IN recent years, a marked increase in knowledge about the levels of circulating lipoprotein classes, their structural components, and physical organization has taken place. A new appreciation of the dynamic metabolism of lipoproteins in humans and in experimental animals has been gained from studies of the various nascent lipoproteins, the sequential steps of lipolysis that take place among and give rise to circulating lipoproteins, and the various exchanges of constituents among classes. It is not possible to do more than summarize major advances, but several recent excellent reviews may be consulted for details (Smith et al., 1978; Fredrickson et al., 1978; Schaefer et al., 1978a). Triglyceride-rich very low density lipoproteins (VLDL) are secreted by the liver and to some degree by the intestine and are acted on by lipoprotein lipase (LPL), leading to progressive lipolysis, loss of triglyceride, and conversion to a smaller cholesterol ester (and cholesterol)-rich lipoprotein class known as low density lipoprotein (LDL). Chylomicrons are large fat-containing particles resulting from intestinal absorption of dietary fat and through a similar action of LPL are converted to so-called "remnant" particles, rich in esterified and free cholesterol. Another class of lipoprotein, high density lipoproteins (HDL), apparently is derived from both intestinal and hepatic sources. HDL not only exchange certain proteins and phospholipids with VLDL, but also apoprotein C-II of the subclass of HDL called HDL₂ seems to activate LPL, thereby favoring the conversion of VLDL to LDL (Fredrickson et al., 1978; Tall and Small, 1978).

Epidemiological studies have demonstrated that LDL levels are positively associated with risk of clinical sequelae of atherosclerosis and that HDL levels are negatively associated with risk; i.e., they may be protective. VLDL levels are not usually an independent risk factor for heart disease but add risk to those with elevated LDL levels and probably act independently at extremely high levels, as occurs in diabetes mellitus (Kannel et al., 1979). Many studies have been directed at identifying those circumstances in humans that favor elevation or depression of one or another of these lipoprotein classes in the hope of devising therapeutic interventions. Furthermore, intense investigations are being conducted on genetic diseases in which certain lipoprotein classes are reduced or absent, such as abetalipoproteinemia, lecithin: cholesterol acyltransferase deficiency, and Tangier disease.

Another aspect of the problem of atherogenesis, however, that has received less attention is one that can be called the "tissue" contribution to lipoprotein metabolism, in particular, catabolism. For example, it is agreed that LPL is an enzyme functional to a large extent at the vascular endothelial surface at which site interaction with lipoprotein occurs (Fielding and Havel, 1977). The enzyme is present in a variety of tissues, including skeletal and heart muscle, adipose, lung, and lactating mammary tissue, and presumably is most active in the endothelial cells of the vascular component of these tissues. However, remaining to be explained are the presence of enzyme in the parenchymal cells of these tissues, the release of only a portion of total tissue enzyme by heparin, and the variation in activity of the functional enzyme by as much as 10-fold in the different sites (Fielding and Havel, 1977). Thus, whereas we can point with considerable certainty to the physiological role for this enzyme in lipoprotein metabolism, we know much less about the tissue mechanisms regulating synthesis, storage, and release of LPL.

A strong case can be made for an inverse relationship between amounts of LPL in certain tissues and the levels of a circulating lipoprotein substrate,
Lipoprotein Lipase and Catabolism of VLDL and Chylomicrons occurs in vitro at pH 8.0-8.6 and is attributed to the activity of LPL (Fielding and Havel, 1977). The functional site is believed to be on or near the endothelial surface because perfusion of the vascular bed of a tissue is sufficient to demonstrate lipolytic activity. The enzyme has been found in both large elastic vessels, such as aorta (Corey and Zilversmit, 1977), and the capillary beds of a variety of tissues, including the brain (Brecher and Kuan, 1979). Although this enzyme has been reported to be much more abundant in the capillary bed than in large vessels (Fielding and Havel, 1977), the frame of reference for this comparison is not convincing.

Considerable evidence has accumulated to justify the conclusion that the activity of LPL influences the concentration of plasma triglycerides in humans and in experimental animals. In a recessively inherited form of human hypertriglycerideremia, lipolytic activity in postheparin plasma is practically absent, and no LPL is found in the adipose tissue. The finding of normal levels of hepatic LPL implicates the absence of adipose tissue LPL in the pathogenesis of this disease (Fielding and Havel, 1977).

