochemistry,* 14, 3057, 1975.
42. **Newman, G. C. and Huang, C.,** Structural studies on phosphatidylcholine-cholesterol mixed vesicles, *Biochemistry,* 14, 3363, 1975.

43. **Scanu, A. M., Lagocki, P. I., and Chung, J.,** Effect of apolipoprotein A-II on the structure of high density lipoproteins: relationship to the activity of lecithin:cholesterol acyl transferase in vitro, *Ann. N.Y. Acad. Sci.,* 348, 160, 1980.
44. **Chajek, T., Aron, L., and Fielding, C. J.,** Interaction of lecithin: cholesterol acyltransferase and cholesteryl ester transfer protein in the transport of cholesteryl ester into sphingomyelin liposomes, *Biochemistry,* 19, 3673, 1980.
45. **Chung, J., Abano, D., and Scanu, A. M.,** Mass:activity distribution of lecithin:cholesterol acyltransferase in plasma lipoproteins of human plasma, *Fed. Proc. Fed. Am. Soc. Exp. Biol.,* 40(Abstr. 902), 1696, 1981.
46. **Zilversmit, D. G., Hughes, L. B., and Balmer, J.,** Stimulation of cholesterol ester exchange by lipoprotein-free rabbit plasma, *Biochim. Biophys. Acta.* 409, 393, 1975.
47. **Pattnaik, N. M., Montes, A., Hughes, L. B., and Zilversmit, D. B.,** Cholesteryl ester exchange protein in human plasma: isolation and characterization, *Biochim. Biophys. Acta,* 530, 428, 1978.
48. **Chajek, T. and Fielding, C. J.,** Isolation and characterization of a human serum cholesteryl ester transfer protein, *Proc. Natl. Acad., Sci. U.S.A.,* 75, 3445, 1978.
49. **Morton, R. E. and Zilversmit, D. B.,** The separation of apolipoprotein D from cholesteryl ester transfer protein, *Biochim. Biophys. Acta,* 663, 350, 1981.
50. **Sniderman, A., Teng, B., Vezina, C., and Marcel, Y. L.,** Cholesteryl ester exchange between human high and low density lipoproteins mediated by a plasma protein factor, *Atherosclerosis,* 31, 327, 1978.
51. **Barter, P. J., Rajaram, O. V., and White, G. M.,** Isolation and characterization of a serum triglyceride transfer protein, *Circulation,* 60(Suppl. II-17, Abstr. 452), 1979.
52. **Ihm, J., Harmony, J. A. K., Ellsworth, J., and Jackson, R. L.,** Simultaneous transfer of cholesteryl ester and phospholipid by protein(s) isolated from human lipoprotein-free plasma, *Biochem. Biophys. Res. Commun.,* 46, 1493, 1980.
53. **Fielding, P. E. and Fielding, C. J.,** A cholesteryl ester transfer complex in human plasma,*Proc. Natl. Acad. Sci. U.S.A.,* 77, 3327, 1980.

53a. **Fielding, C. J. and Fielding, P. E.,** Regulation of human plasma lecithin:cholesterol acyltransferase activity by lipoprotein acceptor cholesteryl ester content, *J. Biol. Chem.,* 256, 2102, 1981.

54. **Forte, T., Norum, K. R., Glomset, J. A. and Nichols, A. V.,** Plasma lipoproteins in familial lecithin: cholesterol acyltransferase deficiency: structure of low and high density lipoproteins as revealed by electron microscopy, *J. Clin. Invest.,* 50, 1141, 1971.
55. **Blomoff, J. P.** High density lipoproteins in cholestasis, *Scan. J. Gastroenterol.,* 9, 591, 1974.
56. **Marcel, Y. L., Vezina, C., Edmond, D., and Suzel, G.,** Heterogeneity of human high density lipoprotein:presence of lipoproteins with and without apoE and their roles as substrates for lecithin: cholesterol acyltransferase reaction, *Proc. Natl. Acad. Sci. U.S.A.,* 77, 2969, 1980.
57. **Marsh, J. B.,** Apoproteins of the lipoproteins in a non-recirculating perfusate of rat liver, *J. Lipid Res.,* 17, 85, 1976.
58. **Green, P. H. R., Tall, A. R., and Glickman, R. M.,** Rat intestine secretes discoid high density lipoprotein, *J. Clin. Invest.,* 61, 528, 1978.
