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## Lipoprotein Metabolism

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Volume Editor
*S. Eisenberg*, Jerusalem

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# Contents

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# Introductory Remarks

*S. Eisenberg*

Circulating lipoproteins are lipid-carrying particles of the blood plasma. Operationally, they are subdivided into several species: chylomicrons, very-low-density (VLDL), intermediate-density (IDL), low-density (LDL), and high-density (HDL) lipoproteins. Each of the plasma lipoproteins is a complex particle of finite dimensions and composition. Different lipoproteins, however, differ markedly in size and lipid content. For example, HDL particles are of a diameter of about 80–100 Å; the chylomicrons may attain a diameter of several microns. Hence, the amount of lipid transported in one chylomicron particle exceeds that in one HDL particle by tens of thousands. In contrast, the number of HDL particles circulating in the plasma exceeds that of all other lipoproteins by many folds. Within each lipoprotein, moreover, there exists a spectrum of particles differing in size, weight and composition. Some of these differences reflect changes induced by metabolism, when lipoproteins loose – or gain – lipids and apoproteins. Other differences represent true heterogeneity of lipoprotein fractions isolated from plasma. Whereas the striking differences among lipoproteins are not reflected by the conventional expression of plasma lipid and lipoprotein lipid levels, they are essential for the understanding of lipid transport in lipoproteins. Thus, interactions among lipoproteins, and between lipoproteins and enzymes or cells can be fully understood only when the lipoproteins are regarded as particles.

The number of protein and lipid constituents assembled together in any lipoprotein particles is very large. About 10–12 different proteins (the apolipoproteins) were identified so far in lipoproteins. These include three A proteins (apoA-I, apoA-II and apoA-III), apoB, three C proteins (apoC-I, apoC-II and apoC-III), apoD, several polymorphic forms of apoE, and apoF. Major lipid constituents are triglycerides, phospholipids, unesterified cholesterol and

cholesterol ester. Fatty acids, di- and monoglycerides and several different phospholipid species (lecithin, sphingomyelin and small amounts of other glycerophosphatides) are also present. The lipoproteins are not only complex particles, but are also in a dynamic equilibrium among themselves and with blood and tissue cells. Apoproteins and lipids do exchange among lipoproteins. The lipids exchange also between lipoproteins and cells. Lipoproteins, moreover, are donors and recipients of apoproteins and lipids and continuously change their composition and character during metabolism. Some lipoproteins are produced in the plasma, at least in part, and represent dissociation 'units' or breakdown products of the VLDL and chylomicrons. Consequently, change in level, structure, composition or metabolism of any lipoprotein family may affect the levels, structure, composition or metabolism of other lipoproteins.

Circulating lipoproteins are highly ordered particles. It is usually believed that apolar lipids – triglycerides and cholesterol esters – occupy the core of the lipoproteins, and that proteins, phospholipids and unesterified cholesterol constitute the surface ('lipid core' model). Several molecular models of lipoproteins have been recently proposed. All models use the general 'lipid core' concept for the structure of lipoproteins. Yet, newly secreted ('nascent') lipoproteins are different in composition and structure from circulating lipoproteins, and active lipid transport affects predominantly the core compartment of the lipoproteins. In order to conform with the general 'lipid core' model, newly secreted lipoproteins entering the circulation or lipoproteins altered during fat transport, must change and reorganize. This process involves either the core, or the surface (or both) of the lipoproteins. Therefore, structural considerations must be integrated into each of the many events occurring during lipid transport in lipoproteins.

Quantitatively and qualitatively, the main lipid transported in lipoproteins is triglyceride of exogenous (dietary) and endogenous origin. The triglyceride carried in lipoproteins is utilized by many tissues and may be stored in the adipose tissue. Whether lipoproteins play a role in the transport of other lipids – an in particular cholesterol – is uncertain. On theoretical grounds, lipoproteins may be involved with centripetal cholesterol transport, i.e. from tissues to sites of utilization. Failures in transporting lipids result inevitably in severe pathology, as seen in the rare diseases of impaired synthesis of lipoproteins (i.e. abetalipoproteinemia) or in the much more common hyperlipoproteinemia disease states. Lipid transport in lipoproteins is mediated through the activity of several enzyme systems, and through interactions of the particles with tissue cells. Triglyceride transport is dependent on the

