THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 257, No. 24, Issue of December 25, pp. 14968-14972, 1982
Printed in U.S.A.

Selective Incorporation of Polyunsaturated Fatty Acids into Phosphatidylcholine by Rat Liver Microsomes*

(Received for publication, July 19, 1982)

William E. M. Lands‡§, Makoto Inoue¶, Yoshihiro Sugiura¶, and Harumi Okuyama¶

From the §Department of Biological Chemistry, University of Illinois Medical Center, Chicago, Illinois 60612 and the ¶Department of Biological Chemistry, Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya, 467 Japan

The various polyunsaturated fatty acids found in cellular lipids are transferred from their coenzyme A thiol esters to phospholipid acceptors with greatly different maximal velocities. The very low apparent K_m values for the thiol esters in acylating 1-acylglycerol-3-phosphocholine could not be directly measured, but their values could be estimated relative to that for arachidonate. The competitive effectiveness of various polyunsaturated acyl-CoAs was estimated by measuring the equivalent concentrations which allow incorporations equal to arachidonoyl-CoA. These values help predict the way in which various polyunsaturated acyl-CoAs may be selectively esterified to membrane lipids by the 1-acylglycerol 3-phosphocholine (1-acyl-GPC) acyltransferase system of liver microsomes. The acyl-CoA esters of saturated acids, as well as those for 22:1, 22:2, and 22:3, had negligible ability to compete for the active sites of the 1-acyl-GPC acyltransferase system. The acyl-CoA esters of arachidonate (20:4n-6), eicosatrienoate (20:3n-6), eicosapentaenoate (20:5n-3), and both isomers of linolenate (18:3n-6 and n-3) were handled preferentially by the 1-acyl-GPC acyltransferases. The system from liver has a high selectivity for unsaturated acids but does not appear to discriminate among the polyunsaturated acids of the n-6 and n-3 series that serve as precursors of prostaglandins and leukotrienes.

Polyunsaturated fatty acids found in higher animals can be grouped into three categories on the basis of their biosynthetic precursors: the oleate(n-9), linoleate(n-6), and linolenate(n-3) series (1). In mammalian tissues the double bond nearest the methyl end of the chain is not altered when new double bonds are inserted toward the carboxyl terminal, and the various fatty acids in the three categories are not metabolically interchangeable. Some of the polyunsaturated fatty acids may be converted to physiologically potent autacoids, but others are not (2, 3); some fatty acids may even inhibit the conversion of precursor acids to autacoids (4, 5). Consequently, the relative proportions of precursor and inhibitor acids at the 2-position of phospholipids may have an important influence upon cellular physiology.

The incorporation of polyunsaturated fatty acids into the 2-position of phospholipids is catalyzed by acyl-CoA:1-acyl-GP[1] and acyl-CoA:1-acyl-GPC acyltransferase systems. Studies on the specificities of these acyltranserase systems in liver have indicated that arachidonate and related long chain polyunsaturated acids may be esterified mainly by the 1-acyl-GPC acyltransferase system whereas monoene and diene fatty acids may be incorporated into phospholipids mainly by the 1-acyl-GP acyltransferase system (6). Tracer experiments *in vivo* or with tissue slices have supported this conclusion (7-9). Specificities for various acyl-CoAs as measured by maximal velocities *in vitro* were used successfully to predict the observed acyl chain compositions in erythrocytes (10). Studies with the more complex situation occurring in hepatocytes, however, indicated that relative turnover numbers alone do not clearly indicate the degree to which various fatty acids are esterified *in vivo* (6, 11) and that a consideration of K_m values is necessary. For example, oleate and arachidonate exhibited comparable maximal velocities *in vitro*, but the latter seems to be preferentially esterified in the acylation of 1-acyl-GPC *in vivo* (6, 9). The selectivities of acyltransferase systems that have been determined in the presence of mixtures of acyl donors and suboptimal concentrations of acceptors provide values that seem to be more closely correlated with *in vivo* phenomena (11-13). The design of those competitive studies requires mixtures of labeled acids, preferentially as CoA esters, such as [^{14}C]acyl-CoA and [^{3}H]acyl-CoA. Unfortunately, the labeled derivatives available at present do not allow many combinations of polyunsaturated acyl-CoAs for such assays. Thus, an alternate method is needed to evaluate the selective aspects of the competitive incorporation of various fatty acids into phospholipids.

