George Lyman Duff Memorial Lecture

Role of the Liver in Atherosclerosis

Richard J. Havel

George Lyman Duff was a pioneer in relating the quality as well as the quantity of plasma lipoproteins to experimental atherosclerosis. His investigations and those of John Gofman foreshadowed much contemporary research on the interactions of lipoproteins with cells and other components of the arterial wall. This current research has, I believe, put to rest past criticisms of the meetings of our Council, at which it seemed to some that the artery is hardly involved in the pathogenesis of atherosclerosis. Perhaps I can then be forgiven for taking as the subject of this lecture the role of the liver in lipoprotein metabolism and atherosclerosis, and for daring to suggest that if the liver would only do correctly its job of secreting the "right" kinds of lipoproteins and of taking up, catabolizing, and excreting lipoprotein components, it would be unnecessary for us to be so concerned about the mechanisms by which lipoprotein-cholesterol accumulates in the arterial intima.

It is a truism that laboratory animals fed standard chow diets have low levels of atherogenic lipoproteins and do not develop atherosclerosis, whereas lipid accumulates in arteries and proliferative, space-occupying lesions develop when these animals are fed unnatural diets, sometimes aided by auxiliary measures that perturb hepatic cholesterol metabolism.

My introduction to this field was in Joseph Bragdon's laboratory at the National Heart Institute in 1953. Bragdon, a pathologist, had read a report that Alaskan ground squirrels have rather high serum lipid levels and he undertook a systematic investigation of another species of ground squirrel native to the northern Rocky Mountains, Citellus colombianus. These animals did not develop hypercholesterolemia with cholesterol-feeding. However, in the rather dark and frequently cool basement laboratory that we occupied, they seemed to think that winter was coming and many of them rapidly gained weight and had visibly lipemic serum, whether they were eating low-fat or high-fat vegetable diets. In some of them, triglyceride levels reached several thousand mg/dl and they had creamy serum. When the animals were caused to hibernate by placing them in the cold room, they lost weight and their serum triglyceride levels fell rapidly. Some of the lipemic animals had oil red O-positive extracellular deposits in the aorta, coronary arteries, and heart valves, and two of them had masses of foam cells beneath the endothelium, even though they had quite high density lipoprotein (HDL) levels, as estimated by analytical ultracentrifugation.

What struck me was Bragdon's observation that serum triglyceride levels were correlated with the extent of fatty infiltration of the liver, which in one case reached a level of 44% of gross liver weight, and also that both the severity of the fatty liver and the hypertriglyceridemia were correlated with the rate of body weight gain. Joe and I talked then about a junket to Alsace-Lorraine to study the fatty liver and (we assumed) the hyperlipemia of the force-fed goose.

Lipoprotein Synthesis and Secretion

Some may not fully appreciate that in 1953 there was little understanding of the sites or mechanisms of lipoprotein biosynthesis, and we knew essentially nothing about apolipoproteins. Much later, when we did know something about lipoprotein structure and metabolism, I encountered in fetal guinea pigs a phenomenon reminiscent of Bragdon's early observations in ground squirrels. In these fetal animals, which rapidly gain weight during the last part of gestation and are born sufficiently mature that they do not need to suckle to survive, Thomas Bahmer and I studied the transplacental transfer of free fatty acids. We showed that a large fraction of fatty acids derived from maternal adipose tissue is transferred to the fetal liver and esterified to form triglycerides, causing fatty liver and hyperlipemia. By electron microscopy, the secretory vesicles of the Golgi apparatus of hepatocytes were seen to be filled with giant, very low density lipoproteins (VLDL) (the size of chy-
Figure 1. Transmission electron photomicrographs of fetal guinea pig liver at 68 days of gestation. A. Golgi region of hepatocyte, showing dilated Golgi cisternae and secretory vesicles containing lipoprotein particles up to 2000 Å in diameter, × 35,000. B. Portions of two hepatocytes, with extracellular regions between the cells and at the sinusoidal front filled with lipoprotein particles of similar size to those in the Golgi apparatus. Several large fat droplets are present in the cell on the left. Arrow indicates possible point of fusion of secretory vesicle with plasma lemm.