Another example of the inverse relationship between tissue LPL and circulating levels of VLDL and chylomicrons is seen when carbohydrates are ingested by a variety of mammalian species, including humans. The activity of adipose tissue LPL increases; heart LPL is also increased but less so. The increase is thought to be due to induction of enzyme by insulin, a response seen not only in adipose tissues removed from animals (Garfinkel et al., 1976) but one that has also been demonstrated recently in vitro, using cultured 3T3-L1 cells (Eckel et al., 1978). Insulin augmented both synthesis and membrane release of the enzyme. In insulin-deficient diabetic subjects, plasma VLDL levels are increased and both basal amounts of LPL and its induction by carbohydrate are deficient (Table 1); this is true of both adipose tissue (Pykalisto et al., 1975) and hepatic enzymes (Elkeles and Hambley, 1975).

**Lipoprotein Lipase and Catabolism of VLDL and Chylomicrons**

Catabolism of triglyceride carried in VLDL and chylomicrons occurs in vitro at pH 8.0-8.6 and is attributed to the activity of LPL (Fielding and Havel, 1977). The functional site is believed to be on or near the endothelial surface because perfusion of the vascular bed of a tissue is sufficient to demonstrate lipolytic activity. The enzyme has been found in both large elastic vessels, such as aorta (Corey and Zilversmit, 1977), and the capillary beds of a variety of tissues, including the brain (Brecher and Kuan, 1979). Although this enzyme has been reported to be much more abundant in the capillary bed than in large vessels (Fielding and Havel, 1977), the frame of reference for this comparison is not convincing.

### Table 1 Summary of Relationship between Circulating Lipoprotein and Tissue Lipolytic Enzymes in Males

<table>
<thead>
<tr>
<th>Lipolytic Enzymes in Males</th>
<th>Insulin-deficient diabetes mellitus</th>
<th>Exercise</th>
<th>Hypothyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL activity (endothelial)</td>
<td>Adipose tissue</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>?</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Chylomicron clearance rate</td>
<td>↓</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>Serum triglyceride (VLDL) level</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>ACE activity</td>
<td>Aortic smooth muscle</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Fractional catabolic rate of LDL</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Total serum cholesterol level</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>LDL intravascular pool</td>
<td>↑</td>
<td>—</td>
</tr>
</tbody>
</table>

Studies on LPL and VLDL have been carried out on rats, humans, or both, and in some instances, e.g., VLDL-LPL relationship in hypothyroidism, important species differences exist. See text for these details and sources of these results. All studies of ACE and LDL summarized here have been done on rats only. Symbols: ↑ = increased; ↓ = decreased; — = unchanged; ? = unknown.
et al., 1977) and skeletal muscle (Lithell et al., 1978) demonstrated increase in clearance of circulating triglyceride, increased amounts of adipose tissue (Taylor et al., 1976; Nikkilä et al., 1978); this triglyceride lowering is found even when caloric intake is increased to maintain body weight (Gyntelbert et al., 1977). In addition, increased LPL activity is found in the adipose tissue and skeletal muscle of these male runners (Nikkilä et al., 1978), and total calculated enzyme in these tissues is increased by factors of 2.7 and 1.7, respectively, over normal sedentary levels; increased skeletal muscle LPL also has been reported in exercised rats (Borensztajn et al., 1975). With respect to the mechanism of increased LPL in exercise, serum insulin levels were reduced in runners, but increased tissue sensitivity to insulin with exercise is still a possible explanation (Nikkilä et al., 1978). Other explanations include an increased caloric intake by runners or perhaps a direct hemodynamic effect of exercise on endothelial metabolism. The increased circulating HDL level seen with exercise may reflect the increased lipolysis of VLDL triglyceride (see review by Tall and Small, 1978).

Hypothyroidism in humans is another example of the inverse correlation between adipose tissue content of LPL and circulating triglyceride (VLDL) levels. Hypertriglyceridemia found in hypothyroidism is associated with significantly reduced adipose tissue LPL content and decreased postheparin lipolytic activity (Pykälästö et al., 1976). Treatment with thyroxine restores all values toward normal with the lowered triglyceride inversely correlated with increased tissue LPL. In rats, the results are different. Neither circulating triglyceride levels nor tissue LPL are greatly influenced by the hypothyroid state (Redgrave and Snibson, 1977).