This communication reports experiments which determine the relative competitive effectiveness of various polyunsaturated fatty acids as substrates for the 1-acyl-GPC acyltransferase system of rat liver microsomes by a method involving nonlabeled polyunsaturated fatty acyl-CoA esters.

MATERIALS AND METHODS

Fatty acids were obtained from Nuchek Preps, and [1-^{14}C]arachidonic acid was obtained from New England Nuclear. The coenzyme A thiol esters of polyunsaturated acids and [^{14}C]arachidonate were synthesized according to a previously described modification (14) of Seubert's procedure (15). Santoquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) was used as an antioxidant in an amount of 1% (w/w) of the weight of fatty acids. [^{3}H]Phosphatidylcholine was prepared biosynthetically by growing *Saccharomyces cerevisiae* for 20 h with

* This research was supported in part by a Scientific Research Grant from the Foundation for the Promotion of Research of Medicinal Resources, Ministry of Education, Science and Culture of Japan, a Fellowship from the Japan Society for Promotion of Science, and a Visiting Scientist Award from the United States-Japan Cooperative Science Program of the National Science Foundation and National Science Foundation Grant PCM 80-15638. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom reprint requests should be sent. Present address, Department of Biological Chemistry, University of Illinois at Chicago, Chicago, IL 60612.

[1] The abbreviations used are: 1-acyl-GP, 1-acyl-*sn*-glycerol 3-phosphate; 1-acyl-GPC, 1-acyl-*sn*-glycerol 3-phosphocholine; diacyl-GPC, 1,2-diacyl-*sn*-glycerol 3-phosphocholine; EqC, equivalent concentration.

[2-^{3}H]glycerol (New England Nuclear) followed by extraction and separation of lipids by thin layer chromatography. 1-Acyl-*sn*-[2-^{3}H] glycerol 3-phosphocholine was prepared by hydrolysis of the isolated [^{3}H]phosphatidylcholine with phospholipase A_2 (*Crotalus adamanteus* venom). The purity of the [^{3}H]1-acyl-GPC was at least 98% as judged by thin layer chromatography on Silica Gel H with chloroform/methanol/water (65:25:4) and chloroform/methanol/acetic acid/water (25:15:4:2) as solvents.

The incubation mixture for the incorporation of fatty acids consisted of 89 μM [^{3}H]1-acyl-GPC (4400 cpm/nmol under dual label counting conditions), 36 μM [^{14}C]arachidonoyl-CoA (2000 cpm/nmol, varying concentrations of another acyl-CoA, and 20 μg of microsomal protein in 0.3 ml of 0.1 M Tris-HCl (pH 7.4). The esterification reaction was linear at 25 °C for 10 min under the assay conditions. Routinely, the reaction was stopped after 7 min by adding 4 ml of chloroform/methanol (1:1), 2 ml of chloroform, and 1 ml of water. The extracted lipids in the chloroform layer were separated by thin layer chromatography on Silica Gel H with chloroform/methanol/acetic acid/water (25:15:4:2) as solvent. The area of the plate containing the product, diacyl-GPC, was scraped into a vial and counted in a toluene-Triton X-100/water system (16) with a liquid scintillation spectrometer (Searle Mark II). The ^{3}H to ^{14}C ratio was determined by the channels ratio method following correction for background and crossover. Settings of Range A200, levels 4–25 for the ^{3}H channel, and Range D-500, levels 8–∞ for the ^{14}C channel limited the crossover of ^{3}H into the ^{14}C channel and ^{14}C into the ^{3}H channel to 0.12 and 13%, respectively.

Arachidonate incorporation indicated by ^{14}C radioactivity in the diacyl-GPC was expressed as a percentage of the total acylation of 1-acyl-GPC, which was indicated by ^{3}H radioactivity in the diacyl-GPC fraction.

RESULTS

Under conditions that permitted the optimal rate of acylation of 1-acyl-GP, the CoA thiol esters of the unsaturated 18-carbon acids were the best substrates (Table I) followed by those of the saturated 16- and 18-carbon acids and the unsaturated 20-carbon acids. In contrast, the acylation of 1-acyl-GPC was most rapid with the CoA esters of 20:3n-6, 20:4n-6, and 20:5n-3, which were faster than the rates for the unsaturated 18-carbon acids. The saturated acyl-CoAs and 22-carbon acyl-CoAs with four and six double bonds were relatively poor substrates, and the 22-carbon acyl-CoAs with 1 to 3 double bonds exhibited almost no activity for the acylation of either 1-acyl-GP or 1-acyl-GPC. The esterification rates with acyl-GPC were not altered appreciably by reducing the thiol ester concentrations from 100 μM to 5 μM. Thus the apparent K_m values were less than 5 μM, in accord with earlier observations (11, 17), and the rates observed with only one acyl-CoA present are essentially V_{max} values.

