Molecular species of lecithins from erythrocytes and plasma of man

L. MARAI and A. KUKSIS

Department of Biochemistry and the Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada

ABSTRACT The lecithins of the plasma and erythrocytes of man were isolated by thin-layer chromatography, and the major molecular species were identified and quantitatively estimated by combined thin-layer and gas–liquid chromatography and specific enzymic hydrolyses. Using these techniques we could identify over 60 molecular species, accounting for some 98% of the total lecithin, in both plasma and cells, but only about 30 of them occurred in concentrations over 1%.

The molecular species of lecithins in the cells and plasma were qualitatively similar; quantitatively, large differences were noted among and within the various classes of unsaturation. In the same blood, the erythrocyte lecithins contained 8–20 times as high a percentage of saturated lecithins and nearly twice as high a percentage of monounsaturated lecithins as did plasma lecithins. The differences in the relative amounts of a particular molecular species within a class of unsaturation were, however, most pronounced among the polyunsaturated lecithins.

These results suggest that plasma and red cells possess distinct lecithin populations and that complete equilibration of the intact molecules between the two media is unlikely.

SUPPLEMENTARY KEY WORDS fatty acids · positional distribution · diglycerides · diglyceride acetates

FROM A REVIEW of the available experimental data it has been concluded (2) that each animal tissue has a capacity for a more or less independent synthesis of phospholipids. Only certain precursors may be received through the plasma, and intact circulating phospholipid molecules are seldom assimilated. A similar tissue independence is demonstrated in the catabolism of the phospholipids, which are broken down in situ and are not transported elsewhere for disposal.

The membrane of the red blood cell seems to be an exception as it has not been shown to contain any elaborate synthetic or degradative mechanisms for the modification of its phospholipids, although limited exchanges of specific fatty acids in certain lipid classes have been reported (3). Indeed, on the basis of an observed complementary growth and decay of phospholipid specific activity between the cells and the plasma in vitro, it has been suggested (4) that the plasma phospholipid is in simple diffusion equilibrium with the same phospholipid within or at the surface of the cells. Thus far, however, only plasma albumin-bound lysolecithin has been specifically shown (5–7) to be taken up by the erythrocytes while undergoing a simultaneous acylation to a lecithin.

As a basis for further metabolic studies a detailed analysis has been made of the molecular species of lecithins, which are the major phospholipids of both plasma and erythrocytes of man. Since the publication of this study in an abstract form (1), a similar analysis of the lecithin species from the erythrocytes and plasma of man and rabbit has been reported by Van Golde, Tomasi, and Van Deenen (8).

MATERIALS AND METHODS

The methods and general experimental conditions were similar to those previously described (9), except as noted below. All solvent evaporations and enzymic and chemical transformations were performed under nitrogen. A few crystals of purified quinol were added as an antioxidant during extraction and storage of lipids.

Presented at the 10th Annual Meeting of the Canadian Federation of Biological Societies held in Montreal, Quebec, July 1967. An abstract has appeared (1).

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; C_{14}–C_{22}, fatty acids with 14–22 carbon atoms; C_{32}–C_{46}, diglycerides or diglyceride acetates with a total number of fatty acid carbon atoms (including those of acetic acid) of 32–46. Fatty acids designated by chain length: number of double bonds.

Blood Samples

Four samples (500 ml each) of citrated whole blood from healthy male donors (25–40 yr old) were obtained from the Toronto General Hospital Blood Bank within 1 month of collection. These were separated into the plasma and erythrocyte fractions by means of refrigerated (4°C) centrifugation (2000 *g* for 30 min). The lipids of both red cells (10) and plasma (9) were immediately extracted with chloroform–methanol 2:1. At least one aliquot of each lipid extract was processed immediately as described below, while the rest of the total lipid extract was stored at −20°C for a maximum of 1 wk.

Isolation of Lecithins

The lecithins were isolated by TLC on Silica Gel G (9). Usually six to eight plates were run at a time in order to obtain sufficient material for convenient analysis (10–20 mg). The lecithins were recovered from the plates by elution of the silica gel scrapings with several portions of chloroform–methanol 1:9. The eluted lecithins had a fatty acid composition indistinguishable from that obtained by transmethylation of the lecithin zone in the presence of silica gel. The lecithin was free of the common phospholipid contaminants as shown by rechromatography in the TLC system of Skipski, Peterson, and Barclay (11) and by the spot tests they suggested.

Enzymic Hydrolyses

The hydrolyses of the lecithins with phospholipases A and C and of the diglyceride acetates with pancreatic lipase were performed as previously described (9), except that a few crystals of quinol were added as an antioxidant.

Preparation and Resolution of Diglyceride Acetates

The diglyceride acetates were prepared from free diglycerides by acetylation, and the acetates were purified by TLC as described (9). The recovered diglyceride acetates had a fatty acid composition which, except for minor losses of polyunsaturates, was indistinguishable from that of the original lecithins.

