

Transport of lysolecithin by albumin in human and rat plasma

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SUMMARY Lysolecithin comprises 9.6 and 21.5% of the phospholipids of the plasma of man and rat, respectively. Ultracentrifugal and gel filtration studies showed that the major portion of the lysolecithin is not found together with the other phospholipids in the plasma lipoproteins. By zone electrophoresis, gel filtration, and ammonium sulfate fractionation, it was found that lysolecithin was consistently associated with albumin fractions. Immunoelectrophoretically homogeneous rat albumin was prepared. It contained 0.5 mg of lipid phosphorus per g of protein; 98.3% of this lipid was lysolecithin. It is concluded that lysolecithin is transported in plasma bound to albumin.

KEY WORDS lysolecithin · albumin · complex · gel filtration · immunoelectrophoresis · block electrophoresis · transport · plasma · rat · man · lipoproteins · $d > 1.21$ proteins

LYSOLECITHIN WAS FIRST shown to be a normal constituent of plasma in man by Phillips (1) and in the rat by Newman, Lin, and Zilversmit (2). The manner in which lysolecithin is transported in plasma has not been clearly defined. Earlier, Havel, Eder, and Bragdon (3) had demonstrated the presence of a significant amount of lipid phosphorus in the protein fraction ($d > 1.21$) obtained after removal of the lipoproteins from human plasma by ultracentrifugation. Preliminary electrophoretic studies of this fraction indicated that the lipid phosphorus migrated with albumin or α_1 -globulin (3, 4). Phillips (5) subsequently separated the phospholipids in this fraction and found the major component to be lysolecithin with lesser amounts of lecithin, sphingolipids, and cephalin. Data are presented here showing that the major portion of the lysolecithin in plasma is bound to albumin.

METHODS

Blood was obtained from nonfasted adult male subjects and Sprague-Dawley male rats obtained from Holtzman Laboratories (Madison, Wis.). Heparin or EDTA was used as anticoagulant and the plasma was separated immediately by centrifugation at 5–10°. Only freshly obtained plasma was used in the studies reported. Ultracentrifugal separations were performed by the method of Havel et al. (3). Block electrophoresis was performed in a supporting medium of Pevikon C-870 (Fosfatbolaget, Stockholm, Sweden) using 0.04 M barbital buffer, pH 8.6, as described by Müller-Eberhardt (6). Gel filtration on Sephadex G-200 was performed as described by Porath (7) and Flodin and Killander (8, 9). Plasma lipids were extracted with 21 volumes of chloroform-methanol 2:1 and washed with 4 volumes of distilled water as described by Folch, Lees, and Sloane Stanley (10). Immunoelectrophoresis was performed by the micromethod of Scheidegger (11) in 1% Noble Agar with 0.04 M barbital buffer, pH 8.2. Goat anti-rat serum (Hyland) was used for immunoprecipitation.

Thin-layer chromatography on Silica Gel G (Brinkmann Instruments, Westbury, N.Y.) was performed essentially by the method of Stahl (12) as summarized by Mangold (13). For phospholipids a developing solvent of chloroform-methanol-water 72:24:4 in the presence of hexane vapor was used.¹ The sample was divided into three equal aliquots and each was applied to a separate zone of a single plate. After developing, individual fractions were made visible by brief exposure to iodine vapor and were removed by scraping with a razor blade. The scrapings were digested and analyzed for phosphorus by

¹ The presence of hexane vapor was found to be useful in the simultaneous separation of neutral lipids and phospholipids and enhanced resolution of the phospholipids (M. Rapport and N. Alonzo, personal communication).

the method of Beveridge and Johnson (14). The presence of silicic acid did not significantly alter the blank. The sensitivity of the method was increased by extraction of the blue complex (reduced molybdenum) with 0.24 volume of butanol and measurement of the optical density in a Coleman Jr. Spectrophotometer at 660 m μ . More than 90% of the applied lipid phosphorus could be recovered in this manner. Individual values of major components (>10% of the total sample) were within 6% of the mean of triplicate determinations.