TABLE I

Properties of polyunsaturated acyl-CoAs as substrates of 1-acyl-GP and 1-acyl-GPC acyltransferase systems

Maximal velocities (averages of three separate determinations) were determined spectrophotometrically (22). The incubation mixture consisted of 20 μM acyl-CoA, 50 μM 1-acyl-GP or 150 μM 1-acyl-GPC, 1 mM 5,5′-dithiobis-(2-nitrobenzoic acid), and 0.2 mg/ml of microsomal protein in 1 ml of 0.1 M Tris-HCl (pH 7.4). Equivalent concentrations (EqC_{25}) were determined graphically as described in Fig. 1. Concentrations of acyl-CoAs that caused 50% inhibition (IC_{50}) were determined from plots of the rate of acylation of [^{3}H]1-acyl-GPC *versus* the logarithm of the concentration of added acyl-CoA (see Fig. 4).

Acyl-CoA	1-Acyl-GP acyltransferase (maximal velocity)	1-Acyl-GPC acyltransferase			
		Maximal velocity	EqC	Competitive effectiveness	IC_{50}
	nmol/min/mg	*nmol/min/mg*	*μM*	*36/EqC*	*μM*
16:0	45	14	>500	~0	>200
18:0	35	11	>500	~0	>200
18:1(n-9)	61	51	140	0.25	>200
18:2(n-6)	60	56	50	0.72	>200
18:3(n-3)	75	45	25	1.4	>200
18:3(n-6)	[a]	28	20	1.8	>200
20:2(n-6)	16	35	[a]	[a]	>200
20:3(n-3)	18	21	300	0.12	>160
20:3(n-6)	19	79	45	0.80	>200
20:4(n-6)	15	72	35	1.0	>200
20:5(n-3)	13	72	22	1.6	>200
22:1(n-9)	~0	~0	>500	~0	[a]
22:2(n-6)	0.4	0.4	>500	~0	48
22:3(n-3)	0.4	0.4	>500	~0	130
22:4(n-6)	9	5	52	0.69	32
22:6(n-3)	8	14	200	0.18	180

[a] Values were not determined.

The following experiments were designed to estimate the relative ability of acyl-CoAs to compete with each other as substrates and/or act as inhibitors of the 1-acyl-GPC acyltransferase system. Varying concentrations of acyl-CoAs were incubated with rat liver microsomes, [^{3}H]1-acyl-GPC, and [^{14}C]arachidonoyl-CoA. The total acylation was estimated by measuring the amount of ^{3}H label in diacyl-GPC, the rate of incorporation of arachidonate by ^{14}C in diacyl-GPC, and the rate of incorporation of the other acids by the difference between ^{3}H and ^{14}C. The incorporation of [^{14}C]arachidonate relative to [^{3}H]1-acyl-GPC decreased when the concentration of the other unsaturated acyl-CoA was increased as illustrated by the examples shown in the semilogarithmic plots of Fig. 1.

Equal amounts of labeled arachidonoyl-CoA and the nonlabeled acyl-CoA were esterified when the incorporation of ^{14}C-arachidonate was 50% of that for the [^{3}H]1-acyl-GPC. For convenience in comparing different acids, an EqC was defined as the concentration of the other acyl-CoA which permitted the 36 μM arachidonoyl-CoA to comprise only 50% of the acids involved in converting acyl-GPC to diacyl-GPC (see Fig. 1). Smaller EqC values occur with the more effective substrates, and thus the value of the ratio 36/EqC indicates the competitive effectiveness of various acyl-CoAs as substrates relative to arachidonate. The wide range of values (from almost zero to 1.8; Table I) indicates that the effectiveness of each individual unsaturated acyl-CoA must be considered independ-