activity of an acylhydrolase enzyme system, the lipoprotein lipase (LPL). The enzyme(s) was (were) originally described as heparin-releasable hydrolytic activity which decreases the turbidity of triglyceride-rich lipoprotein solutions. Subsequent studies have shown that it is composed of at least two species of enzymes, of hepatic and extrahepatic origin, and contains activities against triglycerides, monoglycerides and glycerophosphatides. The enzymic activity released into the circulation by heparin is therefore heterogenous. A second enzyme system found in plasma and active towards lipoprotein lipids is the lecithin:cholesterol acyltransferase (LCAT). The enzyme transfers an acyl group from lecithin molecules to unesterified cholesterol to form lysolecithin and cholesterol ester molecules. The combined activity of the two enzyme systems, the LPL and LCAT, affects therefore all lipid classes in lipoproteins. Since changes induced in lipoprotein lipids by either enzyme affects also the apoprotein profile of the lipoproteins, these enzymes should be regarded as lipoprotein-metabolizing systems rather than enzymes with activities limited towards their respective substrates.

Another complexity of the lipoprotein system stems from uncertainties concerned the origin of major lipoprotein species and subspecies. Chylomicrons and VLDL are undoubtedly assembled and secreted from tissues, intestine and liver. There is however great doubt whether LDL and HDL are also primary secretory products of cells. It is significant to note that neither LDL nor HDL has been identified as yet inside tissue cells. Recently, it has been established that LDL is formed in the circulation following the delipidation of VLDL. This path may account for all the circulating LDL in the plasma of humans and animals, i.e. monkeys, rats and guinea pigs. Thus, at least one of the major circulating lipoproteins is normally *not* secreted from cells and cannot be regarded as a primary 'lipid transport' vehicle. More recently, the question whether HDL is secreted by cells has also been challenged. The principal HDL apoproteins, apoA-I and apoA-II (in human), are synthesized in intestinal absorptive cells and are secreted with intestinal VLDL and chylomicrons. These apoproteins, as well as phospholipids, unesterified cholesterol and additional apoproteins (apoC and ?apoE) are freed from the triglyceride-rich lipoproteins during triglyceride transport. The possibility therefore that some, or all, of the circulating HDL is derived from surface constituents of chylomicrons and VLDL must be seriously considered. According to this view, HDL may represent the final form of 'surface remnants' generated during the metabolism of triglyceride-rich lipoproteins and is formed in the plasma compartment. Whereas this view is far from being proven, it explains many observations of the relationships among plasma

lipoproteins and between the levels of lipoproteins and the levels of the lipoprotein-metabolizing enzymes. It furthermore enables to regard all plasma lipoproteins as constituents of one process, the transport of triglycerides.

An exciting facet of lipid transport in lipoproteins and lipoprotein metabolism has been discovered when quantitative and qualitative approaches for the study of the interaction of lipoproteins with tissue cells were developed. Again, complex processes take place. Partially hydrolyzed triglyceride-rich lipoproteins (so-called 'remnant' particles) interact avidly with liver cells; LDL in contrast, readily interacts with many cell lines (fibroblasts, smooth muscle cells) but not with hepatocytes. HDL may be metabolized differently in different animal species. Some of these interactions result in internalization and degradation of the particles; in others, the lipoproteins may donate or extract lipids from the cells. With either process, the interaction results in profound effects on the cellular lipid-metabolizing apparatus. In turn, the cells change the number of their lipoprotein recognition sites and thus regulate the extent of further interactions with lipoproteins. The nature of the interaction between lipoproteins and cells has been partially elucidated. Specific cell receptors are involved with these interactions (i.e. the LDL receptor). However, to what extent are the receptors specific for a lipoprotein family is yet unknown, as are their physiological role in lipid transport and lipoprotein metabolism.

It is the purpose of this volume to describe the known, and discuss the unknown phenomena occurring during the life span of a lipoprotein particle. This volume begins with a description of the enzymes involved with lipoprotein metabolism. The second part discusses the complex process of fat transport in lipoproteins. The final chapters discuss lipoprotein degradation and the possible mechanisms of action of drugs on lipoproteins and their metabolism. Many distinguished scientists have contributed to the book. I am honored to acknowledge these contributions. It is only by the effort of the authors that a description of fat transport in lipoproteins, as is currently known, could be achieved.

