J. Phys. Chem. 61, 271.
Mathieson, A. M., and Welsh, H. K. (1952), *Acta Crystallogr., Sect. A, 5*, 599.
Mattice, W. L., and Mandelkern, L. (1970), *J. Amer. Chem. Soc. 92*, 5285.
Mattice, W. L., and Mandelkern, L. (1971a), *J. Amer. Chem. Soc. 93*, 1769.
Mattice, W. L., and Mandelkern, L. (1971b), *Biochemistry 10*, 1926.
McBride, O. W., and Harrington, W. F. (1967), *Biochemistry 6*, 1499.
McIntyre, D., Wims, A., Williams, L. C., and Mandelkern, L. (1962), *J. Phys. Chem. 66*, 1932.
Miller, W. G., Brant, D. A., and Flory, P. J. (1967), *J. Mol. Biol. 23*, 67.
Miller, W. G., and Goebel, C. V. (1968), *Biochemistry 7*, 3925.
Nemethy, G., Leach, S. J., and Scheraga, H. A. (1966), *J. Phys. Chem. 70*, 998.
Ooi, T., Scott, R. A., Vanderkooi, G., and Scheraga, H. A. (1967), *J. Chem. Phys. 46*, 4410.
Orofino, T. A., and Flory, P. J. (1957), *J. Chem. Phys. 26*, 1067.
Piez, K. A., and Gross, J. (1960), *J. Biol. Chem. 88*, 2041.
Pyun, C. W., and Fixman, M. (1966), *J. Chem. Phys. 44*, 2107.
Ramachandran, G. N., Ramakrishnan, C., and Sasisekharan, V. (1963), *J. Mol. Biol. 7*, 95.
Ramachandran, G. N., Ramakrishnan, C., and Venkatachalam, C. M. (1965), *Biopolymers 3*, 591.
Ramachandran, G. N., and Sasisekharan, V. (1968), *Advan. Protein Chem. 23*, 283.
Sasisekharan, V. (1959), *Acta Crystallogr., Sect. A, 12*, 903.
Schachman, H. K. (1957), *Methods Enzymol. 4*, 32.
Schimmel, P. R., and Flory, P. J. (1967), *Proc. Nat. Acad. Sci. U. S. 58*, 52.
Schimmel, P. R., and Flory, P. J. (1968), *J. Mol. Biol. 34*, 105.
Schroeder, W. A., Kay, L. M., Legette, J., Honnen, L., and Green, F. C. (1954), *J. Amer. Chem. Soc. 76*, 3556.
Scott, R. A., and Scheraga, H. A. (1966), *J. Chem. Phys. 45*, 2091.
Takahashi, A., Mandelkern, L., and Glick, R. E. (1969), *Biochemistry 8*, 1673.
Tonelli, A. E., and Flory, P. J. (1969), *Macromolecules 2*, 225.
Williams, J. W., Van Holde, K. E., Baldwin, R. L., and Fujita, H. (1958), *Chem. Rev. 58*, 715.
Yan, J. F., Momany, F. A., Hoffman, R., and Scheraga, H. A. (1970), *J. Phys. Chem. 74*, 420.

Identification of Phospholipase A_1 and A_2 in the Soluble Fraction of Rat Liver Lysosomes*

Richard Franson, Moseley Waite, and Mariano LaVia

ABSTRACT: Rat liver lysosomes were isolated by sucrose density gradient centrifugation from rats previously injected with Triton WR-1339. As measured by acid phosphatase activity, the lysosomes were purified 32-fold over the homogenate with an average yield of 6.0%. Mitochondrial, microsomal, and peroxisomal contaminations were each less than 0.05% of the total activity of the homogenate. When the lysosomes were incubated at pH 4.0 with 1.0 mM EDTA, [^{14}C]linoleic acid and [^{14}C]monoacylglycerophosphorylethanolamine were produced from 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine. After osmotic rupture of purified lysosomes the phospholipases (A_1 and A_2) were in the soluble fraction entirely. The two phospholipases were not inhibited to the same extent by increasing concentrations of Ca^{2+} or EDTA. Phospholipases A_1 and A_2 were separated by gel filtration on Sephadex G-200.