Lipemicrons normally produced after a fat-rich meal) and similar VLDL were seen in the space of Disse (Figure 1); both were comparable to the animals' serum VLDL. We concluded that the hyperlipemia in these animals, in which no large chylomicrons could have been formed, arose from overloading of the liver with maternal fatty acids, which were subsequently transported, as VLDL-triglycerides, to adipose tissue for use by the animals after birth. I was by then convinced that the liver has a tremendous capacity to secrete triglycerides and, like the gut, can make bigger VLDL when this is necessitated by the fat load. It seems to make no difference whether the fat comes directly from the diet, from preformed free fatty acids, or from hepatic lipogenesis when large amounts of carbohydrates are ingested.

In the late 1960s, Robert Hamilton, Albert Jones, and others defined the pathway of synthesis and secretion of VLDL in hepatocytes (Figure 2). The nonpolar cores of the VLDL that the liver secretes usually contain mainly triglycerides. In species that store large amounts of cholesteryl esters in the liver during cholesterol feeding, including rabbits, guinea pigs, some primates, and (with auxiliary measures) rats, the liver then secretes VLDL with cholesteryl ester-enriched cores. Evidently, the liver has the capacity to package and secrete in VLDL whichever nonpolar lipid is available.
Figure 2. Pathway of assembly and secretion of VLDL in hepatocytes. The lipid particle, which is assembled in the smooth endoplasmic reticulum (SER), is thought to acquire its protein components, including apo B which is synthesized in the rough endoplasmic reticulum (RER), at the smooth-surfaced termini of the latter. The nascent VLDL are transported to the Golgi apparatus, where glycosylation of the proteins is completed, and concentrated in secretory vesicles. The latter migrate to the cell surface and fuse with the plasmalemma, releasing the particles into the hepatic sinusoid. (Reprinted from reference 4, with permission of the publisher.)

Figure 3. The composition of the core of VLDL and IDL isolated from blood plasma of guinea pigs is drastically altered by feeding the animals a diet containing 1% cholesterol by weight. In animals fed a low-fat chow, these lipoproteins contain very small amounts of cholesteryl esters (CE). In cholesterol-fed animals, cholesteryl esters replace much of the normally predominant triglycerides (TG) in the core of the particles. The size of VLDL and IDL is not appreciably changed by cholesterol-feeding. (Graph prepared from data in reference 6.)

Hepatic Uptake of Lipoproteins

It has been known since the early 1960s that free fatty acids derived from chylomicron triglycerides, like free fatty acids derived from adipose tissue, can be esterified to form triglycerides in the liver and then exported in VLDL. Dietary cholesterol, however, enters the liver with chylomicron remnants. The early work of Paul Nestel, working with me in San Francisco, and the later studies of Emmett Bergman in sheep and dogs and those of Trevor Redgrave, who first isolated chylomicron remnants from functionally eviscerated rats, defined the two-step pathway of chylomicron metabolism and showed that the liver is the major and obligatory site of uptake of dietary cholesterol. Subsequently, it was found that hepatic VLDL are also metabolized by an analogous pathway, the outlines of which had become apparent by 1974 (Figure 4). At the same time, it became apparent that lipoproteins can be catabolized by receptor-dependent endocytosis as a result of the discovery of the LDL receptor by Michael Brown and Joseph Goldstein. We also knew that not all VLDL remnants enter the liver, but that some are further processed to form low density lipoproteins (LDL). Finally, we knew that one of the major differences between VLDL remnants, which Ole Mjos isolated from functionally eviscerated rats, and LDL was that LDL lack the arginine-rich protein discovered by Virgie and Bernard Shore, now known as apolipoprotein E. Some of the other features of VLDL metabolism, which are analogous to those of chylomicrons, including the acquisition of the cofactor protein for lipoprotein lipase (apo C-II) and other C apoproteins from HDL, are also shown in this 1974 model.