59. **Glickman, R. M. and Green, P. H. R.,** The intestine as a source of apolipoprotein A-I, *Proc. Natl. Acad. Sci. U.S.A.,* 74, 2569, 1977.
60. **Green, P. H. R., Glickman, R. M., Sandek, C. D., Blum, C. B., and Tall, A. R.,** Human intestinal lipoproteins: studies in chyluric subjects, *J. Clin. Invest.,* 64, 233, 1979.
61. **Glomset, J. A., Mitchell, C. D., King, W. C., Applegate, K. A., Forte, T., Norum, K. R., and Gjone, E.,** In vitro effects of lecithin:cholesterol acyltransferase on apolipoprotein distribution in familial lecithin:cholesterol acyltransferase deficiency, *Ann. N.Y. Acad. Sci.,* 348, 224, 1980.
62. **Glangeaud, M.C., Eisenberg, S., and Olivecrona, T.,** Very low density lipoprotein: dissociation of apolipoprotein C during lipoprotein lipase induced lipolysis, *Biochim. Biophys, Acta* 486, 23, 1977.
63. **Patsch, J. R., Gotto, A. M., Olivecrona, T., and Eisenberg, S.,** Formation of high density lipoprotein-like particles during lipolysis of very low density lipoproteins in vitro, *Proc. Natl. Acad. Sci. U.S.A.,* 75, 4519, 1978.
64. **Yukawa, S. and Scanu, A. M.,** In vitro effect on human serum lipoproteins by increasing amounts of exogenous very low density lipoprotein (VLDL) in the absence and presence of lipoprotein lipase (LPL), *Circulation,* 62 (Suppl. III-80, Abstr. 290), 1980.
65. **Anderson, D. N., Nichols, A. V., Forte, T. M. and Lindgren, F. T.,** Particle distribution of human serum high density lipoproteins, *Biochim. Biophys, Acta,* 493, 55, 1977.
66. **LaRosa, J. C., Levy, R. I., Windmueller, H. G., and Fredrickson, D. S.,** Comparison of the triglyceride lipase of liver, adipose tissue, and post heparin plasma, *J. Lipid Res.,* 13, 356, 1972.
67. **Jansen, H., Van Zuylen-Van Wiggen, A., and Hulomann, W. C.,** Lipoprotein lipase from heart and liver: an immunological study, *Biochem. Biophys. Res. Commun.,* 55, 30, 1973.
68. **Sauan, J. and Stokke, K. T.,** Plasma estrogens and hepatic lipase in postheparin plasma with special reference to liver disease, *Clin. Chim. Acta,* 92, 101, 1979.

69. **Kuusi, T., Kinnunen, P. K. J., and Nikkila, E. A.,** Hepatic endothelial lipase antiserum influences rat low and high density lipoproteins in vivo, *FEBS Lett.*, 104, 384, 1979.
70. **Huttunen, J. K., Ehnholm, C., Kekki, M., and Nikkila, E. A.,** Post-heparin plasma lipoprotein lipase and hepatic lipase in normal subjects and in patients with hypertriglyceridaemia, *Clin. Sci. Mol. Med.*, 50, 249, 1976.
71. **Bengtsson, G. and Olivecrona, T.,** The hepatic heparin releasable lipase binds to high density lipoproteins, *FEBS Lett.*, 119, 290, 1980.
72. **Kuusi, T., Saarinen, P., and Nikkila, E. A.,** Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoproteins in man, *Atherosclerosis*, 36, 589, 1980.
73. **Jansen, H., Van Tol, A., and Hulsmann, W. C.,** On the metabolic function of heparin-releasable liver lipase, *Biochem. Biophys. Res. Commun.*, 92, 53, 1980.
74. **Van Tol, A., Van Gent, T., and Jansen, H.,** Degradation of high density lipoprotein by heparin-releasable liver lipase, *Biochem. Biophys. Res. Commun.*, 94, 101, 1980.
75. **Eisenberg, S., Schun, D., Goldman, H., and Olivecrona, T.,** Comparison of the phospholipase activity of bovine milk lipoprotein lipase against rat plasma very low density and high density lipoprotein, *Biochim. Biophys. Acta*, 531, 344, 1978.
