PRELIMINARY NOTES
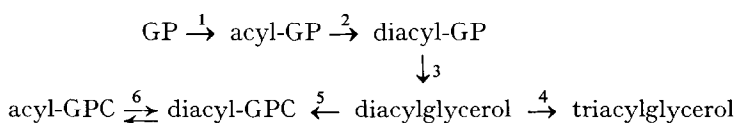
Incorporation of long-chain and polyunsaturated acids into phosphatidate and phophatidylcholine

Glycerol is incorporated into the glycerolipids of many tissues by the pathway described by KENNEDY and his co-workers[1,2]. These reactions (Reactions 1–5) provide diacylglycerols that may then form either trialcylglycerols or phosphatidyl derivatives.

$$GP \xrightarrow{1} \text{acyl-GP} \xrightarrow{2} \text{diacyl-GP}$$
$$\downarrow 3$$
$$\text{acyl-GPC} \underset{\xrightarrow{6}}{\rightleftarrows} \text{diacyl-GPC} \xleftarrow{5} \text{diacylglycerol} \xrightarrow{4} \text{triacylglycerol}$$

One phenomenon not fully explained at the present is that mammalian tissue phospholipids contain a much higher content of long-chain polyunsaturated acids at the 2-position than do the triacylglycerols[3,4]. In fact, MATTSON AND VOLPENHEIN[5] found in plant seed oils that acids with a chain length of more than 18 carbon atoms, regardless of their ethylenic bond content, were esterified almost exclusively at the primary position(s). One explanation would be that Reaction 4 selects only those diacylglycerols that do not contain polyunsaturated acids in accord with an earlier suggestion[2]. Alternatively, Reactions 1 and 2 may not effectively use these acids and thus produce diacylglycerols containing predominantly palmitate, oleate and linoleate. Polyunsaturated acids could then enter phosphatides via acyltransferase-catalyzed reactions (Reaction 6) with the monoacyl derivatives[6,7]. The following experiments were designed to test the selectivity of the transfer of these acids in Reaction 2 in comparison to that for Reaction 6.

1-Acyl-GP was prepared by phosphorylation of monooleoylglycerol with $POCl_3$ in chloroform[8] and was isolated as the ethanol-insoluble sodium salt (phosphorus: ester = 1.2). Acyl-CoA derivatives were prepared by condensation of the acid chloride with CoA as described by REITZ et al.[9]. Acid chlorides were obtained commercially or by treating the free acids (Hormel Institute) with oxalyl chloride. Quantitative gas chromatography of the methyl esters prepared from acyl-CoA's indicated purities of greater than 95% for the 20:3, 20:4, 20:5 and 22:1 and greater than 90% for the 22:6 thiol esters. All other CoA thiol esters were more than 99% pure. 1-Acyl-GPC was prepared by treatment of egg phosphatidylcholine with phospholipase A (ref. 10) and was isolated by column chromatography on silicic acid. Rat and guinea-pig liver microsomes were prepared as described earlier[6] and stored at −15° in 0.25 M sucrose–1 mM EDTA (25–40 mg protein/ml). Rat liver microsomes were treated with 0.1 vol. 10 mM DFP in propylene glycol for 30 min at room temperature prior to the assay for acyltransferase activity. Acyl-transfer reactions were assayed by following the increase in absorbance at 412 mμ produced by the reaction of the released mer-

TABLE I

EFFECT OF ACYL-CoA, ACYL-GP AND PROTEIN CONCENTRATIONS ON 1-ACYL-GP: ACYL-CoA ACYL-TRANSFERASE RATES

The reaction mixture contains 1 μmole DTNB, 90 μmoles Tris–chloride (pH 7.4), 0.20 mg protein (or as indicated) and 19 mμmoles acyl-CoA (or as indicated) and 56 mμmoles acyl-GP (or as indicated) in a total volume of 1.0 ml. The acyl-transfer rates are expressed in mμmoles/min per mg.

