Biochimica et Biophysica Acta, 296 (1973) 563–571

BBA 56208

SELECTIVE TRANSFERS OF ACETYLENIC ACIDS TO FORM LECITHINS

YOICHI TAMAI[a], WILLIAM E. M. LANDS[a]*, J. A. BARVE[b] and FRANK D. GUNSTONE[b]

[a]*Department of Biological Chemistry, The University of Michigan, Ann Arbor, Mich. 48104 (U.S.A.) and* [b]*Department of Chemistry, St. Andrews University, St. Andrews (Great Britain)*

(Received September 28th, 1972)

SUMMARY

Although acetylenic acids are not normally found in mammalian tissues, octadecynoyl-CoA esters can serve as substrates for rat liver microsomal acyl-CoA: phospholipid acyltransferases. The relatively high rate of transfer to the 2-hydroxyl of 1-acylglycerol-3-phosphorylcholine by the 5-, 9-, and 12-positional *cis*, *trans* and *yne* isomers of acids supports the concept that π-bonds at these positions facilitate esterification irrespective of configuration. The preferred positional isomers for transfer to the 1-hydroxyl, however, are different for the *cis* and *yne* derivatives, indicating a sensitivity of the enzyme(s) to configuration. In the latter transfer, an alternating selectivity occurred with the acetylenic isomers between 8 to 13 that was opposite to the pattern for the *cis*-octadecenoyl transfers.

INTRODUCTION

Although unsaturated fatty acids may be generally regarded as desirable constituents of cellular lipids, the physicochemical properties that make them desirable are not clearly demonstrated. Acyl-CoA:phospholipid acyltransferases[1] have relatively high selectivities[2] for transfer to both the 1- and 2-position of phosphoglycerides and these selectivities show a good correlation with the naturally-occurring distributions of acids between these positions[3–5]. Since the two positions often contain saturated and unsaturated esters, respectively, the transferase activity forming the primary ester may select for some property of saturated acids and that forming the secondary ester for some properties of unsaturated acids.

In our studies of the criteria by which an acyl chain may be selected for incorporation into phospholipids, we have examined positional isomers of *cis*-[5,6] and *trans*-ethyleneic acids (ref. 7) as well as *cis*-cyclopropane derivatives[8]. A significant property of naturally-occurring saturated acids seems to be the absence of *cis*-9 or *cis*-11 configurations which are not favored by the transferase activity to the 1-position. Unsaturated derivatives, that are normally esterified to the 2-position, seem to have π-bonds at particular locations along the hydrocarbon chain with less regard

* To whom correspondence should be sent.

to the configuration of the unsaturated group. The continued observation of a very selective and sensitive structural and configurational interaction between the hydrocarbon chains of the acyl-CoA substrates and the catalytic proteins provides further insight into the nature of lipid–protein interactions. We hope that this model system will aid in defining the criteria that make unsaturated acids so valuable to living cells. The present report concerns acetylenic derivatives that contain π-bonds in a configurational arrangement different from that for the ethylenic acids.

MATERIALS AND METHODS

Synthesis of octadecynoic acids

15 of the 16 possible octadecynoic acids previously prepared and examined by Gunstone and co-workers[9-11] were made available for this study.

Synthesis of acyl-CoA derivatives

The acyl-CoA esters of the octadecynoic acids were synthesized from the free fatty acids by the slight modifications of Seubert's procedure[12] similar to those described by Okuyama *et al.*[8] and Reitz *et al.*[5]. The free acids (5 mg) were converted to acyl chloride by adding oxalyl chloride (about 0.5 ml) and removing the excess of oxalyl chloride completely under a stream of N_2 gas on a steam bath. This procedure was repeated three times. The oily residue was dissolved in 0.5 ml of freshly distilled tetrahydrofuran. The acid chloride solution was added in 0.1-ml portions to a solution of CoA (5 mg) dissolved in 5 ml of a mixture of tetrahydrofuran and water (7:3, v/v), while the pH was maintained carefully at 7.5–8.0 by dropwise addition of 1 M NaOH. The solution was stirred vigorously with a magnetic bar and the formation of thiolester was followed by measuring the decrease in mercaptan[13,14]. The reaction mixture was finally adjusted to pH 4.5–5.0 with a few drops of 10 % $HClO_4$ and the tetrahydrofuran was removed under a stream of N_2 gas. The solution was transferred to a centrifuge tube, adjusted to 2 ml with water, and the thiolesters were precipitated while cooling in an ice bath by adding 10 % $HClO_4$ to make the final concentration 1 %. The white precipitate was collected by centrifugation and then washed three times with light petroleum–diethyl ether (1:1, v/v). The white residue thus obtained was dissolved in 2 ml of water with the pH adjusted carefully to 5 with 5 % $NaHCO_3$. Any insoluble material still present at this pH was removed by centrifugation. The concentration of acyl-CoA was determined with the usual $A_{232\,nm}/A_{260\,nm}$ assay[12].