76. **Simpson, E. R., Bilheimer, D. W., MacDonald, P. C., and Porter, J. C.,** Uptake and degradation of plasma lipoproteins by human choriocarcinoma cells in culture, *Endocrinology*, 104, 8, 1979.
77. **Carr, B. R., Porter, J. C., MacDonald, P. C., and Simpson, E. R.,** Metabolism of low density lipoprotein by human fetal adrenal tissue, *Endocrinology*, 107, 1034, 1980.
78. **Kovanen, P. T., Faust, J. R., Brown, M. S., and Goldstein, J. L.,** Low density lipoprotein receptors in bovine adrenal cortex. I. Receptor-mediated uptake of low density lipoprotein and ultilization of its cholesterol for steroid synthesis in cultured adrenocortical cells, *Endocrinology*, 104, 599, 1979.
79. **Kovanen, P. T., Schneider, W. J., Hillman, G. M., Goldstein, J. L., and Brown, M. S.,** Separate mechanisms for the uptake of high and low density lipoproteins by mouse adrenal gland in vivo, *J. Biol. Chem.*, 254, 5498, 1979.
80. **Andersen, J. M. and Dietschy, J. M.,** Regulation of sterol synthesis in 15 tissues of rat, *J. Biol. Chem.*, 252, 3652, 1977.
81. **Andersen, J. M. and Dietschy, J. M.,** Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary and testis of rat, *J. Biol. Chem.*, 253, 9024, 1978.
82. **Brown, M. S., Kovanen, P. T., and Goldstein, J. L.,** Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex, *Recent Prog. Horm. Res.* 35, 215, 1979.
83. **Balasubramaniam, S., Goldstein, J. L., Faust, J. R., Brunschede, G. Y., and Brown, M. S.,** Lipoprotein-mediated regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesteryl ester metabolism in the adrenal gland of the rat, *J. Biol. Chem.*, 252, 1771, 1977.
84. **Faust, J. R., Goldstein, J. L., and Brown, M. S.,** Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultural mouse adrenal cells, *J. Biol. Chem.*, 252, 4861, 1977.
85. **Brown, M. S., Kovanen, P. T., and Goldstein, J. L.,** Regulation of plasma cholesterol by lipoprotein receptors, *Science*, 212, 628, 1981.
86. **Gwynne, J. T., Mahaffee, D., Brewer, H. B., Jr., and Ney, R. L.,** Adrenal cholesterol uptake from plasma lipoproteins: regulation by corticotropin, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 4329, 1976.
87. **Henderson, J. F.,** Studies on fatty liver induction by 4-amino-pyrazolopyrimidine, *J. Lipid Res.*, 4, 68, 1963.
88. **Kita, T., Beisiegel, V., Goldstein, J. L., Schneider, W. J., and Brown, M. S.,** Antibody against low density lipoprotein receptor blocks uptake of low density lipoprotein (but not high density lipoprotein) by the adrenal gland of the mouse in vivo, *J. Biol. Chem.*, 256, 4701, 1981.
89. **Kovanen, P. T., Goldstein, J. L., Chapel, D. A., and Brown, M. S.,** Regulation of low density lipoprotein receptors by adrenocorticotropin in the adrenal gland of mice and rats in vivo, *J. Biol. Chem.*, 255, 5591, 1980.
90. **Gwynne, J. T. and Hess, B.,** The role of high density lipoproteins in rat adrenal cholesterol metabolism and steroidogenesis, *J. Biol. Chem.*, 255, 10875, 1980.
91. **Chen, Y-D., Kroemer, F. B., and Reaven, G. M.,** Identification of specific high density lipoprotein-binding sites in rat testis and regulation of binding by human chorionic gonadotropin, *J. Biol. Chem.*, 255, 9162, 1980.
92. **Gwynne, J. T. and Hess, B.,** Binding and degradation of human ^{125}I HDL by rat adrenocortical cells, *Metab. Clin. Exp.*, 27, 1593, 1978.
93. **Wu, J. D., Butler, J., and Bailey, J. M.,** Lipid metabolism in cultured cells. XVIII. Comparative uptake of low density and high density lipoproteins by normal, hypercholesterolemic and tumor virus-transformed human fibroblasts, *J. Lipid Res.*, 20, 472, 1979.