A similar pattern of increased tissue LPL and decreased plasma triglyceride also is found after treatment of patients with clofibrate. Concomitant with a fall in plasma triglyceride and a demonstrated increase in clearance of circulating triglyceride, increased amounts of adipose tissue (Taylor et al., 1977) and skeletal muscle (Lithell et al., 1978) LPL enzymes are found. Serum insulin levels are lower after clofibrate treatment (Lithell et al., 1978) but, again, increased tissue sensitivity may be the explanation (Ip et al., 1976). Stimulation of the enzyme also could have come about by reduction of intracellular free fatty acid levels in adipose tissue or by a direct inductive effect of the drug on LPL itself.

Therefore, it seems likely that under a variety of circumstances an inverse relationship between tissue LPL, predominantly located in vascular endothelial cells, and circulating VLDL triglyceride levels can be found (Table 1). In addition, the inverse relationship found between HDL and VLDL plasma levels suggests that the former lipoprotein class also is tied in to the enzyme-VLDL relationship by one or more of several possible mechanisms (Tall and Small, 1978).

The mechanisms by which these relationships influence development of vascular disease remain unidentified. In a recent study, Corey and Zilversmit (1977) examined the effect on aortic LPL activity of cholesterol feeding of rabbits. Confirming first the extremely low level of LPL in the normal aorta, they demonstrated a positive linear relationship between aortic LPL level and aortic cholesterol content, levels of enzyme in some instances being increased 40-fold with extensive disease. Heart LPL and cholesterol contents also were increased. These results were thought to be compatible with a hypothesis proposed by Zilversmit (1973) in which LPL activity at the endothelial surface would act to promote lipid deposition in arteries. This intriguing proposal implicates LPL activity in the generation of smaller cholesterol-rich remnants from chylomicrons, which would be taken up quickly by the vessel wall; circulating remnant or LDL levels would not necessarily reflect a much higher atherogenic concentration near the blood vessel wall. Measurements of triglyceride levels in individuals kept in the fasting state would underestimate the atherogenic potential of their circulating lipids (Zilversmit, 1973). Consistent with this theory is the finding by Bierman et al. (1973) that “remnants” were taken up much more avidly than were VLDL particles by cultured rat aortic smooth muscle cells and, once internalized, were degraded more slowly. It is not yet clear how to reconcile this theory with the apparent greater level of LPL in peripheral vascular beds which are, of course, not vulnerable to atherogenesis. However, it is interesting that an enzyme activity physiologically appropriate for the capillary bed of a peripheral tissue such as adipose tissue can perhaps be deleterious when present in the endothelium of large vessels.

One problem that arises is the determination of total enzyme activity relative to the presumed substrate load. The tissue activity usually is expressed on the basis of tissue weight, almost never on the basis of DNA. Rarely, an attempt is made to express the enzyme activity/unit surface area, as done by Corey and Zilversmit (1977). Nikkilä et al. (1978) have attempted to calculate total body LPL activity...
on the basis of estimates of total body muscle mass and adipose tissue. Normal females were estimated to have more than two times the whole-body LPL activity of males, and more than 80% was present in adipose tissue as opposed to about 60% in males. However, even these authors agree that information about body composition is so sparse that their calculations of these values in runners probably were incorrect (Nikkila et al., 1978).

It is interesting in this regard to consider the surface area of the vascular tree available for endothelial LPL-lipoprotein interactions. I have calculated endothelial surface area of various portions of the cardiovascular tree in a 70-kg man (see Table 2). These calculations are based on published estimates of cross-sectional areas of (Berne and Levy, 1977) and blood distribution volumes to (H. Bazett [Burton, 1972]; Detweiler, 1973) various vascular beds, assuming a total blood volume of 5 liters. Extrapolating from the geometry of the mesenteric vascular bed of the dog [F. Mall (Burton, 1972)] to the adult man and using average values of wall lumen and endothelial and medial thicknesses (Burton, 1972), the number and total circumference of each vessel type could be calculated. The volume and weight (assuming water density and with no correction for connective tissue) of each vessel type then was derived easily. These data are admittedly crude estimates, particularly so for veins, which contain loosely packed smooth muscle cells in a rich connective tissue stroma. However, it is reassuring to note that the total capillary surface area is reported elsewhere to be $6 \times 10^6$ cm$^2$ of which only 25–35%, or about $1.5 \times 10^6$ cm$^2$, are open at any one time in the resting condition (Folkow and Neil, 1971); this figure agrees well with our estimate (Table 2). With respect to the problem of estimating total body LPL activity from isolated measurements, it seems possible that physiological factors may enhance the interaction between substrate (VLDL) and enzyme (LPL) without necessitating a measurable increase in enzyme activity per unit tissue. For example, the doubling of capillary surface due to opening up of previously closed beds found to occur with exercise (Folkow and Neil, 1971) could in itself increase the exposure of VLDL to LPL. Altered cardiovascular physiology associated with obesity or hypertension also could lead to alterations of this enzyme-lipoprotein interaction. Estimation of surface area of various portions of the circulatory tree, such as those shown in Table 2, could facilitate studies of the effects of these and other perturbations of normal physiology.

