

Control of lecithin biosynthesis in erythrocyte membranes

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ABSTRACT The detailed relationship between the relative composition of the potential precursor acids, the esterification rates of their CoA thiol ester derivatives, and the relative composition of the fatty acids in the product, lecithin, which was isolated from normal erythrocytes, suggests that in humans the stromal acyltransferases could be the significant enzymatic factor controlling the fatty acid composition at the 2-position of lecithin in erythrocytes.

KEY WORDS erythrocyte · acyl-CoA:monoacylglycerophosphoryl choline acyltransferase · lecithin · lysolecithin · fatty acid composition · plasma free fatty acids · fatty acyl CoA · membrane

FOLLOWING EARLIER WORK ON acyltransferase activity in liver microsomes (2), several investigators examined the manner in which fatty acids are incorporated into the phospholipids of erythrocytes. Oliveira and Vaughan (3, 4) showed that the reaction was similar to that observed in liver in that it required ATP and CoA and that the newly esterified acid was located at the 2-position of the glycerophosphatide. In addition, Robertson and Lands (5) noted that the synthesis of either phosphatidyl choline or phosphatidyl ethanolamine is stimulated appreciably if the corresponding monoacyl precursor is added.

This work raised the interesting possibility that the physical characteristics of the cell membrane could be subject to modification as the phospholipids were modified by the acyl-CoA:monoacylphosphoglyceride acyl-

transferases. The acyltransferase activities were similar in erythrocytes from normal humans and from humans with spherocytosis (5), which suggests that a defect in this enzyme was not the basis for the different membrane properties of the two types of cells, as had been suggested earlier (6). Nevertheless, other reports consistently supported the concept that changes in the diet could alter the fatty acid composition of erythrocyte phospholipids (7, 8) more rapidly than could be accounted for by erythropoiesis (9–11). Alterations in the fatty acid composition of erythrocyte phospholipids can be correlated with changes in the permeability of the cell membrane (12–14).

Such findings prompted us to consider in more detail the possibility that the acyltransferase activity may be an agent controlling the composition of the fatty acids in the stromal phospholipids. Our experience with these enzymes now allows a more quantitative estimate of their total activity and their specificity for transferring the many different fatty acids that may be made available to them. This paper reports the detailed relationship between the relative composition of the potential precursor acids (serum free fatty acids), the esterification rates of their CoA thiol ester derivatives, and the relative composition of the fatty acids in the product, lecithin, which was isolated from normal erythrocytes.

METHODS

Acyl-CoA derivatives were synthesized from the acid chlorides and free CoA by a modification (15) of the method described by Seubert (16). They were stored at -10°C in the presence of Santoquin (Monsanto Chemical Co., St. Louis, Mo.). 1-Acyl-GPC was prepared from egg lecithin by treatment with phospholipase A (*Crotalus adamanteus*) and purified by chromatography on a silicic acid column. 2-Acyl-GPC was prepared by treating 25

A preliminary report of this work has been presented (1).

Abbreviations: GPC, glycerophosphoryl choline; TLC, thin-layer chromatography; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).

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μ moles of pig heart choline phosphoglycerides in 2 ml of chloroform-methanol 2:1 with 1 ml of 0.1 M Tris-HCl buffer (pH 7.4) and 0.7 ml of aqueous I_2 as described earlier (17). After 20 min, 3 ml of ethanol was added, the excess I_2 was reduced with $Na_2S_2O_3$, and the chloroform solution was washed twice with water to remove salts. The lipid extract was evaporated to dryness, washed twice with acetone at 0°C to remove organic and inorganic iodides, and used as a dispersion in water (10 μ moles/ml).

Stromata from the erythrocytes of humans, cows, and rats were prepared by the method of Dodge, Mitchell, and Hanahan (18). Fresh citrated blood was added to an equal volume of 0.109 M sodium phosphate buffer (pH 7.4) and centrifuged at 4000 g for 10 min. The supernatant fluid was decanted, and the erythrocytes were washed two more times with 0.109 M sodium phosphate buffer. The erythrocytes were then hemolyzed in 20 volumes of 0.0072 M sodium phosphate buffer (pH 7.4). The hemolyzed solution was centrifuged at 20,000 g for 30 min, and the stromata were washed three times with 0.0072 M sodium phosphate buffer.

