Effect of liposome composition on the activity of detergent-solubilized acylcoenzyme A:cholesterol acyltransferase

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Abstract Acylcoenzyme A:cholesterol acyltransferase (ACAT) was solubilized from Ehrlich ascites cell microsomes with Triton X-100. After removal of the detergent, ACAT activity per mg protein was reduced by 50 to 65% as compared with untreated microsomes. When this microsomal extract was combined with liposomes composed of cholesterol and egg phosphatidylcholine, the ACAT activity increased 5.4- to 6.7-fold. Under these conditions sucrose density gradient centrifugation indicated that more than 50% of the added lipid was incorporated into vesicles having the same density as the ACAT activity, suggesting the formation of a complex. ACAT activity increased 2.9-fold when the phosphatidylcholine content of the liposomes was raised from 0.5 to 5.0 μmol/mg microsomal protein. By contrast, the ACAT activity increased only 42% when the cholesterol content of the liposomes was raised from 0.17 to 0.57 μmol/mg microsomal protein. Addition of phosphatidylethanolamine to the liposomes produced little change in ACAT activity, whereas the activity was reduced by 25 and 50%, respectively, when sphingomyelin or phosphatidylserine was added. ACAT activity was five times higher when the liposomes were prepared from dioleoylphosphatidylcholine than from saturated phosphatidylcholines, including hydrogenated egg yolk, dimyristoyl or dipalmitoyl phosphatidylcholine. Likewise, the ACAT activity with liposomes made from soybean or egg yolk phosphatidylcholine was almost 3.5-fold greater than with those prepared from the saturated phosphatidylcholines. These results are consistent with the view that the activity of ACAT can be modified by changes in the composition of the membrane lipids with which the enzyme is associated.—**Mathur, S. N., and A. A. Spector.** Effect of liposome composition on the activity of detergent-solubilized acylcoenzyme A:cholesterol acyltransferase. *J. Lipid Res.* 1982. **23**: 692–701.

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Acylcoenzyme A:cholesterol acyltransferase (E.C. 2.3.1.26) (ACAT) catalyzes cholesteryl ester synthesis in mammalian cells (1). The enzyme is tightly bound to microsomal membranes and is recovered from tissue homogenates primarily in the rough endoplasmic reticulum (2). ACAT has several important metabolic roles. It prevents intracellular accumulation of unesterified cholesterol (3), thereby protecting against possible perturbations in membrane fluidity. It also is involved in the storage of the cholesterol that is released in the lysosomes during the degradation of plasma lipoproteins (4). Because it catalyzes cholesteryl ester formation in the arterial wall, ACAT is thought to be involved in the development of the atherosclerotic lesion (5–7). Therefore, it is important to understand the factors that are involved in the regulation of ACAT activity.

During membrane lipid modification studies with Ehrlich ascites cells, we observed that ACAT activity was sensitive to changes in fatty acyl composition of the microsomal phospholipids (8). The ACAT activity of rat liver microsomes also was altered by changes in the microsomal fatty acid composition (9). An increase in polyunsaturated fatty acids increased the ACAT activity of the liver microsomes (9), whereas it reduced the ACAT activity of the Ehrlich cell microsomes (8). These findings suggest that, while the specific responses may vary in different species or tissues, it is possible that ACAT activity in general may be modulated by changes in the composition of the surrounding membrane phospholipids. This interpretation remained open to question, however, because the lipid modifications in both the Ehrlich cells and rat liver were made in vivo, and factors other than the change in membrane lipid composition could have been responsible for the change in enzyme activity. Therefore, the observed correlations between ACAT activity and lipid composition might be coincidental rather than causal.

In an attempt to investigate whether changes in membrane lipid composition can directly affect ACAT activity, we developed a procedure for extracting ACAT from Ehrlich cell microsomes and combining the solubilized extract with liposomes (10). The present communication demonstrates that the activity of this solubilized ACAT

Abbreviations: ACAT, acylcoenzyme A:cholesterol acyltransferase. Fatty acids are abbreviated as chain length:number of unsaturated bonds. Thus 18:1 is an 18 carbon atom fatty acid having one double bond.

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preparation can be modified by changing the phospholipid and fatty acid composition of the liposomes with which the enzyme is complexed.

METHODS

Materials and chemical methods

Egg L-α-phosphatidylcholine, dimyristoyl L-α-phosphatidylcholine, dioleoyl L-α-phosphatidylcholine, hydrogenated egg L-α-phosphatidylcholine, soybean L-α-phosphatidylcholine, bovine brain sphingomyelin, egg phosphatidylethanolamine, bovine brain L-α-phosphatidyl-L-serine, cholesterol, and dithiothreitol were obtained from Sigma Chemical Co. (St. Louis, MO). [1-^{14}C]Palmitoyl coenzyme A (57 Ci/mol) was purchased from New England Nuclear Corp. (Boston, MA). Palmitoyl coenzyme A was supplied by P-L Biochemical Inc. (Milwaukee, WI).