94. **Miller, N. E., Weinstein, D. B., Carew, T. E., Koschinsky, T., and Steinberg, D.,** Interaction between high density and low density lipoproteins during uptake and degradation by cultured human fibroblasts, *J. Clin. Invest.*, 60, 78, 1977.
95. **Kovanen, P. T., Brown, M. S., and Goldstein, J. L.,** Increased binding of low density lipoprotein to liver membranes from rats treated with 17 α-ethinyl estradiol, *J. Biol. Chem.*, 254, 11367, 1979.
96. **Munthe-Kaas, A. C., Berg, T., and Seljelid, R.,** Distribution of lysosomal enzymes in different types of rat liver cells, *Exp. Cell Res.*, 99, 146, 1976.
97. **Nakai, T., Otto, P. S., Kennedy, D. L., and Whayne, T. F., Jr.,** Rat high density lipoprotein subfraction (HDL_3) uptake and catabolism by isolated rat liver parenchymal cells, *J. Biol. Chem.*, 251, 4914, 1976.
98. **Van Berkel, T. J. C., Van Tol, A., and Koster, J. F.,** Iodine-labeled human and rat low-density and high-density lipoprotein degradation by human liver and parenchymal and non parenchymal cells from rat liver, *Biochim. Biophys. Acta*, 529, 138, 1978.
99. **Van Berkel, T. J. C., Koster, J. F., and Hulsmann, W. C.,** High-density lipoprotein and low-density lipoprotein catabolism by human liver and parenchymal and non-parenchymal cells from rat liver, *Biochim. Biophys. Acta*, 486, 586, 1977.
100. **Breslow, J. L., Lothrop, D. A., Clowes, A. W., and Lux, S. E.,** Lipoprotein regulation of 3-hydroxy-3-methylglutaryl coenzyme. A reductase activity in rat liver cell cultures, *J. Biol. Chem.*, 252, 2726, 1977.
101. **Edwards, P. A.,** Effect of plasma lipoproteins and lecithin-cholesterol dispersions on the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase of isolated rat hepatocytes, *Biochim. Biophys. Acta*, 409, 39, 1975.
102. **Ose, L., Ose, T., Norum, K. R., and Berg, T.,** Uptake and degradation of ^{125}I labelled high density lipoproteins in rat liver cells in vivo and in vitro, *Biochim. Biophys. Acta*, 574, 521, 1979.
103. **Ose, L., Ose, T., Norum, K. R., and Berg, T.,** The intracellular distribution of high density lipoproteins taken up by isolated rat hepatocytes, *Biochim. Biophys. Acta*, 620, 120, 1980.
104. **Ose, T., Berg, T., Norum, K. R., and Ose, L.,** Catabolism of (^{125}I) low density lipoproteins in isolated rat liver cells, *Biochem. Biophys. Res. Commun.*, 97, 192, 1980.
105. **Van Berkel, T. J. C., Kruijt, J. K., Van Gent, T., and Van Tol, A.,** Saturable high affinity binding of low density and high density lipoproteins by parenchymal and non-parenchymal cells from rat liver, *Biochem. Biophys, Res. Commun.*, 92, 1002, 1980.
106. **Ose, L., Röken, I., Norum, K. R., Drevon, C. A., and Berg, T.,** The binding of high density lipoproteins to isolated rat: hepatocytes, *Scand. J. Clin. Invest.*, 41, 63, 1981.
107. **Wandel, M., Norum, K. R., Berg, T., and Ose, L.,** Binding, uptake, and degradation of ^{125}I-labelled high-density lipoproteins in isolated non-parenchymal rat liver cells, *Scand. J. Gastroenterol.*, 16, 71, 1981.
108. **Drevon, C. A., Berg, T., and Norum, K. R.,** Uptake and degradation of cholesterol ester-labelled rat plasma lipoproteins in purified rat hepatocytes and non-parenchymal liver cells, *Biochim. Biophys. Acta*, 487, 122, 1977.
109. **Ose, L., Ose, T., Reinertsen, R., and Berg, T.,** Fluid endocytosis in isolated rat parenchymal and non-parenchymal liver cells, *Exp. Cell Res.*, 126, 109, 1980.
110. **Innerarity, T. L., Pitas, R. E., and Mahley, R. W.,** Disparities in the interaction of rat and human lipoproteins with cultured rat fibroblasts and smooth muscle cells, *J. Biol. Chem.*, 255, 11163, 1980.
