Metabolism of lysophosphatidyl ethanolamine and lysophosphatidyl choline by homogenates of rabbit polymorphonuclear leukocytes and alveolar macrophages

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ABSTRACT A comparison has been made between the conversion of ^{32}P-labeled lysophosphatidyl ethanolamine (LPE) and lysophosphatidyl choline (LPC) to their respective acylated and deacylated derivatives by homogenates of rabbit polymorphonuclear leukocytes and alveolar macrophages.

Synthesis of PE by both homogenates and of PC by macrophage homogenates proceeded to about the same extent and is attributed to direct acylation of the lyso compounds. At higher LPC concentrations formation of PC by leukocytes is far greater than by macrophages. The mechanism of this enhanced synthesis of PC, which is brought out by higher substrate concentrations, is believed to be a transfer of the acyl group of one LPC molecule to another.

Under optimal conditions macrophage homogenates deacylated LPE to a greater extent than LPC, while the reverse was true for leukocyte homogenates.

Albumin inhibited deacylation of LPC and its conversion to PC by leukocytes, perhaps by binding the substrate (2 moles of LPC per mole of albumin). Other effects of albumin—stimulation of deacylation and acylation of LPE by macrophages, inhibition of deacylation and acylation of LPE by leukocytes—remain unexplained.

KEY WORDS phospholipids · lyso compounds · metabolism · lysophosphatidyl ethanolamine · lysophosphatidyl choline · rabbit · phagocytic cells · polymorphonuclear leukocytes · alveolar macrophages · homogenates

MONOACYL PHOSPHATIDES manifest membrane-lytic properties. Several mammalian tissues have been shown to contain enzymes that convert these lyso compounds into either diacyl or deacylated derivatives (1–8). Thus the potentially harmful monoacyl phosphatides not only can be broken down to water-soluble glycerol derivatives, but may also participate in a monoacyl–diacylphosphatide cycle that permits renewal of the fatty acid constituents of important membrane components. Such a cycle could be important during phagocytosis, when marked morphologic alterations involving both outer and intracellular membranous structures (9) take place.

Previous studies on phospholipid metabolism by two types of phagocytic cells, polymorphonuclear leukocytes and alveolar macrophages, have indicated that homogenates of both cell types hydrolyze PC and LPC and convert LPC to PC as well as to GPC (7, 8).

This communication presents further observations on the metabolism of lyso compounds by homogenates of granulocytes and lung macrophages; it focuses on a comparison of LPE and LPC as substrates in both the synthesis and breakdown of phospholipids.

The results indicate that these lyso compounds are not metabolized to the same extent by the two homogenates. In addition, the effect of albumin on the reactions under consideration was found to be different for the two cell types.

This paper is part II of a series entitled *Metabolism of Phospholipids by Phagocytic Cells*, of which Part I is ref. (8). A portion of this work was presented at the FASEB meeting held in Atlantic City, New Jersey in April 1966.

Abbreviations: LPE, lysophosphatidyl ethanolamine; LPC, lysophosphatidyl choline; PE, phosphatidyl choline; GPE, glycerophosphoryl ethanolamine; GPC, glycerophosphoryl choline; TLC, thin-layer chromatography.

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MATERIALS AND METHODS

Polymorphonuclear leukocytes were obtained from peritoneal exudates produced in rabbits and alveolar macrophages from rabbit lungs, as previously reported (8, 10, 11). The preparation of ^{32}P-labeled phospholipids was carried out as described in detail before (7, 8).

PE and PC labeled by incubation of rat liver slices with ^{32}P and isolated by preparative TLC were broken down with *Crotalus adamanteus* phospholipase A (12). The ^{32}P LPE and LPC thus obtained were isolated by TLC on silica gel–starch plates (Macherey, Nagel & Co., Düren, Germany) and by radioautography (7, 8). The radio-chemical purity of the ^{32}P LPC and LPE as determined by TLC (see below) was at least 98% and 97% respectively.

Unlabeled LPC and LPE were obtained from Mann Research Labs. Inc. (New York) and Pierce Chemical Company (New York), respectively. No significant impurities could be detected in these compounds by TLC and standard techniques for visualization of lipid compounds. Phospholipid-*P* determinations on weighed samples indicated that no appreciable nonphospholipid material contaminated the commercial lyso compounds.