Protein was estimated by a modification of the Lowry method as described by Lees and Paxman (11). Bovine serum albumin served as the standard. Phospholipid content was determined by the method of Raheja et al. (12), and cholesterol content was determined enzymatically with the commercially available cholesterol oxidase method (Cholesterol Reagent Set, Boehringer-Mannheim Corp., Indianapolis, IN). The latter assay was modified by adding 10 mg of Triton X-100 to the chloroform solution and, after removal of the organic solvent by evaporation under N_2, 1 ml of the commercial reagent was added. The samples were mixed thoroughly, incubated for 1 hr at 37°C with shaking, and the absorbance was measured at 410 nm.

Fatty acid compositions were determined by gas–liquid chromatography. Methyl esters of the fatty acids were prepared using 14% boron trifluoride in methanol. After extraction of the fatty acid methyl esters (13), they were separated by gas–liquid chromatography using a 2 mm I.D. column containing SILAR-10C on 100/200 mesh gas chrom Q (Applied Science Laboratories, Inc., State College, PA). A 5710A Hewlett Packard gas chromatograph with temperature programming from 180 to 210°C was employed for the separation. Peak areas were obtained with a Hewlett Packard 3380A integrator. Peaks were identified with fatty acid methyl ester standards obtained from Supelco Inc. (Bellefonte, PA).

Preparation of liposomes

Phospholipids and cholesterol (2:1, mol/mol) were dissolved in chloroform–methanol 2:1 (v/v) and dried under a stream of nitrogen. The dried lipid mixture was dissolved in 4 ml of hexane, and the hexane was subsequently removed under nitrogen. To this mixture, 0.1 M K_2HPO_4, pH 7.5, containing 1 mM dithiothreitol (incubation buffer) was added to give a final concentration of 32 μmol of phospholipid and 16 μmol of cholesterol per ml. After the lipids were allowed to swell at 40°C for 60 min, the suspension was agitated with a Vortex mixer until all the lipid material went into solution. This mixture was sonicated at 30°C for 15 min under nitrogen, and the resulting vesicles were annealed by incubation at 40°C for 60 min. Unilamellar vesicles were obtained from the supernatant solution after the larger particles were sedimented at 12000 *g* for 10 min.

Solubilization of enzyme

Microsomes were prepared from an Ehrlich cell homogenate as described by Brenneman, Kaduce, and Spector (8). The microsomes were extracted for 2 hr at 4°C with a 2% solution of Triton X-100 in 0.1 M K_2HPO_4, 1 mM dithiothreitol, pH 7.4, and the residue was sedimented at 105,000 *g* for 1 hr. In order to remove the detergent, the supernatant solution was incubated twice with 0.6 g/ml of SM-2 BioBeads. The BioBeads were removed by filtration through glass wool followed by passage of the filtrate through a 0.22 μ Millipore filter. Following this, the soluble microsomal extract was dialyzed twice against 50 volumes of the incubation buffer for 16 hr. About 23 ± 2.2% (mean ± SEM, n = 8) of the total microsomal protein was recovered in the soluble extract. An experiment with radioactive Triton X-100 revealed that after two treatments with BioBeads followed by dialysis, less than 0.01% of the detergent remained in the microsomal extract.

Preparation of the protein-liposome complex

Unless indicated otherwise, liposomes containing cholesterol and microsomal extract (10.8 ± 0.6 μmol phospholipid/mg microsomal protein) were mixed, sonicated using a Branson sonifier at 10 watts output for 15 sec, and incubated at 4°C for 1.5 hr with stirring. After the suspension was centrifuged at 105,000 *g* for 1 hr, any liposomes that floated were removed from the top of the tube. The pellet containing the liposome-microsomal protein complex was dispersed in the incubation buffer to give a suspension containing 2–3 mg protein/ml. This complex of solubilized microsomal extract and phospholipid vesicles contained 58 ± 4.2% (mean ± SE, n = 8) of the protein added as soluble microsomal extract and contained more than 98% of the solubilized ACAT activity. Preliminary experiments indicated that sonication for more than 15 sec resulted in a loss of ACAT activity. For example, the final activity was reduced by 20% when a 2-min sonication period was employed. Freezing and thawing of the liposome-microsomal extract mixture in an attempt to more completely insert the protein into the liposomes (14) resulted in a loss of 80% of the activity of the preparation.

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A sucrose density gradient was employed to isolate the liposome-microsomal protein complex. The complex dispersed in 4 ml was layered on 28 ml of a 12–44% (w/v) linear sucrose gradient prepared in incubation buffer. After centrifugation for 16 hr at 82,500 *g*, 4-ml fractions were collected. The top two fractions were dialyzed against 20 volumes of the incubation buffer, while the remaining fractions were diluted to 22 ml with this buffer and then centrifuged at 105,000 *g* for 1 hr. The pellet from each of these fractions was resuspended in 2 ml of incubation buffer and analyzed for lipids and enzyme activity.

The complex of liposomes and microsomal extract also was isolated by gel exclusion chromatography. A 2% Sepharose column, 2.5 cm × 40 cm, was equilibrated with 0.15 M NaCl. Two ml of liposome-microsome complex containing 16 mg of protein was applied to the column and then eluted with 0.15 M NaCl. Fractions of 3 ml were collected at a flow rate of 25 ml/hr.

The complex of liposomes and microsomal extract was fixed for electron microscopy with 2.5% glutaraldehyde–0.1 M sodium cacodylate buffer, pH 7.2. After post-fixation in 1% osmium tetroxide and 1.5% potassium ferrocyanide, the samples were embedded in Spurr's plastic. Sections were stained with uranyl acetate and examined in a Hitachi H600 electron microscope. Vesicle size was determined from photomicrographs (magnification 39000 to 46000) using a Zeiss MOP-3 digitizer.

