Metabolism of glycerol monoethers in cultured liver cells and implications for monoglyceride pathways

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Abstract A comparative study has been made of the assimilation and metabolism of *rac*-1 and 2-[9,10-^{3}H]-octadec-9-enylglycerol in a clone of epithelial-like cells isolated from rabbit liver. Based on cell protein content, the free glycerol ether isomers attained equal cellular concentrations. As shown by isolation and degradation experiments, however, the incorporation of radioactive 1-monoether was appreciably higher than that of radioactive 2-monoether in both the triacylglycerol and phospholipid fractions. The 1-monoether, unlike the 2-monoether, was also a significant source of esterified fatty acids in both lipid fractions. In addition, the 1-monoether, but not the 2-monoether, was an active precursor of plasmalogens, particularly ethanolamine plasmalogen. In contrast to the 1-monoether, the 2-monoether was a more active precursor of triacylglycerols than it was of phospholipids. The results indicate that in the rabbit liver cells the pathway of complex lipid synthesis from 1-monoether was via 1-alkyl-*sn*-glycerol-3-phosphoric acid and from 2-monoether via 1-alkyl-2-acyl-*sn*-glycerol.

Supplementary key words cell lipids · *rac*-1-octadec-9-enylglycerol · 2-octadec-9-enylglycerol · alkyl-diacyl-glycerol · alkyl-acyl-phospholipids · oxidation of alkyl glycerols · metabolism of alkyl glycerols · plasmalogens

Because of their greater resistance to hydrolysis, the monoether analogues of *rac*-1-monoacylglycerol and 2-monoacylglycerol have been successfully used in studying the monoglyceride pathway of triacylglycerol synthesis in the intestinal epithelium of the whole animal by Swell, Law, and Treadwell (1), and in everted intestinal slices by Kern and Borgström (2). Recently, the employment of 2-[9,10-^{3}H]octadec-9-enylglycerol enabled Schultz and Johnston (3) to demonstrate the hitherto unsuspected existence of the monoglyceride pathway for triacylglycerol synthesis in white and brown adipose tissue. In the microsomal fraction from the fat cells, the 2-monoether was a more potent source of higher glycerides than the *rac*-1-monoether. This discrimination was in agreement with earlier observations of Brown and Johnston (4) and Ailhaud et al (5) that 2-monoacylglycerol is the preferential isomer for diacylglycerol synthesis in homogenates and microsomal fractions of intestinal mucosal cells.

A possible role for the monoglyceride pathway in the lipid metabolism of cultured mammalian cells was based on the following considerations. Experiments with ^3H_2O, [^{14}C]glucose, and [^{14}C]acetate (6–8) have shown that the synthesis of fatty acids in cultured cells is repressed by the serum of the medium. In its presence, approximately two-thirds and one-half of the fatty acids of the cellular triacylglycerol and phospholipid fractions, respectively, are derived from albumin-bound fatty acids (9, 10). Similarly, recent isotopic experiments have indicated that only two-thirds and one-half of the glycerol moieties of the triacylglycerols and phospholipids originate from glucose, glycerol, and amino acids (11). Presumably, the sources of the remaining fatty acids and glycerol are the lipids of the serum lipoproteins (9), or their partial hydrolysis products. Inasmuch as 2-monoacylglycerol seemed a likely candidate for this role, we initiated a study of the metabolism of its monoether analogue, 2-[9,10-^{3}H]-octadec-9-enylglycerol, in our rabbit liver cell, which is rich in triacylglycerols and has an epithelial-like appearance in monolayer cultures (6, 12). Soon after these studies were begun, Lynch and Geyer (13) reported that cultured strain L mouse fibroblast cells extensively converted labeled *rac*-1-oleylglycerol to triacylglycerol and phosphoglycerides when paraoxon was added to the medium to inhibit serum esterase activity. For comparative purposes, we therefore also included *rac*-1-[9,10-^{3}H]octadec-9-enylglycerol in our investigations.