The packed stromata were then suspended in 0.1 M Tris-HCl buffer (pH 7.4). Stromal lipids were extracted by suspending 70 ml of stromata (6 mg protein/ml) in 630 ml of chloroform-methanol 2:1 to which the antioxidant, Santoquin was added. The solution was allowed to stand overnight with occasional swirling. The denatured protein was removed by filtration, and the extract was washed with 80 ml of water. The chloroform layer was taken to dryness in a rotary evaporator. The lipids of rat stroma were extracted in a similar manner, but with one half the amount of materials. A crude lecithin fraction of each lipid preparation was prepared by silicic acid column chromatography (19).

Pure lecithin was isolated by TLC on Silica Gel G with chloroform-methanol-water 65:25:4. Portions of the material (approximately 4 μ moles) were dissolved in 3 ml of ether and hydrolyzed using 0.10 ml of snake venom (from *Crotalus adamanteus*, 10 mg/ml in 0.1 M Tris-HCl buffer, pH 7.4) with 0.05 ml of 0.02 M $CaCl_2$ for 4 hr at room temperature. Under these conditions, over 95% of the lecithin was hydrolyzed. The reaction mixture was dried under N_2 , and the products were separated by TLC to yield free fatty acids and acyl derivatives of GPC.

The free fatty acid band was scraped off the plate and treated (without elution) with 1.5 ml of BF_3 in methanol (14% w/v; Applied Science Laboratories Inc., State College, Pa.) at 100°C in a sealed tube for 5 min by the method of Metcalfe, Schmitz, and Pelka (20). The reaction mixture also contained methyl pentadecanoate as an internal standard for the subsequent gas-liquid chromatographic analysis. After the addition of 1 ml of water and 2 drops of 3 N HCl, the methyl esters were extracted with two 3 ml portions of petroleum ether.

The acyl-GPC band from the plate was added to 1.2 ml of 0.5 N NaOH in anhydrous methanol. Methyl pentadecanoate was added as an internal standard, and the mixture was heated at 100°C in a sealed tube for 5 min. The tube was allowed to cool, and 1.8 ml of BF_3 in methanol was added. The sample was further treated as described above, and the methyl esters were analyzed by gas chromatography. Purified lecithin from egg yolk was hydrolyzed by snake venom under the same conditions to serve as a control for the recovery of fatty acids from the original lecithins. The recovery of the products is shown in Table 1.

The free fatty acids in human, cow, and rat blood plasma were isolated by the method of Dole (21). 5 ml of an extraction mixture (isopropanol-heptane-N H_2SO_4 , 40:10:1) was shaken vigorously with 1 ml of plasma for 1 min. After the mixture had stood for 10 min, 2 ml of heptane and 3 ml of water were added to induce phase separation. A 3 ml aliquot of the upper phase was transferred to a 15 ml tube containing 1 ml of 0.01% thymol blue in 90% ethanol and titrated with 0.01 N NaOH under a stream of nitrogen until the green-yellow end point was reached. The upper heptane layer was removed, and the ethanol layer was washed with petroleum ether to remove contaminating glycerides. The ethanol layer was acidified with 2 drops of 3 N HCl and the free fatty acids were extracted with two 4 ml portions of petroleum ether and esterified with BF_3 in methanol as described above.

The activity of the 1-acyl-GPC:acyl-CoA acyltransferase was estimated by the method described earlier (22). The incubation mixtures contained 50 $m\mu$ moles of 1-acyl-GPC (suspended in distilled water), 32 $m\mu$ moles of fatty acyl CoA, 1 μ mole of DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] neutralized by $NaHCO_3$ in 0.1 ml of distilled water, and erythrocyte stromata (0.10-0.20 mg of protein).

