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PHOSPHOLIPID METABOLISM IN RAT LIVER ENDOPLASMIC RETICULUM

STRUCTURAL ANALYSES, TURNOVER STUDIES AND ENZYMIC ACTIVITIES

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SUMMARY

1. Rough and smooth submicrosomal fractions of normal rat liver were isolated by zonal centrifugation. The distribution and turnover of acyl moieties at the 1 and 2 positions of phosphatidylcholine and phosphatidylethanolamine, the relative rates of degradation of the major phospholipid classes, and several synthetic and hydrolytic enzyme activities associated with lipid metabolism were determined in both subfractions.

2. We found that the fatty acid compositions of phosphatidylcholine and phosphatidylethanolamine in the submicrosomal fractions were very similar. The 1 position is predominantly occupied by saturated fatty acids and the 2 position by unsaturated fatty acids. The relative rates of degradation for the phospholipid classes in the rough microsomes are the same as those in the smooth microsomes. However, the different phospholipid species are degraded differently within a given subfraction. Furthermore, acyl moieties esterified at the 1 position of phosphatidylcholine and phosphatidylethanolamine turned over faster than the acyl moieties esterified at the 2 position.

3. A delicate balance of synthetic and degradative enzymes apparently accounts for the similar gross features of lipid composition and turnover in the two submicrosomal fractions derived from the endoplasmic reticulum. For example, the specific activities of 1-acylglycerol-3-phosphate acyltransferase, palmityl-CoA hydrolase, and palmityl-CoA synthetase are higher in the smooth-surfaced microsomes than in rough-surfaced microsomes. Yet, when measuring *in vitro* exchange (indicative of phospholipase and total acyltransferase activities) with [1-^{14}C]oleic acid, the smooth microsomes incorporated more [^{14}C]oleic acid into phosphatidylethanolamine than the rough microsomes, but essentially no difference was observed for other lipid classes within the two submicrosomal fractions. The results obtained *in vivo* and *in vitro* are consistent with the concept that the endoplasmic reticulum is a mosaic array of structural lipoproteins and enzymic proteins in a dynamic state of heterogeneous turnover.

INTRODUCTION

The endoplasmic reticulum of rat hepatocytes can be divided into rough- and smooth-surfaced components. Studies from other laboratories[1,2] have indicated that it is a dynamic structure with constituents and enzyme activities that are continually changing. Using ^{14}C- and ^{3}H-labeled amino acids, Omura *et al.*[1] and Arias *et al.*[2] have shown that the total proteins of both rough and smooth membrane fractions have identical turnover rates with a mean half-time of 2.0 days, whereas the relative rates of degradation of individual protein species are heterogeneous.

Composition and turnover of lipid constituents of the endoplasmic reticulum have been investigated from several points of view[1,3,4]. Glaumann and Dallner[3] found that the rough and smooth microsomal fractions have similar phospholipid compositions, but that a subfraction of the smooth microsomes incorporated less [2-^{3}H]glycerol and ^{32}P into phospholipids than the other microsomal fractions. Omura *et al.*[1] demonstrated that total lipids of rough microsomal membranes have the same turnover rate as in smooth microsomal membranes. Holtzman *et al.*[4] have shown that the uptake of ^{32}P into the major phospholipids is the same for rough and smooth microsomal membrane fractions, but differs for each phospholipid class within a given subfraction. However, detailed studies were not carried out on the rate of degradation and fatty acid analysis of individual phospholipid classes in the smooth and rough endoplasmic reticulum.

The present study* was undertaken to evaluate the distribution and turnover of acyl moieties at the 1 and 2 positions of phosphatidylcholine and phosphatidylethanolamine and the degradation of the major phosphoglycerides of the smooth and rough endoplasmic reticulum of normal rat liver. In addition, the distribution of several key enzymes in lipid metabolism (1-acylglycerol-3-phosphate acyltransferase, acyl-CoA synthetase, acyl-CoA hydrolase) and reactions with [1-^{14}C]oleic acid, an indicator of acyltransferase and phospholipase activities, were determined for the submicrosomal fractions. The data obtained support the concept[1] that the endoplasmic reticulum represents a mosaic arrangement of lipoproteins in which all components, including individual portions of molecules, are synthesized and degraded at independent rates.