Assay of enzyme activity

The incubation medium contained 0.2 mg of microsomal protein, 50 nmol of defatted bovine serum albumin, 6 nmol of palmitoyl CoA, 0.05 μCi of [1-^{14}C]palmitoyl CoA, 0.1 M K_2HPO_4, and 1 mM dithiothreitol, pH 7.5, in a final volume of 0.5 ml. Unless stated otherwise, incubations were done with shaking at 30°C for 10 min and terminated by addition of 3 ml of chloroform–methanol 2:1 (v/v). Lipids were extracted in the chloroform layer (15), and the cholesteryl esters were isolated by thin-layer chromatography on silica gel G using a solvent system containing hexane–diethyl ether–methanol–acetic acid 170:40:2:2. The cholesteryl esters were visualized by exposure to iodine vapor and after the iodine had sublimed, the band was scraped into vials containing 10 ml of Budget-Solve scintillator solution. Palmitoyl CoA hydrolase activity was assayed under the same conditions, except that the free fatty acid band from the thin-layer chromatogram was scraped into vials containing scintillator solution. Radioactivity was measured by liquid scintillation counting using a Beckman LS7000 spectrometer. Quenching was monitored with a ^{137}Cs external standard.

RESULTS

ACAT activity

Table 1 illustrates the effect on microsomal ACAT activity of solubilization and addition of liposomes. As compared with intact microsomes, the soluble preparation, after removal of more than 99% of the Triton X-100, contained only 35–50% as much ACAT activity per mg protein. In terms of total measurable activity, the soluble extract contained 8–12% of the total activity present in the microsomes before extraction. These values probably underestimate the total amount of ACAT actually extracted from the microsomes because, as described below, the activity of the soluble preparation could be enhanced considerably by adding lipid. When the soluble material was combined with liposomes containing egg phosphatidylcholine and cholesterol, however, the ACAT activity was 5.4- to 6.7-fold higher per mg of protein than that of the solubilized extract and 2.2- to 2.8-fold higher per mg of protein than that of the intact microsomes. Based on these activity values, from 32 to 37% of the microsomal ACAT activity was recov-

TABLE 1. Enhancement of ACAT activity in solubilized preparations by addition of liposomes.

Enzyme Preparations	Lipid Content		Protein Content	ACAT Activity			Palmitoyl CoA Hydrolase Activity[b]
	Cholesterol	Phospholipid		12 μM	35 μM	Total[a]	
	nmol/mg protein		*mg*	*pmol/mg protein/min*		*pmol/min*	*pmol/mg protein/min*
Microsomes	52 ± 4[c]	141 ± 10[c]	100	45 ± 9[c]	57 ± 14[d]	5700	139
Microsomal extract[e]	122 ± 9	332 ± 30	23	15 ± 4	30 ± 6	690	194
Microsomal extract + liposomes[f]	348 ± 50	917 ± 94	13	101 ± 26	164 ± 24	2130	249

[a] Based on the values obtained with 35 μM palmitoyl CoA.
[b] The palmitoyl CoA concentration was 35 μM and the albumin concentration was 100 μM.
[c] Mean ± SE of determinations with five separate enzyme preparations.
[d] Mean ± SE of determinations with three separate enzyme preparations.
[e] Triton X-100 has been removed by incubation with BioBeads SM-2.
[f] Liposomes contained egg phosphatidylcholine and cholesterol, 2:1 mol/mol.

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ered in the soluble material. There was 2.8- and 6.5-fold more lipid per mg protein in the liposome complex than in the soluble extract and intact microsomes, respectively. However, the molar ratio of cholesterol to phospholipid was almost the same in each preparation. By electron microscopy (**Fig. 1**), the complex of liposomes and microsomal extract appeared as bilayer vesicles. Measurement of the vesicles with a digitizer indicated that their average diameter was 505 ± 50 Å. No multilayer vesicles were noted. Column chromatography of this material on 2% Sepharose indicated that the ACAT activity eluted in the void volume. This is consistent with the enzyme being contained in a large vesicular complex.

A corresponding experiment was carried out regarding palmitoyl CoA hydrolase activity, and the results also are shown in Table 1. As opposed to the ACAT result, the palmitoyl CoA hydrolase activity of the soluble extract, after removal of the Triton X-100, was 40% higher per mg of protein than that of the microsomes. Although addition of the liposomes also increased the palmitoyl CoA hydrolase activity of the soluble extract, the increase was only 28%. By contrast, the ACAT activity of the extract was increased 5.4-fold at the corresponding palmitoyl CoA concentration, 35 μM.

