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EFFECTS OF ETHYLENIC BOND POSITION UPON ACYLTRANSFERASE ACTIVITY WITH ISOMERIC *CIS*-OCTADECENOYL COENZYME A THIOL ESTERS*

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SUMMARY

The specificity of the acyl-CoA:phospholipid acyltransferases has been studied using the 16 positional isomers of *cis*-octadecenoic acid. The results showed that the acyl-transferases acting at both the 1- and 2-positions of acyl-glycero-3-phosphoryl-choline (acyl-GPC) discriminated between the acyl-CoA isomers in quite different ways.

1. Acyl-CoA:1-acyl-GPC acyltransferase activity showed a distinct preference for the 9-, and 12-isomers. Of these three, the 9-octadecenoate (oleate) was the preferred substrate having a rate of 98 nmoles/min per mg.

2. Acyl-CoA:2-acyl-GPC acyltransferase reacted more rapidly with the 8-, 10-, 12-, 13- and 15-isomers, and of these the 12-octadecenoate had the fastest rate (121 nmoles/min per mg).

3. As the enzymes were allowed to age at 4°, the activity was lost at slightly different rates for each isomer. The enzyme(s) esterifying the 1-position seemed to loose activity fairly uniformly with all isomers so that a similar pattern of reactivities was observed over a period of several days. The enzyme(s) esterifying the 2-position, however, differed in that after 2 days, the rate for the 9-isomer had dropped below that for the 12-isomer. This result suggests that different enzymes may exist for different acyl-CoA isomers.

4. High concentrations of sucrose (0.8 M) tended to stabilize the activities with the 9- and 12-isomers, but did not change the fact that the activity for the 9-isomer was lost more rapidly. Albumin, contrary to our expectations, increased the rate of loss of activity.

5. The enzyme activities were purified 10- to 15-fold above that of the crude tissue homogenate by treating the microsomal particles with sodium deoxycholate and albumin.

Abbreviations: GPC, glycero-3-phosphorylcholine; DTNB, 5,5'-bisdithio(2-nitrobenzoic acid).

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6. Acyltransferase rates for the esterification of the naturally occurring 7-, 9-, 11-, and 13-octadecenoates to position 1 and 2 of diacyl-GPC indicated a preferred position for each acid which is in accord with that reported for the distribution of these monoenoic acids in phospholipids isolated from rat liver.

INTRODUCTION

The acyltransferase activities for the complete series of methylene interrupted *cis,cis*-octadecadienoic acids were recently reported[1]. The rates of reaction seemed to be influenced by combined effects of both ethylenic bonds rather than the existence of one ethylenic bond at some particular position.

LANDS *et al.*[2] have shown that the distribution *in vivo* as well as *in vitro* of fatty acids between position 1 and 2 of diacyl-glycero-3-phosphorylcholine (diacyl-GPC) was influenced by the location and configuration of the ethylenic bond(s) within the fatty acid. JEZYK AND LANDS[3] showed that the acyltransferase-catalyzed esterification of the 1-position of the glycerolipid was dependent on the configuration of the 9-ethylenic bond, whereas the esterification of the 2-position was more dependent on the configuration of the 12-ethylenic bond. The remarkable selectivity of the enzymes in response to the different positional isomers led us to investigate the acyltransferase specificities further by using the complete series of *cis*-octadecenoic acids. This simpler series would avoid combined effects of two bonds in one acyl chain. The results showed that the acyltransferases esterifying the 1- and 2-positions of acyl-GPC discrimated between the positional isomers of octadecenoyl-CoA in quite different ways. The relative activities for the 8-, 9-, and 10-isomers were surprisingly similar to those for the 8,11-, 9,12-, and 10,13-octadecadienoates.

In particular, the isomer containing a 12,13-ethylenic bond, which could be regarded as enabling an octadecanoic acid to serve as an essential fatty acid, was handled in a manner quite different from the other positional isomers.