Cholesterol analyses were performed by the method of Abell, Levy, Brodie, and Kendall (15). Protein analyses were performed by the method of Lowry (16). Ester-bond analyses were performed by a modification of the method of Antonis (17). Methyl esters were prepared for gas-liquid chromatography by the method of Stoffel, Chu, and Ahrens (18) and purified by thin-layer chromatography using a developing solvent of hexane-ethyl ether-acetic acid 83:16:1. The methyl esters were fractionated by gas-liquid chromatography using an ethylene glycol succinate polyester column at 181° under 30 psi of Argon; a Barber-Colman, model 10, apparatus equipped with a ⁹⁰Sr ionization detector was used. The phospholipid reference standards were prepared from beef liver and cardiac muscle and were generously supplied by Drs. M. Rapport and E. Gottfried.

Ammonium sulfate fractionation was performed by addition of increasing amounts of a saturated solution of ammonium sulfate to the d > 1.21 fraction previously dialyzed against Ringer's solution. The mixture was centrifuged at 2000 rpm for 1 hr at 10° and the precipitate and supernatant fraction were analyzed for protein and lipid phosphorus. Rat albumin was prepared by the method of Schwert (20) in which the d > 1.21 plasma proteins were precipitated with an equal volume of 10% trichloroacetic acid and the precipitate was extracted with 80% ethanol (2.5 ml/ml of d > 1.21 protein solution). The alcohol was removed by successive dialyses against water, saline, and finally water. The precipitate was discarded and the clear supernatant solution containing the albumin was concentrated by vacuum dialysis.

RESULTS

Content of Phospholipid in the d > 1.21 Proteins

Analysis of the d > 1.21 proteins from six normal, adult male subjects yielded a mean concentration of 1.04 mg of lipid P per 100 ml of plasma, with a range of 0.91–1.17 mg/100 ml (Table 1). This represented 9.2% of the total lipid P in the plasma. These samples contained less than 0.7% of the total serum cholesterol. Pooled rat d > 1.21 proteins yielded essentially the same value for lipid P (1.1 mg/100 ml of plasma).

TABLE 1 DISTRIBUTION OF LIPID PHOSPHORUS AND CHOLESTEROL IN D < 1.21 AND D > 1.21 FRACTIONS OF HUMAN PLASMA

Subject	Lipid P		Cholesterol	
	d < 1.21	d > 1.21	d < 1.21	d > 1.21
	mg/100 ml plasma		mg/100 ml plasma	
1	9.52	0.99	144	1.0
2	12.2	1.15	234	0.5
3	10.7	1.06	157	0.5
4	10.6	0.98	155	0.5
5	11.7	1.17	190	0.8
6	8.65	0.91	126	0.5
Mean \pm SEM	10.6 \pm 0.5	1.04 \pm 0.04	168 \pm 16	0.6 \pm 0.1

Identity of the Phospholipid in the d > 1.21 Proteins

Analyses of the lipids extracted from d > 1.21 proteins of the rat and separated by thin-layer chromatography showed 82.8–88.3% of the lipid P to be lysolecithin. An additional 4.8–12.8% was lecithin. The material designated lysolecithin had the same mobility (" R_F ") as the reference lysolecithin. It yielded a pink color with Dragendorff's reagent (choline-positive) and did not react with ninhydrin. It had an ester:phosphorus molar ratio of 0.99. Gas-liquid chromatography of the methyl esters of the fatty acids prepared from a sample of rat lysolecithin revealed the following fatty acid distribution: palmitate 45.1%, stearate 32.5%, linoleate 12.9%, oleate 4.6%, arachidonate 3.3%, others 1.6%. Lecithin isolated from the same plasma had a very different fatty acid composition: palmitate 35.7%, stearate 21.0%, linoleate 22.0%, oleate 5.7%, arachidonate 13.8%, and others 1.8%.

A single pool of human plasma and the protein fractions prepared from it were analyzed for phospholipid composition (Table 2). The phospholipids of the d > 1.21 fraction comprised 71% lysolecithin and 18% lecithin. On the other hand, the d < 1.21 fraction contained only 3.5% lysolecithin as compared with 65% lecithin. Of the total lysolecithin recovered in the fractions, 75% was recovered in the d > 1.21 fraction. However, it should be noted that the amount of lysolecithin recovered was appreciably greater than the amount present in the original plasma, presumably because lysolecithin was formed from lecithin during ultracentrifugation. Assuming this breakdown to be random, the newly formed lysolecithin would be largely in the d < 1.21 fraction. The absolute amount of lysolecithin recovered in the d > 1.21 fraction, therefore, would represent as much as 96% of the original lysolecithin present in native plasma.

