Synthesis of molecular species of glycerophospholipids from diglyceride-labeled brain microsomes

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Abstract Selectivity of CDP-choline: diacylglycerol choline phosphotransferase and CDP-ethanolamine: diacylglycerol ethanolamine phosphotransferase for molecular species of diglyceride has been studied in rat brain microsomes in vitro. Diglyceride-labeled microsomes were prepared by incubation with labeled *sn*-glycerol-3-phosphate; the microsomes were then incubated with CDP-choline or CDP-ethanolamine for different time intervals. Experimental data extrapolated to zero-time incubation were taken into account for evaluating species specificity. A small selectivity for diglyceride species has been demonstrated for the choline phosphotransferase, but the ethanolamine phosphotransferase was found to convert hexaenoic diglyceride into phospholipid at the highest rate. — **Roberti, R., L. Binaglia, and G. Porcellati.** Synthesis of molecular species of glycerophospholipids from diglyceride-labeled brain microsomes. *J. Lipid Res.* 1980. **21:** 449–454.

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Glycerolipid synthesis in animal tissue is regulated at different levels of its metabolic sequences. In this connection, the glycerol phosphate acyl transferase is rate-limiting in phosphatidate synthesis (1), although the rate of the reactions catalyzed by the liver microsomal phosphatidate phosphohydrolase (E.C. 3.1.3.4) and the brain cytidylyltransferase activities (E.C. 2.7.7.14 and E.C. 2.7.7.15) in vitro are the lowest in the overall synthetic pathway (2, 3). The rate of diglyceride utilization through the diglyceride acyltransferase (E.C. 2.3.1.20), the choline and the ethanolamine phosphotransferases (E.C. 2.7.8.2 and E.C. 2.7.8.1) and the diglyceride kinase reaction seems, on the other hand, strictly related to the nature of the fatty acyl moiety composition (4–10). The work of Åkesson and Sundler (11) provides in this respect an excellent outline of the overall mechanism for glycerolipid synthesis in the hepatocyte.

Phosphoglyceride formation in the brain has also been extensively studied, and interesting relationships among diglyceride, phosphatidic acid, and phospholipid have been described both in vivo and in vitro (12–18). One of the most interesting problems which has emerged from these last studies is how the steady-state composition of brain lipids is controlled by the mechanisms involved in their metabolism. Brain phosphatidic acid, diglyceride and phospholipid show, in this connection, a quite different fatty acid composition (16–18), which cannot be simply explained by the intervention of transacylase mechanisms. Many data, on the other hand, strongly suggest the existence of metabolic lipid compartmentation that could fit with the analytical results (17, 18).

Taking into account previous results concerning the synthesis of phosphatidic acid molecular species and their dephosphorylation to diglyceride (16), the present work attempts to answer in somewhat more detail the following question: is there a species selectivity of the choline and ethanolamine phosphotransferase reactions? To answer this question, experiments have been planned in which the time course of phospholipid synthesis from endogenous lipid precursors was studied.

EXPERIMENTAL

Chemicals

ATP, CoA, unlabeled *sn*-glycerol-3-phosphate, CDP-choline and CDP-ethanolamine were purchased from Boehringer, GmbH (Mannheim, West Germany). [U-^{14}C]-*sn*-glycerol-3-phosphate was obtained from NEN Chemicals, GmbH (Frankfurt, West Germany). Other chemicals were from Merck (Darmstadt, West Germany). Solvents were glass-distilled before use; ethyl ether and chloroform were supplemented with 0.01% 2,6-di-*tert*-butyl-4-methyl-phenol, as antioxidant.

Preparation of membrane-bound diglyceride

Membrane-bound phosphatidic acid was prepared by incubating rat brain microsomes with [U-^{14}C]-*sn*-glycerol-3-phosphate, as previously described (16). The incubation mixtures (0.5 ml of final volume) contained 6 mM ATP, 0.2 mM CoA, 4 mM $MgCl_2$, 100 mM K-phosphate buffer (pH 7.0), 1.4 mM [U-^{14}C]-*sn*-glycerol-3-phosphate (sp act 10 Ci/mol), 70 mM NaF, and about 3 mg of microsomal protein/ml.