MATERIAL AND METHODS

Synthesis of octadecenoic acids

All of the *cis*-octadecenoic acids have been prepared and their purity checked by gas–liquid chromatography, by infrared spectroscopy, and by oxidative degradation[4]. The impurities in the preparations include traces of stearate, *trans* isomers, and positional isomers. The 2-octadecenoic acid, prepared from palmitic acid *via* the C_{15}-bromide and C_{17}-alkyne, was 95% pure. The 3-isomer (91–93% pure) was obtained from 3-octadecynol, itself prepared by condensation of the lithium derivative of 1-hexadecyne with ethylene oxide. The 4- to 9-octadecenoic acids were formed from condensation of the dimethylamides of alkynoic acids with an alkyl bromide, and the 10- to 12-isomers were prepared from 1-alkynes and ω-halogeno acid amides. All of these acids contained less than 1% impurity. The remaining acids (13- to 17-octadecenoic acids) were obtained from the corresponding C_{12}-acids (7- to 11-dodecenoic acids) by using the enamine process for chain-extension by six carbon atoms. These acids were contaminated mainly with their *trans* isomers and stearic acid: 13-octadecenoic acid (approx. 10%); 14-octadecenoic acid (4–5%); 15-octadecenoic acid

(5%); 16-octadecenoic acid (2–4%); and 17-octadecenoic acid (6%). The methyl esters of these 5 acids were separated from the contaminants by chromatography on $AgNO_3$-impregnated thin-layer plates. After the thiol esters had been prepared, the purity of the derivatives was checked by transesterification and gas chromatography on a column of 10% ethylene glycol succinate at 198°. All CoA thiol esters were greater than 97% pure except the 2- and 3-octadecenoates.

After the CoA thiol esters had been prepared, gas chromatography showed that these two isomers were contaminated by three additional components which amounted to 50–60% of the total sample.

Synthesis of acyl-CoA derivatives

The acyl-CoA thiol esters of the *cis-n*-octadecenoic acids were synthesized by the micromodification of SEUBERT's procedure[5] described by REITZ *et al.*[1]. 30 μl of each methyl ester were saponified in a solution of tetrahydrofuran–methanol (1:1, by vol.) containing 10% KOH by heating it for 1 h in a sand bath (80°) and then allowing it to stand overnight at room temperature. The solution was acidified to about pH 1–2 with additions of 3 M HCl, and the free fatty acids extracted with hexane. The acid chlorides were prepared by treatment with oxalyl chloride and then used to form acyl-CoA as previously described[1]. The acyl-CoA derivitives were quantitatively determined by comparing the phosphorus content[6] and the absorbance at 260 and 232 mμ (ref. 5) with the spectrophotometric measurement of the amount of mercaptan released by acyltransferase systems[7]. Agreement between these values indicated that the synthesized material was 100% ($\pm$3%) reactive.

Enzymatic studies

Liver microsomal preparations from rats and pigs were prepared by collecting the particles that sedimented between $20000 \times g$ (20 min) and $100000 \times g$ (60 min) from tissue homogenates in 0.25 M sucrose containing 1 mM EDTA. These particles were rehomogenized once with 0.25 M sucrose containing 1 mM EDTA, centrifuged for $9 \cdot 10^6 g \cdot$ min, resuspended and stored at −15°. The protein content was estimated using a nomograph based on the values given by WARBURG AND CHRISTIAN[8]. The rat liver microsomal protein used in earlier studies (see Table II) was treated with DFP to inhibit the high "hydrolase" activity[7].

Further purification of the enzymes was obtained by treating the microsomal protein with sodium deoxycholate and albumin. 20 mg of sodium deoxycholate in 1 ml of 0.25 M sucrose containing 1 mM EDTA was stirred with 60 mg of microsomal protein (2–3 ml) for 5 min, and then 60 mg of albumin in 2 ml of 0.25 M sucrose containing 1 mM EDTA was added and stirred for 10 min. This solution was layered over 1.5 ml of 0.5 M sucrose containing 1 mM EDTA and centrifuged for $9 \cdot 10^6 g \cdot$ min. The 0.5 M sucrose fraction was combined with the pellet, homogenized, and resedimented for $9 \cdot 10^6 g \cdot$ min. This treated microsomal protein resulted in a 10–15-fold increase in acyltransferase activity above that in the crude liver homogenate.