The yield was generally 25–50 % based on the initial amount of CoA. With some of the acyl-CoA isomers, the amount of CoA released by the microsomal acyltransfer system[14] was compared with the value obtained by $A_{232\,nm}/A_{260\,nm}$ assay. The agreement between these two values indicated that the synthesized material was 100 % reactive.

Preparation of microsomes

Male Sprague–Dawley rats (175–225 g), which had been fasted for 24 h, were sacrificed by decapitation, and the livers were perfused with cold 0.9 % NaCl (pH 7.4) containing 1 mM EDTA. After weighing, the livers were homogenized in 3 vol. of 0.25 M sucrose containing 1 mM EDTA (pH 7.4) using a Dounce ball-type homogenizer. The microsomal fractions which were used as an enzyme source were obtained

as the particulate fraction that sedimented between 20000 × *g* (20 min) and 100000 × *g* (90 min). In some experiments, microsomes suspended in 0.25 M sucrose containing 1 mM EDTA were further treated with 5 % sodium deoxycholate and albumin as described by Okuyama *et al.*[8]. In the present experiments, both EI and EII fractions[8] were combined and subjected to the assay. Because the enzyme preparations obtained by deoxycholate treatment were relatively labile as discussed by Reitz *et al.*[5] the enzyme was freshly prepared throughout the experiments unless otherwise indicated.

The protein content was estimated with a nomograph based on the values given by Warburg and Christian[15].

Preparation of phospholipid substrates

1-Acylglycerol 3-phosphorylcholine was prepared by venom-catalyzed hydrolysis of diacylglycerol 3-phosphorylcholine from egg and the 1-linoleoylglycerol 3-phosphorylethanolamine was prepared similarly from dilinoleoylglycerol 3-phosphorylethanolamine which was obtained by a transphosphatidylation reaction[16] of dilinoleoylglycerol 3-phosphorylcholine[7]. The products were purified by DEAE-cellulose and silicic acid column chromatography, and provided a single spot thin-layer chromatography.

2-Acylglycerol 3-phosphorylcholine and 2-acylglycerol 3-phosphorylethanolamine were prepared freshly before use by iodine cleavage of the corresponding alkenylacyl derivative isolated from beef heart with only slight modification of the procedures described by Lands[17], and Eibl and Lands[18].

Assay system

A typical acyltransferase reaction mixture contained 0.8 ml of 0.1 M Tris–HCl buffer (pH 7.4), 0.1 ml of 0.01 M 5,5′-dithio-bis-(2-nitrobenzoic acid), 20 nmoles of acyl-CoA, 0.2 mg of microsomal protein (or 0.1 mg of deoxycholate-treated microsome), and 0.1 ml of monoacylphosphoglyceride as the acceptor. The amount of acceptor was 175 nmoles of 1-acylglycerol 3-phosphorylcholine, 75 nmoles of 2-acylglycerol 3-phosphorylcholine, 200 nmoles of 1-linoleoylglycerol 3-phosphorylethanolamine or 75 nmoles of 2-acylglycerol 3-phosphorylethanolamine. The acyl-transfer rates were continously recorded in a spectrophotometer with the use of 5,5′-dithio-bis-(2-nitrobenzoic acid)[13] to measure the released CoA as described earlier[14]. The endogenous rate observed without added acylglycerol 3-phosphorylcholine or acylglycerol 3-phosphorylethanolamine was substracted to give a net acyl-transfer rate.

RESULTS AND DISCUSSION

Table I gives the acyl-transfer rates for acylglycerol 3-phosphorylcholine when the concentrations of isomeric acyl-CoA esters were varied. The velocity observed for each isomer was relatively constant over the range of 10–40 μM with a small inhibitory effect sometimes noted at 40 μM. On the other hand, acyl-CoA hydrolase activity increased to a high rate with the increasing concentrations of acyl-CoA in agreement with the earlier reports of a high apparent K_m for the hydrolase activity[1]. The acyl-transfer rate decreased and acyl-CoA hydrolase activity increased to a great degree with increasing concentrations of the Δ10 acyl-CoA. These unexpected findings observed for the Δ10-isomer will be discussed later in regard to its low transfer rate.