EXPERIMENTAL PROCEDURE

Materials

Female Charles River (CD strain) rats weighing 150–250 g, fed *ad libitum* on a Dietrich and Gambrill Laboratory Diet, were used for all experiments. *Crotalus adamanteus* venom containing phospholipase A was obtained from Ross Allen's Reptile Institute, Inc. Phospholipase D was purchased from General Biochemicals. Lipid standards were purchased from The Hormel Institute and Supelco, Inc. New England Nuclear Corp. supplied the following compounds: [2-^{14}C]glycerol (spec. act. 13.6 μCi/μM), [2-^{3}H]glycerol (spec. act. 836 μCi/μM), sodium [2-^{14}C]-acetate (spec. act. 52.3 μCi/μM), sodium [2-^{3}H]acetate (spec. act. 100 μCi/μM), and [1-^{14}C]oleic acid (spec. act. 25 μCi/μM). 1-Acylglycerol-3-phosphate was prepared from phosphatidylcholine (egg yolks) by treating with phospholipase A and subsequently with phospholipase D (ref. 6).

* Part of the results has been reported in a preliminary form[5].

Isolation of submicrosomal fractions

The rough and smooth submicrosomal fractions were separated by zonal centrifugation[7]. The contents of tubes from the peak of each submicrosomal fraction were diluted with 0.005 M Tris–HCl buffer (pH 7.5) and then centrifuged at 105000 × *g* for 60 min. The pellets were suspended in a solution (pH 7.4) of 3 mM Tris–HCl, 1 mM EDTA and 0.1 mM dithiothreitol, and stored in a liquid-nitrogen freezer at −70 °C until analyzed. The purity of each fraction was verified by enzyme markers and electron microscopy as reported earlier[7].

Chromatography of phospholipids and positional analysis of fatty acids

Total lipids were extracted from rough and smooth microsomal suspensions by the procedure of Bligh and Dyer[8]. The neutral lipid and phospholipid fractions were separated on silicic acid columns[9] and the percentage of each fraction was determined by weighing. Individual phospholipid classes were purified by preparative thin-layer chromatography on Silica Gel HR. All samples were chromatographed with standards in two solvent systems: chloroform–methanol–acetic acid–saline (50:25:8:4, by vol.) and chloroform–methanol–NH_4OH (60:35:8, by vol.). The lipid classes, including the standards, were visualized by iodine vapor. Phospholipid phosphorus on silica gel was measured by the method of Rouser *et al.*[10].

We used phospholipase A to hydrolyze the fatty acids from the 2 position of each phospholipid class[11]. The lysophosphatides and fatty acids were separated on Silica Gel G layers by first developing the chromatogram approximately 15 cm in hexane–diethyl ether–acetic acid (80:20:1, by vol.); the chromatogram was then developed a second time in chloroform–methanol–acetic acid–saline (50:25:8:4, by vol.) until the solvent front reached the bottom edge of the fatty acid area observed after the first development. The purified lysophosphatides and fatty acids were treated with H_2SO_4–methanol to form methyl ester derivatives. These fractions were analyzed by gas–liquid chromatography. Conditions for gas–liquid chromatography were similar to those described by Cotman *et al.*[12] except that the column was temperature-programmed from 170–200 °C at 2 °C/min.

In vivo tracer experiments

All labeled compounds were administered intraperitoneally to rats in a volume of 1 ml of 0.9% NaCl. Specific dosages and times of administration are given in legends to tables. The turnover rates of the various phospholipids in the two submicrosomal fractions were tested by using a technique whereby two time points in a radioactivity decay curve for the same animal are determined by administering the ^{14}C and ^{3}H form of the same precursor[2]. With ^{3}H radioactivity representing the initial time point and ^{14}C radioactivity representing the decay time point, the lipids with the most rapid rate of turnover have the highest ratio of $^{3}H/^{14}C$; this value is referred to as relative degradation rate constant.