### Lysosomal Lipases and Catabolism of LDL

ACE, one of several lysosomal lipases, must be considered in any schema that attempts to relate tissue catalytic activity to serum lipoprotein levels. This lipase is optimally active at an acid pH (usually 4.2 or 4.5) and has been localized unequivocally to the lysosome, the membrane-bounded digestive organelle of the cell (Takano et al., 1974). Furthermore, it has been shown to be involved integrally in the catabolism of cholesteryl ester-rich LDL (Basu et al., 1976). To date, it has not yet been possible to separate the ACE hydrolytic activity from an acid triglyceride hydrolitic activity in vascular tissue (Brecher et al., 1978). The enzyme has been found in aortic tissue of several mammalian species, including humans (Fowler and Wolinsky, 1980) and has been described recently in rabbit brain microvessels (Brecher and Kuan, 1979); variations in activity with vessel site have not yet been reported. In addition, an ACE has been found in macrophages (Werb and Cohn, 1972), liver (Nilsson et al., 1973), lymphocytes (Coates et al., 1979), and kidney (Wolinsky, H., unpublished observations).

It is estimated that, each day, normal humans must catabolize 1.0 g of apoprotein $\beta$ in LDL, which corresponds to 1.5 g of cholesterol, at least two-thirds of which is esterified (Langer et al., 1972). Unlike VLDL, which is converted largely to LDL while in the circulation, LDL seems to represent an end point in lipoprotein catabolism and must be internalized by cells and dealt with directly by tissue hydrolases, including cathepsins and lipases. In humans, 90% or more of VLDL is converted to LDL which then is catalyzed by tissues; in the rat

| TABLE 2 Estimated Dimensions and Weights of the Vascular Tree in a 70-kg Man |
|---------------------------------|------|-------|------|
|                                 | Smooth muscle |
| Endothelium                     | Volume (cm$^3$) | wt (g) |
| Total surface area (cm$^2$)     | Volume (cm$^3$) | wt (g) |
| Aorta                           | 156  | 0.016 | 31   |
| Large arteries                  | 3,333| 0.333 | 333  |
| Small arteries                  | 14,473| 1.447 | 290  |
| Arterioles                      | 261,337| 26.134| 497  |
| Total                           | 279,299| 27.300| 1151 |
| Capillaries                     | 1,517,295(resting)| 151.73 |  |
| Venules                         | 879,989| 87.999| 352  |
| Small veins                     | 32,655| 3.266 | 163  |
| Large veins                     | 6,836 | 0.684 | 342  |
| Vena cava                       | 177  | 0.018 | 27   |
| Total                           | 919,657| 91.967| 884  |
only 10-20% is so converted with most of the VLDL recycled to liver for disposal (Schaefer et al., 1978a). Goldstein and Brown (1977) have suggested that LDL represents a normal circulating storage "bank" to supply tissues with cholesterol for ordinary cellular needs without necessitating local cholesterol biosynthesis. Internalization of LDL by cells leads to fusion of the endocytic vesicle with lysosomes, hydrolysis of the ester, and provision of free cholesterol for cellular needs. Although a plausible attempt to integrate LDL receptor activity, lysosomal hydrolysis, and circulating lipoprotein levels, this hypothesis does not deal directly with several related questions. For example, it does not account for the particular predisposition of vascular tissue to atherosclerosis, nor does it specify which tissues are responsible for disposal at normal or elevated circulating LDL levels. Second, although individuals with familial hypercholesterolemia would be expected to have unrestrained lipid synthesis due to absent LDL receptor modulation, metabolic studies to date indicate that, in fact, it is the catabolic rate and not the synthetic rate that is primarily apace in these patients (Schaefer et al., 1978a). Finally, the basic mechanism by which tissues become overloaded with lipid in human familial hypercholesterolemia, where specific receptors are absent, remains to be explained fully. Goldstein et al. (1979) have proposed that the macrophage is the dominant cell involved in lipid overload of tissues of these patients. They have postulated a backup "scavenger" role for this cell that results in massive overloading via specific binding sites that recognize acetylated but not native LDL.