As shown in **Fig. 2**, the microsomal extract, liposomes, and liposomes combined with microsomal extract were analyzed on a sucrose gradient. Following removal of the Triton X-100, 70% of the ACAT activity present in the microsomal extract migrated on the gradient between the densities of 1.08 and 1.14 g/ml (Fig. 2A). Likewise, 64% of the cholesterol and 55% of the phospholipid in the extract also was recovered in this density range. These findings indicate that the ACAT activity extracted from the microsomes probably formed a complex with microsomal lipids after removal of the detergent. A sucrose density gradient profile of the liposomes, shown in Fig. 2B, indicated that 95% of the phospholipid and 99% of the cholesterol remain at the top, in the density range of 1.02 to 1.06 g/ml. Fig. 2C shows the gradient profile after the liposomes are combined with the microsomal extract. Under these conditions, 80% of the ACAT activity recovered remained in the density range of 1.08 to 1.14 g/ml. Sixty-five % of the phospholipid and 50% of the cholesterol also migrated in this density range, and

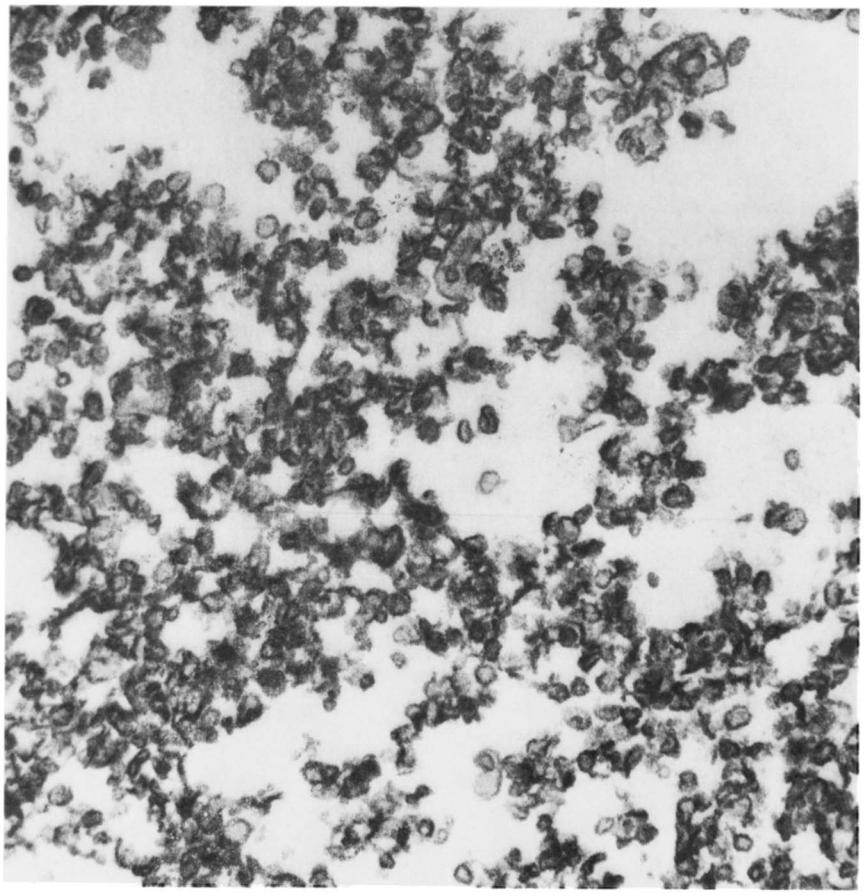

Fig. 1. Electron micrograph of the complex of liposome and microsomal extract containing ACAT activity. The magnification is ×46200.

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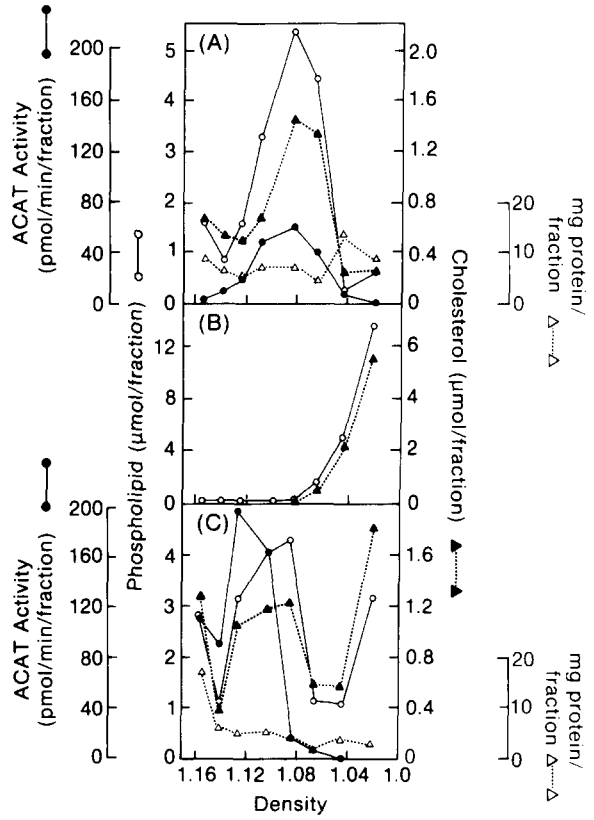

Fig. 2. Sucrose density fractionation of (A) microsomal extract, (B) liposomes without added protein, and (C) microsomal extract combined with liposomes. A 4-ml sample was applied over a 28 ml linear sucrose gradient (12–44%), and 4-ml fractions were collected. These fractions were assayed for phospholipid and cholesterol content and, in the case where microsomal protein was added, for ACAT activity.

only 20 to 30% of these lipids remained at the top of the gradient. Therefore, at least 50% of the added liposomal lipids acquired a higher density. The most direct explanation of this finding is that many of the liposomes took up protein from the microsomal extract, perhaps by fusion, forming more dense lipid-protein complexes. Since much of the ACAT activity also migrated in this density range, it is reasonable to assume that a considerable amount of the enzyme became associated with the added lipid.

