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THE ACYLATION OF 1-ACYLGLYCERO-3-PHOSPHORYLCHOLINES BY RAT-LIVER MICROSOMES

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SUMMARY

1. The transfer of acyl groups from acyl-coenzyme A derivatives to phosphatidylcholine by rat-liver microsomes was found to be significantly stimulated by the addition of synthetic 1-acylglycero-3-phosphorylcholines. Unsaturated acyl chains were transferred in preference to saturated ones, particularly when mixtures of CoA esters were present.

2. Of the series of 1-acylglycero-3-phosphorylcholines studied, (carrying decanoate, myristate, stearate, oleate or linoleate) only 1-decanoylglycero-3-phosphorylcholine was found to be a poor acceptor in the acyl-transfer reaction. The fatty acyl constituent of the other lysolecithins studied exhibited limited influence on the rate of acyl transfer.

3. Mixtures of various 1-acylglycero-3-phosphorylcholines were incubated with rat-liver microsomes in the presence of either labeled saturated or mono-unsaturated acyl-CoA derivatives. The radioactive lecithins synthesized were converted by phospholipase C (EC 3.1.4.3) into diglycerides which were separated in individual molecular species. The results demonstrated that under these conditions no preferential acylation of saturated, mono- or di-unsaturated lysolecithin occurred with either laurate, palmitate or oleate.

4. These findings are discussed with respect to the distribution of fatty acyl moieties in rat-liver lecithin.

INTRODUCTION

Saturated and poly-unsaturated fatty acids were found to be incorporated into the 1- and 2-positions, respectively, of lecithin in homogenates and mitochondrial and microsomal fractions of rat liver[1,2]. SCHERPHOF AND VAN DEENEN[3] demonstrated that the uptake of fatty acids under the conditions of these experiments proceeds mainly by a conversion of endogenous lysophosphoglycerides rather than by a *de novo* synthesis *via* acylation of glycerol 3-phosphate to phosphatidic acid. The enzymic acylation of lysolecithins was first found to occur in rat-liver microsomes by LANDS[4]. Both 1-acyl and 2-acyl isomers of lysophosphoglycerides appeared to be converted

into the corresponding diacyl analog. When mixtures of isomeric lysophosphoglycerides were used as substrates in the acylation reaction, the saturated acids were directed mainly to the 1-position whereas unsaturated acids favoured the 2-position, compatible with the distribution pattern of saturated and unsaturated acyl moieties that is known to prevail in liver phosphoglycerides[5,6]. Further detailed studies of LANDS *et al.*[7,8] showed an agreement between relative rates of acyl-CoA:lysolecithin acyltransferase activities for the esterification of various acids at the 1- and 2-positions and the positional distribution ratio of these acids in liver lecithins. Recently, however, STOFFEL, SCHIEFER AND WOLF[9] arrived at the conclusion that the microsomal acyltransferases showed no specificity for various natural or unnatural acyl-CoA derivatives when tested with 1-acylglycero-3-phosphorylcholine as acceptor*. This paper deals with the selectivity of the acyltransferases of rat-liver microsomes for the transfer of both saturated and unsaturated fatty acyl moieties from their CoA derivatives to 1-acylglycero-3-phosphorylcholines. Various synthetic 1-acylglycero-3-phosphorylcholines containing fatty acid constituents differing in both chain length or degree of unsaturation were utilized to establish whether differences exist in metabolic behaviour between the various molecular species of lysolecithin.

METHODS

For the preparation of acyl-CoA derivatives, essentially the method of GOLDMAN AND VAGELOS[10] was followed. Radioactive fatty acids were purchased from the Radiochemical Centre (Amersham, Great Britain). Fatty acids were obtained from Fluka A.G. or the Hormel Institute.

Microsomal preparations were obtained by differential centrifugation of freshly prepared 10% homogenates in 0.25 M sucrose containing 0.02 M Tris chloride buffer (pH 7.2). The subcellular particles that sedimented between $15000 \times g$ (15 min) and $100000 \times g$ (60 min) were collected and resuspended in 0.1 M Tris (pH 7.2) in 0.125 M KCl. The protein content of the microsomal suspensions was determined according to the method of LOWRY[11].