111. **Mahley, R. W. and Innerarity, T. L.,** Interaction of canine and swine lipoproteins with the low density lipoprotein receptor of fibroblasts as correlated with heparin/manganese precipitability, *J. Biol. Chem.*, 252, 3980, 1977.
112. **Mahley, R. W., Innerarity, T. L., Pitas, R. E., Weisgraber, K. H., Brown, J. H., and Gross, E.,** Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification or arginyl residues in arginine-rich and B apoproteins, *J. Biol. Chem.*, 252, 7279, 1977.
113. **Goldstein, J. L. and Brown, M. S.,** The low density lipoprotein pathway and its relation to atherosclerosis, *Ann. Rev. Biochem.*, 46, 897, 1977.
114. **Chao, Y., Windler, E. E., Chen, G. Ch., and Havel, R. J.,** Hepatic catabolism of rat and human lipoproteins in rat treated with 17 α-ethinyl estradiol. *J. Biol. Chem.*, 254, 11360, 1979.
115. **Kita, T., Brown, M. S., Watanabe, Y., and Goldstein, J. L.,** Deficiency of low density lipoprotein receptors in liver and adrenal gland of the WHHL rabbit, an animal model of familial hypercholesterolemia, *Proc. Natl. Acad, Sci. U.S.A.*, 78, 2268, 1981.
116. **Bachorik, P. S., Kwiterovich, P. O., and Cooke, J. C.,** Isolation of a porcine liver plasma membrane fraction that binds low density lipoproteins, *Biochemistry*, 17, 5287, 1978.
117. **Ose, L., Röken, I., Norum, K. R., and Berg, T.,** The effect of ammonia, chloroquine, leupeptin, colchicine and cytocholasin B on degradation of high density lipoproteins in isolated rat hepatocytes, *Exp. Cell Res.*, 130, 127, 1980.
118. **Quarfordt, S., Hanks, J., Jones, R. S., and Shelburne, F.,** The uptake of high density lipoprotein cholesteryl ester in the perfused rat liver, *J. Biol. Chem.*, 225, 2934, 1980.

119. **Jakoi, L. and Quarfordt, S. H.,** Alterations of rat hepatic cholesterogenesis by heterologous lipoproteins, *J. Biol. Chem.*, 252, 6856, 1977.
120. **Schwartz, C. C., Halloran, L. G., Vlahcevic, Z. R., Gregory, D. H., and Swell, L.,** Preferential utilization of free cholesterol from high density lipoproteins for biliary cholesterol secretion in man, *Science*, 200, 62, 1978.
121. **Halloran, L. G., Schwartz, C. C., Vlahcevic, Z. R., Hisman, R. M., and Swell, L.,** Evidence for high-density lipoprotein-free cholesterol as the primary precursor for bile acid synthesis in man, *Surgery*, 84, 1, 1978.
122. **Rothblat, G. H., Arbogast, L. Y., and Ray, E. K.,** Stimulation of esterified cholesterol accumulation in tissue culture cells exposed to high density lipoproteins enriched in free cholesterol, *J. Lipid Res.*, 19, 350, 1978.
123. **Nilsson, M. and Berg, T.,** Uptake and degradation of formaldehyde-treated ^{125}I-labelled human serum albumin in rat liver cells in vivo and in vitro, *Biochim. Biophys, Acta*, 497, 171, 1977.
124. **Goldstein, J. L., Ho, Y. K., Basu, S. K., and Brown, M. S.,** Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 333, 1979.
125. **Mahley, R. W., Innerarity, T. L., Weisgraber, K. H., and Oh, S. Y.,** Altered metabolism (in vivo and in vitro) of plasma lipoproteins after selective chemical modification of lysine residues of the apoproteins, *J. Clin. Invest.*, 64, 743, 1979.
126. **Stein, Y., Stein, O., and Goren, R.,** Metabolism and metabolic role of serum high density lipoproteins, in *High Density Lipoproteins and Atherosclerosis*, Gotto, A. M., Jr., Miller, N. E., and Oliver, M. F., Eds., Elsevier, Amsterdam, 1978, 37.
127. **Miller, N. E., Weinstein, D. B., and Steinberg, D.,** Uptake and degradation of high density lipoprotein: comparison of fibroblasts from normal subjects and from homozygous familial hypercholesterolemic subjects, *J. Lipid Res*, 19, 644, 1978.