The 2-acyl-GPC:acyl-CoA acyltransferase activity was assayed by incubating human erythrocyte stromata (0.45 mg of protein) with 2-acyl-GPC (50 $m\mu$ moles), 1- ^{14}C -stearoyl CoA (64 $m\mu$ moles), and 0.5 ml of 0.1 M Tris-HCl buffer (pH 7.4) for 40 min at room temperature. After incubation, 15 ml of chloroform-methanol 2:1 and 5

TABLE 1 RECOVERY OF PRODUCTS FROM SNAKE VENOM HYDROLYSIS OF LECITHIN

Experiment	Fatty Acid	Acyl-GPC	Recovery
	μ moles		%
1	2.57	2.10	104
2	2.15	1.87	90
3	2.20	1.98	94

Each sample of lecithin contained 4.47 μ moles of fatty acids. After hydrolysis of lecithin by snake venom, the products, acyl-GPC and fatty acids, were isolated by TLC and converted to methyl esters in the presence of an internal standard for analysis by GLC.

$m\mu$ moles of pig heart choline phosphoglyceride (as a carrier) were added. The solution was washed twice with 4 ml of water the the chloroform layer was evaporated. The remaining incubation product was dissolved in 5 ml of ether-benzene 1:1 and fractionated by chromatography on 2.7 g of silicic acid. Free fatty acid (derived from ^{14}C -acyl CoA by a nonspecific hydrolase of the human blood stroma), ^{14}C -acyl CoA, and lecithin were eluted with 40 ml of benzene-ether 1:1, 40 ml of ether-ethanol 1:1, and 40 ml of methanol, respectively. 20-ml fractions were collected in scintillation vials. The fractions were dried at 90°C , and 7 ml of scintillation fluid—4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene in 1 liter of toluene—and 0.1 ml of Hyamine [*p*-(diisobutylcresoxy-ethoxyethyl)dimethyl benzyl ammonium hydroxide] was added to each sample. The samples were counted in a Packard liquid scintillation counter, model 314-DC. The specific activities of ^{14}C -stearoyl CoA and ^{14}C -linoleoyl CoA were 200 and 170 cpm/ μ mole, respectively. In a control experiment, the stromata were incubated without 2-acyl-GPC. The difference between the radioactivities in corresponding methanol-eluted fractions was used as a measure of the synthesis of lecithin.

Additional control experiments included erythrocyte stromata incubated with ^{14}C -linoleoyl CoA and 1-acyl-GPC; and rat liver microsomes (0.45 mg of protein, treated with 10^{-4} M diisopropylfluorophosphate) incubated with ^{14}C -stearoyl CoA and 2-acyl-GPC. The radioactive lecithins synthesized in these systems were extracted, chromatographed, and counted as described for the experimental samples. As a check on the position of the incorporated stearate and linoleate, the synthesized phospholipids were extracted from the incubation mixture, 3 μ moles of pig heart lecithin was added as a carrier, and the lecithin band was purified by TLC in chloroform-methanol-water 65:25:4. One half of the radioactive lecithin was hydrolyzed by venom as described above, and the other half was saved as a control for the subsequent chromatography. Each sample was analyzed by chromatography on a silicic acid column (2.7 g) eluted with chloroform, 40 ml, chloroform-methanol 3:2, 100 ml, and methanol, 60 ml; 20-ml fractions were collected. The free fatty acid derived from the 2-position of the lecithin was eluted in the chloroform fraction and the lysolecithin was eluted in the methanol fraction.