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# Hepatic Triglyceride Lipase in Tissue and Plasma

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## *Introduction*

The clearance of circulating lipoprotein triglyceride is mediated by lipolytic activities at or near the surface of the capillary endothelial wall. These enzymes are probably bound to the endothelium since they are not normally measurable in plasma. However, within minutes after the intravenous injection of heparin, lipolysis is markedly stimulated and a group of lipolytic activities can be assayed. These together are referred to as postheparin lipolytic activity (PHLA). The lipase activities of postheparin plasma have been shown to hydrolyze many classes of lipids including triglycerides, di- and monoglycerides, phospholipids and long-chain fatty acyl coenzyme A derivatives [*Greten et al.*, 1969; *Vogel and Zieve*, 1964; *Greten et al.*, 1970; *Ehnholm et al.*, 1975; *Jansen and Hülsman*, 1973, 1974]. Clinical interest in PHLA derives from its potential use in assessing lipoprotein-clearing capacity. PHLA consists of at least two triglyceride lipases with different molecular properties and different sites of origin namely hepatic triglyceride lipase (H-TGL) and extrahepatic triglyceride lipase or lipoprotein lipase (LPL) [*LaRosa et al.*, 1972; *Fielding*, 1972; *Greten et al.*, 1972; *Augustin et al.*, 1978]. Any clinical or pharmacological study reporting decreased PHLA has to account for the presence of these two lipases in postheparin plasma. The full understanding of triglyceride clearance from plasma requires thorough knowledge of the characteristics and availability of lipases *in situ*. At present, however, metabolic and diagnostic studies of these individual lipase activities in the intact subject must depend on the selective measurement after release into plasma by heparin or similar polyanions.

Plasma H-TGL most probably originates from liver plasma membranes. However, at present no data are available as to the particular site of enzyme

synthesis. Recently, evidence was provided that a triglyceride lipase is synthesized in the liver and is delivered to the cell surface through a vesicular transport system [*Chajek et al.*, 1977]. Almost all liver cell compartments contain triglyceride lipase activities which have been partially purified and characterized during the last decade. An attempt is made therefore, to review the characteristics of the different lipase preparations from liver cell fractions for comparison with the plasma H-TGL. Clinical disorders associated with lipoprotein abnormalities will then be discussed in the light of altered H-TGL activity and the functional role of this enzyme in lipoprotein metabolism will be evaluated.

*Lipase in Liver Mitochondria*

Since the mitochondrion is the site of intracellular fatty acid oxidation, it is conceivable that lipases at this location provide substrates for mitochondrial oxidation. Mitochondria obtained by cell fractionation of rat liver homogenates were reported to contain a phospholipase A activity which catalyzes the hydrolysis of the 1-acyl fatty acid, thus giving rise to the 2-acyl-lysoderivatives [*Waite and Van Deenen*, 1967]. *Scherphof and Van Deenen* [1965] reported that both $^{32}$P-phosphatidylcholine and, at a greater rate, $^{32}$P-phosphatidylethanolamine could be hydrolyzed by this enzyme preparation. In accordance with the finding that highly purified pancreatic lipase catalyzes the hydrolysis of phospholipids [*De Haas et al.*, 1965], the mitochondrial enzyme was also found to exhibit lipase activity with sonicates of a triglyceride and phosphatidylethanolamine mixture [*Waite and Van Deenen*, 1967]. Whereas phospholipid hydrolysis was found to be completely inhibited by EDTA, triglyceride hydrolysis was not affected even at high EDTA levels. In contrast, lipase activity decreased up to 50% by the addition of *p*-chloromercuribenzoate in the assay medium while phospholipase $A_2$ activity remained unaffected by this inhibitor. These experiments suggested that phospholipids and triglycerides are probably hydrolyzed by separate enzymes.

