It is an honor and privilege to present the 1982 Duff Memorial Lecture. I should start by confessing that for some years I have felt a bit like a pretender in the field of atherosclerosis research. To be sure, I have been very much involved in lipid and lipoprotein research and so, to the extent that lipoproteins are involved in atherogenesis, I could justify my participation in the proceedings of the Council. But the voice of G. Lyman Duff himself made me uneasy. In a classic review he wrote in 1951 together with one of our outstanding past Chairmen, Gardner McMillan, he referred to overly enthusiastic champions of the lipid hypothesis as follows: "So popular has this view [the lipid hypothesis] become that the casual reader of recent literature might wonder whether some authors conceive of an atherosclerosis so independent of the substrate of the vessel wall that it may occur in the absence of the blood vessels themselves." I hope that I am not one of those authors, but you can see that I would have felt uncomfortable presenting a Duff Lecture in which the cells of the artery wall did not make an appearance. I am relieved to be able to assure you that they will appear — even an intact artery.

Duff was one of the greats among the pioneers working with the complex problem of atherosclerosis. Anyone who has read his papers knows how carefully and how cleverly he designed his experiments, how critically he interpreted the results. Not everyone was as clever or as critical. A lot of research that wrapped itself in the atherosclerosis flag was of at least questionable importance. For example, I believe I recall a paper presented about 30 years ago before the American Society for the Study of Arteriosclerosis (the parent society of this council) dealing with atherosclerosis in cholesterol-fed rabbits. The authors had compared the severity of lesions in animals fed fried eggs with that in animals fed equal numbers of poached eggs! I think it is fair to say that the field in the 1950s was still in a formative stage. Yet Duff and his collaborators were among a small but centrally important number who asked good questions that could be approached with the tools then available. While preparing this lecture I looked back at Duff's papers, including a remarkable 89-page review he published in 1935. In it he clearly formulated many of the fundamental questions about atherogenesis, although obviously not in terms of the biochemical and cell biological paradigms within which we work today. That review is carefully written, comprehensive, and provocative. Unfortunately, too many of Duff's questions still remain incompletely answered.

The reasons for the difficulty in making progress at that time are not hard to find. The lesion is complex and the time course of its evolution so long that it was — and still is — frustrating to try to design and execute critical studies. The experimental tools and the basic science foundations were simply not yet available. Consequently, investigators turned their attention to riper problems, and rightly so. There were, after all, a few good ones around — such as how proteins are biosynthesized, how cell membranes are constructed, the deciphering of the genetic code, and other not unimportant questions. Things are somewhat different today, in large part precisely because of the enormous progress in answering some of those not unimportant questions. The advances in biochemistry and cell biology have given us the concepts and the techniques with which to frame rel-
evant questions about the atherogenic process. Specific hypotheses can now be formulated and the experiments to test them can be designed and executed. There is a sense of excitement in the air as investigators from various established, sophisticated disciplines begin to work on — or at least flirt with — the problem of atherogenesis. There may be a loose analogy here with the story of the man who was hunting around under the street lamp outside his garage one night. A neighbor stopped and asked him what he was looking for.

"My car keys."
"When did you have them last?"
"Well, I think I dropped them in the garage."
"Then why are you looking for them out here?"
"Because the light is much better out here."

The moral, of course, is that if you really want to find the keys you eventually have to go to where the problem is even if the light isn't too good.

The Lipid Hypothesis, the Endothelial Injury Hypothesis, and the Unified Hypothesis

The Lipid Hypothesis

To begin at the beginning, let me defend the thesis that lipoproteins, most specifically low density lipoproteins (LDL), can be a sufficient cause of atherosclerosis. (Note that I say sufficient but not necessarily necessary.) Many different lines of evidence support this proposition, but without doubt the genetic evidence is the strongest. Patients with familial hypercholesterolemia have a monogenic disorder long known to be inherited in classical Mendelian fashion, and they suffer from galloping atherosclerosis. The homozygotes not infrequently suffer their first myocardial infarction before adolescence and seldom survive to age 30. The specific genetic lesion has been established by the classic work of Goldstein and Brown as a loss of functional LDL receptors. The genetic argument has recently been strengthened by the development in Japan of a unique strain of LDL-receptor-deficient rabbits, the Watanabe or WHHL strain. Professor Watanabe was kind enough to send us some of these remarkable animals, and we have been studying their lipoprotein metabolism, as discussed below. These animals have enormously elevated plasma cholesterol levels, above 400 mg/dl, and this on an ordinary rabbit chow diet (zero cholesterol). As early as 6 months of age, they show moderately severe aortic lesions, with marked intimal thickening and many foam cells (Figure 1). They even develop tendon xanthomas (Figure 2). Their receptor deficiency has been documented in several ways:

1. Studies of cultured skin fibroblasts in Dr. Watanabe's laboratory.
2. Studies of cultured hepatocytes in our laboratory.

The fractional catabolic rate for LDL degradation in vivo in these rabbits is about one-third normal, consonant with the functional absence of receptors.

Figure 1. Microscopic section of the aorta of a 6-month-old rabbit with LDL receptor-deficiency (WHHL rabbit). Araldite section, toluidine blue staining. L = lumen of vessel; F = one of many foam cells; Bar = 10 μm.
I think this animal is going to prove an invaluable model not only for studies of the role of the receptor in LDL metabolism but also as a model for experimental LDL-induced atherosclerosis.

So, arguing from the human receptor deficiency and its rabbit counterpart, I submit that any and all phenotypic consequences of the monogenic disorder — a receptor deficiency — must flow somehow from that primary gene defect (Figure 3). The most proximate effect is to slow down the rate of LDL removal from the plasma compartment. This leads to elevation of LDL levels, which in turn must somehow lead on to atherogenesis. As indicated, both the patients and the rabbits also overproduce LDL, but there is good reason to believe that that also stems from the receptor deficiency. I am not able to see any holes in this genetic argument and must conclude that the case is proved, at least in this limited arena: LDL can be, in itself, a sufficient (but not necessarily a necessary) cause of atherosclerosis.

Would a comparable degree of LDL elevation on some other metabolic basis have equally drastic consequences? Well, we simply never see patients other than homozygotes with levels nearing 1000 mg/dl, so no comparison is possible. However, the coronary mortality experience of the heterozygotes is not out of line with that of the few individuals who have comparably high LDL levels on some other basis. The LDL in patients with familial hypercholesterolemia does not differ much from normal LDL except in total concentration. As Dr. Myant and his group showed, LDL from a homozygote when injected into a normal subject has a normal half-life. Is there perhaps some critical threshold below which elevated LDL levels are not threatening? The epidemiologic data do not demonstrate any such discontinuity in the curves relating coronary risk to cholesterol level. For example, in the Oslo study of Westlund and Nicolaysen, the relationship is smooth and still rising above 350 mg/dl and still falling (although less steeply) at 200 mg/dl.

