Quiocho, F. A., Miller, D. M., Newcomer, M. E., Guilliland, G. L., Pflugrath, J. W., & Charletta, D. A. (1980) *J. Supramol. Struct., Suppl. 4*, 76.
Redfield, A. G., & Gupta, R. K. (1971) *J. Chem. Phys. 54*, 1418–1419.
Robertson, D. E., Kroon, P. A., & Ho, C. (1977) *Biochemistry 16*, 1443–1451.
Stoesz, J. D., Malinowski, D. P., Redfield, A. G. (1979) *Biochemistry 18*, 4669–4675.
Szmeleman, S., Schwartz, M., Silhavy, T. J., & Boos, W. (1976) *Eur. J. Biochem. 65*, 13–19.
Wada, A., & Ueno, Y. (1964) *Biopolym. Symp. 1*, 343–364.
Weiner, J. H., & Heppel, L. A. (1971) *J. Biol. Chem. 246*, 6933–6941.

Interaction of Oleoyl Coenzyme A with Phospholipid Bilayers†

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ABSTRACT: The effect of oleoyl coenzyme A (CoA) on three phospholipid bilayer systems, human red blood cell ghosts, egg yolk lecithin dispersions, and unilamellar lecithin vesicles, was studied. Addition of oleoyl-CoA to sealed, right-side-out, human red blood cell ghosts resulted in a loss of latent NADH–cytochrome *c* oxidoreductase activity. The turbidity of lecithin dispersions decreased as a result of the addition of oleoyl-CoA in a concentration-dependent manner. This decrease in turbidity was influenced by the mode of addition of oleoyl-CoA to the phospholipid, and the most pronounced decrease was observed when oleoyl-CoA was dried together with the lecithin prior to resuspension in an aqueous solution. The presence of cholesterol (lecithin:cholesterol molar ratio 2:1) diminished the effect of oleoyl-CoA on the turbidity of the lecithin dispersions. Addition of oleoyl-CoA to unilamellar vesicles, which contained 5,6-carboxyfluorescein, increased the leakage of the dye from the vesicles in a concentration-dependent manner. This effect was diminished when cholesterol was incorporated into the vesicles (lecithin:cholesterol molar ratio 2:1). The interaction of oleoyl-CoA with lecithin was further studied by preparing mixtures where the lipids were dried together prior to sonication and had lecithin:oleoyl-CoA molar ratios of either 100:1 or 10:1. The resulting complexes were characterized by gel filtration and sucrose density gradient ultracentrifugation. Oleoyl-CoA was associated with particles having a size indistinguishable from that of unilamellar vesicles. At the higher oleoyl-CoA concentration, the complex formed was readily detected by density gradient ultracentrifugation because of the increased particle density. Addition of albumin to the mixtures caused dissociation of oleoyl-CoA from the vesicles but did not result in vesicle disruption under the conditions employed. The results show that oleoyl-CoA forms stable complexes with phospholipid bilayers and suggest that such complexes modify the permeability of the bilayer system.

Long-chain acyl-CoA[1] molecules have been proposed to have a regulatory role in intracellular metabolism. Support for such an effect is based primarily on the in vitro effect of either palmitoyl- or oleoyl-CoA on the activity of several enzymes localized in the cytosol (Kawaguchi & Bloch, 1976; Edgar & Bell, 1979). The physiological significance of such regulation is controversial since long-chain acyl-CoA forms micelles at concentrations close to that required for the inhibition of enzyme activity (Zahler et al., 1968), suggesting that the inhibition observed in vitro may be due to nonselective detergent effects. Recent studies have shown inhibition of several enzymes by palmitoyl-CoA at concentrations below its putative critical micellar concentration (Edgar & Bell, 1979) and where the effect of the CoA derivative was distinguished from that of other detergents (Wititsuwannakul & Kim, 1977; Hsu & Powell, 1975; Kawaguchi & Bloch, 1974), suggesting that acyl-CoA may have a regulatory role in vivo.

An understanding of what actually occurs in vivo is complicated by observations showing that intracellular proteins exist that bind long-chain acyl-CoA (Mishkin & Turcotte, 1974; Jamdar, 1979) and may prevent micelle formation. Further, a recent report indicated that the critical micellar concentration of palmitoyl-CoA was above 30 μM and the aggregation number was between 20 and 40 (Powell et al., 1981). These values are different than the previously reported values of 3–4 μM and 1000 (Zahler et al., 1968). Finally, there is no direct evidence on the state of long-chain acyl-CoA monomers, or micelles, bound to proteins or membranes within the cell. Most previous studies on acyl-CoA–enzyme interactions were performed with soluble enzymes often found in the cytosol. It is now known that many of the enzymes that utilize long-chain acyl-CoA as substrates are membrane bound and are localized on the outer (cytoplasmic) side of sealed microsomal vesicles (Bell et al., 1981). Therefore, acyl-CoA must interact with the membrane prior to or during its metabolism. Previous studies showed an association between palmitoyl-CoA and liver microsomes (Lamb & Fallon, 1972). Recent studies by Polokoff & Bell (1978) suggested that at physiological concentrations, palmitoyl-CoA can penetrate rat liver microsomes to become accessible to an ethanol acyltransferase, shown to be located on the inner (lumenal) side of the microsomes. That study and those of Jamdar (1979) and Lichtenstein & Brecher (1980) have shown that acyl-CoA can alter the permeability of rat liver microsomes as reflected by loss of latent mannose-6-phosphatase activity.

The precise nature of the interaction between long-chain acyl-CoA and biological membranes is not understood. This study describes the interaction of oleoyl-CoA with human red

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[1] Abbreviations: 5,6-CF, 5,6-carboxyfluorescein; CoA, coenzyme A; NADH, reduced nicotinamide adenine dinucleotide; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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blood cell ghosts, multilamellar liposomes, and unilamellar vesicles.

Experimental Procedures

Materials. [1-^{14}C]Oleoyl-CoA (sp act. 59 mCi/mmol), [7-^{3}H]cholesterol (sp act. 11 Ci/mmol), and dipalmitoylphosphatidyl[*methyl*-^{3}H]choline (sp act. 27 Ci/mmol) were obtained from the New England Nuclear Corp. (Boston, MA). [2-*palmitoyl*-9,10-^{3}H]Dipalmitoylphosphatidylcholine was purchased from Applied Science, Inc. (State College, PA). Purity of the radiolabeled phosphatidylcholine and oleoyl-CoA was assessed by thin-layer chromatography by using chloroform–methanol–water (65:25:4 v/v/v) or 1-butanol–acetic acid–water (5:2:3), respectively, as developing solvents. In each case, greater than 95% of the radioactivity migrated as a single spot corresponding to a nonradiolabeled standard.

Egg yolk lecithin was purchased from Lipid Products (Nutley, United Kingdom), cholesterol from Applied Science Laboratories, Inc. (State College, PA), and 5,6-carboxyfluorescein (5,6-CF) (catalog no. 9953) from Eastman Kodak Co. (Rochester, NY). Oleoyl-CoA, acetyl-CoA, NADH, cytochrome *c*, and bovine serum albumin (crystallized, lyophilized, essentially fatty acid free, catalog no. A-7511) were all obtained from Sigma Chemical Co. (St. Louis, MO). Water was doubly distilled in glass.