Phosphatidate-labeled microsomes (5–10 mg protein) were pelleted by centrifugation at 104,000 *g* for 1 hr at 0–4°C, the pellet was washed with 0.32 M sucrose/2 mM β-mercaptoethanol solution and resuspended in this medium. The conversion of membrane-bound phosphatidic acid into diglyceride was achieved by incubating phosphatidate-labeled microsomes in 0.32 M sucrose–2 mM β-mercaptoethanol (5 mg protein/ml) at 37°C for 30 min. Diglyceride-labeled microsomes were used for studying the species-selectivity of the choline phosphotransferase and ethanolamine phosphotransferase reactions.

Phospholipid synthesis

The microsomal suspension resulting from a 30 min incubation of phosphatidate-labeled microsomes with the membrane-bound phosphatidate phosphohydrolase was supplemented with 10 mM $MnCl_2$, and 1 mM of either CDP-choline or CDP-ethanolamine. The pH was adjusted to 8.0 by adding 50 mM Tris-HCl buffer. Each incubation mixture contained 1.8 mg of microsomal protein in a final volume of 0.5 ml. The mixtures were incubated at 37°C for different time intervals, and the reactions stopped at given times by the addition of 5 ml of chloroform–methanol 1:1 (by vol).

Microsomes were kept at 2–4°C, except during incubation.

Lipid analyses

Lipid was extracted from microsomes as previously reported (16). Procedures for preparative chromatography of phosphatidylethanolamine, phosphatidylcholine, diglyceride, triglyceride, and phosphatidic acid have been reported previously (16, 17).

Diacylglycerol and phosphatidylcholine molecular species differing in the degree of unsaturation were separated on silver nitrate-impregnated silica gel-G plates, as 1,2-diacyl-3-acetyl derivatives (16), and phosphatidylethanolamine molecular species as *N*-acetyl-*O*-methyl derivatives according to Sundler and Åkesson (19).

Automatic radiochromatoscanning, co-chromatography with authentic lipid standards, and gas–liquid chromatography of the fatty acids methyl esters were used for identification of lipid spots on the plates. Complete phospholipid recovery was achieved by repeated elutions with methanol of the silica layer which was scraped and packed in Pasteur pipettes. Neutral lipids and dimethylphosphatidate were eluted from such silica layers with chloroform–methanol 2:1 (by vol), after washing the silicic acid with 0.9% saline.

General

Protein was estimated according to Lowry et al. (20). Phospholipids were quantitated by measuring the phosphorus content according to Bartlett (21). Diglyceride glycerol was measured by a fluorimetric determination (22) on a MPF-3 model Perkin Elmer fluorescence spectrophotometer. Gas–liquid chromatography of fatty acids was done with a Carlo Erba (Milan, Italy) model GV Fractovap Instrument equipped with flame ionization detectors (17). Automatic radiochromatoscanning on a Packard model 7201 radiochromatogram scanner was used to localize labeled lipids after thin-layer chromatography. Radioactivity was measured in a model 3330 Packard Tri-Carb liquid scintillation spectrometer, as previously described (16).

RESULTS

The mass distribution of rat brain microsomal lipids has been determined. Saturated, monoenoic, dienoic plus trienoic, tetraenoic plus pentaenoic and hexaenoic molecular species represent 1.7%, 4.4%, 18.0%, 49.8%, and 26.2% of the membrane phosphatidylethanolamine, respectively (mean values from four determinations). The mass distribution of phosphatidylcholine among the molecular species was quite different: saturated, monoenoic, dienoic plus trienoic, tetraenoic, pentaenoic and hexaenoic accounting for 20.6%, 38.6%, 2.5%, 12.6%, 4.8%, and 20.9%, respectively.

If a comparison with the mass distribution of brain microsomal diglycerides is made (16), large differences are evident. Tetraenoic, pentaenoic and hexaenoic molecular species (56.6%, 11.2% and 6.6%, respectively) represent the highest fraction of membrane diglycerides; saturated, monoenoic and dienoic plus trienoics account for 14.7%, 7.2% and 3.6% of the lipid class, respectively (16).

After incubation of brain microsomes with labeled glycerol phosphate, lipid labeling was found only in phosphatidic acid. **Fig. 1** shows that no radioactivity was present in phospholipids other than phosphatidic acid, also after the phosphatidate phosphohydrolase incubation, while consistent labeling was found in diglyceride.