Addition	Acyl-CoA							
	16:0		18:1		20:2		20:5	
	Rat	Guinea pig	Rat	Guinea pig	Rat	Guinea pig	Rat	Guinea pig
Acyl-CoA (mμmoles)								
14	28	22	21	21	9	—	10	5
19	27	25	26	26	9	—	11	6
30	22	16	21	22	8	—	10	6
50	20	15	22	15	6	—	10	4
75	16	20	18	19	7	—	9	5
100	4	12	13	17	5	—	8	6
Acyl-GP (mμmoles)								
28	17	—	17	—				
35	—	33	—	19				
49	28	39	30	—				
63	—	38	27	47				
91	25	38	29	45				
105	23	36	23	50				
135	—	28	20	36				
Protein (mg)								
0.10	24	32	37	48			11	9
0.20	30	29	34	45			13	7
0.30	29	30	36	35			11	7
0.40	21	20	25	31			9	6

captan with DTNB[11]. The reaction was initiated by adding acyl-CoA after a 2-min preincubation of the protein. Control incubations were conducted for each acyl-CoA in the absence of acyl-GP and the observed endogenous rate (never greater than 5 mμmoles/min per mg) was subtracted from the rate observed in the presence of acyl-GP. 1-Acyl-GPC: acyl-CoA acyltransferase was measured in the same manner except 30 mμmoles of acyl-CoA and 175 mμmoles of 1-acyl-GPC were used.

To insure that the rates observed with different acyl-CoA's were optimal, the effect of varying amounts of acyl-CoA, acyl-GP and protein was determined. These results are summarized in Table I. Various concentrations of 4 different thiol esters were tested and found to give optimal rates over a wide range of concentrations. In agreement with our earlier results[6], high concentrations (75–100 μM) yielded lower rates of esterification with acyl-GP although the effect varied with different acyl-CoA's. Acyl-GP was rate limiting at lower concentrations and caused some inhibition at high concentrations. With low levels of acyl-CoA (19 μM) and acyl-GP (55 μM) the assay gave a linear response to protein in the range of 0.10 to 0.30 mg of protein (Table I). Particular attention was given to the above variables since earlier work[6] indicated that a non-linear relationship can occur with some thiol esters. This effect may explain the different values for rat liver[6]. Some variation was observed in the absolute turnover numbers for different batches of the particulate enzyme preparation in this work, but the standard errors presented in Table II are sufficiently small to allow useful comparisons among the various acid derivatives.

Acyl transferase rates for a series of acyl-CoA's with 1-acyl-GP and 1-acyl-GPC

are given in Table II. In accord with earlier findings[6], guinea-pig microsomes yielded slightly lower rates of esterification to 1-acyl-GPC when compared to rat liver. With the exception of 18:0, the acyl-CoA's containing 16 or 18 carbon atoms were readily esterified to the 2-position of acyl-GP regardless of their degree of unsaturation whereas acyl-CoA's containing 20 or 22 carbon atoms were not. The polyunsaturated acyl-CoA's were, however, relatively better substrates in both transfers to the 2-position than their corresponding saturate or monoene analogs. Furthermore, the long-chain polyunsaturated acyl-CoA's containing 20 carbon atoms were more rapidly esterified by rat liver microsomes to 1-acyl-GPC than to acyl-GP.

TABLE II

RELATIVE RATES OF ACYL TRANSFER TO ACYL-GP AND ACYL-GPC

The reaction mixture contained 1 μmole of DTNB, 90 μmoles of Tris–chloride (pH 7.4), 0.20 mg of protein and either 19 mμmoles acyl-CoA *plus* 56 mμmoles 1-acyl-GP or 25 mμmoles of acyl-CoA *plus* 175 mμmoles 1-acyl-GPC in a total volume of 1.0 ml. The values presented (mμmoles/min per mg microsomal protein) are the averages of assays (number in parentheses) with at least 3 different preparations of the particulate fraction. Each assay involved a continual recording of the products formed and thus each rate represents a large number of recorded observations.