Enzymic incubations

Acyl-CoA synthetase was assayed[13] in an NH_2OH–Tris–KF mixture at pH 7.4 (500 mM, 25 mM and 100 mM, respectively) containing 15 mM ATP, 1.2 mM CoA, 30 mM freshly prepared cysteine neutralized with 40 mM Tris, 4 mM $MgSO_4$, and 0.1–0.4 mg protein in a final volume of 1 ml. The reaction was initiated by the

addition of 0.4 mM potassium palmitate. Each sample was incubated for 60 min at 37 °C.

1-Acylglycerol-3-phosphate acyltransferase was assayed in 50 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA, 2.35 μM [1-^{14}C]palmityl-CoA (0.4 μCi), 6 μM 1-acylglycerol phosphate, and 0.01–0.05 mg protein in a final volume of 3.0 ml. Acyl-CoA hydrolase was assayed in 50 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA, 14.8 μM [1-^{14}C]palmityl-CoA (1 μCi), and 0.01–0.05 mg protein in a final volume of 3 ml. Samples were incubated for 5 min at 37 °C. Incorporation of [1-^{14}C]oleic acid into phosphatidylcholine, phosphatidylethanolamine, and the phosphatidylserine–phosphatidylinositol fraction was quantitated for both smooth and rough microsomes. The complete incubation mixture contained 0.2 μmole [1-^{14}C]oleic acid (0.25 μCi), 0.02 M ATP, 0.025 M $MgCl_2$, 0.0025 M $CaCl_2$, 0.0005 M CoA, 0.2 M NaF, 0.05 M Tris–HCl buffer (pH 7.4), and 0.3–2.0 mg protein in a final volume of 3.0 ml. Each sample was incubated for 30 min at 37 °C. Reactions were terminated by extracting the lipids from the incubation mixture by the Bligh and Dyer[8] procedure. The enzyme activities were determined by radioassay of the products separated by thin-layer chromatography in the systems described in the legend of Table IX. All *in vitro* enzyme assays were done at two or more concentrations of proteins to establish zero order kinetics. The reaction was linear with time at the protein concentrations used.

Radioassay

The radioactivity in individual phospholipid classes was determined by two techniques. Each phospholipid class was purified by preparative thin-layer chromatography and a portion used directly for radioassay, or the distribution of radioactivity along the entire chromatographic lane was determined quantitatively by zonal scanning in 5-mm increments[14,15]. The phospholipids were resolved on 250-μm layers of Silica Gel HR in a solvent system of chloroform–methanol–acetic acid–saline (50:25:8:4, by vol.). Methyl esters of the fatty acids from the purified phosphatidylcholine and phosphatidylethanolamine were isolated as described above and the radioactivity determined.

Free fatty acids in the lipids of the supernatant were isolated and purified by thin-layer chromatography in a solvent system of hexane–diethyl ether–acetic acid (80:20:1, by vol.). The methyl esters of the fatty acids were prepared and then separated according to their degree of unsaturation by argentation chromatography[16] on 250-μm layers of Silica Gel G (containing 20% $AgNO_3$) in a solvent system of hexane–chloroform (80:20, by vol.). Chromatographic areas corresponding to standards of saturated and unsaturated methyl esters of fatty acids were transferred directly into scintillation vials containing scintillation solvent[15] and radioassayed in a Packard liquid scintillation spectrometer (Model 3320). All the other radioactive samples were counted under the same conditions.

RESULTS

Lipid composition

Rough- and smooth-surfaced microsomes from rat liver are very similar in lipid composition. The phospholipids comprise 85% of the total lipids present

in each of the submicrosomal fractions. Phosphatidylcholine (65%) and phosphatidylethanolamine (20%) are the two main phospholipids, whereas the others, phosphatidylserine, phosphatidylinositol, and sphingomyelin, account for less than 15% of the total. These results are similar to the published reports of others[3,17] who prepared the subfractions by other procedures.