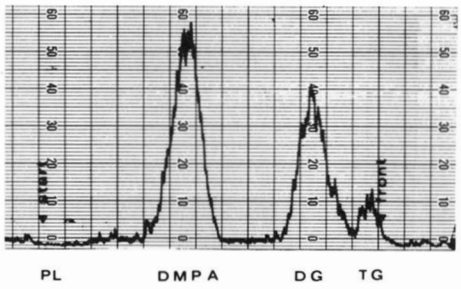

Fig. 1. Radiochromatogram of labeled microsomal lipids isolated by thin-layer chromatography after 30 min incubation with the microsomal phosphatidate-phosphohydrolase. Phosphatidate-labeled microsomes were incubated at 37°C in 0.32 M sucrose–2 mm mercaptoethanol. After incubation, the microsomal lipids were extracted, treated with diazomethane, and then chromatographed on a silica gel G plate developed in ethyl ether–petroleum ether (bp 40–70°C)–acetic acid 80:20:1 (by vol). DMPA, dimethyl phosphatidate; DG, diacylglycerol; TG, triglyceride; PL, phospholipid. No trace of labeled phospholipid appears on the chromatogram.

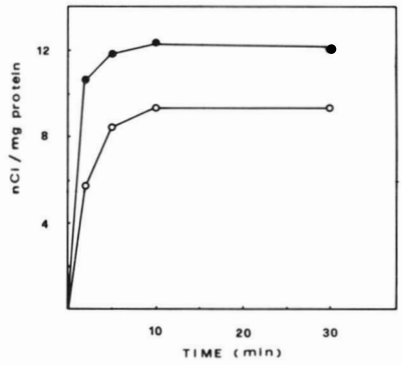

Fig. 2. Conversion of endogneously labeled diglyceride into phosphatidylcholine (● —— ●) and phosphatidylethanolamine (○ —— ○). Microsomes containing 31 nCi of [^{14}C]diglyceride, 13 nCi of [^{14}C]-triglyceride, and 52 nCi of [^{14}C]phosphatidic acid per mg of protein were incubated for different time intervals with saturating concentrations of CDP-choline or CDP-ethanolamine.

Diglyceride-labeled microsomes were used to study the choline phosphotransferase and ethanolamine phosphotransferase reactions. **Fig. 2** shows the time course of the incorporation of endogenously labeled diglycerides into phospholipids after separate incubations of labeled microsomes with CDP-choline or CDP-ethanolamine.

After 5 min of incubation of the labeled microsomes with CDP-choline, the conversion of diglyceride into phosphatidylcholine was 37% and reached the maximum value of 38.5% after 10 min. Similarly, the conversion of endogenously labeled diglyceride into phosphatidylethanolamine following incubation of microsomes with CDP-ethanolamine was 28% at 5 min and reached the maximum value of 31% after 10 min of incubation. After incubation of diglyceride-labeled microsomes with CDP-choline, some radioactivity was found also in phosphatidylethanolamine. Likewise, labeled phosphatidylcholine was found after incubation of microsomes with CDP-ethanolamine. These results may be due to the presence of endogenous levels of CDP-choline and CDP-ethanolamine, or to the participation of the reverse reaction (23). CDP-choline could be formed in fact by this last reaction during incubation of microsomes with CDP-ethanolamine and could then be used in the choline phosphotransferase reaction.

Table 1 shows the distribution of label among molecular species of phosphatidylcholine after incubation of diglyceride-labeled microsomes with CDP-choline for different time intervals. If comparison is made between the percent of the label distribution among the molecular species of phosphatidylcholine and that of the incubated diglyceride, comparable values are found for all species, except for saturated and monoenoics, at incubation time extrapolated to zero. The initial value of the percent labeling of saturated phos-

TABLE 1. Distribution of ^{14}C (%) among the molecular species of phosphatidylcholine after incubation of diglyceride-labeled microsomes with CDP-choline[a]

			Incubation Time (min)			
	DG[b]	t_0[c]	2	5	10	30
Saturated	24.7 ± 1.6	19.5	22.1 ± 1.0	25.6 ± 2.4	28.5 ± 1.5	29.0 ± 3.4
Monoenoic	23.5 ± 2.0	29.5	28.0 ± 1.5	26.4 ± 1.8	26.6 ± 2.4	26.8 ± 5.9
Dienoic + trienoic	2.9 ± 1.4	3.5	3.6 ± 0.5	4.1 ± 0.7	2.9 ± 0.4	7.7 ± 4.1
Tetraenoic	34.4 ± 4.0	35.0	33.1 ± 0.8	29.8 ± 2.1	26.0 ± 1.2	24.6 ± 0.4
Pentaenoic + hexaenoic	14.3 ± 4.0	12.5	13.2 ± 0.9	14.1 ± 2.6	16.0 ± 1.9	11.9 ± 3.0

[a] Diglyceride-labeled microsomes were prepared by incubating the membranes with [U-^{14}C]-glycerol-3-phosphate and by the successive dephosphorylation of the labeled phosphatidate. CDP-choline was incubated at 1 mM final concentration. See text for additional information.