Two proteins with lysophospholipase activity – lysophospholipase I and II – were isolated to homogeneity from beef liver homogenates [*De Jong et al.*, 1974]. The two enzyme preparations could be further characterized by different molecular weights, different pI and pH optima. Calcium and EDTA had no significant influence on both activities. Both lysophospholipases exhibited general esterolytic propertis. Lysophospholipase I showed a bimodal distribution with highest specific activity in the cytosol and the mitochondrial

matrix and was virtually absent in lysosomes and microsomes. Lysophospholipase II appeared to be a membrane-bound enzyme with its highest specific activity in the microsomal fraction [*Van den Bosch and De Jong*, 1975; *De Jong et al.*, 1976]. No data are available on the lipase activity of these preparations against long-chain triglycerides under comparable assay conditions.

Recently, a lipase activity has been purified 77-fold from rat liver mitochondria using gel chromatography of sonically disrupted mitochondrial material [*Claycomb and Kilsheimer*, 1971]. On Sephadex G-50 and G-200 the enzymatic activity eluted with the void volume, indicating a molecular weight greater than 800,000. This might be due to a large lipid-protein complex and no further investigations on enzyme purity or molecular properties were done in this study. Hydrolysis of triglycerides increased as the chain length of the esterified fatty acid decreased. The activity against tributyrin was more than 100 times greater than against tripalmitin. Partial glycerides were hydrolyzed more readily than the corresponding triglycerides with the exception of tripalmitin. This enzyme preparation, however, did not hydrolyze phosphatidylcholine.

The mitochondrial phospholipase $A_2$ activity has been the subject of several recent publications [*Waite et al.*, 1966; *Nachbaur and Vignais*, 1965; *Waite and Sisson*, 1971; *Nachbaur et al.*, 1972]. This activity has been attributed mainly, but not exclusively, to the outer membrane. In the studies reported so far, different substrates and emulsification procedures have been used. Therefore, comparison of these activities is still difficult and must await further purification of enzyme proteins in order to differentiate between existing enzyme entities of more than one active site on one lipase molecule. In this connection, the recent work of *Okuda and Fujii* [1968], who demonstrated the interconversion of the rat liver esterase into lipase by sonication with lipid, might be of interest.

### *Lipolytic Activities in Hepatic Microsomes*

Monoglyceride lipase activity had been demonstrated in intestinal mucosal microsomes with a possible role in the intracellular completion of fat digestion [*Senior and Isselbacher*, 1963]. A similar enzyme was also found in liver microsomes [*Carter*, 1967]. In comparison with the soluble fraction, the specific activity of this preparation was 10–20 times higher as a monoglyceride lipase and 3–4 times higher as a triglyceride hydrolase. The pH optimum was between 8.6 and 9.0 with monolaurin emulsified in ethanol as substrate. Using

substrates emulsified in gum arabic, these results could later be confirmed by others [*Biale et al.*, 1968; *Guder et al.*, 1969]. With whole liver homogenates as the enzyme source, *Biale et al.* [1968] demonstrated substrate hydrolysis in the order monobutyrin >tributyrin >mono->> di- > triolein. This preference for certain substrates was unchanged with isolated homogenate sediments, microsomes or the soluble fraction. The ratio between optimal activity towards monoglycerides to that towards triglycerides varied between different tissues and led the authors to the conclusion that distinct enzyme entities may be responsible for the cleavage of full and partial glycerides in the tissues investigated. Both activities were predominantly located in the microsomal fraction although the possibility that they were derived from other cell compartments could not completely be ruled out. *Guder et al.* [1969] confirmed these results with similar substrate preparations. Since lipolysis of triolein at pH 8.5 yielded a molar ratio of fatty acids:glycerol lower than 3, it was suggested that not all fatty acids were released into the medium, but might have entered other pathways such as transacetylation reaction. However, this possibility remains hypothetical for the rather crude preparation studied.

An esterase has subsequently been purified 254-fold from rat liver microsomes by acetone and ammonium sulfate precipitation and hydroxyapatite column chromatography [*Hayase and Tappel*, 1969]. This enzyme hydrolyzed glycerol 1-monodecanoate at a rapid rate at alkaline pH. Hydrolysis rates for corresponding di- and triglycerides were one third and one hundredth of that of glycerol 1-monodecanoate. All substrates were emulsified in gum arabic. The enzyme exhibited little activity against long-chain fatty acid mono-, di- and triglycerides. Thus, this esterase activity has a broad substrate specificity which overlaps that of monoglyceride lipase. The enzyme preparation was far from being purified to homogeneity, and activity against phospholipids was not studied.