Figure 4. This 1974 model of VLDL metabolism has proven to be generally correct. Nascent VLDL contain apo B (○), apo E (▲), here called hi ARG, and C apoproteins (△). The role of apo C-II as a cofactor for lipoprotein lipase was then known, as was that of HDL as a reservoir for C apoproteins, but the general role of C apoproteins in preventing the premature uptake of nascent VLDL prior to formation of remnant particles, was discovered later; so was the function of apo E as a ligand for lipoprotein receptors and the definitive demonstration that VLDL remnants are taken up into hepatocytes by receptor-mediated endocytosis. (Reprinted from reference 10, with permission of the publisher.)
It is now appreciated that the uptake of chylomicron remnants by the liver is a receptor-mediated, endocytic event and that apo E has a crucial role in this process. We know that:

1. HDL containing only apo E compete for uptake of chylomicron remnants in perfused rat livers.
2. The addition of apo E to chylomicrons from estradiol-treated rats increases their uptake into perfused rat livers.
3. Particles resembling chylomicron remnants (which contain apo B-48) accumulate in the blood plasma of persons with familial dysbetalipoproteinemia.
4. The removal of apo B-48 from plasma is grossly impaired in persons with familial dysbetalipoproteinemia caused by at least two mutations of apo E.

A modern version of chylomicron metabolism is shown in Figure 5. Several years ago in his Duff lecture, Donald Zilversmit presented evidence that chylomicron remnants, enriched in cholesterol, are responsible for the hypercholesterolemia of cholesterol-fed rabbits. His subsequent studies with Katherine Thompson have shown that the particles that accumulate probably represent remnants of VLDL, secreted from the cholesterol-loaded livers of these animals. They found that chylomicron remnants are removed normally by perfused livers of cholesterol-fed rabbits, whereas the cholesterol-rich particles that accumulate in their plasma are removed slowly.

The nature of the particles that accumulate in cholesterol-fed animals can also be evaluated by determining the species of apo B that they contain. Jean-Louis Vigne and I (Vigne JL, Havel RJ, unpublished data) have found that mesenteric lymph chylomicrons from both chow-fed and cholesterol-fed rabbits contain apo B-48, the form of apo B secreted from the intestine of rats and humans. By contrast, the cholesterol-enriched VLDL of nonfasted, cholesterol-fed rabbits, like the VLDL secreted from their perfused livers, contain predominantly apo B-100 at all times after cholesterol feeding (Figure 6). The accumulation of cholesterol in the liver evidently has two effects: 1) secretion of cholesterol-enriched VLDL; and 2) down-regulation of hepatic LDL receptors. Both of these contribute to the accumulation of cholesterol in the blood. Only in the alloxan-diabetic, cholesterol-fed rabbit, a model introduced by Duff and McMillan do triglyceride-rich particles that contain mainly apo B-48 accumulate in appreciable amounts (Vigne JL, Havel RJ, unpublished data). These large particles are probably those that Duff

**Figure 5.** Chylomicron pathway. Chylomicrons secreted from absorptive cells of the small intestine transport dietary triglycerides (gray region of particle) and cholesterol, as cholesteryl esters (darker region of particle). Most of the triglycerides are hydrolyzed in extrahepatic tissues by lipoprotein lipase. The resulting remnant particles, depleted of triglycerides and most surface lipids and proteins (but retaining the cholesteryl esters, apo B-48, and apo E), are taken up into hepatocytes after binding to a chylomicron remnant receptor. Lysosomal catabolism releases the cholesterol, which thereby regulates a number of key processes in this cell that influence lipoprotein biosynthesis and catabolism, including cholesterol synthesis, cholesterol esterification, bile acid synthesis, and the activity of LDL receptors. (Reprinted from reference 21, with permission of the publisher.)
found to be less atherogenic and which Peter Brecher and his associates\(^2\) have shown to have less capacity to induce foam cell formation when incubated with macrophages than the remnant-like VLDL that accumulate in non-diabetic, cholesterol-fed rabbits (in whom lipoprotein lipase is normally active). In the alloxan-treated animals, apo B-100 remains the predominant form of apo B secreted by the liver (Figure 7).