[b] Distribution of label among molecular species of diglyceride expressed as percent of the class labeling ±standard deviation (mean of four experiments).

[c] Percent labeling of the molecular species of phosphatidylcholine obtained by extrapolating to zero incubation time the mean values from four experiments.

phatidylcholine species is in fact smaller than that of saturated diglyceride species while the opposite is evident for the monoenoics.

Table 2 shows the label distribution among the molecular species of phosphatidylethanolamine after incubation of diglyceride-labeled microsomes with CDP-ethanolamine for different time intervals. Large differences are evident in comparing the percent distribution of the label among the molecular species of the incubated diglyceride with those of phosphatidylethanolamine extrapolated to zero time of incubation. Phospholipid monoenoic and tetraenoic species are considerably less labeled than the diglyceride species, if the percent of the respective class labeling is considered. Polyenoic diglyceride species are, on the other hand, preferentially and rapidly converted into phosphatidylethanolamine, if a similar comparison is made.

By increasing the incubation times of the experiments presented in Table 1 from 2 to 30 min, a noticeable decrease of the percent of labeling of the tetraenoic phosphatidylcholine is evident, while a constant increase is seen for the saturated and the dienoic plus trienoic species. In the same range of incubation time, the percent distribution of the label among the phosphatidylethanolamine species is quite similar to that of the incubated diglycerides (Table 2).

DISCUSSION

Rat brain microsomes contain phosphatidic acid, diglyceride and phospholipids with very different fatty acid and molecular species composition. Saturated and monoenoic phosphatidylethanolamines represent only a small percent of this class of lipid, while saturated phosphatidic acid and phosphatidylcholine represent about 20%, and monoenoics about 38%, of the respective classes. Tetraenoic molecular species are the most abundant among diglyceride and phosphatidylethanolamine species, but their content in phosphatidic acid and phosphatidylcholine is relatively small.

It is interesting to speculate on the metabolic basis of these variations. The different composition of microsomal lipid molecular species could be explained either by species selectivity during the biosynthetic pathway or by the participation of other mechanisms involved in the regulation of the steady-state composition of membrane lipids (10, 15–18).

Kanoh and Ohno (7) described for liver microsomes a lack of selectivity for the conversion of membrane diglycerides produced by a reverse reaction into phosphatidylcholine, with only a small preference for the utilization of dienoic species. In the same study, a strong selectivity was exhibited by the ethanolamine phosphotransferase for the hexaenoic diglycerides.

A consistent selectivity of choline phosphotransferase for dienoic diglycerides was confirmed by Fallon, Barwick, and Lamb (8), while a relative exclusion was found for highly saturated diglyceride species.

In a previous study (16), the synthesis of phosphatidic acid from labeled glycerol phosphate was examined in vitro in rat brain microsomes and the initial rate of synthesis for the individual molecular species was evaluated. Without adding exogenous fatty acids, the initial rate of synthesis of tetraenoic and monoenoic phosphatidic acids was found to be the highest among all the molecular species. The distribution of label among diglyceride species obtained by dephosphorylation of membrane-bound phosphatidic acid

TABLE 2. Distribution of ^{14}C (%) among the molecular species of phosphatidylethanolamine after incubation of diglyceride-labeled microsomes with CDP-ethanolamine[a]

			Incubation Time (min)			
	DG[b]	t_0[c]	2	5	10	30
Saturated	24.7 ± 1.6	17.5	17.8 ± 1.2	20.2 ± 2.7	20.1 ± 4.7	21.9 ± 3.3
Monoenoic	23.5 ± 2.0	7.5	13.7 ± 1.6	22.9 ± 0.7	25.7 ± 4.2	23.2 ± 0.5
Dienoic + trienoic	2.9 ± 1.4	4.5	4.3 ± 0.5	4.7 ± 0.5	4.2 ± 0.5	4.9 ± 0.6
Tetraenoic	34.4 ± 4.0	25.0	25.9 ± 1.4	25.5 ± 1.8	26.7 ± 3.0	31.4 ± 2.7
Pentaenoic + hexaenoic	14.3 ± 4.0	45.5	38.2 ± 1.3	26.7 ± 1.7	23.2 ± 2.4	18.4 ± 1.3

[a] Diglyceride-labeled microsomes were prepared by incubating the membranes with [U-^{14}C]-glycerol-3-phosphate and by the successive dephosphorylation of the labeled phosphatidate. CDP-ethanolamine was incubated at 1 mM final concentration. See text for additional information.