Preparation of Lecithin–Oleoyl-CoA Dispersions. Egg yolk lecithin liposomes were prepared by dissolving the designated amount of phospholipid in chloroform–methanol (2:1 v/v). The sample was evaporated under a stream of nitrogen and then pumped under vacuum with a freeze-drying apparatus for 1 h to remove residual organic solvent. The sample was then resuspended in a 0.02% sodium azide solution by vigorous stirring on a vortex-type apparatus for 2 min. Typically, 6 mg of lipid was dried in 25 × 75 mm test tubes and then resuspended in 2 mL of aqueous solution. Preparations containing cholesterol (lecithin:cholesterol molar ratio 2:1) were prepared similarly except that the appropriate amount of cholesterol was dissolved with the lecithin in the organic solvent prior to evaporation under nitrogen.

Oleoyl-CoA was added to the lecithin by three different procedures: (1) Oleoyl-CoA, dissolved in a small volume of water, was added to the chloroform–methanol (2:1 v/v) solution containing the egg yolk lecithin (and cholesterol, when designated); the resulting mixture was immediately evaporated under nitrogen, dried further in the freeze-drying apparatus, and finally resuspended in aqueous solution. (2) Oleoyl-CoA was included in the aqueous solution used to resuspend the dried lipids. (3) Oleoyl-CoA was added directly to the aqueous preparation subsequent to resuspension of the lipid from a concentrated stock solution containing 4.6 mM oleoyl-CoA in 0.01 M sodium acetate buffer, pH 6.0.

Preparation of Unilamellar Vesicles Containing 5,6-CF. Vesicles were prepared according to minor modifications of previously described procedures (Weinstein et al., 1977; Szoka et al., 1979). Egg yolk lecithin (6 or 60 mg) was dissolved in chloroform–methanol (2:1 v/v); the solution was dried as described above and then resuspended in 6 mL of a solution containing 0.25 M 5,6-CF, 0.1 M NaCl, 0.02% sodium azide, and 0.01 M Tris, pH 7.4. The resulting suspension was sonicated continuously in a Branson W-350 apparatus under a stream of nitrogen for 10 min (Brecher et al., 1977) and then centrifuged at 4 °C at 140000*g* for 60 min. The upper 4 mL of the centrifuged preparation was removed and used for subsequent studies. All subsequent manipulations of the 5,6-CF-containing vesicles were done at 4 °C to minimize leakage.

Vesicles containing cholesterol were prepared by adding cholesterol (1.5 or 15 mg) to the lecithin dissolved in organic solvent so that the molar ratio of lecithin to cholesterol was 2:1. Following removal of the organic solvent, the dried lipids were resuspended in the 5,6-CF solution as described above and sonicated for 1 h in a Branson W-350 sonifier with a 50% pulse setting under a stream of nitrogen. The resulting suspension was then centrifuged at 140000*g* for 1 h, and the upper 4 mL was removed and used for subsequent treatment.

The vesicle preparations containing entrapped 5,6-CF were applied to a Sephadex G-50 column (0.9 × 20 cm), and the vesicles eluting at the void volume were collected (Guo et al., 1980). The column was preequilibrated and eluted with a solution containing 0.1 M NaCl, 0.02% sodium azide, and 0.01 M Tris, pH 7.4. The 5,6-CF-containing vesicles were stored at 4 °C in dialysis tubing, which was suspended in the elution buffer, and were used within 3 days of preparation. In separate experiments, it was established that the elution profile of the vesicles containing 5,6-CF was virtually identical with that of vesicles lacking the dye with respect to the eluted lecithin.

Vesicle preparations lacking 5,6-CF were made exactly as described above except that 5,6-CF was omitted from the aqueous solution used to resuspend the dried lipids and the filtration through Sephadex G-50 was not performed. For the experiments shown in Figures 6 and 7, all lipids were dried together prior to sonication. Labeled lecithin, cholesterol, or oleoyl-CoA was routinely mixed with the corresponding unlabeled lipid to monitor recovery and to calculate molar ratios based on the initial specific activities.

Measurement of Turbidity of Lecithin–Oleoyl-CoA Preparations. Turbidity was determined by measuring the absorbance at 640 nm in a Coleman spectrophotometer. Initial measurements were made 5 min following the addition of oleoyl-CoA. Tubes were then shaken continuously for the designated time at ambient temperature (22–24 °C) with a wrist-action shaker moving at about 50 oscillations/min.

Measurement of 5,6-CF Release from Unilamellar Vesicles. Release of 5,6-CF was measured by fluorescence with a Perkin-Elmer fluorescence spectrophotometer (MPI-2A) at excitation maximum and emission maximum of 490 and 520 nm, respectively (Weinstein et al., 1977). Oleoyl-CoA was added from a concentrated stock solution (4.6 mM) to cuvettes containing 3 mL of a solution with the designated amount of vesicles, and fluorescence was read at selected time intervals. Total fluorescence for each sample was determined at the conclusion of each experiment by adding Triton X-100 to a final concentration of 0.1%. A control sample for monitoring leakage was included in all experiments for all vesicle preparations. All measurements were made at 25 °C. The percentage of 5,6-CF released was calculated by subtracting the fluorescence of the blank sample at the designated time interval from the total fluorescence of the experimental sample, dividing this figure by the total fluorescence in the cuvette as determined by the addition of 0.1% Triton X-100, and multiplying by 100.

Measurement of NADH–Cytochrome c Oxidoreductase in Human Red Blood Cell Ghosts. Human red cell ghosts were prepared by using the procedures outlined by Hanahan & Ekholm (1974). The membranes were sealed (right side out) by incubation in 150 mM sodium chloride and 5 mM sodium phosphate, pH 8.0, for 40 min at 37 °C (Steck & Kant, 1974). NADH–cytochrome *c* oxidoreductase activity was determined by incubating the designated amount of membrane protein, 0.2 mM β-NADH, 0.25 mg of cytochrome *c*, 150 mM sodium chloride, and 5 mM sodium phosphate, pH 8.0, in a total