The various 1-acylglycero-3-phosphorylcholine substrates were prepared by snake venom *(Crotalus adamanteus)* degradation of the corresponding diacyl compounds, synthesized by acylation of glycero-3-phosphorylcholine with acyl chlorides as described by BAER AND BUCHNEA[12]. Gas–liquid chromatography revealed that the lysolecithins contained over 98% of the indicated fatty acids, except the 1-decanoylglycero-3-phosphorylcholine, which showed a 5% contamination each of 1-lauroyl- and 1-myristoylglycero-3-phosphorylcholine.

The enzyme assay of acyl-CoA: phospholipid transferase was performed in a standard incubation mixture containing, unless otherwise stated, the following components: 200 mμmoles of 1-acylglycero-3-phosphorylcholine (pipetted from a clear emulsion with a concentration of 2 μmoles/ml, obtained by ultrasonication), 60 mμmoles of [1-^{14}C]acyl-CoA (spec. act. 2.85 μC/μmole), 10 μmoles of $MgCl_2$, 50 μmoles of Tris buffer adjusted with HCl to pH 7.2 and 200 μg of microsomal protein in a final volume of 1.0 ml. Incubations were carried out in a shaking water bath at 37° and after appropriate times the reactions were stopped by addition of 3 vol. of

* However, see NOTE ADDED IN PROOF on p. 623.

methanol–chloroform (2:1, v/v). Lipids were extracted 3 times according to the method of BLIGH AND DYER[13]. Extraction was improved by thorough mixing with the aid of a Vortex mixer. Combined extracts were evaporated to dryness and dissolved in 0.5 ml methanol. Known quantities were applied to silica-impregnated papers. After development of the chromatograms and staining with Rhodamine 6 G phospholipid spots were cut out and transferred into counting vials containing 16 ml of a scintillation solution (5 g 2,5-diphenyloxazole (PPO) and 0.3 g dimethyl 1,4-bis-(5-phenyloxazolyl-2)-benzene (POPOP) per l toluene). The radioactivity of the samples was measured with a liquid scintillation spectrometer (Packard Tri-Carb), using a channel ratio method for quenching corrections. Enzyme activities were calculated from the observed count rates and the known specific activities of the acyl-CoA derivatives.

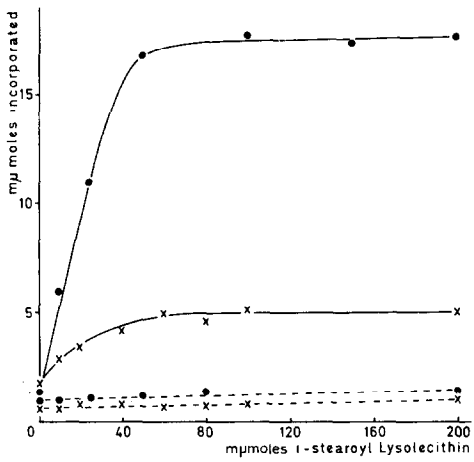
When lecithin was to be isolated for further investigations, (*e.g.* phospholipase A or C degradations), total lipid extracts were subjected to thin-layer chromatography. After development in chloroform–light petroleum–acetic acid (65:33:2, by vol.) to remove neutral lipids, the phospholipids were separated from each other in a second run with chloroform–methanol–water (65:35:4, by vol.) as developing solvent. Phospholipase A and C degradations were done in ether medium as described previously[14]. Diglycerides obtained by phospholipase C degradation of lecithins were separated into various molecular species on thin-layer plates coated with silica gel impregnated with $AgNO_3$ (refs. 15, 16).