Abbreviations: *rac*-1- and 2-[9,10-^{3}H]octadec-9-enylglycerol are referred to as *rac*-1- and 2-monoether, respectively.

Finally, the metabolic reactions of glycerol ethers in mammalian cells are of interest in their own right. This is particularly so in view of the discovery by Snyder, Cress, and Stephens (14), Wood and Snyder (15), and Bollinger (16) that the ether bond is present in appreciable amounts in the triacylglycerol fraction of a number of human and animal tumors.

METHODS

Cell culture

Rabbit liver cells (12) were plated in replicate 6-cm Petri dishes and the initial cell protein determined as previously described (9). The stock medium in triplicate dishes was then replaced with 5 ml of our modification of Eagle's medium (17) containing 20% rabbit serum, and either the *rac*-1- or 2-radioactive monoether. Both media were replaced daily with appropriate fresh media, pH 7.4, and the cells were grown at 37.5°C in a constant atmosphere of 5% CO_2 in air (17). Media were sterilized by filtering through a 02 porosity Selas candle (Selas Flotronics, Spring House, Pa.). Rabbit serum was obtained from Colorado Serum Co., Denver, Colo.

Radioactive monoethers

The *rac*-1- and 2-[9,10-^{3}H]octadec-9-enylglycerol preparations, synthesized by the procedures of Wood and Snyder (18), were kindly provided by Dr. John M. Johnston, Department of Biochemistry, The University of Texas (Southwestern) Medical School. The radioactive monoethers were repurified on boric acid–silica gel G (E. Merck, Darmstadt) preparatory thin-layer plates (19) developed in chloroform–methanol 98:2 (v/v) (18). The radioactive monoethers were scraped from the plates, eluted with chloroform–methanol 2:1 (v/v), filtered, counted, and diluted with the corresponding nonradioactive monoether to obtain specific activities of 1.34–1.55 × 10^6 dpm/μmole. The preparations were stored in hexane at −20°C. The final *rac*-1-monoether preparation contained 98.7% of radioactive 1-monoether and 0.25% of radioactive 2-monoether. The 2-monoether preparation contained 96.8% of radioactive 2-monoether and 2.4% of radioactive 1-monoether. When the 2-monoether spot from the latter preparation was rechromatographed, approximately 2.5% of the ^{3}H again appeared in the 1-monoether spot. No correction was made for this contamination in the cell experiments. Scintillation counting and determination of dpm were carried out as previously described (11).

Labeling of serum

Nine to 10 μmoles of radioactive monoether in 10 ml of hexane were evaporated under N_2 in a 125-ml Erlenmeyer flask and bound to 20 ml of rabbit serum by shaking under N_2 at 80 rpm on a platform shaker for 165 min at room temperature. Radioactive uptake of the serum ranged from 75 to 80%, of which 90% passed through the Selas candles.

Isolation and fractionation of cell lipid

Cells were washed free of medium, their lipids were quantitatively extracted (20), and protein was determined (21) on the lipid-free residue as previously described. Lipid samples weighing 0.5–1.8 mg were separated into five fractions by silicic acid column chromatography (20) except that an additional diacylglycerol–cholesterol eluate was collected prior to the monoacylglycerol fraction, and the two phospholipid eluates were collected in 8 and 7 g portions of methanol. All fractions were dried and weighted to ±5 μg. The first methanol fraction, which contained over 90% of the cellular phospholipids, was washed three times, with complete mixing, by the procedure of Folch, Lees, and Sloane Stanely (22), filtered, evaporated to dryness under N_2, reweighed, and stored in chloroform at −20°C. The other lipid fractions were stored at −20°C in their eluting solvents.

