Atherogenesis: A Postprandial Phenomenon

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SUMMARY The hypothesis that plasma chylomicrons in persons who ingest a cholesterol-rich diet are atherogenic is evaluated. Evidence is presented that in humans, and experimental animals, chylomicron remnants as well as low-density lipoproteins are taken up by arterial cells. In persons who do not have familial hyperlipoproteinemia, atherogenesis may occur during the postprandial period. Research directions that may contribute to the evaluation of chylomicron remnants as a risk factor for atherogenesis are discussed. Lipoprotein studies after administration of a test meal containing fat and cholesterol are urgently needed.

THE VIEW that coronary heart disease, or arteriosclerosis, is a multifactorial disease has always disturbed me—not because it isn’t important to uncover the conditions that aggravate this disease, but because as a basis for scientific inquiry I feel that it may be a very long time before we can formulate a model that will be both scientifically sound as well as aesthetically pleasing.

In some way nearly every process around us is multifactorial. Even a simple discussion about the weight of a gas can be conducted in multifactorial terms. The weight depends, of course, on its volume, pressure, temperature, molecular weight and the gravitational force. Even in this simple analysis at least five factors determine the outcome of the inquiry. If we analyzed the question in terms of molecular, atomic or subatomic physics the number of factors involved in answering the inquiry would probably be increased manifold.

Therefore, we should not be discouraged by the multitude of possible etiological and risk factors that compete for priority status. It is highly unlikely that we shall have to control each and every one of them to prevent the clinical manifestations of arteriosclerosis.

However, it is still not possible to present a unitary view of the atherogenic process. Consequently, this presentation deals only with selected early manifestations of arterial pathology, i.e., lipid accumulation, and lends more heavily on my own work than is warranted by its importance. I shall, however, synthesize our findings with those of others in support of the view that arterial lipid accumulates as the result not only of abnormally high concentrations of low-density lipoproteins (LDL) in the blood plasma, but also as a consequence of the normal process of lipid absorption and transport. This normal process may be innocuous in persons who ingest low-fat diets, but is probably pathogenic in those who consume a diet rich in fat and cholesterol.

Lipid and Atherosclerosis

Cholesterol and Atherosclerosis

Most scientists working in the atherosclerosis area see cholesterol as a pivotal element in the etiology of cardiovascular disease. At least five areas of research contribute to this view. In 1910, Windaus described the presence of cholesterol in the lesions of diseased arteries. Since then, many studies have confirmed that free and esterified cholesterol accumulate in the aorta, coronary arteries, cerebral vessels and other large arteries at different rates in different persons or in different population groups. In persons with premature, genetically determined hypercholesterolemia, arterial cholesterol accumulates more rapidly and clinical manifestations of cardiovascular disease appear earlier in life. Epidemiological studies have repeatedly shown high degrees of correlation between the intake of cholesterol and other lipids with the prevalence of coronary heart disease in various populations. Animal studies show, almost without exception, that experimentally induced hypercholesterolemia is followed by the accumulation of cholesterol in the large arteries, and that under certain conditions even more advanced manifestations of arteriosclerosis are produced. Finally, the more recent work on cultured cells shows that certain lipoproteins from normal or from hypercholesterolemic serum are rapidly taken up by arterial smooth muscle and endothelial cells and by skin fibroblasts.

Low-density Lipoproteins and Atherosclerosis

If we now examine the basis for the assertion that LDLs (1.019 < d < 1.063) are causative in atherogenesis we see a similar set of arguments. The LDL is the principal carrier of cholesterol in the plasma of persons with plasma cholesterol concentrations higher than 150 mg/dl. The apoprotein of

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LDL, apo-B, is found in the arterial lesion. Familial hyperbetalipoproteinemia, in which high levels of LDL are present prematurely, is associated with premature atherosclerosis and premature coronary heart disease. LDLs are usually elevated in experimental animals that eat atherogenic diets. Finally, smooth muscle cells and skin fibroblasts exposed to lipoprotein-deficient serum in culture become sensitized to the effect of LDLs. These effects include the binding, ingestion and degradation of these lipoproteins and the regulatory effects of these lipoproteins on cholesterol biosynthesis, cholesterol esterification and regeneration of LDL receptors.

Very Low Density Lipoproteins and Atherosclerosis

The role of very low density lipoproteins (VLDL) in atherogenesis is less well established. However, some epidemiological studies have shown significant correlations between hypertriglyceridaemia and coronary heart disease. The apoproteins characteristic of VLDL (apo-C and apo-B) have been found in human arterial lesions. In hypertriglyceridaemic patients apo-B and apo-C of surgically removed samples of aortas and iliac arteries contained twice as much of these apoproteins as in samples from normolipidaemic patients. Plasma VLDL is the precursor of plasma LDL, and some of the atherogenic properties that I shall ascribe to chylomicrons probably also apply to VLDL.

