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# SOME STUDIES ON THE METABOLISM OF PHOSPHOLIPIDS IN GOLGI COMPLEX FROM BOVINE AND RAT LIVER IN COMPARISON TO OTHER SUBCELLULAR FRACTIONS

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SUMMARY

1. Golgi complex, rough and smooth microsomes, plasma membranes, mitochondria, and nuclei from bovine liver were isolated and the purity assessed using specific marker enzymes.

2. Cholinephosphotransferase and acyl-CoA:1,2-diacyl-*sn*-glycerol acyltransferases were found to be absent in the Golgi complex, mitochondria, plasma membrane, and nuclei. The *de novo* synthesis of lecithin and triacylglycerols is apparently localized exclusively in the smooth and rough endoplasmic reticulum.

3. Acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase was found to be localized mainly in rough and smooth microsomes from bovine and rat liver; though also significant activity was found in the Golgi complex of bovine liver. On the other hand, no significant acylation of 1-acyl-*sn*-glycero-3-phosphorylcholine could be shown in the Golgi system from rat liver.

4. Phosphatidylglycerol synthesis was found to take place predominantly in rat liver mitochondria. Significant synthesis of this phospholipid was, however, also found in rough and smooth microsomes and in Golgi of rat liver.

5. The Golgi complex of rat liver was shown to contain both phospholipase $A_1$ and $A_2$ activities acting on exogenous phosphatidylethanolamine, the activity of the former being somewhat larger.

## INTRODUCTION

Much work has been done in the past few years by many investigators on the intracellular distribution of enzymes involved in the biosynthesis and catabolism of phospholipids. *De novo* synthesis of lecithin and phosphatidylethanolamine has been shown to occur in rat liver predominantly in the endoplasmic reticulum[1]. Various authors have demonstrated that mitochondria[2,3] and plasma membranes[4] do not have the capacity for the *de novo* synthesis of lecithin and phosphatidylethanolamine. Also the synthesis of triacylglycerols[1,3] was reported to be confined to the endoplasmic reticulum and does not take place in mitochondria.

Phosphatidylglycerol biosynthesis *via* the CDP-diglyceride pathway has been

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found to occur predominantly in the mitochondria[5] though substantial synthesis of this phospholipid has been reported recently to occur in plasma membranes[4] and endoplasmic reticulum[6]. Partial synthesis of lecithin and phosphatidylethanolamine *via* the acylation of their monoacyl derivatives has been shown to be localized in endoplasmic reticulum[3,7] and the outer membrane of mitochondria[3,8,9]. Some conflicting evidence exists on the question whether or not plasma membranes are equipped with such an acylation mechanism[10,11].

Phospholipases have been shown to be present in mitochondria[12,13], microsomes[14,15], plasma membranes[4,16,17], and lysosomes[18–20,41].

At present, however, no information is available on the question whether or not the Golgi system of liver possesses the enzyme systems described above. Only recently a paper was published by MORRÉ *et al.*[21] on the metabolism of lecithin in Golgi apparatus isolated from onion stems. These authors found that the Golgi apparatus from this plant source can synthesize *in vitro* lecithin.

The present work concerns the question whether or not the Golgi system of beef and rat liver, when compared to smooth and rough microsomes and other subcellular organelles, possesses the enzymes involved in the *de novo* synthesis of lecithin and triacylglycerols, acylation of monoacyl phosphoglycerides, and *de novo* synthesis of phosphatidylglycerol. Moreover, it was investigated whether phospholipases are present in the Golgi apparatus from liver.

## EXPERIMENTAL

### *Isolation of Golgi and other subcellular fractions from beef and rat liver*

Plasma membranes[22], rough and smooth microsomes, nuclei, mitochondria[23], and Golgi membranes[24] were prepared from bovine liver as described previously. Rough and smooth microsomes were prepared from a pool of 4 rats by our modification[22] of the method of DALLNER[25].