The diglyceride acetates were resolved on the basis of total number and distribution of double bonds per molecule by argentation TLC (9). An interesterified mixture of equal weights of trimyristin and triolein was applied as a spot on one side of the plate and served as a reference standard for the saturated, monoene, diene, and triene bands. The polyene bands were similarly located by reference to the diglyceride acetates prepared from rat liver lecithins (12). The plates were developed one or more times in a solution of 0.8–1.2% (v/v) methanol in chloroform. The acetates corresponding to the various groups of unsaturation were separately recovered by repeated extraction of the gel scrapings with diethyl ether–methanol–acetic acid 60:40:1. The extracts were washed with distilled water and the organic solvents were evaporated under a stream of nitrogen, small amounts of petroleum ether being added to remove the last traces of water by azeotropic distillation.

The ratios of the diglyceride acetates among and within the various bands were determined by GLC with tridecanoin as an internal standard (9).

Analysis of Fatty Acids

The fatty acids were methylated and quantitatively estimated by GLC. The identities of the unsaturated acids were confirmed by rechromatography after silver nitrate TLC and hydrogenation as described (9).

Calculations

The structure of the original lecithins was calculated by proportional summation and normalization of all the analytical data. The accuracy of the final result was determined by matching the mole percentage composition, and positional distribution of the fatty acids in the original lecithins, against that determined for the derived diglyceride acetates. In a few instances the experimental data were compared to random distribution derived by calculation (9).

RESULTS AND DISCUSSION

The amounts of total phospholipid and lecithin obtained by the Folch, Lees, and Sloane Stanley (13) extraction of the blood fractions were of the order reported in the literature for human plasma (14) and red blood cells (15, 16). On the basis of an average molecular weight of 781, the lecithin content of plasma was 2.1 μmoles/ml and that of the erythrocytes 1.3 μmoles/ml of packed cells. Previous studies (16) had revealed an average of 12.1×10^9 red cells/ml in samples prepared as described. The amount of lecithin associated with one human red cell is therefore about 1.1×10^{-10} μmole. It was assumed that the isolated lecithins represented all the molecular species present in this tissue.

Separate analyses were attempted on the plasma and cells of four subjects, but because of spillage complete reconstitutions were obtained for only two of them. As far as the analyses were completed (at least to the stage of silver nitrate TLC), however, there was good agreement among all four subjects.

Fatty Acid Composition

Table 1 gives the over-all composition and the positional distributions of the fatty acids of the plasma lecithins from the two subjects. The corresponding values for the erythrocytes of these subjects are reported in Table 2. The data for the second subject represent an average of two parallel analyses. Both plasma and red blood cells

TABLE 1 COMPOSITION AND POSITIONAL DISTRIBUTION OF FATTY ACIDS OF THE PLASMA LECITHINS OF MAN

Fatty Acids	Subject 1				Subject 2			
	Total	1-*	2-†	Reconst.‡	Total	1-	2-	Reconst.
				moles %				
14:0	1.2	1.6	0.6	1.1	0.4	0.5	0.3	0.4
15:1					0.4	1.5	tr.	0.8
16:0	29.6	59.4	3.4	31.4	33.2	59.8	5.2	32.5
16:1	1.6	1.6	1.2	1.4	1.0	1.2	1.2	1.2
16:2					tr.			
18:0	11.7	23.8	1.2	12.5	13.0	27.8	1.2	14.5
18:1	17.3	7.2	25.6	16.4	12.0	5.8	16.9	11.3
18:2	18.8	4.1	31.8	18.0	18.8	1.6	32.2	16.9
20:0	0.5	1.0	0.2	0.6	tr.	tr.		tr.
20:1	0.8	0.8	0.8	0.8	0.2	0.3	0.3	0.3
20:2	tr.	0.1	0.3	0.2	0.5	0.6	1.1	0.8
20:3	3.2	—	6.2	3.1	3.0		7.1	3.6
20:4	10.4	0.3	17.7	9.0	11.8		23.8	11.9
20:5	0.6	—	1.2	0.6	0.6		1.1	0.6
22:0	tr.	0.1	0.3	0.2	0.6	0.2	1.0	0.6
22:2	0.1	—	0.4	0.2	tr.	tr.		tr.
22:3	0.6	—	1.6	0.8	0.8	0.3	1.3	0.8
22:4	0.2	—	0.6	0.3	0.6		1.0	0.5
22:5	0.6	—	1.6	0.8	0.7		1.4	0.7
22:6	2.8	—	5.3	2.6	2.4		4.7	2.3
24:0					tr.	0.4	0.2	0.3

Fatty acids are designated number of carbon atoms: number of double bonds.
* Fatty acids from the lysolecithins liberated by phospholipase A.
† Free fatty acids liberated by phospholipase A.
‡ Reconstituted composition = (1-acids + 2-acids)/2.