Chylomicrons and Atherosclerosis

The idea that chylomicrons could be atherogenic appears to have been largely overlooked. Although dietary cholesterol and fat, particularly saturated fat, have been designated as atherogenic factors, their role was thought to consist of modifying serum cholesterol concentrations. Furthermore, the few persons identified as hyperchylomicronemics did not have premature clinical signs of atherosclerosis. Yet the fact that chylomicrons are the principal carriers of dietary cholesterol in the bloodstream, and that the first stage of their degradation takes place in contact with the vascular endothelium, led me to examine the possibility that chylomicrons per se might be atherogenic. In 1973 I attempted to reinterpret a variety of experimental studies based on the hypothesis that the interaction of triglyceride-rich lipoproteins with arterial lipoprotein lipase constitutes an atherogenic process. The process is assumed to involve the binding of chylomicrons to the arterial surface, possibly to subendothelial sites exposed by local loss of endothelium, the hydrolysis of triglyceride by arterial lipoprotein lipase and the subsequent internalization of cholesterol-enriched chylomicron remnants by the arterial smooth muscle cells. A somewhat similar view has been expressed by Hülsmann and Jansen. In the following sections I will review only those aspects of lipoprotein metabolism that bring the evidence up to date, and will suggest additional experiments of how the validity of this hypothesis might be tested.

Lipoprotein Metabolism

Interconversions of Lipoproteins

The various lipoproteins of the blood plasma are probably primarily derived from two sources: the liver and the intestine. Both of these organs produce triglyceride-rich lipoproteins. The liver secretes VLDLs in response to a caloric load, whereas the intestine responds more specifically to the presence of dietary fat by secreting chylomicrons. Both lipoproteins are rich in triglycerides, but also contain phospholipids, free and esterified cholesterol and a variety of proteins. Only the apo-B protein appears to be an integral part of the lipoprotein structure, and the other proteins and lipids appear to be subject to exchange or to a net transfer to other lipoprotein complexes. Thus, free cholesterol exchanges readily between all lipoprotein species and even between lipoproteins and cell membranes. Esterified cholesterol, on the other hand, appears to require one or more plasma proteins to accomplish an exchange or net transfer. Exchange of phospholipids is also accelerated by a protein in the d > 1.21 plasma fraction. Apo-A proteins, which may be largely derived from the intestine, apo-E or arginine-rich protein from liver, as well as the apo-Cs, appear to move freely from one lipoprotein to another. Some of these apoproteins are cofactors in the degradation of triglyceride-rich lipoproteins and then appear to be delivered to the higher density fractions in order to be used again by a reciprocal transfer to the newly secreted VLDL or chylomicrons.

The precursor-product relationship between VLDL and LDL has been known for some time, but the quantitative aspects in human subjects were determined first by Sigurdsson et al. In normal subjects the interaction of VLDL with lipoprotein lipase appears to give rise to an intermediate density fraction (IDL) which in turn is degraded by a less well established route to LDL. In normolipidaemic persons the flux of apo-B through these three fractions is approximately equal, indicating quantitative conversions of apo-B from VLDL to IDL and then to LDL. In at least some hypertriglyceridaemic subjects, on the other hand, the flux of apo-B in VLDL exceeds that in the LDL fraction, suggesting that a portion of the VLDL or IDL (S₂ 20–60) may be removed by an additional pathway, possibly by the liver.

Chylomicron Clearance

The sequence of events in the conversion of VLDL to LDL and the possible removal of excess IDL from plasma in hypertriglyceridaemic states seems to have a parallel in the metabolism of chylomicrons. These lipoproteins, carried to the blood via the thoracic duct, interact with the vascular endothelium, where their triglyceride is largely removed by lipolysis. In normal human subjects the half-time for chylomicron removal is 4.5 minutes, which is much faster than that for endogenously produced VLDL. The residual lipoprotein,
or remnant, which still contains some triglyceride, is largely removed by the liver in rats, dogs and sheep. If chylomicrons are loaded with cholesteryl ester derived from dietary cholesterol, the remnants are similarly enriched in this lipid fraction.

During the degradation of chylomicrons by lipoprotein lipase, marked changes in surface chemistry occur. The apo-C proteins, which are transferred from the high-density lipoprotein (HDL) fraction to chylomicrons entering the circulatory system, are gradually removed during the lipolytic stage. Surface phospholipids are partially lost by conversion to lysophospholipids or by transfer to HDL. The fate of the apo-B moiety of the remnant is less well established. Although in normal subjects the apo-B of endogenously produced VLDL is largely converted to LDL, little of the chylomicron apo-B seems to end up in the LDL fraction.