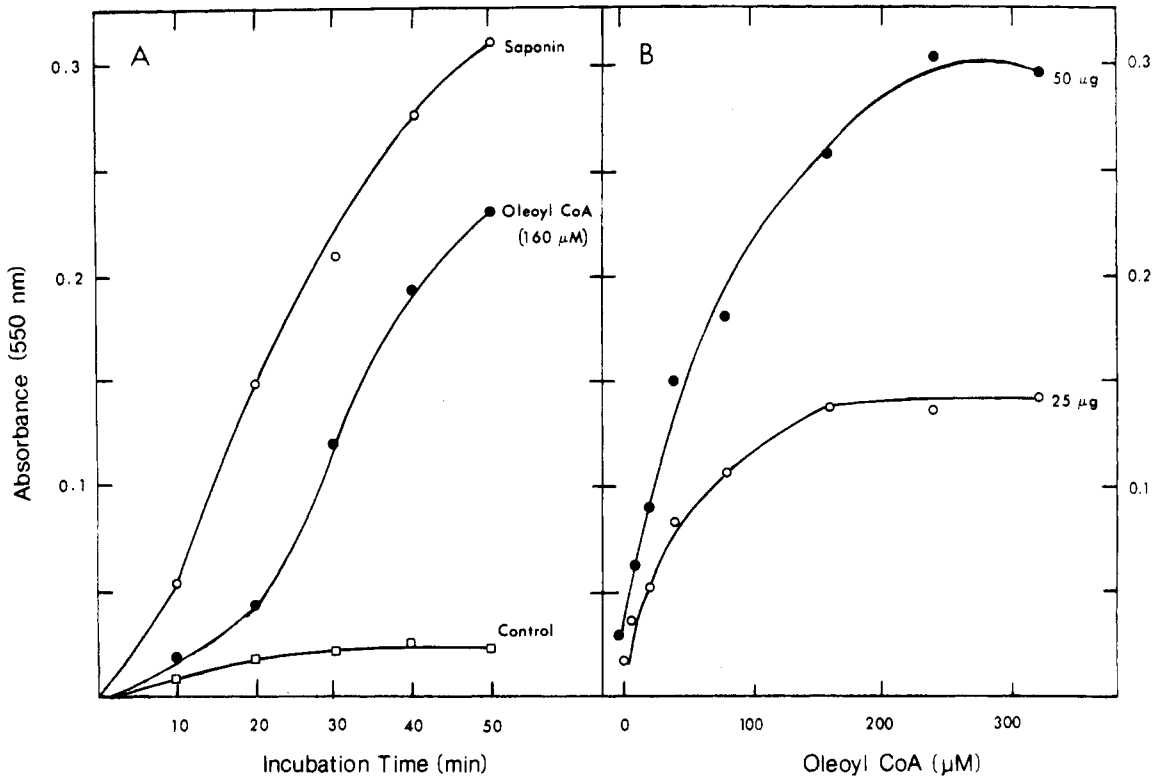

FIGURE 1: Effect of oleoyl-CoA on NADH–cytochrome *c* oxidoreductase in sealed red cell ghosts. The incubation system contained the designated amount of membrane protein, 0.2 mM β-NADH, 0.25 mg of cytochrome *c*, 150 mM NaCl, and 5 mM sodium phosphate, pH 8.0, in a total volume of 0.5 mL. Oleoyl-CoA or saponin was added in volumes not exceeding 10 μL, and the absorbancy at 550 nm was determined at 25 °C. (A) Effect of incubation time and the presence of saponin (0.005%) or oleoyl-CoA on NADH–cytochrome *c* oxidoreductase activity. 50 μg of membrane protein was present in each assay tube. (B) Effect of oleoyl-CoA concentration and amount of membrane protein on NADH–cytochrome *c* oxidoreductase activity.

volume of 0.5 mL and monitoring absorbance at 550 nm (Steck & Kant, 1974).

Gel Filtration. Samples (0.5 mL) were applied to either Sepharose 4B or Bio-Gel A 15-m (2.6 × 28 cm) columns, which were preequilibrated with a solution containing 0.1 M NaCl, 0.02% sodium azide, and 0.01 M Tris, pH 7.4, as indicated in the figure legends. Samples were eluted with the preequilibration buffer at a flow rate of 26–28 mL/h. The void volume and total volume for all columns were determined with dextran blue 2000 and tritiated water, respectively. All column chromatography was performed at ambient temperatures (22–24 °C). Eluted fractions were analyzed for radioactivity with Liquiscint (National Diagnostics) as the scintillation cocktail. Double isotope counting conditions were established such that ^{14}C was counted at 68% efficiency with no crossover of ^{3}H into the ^{14}C channel. Tritium was counted at 27% efficiency with a 14% crossover of ^{14}C into the ^{3}H channel. Data are expressed as the dpm for each of the eluted fractions after correction for crossover.

Sucrose Density Gradient Ultracentrifugation. Samples (200 μL) were layered onto 3.7 mL of an 8–23% continuous sucrose gradient containing 0.1 M NaCl and 10 mM Hepes, pH 7.4. Centrifugation was routinely performed for 16 h at 4 °C with a Beckman SW 60 Ti rotor spun at 310000*g*. Fractions (200 μL) were collected from the bottom of the tubes and analyzed for radioactivity or absorbance determined at 280 nm. Sucrose concentrations of the fractions were determined with a hand refractometer (Bausch & Lomb; range 0–60% sucrose).

Analytical Procedures. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Lipid phosphorus was measured by the method of Bartlett (1959) with a factor of 25 used to estimate phospholipid. Cholesterol was determined by the method of Rudel & Morris (1973). Lipid extracts were obtained by the method of Folch et al. (1957). Thin-layer chromatography was performed on precoated silica gel G plates (Applied Science, Inc., State College, PA). For resolution of major lipid classes, hexane–diethyl ether–acetic acid (70:30:1 v/v/v) was used as a developing solvent. Individual phospholipid classes were separated with chloroform–methanol–water (65:25:4 v/v/v) as the developing solvent.

Results and Discussion

Effect of Oleoyl-CoA on Human Red Cell Ghosts. Recently, it was shown that palmitoyl- or oleoyl-CoA altered the permeability of rat liver microsomes, as reflected by the loss of latent mannose-6-phosphatase activity (Polokoff & Bell, 1978; Jamdar, 1979; Lichtenstein & Brecher, 1980). To determine if analogous effects could be demonstrated for another biological membrane, we investigated the effect of oleoyl-CoA on sealed, right-side-out human red blood cell ghosts by monitoring the latency of an enzyme located on the inner surface of the membranes. Figure 1 shows the effect of oleoyl-CoA on the activity of NADH–cytochrome *c* oxidoreductase. As depicted in Figure 1A, activity was low in the untreated (control) ghosts, indicative of sealed, right-side-out ghosts that are not permeable to either cytochrome *c* or NADH. Addition of saponin, an agent known to interact with cholesterol in the membranes and to produce "leaky" ghosts, resulted in increased enzymatic activity. Oleoyl-CoA (160 μM) also increased the permeability of the ghosts since latent oxidoreductase activity was expressed. This observation was specific for oleoyl-CoA since neither acetyl-CoA nor oleic acid decreased latency at equivalent concentrations to that of oleoyl-CoA (data not shown). As shown in Figure 1B, the

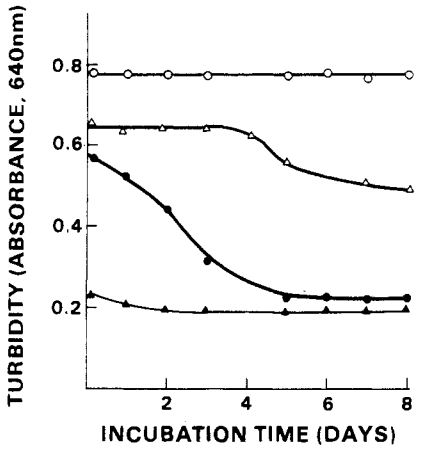

FIGURE 2: Effect of oleoyl-CoA on the turbidity of lecithin dispersions. Egg yolk lecithin dispersions were prepared by dissolving 6 mg of the phospholipid in chloroform–methanol (2:1 v/v), drying under a stream of nitrogen, pumping under vacuum, and resuspending the lipid in 2 mL of 0.02% sodium azide. Oleoyl-CoA, when added, was present at a final concentration of 27.7 μM (molar ratio of lecithin:oleoyl-CoA 137:1). Liposomes without oleoyl-CoA (○); oleoyl-CoA added directly to the liposomes subsequent to resuspension (△); oleoyl-CoA included in the aqueous solution used to resuspend the lecithin (●); oleoyl-CoA codried with the lecithin prior to resuspension in the aqueous solution (▲).