Acyl-CoA	Guinea-pig microsomes		Rat-liver microsomes	
	1-acyl-GP	1-acyl-GPC	1-acyl-GP	1-acyl-GPC
16:0	16.6 ± 0.9 (5)	2.3 ± 0.5 (4)	18.1 ± 1.6 (4)	5.7 ± 1.7 (4)
18:0	9.1 ± 0.8 (6)	2.0 ± 0.5 (4)	9.9 ± 1.6 (4)	1.7 (ref. 7)
18:1 (n–9)	20.7 ± 1.4 (7)	12.4 ± 0.3 (4)	22.7 ± 1.9 (6)	12.4 (ref. 7)
18:2 (n–6)	21.5 ± 0.7 (5)	14.4 ± 0.5 (4)	18.1 ± 1.5 (5)	21.5 ± 0.7 (4)
18:3 (n–3)	18.3 ± 0.5 (5)	11.9 ± 1.3 (4)	19.6 ± 1.5 (4)	14.6 (ref. 7)
20:0	0.6 ± 0.2 (6)	0.3 ± 0.3 (4)	0.4 ± 0.1 (4)	
20:1 (n–9)	0.8 ± 0.3 (6)	1.6 ± 0.1 (4)	3.9 ± 0.4 (4)	1.5 ± 0.6
20:2 (n–9)	4.2 ± 0.4 (6)	9.0 ± 0.9 (4)	3.9 ± 0.5 (4)	16.8 ± 1.9 (4)
20:3 (n–6)	6.2 ± 0.8 (6)	19.5 ± 0.5 (4)	7.3 ± 0.6 (5)	29.2 ± 0.4 (2)
20:4 (n–6)	5.4 ± 0.8 (4)	10.6 ± 0.9 (4)	7.6 ± 0.8 (4)	22.3 ± 1.5 (4)
20:5 (n–3)	5.7 ± 0.9 (5)	15.0 ± 0.3 (4)	6.8 ± 1.0 (3)	29.3 ± 1.4 (4)
22:1 (n–9)	1.6 ± 0.5 (5)	0.2 ± 0.1 (4)	0.8 ± 0.2 (3)	0.0 (4)
22:6 (n–3)	4.2 ± 0.2 (5)	5.8 ± 0.7 (4)	4.2 ± 0.3 (3)	7.3 ± 1.3 (4)

Although the degree of unsaturation of the acyl group of the CoA thiol ester did influence the rate, it appeared to be of somewhat less importance to the acyl-GP: acyl-CoA acyltransferase than the length of the carbon chain. This contrasts to the conclusion of STOFFEL, DE TOMAS AND SCHIEFER[12] "Der Einbau der Fettsäuren war unabhängig von Struktur, Kettenlänge und Zahl der Doppelbindungen". Although our data do not preclude the synthesis of an arachidonoyl-diacylglycerol, they indicate that its rate of synthesis might be small compared to that for oleoyl- and linoleoyl-diacylglycerols. Such a suggestion is in keeping with the observed low content of 20:4 at the 2-position of rat liver triacylglycerols. Comparison of the relative rates of esterification of 20:2, 20:3, 20:4, 20:5 to 1-acyl-GP and 1-acyl-GPC by rat liver particles indicates that a more efficient incorporation of these polyunsaturated fatty acids may occur by the combined actions of phospholipases and acyltransferases upon phosphatidylcholine molecules rather than by way of phosphatidate synthesis *de novo*.

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Biosynthesis of cortisol from 3β, 17α, 21-trihydroxypregn-5-en-20-one by the intact human foetus at midpregnancy

It has been suggested previously that 3β,17α,21-trihydroxypregn-5-en-20-one (17α,21-Δ^5-P) is a normal constituent of human cord blood at term[1]. To explore the fate of this compound in the foetal compartment, we have perfused 2 foetuses at midpregnancy with 50 and 70 μC, respectively, of [7α-^{3}H]17α,21-Δ^5-P*.

The preparation and purification of this compound[2] and the conditions of perfusion[3] have been described elsewhere. The radiochemical purity of the material perfused is indicated in Table I.

TABLE I

RADIOCHEMICAL PURITY OF THE PERFUSED [7α-^{3}H]-3β,17α,21-TRIHYDROXYPREGN-5-EN-20-ONE

0.04 μC of [7α-^{3}H]-17α,21-Δ^5-P were mixed with 25 mg of authentic 3β,17α,21-trihydroxypregn-5-en-20-one.

Solvent	Crystals (disint./min per mg)	Mother liquors (disint./min per mg)
Methanol	3430	3630
Ethanol–benzene	3410	3470

The foetuses were perfused for 30 min. Upon completion of the perfusion, the adrenals were removed, homogenized and extracted by a method described previously[4]. The unconjugated radioactive material (corresponding in both cases to 1% of the perfused dose) was subjected to paper partition chromatography in System 1**,

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Abbreviations and trivial names: DHA: 3β-hydroxyandrost-5-en-17-one; Δ^5-P: 3β-hydroxypregn-5-en-20-one; 17α, Δ^5-P: 3β,17α-dihydroxypregn-5-en-20-one; 17α,21-Δ^5-P: 3β,17α,21-trihydroxypregn-5-en-20-one; cortisol: 11β,17α,21-trihydroxypregn-4-ene-3,20-dione.
* Purchased from New England Nuclear Co., Boston, Mass. (U.S.A.) in the form of the 3,21-diacetate (specific activity: 3.0 μC/μg).
** Systems used for paper partition chromatography at room temperature: No. 1. chloroform/ formamide; No. 2. isooctane–*tert.*-butanol–water (10:9:5, by vol.); No. 3. ethyl acetate–toluene–