Chylomicron Remnant-Liver Interactions

We do not know how the liver differentiates between chylomicrons and chylomicron remnants. Clearly, however, chylomicrons are not readily taken up by the perfused rat liver or by rat liver hepatocytes. Remnants, on the other hand, whether produced by hepatectomized rats, perfused hearts or by soluble lipoprotein lipase, are rapidly ingested by liver cells. Cholesterol uptake from chylomicron remnants is much faster than that from LDL and appears to be a saturable process. Binding of remnants to liver plasma membranes appears not to be inhibited by VLDL and LDL but is reduced in the presence of both chylomicrons and HDL. Carrella and Cooper reported that the binding of chylomicron remnants in the presence of heparin is reduced, but Florén and Nilsson find that preincubation of hepatocytes with heparin increases remnant cholesteryl ester uptake.

Although cholesteryl esters were once thought to be hydrolyzed before entry into hepatocytes, more recent evidence does not support this view. Nilsson failed to find a cholesteryl ester hydrolase in plasma membranes, but found the lysosomal hydrolases to be quite active toward cholesteryl ester and triglycerides. Florén and Nilsson reported an inhibitory effect of chloroquine on cholesteryl ester hydrolysis, indicating that remnant cholesteryl ester may be largely hydrolyzed in lysosomes. The inhibition of remnant cholesteryl ester hydrolysis in the intact hepatocyte by colchicine supports the view that intracellular transport of the remnant may be an essential step in the catabolism of this lipoprotein fragment. Although cholesterol feeding in the rat does not seem to impair the remnant removal mechanisms, this is not the case in the rabbit.

The uptake and metabolism of cholesterol-containing chylomicron remnants by the liver of the intact rat suppresses cholesterol biosynthesis, while LDL or other serum lipoproteins have little or no inhibitory effects. It is interesting, in this connection, that the d < 1.063 of cholesterol-fed rats suppresses cholesterol biosynthesis, which might be taken as evidence that this fraction contains lipoprotein particles of intestinal origin, just as in the cholesterol-fed rabbit.

Hypercholesterolemia from Intestinal Lipoproteins

Studies in Rabbits

The fact that the rabbit responds to cholesterol feeding with massive hypercholesterolemia has been known since the early 1900s. Until recently, however, the cholesterol-laden lipoproteins were assumed to be synthesized primarily in the liver. This was probably a result of the observation that the excess of plasma cholesterol was found predominantly in the VLDL fraction, and the knowledge that in the normal state VLDL is derived mostly from liver. It was assumed that in the cholesterol-fed animal a portion of the excess dietary cholesterol was stored in the liver and that the VLDL secreted by this organ thus became overloaded with cholesterol.

The differentiation between chylomicron remnants and VLDL produced by liver is difficult, because of overlapping sizes and density ranges. Therefore, we devised a technique of labeling rabbit thoracic duct chylomicrons with dietary radioactive retinol. This label, which is incorporated in the chylomicron fraction primarily in the esterified form, remains with the chylomicrons through their degradation to remnants and subsequent uptake by the liver. In the liver the retinyl ester is hydrolyzed and the labeled retinol resecreted in the d > 1.21 fraction. That is, in retinol-fed animals, chylomicron remnants contain labeled retinyl ester, and VLDL produced by liver does not. Figure 1 shows that upon feeding [14C] retinol to a control rabbit and a rabbit fed 1% cholesterol, the control animal contained most of the radioactivity in the d > 1.21 fraction, while the cholesterol-fed rabbit showed most of the label in the VLDL fraction up to 24 hours after administration of the labeled retinol.

When thoracic duct chylomicrons labeled with retinyl ester were injected intravenously into control (fig. 2A) and cholesterol-fed (fig. 2B) recipients, the difference in the metabolism of three VLDL subfractions was immediately evident. In the control animal every VLDL fraction, from the largest particle size (VLDL1) to the smallest (VLDL3), was rapidly removed. In the cholesterol-fed animal, however, the conversion of VLDL1 to VLDL2 and VLDL3 was delayed, and the removal was retarded. This retardation is not related to a slowing of the lipolytic phase of chylomicron degradation. Figure 3 shows that triglyceride disappearance from the bloodstream differed little in cholesterol-fed and control animals, whereas the disappearance of the retinyl ester and cholesteryl ester portions of the chylomicron were greatly retarded. Apparently, a partial blockade exists in removal of cholesteryl ester-laden remnants from the circulation. We do not know if this is caused by a liver malfunction or if compositional differences in
and liver was measured during the following 50 minutes. The top panel in figure 4 shows the curves for total plasma and the middle panel those for labeled retinol and retinyl ester in total liver. Addition of the plasma and liver curves for each animal (lower panel) shows a V-shaped complex of data that appears to be the same for control and cholesterol-fed rabbits. The initial negative slope of this curve represents the rapid clearance of injected chylomicrons. The upswing can be interpreted as the release of remnants from the vascular surfaces. If one assumes that this release is completed within the 50-minute experimental period and that no extrahepatic uptake of chylomicron remnants occurs, the curve should have returned to the initial 100% of dose level. Instead, the curve in panel C rose to about the 60% level, suggesting that an appreciable portion of chylomicron remnant core lipid may have been removed extrahepatically. This interpretation is supported by our observation that in hepatectomized rabbits as much as 43% of chylomicron retinyl and cholesterol esters disappears from the circulation in 1 hour.38 Similarly, Suri et al.36 observed a 50% loss of VLDL protein and cholesterol from the circulation of supradiaphragmatic rats.