Perhaps it would be simpler to take a step back and consider several relevant questions and possibilities. First, where is LDL metabolism occurring? Hay et al. (1971) reported that the liver of the rat could account for the major part of LDL catabolism. In disagreement, more recent studies in the posthepatectomy pig (Sniderman et al., 1974) have shown little if any contribution by this organ to LDL catabolism, and a recent study comparing catabolism in perfused liver with total body catabolism of LDL in the normal rat (Sigurdsson et al., 1978) has concluded that no more than 7% of the total can be attributed to the liver. However, when LDL catabolism is stimulated by estrogen treatment of male rats, the liver seems to be the primary site of augmentation (Chao et al., 1979). Recent use of a method that detects sites of LDL catabolism by retention of the indigestible sucrose moiety of sucrose-linked LDL molecules has begun to yield additional information about the diversity of tissues involved in LDL catabolism (Pittman et al., 1979).

Is it possible that, analogous to VLDL lipolysis by LPL, ACE in vascular tissue, particularly in smooth muscle, is almost totally responsible for LDL catabolism? This seemingly farfetched suggestion prompted calculations which reveal that the arterial side of the vascular tree alone normally contains enough esterase to catabolize approximately 2 g of cholesteryl ester per day or more than the estimated 1.5 g of LDL cholesterol turned over per day. These calculations were based on total arterial tissue mass (smooth muscle) in humans (Table 2), an estimated cell volume of 7.85 \(\times\) 10^{-6} cm³ (Somlyo and Somlyo, 1978), an assumed DNA content of 7 pg/cell, and an estimated specific activity of ACE of 2 mU/mg DNA in human aortic smooth muscle cells (Powlar, S, personal communication). Although this calculation can be considered only a rough estimate, it does demonstrate how important a metabolic tissue the vascular tree can become when considered in aggregate. In fact, when I return to the question of LDL catabolic site(s) later, it will be apparent that it is highly unlikely that the vascular tree plays a major role in total LDL catabolism. Rather, it seems more likely that, for vascular tissue, this enzyme is an important determinant of local susceptibility to lipid deposition.

Let us now turn to the evidence linking the dynamic decay of LDL from the circulation to tissue hydrolase activities. Before proceeding, however, it is important to point out that the circulating LDL-ACE relationship differs from the VLDL-LPL inverse relationship in at least two ways. First, the circulating level of LDL is affected greatly not only by removal rates but also by input from synthesis of LDL, mostly derived from conversion of VLDL, and to a degree by de novo synthesis by the liver (Schaefer et al., 1978a). Thus, the actual level of plasma LDL will give less information pertinent to tissue catabolic capacity than will dynamic studies of LDL removal from the circulation. Second, unlike the case with LPL and VLDL, ACE will interact with LDL only after the latter enters the tissue and is bound to and internalized by the cell. Thus, the level of cellular ACE is best seen as a mirror of a sequence of steps that begins with entrance of LDL into the particular tissue. However, since the fractional catabolic rate reflects irreversible degradation of LDL (Mathews, 1957), it is reasonable to relate actual tissue catabolic capacity to the dynamic removal of LDL from the circulation.