Golgi membranes were prepared by a modification of the single step-gradient procedure described previously[26]. The livers of 4 rats (about 200 g each, male, Holtzman strain) were collected, washed briefly in cold 0.25 M sucrose, blotted, and minced with scissors directly into 3 vol. cold 53 % sucrose containing 0.1 M sodium phosphate (pH 7.1). The mixture was homogenized (3 full strokes) with a Potter–Elvehjem homogenizer with a Teflon pestle with a clearance of 0.026 inch at 1000 rev/min. The entire homogenate was filtered through 2 layers of cheesecloth and the filtrate adjusted to 43.7 % sucrose (Bausch and Lomb refractometer at 25°) by adding 0.25 M sucrose. The homogenate (120 ml) was placed at the bottom of a step gradient in a Ti 14 zonal rotor rotating at 4000 rev./min in a Beckman L-2 65 centrifuge. The rotor had previously been loaded with the following step gradient, using a syringe-type infusion pump (Harvard App. Co.): 135 ml 29 %; 100 ml 33 %; 115 ml 36 % and 180 ml 38.7 % sucrose. About 30 ml homogenizing medium was used as a lower cushion. The gradient was centrifuged finally at 45000 rev./min for 33 min. After the rotor had slowed again to 4000 rev./min, 20 ml fractions were collected from the core by displacement of the rotor contents with 55 % sucrose. Fractions corresponding to the interface between 29 and 33 % sucrose (usually tubes 5–8) were combined, diluted with 1 vol. distilled water and centrifuged at 30000 rev./min in a Spinco No. 30 rotor for 1 h. The Golgi fraction was suspended finally in 0.25 M sucrose. The yield of Golgi vesicles is 10–15 mg protein.

*Enzyme assays*

$Mg^{2+}$-stimulated ATPase, rotenone-insensitive NADH–cytochrome *c* reductase activities, and succinate–cytochrome *c* reductase activities were carried out as described previously[27]. Glucose-6-phosphatase was determined according to SWANSON[28] except that incubations were carried out for 5 and 10 min at 32° and the phosphate released was measured using the method of CHEN *et al.*[29].

Galactosyltransferase was determined as described previously for Golgi fractions from bovine liver[24] and rat liver[26]. Galactosidase (hydrolysis of UDP-galactose) was determined for bovine liver plasma membranes as described[22]. For rat liver fractions, the same method was used except that *N,N*-bis-(2-hydroxythyl)-glycine (Bicine)–HCl, pH 8.6, was used instead of sodium cacodylate, pH 7.2[26]. Aryl sulfatase was carried out by the method of ROY[30].

*Substrates*

1,2-Diacyl-*sn*-glycerols were prepared biosynthetically from rat liver lecithin by degradation with phospholipase C from *Bacillus cereus* (EC 3.1.4.3) as described before[3]. CDP-[$^{14}$C]choline was obtained from Tracer Lab (U.S.A.) and non-labeled CDP-choline was purchased from Sigma (U.S.A.). [9,10-$^{3}H_2$]oleoyl-CoA was synthesized according to the procedure of GOLDMAN AND VAGELOS[31]. 1-Acyl-*sn*-glycero-3-phosphorylcholine and -ethanolamine were prepared from the corresponding diacyl compounds by hydrolysis with snake venom phospholipase A (EC 3.1.1.4)[3].

*sn*-[$^{14}$C]Glycero-3-phosphate was synthesized from [1-$^{14}$C]glycerol and ATP in the presence of glycerokinase (Boehringer, Germany) and purified by the method of CHANG AND KENNEDY[32] as modified by DAVIDSON AND STANACEV[33]. CDP-diglyceride was synthesized as described by PROTTEY AND HAWTHORNE[34]. Both [$^{14}$C]glycero-3-phosphate and CDP-diglyceride were generous gifts of Dr. K. Y. Hostetler from this laboratory. 1-Saturated-2-[1-$^{14}$C]linoleoyl-*sn*-glycero-3-phosphorylethanolamine was prepared biosynthetically as described previously[35].

*Conversion of 1,2-diacyl-sn-glycerols into lecithin*

1,2-Diacyl-*sn*-glycerols were suspended in a concentration of 5 mg/ml in 0.1 M Tris (pH 7.4), being 0.03 % with respect to Tween-20, by means of ultrasonication for 2 × 1 min with a Branson sonifier (Setting 4A). 50 $\mu$l of this suspension of 1,2-diacyl-*sn*-glycerols were added to an incubation mixture consisting of: 10 $\mu$l glutathione (0.1 M); 10 $\mu$l CDP-choline (2 mM), 20 $\mu$l [$^{14}$C]CDP-choline (1 $\mu$C/ml, specific activity 9.3 mC/mmole), 25 $\mu$l of $MgCl_2$ (0.1 M), 85 $\mu$l 0.1 M Tris, pH 7.4 (0.03 % with respect to Tween-20), and 50$\mu$l of the various subcellular fractions (1 or 5 mg protein per ml depending on the fraction).

After incubation in a shaking water bath for 0, 10, 20, and 30 min, respectively, the reactions were stopped by the addition of 1 ml chloroform–methanol (1:2, v/v). Lipids were then extracted by the method of BLIGH AND DYER[36]. The combined chloroform layers were washed three times with methanol–water (1:1, v/v) to remove non-converted [$^{14}$C]CDP-choline. Because it had been demonstrated in a separate experiment that lecithin was the only labeled product which was formed, the chloroform layers were evaporated to dryness, the residues dissolved in 1 ml of methanol, and 0.5 ml aliquots transferred directly into scintillation vials containing 15 ml of 0.5 % PPO (w/v) and 0.03 % (w/v) dimethyl-POPOP per l of toluene. The samples

were counted in a Packard Tricarb liquid scintillation spectrometer using an external standard for quenching corrections.