The conclusion that the upward swing of the labeled retinol curve represents reentry of chylomicron remnants into the circulatory system is supported by a similar resurgence in the plasma VLDL cholesterol-specific activity curves of rabbits dosed intravenously with cholesterol-labeled chylomicrons.38 From these specific activity curves it is possible to estimate how much of the excess VLDL cholesterol in the plasma of cholesterol-fed rabbits is accounted for by chylomicron remnants. In rabbits fed cholesterol for only 4 days, at least two-thirds of the VLDL cholesterol appears to be a result of chylomicron remnants.38 After more prolonged cholesterol feeding this proportion may be higher. Thus, in an animal species that develops arterial lesions quite rapidly in response to cholesterol feeding, the predominant cholesterol-containing lipoprotein in plasma is composed of chylomicron remnants.

FIGURE 1. Distribution of orally administered [14C]retinyl acetate in plasma lipoproteins of control and cholesterol-fed rabbits. Cholesterol (1% of diet) was fed for 17 weeks before isotope dose which was given mixed with a portion of the appropriate diet at time zero. (Unpublished observations: Simmons D, Zilversmit DB).

The remnants account for the impaired removal process.57-59

One other feature of the chylomicron and remnant removal process is illustrated in figure 4. In this experiment, retinyl ester-labeled, cholesterol-enriched chylomicrons were injected intravenously in control rabbits and in rabbits fed cholesterol for 4 days, and the amount of labeled retinol in total plasma volume

FIGURE 2. Distribution of chylomicron [14H]retinyl ester in large (O), middle-sized (∆) and small (□) plasma VLDL particles. [14H]retinol was fed to a donor rabbit and thoracic duct lymph chylomicrons from this donor were injected at time zero in a control and in a cholesterol-fed recipient animal while each was absorbing its appropriate diet.38
Support for the above conclusions is also obtained from a simple cholesterol-feeding experiment. When normal rabbits are given a single meal with 500 mg of cholesterol, the VLDL cholesterol begins to rise within the first few hours (fig. 5). The VLDL level does not return to baseline levels for 24 hours and a second cholesterol-containing meal causes an additional rapid upswing in VLDL cholesterol levels. In our studies, as well as those of others, the VLDL, when subjected to agarose electrophoresis, shows a β mobility. Delipidated VLDL shows a high apo-E content on polyacrylamide gels. These two characteristics are shared with VLDL from patients with broad β disease and with other animal species fed cholesterol-containing diets. Turnover studies of iodine-labeled VLDL appear to be contradictory and suffer from technical faults that make comparisons of the conclusions hazardous. A recent study on LDL apoprotein turnover in rabbits that within 24-48 hours of cholesterol feeding the fractional catabolic rate of this protein decreases to one-fourth of its original value.

Studies in Primates: Human and Nonhuman

The abnormal composition of VLDL in patients with broad β disease (type III hyperlipoproteinemia) has been well established. It appears that the VLDL particles in this condition are spherical, and that cholesteryl ester has largely replaced the triglyceride normally present in the core of the lipoprotein complex. Much of this lipoprotein is found in an intermediate density range (IDL 1.006 < d < 1.019) and is further characterized by a high apo-E content. A study with labeled retinol, similar to that in cholesterol-fed rabbits, shows a retarded clearance of chylomicron remnants. The reasons for the slower clearance are not understood, although altered interaction of surface components with lipases has been postulated. In metabolic studies with iodine-labeled VLDL, an impaired conversion of VLDL to LDL apo-B was observed which appeared to be reversible by estrogen administration.

Impaired remnant clearance may not be the only defect in type III hyperlipoproteinemia, because, in one set of patients, a higher apo-B synthesis was observed. Whatever the mechanisms involved in the etiology of the type III phenotype, patients with this lipoprotein pattern suffer prematurely from ischemic heart and peripheral vascular disease. Topping et al. suggested that the peripheral vascular disease in cigarette smokers may be related to impaired hepatic metabolism of chylomicron remnant, because in perfused livers carbon monoxide appears to reduce chylomicron remnant uptake.