Analysis of lipid fractions

The monoacylglycerol fractions and aliquots of the triacylglycerol fractions, both containing 40 μg of nonradioactive carrier *rac*-1- and 2-monoether, were chromatographed on boric acid–silica gel G plates as described above. Aliquots of the triacylglycerol fraction were also chromatographed on silica gel G plates in hexane–ethyl ether–acetic acid 80:20:1 (v/v). In all chromatographic procedures, the lipid was stained with iodine vapor and scraped into counting vials, unless otherwise specified.

Aliquots of the original triacylglycerol fractions, containing 1–4 × 10^4 dpm and 40 μg of each carrier monoether, were deacylated in duplicate (10). After cooling, 2.25 ml of water was added and the alkaline mixture was extracted three times with 4.0 ml of hexane. It was then acidified with 0.25 ml of 5.0 N H_2SO_4 and reextracted with hexane as described above. The combined recoveries of radioactivity exceeded 99%. Both the alkaline and acid extracts were chromatographed on boric acid–silica gel G plates in chloroform–methanol. In addition, both extracts were chromatographed by the procedure of Wood and Snyder (23) on silica gel plates (Adsorbosil-5, Applied Science Laboratories, Inc., State Colllege, Pa.), previously heated to 110°C for 30 min, and developed in diethyl ether–30% aqueous ammonium hydroxide 100:0.25 (v/v).

Both the phospholipid and triacylglycerol fractions were reduced with $LiAlH_4$ by the procedure of Wood and Snyder (23), modified by further acidification of the final reaction mixture with 1.0 ml of glacial acetic acid. The mixture was then extracted three times with 3.0 ml of diethyl ether with intervening centrifugations at 300 *g* for 5 min. Average recovery of radioactivity from phospholipid and triacylglycerol fractions containing $0.8–9.6 \times 10^4$ dpm was 97%. The reduced preparations were chromatographed on both the boric acid–silica gel G plates and on the silica gel plates (Adsorbosil-5) as outlined above. In control experiments with the radioactive monoethers, deacylation converted 1–3% of the radioactivity to methyl ester plus fatty acid, and reduction converted 2.6% of the radioactivity to alcohol. Appropriate corrections were made for these conversions in the cell experiments.

Aliquots of the phospholipids, to which nonradioactive carrier phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine had been added, were chromatographed on silica gel–magnesium acetate plates (Supelco, Inc., Bellefonte, Pa.) by the two-dimensional procedure of Turner and Rouser (24). The developing solvent in the first dimension was chloroform–methanol–28% aqueous ammonia 13:5:1 (v/v), and in the second dimension chloroform – acetone – methanol – acetic acid – water 3:4:1:1:0.5 (v/v). Radioactive plasmalogens were determined by the two-dimensional chromatographic procedure of Owens (25) in which the plate was sprayed with mercuric chloride after development in the first solvent. The plate was sprayed with 0.2% 2′,7′-dichlorofluorescein in ethanol and the aldehyde, phospholipid, and lysophospholipid spots were scraped into counting vials.

Reference lipids

The following lipids used as reference and carrier compounds were obtained from Supelco, Inc., Bellefonte, Pa.: phosphatidylcholine, phosphatidylethanolamine, ethanolamine plasmalogen, and methyl oleate. Oleyl alcohol, oleic acid, and trioleoylglycerol were obtained from the Hormel Institute, Austin, Minn., and octadecyl aldehyde sodium bisulfite from Aldrich Chemical Co., Milwaukee, Wisconsin. *Rac*-1 and 2-octadec-9-enylglycerol, 1,2- and 1,3-dioleoylglycerol were obtained from Serdary Research Laboratories, Inc., London, Ontario.