Preliminary experiments showed that fatty acids were not limiting in the synthetic reactions studied and therefore no fatty acids were added to the assay mixture.

Homogenates were prepared in 0.25 M sucrose and incubated, and the radiochemical assays were carried out as described in a recent paper (8). Briefly, assay mixtures were extracted in chloroform–methanol by the method of Bligh and Dyer (13), but the extracts were not washed with water (so that water-soluble breakdown products of the ^{32}P-labeled lyso compounds should be retained). The organic solvents in the filtered extracts were evaporated in conical flasks under reduced pressure. The material remaining in the flask was taken up in a few drops of methanol and transferred to a TLC plate. The applied material was separated into various phospholipid fractions in a solvent system consisting of chloroform–methanol–glacial acetic acid–water 110:56:20:10. The ^{32}P monoacyl phosphatide substrate and the diacyl and deacylated derivatives formed in the reaction were scraped off directly into counting flasks. After a solution of scintillator in toluene had been added, radioactivity was determined in a Packard liquid scintillation spectrometer (8).

The extent of conversion was calculated from the percentage of total radioactivity recovered in the products of the reaction and from the specific radioactivity of the added substrate.

The phospholipid compositions of washed lipid extracts (14) and of homogenates of rabbit polymorphonuclear leukocytes and macrophages were examined by TLC. Phospholipids were made visible by exposure to iodine vapor and identified by comparison with appropriate reference substances. After the iodine stains had disappeared, marked areas corresponding to individual spots were scraped off the plate. Phosphorus determinations were carried out directly on the silica gel by the method of Parker and Peterson (15).

The nature of the water-soluble product formed during incubation with ^{32}P LPE or LPC was examined by paper chromatography in two solvent systems: phenol–water 80:20 and propanol–ammonia–water 60:30:20 (16). The paper strips were passed through a strip counter (Baird-Atomic, Inc., Valley Stream, N.Y.) for assay of radioactivity. Identification was limited to determination of the R_f of the radioactive peak as compared to those of unlabeled reference GPC, GPE, and 3-glycerophosphate, detected as described by Dawson (17). Fatty acid titrations were carried out by the procedure of Dole and Meinertz (18). Protein content of homogenates was determined by the method of Lowry, Rosebrough, Farr, and Randall (19).

RESULTS

Phospholipid Content and Composition of Rabbit Polymorphonuclear Leukocytes and Macrophages

Table 1 contains analyses of the total phospholipids and major phospholipid species in homogenates of peritoneal leukocytes and alveolar macrophages. The results are expressed as mμmoles of phospholipid-*P* per mg of homogenate protein. It is evident that macrophages have a higher phospholipid content per mg of protein than do granulocytes. Sphingomyelin, PC, and PE constitute close to 80% of the total recovered phospholipids of the macrophages, but only about 70% of the granulocyte phospholipids. The relative proportions of sphingomyelin, PC, and PE were not markedly different in the two homogenates. Both homogenates contain a few mμmoles of LPC per mg of protein. In the assays to be described no correction has been made for this small amount of en-

TABLE 1 TOTAL AND MAJOR PHOSPHOLIPIDS OF LEUKOCYTES AND MACROPHAGES

	Leukocytes	Macrophages
	mμmoles/mg homogenate protein	
Total phospholipid	183 ± 11.4	297 ± 22.6
Sphingomyelin	35.3 ± 1.7	49.4 ± 9.0
PC*	54.4 ± 3.3	117 ± 8.3
PE*	34.0 ± 2.1	77 ± 13.1

Determinations of total lipid phosphorus and of major phospholipid species separated by TLC (14) were carried out in duplicate on aliquots of washed lipid extracts of seven leukocyte and five macrophage homogenates. Results are given as mean ± SEM.

* No distinction has been made between phosphatidyl and phosphatidal choline and (or) ethanolamine.