*Conversion of 1,2-diacyl-sn-glycerols into triacylglycerols*

50 $\mu$l of a suspension of 1,2-diacyl-*sn*-glycerols (see above) were added to an incubation mixture containing the following components: 10 $\mu$l glutathione (0.1 M), 25 $\mu$l $MgCl_2$ (0.1 M), 10 $\mu$l [9,10-$^3H_2$]oleoyl-CoA (1.75 mM; specific activity 2100 counts/min per nmole), 109 $\mu$l of 0.1 M Tris pH 7.4 (containing 0.03% Tween-20) and 50 $\mu$l of the various subcellular fractions (1 or 5 mg/ml depending on the fraction). After incubation for 0, 5, 10, 20, or 30 min at 37°, the reactions were stopped by the addition of 1 ml of methanol–chloroform (2:1, v/v). After extraction of the lipids according to the procedure of BLIGH AND DYER[36], the triacylglycerols were isolated *via* thin-layer chromatography on 0.5 mm silica plates (20 cm × 5 cm) which were run in hexane–diethyl ether–acetic acid (70:30:1, v/v/v). The triacylglycerol fractions were then scraped from the plate and transferred directly into scintillation vials containing 15 ml of scintillation fluid which had the following composition: 0.7% (w/v) PPO, 0.03% (w/v) dimethyl-POPOP, 10% (w/v) naphtalene per l of toluene with Triton X-100 and water in a ratio of 2:1:0.2 (v/v/v).

*Conversion of 1-acyl-sn-glycero-3-phosphorylcholine into lecithin*

The enzymatic assay of acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase was performed in an incubation medium containing the following components: 25 $\mu$l 1-acyl-3-*sn*-glycero-3-phosphorylcholine (1 mg/ml) taken from a sonicated emulsion in 0.125 M KCl–0.1 M Tris (pH 7.4), 25 $\mu$l $MgCl_2$ (0.1 M), 10 $\mu$l [9,10-$^3H_2$]oleoyl-CoA (1.75 mM), 165 $\mu$l 0.125 M KCl–0.1 M Tris (pH 7.4), and 25 $\mu$l of the various subcellular fractions (1 or 5 mg protein per ml). The incubations were carried out for 0, 1, 2.5, 5 and 10 min at 37° and stopped as described above.

After extraction of the lipids, lecithin was isolated by means of thin-layer chromatography on 0.5-mm silica plates (20 cm × 5 cm) which were developed in chloroform–methanol–water (65:35:4, v/v/v). The lecithin fractions were then scraped from the plates and assayed directly for radioactivity as described above. The conversion of 1-acyl-*sn*-glycero-3-phosphorylethanolamine into phosphatidylethanolamine was assayed in essentially the same way except that 1-acyl-*sn*-glycero-3-phosphorylcholine was replaced by 1-acyl-*sn*-glycero-3-phosphorylethanolamine. Before isolation of phosphatidylethanolamine *via* thin-layer chromatography as described above for lecithin, the plates were pre-run in chloroform–light petroleum (b.p. 40–60°)–acetic acid (65:33:2, v/v/v) in order to avoid contamination with free fatty acids.

*Assay for phosphatidylglycerol biosynthesis*

This assay was carried out in a medium essentially the same as that of KIYASU *et al.*[5]: 50 $\mu$l *sn*-[$^{14}$C]-glycero-3-phosphate (2.7 mM, specific activity 13600 counts/min per nmole), 50 $\mu$l of CDP-diglyceride taken from a sonicated emulsion in water (0.5 mM), 10 $\mu$l mercaptoethanol (1 M), 50 $\mu$l Tris (pH 7.5) (1 M), 140 $\mu$l distilled water and 200 $\mu$l of the various fractions (2.5 mg protein per ml). After appropriate times of incubation at 37°, 50-$\mu$l aliquots were pipetted from the reaction mixture and assayed for radioactivity by the filter paper disc assay as described by GOLDFINE[37]. A single

radioactive lipid product was formed during the reaction which cochromatographed with reference phosphatidylglycerol on silica H thin-layer plates impregnated with 0.4 M boric acid run in chloroform–methanol–water–ammonia (70:30:3:2, by vol.).