The observation that the ACAT activity migrated at a higher density after the liposomes were added (Fig. 2C) than when the microsomal extract itself was put through the gradient (Fig. 2A) was unexpected, for it was thought that the addition of the lipid would reduce the density of the enzyme complex. In order to confirm this result, a second experiment with another microsomal extract was carried out. Similar findings were obtained; the ACAT activity again migrated at a somewhat higher density in the preparation containing liposomes. At present, this finding cannot be explained. It appears as if the active complex which forms when the liposomes are present contains more protein so that it migrates at a higher density than in the microsomal extract itself. In this context, it should be noted that the protein content of the gradient also migrates at a somewhat higher density after the liposomes are added (Fig. 2C) than in the microsomal extract (Fig. 2A). This finding also is unexpected and further attests to the considerable rearrangement of the constituents in the microsomal extract when the material is combined with liposomes.

Kinetic properties of the complex

The kinetics of the ACAT reaction were examined in the liposome-enzyme complex. As seen in the left panel of **Fig. 3,** enzymatic activity was linear for at least 20 min. In this and all other experiments, [1-^{14}C]palmitoyl CoA served as the labeled substrate, and ACAT activity was measured by following the incorporation of the radioactive palmitate group into the cholesteryl ester fraction. The right panel of Fig. 3 illustrates that the reaction was linear relative to protein content under these conditions until the solubilized microsomal protein in the complex exceeded 0.2 mg.

The ACAT activity of the liposome-enzyme complex was dependent upon palmitoyl CoA concentration. As seen in the left panel of **Fig. 4,** the activity increased continuously as the palmitoyl CoA concentration was raised from 5 to 85 μM when the albumin concentration was 100 μM. When the albumin concentration was reduced to 10 μM, however, the activity reached a peak at about 10 μM palmitoyl CoA and then decreased as the palmitoyl CoA concentration was raised further. Because of the apparent dependence on albumin concentration, this relationship was examined in greater detail. The right panel illustrates an experiment in which the palmitoyl CoA concentration was held constant at 14 μM and the albumin concentration was increased from 0 to 400 μM. ACAT activity increased when 10 to 50 μM albumin was added. The activity decreased

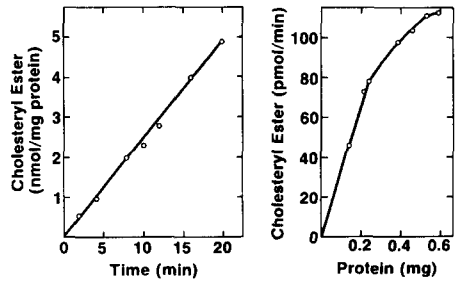

Fig. 3. Effect of incubation time and microsomal protein content on the ACAT activity of the soluble extract complexed with liposomes. The 0.5 ml incubation medium contained 6.34 nmol of [1-^{14}C]palmitoyl CoA and 50 nmol of albumin. In the time study (left side), 0.28 mg of microsomal protein was added. A 10-min incubation time was employed for the protein dependence study (right side). Each value is the mean of three separate determinations.

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when more albumin was added, and pronounced inhibition occurred at albumin concentrations above 100 μM. Presumably, this occurred because excessive binding to albumin prevented the access of adequate amounts of palmitoyl CoA to the enzyme. These effects of albumin and palmitoyl CoA concentration on the ACAT activity of the solubilized preparation are consistent with those observed with rat liver microsomes (16, 17).

Effect of liposomal lipid composition

At a fixed microsomal protein and cholesterol content, the ACAT activity of the complex increased as the phosphatidylcholine content of the added liposomes was raised. This is shown in **Fig. 5** where ACAT activity is seen to increase 3-fold when the ratio of phosphatidylcholine to microsomal protein (μmol/mg) was raised from 0.5 to 5 by addition of egg phosphatidylcholine. By contrast, raising the cholesterol content of the liposomes produced a relatively smaller increase in ACAT activity. As shown in **Fig. 6,** there was a 42% increase in ACAT activity when the cholesterol content of the liposomes relative to microsomal protein (μmol/mg) was raised from 0.17 to 0.57.

The effect of phosphatidylcholine fatty acyl composition on ACAT activity is shown in **Table 2.** In these experiments every attempt was made to keep the total phospholipid and cholesterol content (mol/mol) of the liposome-microsomal extract complexes constant. Some variations could not be avoided, however, and the actual molar ratio ranged from 0.32 to 0.47. Based upon the results in Figs. 4 and 5, phospholipid and cholesterol variations in this range should have very little effect on ACAT activity. As seen in Table 2, the variations in ACAT activity that resulted from altering the type of phosphatidylcholine used to make up the liposomes was considerable, suggesting that something other than the small variation in cholesterol or phospholipid content of the complex was responsible for these differences. As compared with egg phosphatidylcholine, the dimyristoyl,

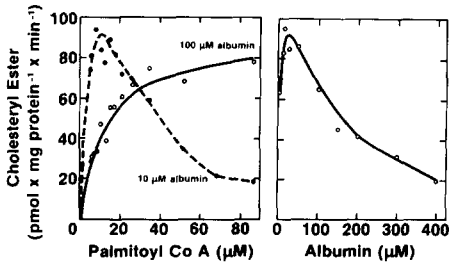

Fig. 4. Effect of palmitoyl CoA and albumin concentrations on ACAT activity in the soluble extract complexed with liposomes prepared from egg phosphatidylcholine and cholesterol. Each incubation mixture contained 0.22 mg of microsomal protein. In the experiment shown in the right panel, the [1-^{14}C]palmitoyl CoA concentration was 14 μM. Each point is the mean of three separate determinations.