With Triton X 100 as detergent, *Assmann et al.* [1973a] demonstrated considerable hydrolytic activities against long-chain fatty acid tri-, di- and monoglycerides. As only small concentrations of di- and monoglycerides accumulated during the reaction, it was suggested that complete hydrolysis of triglycerides was mediated by this enzyme preparation. The enzyme activity was almost completely inhibited by prior incubation with protamine sulfate (1.5 mg/ml) or with NaCl at concentrations of 0.3–0.63 $\mu M$. Higher NaCl concentrations led to a progressive increase of lipase activity.

Several investigators demonstrated phospholipase activity in liver microsomal fractions [*Bjørnstad*, 1966; *Scherphof et al.*, 1966; *Waite and Van Deenen*, 1967; *Nachbaur et al.*, 1972]. As the triglyceride lipase from post-

heparin plasma also contains activity against phospholipids, the possible identity of lipase and phospholipase activities in liver microsomal preparations should be considered. Recently, it has been shown that highly purified pancreatic lipase is capable of specifically attacking the 1-acyl ester of phospholipids which raises the question of enzymes with both lipase and phospholipase activity [*Sarda et al.*, 1964]. Indeed, enzymatic activity obtained from liver microsomes could be shown to preferentially hydrolyze position 1 of phospholipids [*Waite and Van Deenen*, 1967]. In these studies, some lipase activity was also found to be associated with this cell fraction when sonicates of triolein and phosphatidylethanolamine were incubated together. The positional specificity of a microsomal phospholipase $A_1$ was confirmed by *Nachbaur et al.* [1972].

### *Lipase Activity in the Cytosol Fraction*

Lipase activity in the soluble supernatant of rat liver homogenates was detected by *Olson and Alaupovic* [1966] at pH 7.0. This fraction contained 10 times more lipase activity than microsomes and mitochondria with a stabilized Ediol emulsion as substrate. As constituents of this emulsion include monoglycerides and phospholipids and as the production of free fatty acids was not correlated with the disappearance of triglycerides, it is not clear whether the measured hydrolytic rate is due to lipase, esterase or phospholipase action.

Alkaline lipolysis was found to be most active in the soluble part of rat liver homogenates by *Biale et al.* [1968] and *Guder et al.* [1969] with 8–10 times faster hydrolysis of short-chain fatty acid triglycerides than triolein. However, because of several kinetic similarities with the microsomal lipase activity, the authors concluded that the activity found in the 100,000 *g* supernatant of liver homogenates may be released from microsomes into the cytoplasm during the preparation procedure. On the other hand, the possibility that this activity represents a distinct enzyme could not be ruled out [*Mahadevan and Tappel*, 1968]. These results were confirmed by *Assmann et al.* [1973a] who found alkaline lipase activity at pH 8.0 in the cytosol of rat liver cells. The specific activity of this enzyme was far below that found in plasma membranes but as the bulk of cell proteins is present in cytosol, the authors concluded that the majority of alkaline lipase is probably located in this fraction. However, it was not possible to calculate the degree of contamination of the cytosol with lipase of higher specific activity released from other cell compartments. Lipase activity was measured with sonicated triolein, stabilized in Triton X 100, and

the enzyme exhibited activity against tri-, di- and monoglycerides. It was 90% inhibited by prior incubation in NaCl at concentrations of 0.3–0.6 *M* and in protamine sulfate (750 μg/ml).

*Mellors et al.* [1967] found lipase activity against triolein in the soluble fraction of liver cells with a pH optimum around 5.0. The specific activity of this preparation was only 1.5- to 2-fold higher than in the homogenate, and the same activity was again detected in microsomes. Some phospholipase $A_1$ and $A_2$ and mainly lysophospholipase activity was also demonstrated in the soluble fraction of rat liver [*Waite and Van Deenen*, 1967; *Van den Bosch and De Jong*, 1975]. Since some of the activity was always recovered in the particulate fractions, solubilization of the enzyme by the isolation procedure could not be excluded.