Phospholipid substrates were prepared by selective hydrolysis[9] of diacyl-GPC and alkenyl acyl-GPC.

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'-bisdithio(2-nitrobenzoic acid) (DTNB), 30

nmoles of acyl-CoA, 0.2 mg of microsomal protein, and 0.1 ml of acyl-GPC (175 nmoles of 1-acyl-GPC or 200–300 nmoles of 2-acyl-GPC). As described earlier[7], the acyltransferase rates were continuously recorded in a spectrophotometer using DTNB (ref. 10) to measure the released CoA. The endogenous rate observed without added acyl-GPC was subtracted to give a net rate of acyl transfer[7].

RESULTS

Table 1 shows that increasing the concentration of each thiol ester derivative, from 10 to 40 μM, did not significantly affect the rates of acyl transfer. For example, the values for the 12-isomer, which had the fastest rate, ranged from 28–32 nmoles/min per mg protein with microsomal Preparation A. This variation is within the experimental error. Table I also shows that these levels of acyl-CoA saturate the activity of the more highly purified enzyme. Only the 9-, 12-, and 15-isomers were tested with the purified enzyme and their activities remained essentially constant over the range of concentrations. The other members of the series were considered to behave similarly with the purified enzyme preparation, since the activities were

TABLE I

ACYL TRANSFER RATES WITH VARYING CONCENTRATONS OF ACYL-CoA

Microsomal prep. A was treated with DFP[7]. Microsomal preps. B and C were treated with deoxycholate and albumin and not with DFP. With microsomal preparations A and B, the reaction mixture contained 175 nmoles of 1-acyl-GPC, varying concentrations of the indicated acyl-CoA and 0.2 mg protein in a final vol. of 1 ml (see MATERIAL AND METHODS). Reaction mixtures with preparation C contained 200–300 nmoles of 2-acyl-GPC instead of 1-acyl-GPC. Since each assay involved a continual recording of the product formed each velocity reported represents an average of a large number of recorded determination. All values have been corrected for hydrolase activity[7].

Microsomal preparation	*Acyl-CoA isomer*	*Acyl-transfer rate (nmoles/min per mg)*			
		10 μM	*20 μM*	*30 μM*	*40 μM*
A	2	0	0	0	0
	3	0.9	0.4	0.4	0
	4	1.5	1.3	1.2	1.5
	5	2	4	3	3
	6	0	1.0	0.1	0.1
	7	0	0	0	0
	8	1	3	3	5
	9	13	15	15	14
	10	8	10	10	8
	11	6	7	7	7
	12	31	28	32	31
	13	18	18	19	17
	14	4	4	4	4
	15	4	5	5	5
	16	3	4	4	4
	17	4	4	4	4
B	9	52	57	60	60
	12	70	81	85	86
	15	13	12	12	12
C	8	48	48	48	46
	9	13	12	11	9
	11	11	14	12	14
	12	57	61	58	48
	15	31	41	47	47

constant for these three isomers and for all the isomers with the crude enzyme preparation.

The bottom portion of Table I (microsomal preparation C) indicates that the rates of acyl transfer to the 1-position were essentially unaffected by small increases in acyl-CoA concentration. A representative group of the acyl-CoA isomers which had either slow or fast rates was selected. In certain instances, the rates decreased at an acyl-CoA concentration of 40 μM (see the 9- and 12-isomers).