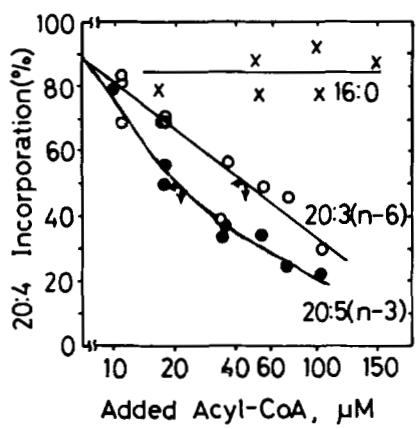

FIG. 1. **Determination of equivalent concentration of acyl-CoA in the acylation of 1-acyl-GPC in rat liver microsomes.** The incubation mixture consisted of 89 μM [^{3}H]1-acyl-GPC (4400 cpm/nmol under dual label counting conditions), 36 μM [^{14}C]arachidonoyl-CoA (2000 cpm/nmol), varying concentrations of other acyl-CoAs, and 20 μg/ml of microsomal protein in 1 ml of 0.1 M Tris-HCl (pH 7.4). The product, diacyl-GPC, was separated by Silica Gel H thin layer chromatography with chloroform/methanol/acetic acid/water (25:15:4:2) as solvent. The incorporation of arachidonate (^{14}C) expressed as per cent of the acylation of [^{3}H]1-acyl-GPC was plotted as a function of the exponential of the concentrations of added other acyl-CoAs. Equivalent concentration was defined as the concentration of other acyl-CoA that permitted to give 50% of the control level of arachidonate incorporation.

ently, and there is no "typical" unsaturated fatty acid. Furthermore, the different EqC values confirm that the V_{max} value determined in kinetic studies with an individual acyl-CoA is not an adequate index of the effectiveness of an acyl chain in the competitive acylations that occur *in vivo*. For example, oleate which has a maximal velocity that is 70% of that for arachidonate showed a competitive effectiveness of only 25% of that of arachidonate. On the contrary, γ-linolenate (18:3n-6) with a lower maximal velocity exhibited a higher competitive effectiveness. Also, 20:3n-6 and 20:5n-3 had maximal velocities similar to 20:4n-6 and showed a similar competitive effectiveness indicating only a very small apparent increase in effectiveness in the acyl transfer reaction with increased degree of unsaturation (20:3<20:4<20:5). However, 22:4n-6 with a maximal velocity only 7% that for arachidonate unexpectedly showed a 69% competitive effectiveness under these conditions.

Even though similar in molecular weight, hydrophobicity, charge, and the CoA moiety, the CoA esters of saturated acids did not compete appreciably with arachidonoyl-CoA for transfer to the 2-position. Palmitoyl-CoA at 20 μM displaced about 10–15% of the total arachidonate esterified, but increasing the concentration to 150 μM caused no further reduction in arachidonate incorporation. Limited incorporation was also exhibited by stearate (18:0) and the oligoenoic 22-carbon acids, 22:1n-9, 22:2n-6, and 22:3n-3 (Fig. 2).

At the present time, we do not know how many enzyme activities are involved in the acylation of 1-acyl-GPC in rat liver microsomes. Several lines of evidence suggest the presence of more than two 1-acyl-GPC acyltransferases (18–21). If only one of the 1-acyl-GPC acyltransferase activities is involved in the acylation of the 2-hydroxyl group with polyunsaturated acyl-CoAs, a simple kinetic equation derived earlier (11) may be applicable to the assay conditions: $v_1/v_2 = S_1/S_2 \times V_1/V_2 \times K_m^2/K_m^1$, where $v_2 = (V_2S_2)/(K_m^2 + S_2)$ represents arachidonate esterification. A plot of v_1/v_2 against S_1/S_2 for 20:4n-6 and 20:3n-6 exhibited a linear relationship from which the ratio of K_m values (K_m^2/K_m^1) can be estimated by dividing the observed value of the slope by V_1/V_2, the ratio of the corresponding maximal velocities that are given in Table I. In this case, the slope of 0.5 for 20:3n-6 (Fig. 3) gave a calculated ratio of 0.45 for $K_m^{20:4}/K_m$ indicating that the K_m for 20:3n-6 is higher than that for 20:4n-6 and that the trienoic acid would have a lower competitive effectiveness. Linoleate, 18:2n-6, also exhibited a linear plot with a slope of 0.5 leading to a ratio of 0.66 in close agreement with the observed competitive effectiveness of 0.72 relative to 20:4n-6. Curvilinear plots with the other unsaturated CoA esters probably reflect multiple enzyme activities. The curvature for 20:5n-3 was less than with many other thiol esters, and the data led to an estimated ratio of 1.2 which was in fair agreement with the observed compet-