These data in cholesterol-fed rabbits are consistent with those that my colleagues and I in San Francisco have obtained in Watanabe heritable hyperlipidemic (WHHL) rabbits in collaboration with Brown, Goldstein, and their colleagues in Dallas. We found that apo B-100 accumulates in VLDL, intermediate density lipoproteins (IDL), and LDL of WHHL homozygotes, but little apo B-48 is found in these lipoproteins.\(^2\) Although these animals essentially lack functional hepatic LDL receptors, their livers take up chylomicron remnants normally.\(^2\) By contrast, the Dallas group has shown that the uptake of VLDL-protein by the liver is greatly retarded.\(^3\) From these studies and those of others,\(^4\) it is now evident that chylomicron and VLDL remnants are removed by distinct hepatic receptors. Uptake of both forms of remnants seems to depend upon apo E. Anton Stalenhoef, working with our group in San Francisco,\(^5\) has studied the metabolism of apo B-48 and apo B-100 of large triglyceride-rich lipoproteins (400–800 Å in diameter) in patients with familial dysbetalipoproteinemia, who have dysfunctional mutant forms of the protein. He found the removal of both B apoproteins from the blood to be grossly impaired. The defect in the structure of apo E in this genetic disorder thus accounts for the accumulation of both types of remnant in the blood.

As I have noted, the lipoproteins that accumulate in WHHL homozygotes include not only LDL, but appreciable amounts of VLDL and IDL as well. The concentration of apo E, like that of apo B-100, is increased severalfold. These findings, taken together with the metabolic studies that I have described in cholesterol-fed and WHHL rabbits, strongly suggest that the hepatic uptake of VLDL remnants, as well as LDL, is mediated by the LDL receptor. In WHHL homozygotes, LDL accumulate not only because their clearance from the blood is impaired, but also because their formation is increased.\(^6\) Toru Kita and David Bilheimer, working with Brown and Goldstein,\(^7\) have suggested that LDL formation is increased because retained VLDL remnants are
gradually converted to LDL. This conclusion is supported by studies of lipoprotein secretion from the liver of WHHL homoygotes carried out by Conrad Hornick and Kita et al. They found that the rate of accumulation of apo B-100 in perfusates of livers from the mutant animals is not increased; furthermore, virtually all the accumulating apo B-100 was in VLDL and almost none in LDL. It therefore appears that the number of VLDL particles secreted is normal but that fewer VLDL remnants are taken up directly by the liver and more are converted to LDL in the absence of a functional LDL receptor.

In humans, Stalenhoef et al. have found that apo B-100 of large VLDL is removed from the blood almost as rapidly as apo B-48 of chylomicrons; little or none of this apo B-100 is converted to LDL. These observations have led us to conclude that the extent to which VLDL remnants are converted to LDL is a function of particle size. The rapid removal of large VLDL by the liver presumably gives little opportunity for further metabolism to yield LDL.

The rapid removal of large VLDL may be related to the presence of more than one molecule of apo E in each particle (Table 1). A number of years ago, Thomas Innerarity, Robert Pitas, and Robert Mahley concluded that HDL particles that contain several apo E molecules bind to multiple LDL receptor sites on cultured human fibroblasts, leading to higher binding affinity of these particles for the receptor than LDL, which presumably bind to the receptor via a single site on apo B. Later, we observed similar high affinity binding of rat VLDL particles containing several molecules of apo E to LDL receptors on liver membranes. The high affinity of such particles can now be understood in terms of the recently described structure of the receptor protein: it contains seven repeated segments of about 40 amino acids in its cysteine-rich N-terminal region. Each of these segments has a highly negatively charged region that probably constitutes a receptor-binding domain. Thus, rat VLDL particles may bind polyvalently to a single receptor, accounting for the high binding affinity and, presumably, the more efficient removal of such particles from the blood. The rapid removal of human chylomicron remnants from the blood may also be a function of polyvalent binding via multiple molecules of apo E on these particles (Table 1).