Fig. 1 indicates the relative acyl transfer rates of the complete series of octadecenoates when being esterified to the 2-position of acyl-GPC. The three curves represent results obtained with different microsomal preparations. In all three cases, three isomers (the 5-, 9-, and 12-octadecenoates) had rates faster than their adjacent isomers. In the more active preparations, the 16- and 17-octadecenoates had rates faster than the 14- and 15-isomers. Curve a, which has the fastest rate for 9-octadecenoate, was obtained with a freshly prepared, purified preparation of microsomal membranes. In this microsomal preparation, the activity for the 9-isomer was 2.4 times that of the 12-isomer; however, in the crude frozen microsomal preparation, Curve c, the activity for the 12-isomer was 3.3 times that of the 9-isomer. When the microsomal preparation used to obtain Curve a was stored 24 h at 4° and the acyltransferase activities measured, Curve b was obtained. The overall pattern of activities was similar to that in Curve a, but attention should be drawn to the interrelationship of the 9- and 12-isomers. The activity for the 9-isomer had decreased approx 50%, whereas that for the 12-isomer decreased only 20%.

The relative instability of the activity for the 9-isomer is further illustrated in Fig. 2. Three samples of microsomes were purified according to the procedure listed in the experimental section; after which, the three pellets were homogenized

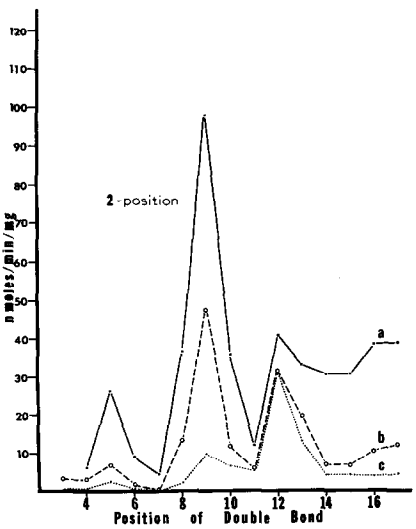

Fig. 1. Acyltransferase specificities of different microsomal preparations from rat liver with 1-acyl-GPC. The reaction mixtures contained 30 nmoles of acyl-CoA, 175 nmoles of 1-acyl-GPC, and 0.1 mg rat liver microsomal protein for Curves a and b, and 0.2 mg protein for Curve c in a final vol. of 1 ml (see MATERIALS AND METHODS). Curve a was obtained with a freshly purified microsomal preparation. Curve b was obtained with the same microsomal preparation after 24 h storage at 4°. Curve c was obtained with a "crude" microsomal preparation.

in 0.8 M, 0.25 M, and 0.25 M sucrose, respectively. The protein content of each was measured and an equal amount of albumin was added to one of the suspensions in 0.25 M sucrose. The activities were measured daily, and the microsomal suspensions were stored at 4°. In all cases, the 12-isomer became the most active substrate by the second day. However, when one compares the three sets of curves, it becomes apparent that even though the cross-over point occurred between the first and second day, there was a distinct difference among the preparations in the rates at which activity was lost. The microsomal preparation which was homogenized in 0.8 M sucrose was more stable towards both substrates than either of the other two preparations. Only 20% of the activity for the 12 isomer was lost during the first 3 days, while during the same time period, 61% and 79% was lost in the 0.25 M sucrose and 0.25 M sucrose *plus* albumin preparations, respectively. The enzyme which transesterifies the 9-isomer was stabilized to a much lesser extent than the enzyme which transesterifies 12-octadecenoate by the 0.8 M sucrose. It had lost 32% of its activity after the first day as compared to 45% and 65% for the enzyme preparation stored in 0.25 M sucrose and 0.25 M sucrose *plus* albumin, respectively.