*Phospholipase A activity towards labeled* 1-*saturated-2-*[1-$^{14}$C]*linoleoyl*-sn-*glycero-3-phosphorylethanolamine*

1-Saturated-2-[1-$^{14}$C]linoleoyl-*sn*-glycero-3-phosphorylethanolamine (specific activity 970 counts/min per nmole) was sonicated in distilled water in a concentration of 2.5 mM. 50-$\mu$l aliquots were taken from this suspension and added to an incubation mixture consisting of 20 $\mu$l $CaCl_2$ (0.1 M), 20 $\mu$l Tris (pH 8) (1 M), 100 $\mu$l distilled water, and 50 $\mu$l of protein (1 mg/ml). The mixtures were incubated for 0, 5, 10, 20, 30 or 60 min, respectively, at 37° and then stopped by the addition of 1 ml methanol–chloroform (2:1, v/v). After the addition of 25 $\mu$l 0.5 M $H_2SO_4$ the lipids were extracted according to the BLIGH AND DYER[36] method. The free fatty acids, phosphatidylethanolamine and monoacylphosphatidylethanolamine were separated by means of thin-layer chromatography on 0.5-mm thin-layer silica plates (20 cm × 5 cm) which were developed in chloroform–light petroleum (b.p. 40–60°)–acetic acid (65:33:2, by vol.) and, in the same direction, in chloroform–methanol–water (65:35:4, by vol.). This procedure accomplishes a very good separation between monoacylphosphatidylethanolamine, phosphatidylethanolamine, and free fatty acids. These fractions were then scraped from the chromatoplates and transferred to scintillation vials containing the following medium: 0.7 % (w/v) PPO, 0.03 % (w/v) dimethyl-POPOP, 10 % (w/v) naphtalene per l of dioxane diluted 5 parts to 1 of water (v/v).

## RESULTS

Table I summarizes the pertinent data on marker enzymes in typical cell fractions used in this study. Glucose-6-phosphatase and rotenone-insensitive NADH–cytochrome *c* reductase, generally used as marker enzymes for endoplasmic reticulum, are found to a rather high extent in preparations of nuclei from liver and may be a constituent of the nuclear membrane[38]. Glucose-6-phosphatase also appears to be high in plasma membrane preparations compared to NADH–cytochrome *c* reductase and may be due to non-specific phosphatases present in plasma membranes. Golgi preparations from bovine liver contain high levels of rotenone-insensitive NADH–cytochrome *c* reductase but little or no glucose-6-phosphatase, so that the latter appears to be a good measure of contamination with endoplasmic reticulum[24]. In rat liver Golgi preparations, estimation of contamination with endoplasmic reticulum can be made using either rotenone-insensitive NADH–cytochrome *c* reductase or glucose-6-phosphatase with similar results[26]. The splitting of uridine diphosphogalactose to yield free galactose is a useful marker for plasma membranes in both rat liver and bovine liver and generally parallels other plasma membrane marker enzymes such as ATPase and 5′-nucleotidase[24, 26]. Galactosyltransferase is a marker enzyme for Golgi membranes in both rat and bovine liver, and the level of this activity in smooth microsomes is probably due to contamination with Golgi[24, 26].

Table II shows the distribution of several enzymes involved in the biosynthesis of lipids among the various subcellular fractions of beef liver. In agreeement with the findings of WILGRAM AND KENNEDY[1], and the recent reports of MCMURRAY AND

TABLE I

ANALYSIS OF LIVER CELL FRACTIONS

| *Assay* | *Beef liver* | | | | | | *Rat liver* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Nuclei* | *Mito-chondria* | *Microsomes* | | *Golgi* | *Plasma membrane* | *Microsomes* | | *Golgi* |
| | | | *Smooth* | *Rough* | | | *Rough* | *Smooth* | |
| Glucose-6-phosphatase | 0.036 | 0.004 | 0.16 | 0.12 | 0.012 | 0.029 | 0.15 | 0.21 | 0.012 |
| Succinate–cytochrome *c* reductase | 0.00 | 0.30 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 |
| NADH–cytochrome *c* reductase* | 0.148 | — | 2.0 | 1.7 | — | 0.091 | 1.2 | 2.4 | 0.25 |
| Galactosidase** | — | — | 36 | 8 | 53 | 350 | 6.1 | 36 | 64 |
| Galactosyltransferase** | — | — | 5 | 3 | 180 | — | 2.8 | 52 | 280 |
| $\mu$g P per mg protein | 38 | 8.5 | 40 | 38 | 43 | 23 | 41 | 30 | 22 |
| % Contamination*** with | | | | | | | | | |
| Smooth endoplasmic reticulum | | | | | | | | | |
| (NADH–cytochrome *c* reductase) | — | — | — | — | — | 5 | — | — | 10 |
| (glucose-6-phosphatase) | — | 2 | — | — | 8 | 18 | — | — | 6 |
| Plasma membrane (galactosidase) | — | — | 10 | 2 | 15 | — | 1 | 7 | 12 |

* Carried out in the presence of 4 $\mu$g rotenone per ml added as 1 mg/ml solution in ethanol.