Nobuhiro Yamada, working with David Shames and me in San Francisco, has used immunoaffinity chromatography on columns containing anti-apo E to characterize VLDL, their remnants, and LDL in normal rabbits and WHHL homoygotes. We reasoned that VLDL and their remnants, which should contain apo E as well as apo B-100, would be retained on the affinity columns, whereas LDL, which contain only apo B-100, would not. We found that more than one-half of the apo B-100 in plasma of normal rabbits binds to such columns. All fractions (VLDL, IDL, and LDL) contain some particles that do not bind to the columns. The unbound fraction increases with density, but even LDL contain an appreciable portion of particles that contain apo E. Similar findings were obtained with lipoprotein fractions from WHHL homoygotes. We have also used immunoaffinity chromatography and multicomartmental analysis to study the conversion of radioiodinated apo B-100 of VLDL to remnants and LDL in normal and mutant rabbits. In normal rabbits, only a small fraction of VLDL particles (containing apo E) appears to be converted to LDL particles that lack apo E; however, all LDL seem to be derived from VLDL. The fraction of the VLDL converted to LDL is increased in WHHL homoygotes, as predicted from the observations of Kita and his associates. However, the overall rate of removal of apo B-100 of VLDL from the blood considerably exceeds that of IDL or LDL. We have therefore considered the possibility that some VLDL particles in WHHL rabbits, which contain more than one molecule of apo E, are removed by their chylomicron remnant receptor, but we cannot exclude the possibility that a small number of LDL receptors, present on hepatocytes of the mutant animals, mediate the clearance of such particles.

Our kinetic studies in normal rabbits also show that IDL particles, which appear to contain only a single molecule of apo E, are removed slowly from the blood, perhaps as slowly as LDL that contain no apo E. Evidently, particles that contain apo E are not invariably removed from the blood more efficiently than LDL. In normal rabbits, the concentration of remnant-like particles, isolated in the density ranges of IDL and LDL, is almost as great as that of particles that contain apo B-100 but no apo E. The apparently large concentration of VLDL remnants in rabbits (relative to "true" LDL) could be related to the very low activity of the hepatic, heparin-releasable lipase in this species (Frost PH, Havel RJ, unpublished data). This enzyme, which hydrolyzes triglycerides and phospholipids in small triglyceride-rich lipoproteins efficiently in vitro, may be involved in the conversion of VLDL remnants to LDL, accompanied by loss of apo E (Figure 8). In this light, the high concentration of putative VLDL remnants in WHHL homoygotes is not surprising. However, humans with homozygous familial hypercholesterolemia also

Table 1. Apo E Content of Human Plasma Very Low Density Lipoproteins

<table>
<thead>
<tr>
<th>Fraction (diameter, Å)</th>
<th>Normal (% of apo B mass)</th>
<th>Endogenous hyperlipemia (% of apo B mass)</th>
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<tr>
<td>900</td>
<td>75</td>
<td>171</td>
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<tr>
<td>680</td>
<td>43</td>
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<td>520</td>
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<td>420</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>320</td>
<td>12</td>
<td>23</td>
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Calculated from data in reference 35.
The formation of LDL from VLDL may involve two lipolytic steps. The first extrahepatic step, catalyzed by lipoprotein lipase, is well recognized. The second, which clearly involves further loss of triglycerides and phospholipids, as well as proteins other than apo B-100, may be catalyzed by hepatic lipase. The removal of both VLDL remnants and LDL from the blood depends mainly upon the activity of LDL receptors. According to this scheme, LDL formation will be promoted by increased activity of hepatic lipase or reduced activity of hepatic LDL receptors.

have increased levels of IDL and remnant-like VLDL, and elevated concentrations of apo E (up to 30 mg/dl) which are proportional to the concentration of plasma total cholesterol (Havel RJ, Bilheimer DW, unpublished data). Moreover, the conversion of IDL to LDL is slowed in human homozygotes. VLDL remnants may contribute to atherogenesis in receptor-deficient humans and rabbits; therefore, it cannot be assumed that LDL are the sole atherogenic lipoprotein in this situation.