RESULTS

Properties of the acyl-CoA: phospholipid acyltransferases

The effect of 1-stearoylglycero-3-phosphorylcholine concentration on the transfer of oleate and palmitate to phospholipids is reproduced in Fig. 1. Addition of lysolecithin increases the incorporation of both the unsaturated and the saturated acid, though the effect is much more pronounced for the former. The incorporation in phosphatidyl ethanolamine is fairly constant and independent of lysolecithin concentration, indicating that the enhanced uptake of fatty acid upon addition of lysolecithin is probably not due to a detergent effect. This is further confirmed by the observations that lysolecithin analogs possessing the same amphiphatic and surface active properties as lysolecithin itself, but lacking a free hydroxyl function, indeed do not stimulate the magnitude of fatty acyl transfer (Table I). In the plateau in Fig. 1 only about 30% of the acyl group of added acyl-CoA was found to be incorporated into lecithin. No appreciable transfer to other lipids could be detected. However, rat-liver microsomes contain an active acyl-CoA hydrolase, causing a decrease in substrate concentration[5,9]. Addition of new substrate gave rise to a further linear increase of incorporation, indicating that no significant loss of transferase activity took place during the incubation period.

The amount of fatty acyl transferred to phospholipid was proportional to the acyl-CoA concentration (Fig. 2) up to about 60 mμmoles/ml, both for palmitate and oleate. Higher concentrations were found to cause inhibition of acyl transferase, in agreement with the observations of LANDS AND MERKL[5] and of AILHAUD AND VAGELOS[17] on the acyltransferases of rat-liver microsomes and *Escherichia coli* membrane preparation, respectively.



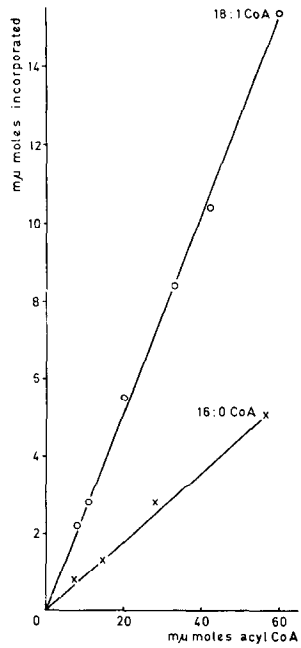


Fig. 1. Influence of lysolecithin concentration on the incorporation of fatty acids in phospholipids. Transferred quantities were determined after incubation of a standard reaction mixture for 30 min at 37° as described under METHODS, except that varying lysolecithin concentrations were used. ●——●, incorporation of [^{14}C]oleic acid into lecithin; ●– – –●, incorporation of [^{14}C]oleic acid into phosphatidyl ethanolamine; ×——×, incorporation of [^{14}C]palmitic acid into lecithin; ×– – –×, incorporation of [^{14}C]palmitic acid into phosphatidyl ethanolamine.

Fig. 2. Effect of the amount of acyl-CoA on the incorporation of acyl chains into lecithin. Standard reaction mixtures containing varying acyl-CoA concentrations were incubated for 20 min at 37°.

An optimal transfer of oleate to lecithin for the microsomal acyltransferase was measured at pH 7 (Fig. 3).

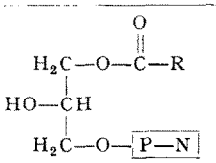
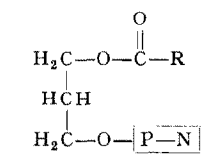
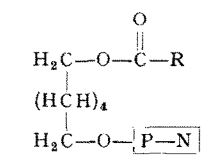
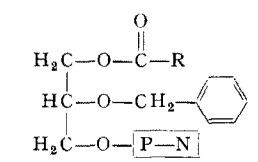
Influence of various 1-acylglycero-3-phosphorylcholines on fatty acyl transfer