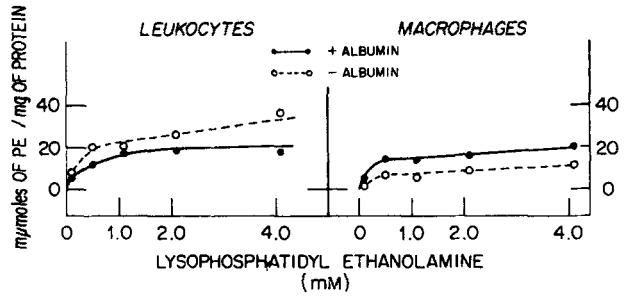

FIG. 1. Effect of increasing concentrations of LPE on formation of PE in the presence and absence of albumin. Assay mixtures of 0.5 ml contained 2 μmoles of ATP, 0.1 μmole of CoA, 2 μmoles of $MgCl_2$, 2.5 μmoles of phosphate buffer at pH 7.2, the indicated concentrations of LPE, 0.1 ml of either leukocyte or macrophage homogenate and 0.3 μmole of albumin (when added); they were incubated for 30 min at 37 °C under gentle agitation. The reaction was terminated by addition of chloroform–methanol 1:1 (13).

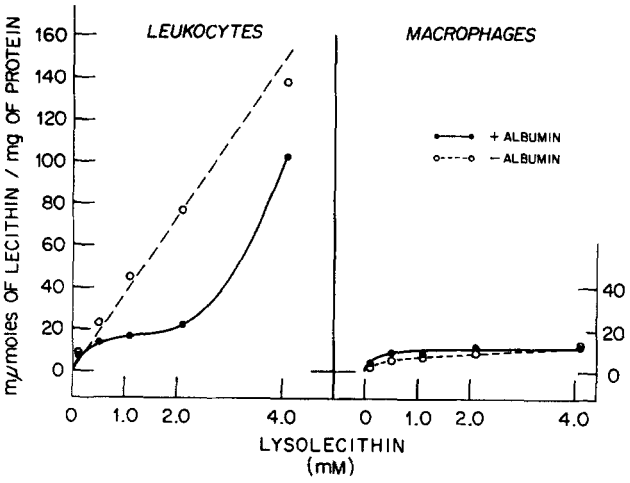

FIG. 2. Effect of increasing concentration of LPC on formation of PC in the presence and absence of albumin. The assay was carried out as described under Fig. 1, except that the substrate was LPC.

dogenous LPC. No attempt was made to recover phosphatidyl serine, phosphatidyl inositol, or phosphatidic acid which were of no immediate importance to these studies. Karnovsky and Wallach have reported that these three phospholipid components constitute only about 4% each of the total phospholipids of guinea pig polymorphonuclear leukocytes (20). They also found that PC, PE, and sphingomyelin comprised the bulk of the phospholipids, although with a somewhat different distribution.

Comparison of Formation of Diacyl Derivatives from LPE and LPC

LPC in the serum occurs bound to albumin (21). The assimilation of circulating LPC by intact tissues (22) presumably takes place, therefore, by transfer from albumin to cellular enzyme(s). To create conditions in our homogenate experiments comparable to those in studies on intact cells (unpublished experiments), we added albumin to the assay mixtures. However, as will become apparent, the role of albumin in the reactions under consideration is quite complex.

Earlier experiments have provided evidence for direct acylation of LPC by homogenates of polymorphonuclear leukocytes and alveolar macrophages (8). This reaction occurs optimally at approximately physiologic pH and is stimulated by ATP and CoA. The mean values of the results obtained in experiments on LPE and LPC carried out under these conditions are presented in Figs. 1 and 2. The effect is shown of increasing concentrations of lyso compounds on the production of diacyl glycerophosphatides in the presence and absence of defatted albumin.

The amount of PE synthesis per mg of homogenate protein in the presence of albumin is approximately the same for the two cell types (Fig. 1) and also closely similar to the amount of PC synthesized by macrophage homogenates (Fig. 2). Except at lower concentrations, increasing amounts of substrate in the assay mixture do not increase the amount of the diacyl compound formed by macrophages, or of PE by leukocytes. In contrast, lecithin synthesis by leukocyte homogenates increases markedly with substrate concentrations.