Mellors and Tappel (1967) reported the first lysosomal phospholipase from rat liver. This enzyme had an acid pH optimum, hydrolyzed both the C-1 and C-2 fatty acid ester linkages of phosphatidylcholine and phosphatidylethanolamine, and was found in both soluble and particulate fractions of lysosomes. Stoffel and Greten (1967) and Mellors *et al.* (1967) confirmed the presence of a phospholipase A with optimal activity in the acid pH range. These reports were contradictory, however, with regard to pH optima and Ca^{2+} requirement.

On the basis of selective inhibition studies, Stoffel and Trabert (1969) suggested the presence of two soluble phospholipases with acid pH optima. Waite *et al.* (1969) confirmed the presence of lysosomal phospholipase(s) A active at pH 4.5 and reported the presence of a phospholipase A in the lysosomal preparation which was stimulated by Ca^{2+} ions and was active in the neutral pH range. The localization of the Ca^{2+}-stimulated enzyme was uncertain since the distribution of this activity did not parallel the major activity found in either the lysosomes or the mitochondria. Recently Rahman *et al.* (1970) reported a Ca^{2+}-stimulated phospholipase A_2 from the particulate fraction of rat liver lysosomes. Nachbaur and Vignais (1968) and Waite (1969) described a Ca^{2+}-stimulated phospholipase A_2 in the outer membrane of mitochondria which is similar to the particulate lysosomal enzyme reported by Rahman *et al.* (1970). This observation suggests that mitochondrial contamination could account for the activity found by Rahman *et al.*

* From the Departments of Biochemistry and Pathology, The Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27103. *Received December 7, 1970.* Supported by U. S. Public Health Service Grant AM 11799 from the National Institutes of Health.

In this study rat liver lysosomes were isolated by sucrose gradient centrifugation. Biochemical purity was measured by marker enzymes for lysosomes, mitochondria, peroxisomes, and endoplasmic reticulum. Both [^{14}C]linoleic acid and [^{14}C]monoacylglycerophosphorylethanolamine were formed from 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine by enzymes in the lysosomal soluble fraction at pH 4.0. No Ca^{2+}-dependent phospholipase A activity was found in either lysosomal soluble or particulate fractions at pH 7.5. Two soluble phospholipases (A_1 and A_2) were separated by gel filtration on Sephadex G-200.

Experimental Section

Preparation of Lysosomes. Three male Wistar rats (150–200 g) were injected intraperitoneally with 170 mg of Triton WR-1339 3.5 days prior to sacrifice. Rats were killed and the livers were perfused with 60 ml of ice-cold 0.25 M sucrose. After perfusion the livers were rapidly removed, minced, and homogenized with a Potter-Elvejhem homogenizer in a total volume of 200 ml of 0.25 M sucrose. All steps were performed at 0–4°. Nuclei and cell debris were sedimented by centrifugation at 1000*g* for 10 min. The supernatant fluid was decanted and was centrifuged at 10,000*g* for 20 min. The resulting supernatant fluid was decanted and the particulate fraction (P-1) was resuspended in 45 ml of 1.32 M sucrose. The discontinuous sucrose gradient consisted of 6 ml of the sample resuspended in 1.32 M sucrose, 10 ml of 0.94 M sucrose, 10 ml of 0.82 M sucrose, and 10 ml of 0.25 M sucrose. Samples were centrifuged at 24,000 rpm (100,000*g*) for 2 hr in a Beckman SW-27 swinging-bucket rotor. After centrifugation, lysosomes which had risen to the 0.94–0.82 and 0.82–0.25 M sucrose interfaces were collected with a Pasteur pipet and were combined. Purified lysosomes were osmotically ruptured by dialysis for 12 hr against 6 l. of 1 mM $NaHCO_3$ and 1 mM EDTA at pH 7.2. Dialyzed lysosomes were then centrifuged at 100,000*g* for 30 min to yield the lysosomal soluble and particulate fractions. The soluble lysosomal preparation (0.20 mg of protein/ml) was concentrated to 1.0 mg of protein/ml using Aquacide. The concentrated preparation (8 ml) was filtered through a Sephadex G-200 column (2.5 × 90 cm) equilibrated with 0.05 M Tris (pH 7.4).