Nature of the d > 1.21 Protein Carrier of Lysolecithin

The d > 1.21 proteins were fractionated by several methods in order to identify the protein carrier of

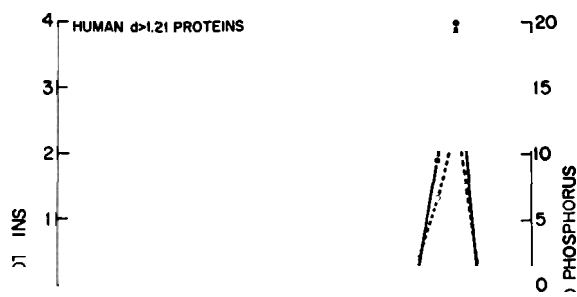


FIG. 1. Pevikon block electrophoresis of $d > 1.21$ plasma proteins from man and rat. Fractions were cut from the cathodal end of the block at 1 cm intervals and analyzed for protein and lipid phosphorus.

lysolecithin. Pooled $d > 1.21$ proteins from man and rat were dialyzed against barbital buffer, 0.04 M, pH 8.6 and subjected to block electrophoresis on a supporting medium of Pevikon. The results, as shown in Fig. 1, demonstrate that the phospholipid migrates with albumin. The albumin-bound phospholipid was isolated and identified by thin-layer chromatography as lysolecithin (Fig. 2).

Whole serum and $d > 1.21$ protein fractions were separated by gel filtration on Sephadex G-200. With whole serum (Fig. 3) the lipid phosphorus appeared both with the first and third protein peaks. The phospholipids present in the first protein peak (tubes 11-18) represented the plasma lipoproteins. The lipid phos-

TABLE 2 DISTRIBUTION OF PHOSPHOLIPIDS IN POOLED HUMAN PLASMA (EDTA) AND IN PROTEIN FRACTIONS PREPARED FROM THIS PLASMA BY ULTRACENTRIFUGAL FLOTATION

	Plasma	$d < 1.21$	$d > 1.21$
	<i>mg of lipid P per 100 ml plasma</i>		
Lysolecithin	0.76	0.24	0.73
Lecithin	5.47	4.55	0.19
Sphingolipids	1.38	1.21	0.03
Others*	0.19	0.98	0.08
Total	7.80	6.98	1.03

* Includes lipid phosphorus remaining at the origin, phosphatidyl ethanolamine, and phospholipids with an R_F greater than phosphatidyl ethanolamine.

phorus present in the third protein peak (tubes 22-29), which consists predominantly of albumin, represented 17% of the total lipid phosphorus of the serum and by thin-layer chromatography was 95% lysolecithin. This corresponds almost exactly with the amount of lysolecithin found in the $d > 1.21$ fraction, and demonstrates that in whole serum the lysolecithin is bound to albumin. When $d > 1.21$ proteins were fractionated by gel filtration on Sephadex G-200 (Fig. 4), the lipid phosphorus was present entirely in the albumin peak and the other fractions contained no phospholipids. This indicates that this fraction is free from lipoproteins and constitutes further evidence that the predominant phospholipid in this fraction, lysolecithin, is bound to albumin.

Confirmatory evidence for the nature of the carrier protein for lysolecithin was obtained by the fractionation of rat $d > 1.21$ proteins by means of ammonium sulfate (Fig. 5). Protein-bound phospholipid began to be precipitated at 45-50% saturation and the phospholipid-protein ratio in the supernatant solution achieved a maximum between 50 and 55% saturation. The solubility of the carrier protein in ammonium sulfate solu-