The transfer of stearate appears to be stimulated to a limited extent only by the addition of 1-acylglycero-3-phosphorylcholines (Fig. 4). Although there are small differences to be noted between the various acceptors tested, both saturated and unsaturated 1-acylglycerol-3-phosphorylcholines promote the incorporation of stearate into lecithin. In principle the same is true for palmitate transfer. The small conversion of the various 1-acylglycero-3-phosphorylcholines with these long-chain saturated acyl groups into lecithin, however, does not allow one to conclude whether the differences observed are meaningful. The short-chain laurate causes much better conversions of 1-acylglycero-3-phosphorylcholines into lecithin. In this respect the behaviour of laurate appears to be intermediate between that of long-chain saturated and that of unsaturated fatty acyl groups. From the experiments with lauroyl-CoA and the unsaturated CoA esters it becomes clear that 1-decanoylglycero-3-phosphorylcholine hardly affects the fatty acyl transfer. On the other hand the transfer of laurate, oleate and linoleate is enhanced considerably by the addition of 1-acylglycero-3-phosphorylcholines carrying long-chain fatty acid constituents. Again only

TABLE I

EFFECT OF LYSOLECITHIN AND SOME STRUCTURE ANALOGS ON OLEATE TRANSFER FROM OLEOYL-CoA TO LECITHIN

Standard incubation mixtures containing the additions as indicated were incubated for 15 min at 37°. R stands for $C_{15}H_{31}$ and P–N indicates the phosphorylcholine moiety. The results are expressed in terms of total mμmoles [^{14}C]oleate transferred to lecithin after correction for the transfer in incubations containing no addition (approx. 1–2 mμmoles). Mean values of two experiments are given.

Addition *Structure*	*Amount (μg)*	*Stimulation of oleate transfer to lecithin (mμmoles)*
	10	7.0
	50	15.1
	100	16.2
	50	0.3
	100	0.5
	200	0.4
	50	0.0
	50	0.3
	100	0.3
	200	0.0

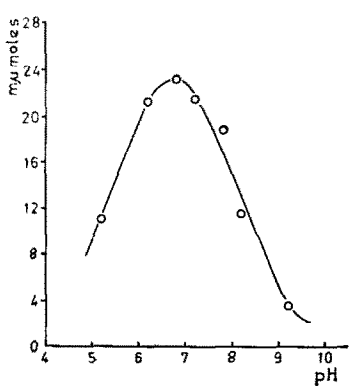


Fig. 3. Influence of pH on the oleoyl-CoA-phospholipid acyltransferase. Each incubation tube contained 4 μmoles of a Tris–maleic acid buffer adjusted to the appropriate pH instead of the Tris–HCl buffer used in the standard procedure. Incubation time was 15 min at 37°.