** At 37°; all others at 32°. Rates expressed as nmoles/h per mg protein; all others as $\mu$moles/min per mg protein.

*** *i.e.* % contamination with smooth endoplasmic reticulum $= \frac{\text{specific activity in fraction}}{\text{specific activity in smooth endoplasmic reticulum}} \times 100$.

TABLE II

DISTRIBUTION OF CHOLINEPHOSPHOTRANSFERASE, ACYL-CoA : 1,2-DIACYL-*sn*-GLYCEROL ACYLTRANSFERASE AND ACYL-CoA : 1-ACYL-*sn*-GLYCERO-3-PHOSPHORYLCHOLINE ACYLTRANSFERASE AMONG VARIOUS SUBCELLULAR FRACTIONS OF BOVINE LIVER

The specific activities are expressed as nmoles product formed per min per mg protein. The relative specific activity of an enzyme in a given fraction is defined as its specific activity in that fraction divided by the specific activity of that enzyme in smooth microsomes. Relative specific activities × 100 are presented. The normalization of the relative specific activity of each cell fraction for phospholipid is based on the lipid phosphorus per mg protein values presented earlier (see refs. 23 and 44). For example bovine liver mitochondria have 5.8 μg lipid-P per mg protein (ref. 23). Hence the phospholipid normalization factor for this fraction would be 38/5.8 = 6.6 where 38 μg P per mg protein is the lipid phosphorus for bovine liver smooth microsomes. The % contamination with smooth endoplasmic reticulum was estimated by glucose-6-phosphatase for Golgi and mitochondria and by NADH–cytochrome *c* reductase for plasma membranes.

| *Bovine liver subcellular fraction* | *Cholinephosphotransferase* | | | *Acyl-CoA : 1,2-diacyl-sn-glycerol acyltransferase* | | | *Acyl-CoA : 1-acyl-sn-glycero-3-phosphorylcholine acyltransferase* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Spec. act.* | *Relative spec. act.* | *Relative spec. act. (phospholipid)* | *Spec. act.* | *Relative spec. act.* | *Relative spec. act. (phospholipid)* | *Spec. act.* | *Relative spec. act.* | *Relative spec. act. (phospholipid)* | *% Contamination with smooth endoplasmic reticulum* |
| Smooth microsomes | 2.43 | 100 | 100 | 2.27 | 100 | 100 | 28.4 | 100 | 100 | — |
| Rough microsomes | 1.34 | 55 | 80 | 1.66 | 73 | 106 | 23.7 | 83.5 | 121 | — |
| Golgi complex | 0.24 | 9.9 | 10.2 | <0.05 | <2 | <2 | 12.2 | 43 | 44 | 8 |
| Nuclei | 0.08 | 3.3 | 42 | 0.13 | 5.7 | 72 | 1.8 | 6.3 | 80 | — |
| Mitochondria | 0.024 | 1.0 | 6.6 | 0.05 | 2.2 | 14.5 | 1.0 | 3.5 | 23 | 3 |
| Plasma membranes | 0.015 | 0.6 | 2.5 | 0.09 | 4 | 16 | 1.2 | 4.2 | 16 | 5 |

DAWSON[2] and SARZALA *et al.*[3] on the distribution of cholinephosphotransferase in subcellular fractions of rat liver, negligible cholinephosphotransferase could be detected in nuclei, mitochondria, or plasma membranes of beef liver. Actually, the relative specific activity of cholinephosphotransferase (with respect to smooth microsomes) in these fractions is even lower than the relative specific activities of glucose-6-phosphatase (in mitochondria) and rotenone-insensitive NADH–cytochrome *c* reductase (in plasma membranes and nuclei). No significant synthesis of lecithin seems to take place in Golgi as well. The relative specific activity of cholinephosphotransferase in this fraction (9.9) hardly exceeds the corresponding value for glucose-6-phosphatase (8). Apparently, cholinephosphotransferase is located almost exclusively in smooth and rough endoplasmic reticulum. The specific activity of this enzyme (based on protein) is lower in rough than in smooth endoplasmic reticulum though the difference is less pronounced when the specific activity is based on $\mu$g lipid-P.