Cholesterol feeding of Patas monkeys produces
lipoprotein alterations that have some resemblance to those seen in type III persons and in cholesterol-fed rabbits. Rhesus monkeys respond to cholesterol feeding with a marked increase in cholesteryl ester-enriched VLDL and IDL. In the LDL fraction an increase in molecular weight, higher cholesteryl ester and lower triglyceride contents have been observed. Similar findings have been reported for M. fascicu-

\[\text{Figure 4. Distribution of labeled retinol in total blood plasma and liver after injection of retinyl ester-labeled chylomicrons.} \]

Recipient rabbits were either controls (serum cholesterol 54 mg/dl) or were fed cholesterol for 4 or 15 days with serum cholesterol concentrations of 192 and 658 mg/dl, respectively. Panel A shows the percent of injected \(^{3}H\)retinol in total plasma volume; panel B shows percent of injected \(^{3}H\) in liver, and panel C shows the sum of \(^{3}H\) in plasma and liver for each animal.

\[\text{Figure 5. Plasma cholesterol in the d < 1.006 fraction of rabbits fed commercial diet supplemented with 500 mg of cholesterol. Time of feeding is indicated by arrows.}\]

\(\text{laris,}^{84}\) in which the molecular weight of the LDL correlated better with coronary atherosclerosis than did the LDL concentration.

Other Animal Species

Even though there is a wide discrepancy between animal species in their response to atherogenic diets, several investigators have produced hypercholesterolemia by diet and/or drug treatments. Mahley et al. studied a large number of lipoprotein changes in dogs treated with cholesterol, bile acids and a thyroid inhibitor.\(^{85}\) These authors suggest that in the hyperresponders the \(\beta\)-migrating VLDL, which contain most of the excess cholesterol, are probably chyomicron remnants. By a similar treatment of rats, \(\beta\)-VLDL with high cholesteryl ester content and elevated apo-E are observed.\(^{86}\) Defective remnant clearance also appears to occur in hypothyroid hypercholesterolemic rats, in which chylomicron cholesteryl ester disappears more slowly than in the controls, while triglyceride clearance is normal.\(^{87}\) Triglyceride clearance of cholesterol-enriched chylomicrons by the perfused rat heart was also unaltered.\(^{88}\)

More than 20 years ago Gidez et al. showed an in-
crease in IDL cholesteryl ester of rats fed 1% cholesterol and 10% olive oil. They noted that oleate accounted for 85% of these cholesteryl ester fatty acids and they concluded that, since cholesteryl ester of control rats contained relatively more polyunsaturated fatty acid, the IDL of the hypercholesterolemic animals were largely derived from liver rather than from the lecithin-cholesterol acyltransferase reaction. Now, it seems likely that the high percentage of cholesteryl IDL might signify an intestinal origin of the IDL fraction, particularly since it has long been known that hypercholesterolemic rabbits have relatively high levels of oleate in their plasma.

There is one additional argument for the intestinal origin of at least some of the lower-density lipoproteins in hypercholesterolemic rat serum. Breslow et al. 92 and Nervi and Dietschy 94 observed that normal rat β lipoprotein (LDL) showed little or no suppression of HMG CoA reductase in hepatocytes and intact liver, respectively. In contrast, the β-migrating lower-density fractions from hypercholesterolemic rat plasma were as effective in suppressing cholesterol biosynthesis as lipoproteins of intestinal lymph, suggesting that these plasma lipoproteins are of intestinal origin.

An additional species in which β-VLDL is found is the cholesterol-fed swine. 91, 92 in which the composition of VLDL resembles that of type III individuals, showing elevated cholesterol ester and apo-E contents with reduced triglyceride. 91 In cholesterol-fed swine 93 with serum cholesterol concentrations of no more than 100 mg/dl, the mitotic index of arterial endothelium, as measured by [3H]-thymidine labeling, increases two- or threefold within 3 days of cholesterol feeding. This suggests that the proliferative factor may well be related to an early product of fat absorption, such as the cholesterol-enriched chylomicron remnant.