RESULTS

Fig. 1 shows the relative rates of diacyl-GPC synthesis from several isomers of linoleoyl CoA when catalyzed by human erythrocyte stromata. The reaction rate, determined with a recording spectrophotometer, was estimated by the increase in absorbance at 413 $m\mu$ due to the reaction of free CoA with DTNB. At this concentra-

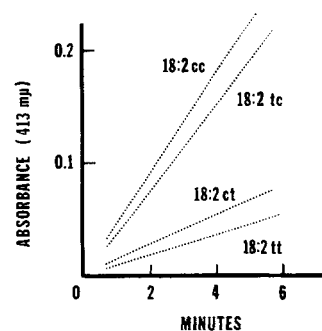


FIG. 1. Relative acyltransferase rates in lecithin synthesis by human erythrocyte stroma. The reaction mixture contained human erythrocyte stromata (0.1 mg of protein), 50 $m\mu$ moles of 1-acyl-GPC, 0.1 ml of DTNB (0.01 M), 32 $m\mu$ moles of the indicated acyl CoA, and Tris-HCl buffer (pH 7.4) to give a final volume of 0.88 ml. Fatty acyl moieties are designated by chain length: no. of double bonds. c = *cis*, t = *trans* (double bond nearest the carboxyl designated first).

tion of enzyme (0.1 mg of protein per ml), no hydrolase activity was observed with any of the acyl-CoA derivatives. Under these conditions, the amount of product formed was proportional to the time of incubation for the first 6 min.

The initial rate of incorporation of linoleoyl CoA into the 2-position of 1-acyl-GPC by human stromata was proportional to the amount of enzyme up to 0.12 mg of protein (Fig. 2). The effect of the concentration of 1-acyl-GPC on the reaction rate with linoleoyl CoA is shown in Fig. 3. The reaction rate was almost constant at substrate concentrations greater than about 50 $m\mu$ moles/0.85 ml and the K_m value was estimated to be about 2×10^{-5} M. In agreement with earlier results (22,23), the K_m value for linoleoyl CoA (and other thiol esters) seems much lower than that for 1-acyl-GPC since the reaction rate was constant at all thiol ester levels above 1×10^{-5} M. The en-

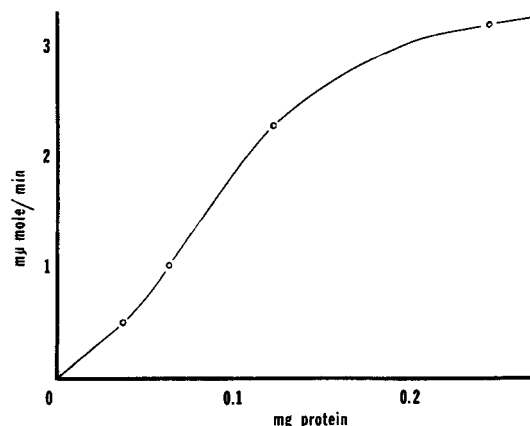


FIG. 2. Incorporation of linoleate into the 2-position of 1-acyl-GPC. The incubation mixture contained 32 $m\mu$ moles of linoleoyl CoA, 50 $m\mu$ moles of 1-acyl-GPC, 0.1 ml of 0.01 M DTNB, enzyme as indicated, and 0.1 M Tris-HCl buffer (pH 7.4) to give a final volume of 0.89 ml.

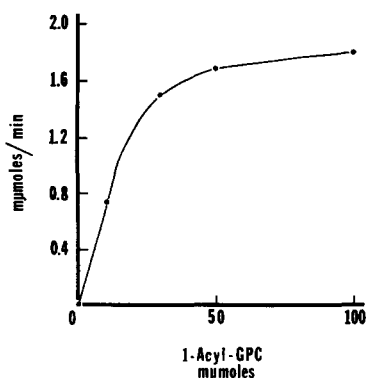


FIG. 3. Effect of 1-acyl-GPC concentration on the rate of reaction with linoleoyl CoA. The reaction mixture contained human erythrocyte stromata (0.12 mg of protein), linoleoyl CoA (32 μ moles), 0.1 ml of DTNB (0.01 M), 1-acyl-GPC as indicated, and 0.1 M Tris-HCl buffer (pH 7.4) to give a final volume of 0.85 ml.

zymes were thus considered to be saturated with substrates in all of the subsequent kinetic work.