128. **Miller, N. E., Weinstein, D. B., and Steinberg, D.,** Binding, internalization, and degradation of high density lipoprotein by cultured normal human fibroblasts, *J. Lipid Res.*, 18, 438, 1977.
129. **Koschinsky, T., Carew, T. E., and Steinberg, D.,** A comparative study of surface binding of human low density and high density lipoproteins to human fibroblasts: regulation by sterols and susceptibility to proteolitic degradation, *J. Lipid Res.*, 18, 451, 1977.
130. **Carew, T. E., Koschinsky, T., Hayes, S. B., and Steinberg, D.,** A mechanism by which high-density lipoproteins may slow the atherogenic process, *Lancet*, 1, 1315, 1976.
131. **Bierman, E. L., Stein, O., and Stein, Y.,** Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture, *Circ. Res.*, 35, 136, 1974.
132. **Stein, O. and Stein, Y.,** Surface binding and interiorization of bomologous and heterologous serum lipoproteins by rat aortic smooth muscle cells in culture, *Biochim. Biophys, Acta*, 398, 377, 1975.
133. **Drevon, C. A., Attic, A. D., Pangburn, S. H., and Steinberg, D.,** Metabolism of homologous and heterologous lipoproteins by cultured rat and human skin fibroblasts, *J. Lipid Res.*, 22, 37, 1981.
134. **Daerr, W. H., Gianturco, S. H., Patsch, J. R., Smith, L. C., and Gotto, A. M., Jr.,** Stimulation and suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in normal human fibroblasts by high density lipoprotein subclasses, *Biochim. Biophys. Acta*, 619, 287, 1980
135. **Emeis, J. J., Vermier, B. J., and Havekes, L.,** Immunoelectron-microscopy of the binding of high density lipoproteins apoproteins to normal and hypercholesterolemic human fibroblasts, *Cell Biol. Intern. Reports*, 5, 133, 1981.
136. **Stein, O., Vanderhock, J., and Stein, Y.,** Cholesterol ester accumulation in cultured aortic smooth muscle cells: Induction of cholesterol ester retention by chloroquine and low density lipoprotein and its reversion by mixtures of high density apolipoprotein and sphingomyelin, *Atherosclerosis*, 26, 465, 1977.
137. **Stein, Y., Glangeaud, M. C., Fainaru, M., and Stein, O.,** The removal of cholesterol from aortic smooth muscle cells in culture and Landschutz ascites cells by fractions of human high density apolipoproteins, *Biochim. Biophys. Acta*, 380, 106, 1975.
138. **Ho, Y. K., Brown M. S., and Goldstein, J. L.,** Hydrolysis and excretion of cytoplasmic cholesteryl ester by macrophages: stimulation by high density lipoprotein and other agents, *J. Lipid Res.*, 21, 391, 1980.
139. **Brown, M. S., Ho, Y. K., and Goldstein, J. L.,** The cholesteryl ester cycle in macrophage foam cells, *J. Biol. Chem.*, 255, 9344, 1980.
140. **Wu, J-D. and Bailey, J. M.,** Lipid metabolism in cultured cells: studies on lipoprotein-catalyzed reverse cholesterol transport in normal and homozygous familial hypercholesterolemic skin fibroblasts, *Arch. Biochem. Biophys.*, 202, 467, 1980.
141. **Bates, S. R. and Rothblat, G. H.,** Regulation of cellular sterol flux and synthesis by human lipoproteins, *Biochim. Biophys. Acta*, 360, 38, 1974.
142. **Stein, O. and Stein, Y.** The removal of cholesterol from Landschütz ascites cells by high density apolipoproteins, *Biochim. Biophys. Acta*, 326, 232, 1973.

143. **Jackson, R. L., Gotto, A. M., Stein, O., and Stein, Y.,** A comparative study on the removal of cellular lipids from Landschütz ascites cells by human plasma apolipoproteins, *J. Biol. Chem.*, 250, 7204, 1975.
144. **Stein, O., Goren, R., and Stein, Y.,** Removal of cholesterol from fibroblasts and smooth muscle cells in culture in the presence and absence of cholesterol esterification in the medium, *Biochim. Biophys. Acta,* 529, 309, 1978.
145. **Fogelman, A. M., Seager, J., Edwards, P. A., and Popják, G.,** Mechanism of induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase in human leukocytes, *J. Biol. Chem.*, 252, 644, 1977.