Besides causing elevated concentrations of atherogenic lipoproteins (which can be taken up into the arterial intima), LDL receptor deficiency may contribute to atherogenesis by impeding the process of reverse cholesterol transport. In mammals that have an active cholesteryl ester transfer protein in blood plasma, such as rabbits and humans, the LDL receptor may be a key participant in reverse cholesterol transport. The cholesteryl esters that are carried by VLDL remnants and LDL in these species are derived largely from the action of lecithin-cholesterol acyltransferase. The findings of many investigators, including those of Christopher and Phoebe Fielding, are consistent with the process of reverse cholesterol transport shown in Figure 9. Cholesterol, derived from cell surfaces or plasma lipoproteins, is esterified on a minor species of HDL, from which it is transferred to other lipoproteins, mainly remnants and LDL, that are taken up primarily into the liver. After lysosomal catabolism, the cholesterol can be excreted into the bile. This process seems to be impeded in many hyperlipidemic states, including familial hypercholesterolemia, as a result of impairment of the transfer step. At least in some cases, this abnormality is related to altered composition of the acceptor particles, VLDL and LDL.

Intracellular Processing of Lipoproteins in Hepatocytes

For several years, my associates and I have been studying the intrahepatic processing of remnants and LDL, especially that mediated by the LDL receptor of hepatocytes. Much of this work has been carried out in ethinyl estradiol-treated rats which, as shown by Petri Kovanen and others in Dallas and by Yu-sheng Chao and several associates in San Francisco, express up to 20 times the normal number of LDL receptors on hepatocytes.

The intracellular pathway, defined originally by autoradiographic studies carried out in collaboration with Albert Jones et al., seems to be the same as that found for other ligands taken up into hepatocytes by receptor-mediated endocytosis. In the course of this work, we found that radioiodinated apo B of LDL accumulates in multivesicular bodies at the bile canalicular pole of the cell, close to the Golgi apparatus. We pursued this observation, with a view to determining the possible significance of this topo-
Figure 10. A negatively stained multivesicular body is shown here at high magnification (x 180,000). Rupture of its membrane (black and white arrow) has permitted the phosphotungstate stain to penetrate and outline the contents. These include collapsed internal vesicles (white arrowheads); numerous electron-lucent particles (250–800 Å in diameter), which represent remnants of triglyceride-rich lipoproteins; a few particles about 200 Å in diameter, which may represent LDL, that were injected into the rat from which the organelles were isolated (black arrowheads); and occasional discoidal structures that may represent early stages of lipoprotein degradation (white arrows). This multivesicular body, from the liver of an estradiol-treated rat, is larger than most observed in normal rats (see text). (Reprinted from reference 50, with permission of the publisher.)
graphical localization for recycling of the receptor and degradation of the lipoproteins. We have now iso-
lated multivesicular bodies from hepatocytes of es-
tradiol-treated rats and have determined some of
their properties. In these animals, the organelles
are filled with triglyceride-rich lipoproteins with the
properties of remnants (Figure 10). This observation
is consistent with autoradiographic studies in untreated
rats, in which we have shown that chyomicrons
and VLDL are processed in the liver by the same
pathway found for LDL. The content lipoproteins of
multivesicular bodies are largely degraded; in-
deed, this organelle seems to represent the last pre-
lyosomal way-station in which endocytosed lipopro-
tins accumulate just before acid hydrolases are
delivered from the nearby Golgi apparatus within pri-
mary lysosomes, which fuse with the multivesicular
bodies.