These results on the distribution of cholinephosphotransferase indicate that rough and smooth endoplasmic reticulum is the locus for lecithin synthesis *de novo*. Golgi complex, nuclei, mitochondria, and plasma membranes are probably highly dependent on the endoplasmic reticulum for the supply of new lecithins, possibly *via* an exchange mechanism as reported by WIRTZ AND ZILVERSMIT[39] and MCMURRAY AND DAWSON[2].

A similar distribution as observed for the lecithin synthesis was found for the synthesis of triacylglycerols from 1,2-diacyl-*sn*-glycerols and acyl-CoA esters. This synthesis seems to be localized almost exclusively in the smooth and rough endoplasmic reticulum. No significant synthesis of triacylglycerols could be observed in Golgi, mitochondria, or plasma membranes. The present data do not fully exclude the synthesis of triacylglycerols in nuclei.

Acyltransferases catalysing the conversion of 1-acyllysolecithin into lecithin were found to occur not only in smooth and rough endoplasmic reticulum, but also in the Golgi system. The relative specific activity of this enzyme in Golgi exceeds that of glucose-6-phosphatase by a factor of 5. It is more difficult to decide from the present data whether or not acyl-CoA: 1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase is present in nuclei and mitochondria because the relative specific activity of this enzyme in these fractions is only slightly higher than that of NADH–cytochrome *c* reductase (nuclei) and glucose-6-phosphatase (in mitochondria). For mitochondria one has to realize, however, as found previously for rat liver mitochondria, that the acyltransferases are located in the outer membrane[3,8,9] which represents only 6–10 % of the protein value of whole mitochondria.

The specific activities can be normalized to approximate for the amount of membrane in the cell fraction by dividing by the value of phospholipid phosphorus per mg protein. A comparison of specific activity based on protein and phospholipid is given in Table II. The only qualitative difference in interpretation is obtained for nuclei. On a membrane basis, the activities shown in the table represent an appreciable specific activity for nuclei compared to the smooth microsomes.

Table III presents data on the distribution of the enzymes discussed above, in smooth and rough microsomes and Golgi from rat liver. Similarly as found in beef liver, no significant *de novo* synthesis of lecithin, as measured by cholinephosphotransferase, could be detected in Golgi from rat liver. The relative specific activity found for acyl-CoA: 1,2-diacyl-*sn*-glycerol acyltransferase in Golgi is even a factor of

TABLE III

DISTRIBUTION OF CHOLINEPHOSPHOTRANSFERASE, ACYL-CoA : 1,2-DIACYL-*sn*-GLYCEROL ACYLTRANSFERASE, ACYL-CoA : 1-ACYL-*sn*-GLYCERO-3-PHOSPHORYLCHOLINE AND ACYL-CoA : 1-ACYL-*sn*-GLYCERO-3-PHOSPHORYLETHANOLAMINE ACYLTRANSFERASE AMONG VARIOUS SUBCELLULAR FRACTIONS OF RAT LIVER

Specific activities and relative specific activities and relative specific activities (phospholipid): see Table II.

| *Rat liver subcellular fraction* | *Cholinephosphotransferase* | | | *Acyl-CoA : 1,2-diacyl-sn-glycerol acyltransferase* | | | *% Contamination with smooth endoplasmic reticulum* |
|---|---|---|---|---|---|---|---|
| | *Spec. act.* | *Relative spec. act.* | *Relative spec. act. (phospholipid)* | *Spec. act.* | *Relative spec. act.* | *Relative spec. act. (phospholipid)* | |
| Smooth microsomes | 2.62 | 100 | 100 | 1.60 | 100 | 100 | — |
| Rough microsomes | 1.66 | 63.5 | 118 | 1.60 | 100 | 186 | — |
| Golgi complex | 0.25 | 9.6 | 13 | 0.04 | 2.5 | 3.4 | 8 |
| Endoplasmic reticulum-rich fraction * | 1.75 | 67 | 84 | 0.75 | 47 | 59 | 49 |

| *Rat liver subcellular fraction* | *Acyl-CoA : 1-acyl-sn-glycero-3-phosphorylcholine acyltransferase* | | | *Acyl-CoA : 1-acyl-sn-glycero-3-phosphorylethanolamine acyltransferase* | | | *% Contamination with smooth endoplasmic reticulum* |
|---|---|---|---|---|---|---|---|
| | *Spec. act.* | *Relative spec. act.* | *Relative spec. act. (phospholipid)* | *Spec. act.* | *Relative spec. act.* | *Relative spec. act. (phospholipid)* | |
| Smooth microsomes | 34 | 100 | 100 | 3.1 | 100 | 100 | — |
| Rough microsomes | 16.3 | 48 | 89 | 1.44 | 46.5 | 87 | — |
| Golgi complex | 1.9 | 5.6 | 7.6 | 0.06 | 2.0 | 2.6 | 8 |
| Endoplasmic reticulum-rich fraction * | 14.8 | 43.5 | 55 | 1.39 | 45 | 57 | 49 |

* Control fraction, isolated from zonal rotor, which is enriched with respect to endoplasmic reticulum and isolated from the same homogenate as the Golgi fraction.