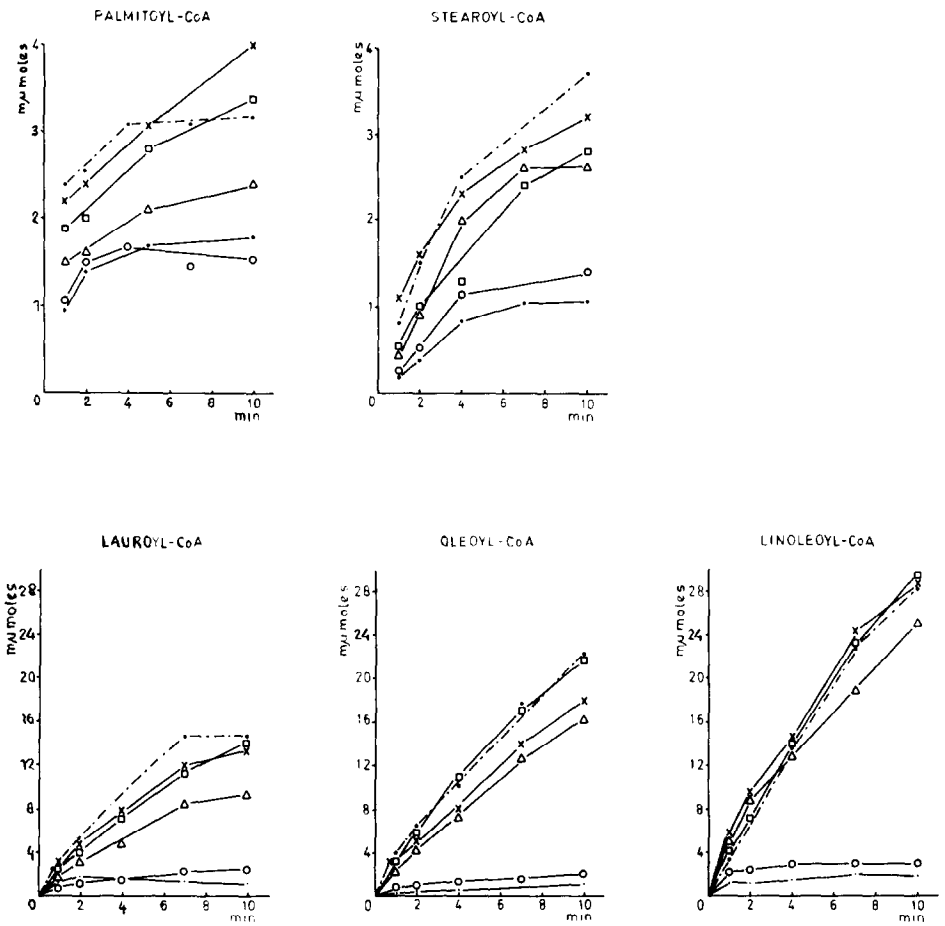


Fig. 4. Time courses for the stimulating effects of 1-acyl lysolecithins on the transfer of various fatty acyl groups from their CoA esters to lecithin. For incubation mixtures see METHODS. ●——●, no lysolecithin; ○——○, 1-decanoyl lysolecithin; ●—·—●, 1-myristoyl lysolecithin; △——△, 1-stearoyl lysolecithin; □——□, 1-oleoyl lysolecithin; ×——×, 1-linoleoyl lysolecithin.

minor differences between the various lysolecithins investigated are observed with respect to the rate of laurate, oleate and linoleate incorporation. Perhaps the 1-myristoylglycero-3-phosphorylcholine and the unsaturated lyso compounds produce the highest stimulating effect. In these acylation studies the acyl groups of the acyl-CoA-esters were found at the 2-position of the lecithins synthesized, as was shown by phospholipase A degradations of the isolated lecithins. In the studies of linoleate transfer, the formation of 1-linoleoyl-2-linoleoyl-glycero-3-phosphorylcholine was found to exceed that of 1-stearoyl-2-linoleoyl glycero-3-phosphorylcholine, in spite of the fact that the latter compound is one of the most abundant species of liver lecithin, whereas the former species is only present in trace amounts[16,18]. In general, it can be concluded from the results presented in Fig. 4, that during the acylation of 1-acylglycero-3-phosphorylcholine with unsaturated CoA-esters the production of lecithins carrying a saturated acyl group at the 1-position is not more favoured than that of lecithins having in this position a mono- or di-unsaturated acyl-constituent.

TABLE II

ACYLATION OF MIXTURES OF 1-ACYLGLYCERO-3-PHOSPHORYLCHOLINES WITH VARIOUS ^{14}C-LABELED ACYL-CoA ESTERS

Incubations were carried out on a scale 3 times the standard procedure for 10 min at 37°. Each tube contained a mixture of equimolar amounts of 1-acylglycero-3-phosphorylcholines as indicated. Abbreviations used: D, M, S, O and L designate 1-decanoyl-, 1-myristoyl-, 1-stearoyl-, 1-oleoyl- and 1-linoleoylglycero-3-phosphorylcholine, respectively. For the separation of the synthesized lecithins into various molecular species see METHODS

Lysolecithin mixture	*Acyl-CoA*	*Distribution of radioactivity (%), among species with indicated number of double bonds*			
		0	*1*	*2*	*3*
D-O-L	Oleate	—	7	50	43
M-O-L	Oleate	—	32	36	32
S-O-L	Oleate	—	26	37	37
D-O-L	Laurate	+*	51	49	—
M-O-L	Laurate	20	40	40	—
S-O-L	Laurate	25	30	45	—
D-O-L	Palmitate	+*	40	60	—
M-O-L	Palmitate	45	32	23	—
S-O-L	Palmitate	26	41	33	—

* Trace amounts of radioactivity (less than 2% of total) present.