Now even if we indict LDL as a sufficient cause, it certainly does not follow that all else is irrelevant. For example, Lyman Duff would immediately ask: “Why do the LDL-induced lesions develop where they do? What are the determining local factors? What is happening at the level of the arterial substratum?” Also we know that there are additional, well-established independent risk factors, notable among them being hypertension, cigarette smoking, and diabetes mellitus. Being independent risk factors, they presumably impinge on the atherogenic process other than by (or in addition to) contributing to hyperlipidemia. Just how they impinge is not known. We shall return to the issue of multiple causality below, but there is one additional point that might be made here about the

![Figure 2. Xanthomas in a 6-month-old rabbit with LDL receptor deficiency. A. Tendon xanthomata on paw. B. Xanthomata on "elbow."](https://example.com/image.png)

![Figure 3. The genetic argument for elevated LDL levels as a sufficient cause of atherosclerosis.](https://example.com/image.png)
sufficiency of hyperbetalipoproteinemia as a cause of atherosclerosis. The 10-year-old child with homozygous familial hypercholesterolemia and the 1-year-old rabbit with receptor deficiency do not have hypertension, obesity, or diabetes mellitus. Nor do they smoke cigarettes! They have only one risk factor, and that's enough.

I find it helpful to consider two possible categories of mechanisms by which lipoproteins may be atherogenic: 1) they may be directly atherogenic as a consequence of their uptake and degradation in the artery wall; or 2) they may act indirectly, by inducing perturbations in one or more biological systems not linked at all (or only indirectly) to lipoprotein uptake and degradation in the artery wall. The first possibility, explicitly or implicitly, underlies the classical lipid infiltration hypothesis. As outlined on the right in Figure 4, elevation of LDL levels is visualized as increasing the rate of LDL infiltration, which in turn increases the rate of cell uptake — into endothelial cells, smooth muscle cells, macrophages, and any other cells present in the artery wall. Here and in what follows we shall deal mainly with and speak of LDL but it should be understood that other lipoprotein fractions (chylomicrons, very low density lipoprotein (VLDL), and especially remnants such as beta-VLDL) are by no means exonerated. They may be just as atherogenic as LDL or even more so. The cells taking up LDL have some capacity to rid themselves of excess cholesterol but that capacity can ultimately be exceeded and then cholesterol and other lipids accumulate. The events beyond that point have never been very clearly defined. How exactly is the accumulation of lipid harmful? To the extent that it contributes to the mass of the lesion, it will of course contribute to stenosis. As pointed out by Small, the composition of the deposited lipids will determine when there will be a phase separation with the appearance of crystalline masses. But does lipid accumulation play an initiating role? For example, does the accumulation of lipid speed up the entry of monocyte/macrophages? Or does it possibly prevent lipid-laden macrophages from leaving the artery wall? Or reduce the capacity of the macrophages to "search and destroy"? Answers to questions like these are needed before the lipid hypothesis can be considered complete.

The Endothelial Injury Hypothesis

Let us turn now to the other major "school" of atherogenesis and look at the endothelial injury hypothesis, outlined on the left in Figure 4. The basic thesis here is that endothelial damage — possibly subtle — causes blood platelets to adhere and aggregate. This causes release of the platelet-derived growth factor (PDGF) discovered by Ross and his coworkers, which stimulates smooth muscle cells to proliferate and to secrete connective tissue matrix elements. Macrophages and endothelial cells have been recently shown to secrete growth factors. Thus, repeated episodes of endothelial damage and repeated bouts of smooth muscle cell replication can lead to the development of the "space-occupying lesion" we recognize as an atheroma.

The Unified Hypothesis

What I would like to persuade you of now is that these two views of atherogenesis, far from representing two contesting "schools" in the classical sense, are better regarded as simply two faces of a unified Janus hypothesis. There are at least six interactions between these two hypotheses that make them almost inseparable (see Figure 4):

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Figure 4. A proposed unified hypothesis linking the endothelial injury "school" and the lipid infiltration "school." See text.
1. Elevated LDL levels may damage endothelial cells. If, then hyperlipidemia could simultaneously initiate the series of events characterizing the lipid infiltration hypothesis and the series characterizing the endothelial injury hypothesis.

2. Hyperlipoproteinemia has been reported to favor platelet aggregation, an effect that might initiate release of platelet-derived growth factor even when endothelial damage was modest.

3. Injury to the endothelium, by removing a transport barrier, will concurrently increase the rate of lipoprotein infiltration, cellular uptake, and degradation at any given plasma lipoprotein level. Direct evidence for this in the case of LDL has recently been provided by studies of Carew et al. in our laboratory.

4. LDL, particularly LDL from hypercholesterolemic animals, can stimulate the growth of smooth muscle cells.

5. Platelet-derived growth factor (and probably other growth factors) increases the expression of LDL receptors on smooth muscle cells and thus increases their rate of uptake of lipid.

6. Stimulation of smooth muscle cell growth may cause the deposition of larger amounts of certain intercellular matrix materials, such as glycosaminoglycans, that can trap LDL in the subintimal space.

In view of these many potential interactions, some firmly established, others needing additional elaboration, we should probably lay to rest the notion that we have to choose sides. Perhaps you recall Jonathan Swift's two tribes in Gulliver's Travels who were constantly at war. One group insisted that soft-boiled eggs should be cracked at the little end, while the other was equally convinced they should be cracked at the big end. Many lives were lost in the endless strife between the Little Endians and the Big Endians. Peace ultimately came when they realized they could compromise and agree to break their eggs in the middle. I hope that most of us are now ready to accept some version of this middle-ground Unified Hypothesis even as we rightly reject the egg altogether.

If we consider the schema shown in Figure 4, we see that there are several input channels that converge on what may be a common final path. Whether the rate of lesion development becomes threatening or not may depend on how many of these independent input channels are operating and at what level. What we are talking about is the basic notion of multiple causality. We all subscribe to the principle of parsimony in science. Given a choice, we prefer to select the simpler hypothesis as did William of Ockam; to explain a given syndrome, we try to identify a single, all-embracing diagnosis. Yet if we stop to reflect, we are fully aware of cause-effect relationships that are not readily reduced in such a way. There is a charming, if apocryphal, tale of an early Pavlovian psychologist who had successfully conditioned fleas to jump upon the command “Jump, flea!” (or the equivalent thereof in Russian). Then he found that if he amputated their legs they no longer responded, so he concluded that the hearing apparatus of the flea is located in the legs. Clearly, at least two “causes” must be simultaneously operative to elicit the flea-jumping phenomenon. I would submit that atherogenesis is at least as complex as a trained flea and that we should be willing to entertain the simultaneous operation of several interactive causes. The remainder of this paper is devoted primarily to LDL and how it may promote atherogenesis, but I yield to no one in my conviction that it is not the whole story.