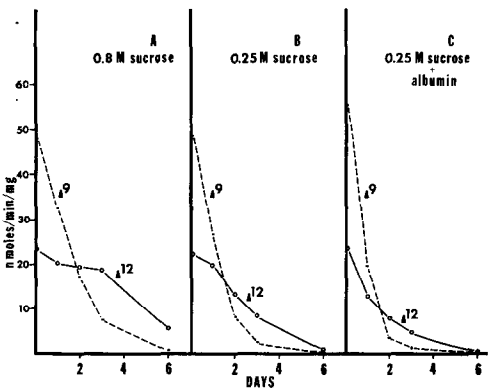

Fig. 2. Loss of activity of 1-acyl-GPC: acyl CoA acyltransferase for 9- and 12-octadecynoyl CoA upon storage at 4°. A. The microsomal preparation was stored in 0.8 M sucrose. B. The microsomal preparation was stored in 0.25 M sucrose. C. The microsomal preparation was stored in 0.25 M sucrose with an added amount of albumin equal to the amount of microsomal protein. The three microsomal preparations were assayed at the time intervals indicated by mixing 30 nmoles of acyl-CoA, 175 nmoles of 1-acyl-GPC, and 0.1 mg microsomal protein in a final vol. of 1 ml (see MATERIALS AND METHODS).

Fig. 3 represents results from studies on the acylation of the 1-position of acyl-GPC using different rat liver microsomal preparations. In all cases, the rates of the 5-, 8-, 10-, 12-, and 15-octadecenoates were faster than either of the two isomers adjacent to them. Curve a was obtained using a freshly-prepared, purified microsomal fraction. With this preparation, the 12-isomer had the highest activity (121 nmoles/min per mg) followed by the 8-isomer (104 nmoles/min per mg). The 10- and 15-isomers had similar activities (67 nmoles/min per mg and 62 nmoles/min per mg, respectively) while the 5-octadecenoate had only one quarter of this activity (17 nmoles/min per mg). Even though the 12-isomer had a faster rate than either of its two adjacent isomers, the 13-octadecenoate had the third fastest acyl transfer rate to the 1-position (93 nmoles/min per mg).

This microsomal preparation was stored at 4° for 3 days; after which the acyl

TABLE II

COMPARISON OF ACYLTRANSFERASE SPECIFICITIES OF RAT AND PIG LIVER MICROSOMALP REPARATIONS FOR THE 1- AND 2-POSITION

The reaction mixture contained 30 nmoles of acyl-CoA, 175 nmoles of 1-acyl-GPC or 200–300 nmoles of 2-acyl-GPC, and 0.2 mg of rat or pig liver microsomes in a final vol. of 1 ml (see experimental section). Each value represents the average of 6 to 10 assays and standard deviations are given. These experiments were done prior to the recognition of the effect that aging has on the microsomes.

Acyl-CoA isomer	*2-position*		*1-position*	
	Rat	*Pig*	*Rat*	*Pig*
2	0.0 ± 0	0	0 ± 0	0 ± 0
3	0.3 ± 0.1	0.1	0.1 ± 0.1	0.9 ± 0.3
4	0.8 ± 0.2	0.3	1.4 ± 0.4	3.1 ± 0.7
5	2.5 ± 0.2	3.5 ± 1.2	8.9 ± 1.4	13.7 ± 2.0
6	0.9 ± 0.2	0.8 ± 0.2	2.6 ± 1.0	5.5 ± 1.2
7	0.0 ± 0	0.0 ± 0	7.7 ± 2.0	18.4 ± 3.5
8	2.3 ± 0.3	2.9 ± 0.7	30.1 ± 3.6	48.7 ± 6.1
9	9.9 ± 0.8	7.0 ± 0.6	6.3 ± 0.9	11.2 ± 2.6
10	6.6 ± 0.6	6.4 ± 1.8	21.4 ± 2.7	22.1 ± 3.9
11	5.7 ± 0.3	5.3 ± 1.7	20.2 ± 3.3	34.9 ± 4.1
12	33.3 ± 1.9	22.2 ± 2.8	33.4 ± 3.2	44.8 ± 2.9
13	13.3 ± 1.2	11.2 ± 2.0	17.3 ± 1.4	28.5 ± 1.5
14	4.3 ± 0.3	5.7 ± 0.8	25.1 ± 2.4	34.8 ± 3.2
15	4.4 ± 0.3	5.8 ± 1.7	15.5 ± 1.8	20.7 ± 1.2
16	3.9 ± 0.3	6.6 ± 1.7	13.2 ± 1.0	16.0 ± 1.4
17	4.3 ± 0.7	3.3 ± 0.7	7.4 ± 1.5	12.0 ± 2.2