**Remnant Formation on Arterial Surfaces**

It is usually assumed that plasma LDL is taken up by the arterial wall by filtration, although uptake by an LDL receptor mechanism also seems possible. 7 The apoprotein of LDL (apo-B) has been identified in diffuse intimal thickening of human arteries as well as in fibrotic plaques. 8-10 More recently, apoproteins characteristic of VLDL and chylomicrons (apo-B and apo-C) have been identified in lesions of human arteries. 8, 9 Although arterial apo-C could have been derived from HDL, the similarity in spatial distributions of apo-B and apo-C suggests that VLDL or chylomicrons may have been the source of these apoproteins. In this section I shall present circumstantial evidence that chylomicrons can be converted to remnants at the arterial surface and that remnants can be incorporated into the intima by endocytosis. 84 Scow et al. 86 have presented evidence of the adsorption of chylomicrons to vascular endothelium, followed by degradation of most of their triglyceride. This process appears to take place in most capillary beds, and could take place in arterial endothelium or smooth muscle cells if these cells contained lipoprotein lipase. Several authors have suggested the presence of this lipase in the arteries of rat, 96 pig, 97, 98 cow, 99 and rabbit. 101 Thus, chylomicrons could be degraded to remnants at, or in, the arterial intima, possibly in areas in which local injury factors had removed or loosened the endothelial layer. Robertson has shown that small chylomicrons (remnants?) adhere near arterial branchpoints when rats are injected with angiotensin II or other vasoactive agents. 102 In perfused rabbit aortas, the uptake of intact triglyceride from chylomicrons has been reported, 103 but it is unlikely that this represents internalization of intact chylomicrons by arterial cells. There appears to be a parallel between the lipoprotein lipase content of an artery and the extent of atheromatosis. When rabbits were fed 0.25-0.50% cholesterol for 2-4 months (fig. 6), the aortic lipoprotein lipase increased in proportion to the aortic cholesterol content. 101 Dissection of the lesioned areas revealed that the increments of lipoprotein lipase were located in the raised fatty lesions. This association of lipoprotein lipase with vascular lipid does not necessarily mean that the increased lipase promoted the lipid accumulation; the lipoprotein lipase may have been brought in as part of the chylomicron remnants. Figure 7, a composite of the information in figure 6 and the previously demonstrated relation between aortic cholesterol content and plasma cholesterol flux into the artery, shows a correlation between arterial lipoprotein lipase and cholesteryl ester influx ($r = 0.9$).

Previously, I have cited studies reporting that the parenteral administration of sulfated mucopolysaccharides reduced the extent of atheromatosis in experimental animals. 18 Since then, Sirtori et al. 104 have shown that the infusion of a mucopolysaccharide of duodenal origin reduces the uptake of labeled VLDL by the aorta of hypercholesterolemic rabbits. In addition, Fielding 105 has demonstrated a reduced uptake of chylomicron cholesteryl ester by the rat heart preperfused with heparin. Such an inhibition could be due to a displacement of lipoprotein lipase from the artery, or could be the result of reduced chylomicron remnant binding to the arterial surface.

In view of this discussion, one might ask why patients with hyperchylomicronemia (type I hyperlipoproteinemia) do not show manifestations of premature coronary heart disease. 8 The explanation may be that these patients show an impaired conversion of chylomicrons to chylomicron remnants. 8 Since it is the remnant, rather than the intact chylomicron, that appears to be taken up by smooth muscle cells, it is not surprising that arteriosclerosis is not a problem in these patients.

The relationship between lipoprotein lipase and atherogenesis has two aspects: 1) lipoprotein lipase activity in the arterial wall may be atherogenic because the lipase stimulates the degradation of chylomicrons adsorbed to the arterial surface, and 2) lipoprotein lipase activity in other vascular beds promotes the formation of circulating remnants which may subsequently be taken up by the arterial wall. Neither one of these aspects may be directly related to lipase ac-
tivity in postheparin plasma, and no conclusions about the atherogenic potential of this lipase activity can be drawn at present.

**Arterial Uptake of Circulating Remnants**

The uptake of chyomicron remnants by the arterial wall could also take place by adsorption of circulating remnants to the arterial surface followed by endocytosis. Although various published reports refer to the atherogenic properties of IDL or $S_r > 20$ fractions and to their avid uptake by a variety of cultured cells, one can only assume that in some, if not all, instances these lipoproteins represent chyomicron or VLDL remnants.

Arterial smooth muscle cells of the rat take up VLDL remnants rapidly and degrade them relatively poorly. Human arterial smooth muscle cells bind and incorporate type III IDL better than LDL. As mentioned previously, the IDL of these patients is largely composed of remnants. Medial smooth muscle cells of rhesus arteries show more cholesteryl ester accumulation when exposed to large LDL derived from hypercholesterolemic serum than when in contact with control LDL. Rabbit aortic medial cells show a similar preference. In human subjects the lipoproteins of the $S_r$ of 20–60 variety are as effective as LDL in suppressing HMG CoA reductase of human fibroblasts, which is
evidence of their uptake by these cells.\textsuperscript{111} The larger VLDLs do not suppress the enzyme, presumably because they are not ingested or degraded. Recently Schönfeld and Pfleger\textsuperscript{112} presented evidence that human VLDL, partially degraded by milk lipoprotein lipase, was a better substrate than the original VLDL for binding and internalization by human fibroblasts.