Enzyme Assays. Phospholipase A was assayed as described by Waite and van Deenen (1967). Phospholipases A_1 and A_2 specifically hydrolyze the fatty acid ester linkages in the C-1 and C-2 positions of phospholipids, respectively, to form their monoacyl analogs. Reaction mixtures in a total volume of 1.0 ml contained (unless otherwise stated) 75 nmoles of 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine (15,000 cpm added as an aqueous ultrasonic suspension), 2 μmoles of EDTA or 2 μmoles of $CaCl_2$, 100 μmoles of buffer (sodium acetate at pH 4.0, Tris-maleate at pH 7.5), and 25–50 μg of protein. Incubation tubes with a mixture of two labeled phosphatidylethanolamines contained a total of 320 nmoles of 1-[^{3}H]palmitoyl-2-acyl-3-glycerophosphorylethanolamine (24,000 cpm) and 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine (9000 cpm). The reaction mixtures were incubated for 15 min at 37°. Reactions were stopped by adding 2 volumes of methanol and the products were extracted by the method of Bligh and Dyer (1959). Radioactive lipids were separated by thin-layer chromatography on silica gel G plates, which were first developed in chloroform–petroleum ether (bp 30–60°)–acetic acid (70:30:2, v/v), and then in chloroform–methanol–H_2O (70:30:4, v/v). Chromatograms were stained with I_2 vapor; the silicic acids that contained the radioactive compounds were each placed in scintillation vials containing Omnifluor scintillation mixture and a thixotropic gel.

Acid phosphatase (EC 3.1.3.2, lysosomal marker) was determined by the method of Gianetto and DeDuve (1955). Inorganic phosphate was measured by the turbidimetric procedure of Eibl and Lands (1969). NADPH–cytochrome *c* reductase (EC 1.6.99.1, microsomal marker) and cytochrome oxidase (EC 1.9.3.1, mitochondrial marker) were assayed spectrophotometrically by the method of Sottocasa *et al.* (1967). Uricase (EC 1.7.3.3, peroxisomal marker) was assayed by the method of Beaufay *et al.* (1964). Monoamine oxidase (EC 1.4.3.4, mitochondrial outer membrane marker) was assayed using [^{14}C]tyramine as described by McCaman *et al.* (1965). Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Electron Microscopy. Lysosomes to be used for electron-microscopic examination were prepared histochemically by the method of Gomori as described by Corrin *et al.* (1969). This method specifically detects acid phosphatase by its reaction with the substrate β-glycerophosphate. The product, P_i, was precipitated with lead, and appears as small electron dense granules at the periphery of lysosomal membranes. The pellets were fixed in osmium, were postfixed in uranyl acetate, and were counterstained with lead citrate and uranyl acetate. Substrate-free reactions on lysosomes from Triton-treated and untreated rats were carried out as controls. Pellets were embedded in ERL-4206 (Spurr, 1969) and thin sections were prepared.

Materials. We synthesized 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine and 1-[^{3}H]palmitoyl-2-acyl-3-glycerophosphorylethanolamine by the procedure of Waite and van Deenen (1967). Triton WR-1339 was purchased from Ruger Chemical Co., New York, N. Y. Aquacide was obtained from Calbiochem, Los Angeles, Calif.

Results

Two lysosomal populations were separated by sucrose gradient centrifugation. One was located at the 0.94–0.82 M sucrose interface while the other had sedimented to the 0.82–0.25 M sucrose interface. As determined by marker enzymes and phospholipase A activities, these populations were identical. Electron micrographs of samples histochemically treated for acid phosphatase (Figure 1) show that the lysosomes which had sedimented to the 0.82–0.25 M sucrose interface (B) are larger in circumference than the lysosomes which had sedimented to the 0.94–0.82 M sucrose interface (A). Probably the difference in size and density is the result of greater uptake of Triton by some lysosomes. Since no other morphological or biochemical differences were noted, the two populations were combined in the studies reported here.

The activity of marker enzymes for lysosomes, mitochondria, microsomes, and peroxisomes was compared in the liver homogenate, the particulate fraction (P-1), and the purified lysosomal fraction. Table I shows that the purified lysosomal fraction contained 0.02% of the microsomal, 0.01% of the mitochondrial, and 0.05% of the peroxisomal activities found in the homogenate. Lysosomes were purified 32-fold over the homogenate in a yield of 6% as measured by acid phosphatase activity. Monoamine oxidase, an enzyme found in the outer membranes of mitochondria (Schnaitman and Greenawalt, 1968), was not detectable in these preparations.