Acylation of mixtures of 1-acylglycero-3-phosphorylcholines

In agreement with the above, when mixtures of saturated and unsaturated lysolecithins were studied, no preferential acylation of the saturated 1-acylglycero-3-phosphorylcholine with unsaturated fatty acyl groups was observed (Table II). When oleoyl-CoA was incubated with rat-liver microsomes and mixtures of lysolecithins existing as either 1-decanoyl-, 1-myristoyl- or 1-stearoylglycero-3-phosphorylcholine together with both 1-oleoyl- and 1-linoleoylglycero-3-phosphorylcholine, it was found that oleate was transferred to almost the same extent to the various lysolecithin acceptors (Table II). Only in the lysolecithin mixture in which the saturated constituent was 1-decanoylglycero-3-phosphorylcholine a low percentage of the incorporated oleate was found in the diglyceride species containing one double bond, indicating that 1-decanoylglycero-3-phosphorylcholine is poorly esterified with oleate so as to form lecithin. A similar behaviour was found for the transfer of laurate and palmitate. These saturated acyl chains are also incorporated into 1-myristoyl-, 1-stearoyl-, 1-oleoyl- and 1-linoleoylglycero-3-phosphorylcholine without significant differences between the various lysolecithin acceptors. Nevertheless, the acylation of lysolecithins carrying unsaturated acyl constituents usually exceeds that of the saturated lysolecithins to some extent. These observations agree with the results presented in Fig. 4, and most probably these effects have to be attributed to solubility factors. Apparently, it makes no difference whether the lysolecithins are added alone or as a mixture. It can be stated from these results that, at least for the acyltransferases of rat-liver microsomes, no clear-cut selective conversion of molecular species into lecithin occurs out of the mixture of the 1-acylglycero-3-phosphorylcholines studied. However, to act as a substrate for the acyl transferases the acceptor apparently needs a long hydrophobic carbon chain of a certain minimum chain length.

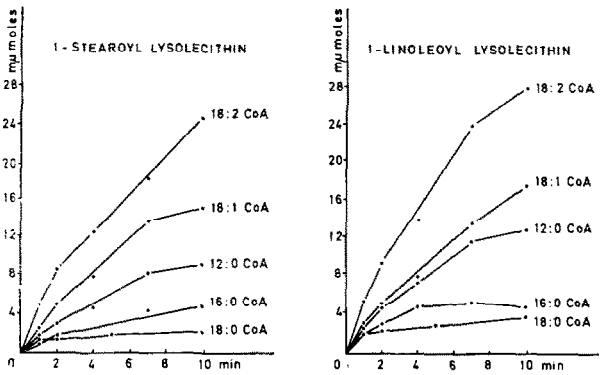


Fig. 5. Comparison of the transfer of various saturated and unsaturated acyl chains from their CoA esters to 1-stearoylglycero-3-phosphorylcholine and 1-linoleoylglycero-3-phosphorylcholine. For conditions see METHODS.

Acylation of 1-stearoylglycero-3-phosphorylcholine with mixtures of acyl-CoA esters

Fig. 5 demonstrates again that neither saturated nor unsaturated fatty acyl-CoA derivatives bring about a preferential conversion of 1-acylglycero-3-phosphorylcholine carrying either stearate or linoleate. However, a selective incorporation of unsaturated fatty acids into lysolecithins containing a free hydroxyl group in the 2-position is apparent. As noticed before, the short-chain saturated lauric acid displays a behaviour intermediate between the behaviours of the saturated and unsaturated acids, suggesting that the acyltransferases are adapted to maintain a popu-