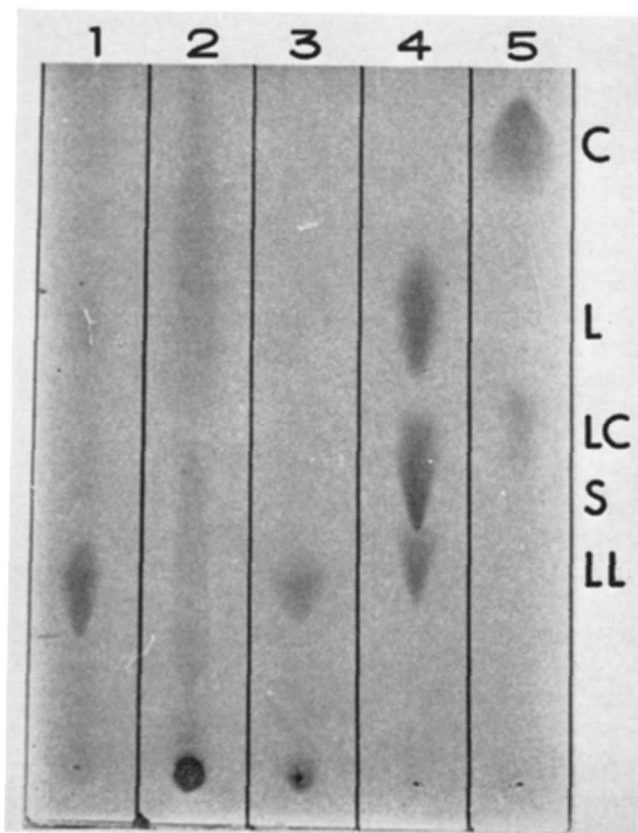


FIG. 2. Thin-layer chromatogram of lipid extracts from (1) human $d > 1.21$ protein; (2) globulins (fractions 5-16) separated by electrophoresis, cf Fig. 1; (3) albumin (fractions 18, 19) separated by electrophoresis, cf Fig. 1; (4) a reference sample containing lysolecithin (LL) sphingolipids (S), and lecithin (L); and (5) a reference sample containing lysocephalin (LC) and cephalin (C).

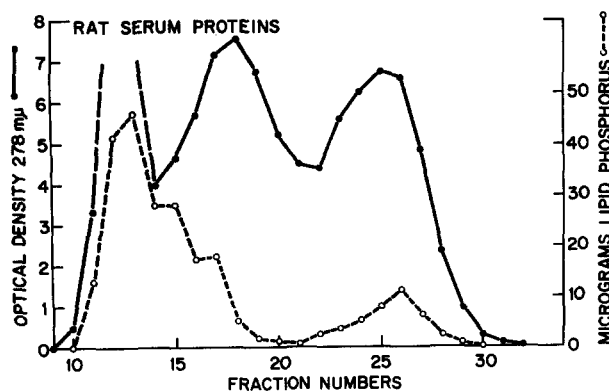


FIG. 3. Gel filtration of rat serum on Sephadex G-200 in Ringer's solution containing 0.02 M tris buffer, pH 7.5. Elution was performed with the same solution.

tions, therefore, corresponds to the known solubility of serum albumin (19). At 55% saturation the ratio of phospholipid to protein in the supernatant solution was more than twice that in the total $d > 1.21$ protein.

Finally, albumin of rat plasma was prepared by extracting with ethanol the trichloroacetic acid precipitate of $d > 1.21$ protein (20). This preparation yielded only a single band by immunoelectrophoresis in agar gel (Fig. 6) and contained 0.5 mg of lipid P per g of albumin. By thin-layer chromatography the phospholipid was found to be 98.3% lysolecithin. These data demonstrate conclusively that lysolecithin is bound to albumin in plasma.

DISCUSSION

Our data confirm the observation that lysolecithin is a normal constituent of plasma, and represents 9.5 and 21.5% of the phospholipids of plasma of man and rats respectively. These values agree well with those reported by Gjone, Berry, and Turner (21) and by Newman et al. (2). The latter authors considered the possibility that lipid extraction and silicic acid chromatography cause significant degradation of lecithin to lysolecithin and concluded that this was negligible with methods similar to ours. We have found, in agreement with Phillips (5), that the major portion of the plasma lysolecithin is present in association with plasma proteins other than the lipoproteins. However, our data show that the fraction associated with the lipoproteins is even smaller than was found by Phillips. It is not possible to exclude the presence of lysolecithin in native lipoproteins, but it should be noted that the conversion of only 5% of the lipoprotein lecithin to lysolecithin during ultracentrifugation could account for the entire lysolecithin content of this protein fraction. This possibility is suggested by experiments such as the one shown in Table 2 in which, although the total recovery of lipid phosphorus after