In macrophages, less PE was formed when albumin was omitted (Fig. 1), in each of four paired experiments, each carried out at all five substrate concentrations. The differences were not statistically significant (as determined by Student's "*t*" test) except at an LPE concentration of 1.1 mM ($P < 0.05$). PC synthesis by macrophages appeared unaffected by omission of albumin (Fig. 2).

The effect of omission of albumin upon synthesis of PE by leukocytes was opposite to that on synthesis by macrophages. In all of six paired experiments, each performed at five substrate levels, slightly more PE was formed in the absence of albumin (Fig. 1). The difference was statistically significant only at 0.5 mM ($P < 0.05$) and 4.1 mM ($P < 0.005$) LPE concentrations.

In the absence of albumin, conversion of LPC to PC proceeds approximately linearly with increasing LPC concentrations (Fig. 2), but in the presence of albumin a plateau is seen at intermediate substrate concentrations. In three paired experiments the differences were statistically significant at 1.1 mM ($P < 0.05$) and 2.1 mM ($P < 0.005$) LPC concentrations.

Comparison of Deacylation of LPE and LPC

Deacylation of these lyso compounds (Figs. 3, 4) was distinctly different for the two homogenates, both in the presence and absence of albumin. Water-soluble reaction products were identified by paper chromatography in two solvent systems as GPC and GPE.

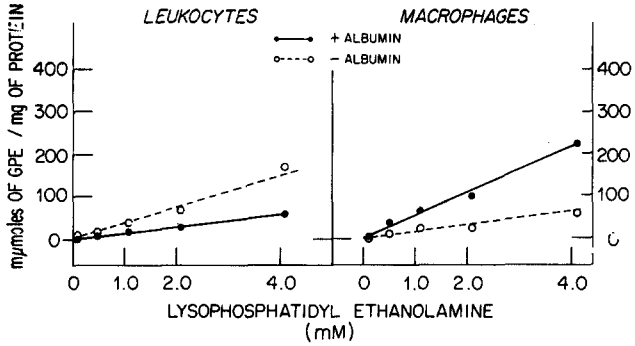

FIG. 3. Effect of increasing concentrations of LPE on formation of GPE in the presence and absence of albumin. Assay mixtures were prepared as described under Fig. 1.

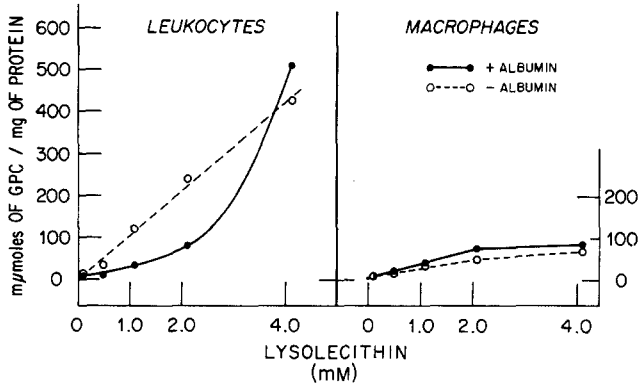

FIG. 4. Effect of increasing concentrations of LPC on formation of GPC in the presence and absence of albumin. For preparation of assay mixtures see Fig. 1.

In the presence of albumin the extent of deacylation of LPE by macrophage homogenates exceeded that by leukocyte homogenates (Fig. 3), while in the absence of albumin the reverse was true. Deacylation was linear with substrate concentration in all four sets of circumstances. Each point represents the mean of at least three assays.

Breakdown of LPC (Fig. 4), when albumin was present in the assay mixture, by leukocyte and macrophage homogenates was approximately the same up to concentrations of 2 mM. At this concentration deacylation by macrophages reached a plateau, whereas hydrolysis of LPC by leukocytes was greatly increased at a substrate concentration of 4.1 mM. Omission of albumin did not appreciably influence hydrolysis of LPC by macrophage homogenates.

The effect of albumin on the deacylation of LPC by leukocytes resembled that on its conversion to PC (Fig. 2), in that at the highest LPC concentration, hydrolysis was similar with and without albumin, while at intermediate concentrations addition of albumin was inhibitory ($P < 0.005$ at 0.5 mM; $P < 0.01$ at 1.1 mM; $P < 0.02$ at 2.1 mM LPC).