RESULTS

Three separate 3-day experiments using different lots of rabbit serum were carried out comparing the uptake and metabolism of radioactive *rac*-1- and 2-monoether in the rabbit liver cells. The average amount of protein per dish at the start of the experiments was 230 μg. In the first experiment, cell growth and lipid content were lower than anticipated (12, 20). The cells grew for 1.4 generations on both isomers, and the cell lipid to protein ratios were 0.31 and 0.25 for the cells grown in the *rac*-1- and 2-monoether media, respectively. In the next two experiments, 2.6 generations were obtained, and the lipid to protein ratios were approximately 0.56 and 0.41 for the *rac*-1- and 2-monoether cells, respectively. Despite these variations in growth and lipid content, the pattern of labeling of various lipid fractions by the *rac*-1-monoether vis a vis the 2-monoether was the same in all experiments. Consequently, the results obtained with each monoether have been averaged. As shown in **Table 1**, the total cell lipid was labeled much more extensively by the *rac*-1- than by the 2-monoether. In agreement with these results, the cellular radioactive accumulation was approximately 15% and 5% of that initially present in the *rac*-1- and 2-monoether media, respectively.

Cell lipid fractionation

Average recoveries of weight and ^3H from the cell lipid after silicic acid column chromatography were 98% and 91%. In view of their small weights and low specific activities, the third eluates of the triacylglycerol fractions were omitted in calculating the triacylglycerol to protein ratios and specific activities given in Table 1. Also, the lipid to protein ratios and specific activities of the phospholipid fractions were corrected for the weight and ^3H losses of approximately 25% and 5%, respectively, produced by the washing procedure (22) described in Methods. As shown in Table 1, both the triacylglycerol and phospholipid fractions of the *rac*-1-monoether cells possessed significantly higher specific activities than did the comparable fractions from the 2-monoether cells. On the other hand, there was no significant difference between the specific activities of the monoacylglycerol fractions, or the specific activities of the diacylglycerol fractions in the cells exposed to the two isomers.

It was instructive to calculate the dpm in the monoacylglycerol fractions per μg cell protein from the following equation: dpm/μg fraction × μg fraction/μg cell protein = dpm in fraction/μg cell protein. When this was done for each experiment, the mean values and standard errors obtained for the *rac*-1-monoether and 2-monoether cells, respectively, were 19.7 ± 1.97 and 24.1 ± 3.73 dpm per μg cell protein.

TABLE 1. Labeling of cell lipids by *rac*-1- and 2-[9,10-^{3}H]octadec-9-enylglycerol

Isomer in Medium	Cell Lipid Fractions									
	Total		Monoacylglycerol		Diacylglycerol[a]		Triacylglycerol		Phospholipid	
	Lipid	Sp Act	Lipid	Sp Act	Lipid	Sp Act	Lipid	Sp Act	Lipid	Sp Act
1	0.477 ± 0.083	324 ± 35.8[b]	0.036 ± 0.004	560 ± 90.7	0.033 ± 0.003	230 ± 33.8	0.240 ± 0.084	309 ± 50.6[b]	0.118 ± 0.007	369 ± 22.2[b]
2	0.357 ± 0.055	161 ± 18.1	0.028 ± 0.003	899 ± 228.7	0.030 ± 0.003	190 ± 70.4	0.141 ± 0.056	147 ± 29.0	0.097 ± 0.007	64 ± 3.8

Rabbit liver cells were grown for three days in 20% rabbit serum medium containing 65 μM of radioactive *rac*-1- or 2-monether. Cell lipid fractions were separated by silicic acid column chromatography and are expressed as μg lipid per μg total cell protein. The specific activities of the monethers, which ranged from 1.34 to 1.55 × 10^6 dpm per μmole in different experiments, have been normalized to 1.0 × 10^6 dpm per μmole. Aliquots of lipid samples were counted in duplicate or triplicate for 10^4 counts, and specific activities are expressed as dpm per μg lipid. All results are mean values and standard errors for three sets of experiments.

[a] Also contains free chloresterol.

[b] As determined by the *t* test (26), *P* values for the differences between mean specific activities of total lipid, triacylglycerol fractions, and phospholipid fractions were <0.02, 0.05, and <0.01, respectively.

Similar calculations for the diacylglycerol fractions gave values of 7.1 ± 0.52 and 5.5 ± 1.92 dpm per μg cell protein for the *rac*-1- and 2-monoether cells, respectively.