transfer rates for the 7-through the 16-isomers were measured again. The results are shown in Curve b. Each isomer seemed to loose activity at slightly different rates, but the same overall pattern of activity was maintained. This same pattern of activity was also obtained with crude, frozen microsomal preparations (Curve c). There was no selective loss of activity for transesterification of the different acids to the 1-position of acyl-GPC.

Table II compares the results of acyltransferase studies with rat liver microsomes to those with pig liver microsomes. This table was included to show that microsomal membranes of different animal species have very similar acyltransferase specificities. In both species, the 5-, 9-, 12-octadecenoates had rates greater than their adjacent isomers when being transferred to the 2-position. When being transferred to the 1-position, the 5-, 8- and 12-octadecenoates had rates faster than their adjacent isomers.

The standard deviations for some of the rates of the acyl-CoA's were quite large (20–30% of the mean) because these studies were done prior to the recognition of the effects of ageing on the acyl transferases. The large deviations were a result of combining values from several different microsomal preparations. It should be pointed out that the deviations in the assays from one microsomal preparation were not large (2–5% of the mean).

In Table III, the rate of transesterification to positions 1 and 2 of acyl-GPC are compared to the melting points of each of the monoenoic acids[4]. When the double bond was located at either end of the carbon chain, the melting points were high (50°); however, their values were alternatively low (12°) and high (24°) from the 5-isomer through the 12-isomer. Interestingly, the rates of acyl transfer to the 1-position followed a similar alternating scheme from the 7- to the 13-isomers. Isomers with ethylenic bonds at even-numbered positions were higher melting and more rapidly

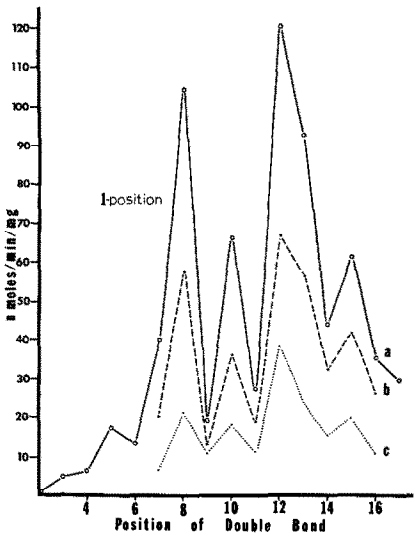

Fig. 3. Acyltransferase specificities of different microsomal preparations from rat liver with 2-acyl-GPC. The reaction mixture contained 30 nmoles acyl-CoA, 175 nmoles 2-acyl-GPC, and 0.1 mg rat liver microsomal protein for Curve a and b, and 0.2 mg protein for Curve c in a final vol- of 1 ml (see MATERIALS AND METHODS). Curve a was obtained with a freshly purified microsomal preparation. Curve b was obtained with the same microsomal preparation after 72 h storage at 4°. Curve c was obtained with a "crude" microsomal preparation.