Positional analysis of acyl moieties in phosphatidylcholine and phosphatidylethanolamine

Both smooth and rough endoplasmic reticulum have identical distributions of fatty acids at the 1 and 2 positions of phosphatidylcholine and phosphatidylethanolamine (Tables I and II). In addition, within each submicrosomal fraction the fatty acid composition of the 1 position of phosphatidylcholine and phosphatidylethanolamine is also similar; both are composed primarily (approx. 90%) of 16:0 and 18:0 fatty acids (Table I). In contrast, more than 90% of the fatty acids in the 2 position of phosphatidylcholine and phosphatidylethanolamine of both submicrosomal fractions are unsaturated (Table II). The percentage of 22:6 acids is much higher in the 2 position of phosphatidylethanolamine than in phosphatidylcholine, whereas the percentage of 18:1 and 18:2 fatty acids is higher in phosphatidylcholine than in phosphatidylethanolamine.

Relative turnover of phospholipid classes

Tables III and IV show the results obtained when [2-^{14}C]glycerol and [2-^{3}H]glycerol were used as lipid precursors of the phosphoglycerides. The total lipids of rough-surfaced microsomes have the same relative degradation rate constant as those of smooth-surfaced microsomes (Table III). Phosphatidylcholine and phosphatidylethanolamine of rough- and smooth-surfaced microsomes have the same degradation rate, but they turn over much more rapidly than the phosphatidylserine-phosphatidylinositol fraction (Table IV).

When sodium [2-^{14}C]acetate and sodium [2-^{3}H]acetate were used as lipid

TABLE I

DISTRIBUTION OF FATTY ACIDS ESTERIFIED AT THE 1 POSITION OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE IN SUBMICROSOMAL FRACTIONS OF RAT LIVERS

Weight percentages given represent the mean of four experiments ± S. E.

Fatty acids	*Phosphatidylcholine*		*Phosphatidylethanolamine*	
	Rough-surfaced microsomes	*Smooth-surfaced microsomes*	*Rough-surfaced microsomes*	*Smooth-surfaced microsomes*
14:0	0.3	0.2	—	—
15:0	0.35 ± 0.05	0.3 ± 0.04	0.5 ± 0.15	0.6
16:0	29.0 ± 2.50	30.5 ± 1.30	22.7 ± 1.20	25.0 ± 2.40
16:1	1.0	0.9 ± 0.04	—	—
17:0	1.7 ± 0.20	1.7 ± 0.15	1.8 ± 0.15	2.4 ± 0.25
17:1	—	0.4	1.0	—
18:0	58.6 ± 1.50	57.3 ± 1.40	58.8 ± 1.10	60.2 ± 2.10
18:1	8.2 ± 1.30	7.9 ± 0.25	12.4 ± 1.40	10.3 ± 0.50
18:2	1.3 ± 0.10	1.0 ± 0.04	1.0 ± 0.15	1.0 ± 0.15
20:4	—	—	1.6	1.4

TABLE II

DISTRIBUTION OF FATTY ACIDS ESTERIFIED AT THE 2 POSITION OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE IN SUBMICROSOMAL FRACTIONS OF RAT LIVERS

Weight percentages represent the mean of four experiments ± S. E.

Fatty acids	*Phosphatidylcholine*		*Phosphatidylethanolamine*	
	Rough-surfaced microsomes	*Smooth-surfaced microsomes*	*Rough-surfaced microsomes*	*Smooth-surfaced microsomes*
16:0	2.1 ± 0.35	2.7 ± 0.20	0.8 ± 0.30	0.9 ± 0.05
16:1	0.8 ± 0.15	0.8 ± 0.05	—	—
18:0	0.8 ± 0.20	1.5 ± 0.20	0.8 ± 0.10	0.7 ± 0.20
18:1	9.1 ± 1.30	11.4 ± 0.30	1.7 ± 0.30	2.3 ± 0.20
18:2	16.5 ± 2.50	19.4 ± 1.90	6.4 ± 1.40	9.3 ± 0.70
18:3	0.6	0.6	—	—
20:3	2.3 ± 0.40	2.7 ± 0.55	2.4 ± 0.25	1.9 ± 0.15
20:4	53.0 ± 4.50	50.0 ± 4.30	52.4 ± 2.70	51.2 ± 1.70
20:5	1.6 ± 0.45	0.9	1.7 ± 0.40	1.5 ± 0.30
22:3 + 24:0	—	—	1.0	0.8
22:4 + 24:1	—	—	1.0	1.1
22:5	0.9	0.6	1.7 ± 0.25	1.9 ± 0.30
22:6	12.8 ± 0.45	9.9 ± 1.40	30.7 ± 1.20	28.8 ± 1.70