TABLE I

ACYL-TRANSFER RATES WITH VARYING CONCENTRATIONS OF ACYL-CoA

The reaction mixture contained 175 nmoles of 1-acylglycerol 3-phosphorylcholine, or 75–100 nmoles of 2-acylglycerol 3-phosphorylcholine, varying concentrations of acyl-CoA, and approximately 10 μl of a rat liver microsomal preparation (0.2 mg of protein) in a final volume of 1 ml. Each number represents an average of two to three assays which were, in themselves, a large series of continued measurements of reaction product and time. All values of the acyl-transfer rates have been corrected for hydrolase activity.

Acyl-CoA		*Hydrolase rate*	*Transferase rate nmoles/min per mg*	
Isomer	*Amount (nmoles)*	*(nmoles/min per mg)*	*2-position*	*1-position*
5	5	4.0	11.0	7.0
	10	4.5	11.5	13.0
	20	6.0	12.0	16.5
	40	7.5	9.0	15.0
6	5	4.5	4.5	28.5
	10	5.0	4.5	38.7
	20	5.0	4.5	42.5
	40	8.0	2.5	37.5
7	5	2.5	5.5	25.5
	10	4.5	3.0	23.0
	20	5.0	1.0	24.5
	40	5.0	4.0	23.5
8	5	4.0	7.5	47.0
	10	5.0	4.5	43.5
	20	5.0	4.5	43.0
	40	5.5	5.0	36.5
9	5	4.5	23.0	41.5
	10	5.5	30.0	46.0
	20	7.5	28.5	45.0
	40	8.0	31.5	44.5
10	5	3.5	11.5	9.0
	10	4.5	11.0	13.5
	20	6.0	11.0	11.0
	40	12.0	8.0	5.5
11	5	4.5	24.5	27.5
	10	5.5	24.5	28.5
	20	6.0	23.0	32.5
	40	7.5	20.0	34.0
12	5	5.0	31.0	12.5
	10	7.5	32.5	18.5
	20	8.5	31.5	21.5
	40	9.5	28.5	22.5
13	5	5.0	16.0	23.0
	10	5.5	19.5	33.5
	20	8.5	15.0	33.0
	40	11.5	15.0	32.0
14	5	6.0	25.5	29.0
	10	8.5	22.5	35.0
	20	12.0	22.0	47.0
	40	15.0	25.0	44.0
15	5	5.0	15.0	37.0
	10	6.0	14.0	43.0
	20	8.0	13.0	43.5
	40	10.0	10.5	42.0

Upon further treatment of the crude microsomes with sodium deoxycholate, the acyltransferase activity increased 2.7 times for 1-acylphospholipid and 1.8 times for 2-acylphospholipid acceptors. This procedure seemed to partially purify the acyltransferase activity for all of the isomer to a similar degree and not provide a selective purification.

Figs 1a and 1b illustrate the relative rates of esterification of the 15-isomers of acetylenic acyl-CoA by comparing the rates for the choline derivatives of monoacyl phospholipids with those for the ethanolamine derivatives. With most of the acyl-CoA isomers used, 1-acylglycerol 3-phosphorylcholine reacted more rapidly than 1-acylglycerol 3-phosphorylethanolamine. Nevertheless, the overall pattern of acyltransfer rates for 1-acylglycerol 3-phosphorylcholine was almost parallel to that for 1-acylglycerol 3-phosphorylethanolamine with the highest activity for the 9- and 12-isomers (Fig. 1a). The various acyltransfer rates for 2-acylglycerol 3-phosphorylcholine were essentially identical with those for 2-acylglycerol 3-phosphorylethanolamine with all isomers examined (Fig. 1b). With the enzyme preparations used, the 1-position of the phospholipids was generally acylated faster than the 2-position, especially with the ethanolamine derivatives. The highest velocities were observed with the 6-, 8-, 9- and 15-isomers. The 12-isomer was found to be transfered at a low rate to the 2-acyl derivatives in contrast to having one of the highest rates observed with the 1-acyl derivatives. The 10-isomer consistently exhibited low acyl-transfer rate