The autosomal dominant disorder, familial type II hyperlipoproteinemia, is characterized by a marked elevation in circulating LDL levels and greatly accelerated atherogenesis (Fredrickson et al., 1978). Studies of the metabolism of LDL in this genetic disorder have shown that the hypercholesterolemia is due primarily to a decreased fractional catabolic rate of LDL* (Langer et al., 1972, Schaefer et al., 1978a) and that the synthetic rate is unchanged. As a result, the half-life of an LDL molecule in the circulation of these individuals is in-

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*The fractional catabolic rate reflects clearance of LDL from the circulation as a percentage of the total. This is usually calculated by following decay of radiodinated LDL from plasma. A biexponential curve is seen, the second component of which is due to irreversible removal and degradation of the iodinated protein (Mathews, 1957).
plasma LDL cholesterol showed a marked impairment of clearance and an increased half-life of this LDL, and increased risk for atherosclerosis in humans. In the insulin-dependent diabetic rat, aortic ACE activity recently has been reported by us to be markedly reduced (Wolinsky et al., 1978a); new studies (Table 1) show that the fractional catabolic rate of $^{125}$I-labeled LDL in diabetic rats is actually increased and that increases in acid lipase activities occur in kidney and liver tissues from these animals (Wolinsky et al., 1980). Furthermore, a stimulatory effect of insulin on receptor-mediated uptake and catabolism of LDL by cultured human fibroblasts is seen (Chait et al., 1978). Thus, the tissue lipase level of nonvascular tissues agrees with the dynamic clearance measurements.

In exercising humans (Wood et al., 1976) and rats (Wolinsky et al., 1980), circulating LDL levels are decreased significantly. Aortic ACE activity is increased in rats on a chronic swimming regime, but liver and kidney acid lipase activities are unchanged (Wolinsky et al., 1979). Clearance of $^{125}$I-labeled LDL from the circulation of these rats also does not differ from that of normal sedentary animals (Table 1; Wolinsky et al., 1980). Again, the unchanged amount of esterase in nonvascular tissues agrees best with the dynamic LDL clearance studies. In exercising mice, lysosomal enzymes of skeletal muscle also are increased, but the acid lipase was not measured specifically (Vihko, 1978).

Hypothyroidism, the third example, is associated with hypercholesterolemia, particularly that of LDL, and increased risk for atherosclerosis in humans (Fredrickson et al., 1978) and experimental animals (Kritchevsky, 1960). Injection of $^{125}$I-labeled LDL into hypothyroid rats with elevated plasma LDL cholesterol showed a marked impairment of clearance and an increased half-life of this lipoprotein (Cnoop-Koopmans and Angel, 1978). In studies of labeled chylomicron clearance in hypothyroid rats (Redgrave and Snibson, 1977), triglyceride was removed quickly from the particles (and adipose tissue LPL was normal or even increased), resulting in “remnants,” but cholesteryl ester clearance from these particles was impaired markedly. Interestingly, liver cholesterol content but not triglyceride content was increased markedly in the hypothyroid animals (Redgrave and Snibson, 1977). Studies from our laboratory show that ACE specific activity in aortic tissue from thyroidectomized rats is decreased only slightly from normal (15%) (unpublished observations), whereas ACE activities in liver and kidney are both decreased markedly (40%) (Coates et al., 1978; Wolinsky, H., unpublished observations). Treatment with thyroid hormone restores LDL clearance and tissue ACE activities to normal. In vitro studies also show a stimulatory effect of triiodothyronine on LDL uptake and catabolism by cultured human fibroblasts (Chait et al., 1979). Again, the ACE activities of liver and kidney change in the same direction and degree so as to agree with the independent dynamic measurement of fractional catabolic rate for LDL; aortic levels of ACE are not so related to the fractional catabolic rate in degree of change.

Other factors have been shown to influence either vascular ACE activity or circulating LDL levels and clearance rates; however, neither kind of data is available in the same model for comparison. Hyperpertension, for example, is a potent stimulus to acid esterase activity in the rat aorta, and the activity returns to normal when the animal is returned to a normotensive state (Wolinsky et al., 1978b). Unlike exercise, however, hypertension results in damage to the wall in association with these enzyme increases (Wolinsky et al., 1979). No information is yet available on LDL clearance in hypertensive humans or experimental animals. On the other hand, treatment of male rats with estrogen causes marked hypolipidemia and increased clearance of $^{125}$I-labeled human and rat LDL (Chao et al., 1979), but no information is available about ACE activities in the various tissues.