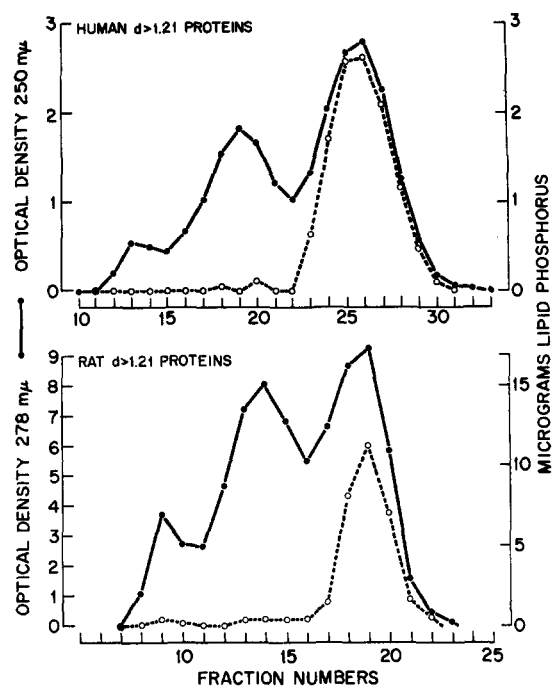


FIG. 4. Gel filtration on Sephadex G-200 of $d > 1.21$ plasma proteins from man and rat. The fractions were dialyzed against 0.3 M NaCl-0.1 M tris buffer, pH 7.5, and eluted with the same buffer.

ultracentrifugation was 103%, the recovery of lecithin was 83% and that of lysolecithin 128%. Glomset (22) has shown that lecithin in the plasma lipoproteins may be converted to lysolecithin via the activity of an acyl transferase in plasma. Although one would expect this reaction to be inhibited by the conditions under which ultracentrifugal separation of lipoproteins is performed, it may proceed to a degree sufficient to account for the results shown in Table 2.

The finding that albumin prepared by electrophoresis, gel filtration, or ammonium sulfate fractionation con-

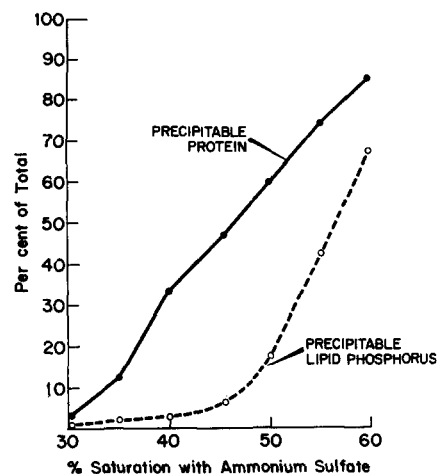


FIG. 5. Precipitation of proteins and of lipid phosphorus from $d > 1.21$ proteins by increasing concentrations of ammonium sulfate.