TABLE I: Characterization of Purified Lysosomes by Marker Enzymes.[a]

Fraction	Protein		Acid Phosphatase		Cytochrome Oxidase		NADPH Cytochrome *c* Reductase		Uricase	
	mg	%	Sp Act.	%	Sp Act.	%	Sp Act.	%	Sp Act.	%
Homogenate	8670.0	100.0	22.9	100.0	287.0	100.0	10.6	100.0	6.1	100.0
Pellet 1 (P-1)	1020.0	11.7	58.7	28.7	856.0	34.0	8.0	8.4	13.0	23.0
Lysosomes	15.4	0.18	732.0	6.0	21.3	0.01	3.7	0.02	3.8	0.05

[a] Specific activities are expressed as nanomoles of product formed per minute per milligram of protein. Per cent of activity is calculated as the total activity of a given fraction divided by the total activity in the crude homogenate times 100. Pellet 1 (P-1) refers to the cytoplasmic granular fraction sedimented at 10,000*g* for 20 min. Lysosomes were isolated from P-1 by sucrose gradient centrifugation. These data are the average of seven experiments.

Seventy-one per cent of the lysosomal protein and forty-one per cent of the recovered acid phosphatase were solubilized by osmotic rupture (Table II). Nearly all of the phospholipase A_1 and A_2 were recovered in the soluble fraction. The phospholipases A in the soluble fraction had optimal activities at pH 4.0 and were inactive above pH 5.5. The particulate fraction had little, if any, phospholipase A activity at either pH 4.0 or pH 7.5 in the presence or absence of 2–10 μmoles of Ca^{2+} or 2 μmoles of EDTA. Hence both the phospholipase A_1 and A_2 in the lysosome are soluble enzymes with optimal activities in the acid pH range.

The lysosomal phospholipase A_1 and A_2 were inhibited by increasing concentrations of Ca^{2+} and EDTA (Figure 2). The inhibition of both phospholipase A_1 and A_2 by Ca^{2+} was greater than that by EDTA at all concentrations tested. However, the two enzymes were not inhibited to the same extent. In the presence of 2 mM Ca^{2+}, [^{14}C]linoleic acid (A_2) release is inhibited 50%, whereas [^{14}C]monoacylglycerophosphorylethanolamine (A_1) release is inhibited less than 5%. Since phospholipase A_2 activity is greatly inhibited by low concentrations of Ca^{2+} (1–2 mM), maximal phospholipase A_1 and A_2 activities were measured in the presence of 1–2 mM EDTA.

Phospholipase A_1 and A_2 activities are linear with time for nearly 20 min (Figure 3A). With increasing protein concentrations the activities are linear to 40–50 μg of protein (Figure 3B). Little increase in the activities is observed above 100 nmoles of phosphatidylethanolamine (Figure 3C). Due to the instability of micelles at higher substrate concentrations, 75 nmoles of phosphatidylethanolamine was used to assay phospholipase A activity. Under these various conditions, phospholipase A_1 activity is greater than that of phospholipase A_2.

The lysosomal-soluble fraction was filtered through a column of Sephadex G-200 (Figure 4) which partially separated phospholipase A_1 from phospholipase A_2. Fraction 30 had the greatest phospholipase A_1 activity whereas fraction

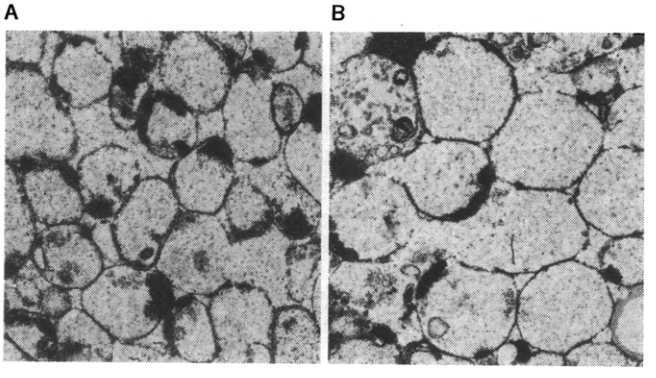

FIGURE 1: Electron micrographs of two populations of lysosomes separated by sucrose gradient centrifugation. Isolation of the fractions was described in the Experimental Section. Lysosomal-rich fractions from the sucrose gradient were made isotonic by dilution with distilled water and the intact lysosomes were sedimented by centrifuging at 10,000*g* for 20 min. Pellets were prepared histochemically for acid phosphatase as outlined in the Experimental Section. Part A is the lysosomal population which sedimented to the 0.94–0.82 M sucrose interface and part B the lysosomal population which sedimented to the 0.82–0.25 M sucrose interface. Substrate-free reactions on lysosomes from Triton-treated and untreated rats were carried out as controls.