4 lower than the value for cholinephosphotransferase in this fraction. This indicates that probably no synthesis of triacylglycerols takes place in Golgi from rat liver in agreement with the findings for Golgi from beef liver. On the other hand, it was found that in Golgi from rat liver, no significant conversion of 1-acyl-*sn*-glycero-3-phosphorylcholine can occur, this in strong contrast to the Golgi fraction from beef liver where significant acyltransferase activity could be detected.

Similarly as found for acyl-CoA: 1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase, no acyl-CoA: 1-acyl-*sn*-glycero-3-phosphorylethanolamine acyltransferase could be detected in Golgi from rat liver. Similar findings on the distribution of these enzymes were observed when acyl-CoA esters were replaced by free fatty acids in the presence of ATP and CoA in the assay system.

In view of the findings described above that the Golgi system apparently does not have the capacity of synthesizing lecithin *de novo*, it was thought of interest to study whether phospholipid synthesis can take place *via* the CDP-diglyceride pathway. Table IV presents the results obtained for the conversion of CDP-diglyceride and [$^{14}$C]glycero-3-phosphate into phosphatidylglycerol in the presence of various subcellular organelles from rat liver. It has been known since the work of KIYASU *et al.*[5] that the synthesis of phosphatidylglycerol takes place mainly in the mitochondrion. Significant synthesis of phosphatidylglycerol in endoplasmic reticulum has been observed by STUHNE-SEKALEC AND STANACEV[6] and recently it was found that also plasma membranes of rat liver have the enzymes required for the synthesis of this phospholipid[4]. The data presented in Table IV show that phosphatidylglycerol synthesis is indeed most active in the mitochondria. In addition significant synthesis of this phospholipid was found to occur not only in rough and smooth microsomes but also in the Golgi fraction.

Several authors have studied in the past few years the subcellular localization of phospholipases in rat liver[12–17, 4]. No information was, however, obtained thus far on the question whether or not Golgi possesses catabolic enzymes acting on phospholipids. Table V shows that rough endoplasmic reticulum contains a phospholipase A acting preferentially on the 1 position of exogenous 1-saturated-2-[1-$^{14}$C]-linoleoyl-*sn*-glycero-3-phosphorylethanolamine. This is in agreement with the recent observations of NEWKIRK AND WAITE[17]. Plasma membranes, on the other hand, contain a

TABLE IV

CONVERSION OF CDP-DIGLYCERIDES AND *sn*-[1-$^{14}$C]GLYCERO-3-PHOSPHATE INTO PHOSPHATIDYLGLYCEROL IN VARIOUS SUBCELLULAR FRACTIONS OF RAT LIVER

| *Rat liver subcellular fraction* | *Spec. act.* * | *Relative spec. act.* ** | *Relative spec. act.* ** *(phospholipid)* | *% Contamination* *** *with mitochondria* |
|---|---|---|---|---|
| Mitochondria | 0.175 | 100 | 100 | — |
| Rough microsomes | 0.023 | 13.1 | 4.6 | <0.2 |
| Smooth microsomes | 0.021 | 12.0 | 2.1 | <0.2 |
| Golgi | 0.019 | 10.9 | 2.6 | <0.2 |

* Specific activity expressed as nmoles [$^{14}$C]glycero-3-phosphate incorporated into phosphatidylglycerol per min per mg protein.

** See legend Table II.

*** Estimated on the basis of succinate cytochrome *c* reductase activity.

phospholipase A acting preferentially at the 2 position of exogenous phospholipids[4]. Golgi membranes appear to be distinct from both microsomes and plasma membranes in that they exhibit about equal levels of both $A_1$ and $A_2$ activity. The ratio of $A_1/A_2$ activity in Golgi appears to be intermediate between rough microsomes (derived almost exclusively from endoplasmic reticulum) and plasma membranes.

Lysosomal contamination of the Golgi and microsome fractions was estimated by aryl sulfatase activity and was found to be less than 1 % in microsomes and about 3 % in the Golgi fractions as compared to purified lysosomes prepared by the method of TROUET[40] as modified by LEIGHTON *et al.*[45]. Lysosomal contamination of the rat liver plasma membrane preparations has been shown previously to be less than 1 %. Under the assay conditions used, only phospholipase $A_2$ activity is measured in lysosomes and is approx. 32 nmoles/min per mg protein[41]. Thus approx. 1 nmole/min per mg protein of the phospholipase $A_2$ activity of the Golgi could be due to lysosomal contamination, so that the ratio of $A_1/A_2$ for Golgi could be as high as 2.4.