**Sites and Mechanisms of LDL Degradation In Vivo**

**Sites of Degradation**

Ten years ago, when our Specialized Center of Research on Arteriosclerosis was established in La Jolla, there was little or no direct evidence with regard to the tissues responsible for degradation of LDL in vivo. Most investigators assumed that degradation must take place exclusively in the liver since only the liver (and the steroidogenic tissues) has the capacity to catabolize or excrete cholesterol. However, Sniderman et al. in La Jolla showed that LDL catabolism continues at a high rate even after total hepatectomy. They clearly established the capacity of extrahepatic tissues to degrade LDL, but could not give any quantification. In fact, the apparent fractional catabolic rate after hepatectomy was enhanced, a paradoxical finding still unexplained. There was at the time no appropriate method available to give quantitative data. The classical A-V difference approach, which works well for materials extracted fairly rapidly from the plasma, cannot be used for LDL. Its half-life is too long and the A-V difference therefore too small to be measured. Even if all LDL degradation took place in the liver, the concentration difference across the liver would be considerably less than 1% and therefore not measurable by any of the available methods. The same difficulty applies in the case of any plasma proteins with half-lives greater than 2 days. Some investigators have measured initial rates of uptake of LDL or other plasma proteins, but such measurements do not necessarily correlate with the rates of irreversible degradation of the protein by those tissues. The rate of equilibration with extravascular pools need not correlate directly and exactly with subsequent rates of uptake into the cells and degradation. On the other hand, at longer time intervals, degradation products will have escaped from the cell, particularly when conventionally iodinated proteins are used, and therefore degradation will be underestimated.

To get around these problems, Ray C. Pittman and I developed a new approach. The principle is
illustrated in Figure 5. As shown on the right, when conventionally iodinated LDL is degraded in the lysosome, the free amino acids, including the labeled iodotyrosine, escape very rapidly from the cell. This is one reason why the amount of radioactivity one finds in a tissue at any given time need not bear any relationship to the rate at which the tissue is degrading the protein. We were at that time using the rate of uptake of $^{14}$C-sucrose to measure rates of fluid endocytosis in cultured cells, a well-established method in cell biology. $^{29}$ Sucrose works for this purpose because it cannot cross the lysosomal membrane at an appreciable rate. Furthermore, there is little or no sucrose activity to degrade it. Consequently, it accumulates in the lysosome. The idea, very simply, was to couple radioactive sucrose covalently to the protein so that each molecule degraded would leave behind in the lysosome one molecule of trapped $^{14}$C-sucrose. One could then inject sucrose-labeled LDL, sacrifice the animal a day or so later, and simply count the amount of sucrose degradation product accumulated in each tissue, which should be in proportion to the amount of LDL degraded in that tissue. If the absolute amount of LDL degraded over the time interval is known from kinetic analysis, one can calculate the absolute amounts of LDL degraded in each tissue. The validity of the method has been demonstrated in both cell culture and in vivo studies. $^{31-33}$ With this method, it has been possible to determine the relative importance of various tissues and cell types in the degradation of LDL under physiologic conditions in vivo. It should be noted that the method is perfectly general, applicable to the study of any plasma protein. Baynes and Thorpe $^{34}$ have utilized the same principle in studies of the sites of degradation of serum albumin in the rat, labeling it by conjugation with raffinose; Yedgar et al. $^{35}$ have utilized the $^{14}$C-sucrose method for studying albumin degradation in the rabbit.

In three animal species — the pig, $^{32}$ rat, $^{33}$ and rabbit $^{8}$ — we have found the liver to be, by all means, the predominant tissue responsible for degradation of plasma LDL. In all three species, about 50% of total LDL turnover was attributable to hepatic degradation. No other single organ even approached the liver in its contribution to overall LDL catabolism. Therefore, changes in hepatic metabolism of LDL would be expected to have a dominant impact on plasma LDL levels. Studies in cultured hepatocytes, $^{36,37}$ in partially purified liver plasma membranes, $^{38-40}$ and in perfused livers $^{41}$ establish the presence of a high-affinity LDL receptor in the liver. That this receptor is the same gene product as the LDL receptor in fibroblasts and other extrahepatic tissues is shown by its absence from the liver cells of the genetically receptor-deficient WHHL rabbit. $^{6}$ Studies over the last 2 or 3 years, ably reviewed recently by Brown et al. $^{42}$ clearly establish that hepatic LDL catabolism is under regulation. Bile acid sequestrants are effective, in part, because they induce the expression of the hepatic LDL receptor. $^{43}$ Inhibitors of HMG-CoA reductase can also induce the hepatic receptor, $^{37,43}$ but they probably act also, in part, by decreasing lipoprotein production.

Just as important as the fact that 50% of LDL degradation takes place in the liver is the fact that 50% takes place extrahepatically. If LDL degradation in humans is more or less like that in the experimental
animals studied to date, the degradation of LDL in extrahepatic tissues delivers about 1 g of cholesterol every day. Since these tissues (other than the steroidogenic tissues) have no machinery for catabolizing cholesterol, this extra "load" of cholesterol (together with any cholesterol synthesized de novo) must somehow be transported back to the liver for excretion. This necessity for "reverse cholesterol transport" has obvious implications with regard to atherogenesis. A failure in that mechanism could lead to a progressive accumulation of cholesterol in any of the extrahepatic tissues, most significantly, in the aorta.