Possible Nature of Inhibitory Effect of Albumin on Deacylation of LPC by Leukocyte Homogenates

Since at a substrate concentration of 4.1 mM inhibition by albumin of deacylation of LPC was less pronounced than at lower concentrations, it seemed possible that binding of substrate to albumin accounted for the observed inhibition, especially in view of the demonstrated binding of LPC to albumin (21).

This possibility was tested in the following manner. Lysolecithinase activity was assayed by determination of fatty acid release (23) at increasing LPC concentrations in the absence and presence of albumin. The results of one such experiment have been plotted in Fig. 5 according to Lineweaver and Burk (24). The solid line indicates the results obtained in the absence of albumin. As the albumin concentration is raised the point at which inhibition becomes manifest is shifted toward higher substrate concentrations. The extent of inhibition appears to be approximately the same for the three albumin levels as judged by the roughly parallel course of the lines that branch off from the line corresponding to the uninhibited reaction. If the inhibitory effect of albumin on deacylation is attributable to a reduction in effective substrate concentration because of the action of binding sites present on the albumin that compete for substrate with the enzyme under consideration, these results should allow an estimate of the number of binding sites involved.

Table 2 contains the raw data of the experiment described graphically in Fig. 5. By using the percentage conversion of the available substrate in the absence of albumin (column 3), we can calculate the reduction in fatty acid release that would be attributable to albumin if each molecule of albumin sequesters one, two, or any chosen number of molecules of LPC.

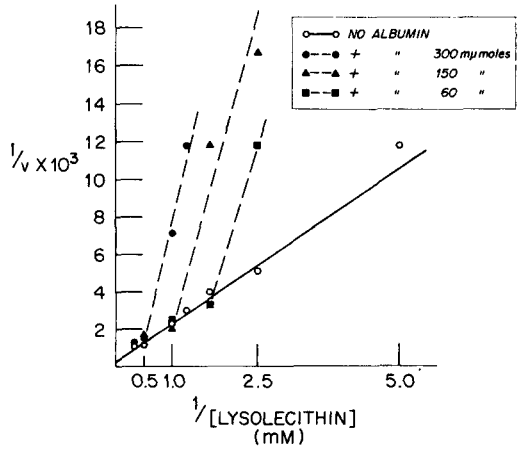

FIG. 5. Inhibitory effect of albumin on fatty acid release from lysolecithin by leukocyte homogenates. For preparation of assay mixtures see footnote to Table 2.

TABLE 2 EFFECT OF ALBUMIN UPON RELEASE OF FATTY ACID FROM LYSOLECITHIN BY LEUKOCYTE HOMOGENATES

Lysolecithin Added (1)	No Albumin Added: Fatty Acid Released (2)	No Albumin Added: % of Substrate Converted (3)	Albumin Added: 300 mμmoles: Substrate Available Calcd (4)	300 mμmoles: Substrate Converted Calcd (5)	300 mμmoles: Substrate Converted Found (5)	Albumin Added: 150 mμmoles: Substrate Available Calcd (6)	150 mμmoles: Substrate Converted Calcd (7)	150 mμmoles: Substrate Converted Found (7)	Albumin Added: 60 mμmoles: Substrate Available Calcd (8)	60 mμmoles: Substrate Converted Calcd (9)	60 mμmoles: Substrate Converted Found (9)
mμmoles	*mμmoles*			*mμmoles*			*mμmoles*			*mμmoles*	
3000	975	32.5	2400	780	835						
2000	835	42	1400	610	670	1700	740	640			
1000	445	44.5	400	175	140	700	305	390	880	380	420
800	334	42	200	85	85						
600	250	42	—	—	—	300	130	85	480	210	300
400	195	49	—	—	—	100	50	60	280	120	85
200	84	42	—	—	—	—	—	—	80	35	—

Each assay mixture contained 1 mM phosphate buffer at pH 7.2, 0.1 ml of homogenate representing 2.8 mg of protein, lysolecithin and albumin in the indicated amounts, and water to give a final volume of 1.0 ml. Incubation was carried out at 37°C for 30 min. [The reaction proceeds linearly during this period of time. Maximal activity is attained at a substrate concentration of about 5 mM (unpublished observations).] Enzymatic activity was terminated by addition of 5 ml of isopropyl alcohol. Fatty acid release was determined by titration (18).