[b] Distribution of label among molecular species of diglyceride expressed as percent of the class labeling ±standard deviation (mean of four experiments).

[c] Percent labeling of the molecular species of phosphatidylethanolamine obtained by extrapolating to zero incubation time the mean values from four experiments.

was very close, on the other hand, to that of the incubated phosphatidic acid (16).

In the present study, the results obtained from experiments on the utilization of endogenously-labeled diglyceride in the choline and ethanolamine phosphotransferase reactions are reported. The results in Table 1 and 2 lead to different conclusions, depending on the different fate of the diglyceride produced from the endogenously labeled phosphatidic acid. It can be assumed, in fact, that labeled diglyceride can mix rather well with the rest of membrane diglyceride. If this is true, the tetraenoic molecular species of membrane diglycerides would have the lowest specific activity in comparison with the other species, and then the high value of the percent labeling of the tetraenoic phosphatidylcholine should indicate a strong specificity of the choline phosphotransferase for these species. A similar consideration can be advanced for the conversion of the tetraenoic and polyenoic diglycerides into phosphatidylethanolamine. In this respect, the specificity of the ethanolamine phosphotransferase is the lowest for the monoenoic species of diglyceride.

This analysis of the experimental results refers to a thermodynamic distribution of diglycerides in the membrane, whatever their metabolic source. Such an assumption, in analogy with the rapid trans-bilayer movement of diglyceride, recently demonstrated for human red cell membranes (24), can be made, if the diglyceride produced in restricted areas of the membrane is assumed to undergo lateral diffusion. However, we have obtained evidence that the diglycerides synthesized in rat brain microsomal membranes from labeled glycerol phosphate through the acyl transferase and phosphatidate phosphohydrolase reactions, under the experimental conditions used in this study, do not migrate from the areas were they are produced.[1]

This could lead to a different evaluation of the experimental results reported in this paper. By making this assumption, in fact, a direct comparison between the percent labeling of the incubated diglyceride species and that of the phospholipid produced provides us with information relative to the species selectivity. More precisely, a small selectivity is evident for the synthesis of phosphatidylcholine species, since monoenoic and dienoic species are preferred to the others for phospholipid synthesis. Phosphatidylethanolamine synthesis, on the other hand, seems to be regulated by a higher specificity. Hexaenoic diglyceride species are in fact very quickly converted into lipid, their percent labeling among phosphatidylethanolamine species at t_0 incubation time reaching 45.5% of the whole class.

In conclusion, in addition to the metabolic events capable of modifying the membrane lipid structures, a certain species selectivity of the ethanolamine phosphotransferase and choline phosphotransferase activities has to be taken into account for understanding the homeostasis of membrane lipids.

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[1] Roberti, R., L. Binaglia, and G. Porcellati. Unpublished observations.