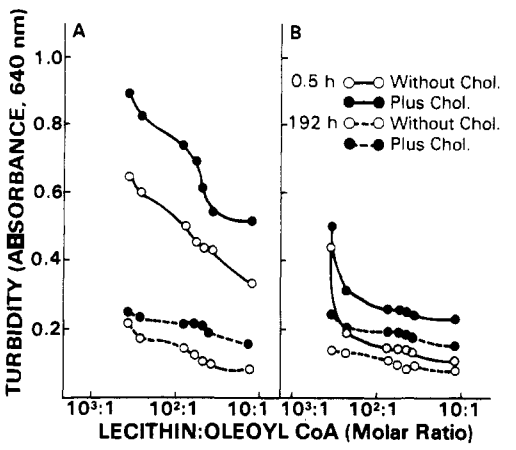

FIGURE 3: Effect of oleoyl-CoA concentration on the turbidity of lecithin dispersions prepared in the absence or presence of cholesterol. Lecithin dispersions without cholesterol were prepared as described in the legend to Figure 2 (also see Experimental Procedures). Preparations containing cholesterol (lecithin:cholesterol molar ratio 2:1) were prepared similarly except that the appropriate amount of cholesterol was dissolved with the lecithin in the organic solvent prior to evaporation under nitrogen. All samples contained 3.8 mM lecithin and when no oleoyl-CoA was present had an average absorbance of 0.95 at 640 nm. (A) The designated amount of oleoyl-CoA was included in the aqueous solution used to resuspend the dried lipids. (B) Oleoyl-CoA was codried with the lecithin prior to resuspension in the aqueous solution.

ability of oleoyl-CoA to affect latency was dependent on the ratio of membrane protein to oleoyl-CoA and the oleoyl-CoA concentration within the range investigated. In order to establish that the loss of latency was not attributable to detergent effects of oleic acid or lysolecithin inadvertently generated in the system, we established in separate experiments that labeled [1-^{14}C]oleoyl-CoA and dipalmitoylphosphatidyl[*methyl*-^{3}H]-choline were not degraded enzymatically by the ghosts during the 50-min incubation time used for the assay.

The data suggest that the loss of latency for NADH–cytochrome *c* oxidoreductase activity caused by oleoyl-CoA can be attributed to increased permeability of the ghosts to the substrate so that contact with the enzyme, located on the inner surface of the ghosts, can be made. These data extend our previous studies, reporting an analogous effect of oleoyl-CoA on the latency of mannose-6-phosphatase, located on the luminal surface of sealed microsomal vesicles from rat liver (Lichtenstein & Brecher, 1980). It is interesting to note that oleoyl-CoA produced similar effects in membrane systems with different cholesterol:phospholipid molar ratios (microsomes, 0.2; red cell ghosts, 0.8).

Effect of Oleoyl-CoA Added to Lecithin Dispersions. A possible explanation for the effects of oleoyl-CoA on membrane-associated enzymes could involve a direct interaction with either the lipid or protein components. Although numerous studies have shown interactions of long-chain acyl-CoA molecules with proteins (mostly soluble enzymes), there is very little information on acyl-CoA–phospholipid interactions. Therefore, we performed studies to determine the effect of oleoyl-CoA on several model systems containing phospholipid bilayers.

The effect of oleoyl-CoA on the turbidity of egg yolk phosphatidylcholine dispersions is shown in Figure 2. The molar ratio of lecithin to oleoyl-CoA was approximately 137:1, and the actual concentration of phosphatidylcholine was 3.8 mM. At that concentration, the turbidity of the suspension could be measured directly in a spectrophotometer at an absorbancy of 640 nm. When the lecithin suspension was prepared in the absence of oleoyl-CoA, the turbidity remained essentially unchanged, even after 8 days of gentle agitation at ambient temperature (22–25 °C). Addition of oleoyl-CoA to the lecithin caused a decrease in turbidity, but the rate at which this decrease occurred was dependent on the procedure by which oleoyl-CoA was added. When oleoyl-CoA was added subsequent to the resuspension of the lecithin, there was a gradual decrease in turbidity with time. If the oleoyl-CoA was present in the aqueous solution used to resuspend the dried lecithin, the decrease in turbidity was more rapid, and changes in turbidity were not seen after the fifth day. If a comparable amount of oleoyl-CoA was mixed with the phospholipid and both lipids were dried together prior to resuspension in the aqueous solution, a rapid decrease in turbidity was observed within 5 min of resuspension, followed by a slight but detectable decrease during the next 2 days. The greater effectiveness of oleoyl-CoA in decreasing turbidity when it was codried with the phospholipid prior to resuspension in water was also observed when the absolute concentration of both lipids was increased or decreased 5-fold but the molar ratios were maintained at 137:1 (data not shown).

The influence of oleoyl-CoA concentration on the turbidity of lecithin dispersions prepared in the absence or presence of cholesterol (molar ratio 2:1) is shown in Figure 3. When measurements were made 0.5 h after oleoyl-CoA was added, turbidity was decreased in proportion to the amount of oleoyl-CoA present, regardless of the mode of addition. Consistent with the data in Figure 2, when oleoyl-CoA was codried with the phospholipid prior to resuspension, the decreased turbidity observed at the early time interval was more pronounced than if oleoyl-CoA was added after the lecithin was resuspended. When cholesterol was present with the lecithin, turbidity also was decreased in the presence of oleoyl-CoA, but the decrease was consistently less than that observed for the preparations lacking cholesterol. After 8 days of incubation, differences in turbidity caused by the mode of addition of oleoyl-CoA were not marked, but cholesterol-containing preparations still were slightly more turbid than those lacking cholesterol.

To assess the extent of hydrolysis of either oleoyl-CoA or phosphatidylcholine during the incubation, we prepared mixtures of oleoyl-CoA and phosphatidylcholine containing [1-

^{14}C]oleoyl-CoA and dipalmitoylphosphatidyl[*methyl*-^{3}H]-choline, and the amount of [^{14}C]oleic acid or [*methyl*-^{3}H]-lysolecithin was determined by thin-layer chromatography of lipid extracts obtained from aliquots of reaction mixtures at several time intervals. No detectable breakdown of either oleoyl-CoA or lecithin was observed after 2 h of incubation, under conditions where appreciable decreases in turbidity could be measured. After 5 days of incubation using several different concentrations of both lipids, breakdown of oleoyl-CoA to oleic acid was less than 2%, and formation of lysolecithin from lecithin was less than 8%. Additionally, when either oleic acid (1–100 μM) or lysolecithin (1–500 μM) was dried with the phosphatidylcholine (3.8 mM) and resuspended as described, no measurable decrease in turbidity was observed after a 6-day period. The above experiments indicated the minor degradation of oleoyl-CoA or lecithin did not contribute significantly to the decrease in turbidity caused by oleoyl-CoA.

The effects of oleoyl-CoA on lecithin dispersions are analogous to those reported for bile salt–lecithin systems. When egg yolk lecithin and bile salts are dried together in the appropriate proportions, clear solutions occur almost immediately following hydration (Small et al., 1966; Small, 1971). However, if a micellar solution of sodium cholate is added to a suspension of multilamellar egg yolk lecithin liposomes, the clearing takes several hours. Presumably the reason for this is that the bile salt must penetrate the lecithin bilayer and peel layers of lecithin off into bimolecular aggregates. In contrast, if the bile salt is incorporated into the dried system, then water penetrates easily, forming small bimolecular aggregates and a "clear" micellar solution (Small, 1971).

By analogy, when an aqueous solution of oleoyl-CoA is added to a suspension of multilamellar liposomes, the conversion of these large particles (which appear as a turbid suspension) to small, presumably unilamellar vesicles depends on the ability of oleoyl-CoA to penetrate and gradually disrupt the outermost leaflets of the liposomes, a process that takes days to accomplish. If an aqueous solution of oleoyl-CoA is added to dried egg yolk lecithin, which is predominantly in the $L_{\beta'}$ form (Tardieu et al., 1973; Loomis et al., 1974), then both swelling of lecithin and penentration of oleoyl-CoA into the outer leaflet interfacing with the aqueous system occur simultaneously. The liposomes formed by this process are presumably smaller than those formed by lecithin alone, as indicated by the lesser turbidity at the beginning of the experiment as shown in Figure 2. However, the process is probably similar to disruption of pure liposomes and takes several days to complete. When oleoyl-CoA is dried together with lecithin, it is probably dispersed in the dried lecithin. Although the exact lattice of this dry mixture is not known, the oleoyl-CoA is presumably incorporated into the bilayered $L_{\beta'}$ structure. The large and highly charged polar groups may allow water to penetrate rapidly (as in the case of the codried bile salt–lecithin system), which produces small particles having low turbidity.