Let's now look at the results with 14C-sucrose LDL from a different point of view. Which tissues are most active in the catabolism of LDL per gram weight of tissue? As shown in Table 1, in each of the three species thus far studied the adrenal was more active per unit weight than any other single tissue. The hierarchy is very similar among the three species studied. Thus, the adrenal, the gonads, and the liver — the three tissues having the ability to further metabolize cholesterol — were the tissues most active in uptake and degradation of LDL. These results, obtained under physiologic conditions in vivo, are consonant with the demonstrated ability of steroidogenic cells of several species to utilize LDL cholesterol as precursor.44-50 From 14C-sucrose-LDL studies in pigs, it was calculated that LDL uptake and degradation was delivering daily to the adrenals an amount of cholesterol nearly equivalent to the maximum rate of steroidogenesis in ACTH-stimulated glands.32 In the rat (and perhaps only in the rat) HDL also supplies a significant fraction of the cholesterol used for gonadal and adrenal steroidogenesis.45,47 When we compare our in vivo data on the tissues most active in LDL degradation with the results of studies of LDL receptor distribution in isolated tissues, we find reasonably good concordance.39 The adrenal, the gonads, and the liver are, in fact, the tissues in which the density of LDL receptors on the plasma mem-

brane has been found to be the highest. This strongly suggests, but does not prove, that a large component of the uptake and degradation of LDL in these tissues is mediated by the receptor under physiologic conditions.

Table 1. Ranking of Tissues Most Active in Irreversible Degradation of LDL In Vivo (Activity Per Unit Weight)

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Rabbit</th>
<th>Rat</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Adrenal</td>
<td>Adrenal</td>
<td>Adrenal</td>
</tr>
<tr>
<td>2nd</td>
<td>Spleen</td>
<td>Liver</td>
<td>Liver</td>
</tr>
<tr>
<td>3rd</td>
<td>Liver</td>
<td>Ovary</td>
<td>N.G.*</td>
</tr>
<tr>
<td>4th</td>
<td>Ovary</td>
<td>Spleen</td>
<td>Spleen</td>
</tr>
<tr>
<td>5th</td>
<td>Adipose tissue</td>
<td>Adipose tissue</td>
<td>Adipose tissue</td>
</tr>
</tbody>
</table>

As indicated in the text, the liver as an organ accounts for about one-half of total body LDL degradation; it is also highly active on a "per gram" basis. The adrenal, while most active on a "per gram" basis, obviously accounts for only a small fraction of total body LDL degradation because of its small size.

*N.G. = no gonads; removed at a tender age by previous owners.

Mechanisms of LDL Uptake

As indicated in Figure 6, total body LDL degradation can be considered in two major categories: that dependent on the Brown-Goldstein LDL receptor, and that occurring via alternative mechanisms. In the latter category there appear to be alternative high affinity mechanisms available to modified forms of LDL but not to native LDL itself. So far, these alternative high affinity mechanisms have been demonstrated only in macrophages and endothelial cells.51,52 The low affinity mechanisms include fluid endocytosis and adsorptive endocytosis. The former is, of course, totally nonsaturable, involving only the uptake of microdroplets of surrounding tissue fluid with whatever concentration of LDL happens to be present. Fluid endocytosis alone, however, does not appear to be adequate to account for the observed rate of LDL uptake and degradation by fibroblasts lacking the LDL receptor.53 Thus, it becomes necessary to postulate an adsorptive element although the nature of that adsorption remains to be delineated. Studies in cultured fibroblasts of patients with homozygous familial hypercholesterolemia54 and studies in cultured rat hepatocytes using human LDL55 (which is poorly recognized by the rat receptor) suggest that the metabolic consequences of uptake by nonspecific pathways may differ from the consequences of uptake via the high affinity LDL receptor. These findings have recently been reviewed elsewhere.56 What implications this may have for atherogenesis remains to be determined. Later we will return to some further discussion of these various mechanisms available for LDL uptake. First, however, let us review what we know about which mechanisms are operative under physiologic conditions in vivo.
Mahley and coworkers\textsuperscript{57} showed that recognition of LDL by its specific receptor is abolished if the LDL is chemically modified, for example, by reductive methylation of the lysine epsilon amino groups. If the LDL receptor-independent mechanisms remain unaffected by these chemical alterations, then the difference between the rate of catabolism of native and of chemically modified LDL should give the rate of metabolism attributable to the LDL receptor-independent pathways (Figure 7). This underlying assumption of equivalence with regard to all other pathways is not yet established and so conclusions must be considered tentative at this point. From the difference between the fractional catabolic rates of native and methylated LDL in the intact rat it was estimated that 52% of total body LDL degradation occurred by way of the LDL receptor.\textsuperscript{58} The same principle was then applied to each individual tissue, by comparing the uptake of native and methylated LDL labeled with \textsuperscript{14}C-sucrose. This permitted an estimation of the LDL receptor-dependent uptake on a tissue-by-tissue basis. As shown in Table 2, the calculated receptor-mediated uptake in liver, adrenal, and ovary were all about the same — 65% of total — while that in the intestine was somewhat less, and the fraction in other tissues still less. Because the liver accounts for such a large fraction of total body LDL degradation, it is understandable that the values for receptor-dependent uptake in the whole body will closely reflect the percentage of receptor-dependent uptake in the liver.

A second approach for estimating the amount of LDL receptor-dependent uptake in tissues in vivo was made by capitalizing on the availability of the receptor-deficient rabbit, discussed above. Here we have a model in which no LDL degradation, or practically none, occurs by way of the LDL receptor pathway. Thus, the fractional catabolic rate in the WHHL rabbit represents only uptake that is independent of the LDL receptor, while that in the normal rabbit represents the sum of LDL receptor-dependent and LDL receptor-independent uptake. Thus, the approach is in principle analogous to that used when comparing the metabolism of native LDL and blocked LDL in the normal animal (Figure 7). We found\textsuperscript{8} that the fractional catabolic rate of LDL in the WHHL rabbit was only about one-third that in control animals. Based on the assumption that the LDL receptor-independent pathways are unaffected by deletion of the LDL receptor, we conclude that about two-thirds of the LDL degradation occurring in the normal rabbit is LDL receptor-dependent. The validity of the assumption that pathways other than the receptor pathway are unaffected in the WHHL rabbit is supported by studies using chemically blocked forms of LDL. Witzum and coworkers\textsuperscript{59} have shown that glucosylation of LDL blocks its recognition by the high affinity receptor. Whereas cyclohexanedione-treated LDL is not completely stable, reductively glucosylated LDL made using cyanoborohydride is. Kesaniemi et al.\textsuperscript{60} have therefore used glucosylated LDL to determine LDL receptor-independent degradation in man. Reductively methylated LDL, for reasons yet to be established, disappears more rapidly than native LDL in man.\textsuperscript{61} In normal rabbits the fractional catabolic rate of glucosylated LDL was much less than that of native LDL, but in the WHHL rabbit native and glucosylated LDL disappeared at almost exactly the same rate.\textsuperscript{62} The results imply that the LDL receptor-independent pathways are unaffected in the WHHL rabbit. These findings are in general agreement with those of Bilheimer and coworkers\textsuperscript{5} who compared the catabolism of native and reductively methylated LDL in normal and WHHL rabbits.