Although appreciable acyltransferase activity was found for 1-acyl-GPC, the reaction rate with 2-acyl-GPC could not be observed with the spectrophotometric assay and was almost negligible when measured with radioactive tracers (Table 2). Control incubations indicate that the stearoyl CoA and 2-acyl-GPC were suitable substrates when an appropriate enzyme was used, but acyl-transfer reactions to the 1-position of 2-acyl-GPC were not appreciably catalyzed by the human stromata. The slight increase in radioactivity at the 1-position (right-hand column, Table 2) above the control value for

TABLE 2 INCORPORATION OF FATTY ACIDS INTO THE UN-ESTERIFIED POSITION OF ISOMERS OF MONOACYL-GPC IN THE PRESENCE OF HUMAN ERYTHROCYTE STROMATA OR RAT LIVER MICROSOMES

Enzyme Source	Substrates		Radioactive Products		
	Acyl CoA	Acyl-GPC isomer	Lecithin fraction	Free Acid	Acyl-GPC
Erythrocyte	18:2	None	194 (200)	116	21
		1-acyl	4951 (4792)	2893	0
	"	None	242 (224)	62	17
		2-acyl	498 (424)	97	41
	18:0	None	403 (376)	58	16
		2-acyl	439 (596)	56	190
Liver	18:0	None	278 (728)	106	128
		2-acyl	2630 (4408)	170	1060

The incubation mixture contained 1- or 2-acyl-GPC (50 μ moles), $1\text{-}^{14}\text{C}$ -linoleoyl or $1\text{-}^{14}\text{C}$ -stearoyl CoA (64 μ moles, 170 and 200 cpm/ μ mole, respectively), enzyme preparation (0.45 mg of protein), and 0.5 ml of 0.1 M Tris-HCl buffer (pH 7.4) in a total volume of 0.65 ml. The incubation time was 40 min (room temperature). The lecithin fraction was hydrolyzed by snake venom and analyzed by silicic acid column chromatography. The results in parentheses indicate results from additional experiments.

stearate (174 cpm) represents 0.07 μ mole/minute per mg of protein.

The specificities for the transfer of several fatty acids into the 2-position of 1-acyl-GPC with human, cow, and rat erythrocyte stromata are shown in Table 3. Each preparation had good transferase activity with the *cis*-unsaturated derivatives and lower activity with the saturated thiol esters. Some differences in the specificity of the three enzyme preparations were observed. For example, with the rat enzyme, the esterification rate with arachidonoyl CoA is twice as high as that with human stromata but only half as great with palmitoleoyl CoA and oleoyl CoA. These differences seem to have some relationship to the fatty acid composition of the 2-position of the stromal lecithins of the corresponding animals, although there is no direct correlation. These compositions are compared in Table 4. The composition of the plasma unesterified fatty acids is also included for comparison. The relative abundance of a particular acid in this fraction is even less closely correlated with that found at the 2-position of the lecithin than are the acyl-transfer rates.

DISCUSSION

Earlier studies with intact human erythrocytes (4), hemolysates (5), and stromal preparations (4) showed that exogenous acids were incorporated almost entirely at the 2-position. The very low acyltransferase activity with

TABLE 3 RATE OF ACYL-CoA ACYL TRANSFER INTO THE 2-POSITION OF 1-ACYL-GPC IN THE PRESENCE OF STROMATA FROM HUMAN, RAT, AND COW ERYTHROCYTES

Acyl CoA	Human	Rat*	Cow
<i>μmoles/min per mg of protein</i>			
12:0	3.0	3.4	2.8
14:0	2.7	3.0	2.7
16:0	2.0	1.6	2.2
16:1	7.5	4.4	3.9
18:0	0.7	0.7	0.7
18:1c	5.3	2.5	5.0
18:1t	3.7	6.0	—
18:2cc	14.0	14.0	8.5
18:2ct	3.9	6.6	—
18:2tc	11.4	13.3	—
18:2tt	2.4	3.4	—
18:3	12.3	12.3	8.5
20:4	8.1	15.5	6.4

Fatty acyl moieties are designated by chain length: no. of double bonds. c = *cis*, t = *trans* (double bond nearest the carboxyl group designated first).