Additional studies were performed to characterize the particles formed when oleoyl-CoA was codried with lecithin. Figure 4 shows the elution profile of a codried mixture containing [^{3}H]lecithin and [1-^{14}C]oleoyl-CoA that was mixed with water 1 h prior to application onto the Bio-Gel A 15-m column. The mixture was clear before chromatography, and the molar ratio of phospholipid to oleoyl-CoA was 12:1, respectively. Lecithin eluted in two distinct peaks: the larger peak (83% of the lecithin) corresponded to the void volume, and the smaller peak eluted at a position corresponding in size to a particle somewhat smaller than unilamellar lecithin

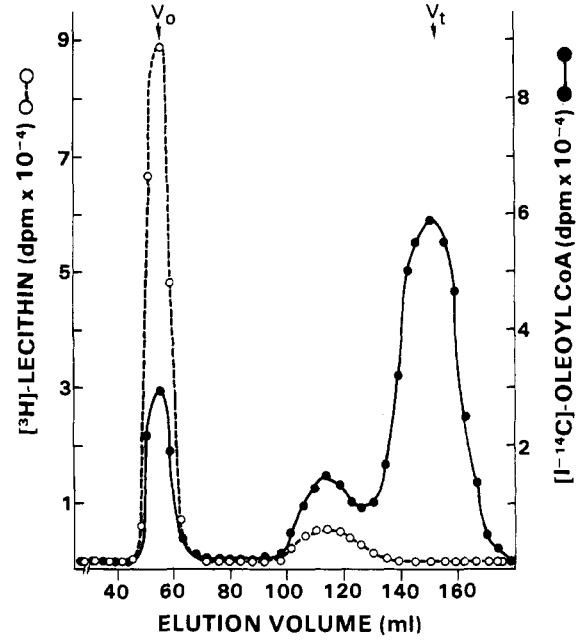

FIGURE 4: Bio-Gel A 15-m chromatography of a lecithin–oleoyl-CoA dispersion (lecithin:oleoyl-CoA molar ratio 12:1). Samples contained 3.43 mM egg yolk lecithin, [2-*palmitoyl*-9,10-^{3}H]dipalmitoylphosphatidylcholine (0.66 μCi/mL), and [1-^{14}C]oleoyl-CoA (0.25 μCi/mL). Lipids were codried prior to resuspension in the elution buffer and were gently shaken for 1 h prior to gel filtration.

vesicles prepared by sonication. Some oleoyl-CoA coeluted with each of the lecithin peaks, but most oleoyl-CoA (67%) eluted at the total volume. Oleoyl-CoA alone, when applied to the column, eluted as a single peak at the total volume. Recovery of labeled lecithin and oleoyl-CoA after gel filtration was 79% and 85%, respectively. These recovery values were representative of several other experiments performed in a similar manner. The molar ratio of lecithin to oleoyl-CoA in the peaks corresponding to the void volume and the fraction eluting at 115 mL was 83:1 and 13:1, respectively.

The data in Figure 4 suggest that several particles of different size are formed from the codried mixtures. It is important to note that if phospholipid dispersions not containing oleoyl-CoA (multilamellar liposomes) are applied to the column, the lecithin absorbs to the gel and does not elute at all. Therefore, in order to focus on the effects of oleoyl-CoA on a single, well-characterized population of particles, we initiated experiments with unilamellar vesicles prepared by sonication.

Effect of Oleoyl-CoA on Unilamellar Lecithin Vesicles. Leakage of 5,6-CF from unilamellar vesicles has been used to demonstrate the relative stability or permeability of these particles in the presence of various perturbing agents (Guo et al., 1980; Kirby et al., 1980). Vesicles containing 5,6-CF were prepared by sonication in either the absence or presence of cholesterol. Figure 5A shows the rate of 5,6-CF release from both types of vesicles incubated with or without 320 μM oleoyl-CoA. Leakage of dye occurred in the absence of oleoyl-CoA and was more pronounced in vesicles prepared without cholesterol. Addition of oleoyl-CoA increased the leakage rate in both vesicle preparations, but the cholesterol-containing vesicles clearly were more resistant to the effect of oleoyl-CoA, since 5,6-CF release occurred at a slower rate.

The influence of different concentrations of oleoyl-CoA on release of 5,6-CF from vesicles prepared with or without cholesterol is shown in parts B (30 min after addition) and C (6 h after addition) of Figure 5. The data in parts B and

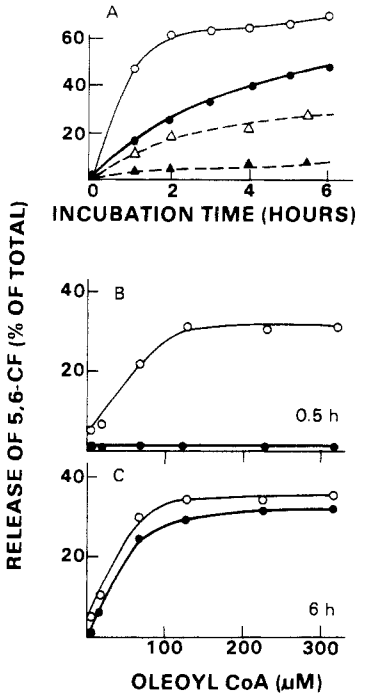

FIGURE 5: Effect of oleoyl-CoA on the release of 5,6-CF from unilamellar vesicles prepared in the absence and presence of cholesterol. Vesicles were formed by sonication as described under Experimental Procedures and were diluted prior to the addition of oleoyl-CoA so that the total amount of 5,6-CF in each cuvette would not exceed 100 μM when fluorescence was measured at excitation maximum and emission maximum of 490 and 520 nm, respectively. This resulted in a final lecithin concentration of 84.7 μM. Unilamellar vesicles containing lecithin alone (○; △); vesicles containing lecithin:cholesterol in a molar ratio of 2:1 (●; ▲). (A) Time course of the leakage of 5,6-CF from vesicles incubated in the absence of oleoyl-CoA (△; ▲); vesicles incubated in the presence of 320 μM oleoyl-CoA (○; ●). (B) Effect of oleoyl-CoA on the release of 5,6-CF 30 min after addition of oleoyl-CoA. (C) Effect of oleoyl-CoA on release of 5,6-CF 6 h after addition of oleoyl-CoA.

C of Figure 5 are corrected for leakage occurring in the absence of oleoyl-CoA with paired controls. After a 30-min incubation, clear-cut differences were observed between the two vesicle preparations. Leakage of 5,6-CF from the lecithin vesicles was produced by oleoyl-CoA in a concentration-dependent manner up to 120 μM, and at higher concentrations no additional leakage was observed. In contrast, no detectable leakage of 5,6-CF due to oleoyl-CoA occurred from the cholesterol-containing vesicles.

Following a 6-h incubation period, leakage occurred from both vesicle preparations at all concentrations of oleoyl-CoA tested, but values for the cholesterol-containing vesicles were consistently 80–90% of those measured for vesicles prepared without cholesterol. Since leakage in the presence of a relatively high concentration of oleoyl-CoA was not complete, at least when compared to dye release in the presence of Triton X-100, it was possible that oleoyl-CoA mediated its effect by interacting with, but not disrupting, the vesicles.

Complex Formation of Oleoyl-CoA with Unilamellar Lecithin Vesicles. To determine directly if complexes between oleoyl-CoA and unilamellar vesicles could be demonstrated, we prepared cosonicated mixtures of oleoyl-CoA and lecithin and characterized them by gel filtration and sucrose gradient ultracentrifugation.