Location of radioactivity in monoacyl and diacylglycerol fractions

Monoacylglycerol fractions containing 5–7 × 10^4 dpm plus carrier *rac*-1- and 2-monoether, were chromatographed on boric acid–silica gel plates (18, 19). Over 95% of the ^{3}H present in all samples was located in the monoether spot that corresponded to the isomer present in the medium. Apparent isomerization to the opposite monoether accounted for less than 1.2% of the ^{3}H.

Diacylglycerol fractions containing 5.5 × 10^3 dpm, plus carrier 1,2- and 1,3-dioleoylglycerol, were chromatographed on silica gel G plates in chloroform–methanol–acetic acid 98:2:1 (v/v). The carrier spots together with a fainter spot preceding, but contiguous with, 1,3-dioleoylglycerol contained 95% of the ^{3}H.

Locations of radioactivity in triacylglycerol fraction

Thin-layer chromatography showed that the triacylglycerol fraction isolated by column chromatography contained no radioactive free *rac*-1- or 2-monoether, or radioactive nonesterified fatty acid. On the boric acid–silica gel G plates, all of the ^{3}H was located in a single spot that corresponded to triacylglycerol. On the silica gel G plates, 85% of the ^{3}H was in a faint spot with an R_f of 0.76 which preceded, but was contiguous with, the triacylglycerol spot of R_f 0.68. The latter spot contained 7% of the ^{3}H and the remainder of the radioactivity was distributed between it and the origin. Apparently alkyldiacylglycerol migrates slightly faster than triacylglycerol on silica gel G in the hexane–diethyl ether–acetic acid 80:20:1 (v/v) solvent system.

TABLE 2. Radioactivity in triacylglycerol fractions

Isomer in Medium	Dpm in Derivatives per μg Triacylglycerol Fraction		
	1-Monoether	2-Monoether	Esterified Fatty Acid[a]
1	289.4 ± 48.62	4.2 ± 1.13	15.3 ± 5.24
2	3.8 ± 0.52	140.4 ± 29.16	<0.5

Samples of triacylglycerol fractions (Table 1) containing 1–4 × 10^4 dpm were deacylated by methanolysis and reduced by $LiAlH_4$, each in duplicate. Lipid soluble products were chromatographed in duplicate as described in Methods, and counted for at least 2 × 10^4 dpm. Results are mean values ± standard errors.

[a] Appeared as long chain alcohol in reduced reaction mixtures.

In each experiment, deacylation of 100–200-μg samples of the triacylglycerol fractions eluted from the columns yielded the anticipated radioactive monoether. In addition, the triacylglycerol fractions from the *rac*-1-monoether cells also yielded appreciable amounts of radioactive fatty acid. Furthermore, equivalent amounts of long chain alcohol were generated when samples of these same fractions were reduced with $LiAlH_4$. The triacylglycerol fractions from the 2-monoether cells, on the other hand, failed to yield significant amounts of either radioactive fatty acid or radioactive alcohol. The experimental results are summarized in **Table 2**. From the specific activities of the triacylglycerol fractions (Table 1) and the average mol wt of 870 for triacylglycerols in the rabbit liver cell (10), it can be calculated that the radioactive *rac*-1- and 2-monoethers were the sources of approximately 27 and 12 moles %, respectively, of the triacylglycerol fractions in the present experiments.