The amount of substrate available was estimated by assuming that each mμmole of albumin [molecular weight of 66,000 (30)] rendered 2 mμmoles of lysolecithin unavailable by binding. The predicted fatty acid release in the presence of different concentrations of albumin was calculated from the percentage conversion in the absence of albumin (column 3).

In columns 5, 7, and 9, the values found for release of fatty acid are compared to those predicted if the number chosen is two, and the available substrate concentrations indicated in columns 4, 6, and 8 are correspondingly reduced. The fairly close agreement between the predicted and experimental values is consistent with the existence on each molecule of albumin of two binding sites which have a greater affinity for LPC than does the active site of lysolecithinase. Once these binding sites are occupied the remaining substrate is apparently entirely available to the reaction.

The experiment described was carried out with albumin that had been treated to remove free fatty acid by the method of Goodman (25). The inhibitory effect of "defatted" albumin was identical with that of untreated bovine albumin, which indicates that the extraction procedure had not freed binding sites for LPC or in any other fashion modified the albumin to produce the observed effect. Phosphorus was determined in phospholipid fractions recovered from thin-layer chromatograms of extracts of treated and untreated albumin. The LPC, sphingomyelin, PC, and PE content of 10 mg (150 mμmoles) of each of the two albumin fractions was respectively (in mμmoles): 12, 0, 14.5, and trace for defatted albumin, and 11.6, 0, 14.8, and trace for untreated albumin. Two-dimensional TLC (first in chloroform–methanol–glacial acetic acid–water 110:58:20:10 and then in chloroform–methanol–ammonia–water 115:45:3.5:3.5) carried out to separate LPE from PC revealed a barely visible spot in the area corresponding to LPE. Switzer and Eder found a closely similar value of 10 mμmoles of LPC per 150 mμmoles of immunoelectrophoretically pure rat albumin; this phospholipid comprised almost all the lipid-*P* associated with this protein (21). The presence of phospholipids other than LPC in the bovine albumin fraction from the Armour Co. may reflect a species difference or an impure protein fraction. These findings show that the minute quantity of phospholipid associated with albumin is not removed by the extraction procedure aimed at removing free fatty acid.

The similarity of the curves obtained for formation of PC and GPC by leukocytes in the presence and absence of albumin suggests that the effect of albumin on PC synthesis can also be ascribed to competition for substrate between albumin and enzyme(s).

Evidence has previously been presented that is consistent with the conclusion that two enzymatic activities concerned with the conversion of LPC to PC reside in polymorphonuclear leukocytes. One of these activities acts optimally at pH 7.4, at low substrate concentrations, and in the presence of added ATP and CoA, and sediments upon centrifugation (8). This enzyme seems to have similar properties to the LPC-acylating enzyme first described in liver by Lands (1). The other activity manifests an acid pH optimum, requires high substrate concentrations but no added ATP and CoA, and does not sediment upon high speed centrifugation (7, 8). The mechanism of PC formation by the reaction it catalyzes appears to be: 2 LPC → PC + GPC (7, 8, 26).

Since at low substrate concentrations direct acylation probably accounts for most PC synthesis (8) and also because inhibition by albumin was either absent or less evident at lower LPC concentrations, albumin might compete in particular with the "soluble" enzyme that requires high substrate concentrations.

TABLE 3 EFFECT OF ALBUMIN ON LECITHIN FORMATION BY A 100,000 *g* SUPERNATANT FRACTION AND SEDIMENT OF LEUKOCYTE HOMOGENATES

	Lecithin Formed in Presence of Albumin / Lecithin Formed in Absence of Albumin
Supernatant fraction	0.4 ± 0.07 (12)
Sediment	1.6 ± 0.46 (8)

In three experiments freshly prepared homogenates were centrifuged at 400 *g* for 10 min. The supernatant fluid was pipetted off and centrifuged at 100,000 *g* for 1 hr. The resulting supernatant fraction was taken off and the sediment was resuspended in 0.25 M sucrose to give a volume equal to the initial volume of the 400 *g* supernatant fraction. The assay was carried out in a mixture containing 2 μmoles of ATP, 0.1 μmole of CoA, 2 μmoles of $MgCl_2$, 2.5 μmoles of phosphate buffer, 0.3 μmole of albumin, and 0.1 ml of the fraction to be tested in a total volume of 0.5 ml. LPC was added in various concentrations, ranging from 0.1 to 4.1 mM. The results are given as mean ± SEM of the indicated number (n) of paired assays.