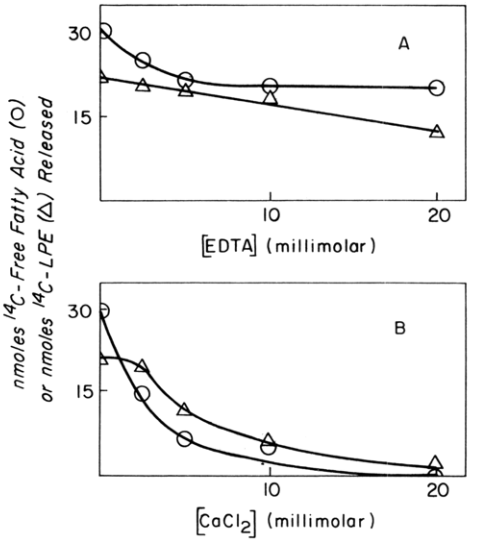

FIGURE 2: Inhibition of phospholipase A activity by Ca^{2+} and EDTA. Incubation mixtures contained 76 μg of lysosomal-soluble protein, 75 nmoles of 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine, 100 μmoles of sodium acetate buffer at pH 4.0, and increasing concentrations of Ca^{2+} or EDTA in a total volume of 1.0 ml. Reaction mixtures were incubated at 37° for 15 min. [^{14}C]FFA (O—O) and [^{14}C]LPE (Δ—Δ) refer to [^{14}C]linoleic acid and 2-[^{14}C]linoleyl-3-glycerophosphorylethanlamine, respectively. All values presented have been corrected for nonenzymatic hydrolysis of substrate. (0.5–1.0 nmoles of FFA or LPE per 15 min.)

TABLE II: Fractionation of Lysosomes by Osmotic Rupture. Acid Phosphatase and Phospholipase A Activities of Lysosomal Soluble and Particulate Fractions.[a]

Fraction	Protein (mg)	Acid Phosphatase		Phospholipase A Products			
				pH 4.0		pH 7.5	
		Sp Act.	%	FFA[b]	LPE[b]	FFA	LPE
Lysosomes	15.4	732	6.0	480	790	29	15
Lysosomal soluble	11.0	428	2.1	370	615	0	0
Lysosomal particulate	4.4	1509	2.9	0	0	12	12

[a] Specific activity is expressed as nmoles of product formed/min per mg of protein for acid phosphatase and as nmoles of product formed/15 min per mg of protein for phospholipase A. Per cent of activity is calculated as the total activity of a given fraction divided by the total activity in the crude homogenate times 100. Phospholipase A activity at pH 4.0 was measured in the presence of 1 μmole of EDTA and at pH 7.5 in the presence of 2 μmoles of Ca^{2+}. These data are the average of five experiments. [b] FFA and LPE refer to [^{14}C]linoleic acid and [2-^{14}C]linoleyl-3-glycerophosphorylethanolamine, respectively.

34 had maximal phospholipase A_2 activity. To demonstrate that the activity in fraction 34 was due to phospholipase A_2 and not the combined actions of the phospholipase A_1 and a lysophospholipase, the enzyme was assayed with a mixture of two labeled phosphatidylethanolamines (1-[^{3}H]palmitoyl-2-acyl-3-glycerophosphorylethanolamines and 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine). This fraction produced equal amounts of [^{3}H]monoacylglycerophosphorylethanolamine and [^{14}C]linoleic acid, in addition to lesser amounts of [^{14}C]monoacylglycerophosphorylethanolamine and [^{3}H]palmitic acid. Since [^{3}H]monoacylglycerophosphorylethanolamine is produced only by a phospholipase A_2, these results are taken as proof of existence of the phospholipase A_2 as suggested by Stoffel and Trabert. However the presence of a lysophospholipase cannot be excluded on the basis of these data.