Further evidence that chylomicron remnants interact with large arteries is the observation that lipoprotein complexing factor, a glycoprotein from rabbit aorta, does not bind normal rabbit LDL, but does interact with rabbit LDL from cholesterol-fed rabbits. This same factor interacts more strongly with human LDL fractions of higher cholesteryl ester contents than with normal LDL.\textsuperscript{113}

\textbf{Remnant Cholesterol Uptake by Other Tissues}

When chylomicrons are injected into glucose-fed rats in which adipose tissue lipoprotein lipase is at high levels, the uptake of chylomicron cholesterol by adipose tissue is twice as high as the uptake in fasting rats, in which lipoprotein lipase levels are known to be low.\textsuperscript{114} Similarly, in mammary tissue the uptake of chylomicron cholesterol is increased in hypercholesterolemic guinea pigs\textsuperscript{115} and shows a parallelism to mammary lipoprotein lipase levels in the rat.\textsuperscript{116, 117} The uptake of rat VLDL remnants by the perfused rat heart is followed by their near-complete catabolism without formation of LDL. In contrast, under the same conditions, LDL was produced from human VLDL.\textsuperscript{118} Fielding\textsuperscript{96} studied the quantitative aspects of chylomicron cholesterol and triglyceride uptake by the perfused rat heart. While 80\% of the triglyceride was removed from the perfusate, 50\% of the chylomicron cholesteryl ester was acquired by the myocardium. The uptake of cholesteryl ester appeared to be nonsaturable and was not inhibited by LDL, but was decreased by HDL. The uptake of cholesteryl ester increased in proportion to the cholesteryl ester content of the chylomicrons. This means that uptake of chylomicron cholesteryl ester by heart tissue would be expected to increase when dietary cholesterol intake is high. In functionally hepatectomized rats some loss of circulating chylomicron cholesteryl ester to extracellular tissues was observed. This is in accordance with our observation that chylomicron cholesteryl ester is partially cleared from the circulation of hepatectomized rabbits.\textsuperscript{98}

\textbf{Relative Atherogenicity of Chylomicron Remnants and LDL}

One might now ask whether chylomicron remnants are as atherogenic as LDL. Recent experiments in our laboratory indicate that they are.\textsuperscript{119} Paired rabbits were fed either a cholesterol-free, low-fat semisynthetic diet, or rabbit chow plus cholesterol in order to match their serum cholesterol concentrations. Both groups developed hypercholesterolemia in the range of 300–600 mg/dl. After 4 weeks of feeding the two diets, the semisynthetic group carried most of its cholesterol as LDL, and the cholesterol-fed group showed mostly VLDL and IDL (fig. 8). At later intervals the differences in lipoprotein patterns, although less pronounced, were maintained. Both groups showed VLDL and IDL compositions in which more cholesteryl ester was present than in the corresponding lipoproteins from control animals. Both groups showed a broad β-migrating lipoprotein band on agarose electrophoresis. Aortic atherosclerosis scores, aortic cholesterol analyses, and histological examinations showed extensive atherosclerosis with no difference between the semisynthetic diet and cholesterol-fed groups. As was shown earlier, the hypercholesterolemia of the cholesterol-fed rabbits is largely due to chylomicron remnants,\textsuperscript{98} whereas the animals fed cholesterol-free, low-fat diets show elevated levels of lipoproteins of endogenous origin. These findings appear to show that cholesterol of exogenous and endogenous origin are equally atherogenic and that, at a given serum cholesterol level, chylomicron remnants contribute as much to arterial lipidosis as do endogenous LDLs and VLDLs.

\textbf{Conclusions and Implications}

I have presented data supporting the view that cholesterol-loaded chylomicrons are atherogenic. Other evidence has been summarized previously.\textsuperscript{18, 94} Although I have emphasized the atherogenic potential of chylomicrons, many of the same arguments would apply equally well to atherogenicity of VLDL produced by liver and intestine. The mechanisms whereby chylomicron or VLDL remnants could provide the building blocks for arterial lesions are twofold:

1) Circulating chylomicron or VLDL remnants may bind and penetrate the arterial surface just like plasma LDLs; and 2) chylomicrons or VLDL may be adsorbed and then degraded to remnants at the arterial surface.

If the first mechanism operates in a given person, then the rate of atherogenesis should be proportional to plasma remnant concentrations. The second mechanism, however, implies no such relationship and would be more difficult to establish experimentally than the first one. In either instance, the reactions leading to endocytosis of remnants by smooth muscle cells may take place at sites where local injury has removed the endothelium; or, they may occur at endothelial surfaces, followed by vesicular transport to subendothelial layers.\textsuperscript{95} Although the proposed mechanisms may operate with or without a concomitant arterial injury, it is possible that the liberation of fatty acid anions during the hydrolysis of chylomicrons on the arterial surface may cause injury.\textsuperscript{18}

The following observations, documented in this article, support the role of chylomicron remnants as atherogenic agents:

1) The cholesterol content of chylomicrons and of chylomicron remnants increases with the cholesterol content of the diet.