REFERENCES

1. Lamb, R. G., and H. J. Fallon. 1974. Glycerolipid formation from *sn*-glycerol-3-phosphate by rat liver cell fractions. The role of phosphatidate phosphohydrolase. *Biochim. Biophys. Acta.* **348:** 166–178.
2. Lloyd-Davies, K. A., and D. N. Brindley. 1975. Palmitate activation and esterification in microsomal fraction of rat liver. *Biochem. J.* **152:** 39–49.
3. Porcellati, G., M. G. Biasion, and G. Arienti. 1970. The incorporation of phosphorylethanolamine into the phospholipids of brain microsomes in vitro. *Lipids.* **5:** 725–733.
4. Parkes, J. G., and W. Thompson. 1973. Synthesis in vivo of phospholipids of liver mitochondria and endoplasmic reticulum from glycerol and fatty acids. *Biochim. Biophys. Acta.* **306:** 403–411.
5. Fallon, H. J., J. Barwick, R. G. Lamb, and H. van den Bosch. 1975. Studies of rat liver microsomal diglyceride acyltransferase and cholinephosphotransferase using microsomal-bound substrate: effects of high fructose intake. *J. Lipid Res.* **16:** 107–115.
6. Sribney, M., C. L. Knowles, and E. M. Lyman. 1976. Regulation of phosphatidylcholine synthesis in rat liver endoplasmic reticulum. *Biochem. J.* **156:** 507–514.
7. Kanoh, H., and K. Ohno. 1975. Substrate-selectivity of rat liver microsomal 1,2-diacylglycerol:CDP-choline (ethanolamine) choline (ethanolamine) phosphotransferase in utilizing endogenous substrates. *Biochim. Biophys. Acta.* **380:** 199–207.
8. Fallon, H. J., J. Barwick, and R. G. Lamb. 1976. Studies on the specificity of the reactions of glycerol lipid biosynthesis in rat liver using membrane-bound substrates. *In* Lipids. Vol. 1. R. Paoletti, G. Porcellati, and G. Jacini, editors. Raven Press, New York. 67–74.
9. Sundler, R., B. Åkesson, and Å. Nilsson. 1974. Effect of different fatty acids on glycerolipid synthesis in isolated rat hepatocytes. *J. Biol. Chem.* **249:** 5102–5107.
10. Holub, B. J., and J. Piekarski. 1978. Suitability of different molecular species of 1,2-diacylglycerols as substrates for diacylglycerol kinase in rat brain microsomes. *J. Neurochem.* **31:** 903–908.
11. Åkesson, B., and R. Sundler. 1977. Factors controlling the biosynthesis of individual phosphoglycerides in liver. *Biochem. Soc. Trans.* **5:** 43–48.
12. O'Brien, J. F., and R. L. Geison. 1974. Incorporation of [2-^{3}H]glycerol into rat brain 1,2-diacyl-*sn*-glycero-3-phosphorylcholine and 1,2-diacyl-*sn*-glycerol molecular species in vivo. *J. Lipid Res.* **15:** 44–49.

13. MacDonald, G., R. R. Baker, and W. Thompson. 1975. Selective synthesis of molecular classes of phosphatidic acid, diacylglycerol and phosphatidylinositol in rat brain. *J. Neurochem.* **24:** 655–661.
14. Hawthorne, J. N., and M. R. Pickard. 1977. Phosphatidate metabolism in stimulated synaptosomes. *Biochem. Soc. Trans.* **5:** 52–55.
15. Binaglia, L., R. Roberti, and G. Porcellati. 1977. Conversion of individual phosphatidates into rat brain lipid in vitro. *Biochem. Soc. Trans.* **5:** 175–178.
16. Binaglia, L., R. Roberti, and G. Porcellati. 1978. A study on the turnover of rat brain phosphatidic acid through the glycerol-phosphate pathway. *In* Enzymes of Lipid Metabolism. Shimon Gatt, Louis Freysz, and Paul Mandel, editors. Plenum Publishing Corporation. 353–366.
17. Porcellati, G., and L. Binaglia. 1976. Metabolism of phosphoglycerides and their molecular species in brain. *In* Lipids. Vol. 1. R. Paoletti, G. Porcellati, and G. Jacini, editors. Raven Press, New York. 75–88.
18. Baker, R. R., and W. Thompson. 1972. Positional distribution and turnover of fatty acids in phosphatidic acid, phosphoinositides, phosphatidylcholine and phosphatidylethanolamine in rat brain in vivo. *Biochim. Biophys. Acta.* **270:** 489–503.
19. Sundler, R., and B. Åkesson. 1973. Chromatographic resolution of phosphatidylethanolamine as *N*-acyl-*O*-methyl derivatives. *J. Chromatography.* **80:** 233–240.
20. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–267.
21. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
22. Laurell, S., and G. Tibbling. 1966. An enzymatic fluorometric micromethod for the determination of glycerol. *Clin. Chim. Acta.* **13:** 317–322.
23. Goracci, G., E. Francescangeli, L. A. Horrocks, and G. Porcellati. 1978. CMP-dependent degradation of membrane-bound brain choline phosphoglycerides. *Ital. J. Biochem.* **27:** 284–286.
24. Allan, D., P. Thomas, and H. R. Michell. 1978. Rapid transbilayer diffusion of 1,2-diacyl-glycerol and its relevance to control of membrane curvature. *Nature.* **276:** 289–290.