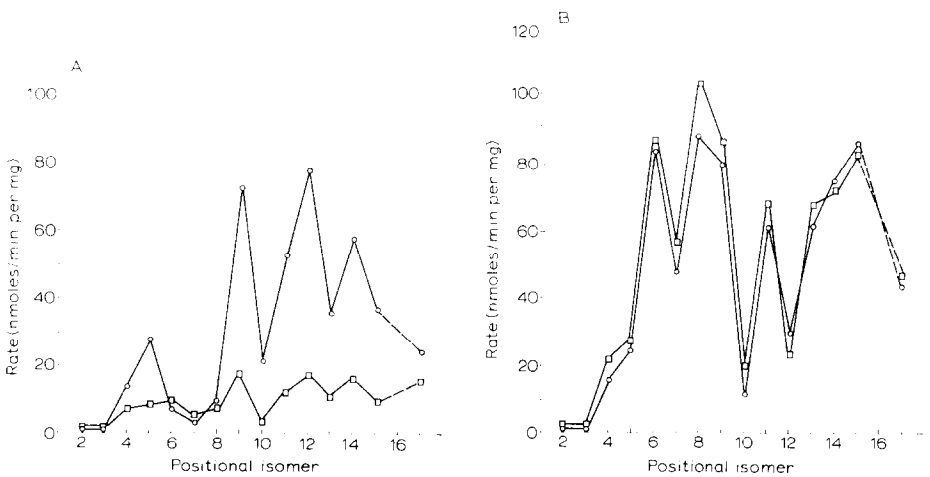

Fig. 1. (A) Acyltransferase specificities of rat liver microsomes with 1-acylglycerol 3-phosphorylcholine or 1-linoleoylglycerol 3-phosphorylethanolamine. The reaction mixture contained 20 nmoles of acyl-CoA, 175 nmoles of 1-acylglycerol 3-phosphorylcholine or 175 to 200 nmoles of 1-linoleoylglycerol 3-phosphorylethanolamine and 0.1 mg of sodium deoxycholate-treated microsomal protein in a final volume of 1 ml. Each rate represents an average of four separate assays, which have been corrected for hydrolase activity. ○—○, acyl-transfer rates to 1-acylglycerol 3-phosphorylcholine; □—□, acyl-transfer rates to 1-linoleoylglycerol 3-phosphorylethanolamine. (B) Acyltransferase specificities with 2-acylglycerol 3-phosphorylcholine or 2-acylglycerol 3-phosphorylethanolamine. The reaction mixture contained 20 nmoles of acyl-CoA, 75–100 nmoles of 2-acylglycerol 3-phosphorylcholine or 2-acylglycerol 3-phosphorylethanolamine and 0.1 mg of sodium deoxycholate-treated microsomal protein in a final volume of 1 ml. Each rate represents an average of four assays, which have been corrected for hydrolase activity. ○—○, acyl-transfer rates to 2-acylglycerol 3-phosphorylcholine; □—□, acyltransferase rates to 2-acylglycerol 3-phosphorylethanolamine.

TABLE II

EFFECTS OF VARYING CONCENTRATIONS OF 10-OCTADECYNOYL-COA ON ACYL-TRANSFER RATES OF PALMITOYL-COA AND LINOLEOYL-COA

The reaction mixtures contained either (A) 175 nmoles of 1-acylglycerol 3-phosphorylcholine or (B) 75 to 100 nmoles of 2-acylglycerol 3-phosphorylcholine, the indicated amount of acyl-CoA and 0.2 mg of rat liver microsomal protein in a final volume of 1 ml. Each transferase velocity is the average of three assays, which have been corrected for hydrolase activity.

(A)

Palmitoyl-CoA (nmoles)	*10-Octadecynoyl-CoA (nmoles)*	*Hydrolase (nmoles/min per mg)*	*Transferase (nmoles/min per mg)*
10	0	6.0	17.7
10	5	6.0	13.0
10	10	7.0	11.9
10	15	6.5	11.5
20	0	9.2	18.0
Palmitoyl-CoA (nmoles)	*12-Octadecynoyl-CoA (nmoles)*		
10	5	5.5	16.0
10	10	8.5	14.0

(B)

Linoleoyl-CoA (nmoles)	*10-Octadecynoyl-CoA (nmoles)*	*Hydrolase (nmoles/min per mg)*	*Transferase (nmoles/min per mg)*
10	0	6.0	23.5
10	5	4.2	23.3
10	10	7.0	23.1
10	20	6.7	22.8
20	0	7.2	25.6
Linoleoyl-CoA (nmoles)	*7-Octadecynoyl-CoA (nmoles)*		
10	5	4.0	20.5
10	10	5.0	20.5

both for monoacylglycerol 3-phosphorylcholine and monoacylglycerol 3-phosphorylethanolamine in contrast to the high rates shown by adjacent positional isomers.