The traffic of chylomicron and VLDL remnants into
multivesicular bodies of rat hepatocytes seems at
first glance to be extraordinary, but our studies of this
organelle are consistent with current knowledge of
lipoprotein catabolism. In the rat, not only virtually all
chyomicron remnants, but more than 90% of VLDL
remnants, are taken up into the liver within minutes
of their secretion. The ability to localize intact
lipoproteins in multivesicular bodies and to isolate
these structures is also clarifying our understanding
of the role of the Golgi apparatus in processing en-
doctyosed macromolecules and of the properties of na-
scent lipoproteins that are delivered to the Golgi ap-
paratus from the endoplasmic reticulum and are finally
concentrated in secretory vesicles. Although the role
of the Golgi apparatus in receptor-recycling
remains to be defined, ligands destined for lysoso-
mal degradation do not themselves enter the Golgi
compartment of the cell. Lipoprotein-filled structures
in the Golgi region of hepatocytes, described by
many investigators, may represent elements of dis-
tinct secretory or endocytic pathways, and we now
recognize that Golgi-rich fractions of livers, as isolat-
ed by common methods, may be seriously contami-
nated by multivesicular bodies.

It is difficult to distinguish secretory vesicles filled
with nascent VLDL from multivesicular bodies filled
with remnant lipoproteins by ordinary electron micro-
scopic methods. Robert Hamilton et al. have used
modified staining techniques that aid this distinction.
Multivesicular bodies, as the name implies, contain a
number of internal bilayer vesicles, about 600 Å in
diameter. In hepatocytes these vesicles are ob-
scured by the content lipoproteins unless the bilayer
is heavily stained. The distinction is easier to make
in livers of estradiol-treated rats because the multi-
vesicular bodies are larger and the Golgi secretory
vesicles smaller than in untreated animals. By use of
a procedure for isolation of "intact" Golgi apparatus
in which the secretory vesicles remain attached to
the flattened cisternae, this organelle can be sepa-
rated from multivesicular bodies. It is thus possible to
isolate and characterize nascent and remnant lipo-
protein populations from the same liver. Application
of these methods promises to provide a new dimen-
sion to our understanding of the processes of hepatic
lipoprotein secretion and catabolism. A current con-
cept of the latter, based upon our studies and those
of investigators who have studied the processing of
other ligands by hepatocytes, is shown in Figure 11.

Role of VLDL Remnants in Atherogenesis

Until recently, it was generally considered that few
VLDL remnants are taken up into the liver as such in
humans. Rather, it was thought that the remnants
(more or less equivalent to intermediate density lipo-
proteins) were first processed to LDL, which are tak-
en up slowly by the liver and other tissues that ex-
press LDL receptors. Recent kinetic studies, how-
ever, suggest that humans may not differ so dras-
tically in this respect from all other mammals,
and that perhaps one-half or more of VLDL may nor-
mally be taken up into the human liver as remnants.

The efficiency of this uptake could be a major deter-
minant of the concentration of atherogenic lipopro-
tins.

Epidemiologic and other clinical observations
have yielded conflicting data concerning the athero-
genicity of VLDL (the concentration of which is close-
ly related to plasma triglyceride levels). In Table 2,1
I have listed some of the structural and metabolic
properties of triglyceride-rich lipoproteins that could
promote their atherogenity. The last, but certainly
not the least, is the activity of hepatic LDL receptors,
which to a substantial extent determines whether
VLDL remnants as well as LDL accumulate in the
blood.

Many years ago, Gofman proposed that small
VLDL and IDL may be more atherogenic than LDL.39
This idea was discounted by a cooperative study of
lipoproteins in patients with coronary heart dis-
ease.56 I do not believe, however, that subsequent
studies, which in the main have measured as "LDL-
cholesterol" the fraction that includes IDL, have
clearly demonstrated that "true LDL" (particles that
contain apo B-100 as the sole protein) are the major
atherogenic lipoprotein in human populations. In an
ongoing intervention study, John Kane, Mary Malloy,
Thomas Ports and I have found that the majority of
asymptomatic familial hypercholesterolemia hetero-
zygotes, whose average age is about 40 years, have
little or no coronary atherosclerosis demonstrable by
angiography. The determinants of premature athero-
sclerosis in these individuals must include factors
other than the concentration of LDL. These could
include additional lipoprotein determinants, such as
the concentration of VLDL remnants.