Finally, the feeding of an atherogenic diet and development of hypercholesterolemia, mainly due to LDL, are associated with lesion formation and increased acid lipase activity in the aorta of experimental animals, including rabbits (Peters et al., 1973; Brecher et al., 1977) and monkeys (Fowler et al., 1980). In the atherosclerotic human aorta, ACE activity also is elevated (Berberian and Fowler, 1979). Corey and Zilversmit (1977) showed concomitant increases in LPL and ACE activities in the same diseased aortic segments obtained from cholesterol-fed rabbits. Information about LDL clearance or even consistently measured circulating LDL levels is not available yet in these various animal models.

A summary of the relationships just described for both LPL and ACE activities in vascular and nonvascular tissues is presented for three of the best studied circumstances (Table 1). The inverse
relationships between LPL levels or chylomicron clearance, on the one hand, and VLDL levels, on the other, seem clear-cut and impressive. Furthermore, it is interesting that both LPL and the ACE in vascular tissue are insulin-responsive and are changed in the same direction by exercise and during development of atherosclerotic disease. When amounts of ACE in two nonvascular tissues are examined with respect to fractional catabolic rates, the dynamic studies and enzyme activities agree in every instance, both showing a significant increase in diabetes mellitus, no change with exercise, and a decrease in hypothyroidism.[The lack of agreement between LDL pool size and fractional catabolic rate results from concomitant changes in steady state conditions of synthesis and degradation, which affect pool size but not fractional catabolic rate (Mathews, 1957).] On the other hand, the enzyme change in vascular tissue does not agree in direction either with enzyme changes in the other tissues or with the fractional catabolic rate (Table 1); in the case of hypothyroidism, aortic enzyme changes in direction but not in degree with other tissues or the fractional catabolic rate. It therefore appears that vascular tissue probably is not a major site of LDL catabolism and may not even play an appreciable role. In fact, it appears unlikely to be related to overall LDL disposal in any predictable way. This postulation of the limited involvement of vascular tissue in lipoprotein catabolism is supported further by the actual measurements of relative specific activities of ACE in three tissues of rats (Fig. 1). Clearly, aortic tissue is far poorer in this acid lipase (and other lysosomal enzymes) by one to two orders of magnitude, which agrees well with the cytochemical evidence for a dramatic scarcity of lysosomes in aortic tissue compared to other tissues (Fig. 1). The similarity of the profiles of pH curves in three tissues further suggests that the same enzyme is being measured in all.

Any attempt to draw the above conclusions must be tempered by several concerns: (1) the number of tissues sampled is small, and it is entirely possible that other tissues could behave still differently or like vascular tissue [of particular interest in this respect is the macrophage in view of its postulated role as a "scavenger cell" for LDL (Goldstein et al., 1979)]; (2) the number of models is few and needs to be expanded to test the proposal; (3) uncertainty as to the degree that in vitro incubation conditions truly reflect the in vivo enzyme environment is always present; and (4) unlike the case with a surface-related enzyme (LPL) acting on a circulating lipoprotein substrate, many steps are interposed between the lysosomal lipase and exposure to the LDL substrate, i.e., endothelial transport processes, LDL entrance into the extracellular space of the wall, and internalization of the molecule by the smooth muscle cell following specific and nonspecific binding. Any one or more of these steps could be rate-limiting and thereby account for the several observed relationships between levels of vascular enzyme and substrate. Unfortunately, little reliable, quantitative information is available about LDL flux into vascular tissue. One isolated report, for example, describes a direct relation between flux of cholesterol into the aorta of alloxan-diabetic rats and serum cholesterol or LDL concentration that does not differ from that of normal animals (Mat-suda and Kalant, 1966); when the circulating level increases, flux increases. Not only are available data of this type extremely limited, but studies of flux of labeled proteins, such as albumin, are plagued with methodological problems (Winlove et al., 1978).

deDuve (1974) has calculated that maximal acid lipolytic activity by cells in the rabbit aorta exceeds by only a factor of two the calculated rates of cholesterol ester influx into aortas from severely hypercholesterolemic animals (Newman and Zilversmit, 1966), but this is acknowledged to be a crude approximation. The many variables affecting rates of influx are exemplified by a recent report describing an apparent effect of insulin on vesicular transport activity of capillary endothelium (Osterby et al., 1978).