TABLE III

ACYLATION OF 1-STEAROYLGLYCERO-3-PHOSPHORYLCHOLINE WITH MIXTURES OF SATURATED AND UNSATURATED ACYL-CoA ESTERS

Indicated quantities of acyl-CoA derivatives and 100 μg 1-stearoylglycero-3-phosphorylcholine were incubated with rat-liver microsomes for 15 min at 37°, either separately or simultaneously. Lecithins were isolated by thin-layer chromatography and treated with methanol–HCl (26 g HCl/l methanol) to convert the fatty acids into their methylesters. These were separated either by gas-liquid chromatography and collected in glass-wool filled tubes or by thin-layer chromatography on plates covered with silica gel impregnated with $AgNO_3$. Either glass-wool filled tubes or spots scraped from the thin-layer plates were transferred into liquid scintillation vials and measured for their radioactivity.

Acyl-CoA mixture	*Saturated/unsaturated ratios (mμmoles)*			*Ratio A/B*
	Amounts added (A)	*Incorporated into lecithin*		
		Incubated separately	*Incubated simultaneously (B)*	
Palmitate/oleate	83/18	7.2/4.7	3.8/5.4	6.6
	55/36	7.0/7.9	2.8/9.1	4.9
	28/72	4.2/10.7	1.4/11.4	3.2
Stearate/oleate	66/13	4.1/4.0	2.8/4.3	7.8
	66/52	4.1/8.3	1.3/7.4	7.0
	34/36	—	2.3/9.7	4.0
	16/52	2.2/8.3	0.9/8.8	3.1
Palmitate/linoleate	59/13	—	3.8/5.6	6.6
	36/38	—	1.4/9.6	6.3
	12/64	—	0.8/11.0	2.7
Stearate/linoleate	34/33	—	2.2/9.2	4.3

lation of molecular species of lecithin possessing particular physicochemical properties. Nevertheless, when the enzymes are provided with only long-chain saturated acyl-CoA esters a considerable amount of these acyl chains are transferred to the 2-position of lecithin, at least if an excess of 1-acylglycero-3-phosphorylcholine is added as fatty acyl acceptor. Apparently, there is no absolute specificity in the acylation, but certainly a fair degree of selectivity exists. To investigate this further rat-liver microsomes were incubated with mixtures of a saturated and an unsaturated acyl-CoA with 1-stearoylglycero-3-phosphorylcholine as acceptor. The results, presented in Table III, show, for instance, that the addition of only 18 mμmoles of oleoyl-CoA to incubation mixtures containing nearly 5 times as much palmitoyl-CoA reduces the incorporation of the latter acid 2-fold. When, for example, 83 mμmoles of palmitoyl-CoA or 18 mμmoles of oleoyl-CoA (ratio 4.6) are incubated separately with the microsomes, 7.2 mμmoles of palmitate or 4.7 mμmoles of oleate are incorporated into lecithin (ratio 1.5), indicating a selectivity for oleate. However, when both acyl-CoA derivatives are mixed and incubated simultaneously with the microsomes, the acids are incorporated in a ratio 0.7, indicating that the selectivity is still further increased by a factor of 2 when the acyl chains compete for the acyltransferases. Under these conditions a relative 6.6 times better esterification of oleate at the 2-position of 1-stearoylglycero-3-phosphorylcholine is observed. The degree of selectivity for this acceptor turns out to be dependent on the relative concentrations of the CoA esters, the preferential transfer of unsaturated acyl chains being relatively most pronounced when low concentrations of unsaturated CoA esters are present. This suggests again that one of the major tasks of the acyltransferases acting on 1-acylglycero-3-phosphorylcholines may be the preservation of a lecithin fraction having unsaturated acyl chains at the 2-positions.