TABLE III

RELATIVE TURNOVER OF TOTAL LIPIDS IN SUBMICROSOMAL FRACTIONS OF RAT LIVERS AFTER THE INTRAPERITONEAL ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED GLYCEROL

Two rats weighing approximately 150 g were injected with 50 μCi (Expt 1) or 100 μCi (Expt 2) of [2-^{14}C]glycerol; 70.5 h later, each rat was injected with 75 μCi of [2-^{3}H]glycerol. The animals were killed 2 h later.

Cell fraction	*Ratio $^{3}H/^{14}C$*	
	Expt. 1	*Expt. 2*
Total microsomes	25.6	15.1
Rough-surfaced microsomes	23.4	14.2
Smooth-surfaced microsomes	24.0	13.3

TABLE IV

RELATIVE TURNOVER OF PHOSPHOLIPID CLASSES IN SUBMICROSOMAL FRACTIONS OF RAT LIVERS AFTER THE INTRAPERITONEAL ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED GLYCEROL

Two rats weighing approximately 150 g were injected with 50 μCi (Expt 1) or 100 μCi (Expt 2) of [2-^{14}C] glycerol; 70.5 h later, each rat was injected with 75 μCi of [2-^{3}H]glycerol. The animals were killed 2 h later. Abbreviations: PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.

Cell fraction	*Ratio $^{3}H/^{14}C$*					
	Expt 1			*Expt 2*		
	PC	*PS + PI*	*PE*	*PC*	*PS + PI*	*PE*
Total microsomes	34	6.8	38	19	2.9	18
Rough-surfaced microsomes	32	6.9	32	20	4.6	19
Smooth-surfaced microsomes	35	7.4	39	19	3.9	18

TABLE V

RELATIVE TURNOVER OF TOTAL LIPIDS IN SUBMICROSOMAL FRACTIONS OF RAT LIVERS AFTER THE INTRAPERITONEAL ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED ACETATE

Two rats weighing approximately 150 g were injected with 200 μCi of sodium [2-^{14}C]acetate; 63 h later, each rat was injected with 500 μCi (Expt 1) or 1 mCi (Expt 2) of sodium [2-^{3}H]acetate. The animals were killed 4 h later.

Cell fraction	*Ratio $^{3}H/^{14}C$*	
	Expt 1	*Expt 2*
Total microsomes	0.86	2.8
Rough-surfaced microsomes	0.81	2.6
Smooth-surfaced microsomes	0.84	2.8

TABLE VI

RELATIVE TURNOVER OF PHOSPHOLIPID CLASSES IN SUBMICROSOMAL FRACTIONS OF RAT LIVER AFTER THE INTRAPERITONEAL ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED ACETATE

Experimental conditions were the same as those described in Table V, except in Expt 3, 250 μCi of sodium [2-^{14}C]acetate and 1 mCi of sodium [2-^{3}H]acetate were injected.

Phospholipid class	*Ratio $^{3}H/^{14}C$*					
	Expt 1		*Expt 2*		*Expt 3*	
	Rough microsomes	*Smooth microsomes*	*Rough microsomes*	*Smooth microsomes*	*Rough microsomes*	*Smooth microsomes*
Sphingomyelin	0.32	0.35	1.7	1.5	1.0	0.9
Phosphatidylcholine	0.89	0.84	3.4	3.5	2.0	1.6
Phosphatidylinositol	0.58	0.52	2.1	1.9	0.7	0.7
Phosphatidylserine	0.43	0.42	2.6	2.8	1.5	1.3
Phosphatidylethanolamine	0.58	0.62	2.5	2.8	1.4	1.8

precursors, the total lipids (Table V) and phospholipid classes (Table VI) of both submicrosomal fractions were found to have the same relative degradation rate constants. However, there is a marked heterogeneity of turnover of the different phospholipid classes within each subfraction of the endoplasmic reticulum (Table VI; phosphatidylcholine > phosphatidylethanolamine, phosphatidylserine > phosphatidylinositol > sphingomyelin).