To test this possibility, we assayed PC synthesis at various LPC concentrations with and without albumin in a 100,000 *g* supernatant fraction (containing predominantly "transferase" activity) and in the sediment (containing predominantly acylating activity). Table 3 shows that the albumin effect is more apparent on PC synthesis by the supernatant fraction than by the sediment ($P < 0.005$). The higher ratios in the series of paired assays of the supernatant fractions were obtained at high substrate concentrations.

DISCUSSION

Although homogenates of polymorphonuclear leukocytes and lung macrophages both convert LPC and LPE to diacyl and deacylated compounds, differences in the extent of the reactions were noted.

In keeping with a recent report from this laboratory, formation of PC was distinctly greater by leukocyte homogenates than by macrophage homogenates when the reaction was studied at higher substrate concentrations (Fig. 2). PE synthesis per mg of protein was, however, closely similar for the two homogenates at all substrate concentrations (Fig. 1). Direct acylation of LPC by the mechanism first described by Lands (1) has been shown to occur in a number of tissues, including leukocytes and macrophages (8). Several investigators have reported that LPE can also serve as substrate in this reaction (5, 6, 22, 27). Unpublished observations in this laboratory, showing that addition of ATP and CoA stimulated conversion of LPE to PE approximately 3-fold in both homogenates and that high-speed centrifugation sedimented most of this activity, suggest that the Lands pathway is indeed the mechanism of synthesis. This contention is strengthened by the close similarity in the extent of conversion of LPC and LPE at lower concentrations (Figs. 1, 2).

Several considerations render it likely that the greater amount of synthesis of PC by leukocytes at higher substrate concentrations can be attributed to the transfer reaction mentioned earlier, in which 2 LPC → PC + GPC (7, 8, 26, 28, 29). Whenever PC synthesis was examined at high LPC concentrations, assays run at pH 5.5 manifested greater conversion than those carried out at pH 7.4 (8). By contrast, PE formation was always much less at acid pH (not shown). The qualitative differences (effects of pH and substrate concentration) in conversion of LPE and LPC to their respective diacyl derivatives also lead to the tentative conclusion that LPE does not serve as a substrate in the "transfer" reaction.

Homogenates of both cell types deacylated LPE and LPC, but the affinity of the enzymes for these lyso compounds was clearly different in the two homogenates. Differences were noted not only for each substrate with regard to one cell type, but also when the two homogenates were compared.

The effect of albumin on the various reactions in the two tissues was also dissimilar. Whereas albumin in the macrophage experiments either did not affect (Fig. 4) or actually stimulated (Fig. 3) deacylation of both substrates [and perhaps also enhanced acylation of LPE to a small extent (Fig. 1)] albumin evidently inhibited these same reactions in leukocyte homogenates. This inhibition was not the same for the two substrates. The kinetic studies (Fig. 5 and Table 2) on fatty acid release from LPC point to competition for the substrate between albumin and the hydrolyzing enzyme. Circumstantial evidence has been presented (Table 2) which suggests that the inhibitory effect of albumin upon PC formation at intermediate LPC concentrations is also due to competition. This competition appears most pronounced with the enzyme that carries out the reaction 2 LPC → PC + GPC.

Since the effects of albumin on hydrolysis by leukocytes of LPE and LPC were different (Figs. 3, 4), in that inhibition of LPE deacylation was still highly significant ($P < 0.001$) at the highest substrate concentration (4.1 mM), it cannot be concluded that a mechanism similar to that invoked for hydrolysis of LPC accounts for this inhibition. Thus far, it is not clear how albumin inhibits deacylation of LPE by leukocytes or how it stimulates deacylation of LPE by macrophage homogenates (Fig. 3).