Location of radioactivity in phospholipid fraction

One hundred μg samples of the phospholipid fractions were reduced and the products chromato-

TABLE 3. Radioactivity in phospholipid fractions

Isomer in Medium	Dpm in Derivatives per μg Phospholipid Fraction				
	1-Monoether	2-Monoether	Vinyl Ether	Aldehyde	Esterified Fatty Acid[a]
1	224.9 ± 22.82	23.1 ± 2.34	19.2 ± 1.35	28.8 ± 1.94	24.6 ± 6.25
2	5.9 ± 1.17	47.7 ± 1.83	1.2 ± 0.07	0.6 ± 0[b]	0.8 ± 0.33

Samples of phospholipid fractions (Table 1) containing 1.0–3.5 × 10^4 dpm were reduced with $LiAlH_4$. Products were chromatographed in duplicate in the two thin-layer systems described in Methods. Aldehyde was derived from the partial splitting of glycerol vinyl ether by acetic acid. Products were counted for at least 2 × 10^4 dpm. Results are means values ± standard errors.

[a] Appeared as long chain alcohol in reduced reaction mixtures.

[b] 1.8 dpm detected in one of three experiments.

graphed on the two thin-layer systems described under Methods. As shown in **Table 3,** the reaction mixtures from the *rac*-1-monoether cells contained, in addition to radioactive monoethers and radioactive alcohol (expressed as esterified fatty acid), appreciable amounts of radioactive glycerol vinyl ether and radioactive long chain aldehyde. Presumably the latter compound arose from the cleavage of vinyl ether when the reaction mixtures were acidified. Consequently, the dpm in vinyl ether plus the dpm in aldehyde represents the total dpm present as glycerol vinyl ether per μg phospholipid. Contrary to the above results, reduction of the phospholipids from the 2-monoether cells yielded only traces of radioactive alcohol, vinyl ether, and long chain aldehyde (Table 3). However, in all experiments approximately 10% of the monoether isomers had undergone isomerization; either in situ or during the $LiAlH_4$ reduction of the phospholipid fractions. By contrast, the isomerization observed in the reduced triacylglycerol fractions was less than 2%. In addition, an average of 14% of the ^3H in the reduced phospholipid fractions remained at the origin on the boric acid–silica gel G plates as contrasted with 5% in the reduced triacylglycerol fractions.

Locations of radioactivity in phosphatidylcholine and phosphatidylethanolamine fractions

Forty to 100 μg of the phospholipid fractions, containing carrier phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, were subjected to two-dimensional thin-layer chromatography (24). In all cases the radioactivity was highest in the phosphatidylcholine followed by the phosphatidylethanolamine fraction. Radioactivity, ranging from 5 to 10% of that present in the phosphatidylcholine fraction, was also detected in the phosphatidylserine fraction.

In the second and third experiments, aliquots of the phospholipid fractions, containing carrier phosphatidylcholine, phosphatidylethanolamine, and ethanolamine plasmalogen, were chromatographed in the two-dimensional mercuric chloride system for the identification of plasmalogens (25). Both the phosphatidylethanolamine and phosphatidylcholine fractions gave rise to radioactive aldehyde. The sum of the radioactivity in aldehyde, lyso derivative, and undegraded phospholipid was approximately the same as that obtained in the respective total phospholipid fractions (24). The results are summarized in **Table 4.** Plasmalogen accumulation was greater in the *rac*-1-monoether than in the 2-monoether cells. Moreover, in the 1-monoether cells, the percentage of plasmalogen present in the phosphatidylethanolamine fraction was much higher than in the phosphatidylcholine fraction.

DISCUSSION

Labeling of the triacylglycerol and phospholipid fractions by the radioactive monoether analogues of

TABLE 4. Radioactivity in phosphatidylcholine and phosphatidylethanolamine fractions

Isomer in Medium	Dpm in Constituent Phospholipid Fraction per μg Total Phospholipid Fraction					
	Phosphatidylcholine Fraction			Phosphatidylethanolamine Fraction		
	Total[a]	Plasmalogen	Lyso PC	Total[a]	Plasmalogen	Lyso PE
1	235.1	15.8	0.9	31.1	17.8	6.3
2	29.1	0.5	0.1	4.1	0.3	1.7
1	180.1	3.6	1.3	69.2	36.4	2.1
2	32.9	2.3	0	11.0	1.6	0

Samples of the phospholipid fractions from two experiments, and containing 4 × 10^3–5 × 10^4 dpm, were chromatographed in the systems for identification of individual phospholipids and plasmalogens described in Methods. Products were counted for at least 5 × 10^3 dpm. Dpm in plasmalogen are taken from the dpm in aldehyde produced by cleavage of the vinyl ether bond by $HgCl_2$. Lyso PC and lyso PE refer to the phospholipid products of this cleavage.