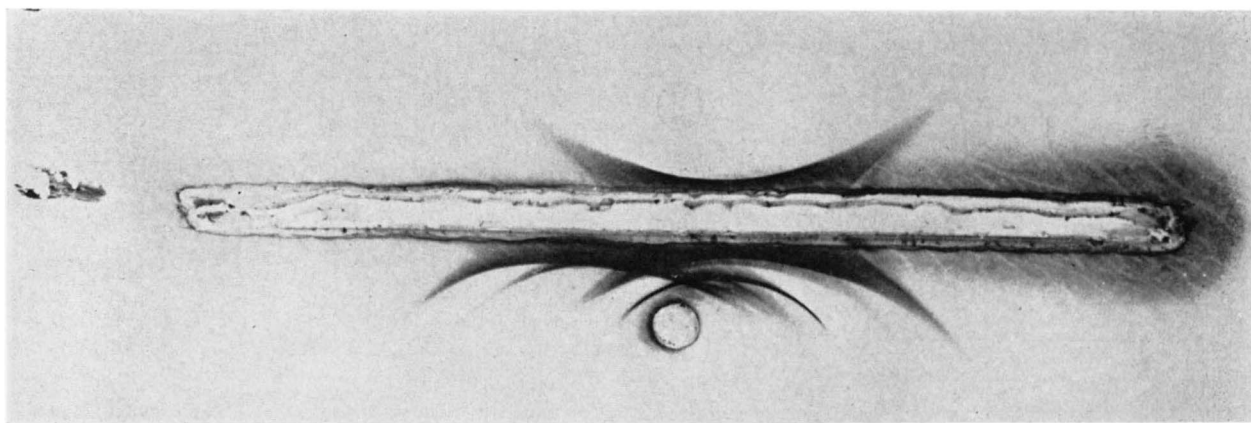


FIG. 6. Immunoelectrophoresis in 1% Noble Agar of rat albumin (top) and rat serum proteins (bottom). Goat anti-rat serum (Hyland) was added to the trough.

sistently contained lysolecithin has strongly suggested that the lysolecithin of the plasma is bound to albumin. Proof of this was obtained by the demonstration that a preparation of rat albumin, which was immunologically homogeneous, contained 98.3% of its phospholipid as lysolecithin. Albumin prepared by the same procedure directly from fresh, whole plasma also contained lysolecithin. However, such a preparation was found to contain significant amounts of lecithin. Undoubtedly, when lipoproteins are present, some of the lecithin they contain is extracted by the alcohol used in this procedure and becomes attached to the albumin. The binding of lecithin by albumin has been demonstrated by Eley and Hedge (23).

Although the phospholipid isolated from albumin prepared from $d > 1.21$ fractions was almost exclusively lysolecithin, the $d > 1.21$ fraction does contain other phospholipids, chiefly lecithin, in amounts between 3 and 5 mg/100 ml. This could be interpreted as indicating the presence of lipoproteins in the $d > 1.21$ fraction. However, experiments utilizing gel filtration on Sephadex G-200 (Fig. 4) show that no phospholipid is recovered where *intact* lipoproteins would be expected to appear. Recently Levy and Fredrickson (24) have obtained evidence that high-density lipoproteins are altered during ultracentrifugation and have found that some of the transformation product is present in the $d > 1.21$ fraction. The phospholipids other than lysolecithin that we have found in the $d > 1.21$ fraction could be constituents of these *altered* high-density lipoproteins.

Since lysolecithin is a potent hemolytic agent, its binding is of physiologic importance. This was suggested by the work of Bergenheim and Fåhræus (25) who, nearly 30 years ago, demonstrated the formation of lysolecithin in incubated, whole blood and noted prehemolytic changes in the shape of the erythrocytes. More recently, Klibansky and DeVries (26) have studied this phenomenon and demonstrated that the changes in erythrocytes

exposed to lysolecithin could be reversed by the addition of albumin which bound the lysolecithin.

A function of the lysolecithin in the plasma has been suggested by the recent observation in several laboratories (27-29) that red blood cells can actively incorporate fatty acids into lecithin in the presence of added ATP and CoA. Van Deenen, De Gier, Houtsmuller, Montfoort, and Mulder (28) demonstrated directly with the use of ^{32}P -labeled lysolecithin that in this reaction lysolecithin is converted to lecithin. Robertson and Lands (29) showed that when lysolecithin was added to such a system there was increased incorporation of labeled precursor into lecithin. Since neither Oliveira and Vaughan (27) nor Robertson and Lands (29) could find phospholipases in the erythrocyte, it seems likely that the lysolecithin which participates in this reaction must come from the plasma. It should be noted, however, that since the amount of lecithin in the erythrocyte is constant there must be continuous transfer of lecithin from the cell as lysolecithin enters. The fate of the lecithin liberated has not been studied, but if the reaction described by Glomset results in the loss of significant amounts of plasma lecithin, sites would be available in the lipoproteins which could bind the lecithin leaving the cells. This would constitute a cycle, with lysolecithin from plasma albumin entering the red cell membrane and forming lecithin, which in turn is released into plasma to combine with lipoproteins, where lysolecithin is again formed and transferred to plasma albumin. Uptake of lysolecithin by cells is not necessarily restricted to erythrocytes. Stein and Stein (30, 31) have recently shown that lysolecithin is taken up and converted to lecithin by heart muscle and by liver. It may be that lysolecithin, because of its solubility in water, is especially suited for transport into tissues where it serves as a precursor for the renewal of cellular lecithin.

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