As pointed out above, the net flux of LDL both in receptor-deficient rabbits and receptor-deficient patients is actually far above normal. In which tissues does all of this LDL receptor-independent degradation take place? What are the mechanisms involved? This apparent paradox of a greater than normal degradation when the receptor is defective led to the logical suggestion that some "scavenger pathway" must be postulated and that the reticuloendothelial (RE) system might be involved.\textsuperscript{63, 64} By utilizing the \textsuperscript{14}C-sucrose-LDL approach in the WHHL rabbit, we were able\textsuperscript{6} to determine the tissue patterns of LDL degradation when all of the degradation is via LDL receptor-independent pathways and compare the results with those in normal rabbits. To our surprise,

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**Table 2. Calculated Percentage of LDL Degradation Via the High-Affinity Receptor Pathway**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rat*</th>
<th>Rabbit†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>53%</td>
<td>62%</td>
</tr>
<tr>
<td>Liver</td>
<td>65%</td>
<td>63%</td>
</tr>
<tr>
<td>Adrenal</td>
<td>65%</td>
<td>92%</td>
</tr>
</tbody>
</table>

*Based on the difference between catabolism of native \textsuperscript{14}C-sucrose LDL and of reductively methylated \textsuperscript{14}C-sucrose LDL (data from ref. 58).
†Based on the difference between catabolism of native \textsuperscript{14}C-sucrose LDL in normal rabbits and that of the same labeled LDL in receptor-deficient (WHHL) rabbits (data from ref. 8).
we found that the patterns were not strikingly different (Figure 8). As in the control rabbits, about 50% of total LDL degradation in the receptor-deficient rabbits occurred in the liver; the fractional contribution of other tissues to LDL degradation was in almost every case very close to that seen in control rabbits.

A question of particular interest was whether the degradation in the liver was attributable to hepatocytes, as it is in control animals, or whether it was shifted toward degradation in the Kupffer cells, which represent a major component of the RE system.

Twenty-four hours after injection of 14C-sucrose LDL, the liver was removed and fractionated to separate parenchymal from nonparenchymal cells. Three different methods were used and all gave the same results (Table 3): 85% or more of the degradation took place in parenchymal cells both in control and WHHL rabbits. The failure to find a predominant role for the Kupffer cell in LDL degradation in these receptor-deficient animals suggests that there is no important overall shunting of LDL catabolism to the RE system even when the receptors are missing. However, this by no means rules out the possibility that such shunting (to tissue-macrophages) may occur in the artery wall and play a crucial role in atherogenesis. As shown below, the fraction of total body LDL degradation attributable to degradation in the artery wall is negligible and could not perceptibly influence overall body patterns of LDL disposal.

**Degradation of LDL in the Aortic Wall**

The rates of LDL degradation in tissues throughout the body are relevant to the problem of atherosclerosis in the sense that the metabolism in each of those tissues helps determine the steady state plasma LDL levels to which the artery wall is exposed. But degradation in the arteries themselves is certainly more immediately relevant to atherogenesis. The lipid accumulating in lesions, particularly early lesions, is found both extracellularly and intracellularly. Moreover, the presence of intact LDL has been

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**Table 3. Percentage of Hepatic Degradation of 14C-Sucrose LDL Attributable to Hepatocytes**

<table>
<thead>
<tr>
<th>Separation method</th>
<th>Normal rabbit</th>
<th>WHHL rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential sedimentation</td>
<td>85.7, 91.8</td>
<td>87.4</td>
</tr>
<tr>
<td>Pronase treatment</td>
<td>92.6, 81.8</td>
<td></td>
</tr>
<tr>
<td>Centrifugal elutriation</td>
<td>69.4</td>
<td></td>
</tr>
</tbody>
</table>

The contribution of hepatocytes to overall hepatic degradation of LDL was calculated assuming that 90% of liver protein is attributable to hepatocytes and 10%, to nonparenchymal cells. Three different methods for separating hepatocytes from nonhepatocytes were used and all gave similar results.
demonstrated using a number of techniques in normal and atheromatous arteries. While careful measurements of the rates of penetration of plasma LDL into the artery wall have been made, most recently by Stender and Zilversmit, it has not been possible to distinguish clearly between entry into the extracellular space, on the one hand, and uptake and degradation by cells of the artery wall, on the other. Once the LDL molecule has been internalized and degraded by a cell, that cell has acquired an increment of cholesterol (and other lipids) that must be dealt with. It must somehow be mobilized and transported back out of the artery wall if atherogenesis is not to progress. Deposition of LDL in the extracellular space (e.g., by binding to connective tissue matrix elements) may also contribute to atherogenesis, but the nature of the removal problem is then probably different. For these reasons and others, it would be valuable to be able to measure cellular uptake and irreversible degradation of LDL and other lipoproteins in the artery wall in vivo. Even more valuable would be the determination of which cell types in the artery wall contribute to such intraarterial degradation.

With the ¹⁴C-sucrose labeling method, at even the highest specific activity of sucrose available, it was difficult to obtain useful data for arteries. However, by counting the entire aorta, some first approximations could be made. In the normal rabbit, aortic degradation, expressed as a percentage of the plasma LDL pool degraded per day, was 0.016. From that value together with the measured plasma LDL pool size, we derived a value of 3.8 μg cholesterol delivered per gram wet weight of aorta per day. This assumes, of course, that apo B (which is what we actually measure) enters cells along with its full complement of cholesterol. This assumption is supported by cell culture studies and also to some extent by the data of Stender and Zilversmit. These investigators measured initial rates of entry into the artery of LDL labeled both in the cholesterol ester moiety and in the apoprotein B. They found a nearly linear correlation between the rates of entry of the two.

In the receptor-deficient atherosclerotic rabbit, we found that the rate of irreversible degradation of LDL in the aorta was 20-fold greater than that in normal rabbits. Moreover, the fractional clearance into the aorta was actually greater than that in normal rabbits. The aorta was the only tissue in which this was the case. Note that if any part of the uptake and degradation in the normal artery occurs by way of the receptor, one should see a lower fractional clearance in the receptor-deficient artery. Even if there were normally no receptor-linked uptake, the fractional clearance in the receptor-deficient artery should be, at most, equal to that in the normal artery. The higher fractional clearance observed suggests at least three possibilities: 1) endothelial permeability may be increased in areas of atherosclerosis; 2) the developing lesions are more highly cellular and contain more actively dividing cells, cells that have been shown to take up LDL more avidly; 3) Some LDL is being converted to a form taken up by way of an alternative high-affinity pathway in the areas of the lesions. We suspect that all three may be operative.