Discussion

Treatment of rats with Triton WR-1339 selectively decreases the density of liver lysosomes, and facilitates their separation from other organelles. With Triton-treated rats two populations of lysosomes, which had identical marker enzyme and phospholipase A activities, were isolated by sucrose gradient centrifugation. Electron micrographs demonstrated only a difference in size which was caused by variability in the uptake of Triton. Other workers have shown that bimodal distribution of lysosomal enzymes demonstrates that two sources of lysosomes exist in normal skeletal muscle (Canonico and Bird, 1970). One group of

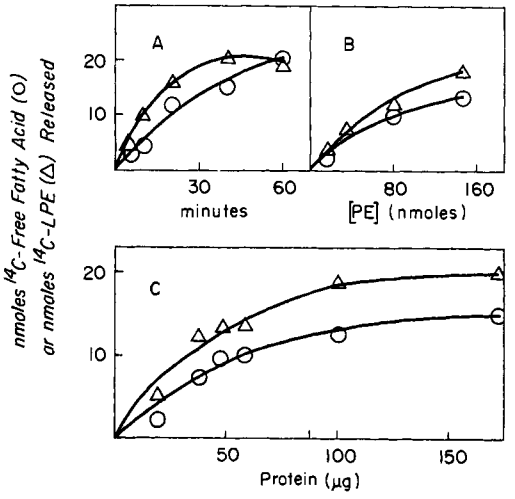

FIGURE 3: Optimal conditions for phospholipase A activity at pH 4.0. Incubation mixtures in part A contained 50 μg of lysosomal-soluble protein, 75 nmoles of 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine (15,000 cpm), 100 μmoles of sodium acetate buffer at pH 4.0, and 2 μmoles of EDTA in a total volume of 1.0 ml. Reaction mixtures in parts B and C were incubated at 37° for 15 min with increasing substrate and protein concentrations, respectively. All other conditions were the same as in part A. [^{14}C]FFA (○—○) and [^{14}C]LPE (△—△) refer to [^{14}C]linoleic acid and 2-[^{14}C]-linoleyl-3-glycerophosphorylethanolamine, respectively. All values presented have been corrected for nonenzymatic hydrolysis of substrate.

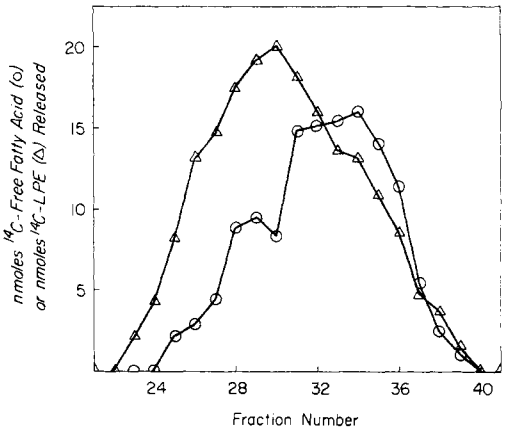

FIGURE 4: Gel filtration of lysosomal soluble preparation on Sephadex G-200. Lysosomal-soluble protein (8 ml; 1 mg of protein/ml) was filtered through a column of Sephadex G-200 (2.5 × 90 cm) equilibrated with 0.05 M Tris at pH 7.2. The first 75 ml of the 150 ml of void volume was discarded. Thereafter, fractions were collected in 6.5-ml aliquots. Reaction mixtures contained 1 ml of each fraction from the column, 75 nmoles of 1-acyl-2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine, 100 μmoles of sodium acetate buffer at pH 4.0, and 2 μmoles of EDTA in a total volume of 1.5 ml. [^{14}C]FFA (○—○) and [^{14}C]LPE(△—△) refer to [^{14}C]linoleic acid and 2-[^{14}C]linoleyl-3-glycerophosphorylethanolamine. All values presented have been corrected for nonenzymatic hydrolysis of substrate.

lysosomes is derived from macrophages and a second group derived from the muscle cells. The possibility exists that the two lysosomal populations observed here may be derived from different cell types within the liver. However, the lack of biochemical variation suggests that the two populations are from the same cell type, presumably the Kupffer cell.