Relative turnover of fatty acids esterified at the 1 and 2 positions of phosphatidylcholine and phosphatidylethanolamine

Table VII shows that fatty acids of phosphatidylcholine have the same relative degradation rate constants in the rough- and smooth-surfaced microsomes when labeled acetate is used as a precursor; the turnover of the fatty acids in phosphatidylethanolamine is also similar for the two submicrosomal fractions. However, for both the choline- and ethanolamine-containing phospholipids it is clear that fatty acids esterified at the 1 position turn over much faster than fatty acids esterified at the 2 position.

TABLE VII

RELATIVE TURNOVER OF FATTY ACIDS ESTERIFIED AT 1 AND 2 POSITIONS OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE IN SUBMICROSOMAL FRACTIONS OF RAT LIVERS AFTER THE INTRAPERITONEAL ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED ACETATE

Experimental conditions were the same as those described in Table V.

Cell fraction	*Ratio $^{3}H/^{14}C$*			
	Expt 1		*Expt 2*	
	1 position	*2 position*	*1 position*	*2 position*
Phosphatidylcholine				
Rough-surfaced microsomes	0.68	0.19	1.98	0.44
Smooth-surfaced microsomes	0.66	0.24	2.03	0.47
Phosphatidylethanolamine				
Rough-surfaced microsomes	0.55	0.20	1.47	0.34
Smooth-surfaced microsomes	0.53	0.24	1.55	0.36

TABLE VIII

RELATIVE TURNOVER OF FREE FATTY ACIDS IN SUPERNATANT OF RAT LIVER AFTER THE INTRAPERITONEAL ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED ACETATE

Experimental conditions were the same as those described in Table V. The supernatant fraction was isolated by zonal centrifugation[7] and then centrifuged at 105000 × *g* for 60 min to remove any particles. In this particular experiment, the lipids were extracted by the procedure of Folch *et al.*[18]; the fatty acids were separated by argentation chromatography[16].

Fatty acids	*Ratio $^{3}H/^{14}C$*	
	Expt 1	*Expt 2*
Saturated	1.37	2.80
Unsaturated	1.08	1.11

The observed ratios of $^{3}H/^{14}C$ in free fatty acids associated with the soluble proteins of the supernatant (Table VIII) indicate the relative turnover of saturated fatty acids is higher than that of unsaturated fatty acids in this fraction and reflect the same results obtained for the diacylphosphoglycerides in the submicrosomal fractions.

Microsomal distribution of enzymic activities associated with lipid metabolism

The distribution of several representative enzymes (1-acylglycerol-3-phosphate acyltransferase, palmityl-CoA synthetase, and palmityl-CoA hydrolase) that participate in lipid turnover was studied in this series of experiments (Table IX); all were always higher in smooth-surfaced microsomes than in rough-surfaced microsomes when the enzyme activity was expressed either as units per mg of protein or per μg lipid phosphorus. However, the magnitude of the difference for palmityl-CoA synthetase between the two microsomal fractions was not as large as that found for the hydrolase and acyltransferase activities.

Another experimental series using [1-^{14}C]oleic acid was designed to estimate the microsomal distribution of a coupled enzyme system involving phospholipase

TABLE IX

DISTRIBUTION OF 1-ACYLGLYCEROL-3-PHOSPHATE ACYLTRANSFERASE, PALMITYL-CoA SYNTHETASE, AND PALMITYL-CoA HYDROLASE IN SMOOTH- AND ROUGH-SURFACED MICROSOMES OF NORMAL RAT LIVER

The numbers in parentheses refer to the number of experiments used to obtain average values indicated under each activity heading. The reaction products of 1-acylglycerol-3-phosphate acyltransferase were isolated on Silica Gel HR layers with chloroform–methanol–NH_4OH (60:35:8, by vol.) The reaction products of palmityl-CoA hydrolase were isolated on Silica Gel G with hexane–diethyl ether–acetic acid (50:50:1, by vol.).