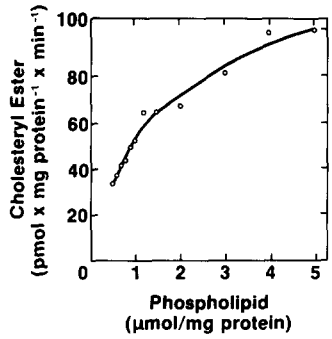

Fig. 5. Effect of phospholipid content of the enzyme-liposome complex on ACAT activity. Egg phosphatidylcholine liposomes were employed, and each preparation contained 107 nmol cholesterol/mg microsomal protein. The incubation medium contained 35 μM [1-^{14}C]palmitoyl CoA and 100 μM albumin.

dipalmitoyl, and hydrogenated egg phosphatidylcholine liposomes reduced the ACAT activity by 70 to 75%. The use of soybean phosphatidylcholine liposomes produced about the same activity as the egg phosphatidylcholine liposomes. The highest ACAT activity was obtained with dioleoyl phosphatidylcholine liposomes, which produced activities that were 30% higher than the egg phosphatidylcholine liposomes. As opposed to ACAT, the activity of palmitoyl CoA hydrolase, which also was solubilized from the microsomes by the Triton X-100 extraction, was not influenced by any of these changes in phosphatidylcholine fatty acyl composition except when dipalmitoyl phosphatidylcholine was used, in which case the hydrolase activity decreased by 35%.

The fatty acid composition of the various liposome-microsomal extract complexes is listed in **Table 3.** Highly saturated complexes resulted when dimyristoyl, dipalmitoyl or hydrogenated egg phosphatidylcholine were employed. The highly saturated compositions clearly indicate that the majority of the fatty acyl groups in these complexes is contributed by the added liposomes and that only a small percentage is derived from the microsomal extract. The majority of the fatty acid in the complex prepared from dioleoyl phosphatidylcholine was monoenoic, primarily 18:1. In this preparation, saturates accounted for only 13% of the total fatty acyl groups. Considerable differences in composition also were observed when the liposomes were prepared from the two natural phosphatidylcholines. For example, the preparation made with egg phosphatidylcholine was enriched with saturated acyl groups, whereas the one made from soybean phosphatidylcholine was enriched with polyunsaturates, especially 18:2. Apparently, differences in fatty acyl composition to the extent produced by these two natural phosphatidylcholines are insufficient to appreciably influence ACAT activity.

The effects of changes in phospholipid head group composition of the liposomal complex on ACAT activity

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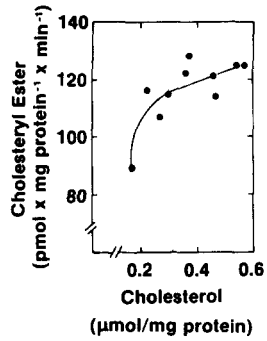

Fig. 6. Effect of the cholesterol content of the enzyme-liposome complex on ACAT activity. Each of the enzyme-liposome complexes contained 0.85 μmol of egg yolk phosphatidylcholine per mg of microsomal protein. The albumin concentration of the incubation medium was 100 μM and the palmitoyl CoA concentration was 10 μM. The lowest cholesterol concentration shown, 0.17 μmol, is the case where no cholesterol was added to the liposomes, the cholesterol present being derived entirely from the microsomal extract.

are shown in **Table 4.** In each case, the ratio of egg phosphatidylcholine to additional phospholipid was 1.0 to 0.5. Again, every attempt was made to keep the molar ratio of cholesterol to total phospholipid constant in each preparation, but this was not possible and the actual ratios varied from 0.43 to 0.62. Little change in ACAT activity occurred when phosphatidylethanolamine was added. By contrast, the addition of sphingomyelin or phosphatidylserine reduced the ACAT activity of the complex by 25% and 50%, respectively. As shown in Table 3, very little change in the fatty acid composition of the liposome-microsomal extract complex was produced by the addition of the second phospholipid component.