TABLE III

COMPARISON OF THE MELTING POINTS OF THE MONOENOIC ACIDS WITH THEIR ACYL TRANSFER RATES

18:1 isomer	*Melting point of acid**	*Transfer rate*	
		Position 1	*Position 2*
2	49.0–50.0	1	0
3	49.5–50.5	5	0.3
4	45.5–46.5	8	6
5	12.5–13.5	17	26
6	28.0–29.0	15	9
7	12.0–13.0	39	5
8	23.5–24.0	105	37
9	10.0–11.0	19	98
10	22.5–23.5	67	36
11	12.5–13.5	28	12
12	27.0–28.0	121	41
13	26.5–27.0	93	33
14	41.5–42.5	44	31
15	40.5–41.5	62	31
16	53.5–54.5	36	39
17	55.5–56.5	30	39

* See ref. 4.

esterified. However, comparing the rates of transesterification to the 2-position, only the 11-, 12-, and 13-isomers followed an alternating pattern somewhat similar to the melting points. It should also be noted that the melting points of the 14- and 15-isomers were essentially identical (41.5° and 40.5°, respectively) as were the acyl transfer rates to the 2-position (31 nmoles/min per mg). As the double bond was placed at the 16- and 17-positions, there was an increase in both the melting points

(53.5° and 55.5°, respectively) and the acyl transfer rates to the 2-position (39 nmoles/min per mg). Here again, the melting points of the acids were essentially identical as were the acyl transfer rates.

DISCUSSION

The results shown in Table I indicate that the acyltransferase rates were not influenced significantly by changing the acyl-CoA concentration from 40 to 10 μM. Since the reaction mixture contains microsomal protein which can bind surface-active materials like acyl-CoA and acyl-GPC, we cannot accurately state the actual concentration of micellar or free monomeric acyl-CoA in the reaction mixture. However, one can say that the enzyme system was sufficiently saturated with substrate so that consumption of substrate during the early part of the reaction would not alter the rate. ZAHLER, BARDEN AND CLELAND[11] have reported a critical micelle concentration for palmitoyl-CoA of 3–4 μM. They stated that "if the free monomeric molecule is the active substrate, Michaelis kinetics will be observed only below the critical micelle concentration, and the initial velocity may be independent of substrate concentration above the critical micelle concentration".

Many studies have shown that the naturally occurring saturated fatty acids are selectively located at the 1-position whereas unsaturated fatty acids are selectively located at the 2-position of phosphoglycerides [12-14]; however, the positional distribution for monoenoic fatty acids is not as clear-cut as that for the saturated and polyunsaturated fatty acids. Data exist which led to the suggestion that the incorporation of monoenoic fatty acids into either the 1- or 2-position of phosphoglycerides is dictated predominately by the nutritional state of the animal[14], thus precluding any real enzyme specificity. The data of LANDS *et al.*[2] and WAKU AND LANDS[15] have shown that the acyltransferases can be used to predict the distribution of fatty acids between the 1- and 2-positions of diacyl-GPC in both rat liver and erythrocyte membranes. Recalculation of BROCKERHOFF AND ACKMAN'S data[16] by multiplying the percentage of a given 18-carbon monoenoic isomer times the percentage of the total monoenoic acids at either the 1- or 2-positions allows the comparison of the amount of each fatty acid at each position. BROCKERHOFF AND ACKMAN[16] have analyzed animal glycerolipids for the positional distribution of several isomers of monoenoic fatty acids which are normal constituents of lipids. Their results clearly indicate that the 7-, 9-, 11-, and 13-octadecenoic acids accumulate to different extents in both the 1- and 2-positions. This distribution could reflect either the amount of each isomer present or enzyme specificity, or more probably a combination of both. There could also be competetion between the monoenes and the other fatty acids present *in vivo* for a given position of diacyl-GPC. The competitive effects which could occur with a mixture of acids has been discussed in some detail by LANDS *et al.*[2]. The present study demonstrates that there is a distinct enzyme specificity which lies in the acyltransferases. In Table IV a comparison is made between the amount of a monoenoic fatty acid present in the 1- and 2-positions of diacyl-GPC of rat liver and the acyltransferase rates for that isomer at these positions. Although the ratio between position 1 and position 2 from the gas–liquid chromatography analysis did not agree quantitatively with that from the acyltransferase analysis, the qualitative comparison was quite similar. Here again, it appears that the acyl transferase activi-

ties can be used as an index to the amount of a given fatty acid which will be found in the 1- and 2-position of isolated diacyl-GPC molecules.