To examine whether the low activity for the acetylenic 10-isomer was due to some inhibitory effect of either a contaminant or to the isomer itself, its effects on palmitoyl-CoA and oleoyl-CoA transfer were examined (see Table II). In addition, the 7- and 12-octadecynoyl-CoA, which showed low transferase rates for 2-acylglycerol 3-phosphorylcholine and 1-acylglycerol 3-phosphorylcholine, respectively, were also examined for comparison. Adding the 10-isomer caused no inhibitory effect on the acylation of 1-acylglycerol 3-phosphorylcholine with linoleoyl-CoA and the velocity of palmitate transfer to 2-acylglycerol 3-phosphorylcholine showed only a slight decrease as the amount of the added 10-isomer increased. Thus, there was no evidence of any inhibitor and the low transfer rates observed for the 7-, 10- and 12-isomers were more likely due to the property of each acid, in itself, not being a favored substrate for the enzyme.

Fig. 2A compares the pattern of selectivity in the acylation of the 2-position

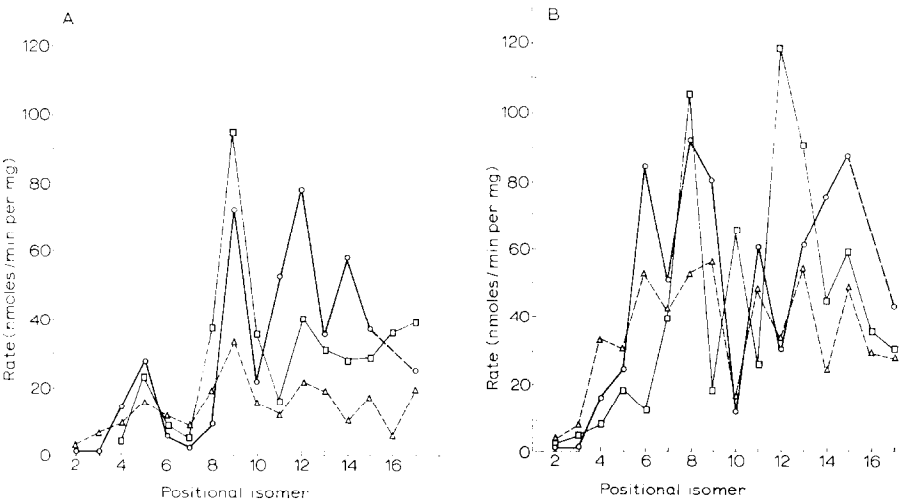

Fig. 2. Comparative study of acyl-transfer rates with three different types of unsaturated acyl-CoA isomers. ○—○, octadecynoyl-CoA; □—□, *cis*-octadecenoyl-CoA[5]; △—△, *trans*-octadecenoyl-CoA[7]. (A) 1-Acylglycerol 3-phosphorylcholine. (B) 2-Acylglycerol 3-phosphorylethanolamine.

in 1-acylglycerol 3-phosphorylcholine among three different isomeric types of unsaturated acyl-CoA esters. The general pattern illustrates that the relative acyl transfer rates are similar for the whole series with all three structural types (except for positional isomers with unsaturated bonds closer to the methyl end). This pattern, however, is different from that obtained for the *cis*-methylene octadecenoyl acids[5].

The data obtained strongly support the idea that the enzyme acylating the 2-position of either 1-acylglycerol 3-phosphorylcholine or 1-acylglycerol 3-phosphorylethanolamine can recognize and respond to the presence of π-bonds at the 5-, 9-, and 12-positions of the fatty acid without being particularly influenced by the acyl chain configuration.

In contrast to the above results, those for the comparative study of the enzyme activity acylating the 1-position of phospholipid among the three types of unsaturated structural isomers are shown in Fig. 2B. The general patterns of acyl transfer differed greatly between acetylenic and *cis*-isomers throughout the series of positional isomers, whereas the selectivities for the *trans*-isomers were almost parallel to those for acetylenic isomers (with the exception of the Δ14-isomer). As discussed in the previous papers by Reitz *et al.*[5] and Okuyama *et al.*[7,8] the enzymes catalyzing the acylation of the 1-position in phospholipids seems to respond to the configuration of the fatty acid. Interestingly, for the isomers from Δ9 to Δ13, the pattern of velocity for octadecynoyl isomers showed a reciprocal relationship to that for *cis*-octadecenoyl isomers. In other words, for those derivatives, the high and low points of the graph were shifting by one isomer between acetylenic and *cis*-isomer series.