DISCUSSION

Previous work with Ehrlich ascites cells and rat liver indicated that microsomal ACAT activity was influenced by changes in the fatty acid saturation of the membrane phospholipids (8, 9). The changes in fatty acid saturation were produced in vivo. Therefore, the possibility existed that the effect on ACAT was a secondary response resulting from exposure of the tissues to an altered lipid environment, rather than being a direct influence of membrane phospholipid composition on the enzyme. To explore this question, we developed a soluble ACAT preparation that was extracted with Triton X-100 from Ehrlich cell microsomes. This preparation was activated when it was combined with liposomes composed of the soybean phospholipid extract, Asolectin, and cholesterol (1, 10). In the present work, we have utilized this system to determine whether the phospholipid composition of the liposomes can exert any effect on the activity of the solubilized enzyme preparation. Because in vivo lipid modification also affects the ACAT activity of rat liver microsomes, it would have been more generally applicable to carry out these studies with an enzyme preparation from liver. However, our repeated attempts to apply the solubilization and reconstitution technique to liver microsomes have been unsuccessful. Therefore, we proceeded with the Ehrlich cell enzyme which consistently regains activity when the solubilized microsomal extract is combined with liposomes. Attempts were made initially to purify the extracted enzyme with respect to both protein and lipids. Removal of the lipids with butanol, however, led to irreversible inactivation. Although gel filtration chromatography produced some purification, the amount of ACAT activity that was recovered was variable (1). Furthermore, in order to test for activity after each chromatographic step, the Triton X-100 had to be removed because the enzyme is inactive in the presence of this detergent. These manipulations led to large losses in total activity, making the procedure impractical in terms of retaining enough material for subsequent study. Because of these difficulties, we decided to work with the Ehrlich cell microsomal extract without further purification.

The sucrose gradient centrifugation studies suggest that some type of complex forms between the added liposomes and the solubilized ACAT. Since the detergent-extracted material contains both phospholipids and cholesterol, it is likely that a complex composed of these lipids and microsomal proteins formed when the Triton X-100 was removed. This is consistent with the observation that the enzyme has an average density of about 1.10 g/ml before addition of the liposomes. When liposomes having a density of 1.02 to 1.06 g/ml were

TABLE 2. Effect of phosphatidylcholine fatty acyl composition on ACAT and palmitoyl CoA hydrolase activities[a]

Phosphatidylcholine	Cholesterol/ Phospholipid	Relative ACAT Activity[b]	Relative Palmitoyl CoA Hydrolase Activity[c]
	mol/mol	*% ± SE*	*%*
Egg yolk	0.44	100	100
Soybean	0.35	95 ± 12	107, 108
Dioleoyl	0.39	130 ± 2	102, 117
Dimyristoyl	0.41	26 ± 5	97, 100
Dipalmitoyl	0.32	26 ± 3	60, 68
Hydrogenated egg	0.47	27 ± 1	85, 97

[a] The incubation medium contained 100 μM defatted albumin and 12 μM palmitoyl CoA.

[b] Mean ± SE of four enzyme preparations. The specific activities of the four ACAT preparations in the presence of egg yolk phosphatidylcholine varied from 188 to 309 pmol/mg protein/min.

[c] Two enzyme preparations were tested, and both values are listed as a percentage of the average value obtained for the egg yolk phosphatidylcholine preparation.

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TABLE 3. Fatty acid composition of ACAT preparations

Fatty Acid	Percentage Composition										
	Microsomes	Triton X-100 Soluble	Egg PC[a]	Soybean PC	Dioleoyl PC	Dimyristoyl PC	Dipalmitoyl PC	Hydrogenated Egg Yolk PC	Egg PC + PE[e]	Egg PC + PS[f]	Egg PC + Sphingomyelin
						%					
Classes[b]											
Saturated	33	32	34	22	13	73	85	71	29	29	27
Monoenoic	23	21	26	17	59	7	3	10	41	42	41
Polyenoic	32	31	39	47	22	10	5	6	27	29	23
Individual acids[c]											
14:0	tr[d]	tr	tr	tr	tr	62.4 ± 3.1	tr	tr	tr	tr	tr
16:0	16.1 ± 3.6	12.0 ± 1.5	19.8 ± 1.2	12.6 ± 2.3	6.4 ± 2.9	4.5 ± 0.9	80.2 ± 0.9	25.5 ± 1.1	14.0	15.0	17.0
16:1	3.0 ± 0.6	2.6 ± 0.8	3.7 ± 1.5	3.5 ± 1.0	1.3 ± 0.2	1.1 ± 0.3	tr	tr	8.5	8.4	9.0
18:0	17.3 ± 0.8	19.9 ± 3.1	14.0 ± 1.7	9.7 ± 2.4	6.6 ± 2.2	5.6 ± 0.8	4.3 ± 0.3	38.3 ± 0.6	14.8	14.3	10.0
18:1	20.3 ± 0.9	18.6 ± 0.5	22.0 ± 1.2	13.0 ± 1.0	57.7 ± 5.5	5.6 ± 0.5	3.2 ± 0.2	10.1 ± 0.2	32.5	33.4	32.2
18:2	19.9 ± 0.9	17.1 ± 1.7	16.6 ± 0.8	36.6 ± 2.4	15.4 ± 1.7	5.3 ± 0.5	2.8 ± 0.1	7.6 ± 0.1	12.4	11.6	11.0
20:4	8.4 ± 0.9	9.2 ± 0.6	5.8 ± 0.7	3.6 ± 1.0	3.3 ± 1.1	2.0 ± 0.6	1.4 ± 0.1	3.9 ± 0.2	7.1	6.0	4.2
22:6	3.4 ± 0.7	4.4 ± 0.1	6.9 ± 1.4	3.6 ± 1.0	3.7 ± 1.3	2.6 ± 0.6	1.0 ± 0.2	1.9 ± 0.4	7.1	6.0	3.1

[a] Phosphatidylcholine (PC) used to prepare liposomes. These fatty acid compositions refer to the final ACAT-liposome complex.
[b] These values do not add up to 100% because there were unidentified acids in each preparation.
[c] Only the major fatty acids are listed. The values are the mean ± SE of four determinations for the individual phosphatidylcholine preparations and the mean of two determinations for the mixed phospholipid preparations.
[d] Trace.
[e] Phosphatidylethanolamine (PE).
[f] Phosphatidylserine (PS).