Cell fraction	*nmoles/min per mg protein*	*nmoles/min per μg lipid phosphorus*
1-Acylglycerol-3-phosphate acyltransferase		
Total microsomes (3)	9.8	0.83
Rough-surfaced microsomes (3)	12.4	1.05
Smooth-surfaced microsomes (3)	21.2	1.49
Palmityl-CoA synthetase		
Total microsomes (3)	5.5	0.53
Rough-surfaced microsomes (3)	5.7	0.46
Smooth-surfaced microsomes (3)	6.8	0.51
Palmityl-CoA hydrolase		
Total microsomes (2)	29.1	2.6
Rough-surfaced microsomes (2)	20.2	1.7
Smooth-surfaced microsomes (2)	33.5	2.5

TABLE X

In vitro INCORPORATION OF [1-^{14}C]OLEIC ACID INTO PHOSPHOLIPIDS OF SMOOTH- AND ROUGH-SURFACED MICROSOMES

Radioactivity is expressed as cpm/μg of lipid phosphorus; average of four experiments. Abbreviations: PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.

Fraction	*Radioactivity*		
	PC	*PS + PI*	*PE*
Total microsomes	140	440	860
Rough-surfaced microsomes	160	430	660
Smooth-surfaced microsomes	140	410	890

and lysophosphatide reacylation activities (Table X). The [1-^{14}C]oleic acid, incubated with submicrosomal fractions of rat liver in the presence of ATP, CoA, Ca^{2+} and Mg^{2+}, was incorporated into individual phospholipids within each fraction at different rates; phosphatidylethanolamine incorporated much more radioactivity than any other phospholipid class under these experimental conditions. The incorporation of ^{14}C activity was elevated in the phosphatidylethanolamine fraction of smooth-surfaced microsomes but no other significant difference was detected for other lipids in the two submicrosomal fractions. Essentially no lysophospholipids could be detected in the submicrosomal fractions either before or after the incubations.

DISCUSSION

We used zonal centrifugation to separate the rough and smooth microsomal subfraction[7]; this method is similar in principle to that described by Dallner[19] in which monovalent cations cause a selective aggregation of rough microsomes[20]. Furthermore, the two subfractions isolated by zonal centrifugation are comparable in metabolic and physical properties to the fractions isolated by the method of Dallner[19] and the Rothschild procedure[21]: (1) The ratios of phospholipids to proteins in the two subfractions are similar to the reported values, (2) The specific activity of NADH-cytochrome reductase is higher in rough microsomal fractions than in smooth microsomal fractions (see ref. 19), and (3) The specific activity of NADPH diaphorase is highest in the smooth microsomal fraction. Our results show that the medium equilibrium densities of the two types of vesicles in aqueous sucrose were 1.15–1.16 for the smooth vesicles and 1.20 for the rough vesicles; similar density values for both types of vesicles were obtained when centrifugation was carried out for 2 h and 12 h. These density values are comparable to the reported values of 1.05–1.18 for the smooth vesicles[21] and 1.18–1.25 for the rough vesicles[22]. However, based on RNA to protein ratio, electron micrographs, and theoretical simulation*, it can be seen that the smooth microsomal fraction is contaminated with free ribosomes. The free ribosomes with an equilibrium density of 1.6 in aqueous sucrose theoretically can be removed by longer centrifugation time or higher centrifugation force, but, unfortunately, smooth microsomes are very unstable and show an irreversible spontaneous aggregation which causes a consequent increase in their density[23]; therefore, the removal of free ribosomes from the smooth microsomal fraction under our conditions of isolation cannot be successfully attained. Since the contamination of the smooth microsomal fraction with free ribosomes does not interfere with the results or interpretations of the experiments we conducted, the smooth microsomal fractions isolated by zonal centrifugation were used throughout this investigation without further purification.