Figure 6A shows the Sepharose 4B elution profile of a suspension containing lecithin (12.7 mM) and oleoyl-CoA (127 μM) that was previously sonicated to form unilamellar vesicles. Both lipids were present during sonication, and tracer amounts of dipalmitoylphosphatidyl[*methyl*-^{3}H]choline and [1-^{14}C]-

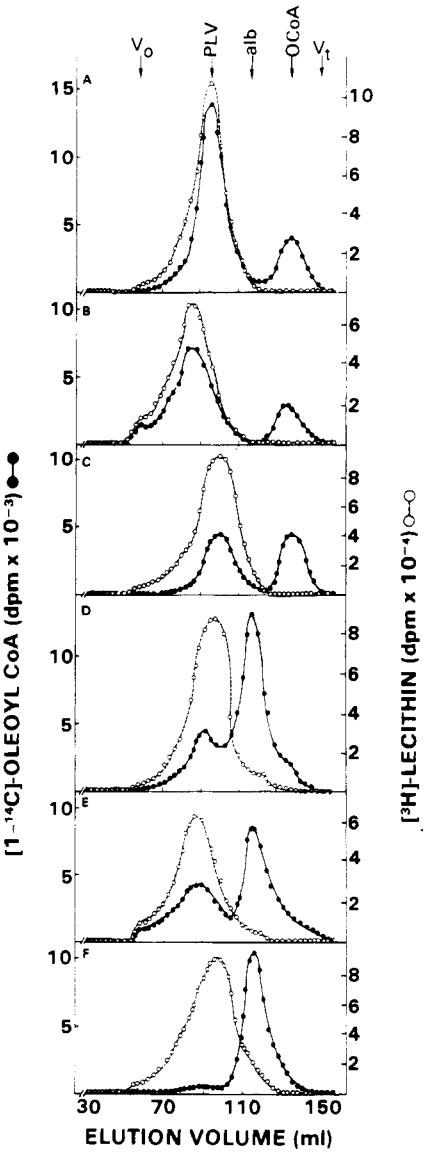

FIGURE 6: Sepharose 4B chromatography of mixtures containing unilamellar vesicles and oleoyl-CoA. Vesicles were prepared with or without cholesterol, and oleoyl-CoA was added prior to sonication. All samples contained 12.7 mM egg yolk lecithin, dipalmitoyl-[*methyl*-^{3}*H*]choline (0.23–0.24 μCi/mL) and [1-^{14}C]oleoyl-CoA (0.054–0.063 μCi/mL). 1-mL aliquots of the sonicated mixtures of lecithin, oleoyl-CoA, or cholesterol, containing the designated molar ratios of each component, were incubated for 1 h in the absence or presence of bovine serum albumin. When albumin was included, it was added in equimolar amounts to that of oleoyl-CoA. Following incubation, 0.5-mL aliquots were subjected to gel filtration. (A) Lecithin:oleoyl-CoA molar ratio 100:1; (B) lecithin:oleoyl-CoA:cholesterol molar ratio 100:1:50; (C) lecithin:oleoyl-CoA molar ratio 10:1; (D) lecithin:oleoyl-CoA:albumin molar ratio 100:1:1; (E) lecithin:oleoyl-CoA:cholesterol:albumin molar ratio 100:1:50:1; (F) lecithin:oleoyl-CoA:albumin molar ratio 10:1:1.

oleoyl-CoA were included. The labeled lecithin eluted as a single peak approximately midway between the void and total volume of the column, which corresponded exactly to the position where unilamellar vesicles prepared in the absence of oleoyl-CoA normally eluted. Approximately 80% of the labeled oleoyl-CoA coeluted with the vesicles, and the remainder eluted slightly before the total volume. Calculated from the specific activities in the peak vesicle region, the molar ratio of lecithin to oleoyl-CoA averaged 137:1. When solutions containing oleoyl-CoA alone (4 or 120 μM) were chromatographed, a single peak, also eluting slightly before the total volume, was observed. Including 4 μM oleoyl-CoA in the

elution buffer did not alter the elution profile of oleoyl-CoA.

The elution profile of a sonicated mixture of lecithin–cholesterol–oleoyl-CoA (molar ratio 100:50:1; lecithin concentration 12.7 mM) is shown in Figure 6B. The lecithin eluted as a larger particle than vesicles that lacked cholesterol, and the peak was broader, suggesting a more heterogeneous particle population. The molar ratio of lecithin:oleoyl-CoA at the peak was 142:1. The labeled oleoyl-CoA eluted predominantly with the cholesterol-containing vesicles, although about 20% of the total oleoyl-CoA again appeared slightly before the total volume.

The elution profile of a sonicated mixture of lecithin (12.7 mM) and oleoyl-CoA at a molar ratio of 10:1 is shown in Figure 6C. All the lecithin eluted as vesicles, but the oleoyl-CoA was about equally distributed between the vesicle region and a peak appearing slightly before the total volume. The molar ratio of lecithin to oleoyl-CoA in the peak region averaged 13:1.

When albumin was added to each of the sonicated preparations described above in equimolar amounts relative to the oleoyl-CoA, incubated for 1 h, and subsequently analyzed by Sepharose 4B chromatography (Figure 6D–F), between 70% and 90% of the labeled oleoyl-CoA was associated with the albumin, whereas the elution profile of the lecithin was essentially unchanged from that of the comparable experiments shown in Figure 6A–C, respectively. The data indicate that oleoyl-CoA was associated with lecithin vesicles after sonication, but this association was affected by the addition of albumin, which is known to bind equimolar amounts of oleoyl-CoA (Lamb & Fallon, 1972; Lichtenstein & Brecher, 1980). Extending the preincubation period of the lecithin–oleoyl-CoA mixtures with the albumin did not alter the elution patterns (data not shown).

The sonicated mixtures of lecithin and oleoyl-CoA were further characterized by sucrose density gradient ultracentrifugation. When lecithin (12.7 mM) and oleoyl-CoA (molar ratio 100:1) were applied onto 8–23% sucrose gradients, both labeled lipids remained at the top of the gradient (Figure 7A). If albumin was added to the mixture in amounts equimolar to the oleoyl-CoA present and then subjected to sucrose gradient ultracentrifugation, approximately 70% of the oleoyl-CoA cosedimented with the albumin, whereas the lecithin again remained at the top (Figure 7C). This was consistent with the observations shown in Figure 6D, indicating that albumin could remove oleoyl-CoA from the vesicles.

Of particular interest was the sedimentation profile of sonicated mixtures of lecithin (12.7 mM) and oleoyl-CoA at a molar ratio of 10:1 (Figure 7B). In this case, both the lecithin and oleoyl-CoA migrated into the gradient, indicating the presence of a particle more dense than that existing when the molar ratio was 100:1. If albumin was added to this mixture, oleoyl-CoA again sedimented with albumin, whereas the lecithin was localized at the top of the gradient (Figure 7D). Sedimentation profiles almost identical with those shown in Figure 7 were observed when lecithin vesicles were prepared containing cholesterol (33 mol %).

The complex formed from a mixture containing a lecithin:oleoyl-CoA molar ratio of 10:1 sedimented into the gradient at a position corresponding to a sucrose concentration of about 16%, and the average molar ratio of lecithin and oleoyl-CoA in this complex was 13:1. In additional experiments, we established that this complex did not sediment further when the centrifugation time was extended to 24 h, suggesting that sedimentation equilibrium was achieved. Thus, the buoyant density of the hydrated, solvated lecithin–oleoyl-CoA complex has the density of 16% sucrose, that is, 1.063 g/mL. Taking the anhydrous density of egg yolk lecithin at 1.015 g/mL (Elworthy, 1959; Small, 1967) and the appropriate weight fraction of lecithin and oleoyl-CoA in a complex with a molar ratio of 13:1, one can calculate an anhydrous density for oleoyl-CoA of about 1.46 g/mL. This is in reasonable agreement with an independent calculation of the anhydrous density of oleoyl-CoA based on the densities of its components (1.41 g/mL).