Recently, Rahman *et al.* (1970) have described a Ca^{2+}-dependent phospholipase A_2 with the optimal activity between pH 7.0–8.0 in the particulate fraction of rat liver lysosomes. Our preparations of lysosomal membranes, however, had little, if any, phospholipase A activity at pH 7.5. These data confirm the suggestion of Waite *et al.* (1969) that the Ca^{2+}-stimulated phospholipase A which appeared in lysosomal preparations was due to an enzyme from a contaminating organelle. It is possible that the activity observed by Rahman *et al.* (1970) is due to contamination of their preparations by outer membranes of mitochondria. Support for this possibility comes from the work of Vignais and Nachbaur (1968) which demonstrated that outer membranes of mitochondria have sedimentation characteristics similar to lysosomal membranes from livers of Triton-treated rats. Furthermore, a Ca^{2+}-dependent phospholipase A_2 with activity at alkaline pH values has been demonstrated in outer membranes of mitochondria (Nachbaur and Vignais, 1968; Waite, 1969).

Our data confirm the conclusion of Stoffel and Trabert (1969) that two phospholipases with acid pH optima exist in the lysosomal-soluble fraction. The separation of two distinct phospholipase A activities reported here provide positive evidence for the existence of at least two phospholipases with different positional specificities. Mahadevan and Tappel (1968) have described a particulate lysosomal lipase with an acid pH optima from rat liver. Our phospholipase A activity is not due to the action of a lysosomal lipase, however, since [^{14}C]tripalmitoylglycerol was not hydrolyzed by our lysosomal-soluble fraction.

The specific activities of phospholipase A_1 and A_2 reported here are 10- to 50-fold higher than those previously reported (Stoffel and Trabert, 1968; Rahman *et al.*, 1970). These differences probably are due to the assay conditions used by these workers. The specific activities reported by us were obtained with optimal assay conditions whereas those obtained by others were not. This conclusion is based on the comparison of conditions reported by them with the results reported here in Figure 3.

Acknowledgment

The excellent technical assistance of Miss Patricia Sisson and Mr. Tilman K. Prater is gratefully acknowledged.

References

Beaufay, H., Jacques, P., Baudhuin, P., Sellinger, O. Z., Berthet, J., and DeDuve, C. (1964), *Biochem. J. 92*, 184.

Bligh, E. G., and Dyer, W. J. (1959), *Can. J. Biochem. Physiol. 37*, 911.

Canonico, P. G., and Bird, J. W. C. (1970), *J. Cell Biol. 45*, 321.

Corrin, B., Clark, A. E., and Spencer, H. (1969), *J. Anat. 104*, 165.

Eibl, H., and Lands, W. E. M. (1969), *Anal. Biochem. 30*, 51.

Gianetto, R., and DeDuve, C. (1955), *Biochem. J. 59*, 433.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Mahadevan, S., and Tappel, A. L. (1968), *J. Biol. Chem. 243*, 2849.

McCaman, R. E., McCaman, M. W., Hunt, J. M., and Smith, M. S. (1965), *J. Neurochem. 12*, 15.

Mellors, A., and Tappel, A. L. (1967), *J. Lipid Res. 8*, 479.

Mellors, A., Tappel, A. L., Sawant, P. L., and Desai, I. D. (1967), *Biochim. Biophys. Acta 143*, 299.

Nachbaur, J., and Vignais, P. M. (1968), *Biochem. Biophys. Res. Commun. 33*, 315.

Rahman, Y. E., Verhagen, J., and Wiel, D. F. M. (1970), *Biochem. Biophys. Res. Commun. 38*, 670.

Schnaitman, C., and Greenawalt, J. W. (1968), *J. Cell Biol 38*, 1.

Sottocasa, G. L., Kuylenstierna, B., Ernster, L., and Bergstrand, A. (1967), *J. Cell Biol. 32*, 415.

Spurr, A. R. (1969), *J. Ultrastruct. Res. 26*, 31.

Stoffel, W., and Greten, H. (1967), *Hoppe-Seyler's Z. Physiol Chem. 348*, 1145.

Stoffel, W., and Trabert, U. (1969), *Hoppe-Seyler's Z. Physiol Chem. 350*, 836.

Vignais, P. M., and Nachbaur, J. (1968), *Biochem. Biophys Res. Commun. 33*, 307.

Waite, M. (1969), *Biochemistry 8*, 2536.

Waite, M., Scherphof, G. L., Boshouwers, F. M. G., and van Deenen, L. L. M. (1969), *J. Lipid Res. 10*, 411.

Waite, M., and van Deenen, L. L. M. (1967), *Biochim Biophys. Acta 137*, 498.