It is evident, however, that albumin, which serves as a carrier for circulating LPC (21), influences the extent to which lyso compounds are metabolized by homogenates of the phagocytic cells examined in these studies. Further work is needed to establish the specificity of the albumin effect and its role in regulating the delivery of these physiologically occurring membrane-lytic agents to the enzymes concerned with their metabolism.

If our conclusions concerning the extent of binding of LPC to albumin are correct, the quantity of LPC found in association with various albumin preparations in the studies of Switzer and Eder (21) and in those reported here would represent a small fraction of the amount that can be bound (the molar ratio of LPC to albumin found in the two studies was between 1:12 and 1:15).

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References

1. Lands, W. E. M. 1960. *J. Biol. Chem.* **235:** 2233.
2. Webster, G. R., and R. H. S. Thompson. 1965. *Nature.* **206:** 498.
3. Stein, Y., and B. Shapiro. 1963. *Biochim. Biophys. Acta.* **70:** 33.
4. Marinetti, G. V., J. Erbland, R. F. Witter, J. Petix, and E. Stotz. 1958. *Biochim. Biophys. Acta.* **30:** 223.
5. Robertson, A. F., and W. E. M. Lands. 1964. *J. Lipid Res.* **5:** 88.
6. Mulder, E., and L. L. M. van Deenen. 1965. *Biochim. Biophys. Acta.* **106:** 348.
7. Elsbach, P., J. W. O. van den Berg, H. van den Bosch, and L. L. M. van Deenen. 1965. *Biochim. Biophys. Acta.* **106:** 338.
8. Elsbach, P. 1966. *Biochim. Biophys. Acta.* **125:** 510.
9. Zucker-Franklin, D., and J. G. Hirsch. 1964. *J. Exptl. Med.* **120:** 569.
10. Elsbach, P., and I. L. Schwartz. 1959. *J. Gen. Physiol.* **42:** 883.
11. Cohn, Z. A., and E. Wiener. 1963. *J. Exptl. Med.* **118:** 991.
12. De Haas, G. H., F. J. M. Daemen, and L. L. M. van Deenen. 1962. *Biochim. Biophys. Acta.* **65:** 260.
13. Bligh, E. S., and W. J. Dyer. 1959. *Can. J. Biochem. Physiol.* **37:** 911.
14. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. *J. Biol. Chem.* **226:** 497.
15. Parker, F., and N. F. Peterson. 1965. *J. Lipid Res.* **6:** 455.
16. Olley, J., and R. M. C. Dawson. 1956. *Biochem. J.* **62:** 5P.
17. Dawson, R. M. C. 1960. *Biochem. J.* **75:** 45.
18. Dole, V. P., and H. Meinertz. 1960. *J. Biol. Chem.* **235:** 2595.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. *J. Biol. Chem.* **193:** 265.
20. Karnovsky, M. L., and D. F. Hoelzl Wallach. 1961. *J. Biol. Chem.* **236:** 1895.
21. Switzer, S., and H. A. Eder. 1965. *J. Lipid Res.* **6:** 506.
22. Stein, Y., and O. Stein. 1966. *Biochim. Biophys. Acta.* **116:** 95.
23. Elsbach, P., and M. A. Rizack. 1963. *Am. J. Physiol.* **205:** 1154.
24. Lineweaver, H., and D. Burk. 1934. *J. Am. Chem. Soc.* **56:** 658.
25. Goodman, D. S. 1957. Science. **125:** 1297.
26. Erbland, J. F., and G. V. Marinetti. 1965. *Biochim. Biophys. Acta.* **106:** 128.
27. Webster, G. R. 1965. *Biochim. Biophys. Acta.* **98:** 512.
28. van den Bosch, H., H. A. Bonte and L. L. M. van Deenen. 1965. *Biochim. Biophys. Acta.* **98:** 648.
29. Mulder, E., J. W. O. van den Berg, and L. L. M. van Deenen. 1965. *Biochim. Biophys. Acta.* **106:** 118.
30. Low, B. W. 1952. *J. Am. Chem. Soc.* **74:** 4830.