DISCUSSION

The results presented in this paper demonstrate, in good agreement with the studies of LANDS and co-workers[5,7], that 1-acylglycero-3-phosphorylcholines are preferentially acylated with unsaturated acyl constituents by rat-liver microsomes. The selectivity of the acyltransferases acting on 1-acylglycero-3-phosphorylcholines is most pronounced under conditions where a competition exists for saturated and unsaturated acyl chains. In rat liver such lysolecithins may be produced by the action of phospholipase A_2, catalyzing the hydrolysis of the fatty acid ester linkage at the 2-ester position of phosphoglycerides. This enzyme was found to be associated to a large extent with the mitochondrial fraction[19] but it has to be noted that the enzyme was rather easily released from this subcellular fraction[20]. Furthermore, it is conceivable that intracellular transport of lysolecithins occurs. Production of 2-acylglycero-3-phosphorylcholine is catalysed by phospholipase A_1, an enzyme recovered mainly in the microsomal fraction of rat liver[14,19]. Conversion of 2-acylglycero-3-phosphorylcholine into lecithin by rat-liver microsomes was demonstrated by LANDS[5,7] to involve preferentially saturated acyl chains. Recent experiments in this laboratory with synthetic lysolecithin containing a labeled fatty acid constituent at the 2-position confirmed this conclusion. Present knowledge on formation and utilization of monoacylphosphoglycerides allows one to formulate a cycle accounting for the independent turnover of fatty acyl moieties at the two positions of rat-liver phosphoglycerides.

The selectivity of the transacylases in rat-liver microsomes appears to be compatible with the non-random distribution of different fatty acid constituents in the lecithin molecules*.

While it can be stated that this mono-diacylphosphoglyceride cycle in rat liver preserves the particular distribution pattern of saturated and (poly)unsaturated acyl chains, the question remains whether this feature is brought about primarily by this pathway or whether it occurs at an earlier stage *viz.* during the *de novo* synthesis of phosphoglycerides[21]. STEIN, STEIN AND SHAPIRO[22] reported a random incorporation of linoleic acid into aortic homogenates from rabbit upon addition of glycero-3-phosphate as acyl acceptor. LANDS AND HART[23] observed a random incorporation of linoleate and stearate into phosphatidic acid of guinea-pig liver, leading to the proposal that the asymmetric location of fatty acid may arise by a redistribution of acyl chains after the introduction of the nitrogenous moiety. Although carried out with the microsomal system of rat liver, some of our observations on the transacylation of various molecular species of 1-acyl lysolecithins are relevant to this problem. No significant differences were observed among the rates of conversion of 1-myristoyl-, 1-stearoyl-, 1-oleoyl- and 1-linoleoylglycero-3-phosphorylcholine into lecithin. This may suggest that the transacylation reaction, at least for these species, is not primarily responsible for the maintenance of non-random distribution of fatty acid constituents in lecithin of rat liver. On the other hand, the possibility can not be excluded that differences exist between the rate of formation of saturated and unsaturated 1-acylglycero-3-phosphorylcholines. Furthermore, the results obtained with 1-decanoylglycero-3-phosphorylcholine indicate that the nature of the acyl constituents present in the lysolecithin in principle may affect the rate of transacylation and it will be of interest to investigate the behaviour of 1-arachidonoylglycero-3-phosphorylcholine. Although it is not possible at the present stage of the investigations to reach final conclusions, it may be mentioned that SCHERPHOF[2] observed that in phosphatidic acid of subcellular fractions of rat liver, palmitate and stearate were incorporated into the 1-position, whereas linoleic acid and linolenic acid were preferentially esterified to the 2-position. The relative contributions of *de novo* synthesis and the redistribution cycle to the introduction of a non-random location of fatty acid constituents needs further investigation not only for different tissues[24], but also on the level of individual molecular species of phosphoglycerides.

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* Our results are in good agreement with similar experiments of Brandt and Lands, reported in the preceding paper of this volume (p. 605–612).

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After the submittance of this paper, a paper of STOFFEL *et al. (Z. Physiol. Chem.* 348 (1967) 882) appeared in which the preferential acylation of 1-acylglycerophosphorylcholines with unsaturated fatty acyl moieties was confirmed.