The shift in the preferred isomer by one carbon atom may reflect a structural phenomenon illustrated by the molecular models in Fig. 3. Although we had originally regarded the acetylenic derivatives as linear analogs, we noted that rotation of the chain adjacent to the acetylenic bond may provide either a "*cis*" or "*trans*" rotameric orientation of the chain. The *cis* rotamer of the 10-*yne* isomer is superimposable on the

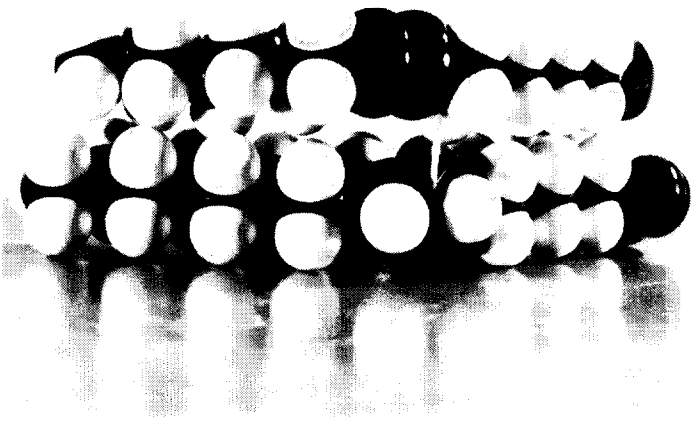

Fig. 3. Structural models of 9-*cis*-octadecenoate and 10-octadecynoate.

cis-9-ethylenic derivative, but not on the *cis*-10-ethylenic compound. The vertical stick in Fig. 3 was placed to indicate that C-10 in the upper acetylenic 10-isomer is directly above C-10 of the *cis*-9-ethylenic compound. This unexpected "frame-shift" causing a configurational similarity between numerically different isomers of the acetylenic and *cis*-ethylenic series of acids lends further support to the concept of a very sensitive detection of configurational details by the acyltransferase acting at the primary position.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the U.S. Public Health Service (N.I.H. AM-05310).

REFERENCES

1 Lands, W. E. M. (1960) *J. Biol. Chem.* 235, 2233
2 Lands, W. E. M. and Merkel J. (1963) *J. Biol. Chem.* 238, 898
3 Lands, W. E. M. and Hart, P. (1965) *J. Am. Oil Chem. Soc.* 43, 290
4 Lands, W. E. M., Blank, M. L. Nutter, L. J. and Privett, O. S. (1966) *Lipids* 1, 224
5 Reitz, R. C., El-Shiekh, M., Lands, W. E. M., Ismail, I. A. and Gunstone, F. D. (1969) *Biochem. Biophys. Acta* 176, 480
6 Reitz, R. C., Lands, W. E M., Christie, W. W. and Holman, R. T. (1968) *J. Biol. Chem.* 243, 2241
7 Okuyama, H., Lands, W. E. M., Gunstone, F. D. and Barve, J. A. (1972) *Biochemistry* 11, 4392 press.
8 Okuyama, H., Lands, W. E. M., Christie W. W. and Gunstone, F. D. (1969) *J. Biol. Chem.* 244, 6514
9 Barve, J. A. and Gunstone, F. D. (1971) *Chem. Phys. Lipids* 7, 311
10 Barve, J. A., Gunstone, F. D., Jacobsberg, F. R. and Winlow, P. (1972) *Chem. Phys. Lipids* 8, 117
11 Gunstone, F. D. and Ismail, I. A. (1967) *Chem. Phys. Lipids* 1, 264
12 Seubert, W. (1960) *Biochem. Prep.* 7, 80

13 Ellman, G. L. (1959) *Arch. Biochem. Biophys.* 82, 70
14 Lands, W. E. M. and Hart, P. (1965) *J. Biol. Chem.* 240, 1965
15 Warburg O. and Christian, W. (1941) *Biochem. Z.* 310, 384
16 Yang, S. F., Freer, S. and Benson, A. A. (1967) *J. Biol. Chem.* 242, 477
17 Lands, W. E. M. (1965) *J. Am. Oil Chem. Soc.* 42, 465
18 Eibl, H. and Lands, W. E. M. (1970) *Biochemistry* 9, 423