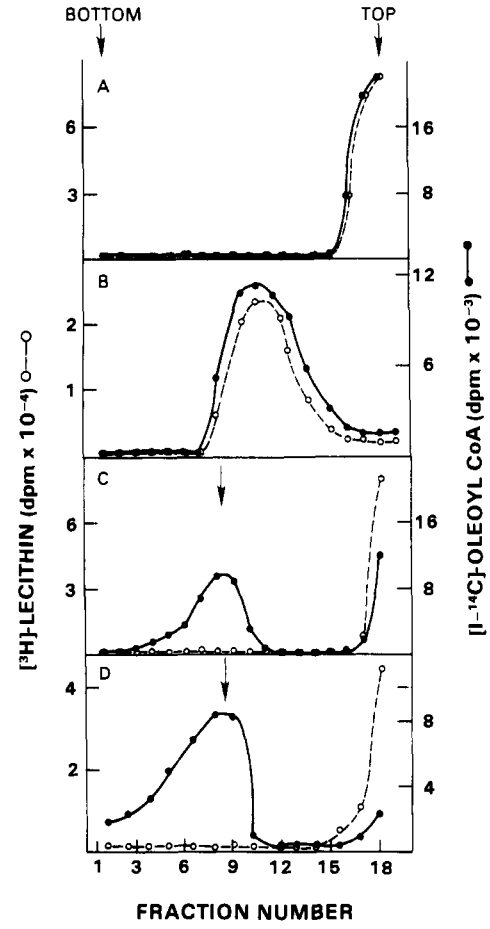

FIGURE 7: Sucrose gradient ultracentrifugation of mixtures containing unilamellar vesicles and oleoyl-CoA. Double-labeled samples of lecithin and oleoyl-CoA were prepared exactly as outlined in the legend of Figure 6. Aliquots (0.2 mL) of the designated mixture were applied to the top of an 8–23% continuous sucrose gradient, spun at 310000*g* for 16 h at 4 °C, and analyzed as described under Experimental Procedures. (A) Lecithin:oleoyl-CoA molar ratio 100:1; (B) lecithin:oleoyl-CoA molar ratio 10:1; (C) lecithin:oleoyl-CoA:albumin molar ratio 100:1:1; (D) lecithin:oleoyl-CoA:albumin molar ratio 10:1:1.

Additional experiments employing 5,6-CF were performed with vesicles containing both oleoyl-CoA and lecithin. Following sonication of a codried dispersion of lecithin and oleoyl-CoA (molar ratio 10:1) in the presence of 0.25 M 5,6-CF and subsequent passage through Sephadex G-50 columns, much of the dye had leaked out of the vesicle. Furthermore, storage overnight in dialysis tubing at 2 °C also resulted in significant additional leakage. These findings were consistent with increased vesicle permeability when oleoyl-CoA was contained in the vesicles.

Our data indicated that the complex was stable during the relatively gentle procedures involved in gel filtration and ultracentrifugation. Although the precise orientation of oleoyl-CoA and vesicle cannot be determined by the techniques used in this study, it seems unlikely that the large and highly charged polar nucleotide would be localized within the bilayer.

We would expect that the acyl chain would be incorporated within the bilayer and the remainder of the molecule would be exposed to the aqueous space. In the situations in which aqueous oleoyl-CoA is added to either an aqueous suspension of multilamellar vesicles or dried egg yolk lecithin, it is reasonable to assume that most of the oleoyl-CoA will be present on the outside leaflet of the unilamellar vesicle, since its presence on the inside would require either total disruption of the bilayer or a flip-flop mechanism through the bilayer, which seems a rather unlikely situation with such a large polar group. However, when water is added to a codried mixture of lecithin and oleoyl-CoA, the possibilities of having oleoyl-CoA within the aqueous space of the vesicle cannot be logically ruled out.

The addition of albumin to unilamellar vesicles at a molar ratio equivalent to that of oleoyl-CoA effectively removed oleoyl-CoA from the vesicle and resulted in an oleoyl-CoA–albumin complex. Under these conditions the addition of albumin did not disrupt the vesicles since the phospholipid remained in a particle having the size and density of the vesicles. Thus, albumin either easily removed the oleoyl-CoA from the outside of the vesicle or removed the oleoyl-CoA and then allowed vesicles to re-form. We think the most reasonable orientation for oleoyl-CoA in these systems is on the outside of the vesicle.

Addition of cholesterol to multilamellar bilayer systems has been shown to alter the properties of the system by several criteria, including resistance to disruption by detergents and decreased permeability to glucose (Demel et al., 1968), ions (Scarpa & de Gier, 1971), and dye (Guo et al., 1980). Our studies show that inclusion of cholesterol with lecithin at a molar ratio of 0.5:1 decreased the rate at which oleoyl-CoA disrupted multilamellar liposomes and reduced the effect of oleoyl-CoA on leakage of dye from unilamellar vesicles. Vesicles containing cholesterol could be characterized by gel filtration and sucrose density gradient ultracentrifugation before and after incubation with oleoyl-CoA, and the data indicated that oleoyl-CoA did associate with the vesicles in a manner similar to that observed with vesicles lacking cholesterol. Thus, the apparent stabilizing effect of cholesterol is not caused by reducing the binding of oleoyl-CoA. In contrast, other studies have shown that apoprotein binding to vesicles is reduced by incorporation of cholesterol (Pownall et al., 1979; Tall & Lang, 1976).

In this study, we demonstrated an effect of oleoyl-CoA on a cellular membrane enriched in cholesterol. The loss of latency of NADH–cytochrome *c* oxidoreductase activity produced by oleoyl-CoA addition to sealed right-side-out human red blood cell ghosts can be attributed to increased permeability of the ghosts to the substrate so that contact with the enzyme, located on the inner surface of the ghost, can be made. In a previous study, we reported an analogous effect of oleoyl-CoA on the latency of mannose-6-phosphatase activity, located on the luminal (inner) surface of microsomal vesicles from rat liver (Lichtenstein & Brecher, 1980). The presence of protein and varied phospholipid composition clearly distinguishes these biological membranes from the model systems, yet it is interesting to note that oleoyl-CoA produced similar effects in membrane systems with different cholesterol:phospholipid molar ratios (microsomes, 0.2; red ghost cells, 0.8).

Recently, it was suggested that the properties of cardiac cells can be altered by the intracellular accumulation of polar lipids such as long-chain acyl-CoA, and such effects may occur during coronary artery occlusion (Katz & Messineo, 1981). Intracellular concentrations of long-chain acyl-CoA were reported to vary between 110 and 152 μM in rat liver (Brunengraber et al., 1978). It is not clear if intracellular acyl-CoA exists transiently as monomers, as micelles, bound to soluble proteins, or associated with membranes. The possibility that localized effects of acyl-CoA derivatives on intracellular membranes can modify membrane function, independent of direct effects on soluble or membrane-bound enzymes, remains to be established.