[a] Total includes dpm in alkyl ether, vinyl ether, and esterified fatty acid groups.

rac-1-oleoylglycerol and 2-oleoylglycerol indicated that the rabbit liver cells contain a monoglyceride pathway for lipid synthesis. Of greater interest, however, were the much higher specific activities of both the triacylglycerol and phospholipid fractions in the cells exposed to the *rac*-1-monoether as compared to those exposed to the 2-monoether (Table 1). This difference could not be accounted for by the inability of the cells to take up and concentrate the 2-monoether, for the cellular concentration of free radioactive 2-monoether was slightly higher than that of free radioactive 1-monoether. Neither was it attributable to the negligible oxidation of the alkyl chain of the 2-isomer, as compared with the 1-isomer, as can readily be seen from inspection of Tables 2 and 3. Since Snyder, Piantadosi, and Malone (27), have shown that the acylation reactions of *rac*-1- and 2-monoalkylglycerol in homogenates of liver and neoplastic tissues are the same as those described for the respective monoacylglycerols in microsomal preparations of the intestinal mucosa (4, 5), our knowledge of the metabolic reactions of the monoacylglycerols, in addition to those of the monoalkylglycerols, should be helpful in elucidating the striking differences in the synthesis of complex lipids from the monoether isomers in the rabbit liver cell.

Brown and Johnston (28) and Sundler and Åkesson (29) observed that the isomers of *rac*-1-monoacylglycerol are converted, without discrimination, to 1,3-diacylglycerol by intestinal mucosal microsomes. The 2-isomer, for which the preparations have a higher affinity, is converted to 1,2-diacylglycerol (4, 5, 29), specifically to 1,2-diacyl-*sn*-glycerol (29). Both of these reactions appear to be catalyzed by a single monoacylglycerol acylase (5). Formation of triacylglycerol from the above compounds occurs without their interconversion (4), and also appears to be catalyzed by a single diacylglycerol transacylase (5). Since 1,2-diacyl-*sn*-glycerol is more rapidly converted to triacylglycerol by intestinal epithelial cell microsomes (5, 29), and since it is also a more direct precursor of phospholipids (30) than the 1,3-isomer, the 2-monoether might well have been expected to be a more potent precursor of these lipids in the epithelial-like rabbit liver cell (6, 12). In fact, just the opposite was the case. This finding indicates that a pathway other than the one involving 1,3-diacylglycerol was active in the synthesis of radioactive triacylglycerol and radioactive phospholipid fractions from radioactive *rac*-1-monoether by the rabbit cell. Parenthetically, Paltauf (31) and Paltauf and Johnston (32) have found that, unlike its acyl analogue, 3-alkyl-*sn*-glycerol is not extensively acylated by either the intact intestine or its mucosal cell microsomes.

In 1962, Pieringer and Hokin (33) observed that a microsomal fraction of brain plus ATP converts *rac*-1- and 2-monoacylglycerol to 1-monoacyl-*sn*-glycerol-3-phosphoric acid. Isomerization of the 2- to the 1-isomer prior to formation of lysophosphatidic acid could not be excluded. These same investigators found that lysophosphatidic acid plus acyl-CoA yields phosphatidic acid in the presence of a cytoplasmic particulate fraction from brain or liver (34). Recently, Rock and Snyder (35) found that 1-alkyl *sn*-glycerol, but not the 2- or 3-*sn*-isomers, was converted to 1-alkyl-*sn*-glycerol-3-phosphoric acid and thence to 1-alkyl-2-acyl-*sn*-glycerol-3-phosphoric acid in the presence of ATP and acyl-CoA by rabbit Harderian gland microsomes.