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TABLE 4. Effect of phospholipid additions on ACAT activity

Phospholipid Added to Egg Phosphatidylcholine[a]	Cholesterol/ Phospholipid	Relative ACAT Activity[b]
	mol/mol	%
None	0.47	100
Phosphatidylethanolamine	0.57	112, 120
Phosphatidylserine	0.62	50, 55
Sphingomyelin	0.43	74, 75

[a] The molar ratio of egg phosphatidylcholine to the added phospholipid was 1:0.5.
[b] Two enzyme preparations were tested and both values are listed.

combined with the microsomal extract, more than half of the added lipid acquired a higher density and migrated in the gradient together with the enzyme activity. This finding suggests that many of the liposomes became associated with ACAT, either by adhering to the surface of the particles containing the enzyme or by actually fusing with them so that the enzyme became associated with these bilayer vesicles. The fact that the density of the ACAT preparation increased somewhat when the liposomes were added indicates that a considerable rearrangement of materials associated with the enzyme occurred when the complex formed.

The extent to which the solubilized microsomal preparation was activated by addition of the liposomes depended primarily upon the type of phospholipids present in the liposomes and the fatty acyl composition of the phosphatidylcholine. This finding provides some direct evidence that changes in the membrane phospholipid composition can affect the activity of ACAT, a regulatory process suggested by the previous in vivo studies (8, 9). With respect to phospholipid fatty acyl composition, the microsomal extract was most active when the added liposomes were prepared from dioleoyl phosphatidylcholine and least active when they were prepared from disaturated phosphatidylcholines. Since Ehrlich cell microsomes enriched in oleic acid exhibit the highest ACAT activities (8), these results with liposomes are consistent with the cellular findings. In this regard, the highest ACAT activities in rat aorta also were produced by feeding an oleic acid-enriched diet (18). Intermediate and roughly equivalent activities were obtained with liposomes composed of either egg or soybean phosphatidylcholine. The variations in fatty acyl composition in the egg and soybean complexes are similar to the extremes that can be produced in the microsomes of the intact cell, the egg preparations being roughly comparable to the most saturated compositions and the soybean preparations to the most unsaturated compositions produced in vivo (8, 9). Differences in saturation of this magnitude are sufficient to alter ACAT activity by almost 50% in the isolated microsome preparations (8, 9). The failure of this degree of change to produce any effect on the activity of the solubilized enzyme could be due to the fact that the fatty acid compositional changes in the liposomes were confined to phosphatidylcholine whereas in the microsomes, they also occurred in the other glycerophospholipids (9).

Except when dipalmitoyl phosphatidylcholine liposomes were added, the changes in the liposomal fatty acid composition did not appreciably affect the acyl CoA hydrolase activity. This indicates that these fatty acid modifications do not exert a generalized effect on microsomal enzymes that utilize acyl CoA. Therefore, it seems unlikely that phospholipid fatty acyl modifications affect ACAT activity primarily by influencing the access of acyl CoA to the enzyme. In this context, acyl CoA hydrolase activity also is not affected in the intact microsomes by the fatty acid modifications that exert a marked effect on ACAT activity (9).

A more plausible explanation is that the phospholipids exert their effect through a physical interaction with ACAT. In this regard, other tightly bound membrane enzymes are sensitive to microenvironmental changes brought about by phospholipid fatty acyl modifications (19–22). Alternatively, the fatty acyl chain composition may influence the accessibility of cholesterol to ACAT, even though the ratio of cholesterol to phospholipid in each of the complexes is quite similar. In support of this possibility, it has been shown that the distribution of cholesterol in liposomes can be influenced by changes in fatty acid composition (23–25). Moreover, in microsomes, not all of the cholesterol is available as a substrate for ACAT (26, 27). If the soluble complexes also contain more than a single cholesterol pool, the distribution of cholesterol among the several pools might be affected by changes in the fatty acyl composition of the phosphatidylcholine, the major phospholipid component. That the type of phospholipid can have an effect on cholesterol utilization by ACAT is suggested by comparing the present result with those reported previously (10). In the experiment shown in Fig. 6, where the liposomes were prepared from egg lecithin, ACAT activity continued to increase somewhat as the cholesterol content of the liposomes was raised. The cholesterol contained in the microsomal extract apparently is sufficient to produce about two-thirds of the maximal ACAT activity in this system. By contrast, a biphasic cholesterol dependence occurred when ACAT was combined with liposomes prepared from Asolectin (10). Small amounts of added cholesterol greatly stimulated ACAT activity in the Asolectin complex, while large amounts produced inhibition (10). Although these two possibilities, lipid-protein interactions or the accessibility of membrane cholesterol, are entirely different processes, the basic mechanism in each case still can be considered as a structural effect of the phospholipid matrix in which ACAT is embedded.

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