Each value is the average of two separate experiments in which each velocity reported represents a continual series of observations on the product formed during the reaction. The incubation mixture contained 0.1–0.2 mg of protein of erythrocyte stromata, 50 μ moles of 1-acyl-GPC, 32 μ moles of acyl CoA, 0.10 ml of 0.01 M DTNB, and 0.70 ml of Tris-HCl buffer (pH 7.4) in a total volume of 0.89 ml.

* The stromata were prepared from the combined erythrocytes of 12 female rats.

TABLE 4 COMPARISON OF THE COMPOSITION OF THE 2-ACYL ESTERS IN ERYTHROCYTE LECITHINS WITH THE COMPOSITION OF THE PLASMA FREE FATTY ACIDS AND ACYL-TRANSFER RATES FOR THE CORRESPONDING CoA THIOL ESTERS

Fatty Acid	(1)	(2)	(1) × (2)	Composition of 2-Acyl Esters*	
	Acyl-transfer Rate	Plasma FFA		Predicted	Observed
	$\mu\text{moles/min per mg}$	$\text{moles } \%$		$\text{moles } \%$	
<i>Erythrocytes from humans</i>					
16:0	2.0	24.8 (22.9)	49.6 (45.8)	9.8 (8.6)	13.8 (13.9)
16:1	7.5	2.7 (3.0)	20.3 (22.5)	4.0 (4.2)	— (2.1)
18:0	0.7	17.0 (16.6)	11.9 (11.6)	2.3 (2.2)	4.2 (3.9)
18:1	5.3	34.1 (34.3)	180.7 (181.8)	35.5 (34.1)	28.3 (27.5)
18:2	14.0	14.4 (16.2)	201.6 (226.8)	39.6 (42.5)	42.7 (34.7)
20:4	8.1	5.5 (5.5)	44.6 (44.6)	8.8 (8.4)	8.3 (14.7)
<i>Erythrocytes from rats</i>					
16:0	1.6	29.4 (25.5)	47.0 (40.8)	7.1 (7.0)	34.6 (33.7)
18:0	0.7	12.6 (16.7)	8.8 (11.7)	1.3 (2.0)	3.8 (1.6)
18:1	2.5	23.5 (21.5)	58.8 (53.8)	8.9 (9.3)	10.8 (13.5)
18:2	14.4	24.4 (24.9)	351.4 (358.6)	53.3 (61.7)	22.3 (30.5)
20:4	15.5	12.5 (7.5)	193.8 (116.3)	29.4 (20.0)	28.5 (20.7)
<i>Erythrocytes from cows</i>					
16:0	2.2	17.3 (15.7)	38.0 (34.5)	10.4 (10.4)	7.6 (8.3)
16:1	3.9	2.5 (2.1)	9.7 (8.2)	2.7 (2.5)	2.7 (1.3)
18:0	0.7	32.9 (34.6)	23.0 (24.2)	6.3 (7.3)	8.4 (6.9)
18:1	5.0	19.7 (36.7)	98.5 (183.5)	26.9 (55.2)	32.9 (53.4)
18:2	8.5	18.7 (6.7)	158.9 (56.9)	43.4 (17.1)	35.6 (18.3)
18:3	8.5	1.3 (1.3)	11.0 (11.0)	3.0 (3.3)	3.8 (1.5)
20:4	6.4	4.2 (2.2)	26.7 (14.0)	7.3 (4.2)	8.9 (6.5)

Values in parentheses represent a second experiment.

* See Discussion section for explanation of the calculation.

2-acyl-GPC noted in this paper suggests that metabolism of lecithin in human erythrocytes may be primarily limited to reactions with 1-acyl-GPC. This suggestion is also compatible with the observation that the predominant isomer of acyl-GPC in human plasma is the 1-acyl rather than the 2-acyl derivative (23). Nevertheless, recent reports of 1-acyl hydrolase activity in plasma after treatment with heparin (24) indicate that 2-acyl-GPC may be available at times to the erythrocyte. Thus, the contrasting report (25) of palmitate incorporation into the 1-position of lecithins may be due to a different physiological state or to the different species studied.