The double isotope technique which was developed by Arias *et al.*[2,24] was used in our experiments to measure the degradation rates of cell components. The method primarily determines the degree of heterogeneity of turnover rates of subcellular components and does not measure absolute rate of degradation. The degradation rates are related to the ratios (relative degradation rate constants) of the two isotopically labeled compounds incorporated. However, the specific activity of the compound under study does not need to be determined; this often is advantageous to use when the compound under study is limited in quantity. Schimke[25] has discussed in detail the assumptions and limitations of this method.

The fatty acid composition of phosphatidylcholine and phosphatidylethanolamine in the two submicrosomal fractions is very similar to that of total rat liver[26,27]. The 1 position is occupied primarily by saturated fatty acids and the 2 position by unsaturated fatty acids. The nonrandom fatty acid distribution observed in our experiments could arise from acylation specificity at the level of *de novo* synthesis of phosphatides[28,29] or by redistribution of the fatty acyl chains *via* phospholipase and acyltransferase reactions[30,31]. Earlier studies by several investiga-

* Based on theoretical simulation (see Appendix), the free ribosomes, mainly polysomes, are the contamination in the smooth fraction isolated under our experimental conditions.

tors[29,32–34] have shown random acylations of glycero-3-phosphate in the cell-free systems. However, Possmayer *et al.*[28] recently demonstrated that acylation of glycero-3-phosphate by microsomes from rat liver yielded phosphatidic acid, phosphatidylethanolamine, and phosphatidylcholine which had an asymmetric distribution of the acyl moieties. Thus, van den Bosch *et al.*[35] proposed that the dissimilar location of saturated and unsaturated fatty acids in phosphatides was brought about during the *de novo* synthesis of these compounds and that the selective acylation of lysophosphatides plays an important role in preserving the asymmetry of the fatty acids during the turnover of the individual components of the phospholipid molecule. This selective distribution of fatty acids is thought to contribute to the physiochemical properties that relate to the function of phospholipids in biological membranes.

The biosynthetic rates of various molecular species of phosphoglycerides in microsomes should be reflected by their relative degradation rate constants. Faster degradation of certain classes indicates that these must also be synthesized more rapidly than others in order to preserve the composition of the endoplasmic reticulum in its equilibrium state. The findings by Holtzman *et al.*[4] support our degradation data; their experiments show that ^{32}P is incorporated into the major phospholipid components to the same extent in rough and smooth microsomes, whereas the rate of incorporation was considerably higher in phosphatidylethanolamine than in the phosphatidylcholine *plus* phosphatidylserine and sphingomyelin within the same submicrosomal fraction.

We have shown in rat liver microsomes that the saturated fatty acids esterified at the 1 position of phosphatidylcholine and phosphatidylethanolamine turn over faster than the unsaturated fatty acids esterified at the 2 position. The mechanisms by which the microsomal lipids are degraded are not clear at present. In general, the rates of turnover for the fatty acids in phospholipids are mainly governed by two factors: (a) The lipolytic enzyme activities that hydrolyze the fatty acids, and (b) The oxidative enzyme activities that degrade the free fatty acids. The main lipolytic enzyme in microsomes catalyzes the hydrolysis of fatty acids from both endogenous and exogenous phospholipids at the 1 position[36–38], whereas lysosomes contain enzymes that hydrolyze both the C-1 and C-2 fatty acid ester linkages of phosphatidylcholine and phosphatidylethanolamine[39,40]. Our data indicate that the saturated fatty acids released were degraded faster than the unsaturated fatty acids (Table VIII).

Our findings on the heterogeneous distribution of microsomal enzymes involving lipids suggest that the catalytic activities of the smooth and rough endoplasmic reticulum are not biochemically equivalent to one another. However, since there is no overall difference in lipid composition or turnover between the two submicrosomal fractions, it would appear that there is a delicate balance of synthetic and degradative enzymes.

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