The limited specific activity of ¹⁴C-sucrose obtainable made it impossible to go much further in studies of arterial degradation. Yet we would like to refine the approach by examining regional differences and even, if possible, uptake by individual cell types. Ray C. Pittman has now made this possible by developing a substitute for sucrose that behaves similarly, in the sense that it is trapped in lysosomes, but that can be labeled with radiiodine to very high specific activities indeed. As shown in Figure 9, the new maker is based on the use of cellobiose, the disaccharide building block of cellulose, which is not degraded by mammalian enzymes. To it is coupled tyramine, which can at that point be iodinated. Finally, this high specific activity ligand, tyramine cellobiose (TC), is coupled covalently to the protein using cyanuric chloride. Switching from ¹⁴C to ¹²⁵I suddenly jumped the sensitivity of our method by almost three orders of magnitude. It has been applied successfully in the study of sites of apo A-I catabolism. In principle the method should now be applicable to studies of proteins present at very low concentrations, including insulin and other polypeptide hormones.

The potential of the method is illustrated by recent studies carried out by Carew et al. comparing rates of LDL degradation in intima with that in the media. Radiiodinated TC-LDL was injected into normal rabbits and the aorta was removed 24 hours later. After thorough washing, pieces of intact aorta were counted and then the intima was gently scraped off and its labeled iodine content measured separately. At this time, most of the radiiodinated material in the intima represented degradation products. As shown in Table 4, almost one-half of the total degradation taking place in the aorta took place in the intima, which represents only a very small fraction of the whole organ. Its activity per milligram of protein was about 50 times that of the rest of the aorta. The high specific activity material used made it possible to get autoradiographs of tissue sections with only a 15-day exposure (Figure 10). These studies, done in collaboration with Roger Marchand in the Depart-

\[
\text{TYRAMINE + CELLOBIOSE} \longrightarrow \text{TYRAMINE-CELLOBIOSE} \\
\text{NaBH₃CN} \\
\text{(T)} \quad \text{(C)} \quad \text{(TC)} \\
\text{TC} \quad \ddagger\ddagger \text{IODGEN} \quad \ddagger\ddagger \text{I-TC} \\
\text{I - TC} \quad \ddagger\ddagger \text{CYANURIC CHLORIDE} \quad \ddagger\ddagger \text{I - TC} \quad \ddagger\ddagger \text{LDL} \\
\]

Figure 9. Schematic outline of the methods used for labeling proteins with ¹²⁵I-tyramine cellobiose.
Table 4. Percentage of Total Aortic Degradation of LDL Occurring in the Intimal Layer (Normal Rabbits)

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Percentage degraded in the intima</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>45%</td>
</tr>
<tr>
<td>23</td>
<td>31%</td>
</tr>
<tr>
<td>30</td>
<td>38%</td>
</tr>
<tr>
<td>31</td>
<td>44%</td>
</tr>
</tbody>
</table>

Mean 39.6% ±3.9% (SD)

Animals received about 200 μCi of 125I-tyramine cellobiose LDL intravenously and were killed at 24 hours for tissue analysis. The systemic circulation was perfused with buffered saline to remove trapped blood. The intimal layer of the opened aorta was removed by gentle swabbing with a cotton swab. An internal control method described elsewhere showed that 87% of the 125I in the stripped intima represented degradation products—not intact LDL.

Another study was done to determine how arterial wall degradation of LDL would be affected by loss of the endothelium. Most investigators have assumed that deendothelialization would allow lipoproteins to enter the artery wall more rapidly and that overall lipoprotein degradation would therefore be enhanced. However, Elspeth Smith and her coworkers find that the free LDL concentration in the subendothelial space is actually greater than that in the plasma, and that the endothelium may prevent LDL from reentering the plasma. If this view is correct, LDL uptake and degradation might decrease after removal of the endothelial barrier. Thomas Carew stripped the endothelium from a short segment of thoracic aorta in normal rabbits using a balloon catheter.

Table 5. Effects of Endothelial Denudation on the Rate of LDL Uptake and Degradation by Rabbit Aorta

<table>
<thead>
<tr>
<th>Aortic segment</th>
<th>Calculated LDL uptake and degradation (μg LDL cholesterol g⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper (intact) segment</td>
<td>2.2 ± 0.55 (n = 6)</td>
</tr>
<tr>
<td>Middle (stripped) segment</td>
<td>7.8 ± 3.2 (n = 5)</td>
</tr>
<tr>
<td>Lower (intact) segment</td>
<td>2.3 ± 0.87 (n = 6)</td>
</tr>
</tbody>
</table>

A balloon catheter was used to strip the intima from an approximately 3-cm segment of the aorta. Two hours later, 125I-tyramine cellobiose LDL was injected and after 24 hours the rabbit was killed for tissue analysis.

Figure 10. Autoradiograph of the aorta of a receptor-deficient rabbit injected 24 hours previously with 125I-tyramine cellobiose LDL. Separate studies of stripped intima show that most of the radioactivity is in degradation products rather than in intact LDL. Grain counts over the width of the aorta showed that about 40% were located over the very narrow intima, in good agreement with the biochemical results shown in Table 4. × 296.
eter. One hour later, $^{125}$I-TC-LDL was injected. At 24 hours, degradation in the stripped segment was compared with that in the intact segments just above and below it. As shown in Table 5, the rate of LDL degradation in the stripped segment (media without intima) was about fourfold that in the media of the control segments.

The potential of this new method is further demonstrated by the autoradiograph shown in Figure 11. A cholesterol-fed rabbit was given 2 mCi of $^{125}$I-TC-LDL and sacrificed 24 hours later. The aorta was slit open, flattened, and stained with Sudan IV. As expected, the lipid stain revealed lesions around the ostia of the intercostal arteries and other aortic branches; the pattern was the classical one with maximum lipid staining in V-shaped areas pointing downstream around the ostia. The left-hand side of Figure 11 is an autoradiograph of the entire aorta laid

Figure 11. Autoradiograph (A) of the aorta of a cholesterol-fed rabbit injected with $^{125}$I-tyramine cellobiose LDL 24 hours previously. The aorta, stained with Sudan IV, shown in B, was apposed to film for 3 days. The concordance between areas in which LDL degradation in most active and those areas showing lipid accumulation is remarkable. The lipid-staining areas near the cut edge of the aorta that do not have corresponding autoradiographic spots represent adventitial fat not removed from an artery. $\times$ 3.
intima-side-down onto a photographic film. The concordance between areas of lipid accumulation (which had been occurring over many weeks on the high cholesterol diet before the time of study) and areas of on-going active LDL degradation is remarkable. The reasons for the high rates of LDL degradation in these areas remains to be determined; we have suggested three possibilities and there are undoubtedly more. It is hoped that this new approach to sensitively assessing lipoprotein degradation in vivo will provide a powerful tool for further studies of patterns of lipoprotein degradation in relation to atherogenesis. Lipoproteins other than LDL can, of course, also be studied, provided suitable methods for labeling the apoprotein of interest, and hopefully only the one of interest, can be devised.