Recent papers (25–27) have confirmed that the incorporation of acids into erythrocyte phosphatides by direct acylation of the monoacyl precursors may be selective, and also that erythrocytes have no appreciable phospholipase A activity. In addition, the observation that L-3-glycerophosphate did not enhance the incorporation of fatty acids into lipids (4, 25) supports the concept that acylation of the monoacyl precursors may be the principal pathway for lecithin formation in the mature cell. Ironically, the earlier work involved a search for unknown acyltransferases to complete a “fatty acid exchange cycle” with the concept that lecithinase activity was ubiquitous (2, 28). It now appears that the acyltransferases are active in a wide variety of tissues and the relative lack of lecithinase activity may require that the

monoacyl derivatives be brought to the tissue from other sources. Such a role for acyl-GPC has also been suggested in other reports (5, 27, 29–32). The erythrocyte provides, then, what appears to be a relatively simple system for lecithin synthesis whereby unesterified fatty acids and acyl-GPC are acquired from the plasma, where they are bound principally to albumin (29, 33, 34). The resulting lecithin may then be retained in the membrane or released (35–37).

Direct comparison of the relative acyl-transfer rate with the relative abundance of an acid at the 2-position of stromal lecithins shows a correlation, although not a quantitative relationship, between the two values (Table 4). Thus linoleate (18:2) is esterified most rapidly and is the most abundant. In general, the unsaturated acids are esterified at a faster rate and are usually more abundant at the 2-position.

In agreement with the results of others (38–40), Table 4 shows that widely different amounts of the different unesterified acids are available to the erythrocyte. To demonstrate the effect that these different levels could have on the type of lecithin formed, we have calculated the product composition based on a relative acyl CoA composition similar to that in the pool of precursor acids. In this way, the relative abundance of an acid times its relative incorporation rate indicates how much of that acid would be present in the lecithin when these two

factors are the major ones concerned. We were very interested in the fact that neither factor alone was a reliable indicator of the acid composition at the 2-position, but when the two were considered together they provided a prediction that was very close to the composition observed.

The inability to find laurate incorporation into erythrocyte phosphatides even though large amounts were fed in the diet (9, 10) cannot be explained entirely on the basis of enzyme specificity since laurate can be esterified at rates comparable to palmitate. Rather, the content of this acid may be limited primarily by a very low level of laurate in the plasma unesterified fatty acids.

An earlier comparison of acyl-transfer rates with relative acid composition in the lecithins of rat liver also gave a close agreement between the predicted and observed values (15). In that report, the abundance of the substrate was not considered since the value calculated was the relative distribution of a given acid between the 1-position and the 2-position of the lecithin molecule. In that case, the value for the relative abundance of the substrate would appear in both the numerator and denominator and thus be eliminated from the calculation. In this report the observed content of an acid in the 2-position is considered independently of the rest of the molecule, and this value is more likely to be dependent upon the relative amounts of the different precursor acids available for synthesis. As with other kinetic analyses, the agreement found in this paper is a condition necessary but not sufficient to prove that these are the major factors controlling the fatty acid composition.

The close agreement between predicted and observed values for so many of the acids, especially in man but also in the case of 18:1 and 20:4 in the rat, makes the inability to predict palmitate and linoleate values in the rat erythrocytes an interesting paradox. Some factor, in addition to those already considered, must be involved in this situation. An obvious possibility is that the erythrocytes have not circulated for a sufficient time to allow the ester at the 2-position of the lecithins to equilibrate with the plasma unesterified acids. A very recent report on the relationship of erythrocyte fatty acids to cell age in the rat concludes that "the concentration of a given acid in the plasma could not have been solely responsible for its rate of uptake or loss, and the cell must have exhibited some specificity in the transacylase process" (41). Whatever additional factors may be recognized in the metabolism of erythrocyte lecithins, the present data suggest that in humans the stromal acyltransferases could be the significant enzymatic factor controlling the fatty acid composition at the 2-position.

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