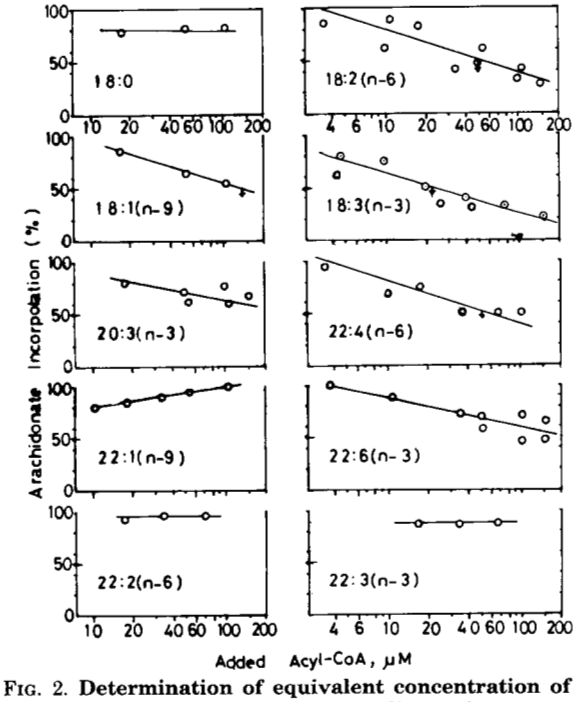

FIG. 2. **Determination of equivalent concentration of acyl-CoA in acylation of 1-acyl-GPC in rat liver microsomes.** Conditions were as noted in the legend for Fig. 1.

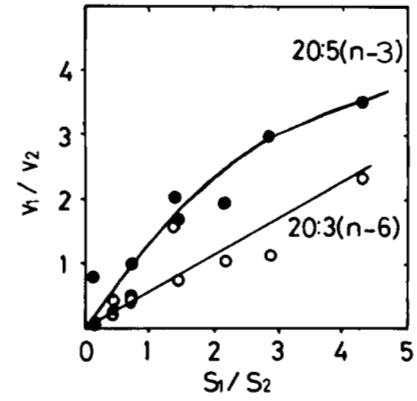

FIG. 3. **Determination of relative K_m values.** The incubation system was the same as in Fig. 1. The ratio of the rates of incorporation of other Acyl-CoA (v_1) and arachidonate (v_2) was plotted against the ratio of the concentrations of other acyl-CoA (S_1) and arachidonoyl-CoA (S_2 = 25 μM in this assay system). The relative K_m values ($K_m/K_m^{20:4}$) shown in Table I were estimated from the slopes and the maximal velocities as described in the text.

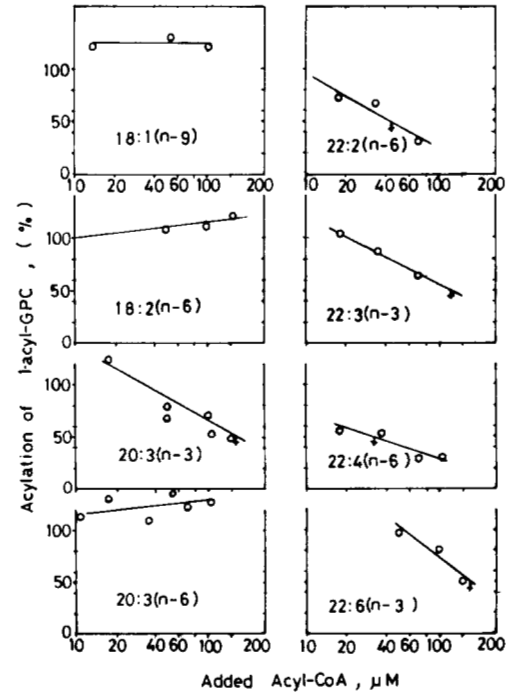

FIG. 4. **Inhibition of overall acyl transfer.** The concentrations causing 50% inhibition of the acylation of 1-acyl-GPC were determined by plotting the total acylation of [^{3}H]1-acyl-GPC (as per cent of control value) *versus* the logarithm of the concentrations of other added acyl-CoAs. The incubation system was the same as described in Fig. 1.

itive effectiveness of 1.6. Similarly, 18:3n-6 had an estimated ratio for $K_m^{20:4}/K_m$ of 2.3 which is in accord with the observed effectiveness that was 1.8 greater than for 20:4n-6. Although reasonable agreement was obtained between these two types of estimates of effectiveness as acyl donors for the four acids noted above, complex curvature with the other acids made further use of the simplified kinetic relationship for v_1/v_2 undesirable.