These reactions provide an explanation for the higher labeling of complex lipids in the rabbit liver cell by *rac*-1-monoether than by the 2-monoether. Furthermore, reisolation of the respective radioactive monoalkylglycerol isomers from the triacylglycerol fraction (Table 2) and the phospholipid fraction (Table 3) is consonant with the existence of two different monoglyceride pathways for complex lipid synthesis in the same liver cells. These pathways may be designated as the more active lysophosphatidic acid pathway from 1-monoacyl-*sn*-glycerol, and the less active 1,2-diacyl-*sn*-glycerol pathway from 2-monoacylglycerol. If, in our experiments, only the 1-*sn*-isomer of the *rac*-1-monoether was active in labeling the complex lipids, as implied by the results of Rock and Snyder (35), its higher activity was achieved at a concentration only half that of the 2-monoether.

Regarding the oxidation of the glycerol-alkyl ether linkage, 5% and 7%, respectively, of the ^3H in the triacylglycerol and phospholipid fractions of the 1-monoether cells was present in the esterified fatty acid, presumably oleic acid (10). Contrarily, in the 2-monoether cells less than 1% of the ^3H was present in this form in either lipid fraction (Tables 2 and 3). These results with living cells are at variance with the results of Snyder, Malone, and Piantadosi (36) who found that the microsomal tetrahydropteridine-dependent cleavage enzyme of Tietz, Lindberg, and Kennedy (37), which oxidizes monoalkylglycerols to free glycerol and long chain aldehydes, exhibits the following preferences towards its isomeric substrates: 2- > 3- > 1-alkyl-*sn*-glycerol. Upon the addition of NAD to the enzyme system, which is located primarily in liver (36), the aldehydes are oxidized to fatty acids. Apparently some other system is responsible for the selective oxidation of the alkyl moiety of the *rac*-1-monoether in the rabbit liver cells. Perhaps, in complex lipids, this group is oxidized sequentially in situ to vinyl ethers and fatty acid

esters. If so, variable rates of oxidation would determine which plasmalogens will accumulate in the cells. It may also be mentioned that Oswald et al. (38) found that 8 hr after rats were injected intraperitoneally with radioactive *rac*-1-0- and 2-0-hexa- and -octadecyl glycerol ethers, very little labeled esterified glycerol ethers or phospholipids were found in the liver. On the other hand, in the intestine, appreciable amounts of glycerol ethers were converted to mono- and diesters, as well as to the alkyl derivatives of phosphatidyl choline, ethanolamine, and serine.

In the present experiments, appreciable vinyl ether formation was detected only in the phospholipid fraction of those cells exposed to the *rac*-1-monoether medium. Here, an average of 15% of the ^{3}H in the phospholipids was located in vinyl ether linkage (Table 3). As was to be expected (39), the radioactive phosphatidylethanolamine fraction contained more plasmalogen than the radioactive phosphatidylcholine fraction. These results on the formation of ethanolamine plasmalogen from the 1-monoether in living mammalian cells are in agreement with those obtained in cell-free systems by Wykle et al. (40) and Paltauf and Holasek (41) who demonstrated the direct conversion of 1-alkyl-2-acyl-*sn*-glycerophosphorylethanolamine to the corresponding plasmalogen by a microsomal mixed-function oxidase in Fisher R-3259 sarcoma and Ehrlich ascites cells (40), and intestinal mucosa cells (41).

Addendum. After submitting this paper, Edwards and Blough (42) reported experiments suggesting that the microsomes of chick embryo fibroblasts directly convert monoglycerides and cytidine diphosphate bases to the appropriate lysophosphatides without diglycerides as intermediates.

This work was supported by a research grant (AM-07162) from the National Institutes of Health.

Manuscript received 16 April 1976 and accepted 30 July 1976.

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