Role of the Macrophage

The foam cell has long been recognized as a characteristic feature of the atheroma and also of xanthomata in skin and tendons but there has been controversy about the origin of these lipid-laden cells. Some have contended, largely on structural grounds, that they represent modified smooth muscle cells that have migrated up into the intima. However, the incubation of cultured smooth muscle cells even in the presence of extremely high concentrations of LDL, while inducing a modest increase in cholesterol content, by no means converts them to foam cells. Furthermore, we might note here that the foam cells found in xanthomata are very much like those found in the atheroma, yet there are no obvious sources of smooth muscle cells in tendons or skin to provide precursor cells. The alternative view about the foam cells has been that some of them, at least, represent tissues histiocyte, presumably derived ultimately from circulating monocytes.

This view is supported by morphologic evidence indicating penetration of monocytes into the artery wall. It has been strongly supported by the findings of Fowler et al. and Schaffner et al. showing that at least many of the foam cells in lesions share membrane receptors and other properties characteristic of the monocyte/macrophage, including the ability to phagocytose red blood cells. However, attempts by Goldstein et al. to convert macrophages to foam cells in vitro by incubation with high concentrations of native LDL were unsuccessful.

Goldstein et al. speculated that LDL in vivo might become altered in some fashion and that the modified form would be taken up more rapidly than the native form. They tried a number of ways to "brutalize" LDL but nothing worked except chemical acetylation. Acetylated LDL was very avidly degraded and it converted macrophages to lipid-loaded cells, very much like foam cells in vivo. The acetylated LDL was taken up by a saturable, specific process, implying the presence of an acetyl-LDL receptor. However, this receptor was not down-regulated as the cholesterol content of the cells increased, in contrast to the behavior of the native LDL receptor. Via et al. have recently succeeded in partially purifying such a receptor from an established line of macrophages. Other chemically modified forms of LDL have also been shown to compete with acetyl LDL for its receptor, including mayelated LDL, acetoacetyl LDL and malondialdehyde-conjugated LDL. These chemically modified forms of LDL have in common that the lysine epsilon amino groups are blocked. These modified forms are recognized less well by the native LDL receptor. They also have in common a marked increase in negative charge. These studies with chemically altered forms of LDL are important in demonstrating that the monocyte/macrophage can accumulate lipid droplets, but the pathophysiologic significance remains unclear since none of these chemically modified forms has been shown to be generated biologically. The potential for generation of malondialdehyde-conjugated LDL has been demonstrated by Fogelman and his collaborators in preliminary studies indicating the generation of such LDL during clumping of platelets, a process that is associated with release of malondialdehyde. Whether conditions in vivo will generate sufficient malondialdehyde to modify significant quantities of LDL remains to be determined.

About 3 years ago Tore Henriksen in Oslo, while studying the toxic effects of LDL on cultured endothelial cells, noted that the electrophoretic mobility of LDL was altered during the incubations. He joined us in La Jolla to test for the possible biological significance of the observed physical change and we were joined in that work by Eileen M. Mahoney. What we found was that overnight incubation of human LDL with either rabbit or human endothelial cells modifies it in some fashion so that the resultant "endothelial cell-modified LDL" (EC-modified LDL) is taken up and degraded four to five times more rapidly than native LDL by macrophages of several kinds and has the following distinctive properties:

**Biological Properties**
1. Uptake and degradation in macrophages increased to three to five times that of control LDL; degradation via native LDL receptor in other tissues reduced.
2. Macrophage degradation specific and saturable; competitive with unlabeled acetyl LDL, malondialdehyde-conjugated LDL, fucoidin, and polyinosinic acid.
3. Cholesterol esterification stimulated and cholesterol content of macrophage increased.

**Physical Properties**
1. Increased electrophoretic mobility (about twice control values).
2. Increased hydrated density (as high as 1.078, i.e., above the normal limit for native LDL).
3. Decrease in cholesterol content, mainly in esterified cholesterol content.
In each study, aliquots of LDL were incubated under identical conditions except in the absence of cells; this no-cell incubation provided “control LDL.” The enhancement of degradation was most evident using resident mouse peritoneal macrophages but could also be demonstrated using the J774 line of macrophages, rabbit alveolar macrophages, or human monocyte/macrophages. The phenomenon has been demonstrated in a totally homologous system—human LDL, human umbilical vein endothelial cells, and human monocyte/macrophages. The uptake of EC-modified LDL is sufficiently rapid to lead to accumulation of cholesterol in macrophages, somewhat less than that obtained with acetyl LDL, but almost as great, and there was an accompanying increase in rates of cholesterol esterification.

Soon after making these observations we naturally tested to see whether the uptake was related to that of acetyl LDL and found that unlabeled acetyl LDL competes with EC-LDL for uptake and vice versa. EC-LDL also competed with malondialdehyde-conjugated LDL. However, attempts to demonstrate malondialdehyde conjugated to EC-LDL were unsuccessful.

The only other cell type with which we have been able to generate LDL with properties like those of EC-LDL are cultured aortic smooth muscle cells. The “smooth muscle-modified LDL” was taken up and degraded about 50% faster than native LDL, i.e., the transformation seems to be less efficient in smooth muscle cells. Incubation of LDL with fibroblasts or hepatocytes did not result in modification.

Accompanying the biological alteration, there was an increase in the electrophoretic mobility and a decided increase in the hydrated density of the LDL. The density, measured using density equilibrium ultracentrifugation, increased progressively and even reached values above those characteristic of normal LDL. Furthermore, the entire peak shifted, suggesting that essentially every molecule in the incubation had undergone a change. Since these studies were done with as much as 100–200 μg LDL per milliliter, it seems unlikely that all this LDL could be processed through the cell. Reckless et al. showed that endothelial cells express a high-affinity LDL receptor but the rates of uptake followed by degradation were very low. However, the possibility that there is rapid endocytosis and subsequent exocytosis (without delivery to lysosomes) should not be ruled out.