146. **Fogelman, A. M., Edmond, J., Seager, J., and Popják, G.,** Abnormal induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase in leukocytes from subjects with heterozygous familial hypercholesterolemia, *J. Biol. Chem.*, 250, 2045, 1975.
147. **Daniels, R. J., Guertler, L. S., Parker, T. S., and Steinberg, D.,** Studies on the rate of efflux of cholesterol from cultured human skin fibroblasts, *J. Biol. Chem.*, 256, 4978, 1981.
148. **Rothblat, G. H. and Kritchevsky, D.,** The secretion of free and ester cholesterol by tissue culture cells: studies with L_{5178} Y and L-cells, *Biochim. Biophys. Acta,* 144, 423, 1967.
149. **Werb, Z. and Cohn, Z. A.,** Cholesterol metabolism in the macrophage. III. Ingestion and intracellular fate of cholesterol and cholesterol esters, *J. Exp. Med.*, 135, 21, 1972.
150. **Glomset, J. A.,** Physiological role of lecithin-cholesterol acyltransferase, *Am. J. Clin. Nutr.*, 23, 1129, 1970.
151. **Miller, N. E.,** Induction of low density lipoprotein receptor synthesis by high density lipoprotein in cultures of human skin fibroblasts. *Biochim. Biophys. Acta,* 529, 131, 1978.
152. **Ritter, M. C. and Scanu, A. M.,** Structural changes in human serum high density lipoprotein-3 attending incubation with blood leukocytes, *J. Biol. Chem.*, 255, 3763, 1980.
153. **Kwok, B. C. P., Dawson, G., and Ritter, M. C.,** Stimulation of glycolipid synthesis and exchange by human serum high density lipoprotein-3 in human fibroblasts and leukocytes, *J. Biol. Chem.*, 256, 92, 1981.
154. **Franson, R., Weiss, J., Martin, L., Spitznagel, J. K., and Elsbach, P.,** Phospholipase A activity associated with the membrane of human polymorphonuclear leukocytes, *Biochem. J.*, 167, 839, 1977.
155. **Ulevitch, R. J., Johnston, A. R., and Weinstein, D. B.,** New function for high density lipoproteins: isolation and characterization of a bacterial lyopolysaccharide-high density lipoprotein complex formed in rabbit plasma, *J. Clin. Invest.*, 67, 827, 1981.
156. **Mathison, J. C. and Ulevitch, R. J.,** The clearance, tissue distribution and cellular localization of intravenously injected lipopolysaccharide in rabbits, *J. Immunol.*, 123, 2133, 1979.
157. **Mathison, J. C. and Ulevitch, R. J.,** Physiologic effects of bacterial lipopolysaccharide (LPS)-lipoprotein complexes, *Fed. Proc. Fed. Am. Sec. Exp. Biol.*, 39, (Abstr. 2154), 674, 1980.
158. **Rifkin, M. R.,** Identification of the trypanocidal factor in normal human serum: high density lipoprotein, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 3450, 1978.
159. **Benditt, E. P. and Eriksen, N.,** Amyloid protein SAA is associated with high density lipoprotein from human serum, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 4025, 1977.
160. **Ericksen, N. and Benditt, E. P.,** Isolation and characterization of the amyloid-related apoprotein (SAA) from human high density lipoprotein, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 6860, 1980.
161. **Hoffmann, J. S., Eriksen, N., and Benditt, E. P.,** Apo-SAA is a fast-clearing HDL apoprotein in the mouse, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 39 (Abstr. 544), 39, 1980.
162. **Malmendier, C. L., Paroutand, P., and Amerychex, J.,** Partial amino acid sequence of three new apolipoproteins isolated from human high density lipoproteins, *FEBS Lett,* 109, 43, 1980.
163. **Jackson, R. L., Kashyap, M. L., and Glueck, C. J.,** Glucose infusions increase plasma levels of amyloid proteins in high density lipoproteins, *Biomedicine,* 33, 245, 1980.
164. **Shore, V. G., Shore, B., and Lewis, S. B.,** Isolation and characterization of two threonine-poor apolipoproteins of human plasma high density lipoproteins, *Biochemistry,* 17, 2174, 1978.