Acknowledgments

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References

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–468.
Bell, R. M., Ballas, L. M., & Coleman, R. A. (1981) *J. Lipid Res. 22*, 391–403.
Brecher, P. I., Pyun, H., & Chobanian, A. V. (1977) *J. Lipid Res. 18*, 154–163.
Brunengraber, H., Boutry, M., & Lowenstein, J. M. (1978) *Eur. J. Biochem. 82*, 375–384.
Demel, R. A., Kinsky, S. C., Kinsky, C. B., & van Deenen, L. L. (1968) *Biochim. Biophys. Acta 150*, 655–665.
Edgar, J. R., & Bell, R. M. (1979) *J. Biol. Chem. 254*, 1016–1021.
Elworthy, P. H. (1959) *J. Chem. Soc.*, 1951–1956.
Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957) *J. Biol. Chem. 226*, 497–509.
Guo, L. S., Hamilton, R. L., Goerke, J., Weinstein, J. N., & Havel, R. J. (1980) *J. Lipid Res. 21*, 993–1003.
Hanahan, D. J., & Ekholm, J. E. (1974) *Methods Enzymol. 31*, 168–172.
Hsu, K. L., & Powell, G. L. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4729–4733.
Jamdar, S. C. (1979) *Arch. Biochem. Biophys. 195*, 81–94.
Katz, A. M., & Messineo, F. C. (1981) *Circ. Res. 48*, 1–16.
Kawaguchi, A., & Bloch, K. (1974) *J. Biol. Chem. 249*, 5703–5800.
Kawaguchi, A., & Bloch, K. (1976) *J. Biol. Chem. 251*, 1406–1412.
Kirby, C., Clarke, J., & Gregoriados, G. (1980) *Biochem. J. 186*, 591–598.
Lamb, R. G., & Fallon, H. J. (1972) *J. Biol. Chem. 247*, 1281–1287.
Lichtenstein, A. H., & Brecher, P. (1980) *J. Biol. Chem. 255*, 9098–9104.
Loomis, C. R., Janiak, M. J., Small, D. M., & Shipley, G. G. (1974) *J. Mol. Biol. 86*, 309–324.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randal, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Mishkin, S., & Turcotte, R. (1974) *Biochem. Biophys. Res. Commun. 57*, 918–926.
Polokoff, M. A., & Bell, R. M. (1978) *J. Biol. Chem. 253*, 7173–7178.
Powell, G. L., Zimmerman, J. K., & Fish, W. W. (1981) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 40*, 1590A.
Pownall, H. J., Massey, J. B., Kusserow, S. K., & Gotto, A. M. (1979) *Biochemistry 18*, 574–579.
Rudel, L. L., & Morris, M. D. (1973) *J. Lipid Res. 14*, 364–366.
Scarpa, A., & de Gier, J. (1971) *Biochim. Biophys. Acta 241*, 789–797.
Small, D. M. (1967) *J. Lipid Res. 8*, 551–557.
Small, D. M. (1971) in *Physical Chemistry of Cholanic Acids in the Bile Acids* (Nair, P. P., & Kritchevsky, D., Eds.) Vol.

1, pp 247–354, Plenum Press, New York.
Small, D. M., Bourges, M. C., & Dervichian, D. Y. (1966) *Biochim. Biophys. Acta 125*, 563–580.
Steck, T. L., & Kant, J. A. (1974) *Methods Enzymol. 31*, 177–178.
Szoka, F. C., Jacobson, K., & Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta 551*, 295–303.
Tall, A. R., & Lang, Y. (1976) *Biochem. Biophys. Res. Commun. 80*, 206–212.
Tardieu, A., Luzzati, V., & Raman, F. C. (1973) *J. Mol. Biol. 75*, 711–733.
Weinstein, J. N., Yoshikami, S., Henkart, P., Blumenthal, R., & Hagins, W. A. (1977) *Science (Washington, D.C.) 195*, 489–490.
Wititsuwannakul, D., & Kim, K. (1977) *J. Biol. Chem. 252*, 7812–7817.
Zahler, W. L., Barden, R. E., & Cleland, W. W. (1968) *Biochim. Biophys. Acta 164*, 1–11.

Cholesterol Biosynthesis and Modulation of Membrane Cholesterol and Lipid Dynamics in Rat Intestinal Microvillus Membranes†

Thomas A. Brasitus and David Schachter*

ABSTRACT: Experiments were performed to test the hypothesis that cholesterol biosynthesis in the rat ileal enterocyte, the major absorptive cell lining the distal epithelium of the small intestine, can modulate the cholesterol content and the motional freedom of the plasma membrane lipids. Decreased sterol biosynthesis in vivo was elicited by feeding sodium taurocholate or by fasting the rats, whereas increased synthesis was induced by biliary ligation or feeding cholestyramine, a bile salt binding resin; these effects were monitored by assay of mucosal 3-hydroxy-3-methylglutaryl coenzyme A reductase. After each procedure, isolated microvillus membranes were examined to determine the lipid composition and the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene. The results demonstrate that variations in cholesterol biosynthesis in vivo can modulate the cholesterol content and the motional freedom of the lipids of the microvillus membrane; similar effects were not observed on the basolateral membrane. The observations suggest that the normal pattern of decreased lipid motional freedom in microvillus membranes of the distal as compared to the proximal small intestine of the rat results from higher rates of cholesterol biosynthesis in the distal mucosa.

There is considerable evidence that many functions of biological membranes are influenced by the composition and physical state of the membrane lipids (Lee, 1975; Melchior & Steim, 1976; Sandermann, 1978). The functional significance of the lipids is further indicated by observations that membranes which differ in function also differ in lipid composition and lipid motional freedom or "fluidity".[1] Such differentiation is illustrated clearly by comparisons of the luminal (microvillus) and contraluminal (basolateral) portions of the plasma membrane of the rat enterocyte, the major absorptive cell of the small intestinal mucosa. These antipodal membranes, which regulate the exchange of substances between organism and environment, differ in ultrastructure (Bloom & Fawcett, 1968; Oda, 1976), enzyme and transport activities (Douglas et al., 1972; Lewis et al., 1975; Murer et al., 1974, 1976), electrophysiological properties (Rose & Schultz, 1971; Okada et al., 1977), and protein components (Fujita et al., 1973). Correspondingly, the membranes differ in lipid composition (Forstner et al., 1968; Douglas et al., 1972; Kawai et al., 1974; Lewis et al., 1975; Brasitus & Schachter, 1980), and lipid molecules of the basolateral as compared to the microvillus membrane have considerably greater motional freedom (Brasitus et al., 1980; Brasitus & Schachter, 1980; Gray et al., 1981).

Although the evidence suggests the existence of regulatory mechanisms which maintain the lipid composition and fluidity characteristic of specific membrane organelles, the precise nature of these mechanisms is largely unknown. Accordingly, the present studies were initiated to examine the role of membrane cholesterol, a component which decreases the motional freedom of bilayer lipids above their transition temperatures (Oldfield & Chapman, 1971). Specifically, we explored the hypothesis that the cholesterol content of rat enterocyte plasma membranes is modulated by the rate of cholesterol biosynthesis in the cell. Several lines of evidence point to this possibility. Studies of the steady-state fluorescence polarization of lipid-soluble fluorophores indicate that the lipid fluidity of rat microvillus membranes is least in the distal (ileal) segment of the intestine and increases in the proximal portion (Schachter et al., 1976; Schachter & Shinitzky, 1977). Corresponding to this distribution, it was found that both the rate of incorporation of precursors into cholesterol and the

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[1] The term "lipid fluidity" as applied to model bilayers and natural membranes is used throughout this report to express the relative motional freedom of the lipid molecules or substituents thereof. When assessed by the estimation of steady-state fluorescence anisotropy of the fluorophore 1,6-diphenyl-1,3,5-hexatriene, changes in the fluorescence anisotropy may be due to alterations in the correlation time and/or maximal hindered anisotropy of the probe. As described previously (Brasitus & Schachter, 1980), we use the terms "lipid fluidity" or "motional freedom" to designate both kinds of alterations.

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