The increase in hydrated density is associated with a decrease in the amount of cholesterol, particularly ester cholesterol, in the EC-modified LDL. The decrease in cholesteryl ester content is just about adequate to account for the increased hydrated density of the molecule. It has not yet been established which physical or chemical change occurring during endothelial cell modification is crucial to the observed biological change.

*Figure 12. Schematic diagram indicating several of the mechanisms available to the macrophage by which it may accumulate lipoprotein lipids and be converted to a foam cell.*
LIPOPROTEINS AND ATHEROSCLEROSIS

The important implications of these findings, of course, relate to the possibility that as LDL enters the artery wall, it may undergo some degree of modification along the lines demonstrated by these in vitro studies (Figure 12). If it does, then it is understandable that the resident macrophages, with their expressed acetyl LDL receptor, might preferentially take up such modified forms of LDL, even though they cannot rapidly take up native LDL. It has been reported that the LDL isolated from artery walls has an increased electrophoretic mobility, and recent studies by Goldstein and co-workers suggest that there may be, at least in atherosclerotic arteries, some population of modified LDL molecules that share properties with acetyl LDL and EC-modified LDL.

The macrophage has a high affinity receptor for another form of lipoprotein that definitely does occur naturally — the beta VLDL. As shown by Mahley and co-workers, this form of VLDL accumulates in the plasma of cholesterol-fed animals of various species. It is also the characteristic "remnant" that accumulates in patients with dysbetalipoproteinemia. Thus, beta VLDL concentrations increase in situations in which there is accelerated atherogenesis. Consequently, it may be highly significant that the macrophage has a high affinity receptor for this lipoprotein, a receptor distinct from the receptor for acetyl LDL/EC-modified LDL. Uptake of beta VLDL is another way in which the foam cell might accumulate its lipid (Figure 12).

Finally, I would like to turn to a new mechanism that may be relevant to foam cell development. Studies by Khoo, Mahoney, and Witztum led to the discovery that the macrophage synthesizes and secretes lipoprotein lipase, and their findings have

---

Figure 13. Macrophage uptake and degradation of $^{125}$-chylomicrons from a patient with apoprotein C-II deficiency in the absence (—•—) and in the presence (—–—) of added apoprotein C-II. At each concentration of chylomicrons, the presence of apo C-II enhanced uptake and degradation of $^{125}$-apoproteins (A) and induced much greater generation of free fatty acids (C) and greater accumulation of cellular triglyceride (E). (Data from Ostlund-Lindqvist et al.)
have been confirmed and extended.94-96 The reason this lipase may have been overlooked for so long is the fact that the secreted enzyme is extremely labile in the medium. Its properties have been shown to be indistinguishable from those of plasma postheparin lipase in almost every respect: a pH optimum of 8.2, a requirement for apoprotein C-II, an inhibition by high salt concentrations, a tight binding to a heparin-Sepharose affinity column, a release from cells on addition of heparin to the medium. Its synthesis and secretion is probably a general property of macrophages; it has been shown to be produced by the J774 line, by alveolar macrophages, by mouse peritoneal macrophages, and by human peripheral monocytes in culture. Interestingly, this seems to be the first lipase recognized and identified as a constitutive secretory product of the macrophage.

The implications with regards to atherogenesis are apparent. This lipase provides the macrophage with new ways in which to facilitate the uptake of lipoproteins. Studies by Ostlund-Lindquist et al.97,98 have demonstrated that the action of the macrophage lipoprotein lipase facilitates uptake of both lipids and apoproteins from VLDL and from chylomicrons. To assess the role of the lipase, lipoproteins from a patient with apoprotein C-II deficiency were utilized. In this way incubation without apo C-II could be used to evaluate uptake not dependent upon lipolytic action; incubations in the presence of C-II provided a measure of the sum of LPL-dependent and LPL-independent uptake. As shown in Figure 13, there was a saturable uptake process even in the absence of apo C-II, indicating receptor-dependent uptake of intact VLDL molecules. The addition of apo C-II, however, speeded the uptake and degradation of VLDL apo-protein, suggesting that remnant particles were being taken into the cell more rapidly. Addition of apo C-II also enhanced accumulation of triglycerides in the cells. Figure 14 illustrates the alternative pathways open to the macrophage for taking triglyceride-rich lipoproteins. The observations of Bates and co-workers99 are in accord with this scheme also.

To sum up, we have to conclude that the macrophage, in addition to its voracious appetite for bacteria and cell debris, also has a voracious appetite for several different kinds of lipoproteins. It can gobble them up whole via specific receptor mechanisms as in the case of beta VLDL, endothelial cell-modified LDL, and related forms when available. In addition to this direct gourmandizing, it can, with the aid of its own internally generated lipoprotein lipase, chew up the triglyceride-rich lipoproteins and take up separately the FFA and other products and then the smaller lipoprotein morsels (remnants) instead of swallowing them whole. A brief summary and a rechristening of the cell are suggested in Figure 15.

Before leaving the Pac-rophage, I want to raise a question that is too seldom discussed. Is this cell a "good guy" or a "bad guy"? At times it seems clear that the macrophage is really our friend. Certainly he is the good guy when it comes to dealing with invading bacteria or debris at sites of infection or wounds. He may be crucially important in body defenses for example, by presenting antigen properly to T-cells. But what about his role in atherogenesis? It is possible that by taking up lipoproteins he actually forestalls damaging effects that might otherwise occur earlier. On the other hand, it is equally possible that, if the macrophage were not so busy taking up lipoproteins, these lipoproteins could be more effectively mobilized and got rid of by other mechanisms available to the artery wall. Further, it is now recognized that the macrophage synthesizes and secretes a growth factor.15,16 Is this process contributing to the development of the ultimate space-occupying lesions, as postulated for platelet-derived growth factor? Or is it possible that these growth factors are somehow helping the artery to confine developing damage and limit the rate of development of lesions? Further research is needed on just what role the macrophage plays in atherogenesis, and we should not conclude that it is a negative one. However, we should not be one bit less interested in further studies of macrophage biology whether the cell is a

Figure 15. Summary of the varieties of lipoprotein gourmandizing available to the omnivorous "Pac-rophage."
"good guy" or a "bad guy." If the cell is a "good guy," we need to find ways to encourage it to enter the artery and do its thing; if it is a "bad guy," then we need to know as much as possible about it so we can develop the appropriate inhibitors, competitors, or "silver bullets."

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Index Terms: atherosclerosis • lipoproteins • macrophages • endothelial cells • lipoprotein lipase • low density lipoprotein • lipoprotein receptors

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