165. **Hessler, J. R., Robertson, A. L., and Chisolm, G. M., III,** LDL-induced cytotoxicity and its inhibition by HDL in human vascular smooth muscle and endothelial cells in culture, *Atherosclerosis,* 22, 213, 1979.
166. **Henricksen, T., Evensen, S. A., and Carlander, B.,** Injury to cultured endothelial cells induced by low density lipoproteins: protection by HDL, *Scand. J. Clin. Lab. Invest.*, 39, 369, 1979.
167. **Beitz, J. and Forster, W.,** Influence of human low density and high density lipoprotein cholesterol on the in vitro prostaglandin I_2 synthetase activity, *Biochim. Biophys. Acta,* 620, 352, 1980.
168. **Srinevasan, S. R., Yost, C., Radhakrishnamurthy, B., Dalferes, E. R., Jr., and Berenson, G. S.,** Lipoprotein-elastin interactions in human aorta fibrous plaque lesions, *Atherosclerosis,* 38, 137, 1981.
169. **Noma, A., Takahashi, T., and Wada, T.,** Elastin-lipid interaction in the arterial wall. II. In vitro binding of lipoprotein-lipids to arterial elastin and the inhibitory effect of high density lipoproteins on the process, *Atherosclerosis,* 38, 373, 1981.
170. **Tracy, R. E., Dzoga, K. R., and Wissler, R. E.,** Sequestration of serum low-density lipoproteins in the arterial intima by complex formation, *Proc. Soc. Exp. Biol. Med.*, 118, 1095, 1965.

171. **Srinivasan, S. R., Yost, K., Radhakrishnamurthy, B., Dalferes, E. R., Jr., and Berenson, G. S.,** Lipoprotein-hyaluronate associations in human aorta fibrous plaque lesions, *Atherosclerosis,* 36, 25, 1980.
172. **Hoff, H. E., Jackson, R. L., Mao, J. T., and Gotto, A. M.,** Localization of low density lipoproteins in arterial lesions from normolipidemics employing a purified fluorescent labeled antibody, *Biochim. Biophys. Acta,* 351, 407, 1974.
173. **Kramsch, D. M., Franzblan, C., and Hollander, W.,** The protein and lipid composition of arterial elastin and its relationship to lipid accumulation in the atherosclerotic plaque, *J. Clin. Invest.,* 50, 1666, 1970.
174. **Smith, E. B. and Smith, R. H.,** Early change in aortic intima, in *Atherosclerosis Reviews,* Vol. 1, Paoletti, R. and Gotto, A. M., Eds., Raven Press, New York, 1976, 119.
175. **Curtiss, L. K., De Heer, D. H., and Edgington, T. S.,** In vivo suppression of the primary immune response by a species of low-density serum lipoprotein, *J. Immunol.,* 118, 648, 1977.
176. **Curtiss, L. K. and Edgington, T. S.,** Differential sensitivity of lymphocyte subpopulations to suppression by LDL-In, an immunoregulatory human serum low density lipoprotein, *J. Clin. Invest.,* 63, 193, 1979.
177. **Fujii, D. K. and Edgington, T. S.,** Direct suppression of lymphocyte induction by the immunoregulatory human serum low density lipoprotein, LDL-In, *J. Immunol.,* 124, 156, 1980.
178. **Chisari, F. V. and Edgington, T. S.,** Lymphocyte E rosette inhibitory factor: a regulatory serum lipoprotein, *J. Exp. Med.,* 142, 1092, 1975.
179. **Chisari, F. V., Routenberg, J. A., Frala, M., and Edgington, T. S.,** Extrinsic modulation of human T-lymphocyte E rosette function associated with prolonged hepatocellular injury after viral hepatitis, *J. Clin. Invest.,* 59, 134, 1977.
180. **Curtiss, L. K. and Edgington, T. S.,** Effect of LDL-In, a normal immunoregulatory human serum LDL, on the interaction of macrophages with lymphocytes proliferating in response to mitogen and allogeneic stimulation, *J. Immunol.,* 118, 1966, 1977.
181. **Schuh, J., Novogrodsky, A., and Haschemeyer, R. H.,** Inhibition of lymphocyte mitogenesis by autoxidized low-density lipoprotein, *Biochem. Biophys, Res. Commun.,* 84, 763, 1978.
182. **Leikin, A. and Scanu, A. M.,** unpublished results.