The 1-acyl-GPC acyltransferase system is relatively unaffected by the detergent action of higher concentrations of the long chain thiol ester substrates. Addition of more than 150 μM palmitoyl-CoA or oleoyl-CoA in addition to the 36 μM arachidonoyl-CoA did not inhibit the acylation of 1-acyl-GPC under the conditions used. However, a few acyl-CoA esters did inhibit the overall acylation of [^{3}H]1-acyl-GPC. The concentrations causing 50% inhibition (IC_{50}) were determined by plotting the total acylation of [^{3}H]1-acyl-GPC *versus* the logarithm of the concentrations of added acyl-CoA (Fig. 4). All five of the 22 carbon acyl-CoAs examined inhibited the acylation of 1-acyl-GPC, whereas all other thiol esters tested showed IC_{50} values above 150 μM. The IC_{50} values for the inhibitory acids do not correlate with the known detergent properties of various acyl-CoAs and may reflect a hitherto unrecognized selective structural interaction with 22-carbon acids rather than a consequence of general detergent properties.

DISCUSSION

The kinetics of acyltransferase systems have not been studied fully due to the inherent difficulties involved: membrane-bound enzymes, micelle formation with substrates, inhibition by higher concentrations of substrates, and binding of substrates to microsomal proteins and lipids (14, 22, 23). These factors have prevented an accurate assignment of the K_m values for acyl-CoA. Our previous (11, 12) and present methods of acyltransferase assay allow an estimate of the relative apparent K_m values for substrates. Although these values will be tentative until we know the number of acyltransferases involved, they indicate that selective competitive aspects of incorporation of the various polyunsaturated fatty acids into phospholipids do occur irrespective of the number of enzymes involved.

The two major parameters of simple enzyme-catalyzed reactions are V_{max} and K_m, and the reaction velocity is dependent upon $V_{max}/(K_m/S + 1)$. Each parameter provides a different insight into the specificity of the reaction. The EqC for acyl-CoAs that were determined by the present method are indirectly related to K_m values. The EqC values provide useful measures of the relative competitive effectiveness of different CoA esters as substrates that the maximal velocities alone cannot provide. By also taking the IC_{50} values into account, the various fatty acids examined can be classified into three types: one type (18:2n-6, 18:3n-6, 18:3n-3, 20:3n-6, and 20:5n-3) effectively competes with arachidonate with comparable V_{max} values and does not inhibit the overall acylation; one type (22:2n-6, 22:3n-3, and 22:4n-6) effectively competes but with low V_{max} values that effectively inhibit the overall acylation; and the last type (16:0 and 18:0) appears to neither compete with arachidonate nor appreciably inhibit the overall acylation. The latter type of acid may readily form some diacyl-GPC by esterifying the small amount of 2-acyl-GPC that forms by spontaneous isomerization of the 1-acyl-GPC substrate. Rapid but limited acylation occurred also with 22:1n-9, 22:2n-6, and 22:3n-3), all acids with a long segment of saturated hydrocarbon (C_{13}) attached to the carboxyl group. These acids seem more likely to be esterified by the 2-acyl-GPC system in a manner resembling the saturated fatty acids. The greater sensitivity of the 2-acyl-GPC acyltransferase(s) to inhibitory detergent effects (24) support this conclusion for 22:1n-9. This acid was better esterified when lower concentrations of its thiol ester were present. The competitive effectiveness values and the IC_{50} values predict the way in which mixtures of acyl-CoAs may be treated by the 1-acyl-GPC acyltransferase system in the more complex situation *in vivo* when the rate-limiting step of the assimilation of polyunsaturated fatty acids is the 1-acyl-GPC acyltransferase system. However, other steps such as membrane transport, activation to form acyl-CoA, and the accessibility of an acceptor pool must also be considered when evaluating the broader aspects of the comparative metabolism of polyunsaturated fatty acids *in vivo*.