In any case, the observed lack of coordination between fractional catabolic rate of LDL and aortic ACE could reflect either that LDL entrance into the wall is limiting or that the sequence of steps beginning with exposure of the cell to LDL and ending with LDL hydrolysis is interrupted. The first possibility seems unlikely, given the nature of the vascular disease, i.e., accumulation of lipid that is derived largely from LDL. The second possibility must perforce result in lipid accumulation in vascular tissue or its cells. Recent studies by Shepherd et al. of humans (1979) and by Mahley et al. of rats and monkeys (1980) support an important role for the LDL receptor in clearance of LDL from the circulation in vivo. Modification of the arginine groups of LDL apoprotein results in marked inhibition of clearance of the molecule compared to unmodified LDL when injected into the circulation. Since in vitro studies have shown interference with LDL-LDL receptor binding by this modification, the delayed clearance of modified LDL appears to implicate the receptor in this step. Although non-receptor-mediated LDL uptake remains the major mechanism of LDL uptake by cells in normal humans, alterations in the receptor-mediated pathway clearly can influence LDL clearance.

Whether it is the specific or nonspecific uptake process or any subsequent step that results in lack of coordination between LDL clearance and ACE activities in vascular tissue, the demonstration of the atypical behavior of this tissue vis-a-vis other tissues bears directly on the question of its special vulnerability to atherogenesis.

Catabolism of HDL

Of the major circulating lipoprotein classes, we probably know least about the metabolism of HDL. HDL is associated with reduced risk of atheroscle-
ACE specific activities in liver, kidney, and aorta of the same rat. Determinations of pH optimums (left) were carried out in acetate buffer; a radiolabeled vesicle as substrate was used in the assay (Brecher et al., 1977). Note the 3-cycle log scale of relative activities. Cytochemical demonstration of dark reaction product when the same three tissues were incubated for acid phosphatase activity (right). The substrate was β-glycerophosphate, and incubation times were 15 minutes for liver and 30 minutes each for kidney and aorta. Liver: p = portal triad; k = Kupffer cell. Kidney: g = glomerulus; p = proximal tubule; d = distal tubule. Aorta: arrows point to sparse lysosomes. All magnifications, 640 X. (Cytochemical preparations courtesy of Dr. Sidney Goldfischer.)

Tangier disease is an autosomal recessive hypolipoproteinemia, characterized in the homozygous state by the near absence of HDL apoproteins and massive deposition of cholesteryl esters in the reticuloendothelial system (Fredrickson et al., 1978). A recent study has suggested that this deficiency is due to a markedly accelerated fractional catabolic rate of the A apoproteins (Schaefer et al., 1978b). Little is known about the nature of the sites or enzymes involved in these processes; it has been suggested that the major site of catabolism of HDL in rats (Eisenberg et al., 1973) and humans (Schwartz et al., 1978) is the liver, but the results in rats have recently been challenged (Sigurdsson et al., 1979). Others have more directly implicated the lysosomes of liver (Rachmilewitz et al., 1972) and kidney (Nakai and Whayne, 1976) in the catabolism of HDL in rats and dogs.

Implications for Atherogenesis

Lipid accumulation in large vessel walls is a central feature of human and experimental atherosclerotic disease. At the tissue level, the problem can be framed in terms of altered endothelial permeability (Fry, 1973) and both extracellular binding (Iverius, 1973) and intracellular accumulation. At the cellular level, lipid accumulation can be addressed as a problem in receptor function (Goldstein and Brown, 1977), vesicular transport, lysosomal enzyme sufficiency (de Duve, 1974), or even relative digestibility of an abnormal LDL molecule (Tall et al., 1978). Regardless of how many of these or other unidentified factors are operative, the net result is an influx-efflux imbalance in the cell and the blood vessel wall. It seems likely that clinical risk factors act by affecting this balance either to the detriment or to the benefit of the cell or tissue, and several specific effects have been suggested.
Blumenfeld, Dr. Lewis Gidez, and Dr. Sidney Goldfischer for their helpful reviews of the manuscript, Dr. Ronald Markle, Dr. the challenge to solve it waiting for those who would provided. The shape of the problem is discernible and sophistication from the added dimensions pro-

interventions, that gain in precision, effectiveness, the synthesis of knowledge seems obvious, however, atherogenesis better and perhaps in devising new

sweeping predictions of pattern have been proposed

two areas of investigation that have tra-

ditionally been addressed to different aspects of the

circulating lipoprotein levels and tissue catabolic

required.

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H Wolinsky

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