In studying the effects of temperature and solvents on the acyltransferases, JEZYK AND LANDS[3] showed that the enzyme which esterifies the 2-position was quite sensitive to the configuration of the 12-ethylenic bond, whereas the enzyme which esterifies the 1-position was more sensitive to the configuration of the 9-ethylenic bond. The activities toward the *cis*-12-ethylenic derivatives were retained while activity towards the *cis*-9-ethylenic bond was lost when ethylenic glycol was added to the system[3]. Even though this report is concerned with positional isomers and not with configurational isomers, similar changes occurred with the activities for the 9- and 12-monoenes during storage at 4°. The *cis*-12-ethylenic bond, which seems to confer essentially to a fatty acid, was recognized by an enzymic activity which was maintained under a variety of environmental conditions in which activity towards

TABLE IV

INCORPORATIONS OF THE NATURALLY OCCURRING MONOENES INTO THE 1- AND 2-POSITIONS OF DIACYL-GPC

Content: see ref. 15. Rate: spectrophotometric assay.

Position esterified in diacyl-GPC	*Location of ethylenic bond*							
	7		*9*		*11*		*13*	
	Content	*Rate*	*Content*	*Rate*	*Content*	*Rate*	*Content*	*Rate*
1	0.49	39	2.73	16	3.15	28	0.56	93
2	0.20	5	8.10	98	1.60	12	0.10	33

other positional isomers was lost; thus, relative comparisons of specific activity need to be evaluated carefully since they represent not one but two different types of enzymic activities which need not necessarily be preserved the same during isolation.

Alternation of physical properties, melting points, molecular volumes *etc.*, of homologous hydrocarbons having odd and even numbers of carbon atoms has been known for many years. This alternation was first thought to be due to the zigzag arrangement of the carbon atoms[17]. However, MALKIN[18,19] showed that the alternation of properties of long chain compounds was due to the tilt of the chain with respect to the plane of the terminal group. HUBER[20] showed that the melting points of the 7- through the 12- octadecenoic acids alternated, and LUTTON AND KOLP[21] showed that the long and the short spacing of X-ray diffractions of this monoene series also alternated with the odd or even placement of the ethylenic bond. These authors also concluded that the alternations of the long and short spacings and melting points were due to the tilt of the molecules. They believed that the odd-numbered ethylenic bond compounds were "perpendicular (or very nearly so)" and that the even ethylenic bond compounds were "tilted with respect to the carboxyl planes". The results from the comparisons in Table III were quite intriguing, in that they show a correlation between the physical constants of the fatty acids and their acyltransferase activities. An important consideration is that the melting points reflect the hydrocarbon chain interactions in the solid phase, whereas the acyltransferase rates are enzymic activities obtained with solvated or possibly micellar forms of derivatives of fatty acids. Even though the alternating melting points and the X-ray diffractions reflect chain–chain interactions, how these interactions would affect the acyltransferase activities is not known.

In early studies on the enzymic activation of amino acids the enzyme was thought to have a very broad specificity[22], but the later data of HOAGLAND, KELLER AND ZAMECNIK[23] suggested the presence of more than one enzyme. This suggestion was subsequently confirmed[24-26], and today it is an accepted fact that there is a specific enzyme for the activation of each amino acid. A similar sequence of events has appeared concerning the specificity of the acid: CoA ligases; however, instead of a ligase for each fatty acid, fatty acid activation is presently grouped into activity for short-(ref. 27), medium-(refs. 28 and 29), and long-chain[30-32] acids. PANDE AND MEAD[32] have recently presented data which led to a suggestion that there are at least two long-chain activating enzymes, one for the saturated and one for the unsaturated fatty acids. The activity lost at different rates as the microsomes were aged could be considered to support the suggestion that each fatty acyl-CoA may have its own acyltransferase. However, further purification and separation of enzymic activities will be necessary to conclusively demonstrate this.

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