Our results make it evident that the 1-acyl-GPC acyltransferase system of rat liver does not incorporate the n-6 and n-3 series of acids in appreciably different ways. Thus both types of fatty acid can be placed at comparable rates into membrane lecithins. The relatively effective acylation of 22:4n-6 compared to other 22-carbon acids suggests a possible special selectivity for this acid and leaves unanswered the question of which enzymes are responsible for the apparently avid retention of 22:6n-3 in mammalian cellular lipids. Recent studies (25, 26) suggest that the special retention of 22-carbon acids might occur more through the selectivity of the ethanolamine phosphotransferase than with the acyl-CoA acyltransferases.

Acknowledgments—We thank Y. Kume for her expert technical assistance.

REFERENCES

1. Klenk, E. (1965) in *Advances in Lipid Research* (Paoletti, R., and Kritchevsky, D., eds) Vol. 3, pp. 1–23, Academic Press, New York
2. Samuelsson, B., Goldyne, M., Granstrom, E., Hamberg M., Hammarstrom, S., and Malmsten, C. (1978) *Annu. Rev. Biochem.* **47,** 997–1029
3. Lands, W. E. M., Pitt, B. W., and Culp, B. R. (1980) *Herz* **5,** 34–41
4. Lands, W. E. M., LeTellier, P. R., Rome, L. H., and Vanderhoek, J. Y. (1973) *Adv. Biosci.* **9,** 15–27
5. Culp, B. R., Titus, B. G., and Lands, W. E. M. (1979) *Prostaglandins Med.* **3,** 269–278
6. Hill, E. E., and Lands, W. E. M. (1968) *Biochim. Biophys. Acta* **152,** 645–648
7. Hill, E. E., Husbands, D. R., and Lands, W. E. M. (1968) *J. Biol. Chem.* **243,** 4440–4451
8. Åkesson, B., Elovson, J., and Arvidson, G. (1970) *Biochim. Biophys. Acta* **210,** 15–27
9. Kanoh, H., and Ohno, K. (1973) *Biochim. Biophys. Acta* **306,** 203–217
10. Waku, K., and Lands, W. E. M. (1968) *J. Lipid Res.* **9,** 12–18
11. Okuyama, H., and Lands, W. E. M. (1972) *J. Biol. Chem.* **247,** 1414–1423
12. Okuyama, H., Yamada, K., and Ikezawa, H. (1975) *J. Biol. Chem.* **250,** 1710–1713
13. Possmayer, F., Scherphof, G. L., Dubbelman, T. M. A. R., van Golde, L. M. G, and van Deenen, L. L. M. (1969) *Biochim. Biophys. Acta* **176,** 95–110
14. Okuyama, H., Lands, W. E. M., Christie, W. W., and Gunstone, F. D. (1969) *J. Biol. Chem.* **244,** 6514–6519
15. Seubert, W. (1960) *Biochem. Prep.* **7,** 80–83
16. Okuyama, H., and Wakil, S. J. (1973) *J. Biol. Chem.* **248,** 5197–5205
17. Barden, R. E., and Cleland, W. W. (1969) *J. Biol. Chem.* **244,** 3677–3684
18. Jezyk, P., and Lands, W. E. M. (1968) *J. Lipid Res.* **9,** 525–531
19. Reitz, R. C., El-Sheikh, M., Lands, W. E. M., Ismail, I. A., and Gunstone, F. D. (1969) *Biochim. Biophys. Acta* **176,** 480–490
20. Okuyama, H., Eibl, H., and Lands, W. E. M. (1971) *Biochim. Biophys. Acta* **248,** 263–273
21. Hasegawa-Sasaki, H., and Ohno, K. (1975) *Biochim. Biophys. Acta* **380,** 486–495

22. Lands, W. E. M., and Hart, P. (1965) *J. Biol. Chem.* **240,** 1905–1911
23. Abou-Issa, H. M., and Cleland, W. W. (1969) *Biochim. Biophys. Acta* **176,** 692–698
24. Merkl, I., and Lands, W. E. M. (1963) *J. Biol. Chem.* **238,** 905–906
25. Kanoh, H., and Ohno, K. (1975) *Biochim. Biophys. Acta* **380,** 199–207
26. Masuzawa, Y., Nakagawa, Y., Waku, K., and Lands, W. E. M. (1982) *Biochim. Biophys. Acta*, in press