*Lipolytic Activities in Liver Lysosomes*

Lysosomes have been shown to contain the apparatus for complete degradation of nucleic acids, proteins and mucopolysaccharides. The ability of rat liver lysosomes to digest various lipids has also been well documented [*Fowler and De Duve*, 1969]. Lysosomes are able to hydrolyze extensively phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidic acid, cardiolipin, tripalmitin, 1.2- and 1.3-dipalmitin and monopalmitin when incubated with these substrates in 0.1 *M* acetate buffer, pH 4.3–4.6. Most substrates were hydrolyzed even in the absence of a detergent with the exception of tripalmitin which was deacylated only in the presence of 5% Triton X 100 and cardiolipin which required 0.1% Triton X 100 for hydrolysis. On the other hand, the detergent inhibited the hydrolysis of phosphatidylethanolamine and monopalmitin. Lysosomes were also found to dephosphorylate phosphatidic acid, α-glycerophosphate and, although rather slowly, the phosphomonoesters of choline, ethanolamine and serine. These data demonstrate that lysosomes possess the ability to digest complex natural materials. After the intravenous injection of nonhemolyzing Triton WR-1339, liver lysosomes are loaded with the detergent and can be removed from the heavier mitochondria by density gradient centrifugation of liver homogenates [*De Duve et al.*, 1955]. During dialysis of these particles against buffer, the membranes rupture and release Triton. Centrifugation then separates lysosomal membranes from the solubilized material. This established procedure yielding membrane-supported and solubilized enzymes, however, does not

rule out solubilization of originally membrane-bound activities by the detergent. Differential characteristics of the two fractions of rat liver lysosomes were first reported by *Stoffel and Greten* [1967]. They found cholesterylester hydrolase and lipase activity mainly in the supernatant whereas phospholipase A activity was almost equally distributed in both the soluble and the particulate fraction. Similar results have then been obtained by several investigators who in general demonstrated the presence of membrane-bound phospholipase and lipase activity which was most active at pH 4.4 [*Mellors and Tappel*, 1967; *Mellors et al.*, 1967; *Guder et al.*, 1969]. This particular lipase preparation was not affected by either protamine sulfate, heparin or NaCl [*Mahadevan and Tappel*, 1968; *Assmann et al.*, 1973a]. In the absence of Triton X 100, the enzyme preparation showed considerable esterolytic activity on monopalmitin, was slightly active on diglycerides with a preference for the 1.2-isomer and had no detectable activity on tripalmitin. In the presence of the detergent (5%), monopalmitin was poorly hydrolyzed whereas considerable amounts of free fatty acids were released from di- and triglycerides with a specificity for the α-position [*Fowler and De Duve*, 1969]. When lysosomes were dispersed in Triton X 100 before assaying, lipase activity was partially inhibited, and the pH optimum of the enzyme was shifted from 5.2 to 4.2 as demonstrated by *Hayase and Tappel* [1970]. These investigators also showed that the esterolytic activity largely depended on the pH of the assay. Osmotic rupture of rat liver lysosomes led to solubilization of phospholipase $A_1$ and $A_2$ activity which could be partially separated by gel chromatography [*Stoffel and Trabert*, 1969; *Franson et al.*, 1971]. These preparations, however, did not hydrolyze triolein.

Some of these conflicting data may be explained by the finding that acid lipase is activated by acid phospholipids [*Kariya and Kaplan*, 1973]. Activation even occurred in the presence of heat-inactivated microsomes using tripalmitin-Triton X 100 dispersions as substrates. The degree of stimulation would then depend on the Triton X 100 concentration in the medium. Heparin and sulfate ions inhibited the activity, but this was partially prevented by phosphatidylserine. The hydrolysis of tripalmitin, dispersed in Triton X 100, taurocholic acid, gum arabic or lysolecithin was activated to a different extent by phosphatidylserine. These results demonstrate that the interpretation of quantitative aspects of lysosomal lipase activity in crude homogenates and partially purified cell organelles is very difficult in the studies reported so far. Several of the data may be rationalized in the light of differential distribution of activities, detergents and endogenous activators.

Rat liver lysosomal lipase has been purified 1,200-fold from crude homo-