## DISCUSSION

The present paper represents the first study on the metabolism of phospholipids of the Golgi complex of beef and rat liver. Until recently, methods were not available to discriminate between Golgi membranes and endoplasmic reticulum and plasma membranes. We have shown, however, that galactosyltransferase is localized in Golgi vesicles in both beef and rat liver, and that enzymes such as glucose-6-phosphatase and galactosidase can be used to estimate contamination with endoplasmic reticulum and plasma membranes respectively[24, 26].

The present study shows that, both in beef and rat liver, the Golgi system does not possess cholinephosphotransferase, the enzyme required for the *de novo* synthesis of lecithin. This enzyme is localized exclusively in rough and smooth surfaced microsomes. Apparently Golgi apparatus as well as mitochondria and plasma membranes are dependent on the endoplasmic reticulum for newly synthesized lecithins. Also the synthesis of triacylglycerols from 1,2-diacylglycerols and acyl-CoA esters was found to be confined to the smooth and rough microsomes. The Golgi apparatus in rat liver has been shown to be involved in the secretion of very low density lipoproteins[42] which contain large amounts of both lecithin and triglycerides. Our results indicate that the lipid portion of the lipoproteins is synthesized in the endoplasmic reticulum rather than in the Golgi apparatus. Thus both lipid and protein must be

TABLE V

ACTIVITIES AND POSITIONAL SPECIFICITY OF PHOSPHOLIPASES A TOWARDS EXOGENOUS 1-SATURATED-2-[1-$^{14}$C]LINOLEOYL-*sn*-GLYCERO-3-PHOSPHORYLETHANOLAMINE IN SOME SUBCELLULAR FRACTIONS OF RAT LIVER

| *Rat liver subcellular fraction* | *Phospholipase $A_2$ activity (nmoles $^{14}$C fatty acid per min per mg protein)* | *Phospholipase $A_1$ activity (nmoles $^{14}$C monoacyl-phosphatidylethanolamine per min per mg protein)* | $A_1/A_2$ |
|---|---|---|---|
| Rough microsomes | 2.23 | 8.9 | 4 |
| Golgi complex | 2.9 | 4.6 | 1.6 |
| Plasma membranes | 6.6 | 1.4 | 0.2 |

transported to the Golgi apparatus in lipoprotein formation. This confirms the work of STEIN AND STEIN[43] who showed autoradiographically that labelled glycerol and palmitic acid are incorporated first in the rough and smooth endoplasmic reticulum of rat liver. The label appeared later in the Golgi apparatus which also contained small osmophilic particles, probably serum lipoproteins. However, the Golgi system of beef liver was shown to possess significant amounts of acyltransferases involved in the conversion of 1-acyl-*sn*-glycero-3-phosphorylcholine into lecithin. This acylation mechanism may enable the Golgi system to introduce subtle changes in the fatty acyl constituents of its phospholipids. Relevant to this observation is the finding that the Golgi system also appears to possess phospholipase A activity.

It is remarkable, however, that no significant acyltransferase activity was found in the Golgi complex of rat liver.

Interesting is the observation that the Golgi complex possesses the enzyme catalysing the conversion of CDP-diglyceride and *sn*-glycero-3-phosphate into phosphatidylglycerol. This enzyme, which is localized predominantly in the mitochondria, has been reported to occur also in endoplasmic reticulum[6] and plasma membranes[4]. Apparently, in contrast to *de novo* synthesis of lecithin which is confined to endoplasmic reticulum, a certain degree of phosphatidylglycerol synthesis can occur in several cell organelles. It would be very interesting to investigate in future studies also the localization of enzymes involved in the biosynthesis of other phospholipids such as sphingomyelin and phosphatidylinositol.

The Golgi complex from both rat and bovine liver is distinct from endoplasmic reticulum in that it lacks cytochrome P-450 and glucose-6-phosphatase activity[24, 26, 44]. Rat liver Golgi also lacks rotenone-insensitive NADH–cytochrome *c* reductase, which further differentiates it from endoplasmic reticulum[24, 44]. We now find further distinctions between Golgi and endoplasmic reticulum. Golgi complex from both rat and bovine liver lack cholinephosphotransferase and acyl-CoA: 1,2-diacyl-*sn*-glycerol acyltransferase activities. Rat liver Golgi also lacks acyl-CoA: 1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferases whereas significant amounts of these enzymes are present in bovine liver Golgi.

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