The role of hepatic LDL receptors in human lipo-
protein catabolism has recently been shown dra-
matically by Bilheimer and his associates in
a young girl with homozygous familial hypercholester-
olemia, in whom the desirable clinical situation re-
quired cardiac transplantation. The child also re-
Figure 11. This diagram, prepared by Robert Hamilton, depicts current concepts of the intracellular processing of endocytosed lipoproteins in hepatocytes. The initial binding occurs at sites on the sinusoidal surface of the cell, from which the receptor-ligand complex moves to a coated pit. Endocytosis ensues and the clathrin coat is rapidly lost. The primary endosomes appear to fuse and then migrate to the bile canalicular pole of the cell, where they are seen as multivesicular bodies (MVB). Primary lysosomes (L¹), derived from the nearby Golgi apparatus, fuse with the multivesicular bodies, converting them to secondary lysosomes (L²), releasing enzymes that hydrolyze the lipid and protein components. The soluble products can then diffuse into the cytoplasm. The membrane of the endosomes, including multivesicular bodies, contains an ATP-drive protein pump, which acidifies the interior of these organelles to a pH of about 5.5. The reduced pH serves to dissociate lipoproteins from the receptors and provides a suitable environment for the action of lysosomal enzymes. After dissociation, the receptor is recycled to the cell surface. The endosomal compartment from which dissociation occurs has been named CURL (compartment of uncoupling of receptor and ligand) and the process of dissociation has been visualized by immunocytochemical techniques for some nonlipoprotein receptors. The precise site of dissociation and the pathway of recycling are poorly defined. BC = bile canaliculus.

Table 2. Triglyceride-Rich Lipoproteins: Potential for Atherogenicity

<table>
<thead>
<tr>
<th>Property</th>
<th>Determinants</th>
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<tr>
<td>Content of cholesteryl esters</td>
<td>Composition of nascent particles, residence time in the blood</td>
</tr>
<tr>
<td>Potential for receptor-mediated endocytosis</td>
<td>Loss of C apoproteins, surface lipid composition, content and reactivity of apo E</td>
</tr>
<tr>
<td>Potential to form LDL</td>
<td>Species of apo B, size/cholesteryl ester content, hepatic lipase activity</td>
</tr>
<tr>
<td>Capacity to accept LCAT-derived cholesteryl esters</td>
<td>Surface cholesterol concentration</td>
</tr>
<tr>
<td>Activity of hepatic vs extrahepatic LDL receptors</td>
<td>Cells' need for cholesterol</td>
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ceived a liver homograft, and has since maintained lipoprotein levels only slightly above normal. Preoperatively, she had grossly elevated levels of IDL as well as LDL and cholesterol-enriched VLDL (Havel RJ, Bilheimer DW, unpublished data).

Conclusions

It is now recognized that the liver has a central role in regulating both the synthesis and the catabolism of the plasma lipoproteins. However, we lack some crucial pieces of information. Although we understand a good deal about the regulation of hepatic triglyceride synthesis and secretion, we still do not know what determines the rate of secretion of VLDL particles (i.e., the rate of synthesis and secretion of apo B). We are aware that hepatic lipoprotein receptors are subject to important regulatory influences related to cholesterol metabolism, but we do not know how the availability of cholesterol to the cell is coupled to receptor activity. We know that hepatic cholesterol homeostasis is regulated in part by biliary secretion of cholesterol and bile acids, but we do not know how these processes are regulated, nor do we understand the basis for the large species differences in the capacity to secrete these substances into the bile in response to the influx of dietary cholesterol in chylomicron remnants. We also know little about the determinants of variation of LDL receptor activity within and among species. Finally, we still do not know whether or how the liver participates in the formation of LDL. These problems pose interesting challenges for investigators of lipid and lipoprotein metabolism. If the accumulation of lipoproteins that contain apo B, with or without accompanying apo E, is as important for atherogenesis as many of us believe, the solution to these problems may be not only interesting, but also quite useful.

References

29. Kitto T, Brown MS, Bilheimer DW, Goldstein JL. Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoprotein in WHHL rabbits. Proc Natl Acad Sci USA 1982;79:5693-5697
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