2) The rabbit, which develops atherosclerosis
rapidly in response to cholesterol feeding, develops hypercholesterolemia primarily as a result of chylomicron remnants.

3) Patients with type III hyperlipoproteinemia have high levels of circulating chylomicron remnants, even in the postabsorptive state.

4) Lipoprotein lipase is present in normal arteries and is increased in proportion to the extent of atheromatosis in cholesterol-fed rabbits.

5) Chylomicron cholesteryl ester is taken up by extrahepatic tissues. In mammary glands and adipose tissue this uptake parallels its lipoprotein lipase content.

6) Chylomicron remnants in rabbits are as atherogenic as endogenously produced LDL and VLDL at equal levels of total serum cholesterol.

7) Several animal species that respond to drug and/or diet treatment with hypercholesterolemia and atherosclerosis accumulate plasma VLDL and/or IDL with high cholesteryl ester/triglyceride ratios, with high levels of apo-E protein and with $\beta$ mobility upon agarose electrophoresis. These properties parallel those of documented chylomicron remnants in human subjects and in rabbits.

If chylomicrons and chylomicron remnants are atherogenic, we must emphasize the study of postprandial conditions. It is nearly a truism to say that in people on high-fat, high-cholesterol diets the arterial wall is exposed to postprandial lipoproteins for a much longer time than to the postabsorptive lipoprotein pattern. Studies that should be done might include a fat-plus-cholesterol tolerance test, with particular attention to the transport and metabolism of VLDL and IDL. The inclusion of moderate or large amounts of cholesterol in the fatty meal may well be essential because, in the rabbit and in several other animal species, fat feeding alone produces only chylomicronemia, whereas the inclusion of cholesterol leads to high levels of cholesterol-rich chylomicron remnants. Some human subjects might develop a transient accumulation of remnants during the postprandial phase of the tolerance test, as the rabbit does; other subjects might clear chylomicron remnants more rapidly, analogous to rats and dogs. The usefulness of casual (nonfasting) plasma samples for screening should be reconsidered. Very high levels of cholesterol in VLDL, or in heparin precipitable lipoproteins, might identify persons at risk for coronary heart disease because of a combination of high-fat, high-cholesterol diets and an impaired ability to clear chylomicron remnants. Such persons may not show any lipoprotein abnormalities in their postabsorptive plasma specimens and may represent a class of people who have myocardial infarction even though their plasma lipids are relatively low.

Even without postprandial hypercholesterolemia, or a postprandial increase in plasma chylomicron remnants, the cholesterol contained in chylomicrons could be deposited by the lipoprotein lipase mechanisms. If this were so, then the several studies in which the feeding of eggs, or other cholesterol-rich foods, did not increase serum cholesterol levels may still not signify that these foods are free of atherogenic effects. The cholesterol problem may be a no-win situation. At high serum cholesterol concentrations a cholesterol-rich diet is clearly undesirable — at normal or low cholesterol concentrations cholesterol-containing foods may still exert an atherogenic effect. If a high-cholesterol diet can exert an atherogenic effect, even though postabsorptive plasma cholesterol levels are not unusually high, we should consider whether obese persons who consume excessive amounts of high-fat, high-cholesterol foods may be at risk primarily because of their excessive cholesterol intakes. In that case, obese persons eating mostly carbohydrate, both within Western society and in less developed areas, may not show an increased tendency toward atherosclerosis and coronary heart disease.

The use of labeled retinol in a cholesterol-fat tolerance test in human subjects appears to increase...
the possibility of detecting remnant clearance defects. Sensitive analytical techniques for retinol ester in whole plasma, or in the lower density lipoprotein fractions, might accomplish the same purpose without radioactive isotopes.

The cholesterol-retinol-fat tolerance test could be combined with cell culture techniques. Postprandial samples, after an oral mixed-lipid load, could be added to skin fibroblasts, lymphocytes or smooth muscle cells to study the interaction of a subject's chylomicron remnants with a cellular model of the artery. In this manner, susceptibility to cellular lipid deposition could be studied with postprandial lipoproteins and cells derived from the subject in whom a risk assessment is desired.

Finally, we should investigate the possibility of finding chemical or biological agents that would inhibit the binding of chylomicrons and/or remnants to basement membranes or areas of injured arteries, thus preventing the atherogenic process at those sites without interfering with the normal chylomicron clearing mechanism at the capillary endothelium.

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