TABLE 2 COMPOSITION AND POSITIONAL DISTRIBUTION OF FATTY ACIDS OF THE ERYTHROCYTE LECITHINS OF MAN

Fatty Acids	Subject 1				Subject 2			
	Total	1-*	2-†	Reconst.‡	Total	1-	2-	Reconst.
				moles %				
12:0					tr.	tr.		tr.
14:0	0.3	1.2	tr.	0.6	0.5	0.4	0.7	0.5
15:0					0.4	0.9	0.4	0.7
16:0	33.8	65.9	5.0	35.5	32.9	67.1	8.8	37.7
16:1	0.7	0.7	1.2	0.9	1.0	1.0	2.1	1.6
16:2					tr.			
18:0	10.9	22.0	1.5	11.7	13.6	20.7	1.1	10.9
18:1	20.2	6.8	35.2	21.0	17.8	6.2	30.6	18.4
18:2	15.0	2.4	29.6	16.0	16.1	2.0	29.2	15.6
20:0	0.7	0.6	0.1	0.4	tr.	tr.		tr.
20:1	tr.		tr.	tr.	0.3	0.1	0.2	0.2
20:2	0.6		0.7	0.4	0.8		1.6	0.8
20:3	1.6		3.0	1.5	2.0		4.0	2.0
20:4	10.7		15.6	7.8	9.3		15.1	7.7
20:5	0.7		1.7	0.8	0.5		0.7	0.4
22:0	0.2	0.4		0.2	0.6	0.2	0.9	0.6
22:2	0.7		0.4	0.2	tr.			
22:3	0.5		0.4	0.2	0.9		1.1	0.5
22:4	0.5		0.9	0.5	0.4		0.5	0.2
22:5	0.7		0.8	0.4	0.6		0.6	0.3
22:6	2.2		3.9	2.0	2.0		2.4	1.2
24:0					0.3	0.8		0.4
24:1					0.2	0.6		0.3

Footnotes as in Table 1.

contain qualitatively the same fatty acids, including most members of the C_{14}–C_{24} series with 0 to 6 double bonds. The lecithins of plasma contain proportionally less oleic and more linoleic acid than the corresponding erythrocytes, with the other acids showing less marked differences. Furthermore, the 1-position in both plasma and red cell lecithins is occupied mainly by saturated fatty acids, although some oleic and linoleic acid can be detected. However, there is a considerable amount of palmitic acid in the 2-position, especially in the erythrocyte lecithins. This distribution is similar to that reported by others for the lecithin fatty acids of serum (17) and erythrocytes (18) of man. Choline plasmalogens account for 0.8% (17) and 1.4% (18), respectively, of human serum and erythrocyte phospholipids. Their removal by TLC (after digestion with phospholipase C) made no discernible difference in the composition of the diglycerides or fatty acids derived from the lecithins.

Molecular Weight Distribution

Table 3 gives the molecular weight distribution of the total lecithins of the plasma and erythrocytes of man as estimated by GLC of derived diglyceride acetates. In both plasma and cells the major constituents of the lecithins are those with one C_{16} and one C_{18} (C_{36}), with two C_{18} (C_{38}), and with one C_{18} and one C_{20} (C_{40}) fatty acid per molecule. The variations in the proportional contributions of the lecithins of different molecular weights reflect differences in both the fatty acid composition and the pairing. Despite detectable differences in the fatty acid composition between the lecithins of the two subjects, the molecular weight distributions of the derived diglycerides are similar. This is not a coincidence due to a large experimental error, however, since parallel analyses showed excellent agreement. Furthermore, a selectivity in the over-all association of the fatty acids in the lecithin molecules is suggested by the differences between the experimental and the random values derived by calculation (9) from the fatty acid composition. As noted for the rat tissue lecithin (9), on a mass basis the unsaturated acids are combined with the saturated acids approximately in the ratio in which the latter occur in the total fatty acid mixture. Table 3 also includes the reconstitution values obtained for the diglyceride acetates by algebraic summation and normalization of the data from argentation TLC. The close agreement between these and the original total indicates that the execution of the detailed analytical procedure

TABLE 3 MOLECULAR WEIGHT DISTRIBUTION OF MIXED DIGLYCERIDES AND DIGLYCERIDE ACETATES DERIVED FROM THE BLOOD LECITHINS OF MAN

Carbon Number*	Subject 1				Subject 2			
	DG†	DGAc†	Random‡	Reconst.§	DG	DGAc	Random	Reconst.
				moles %				
Plasma								
32	tr.	tr.	0.7	tr.	tr.	tr.	0.3	tr.
34	1.0	1.0	10.9	0.6	0.8	0.7	12.1	1.1
35				tr.	tr.	tr.	0.4	tr.
36	41.4	42.3	30.2	41.0	44.6	44.0	30.5	42.5
38	40.3	39.9	32.6	40.8	38.0	38.7	30.2	37.4
39					tr.	tr.	tr.	0.3
40	15.0	14.6	17.5	15.4	15.7	15.5	17.5	16.9
42	2.3	2.2	6.5	2.2	0.9	1.1	7.1	1.8
44			1.3				1.6	
46			0.2				0.3	
Erythrocytes								
32	0.3	0.3	0.2	0.4	0.2	0.4	0.4	0.4
34	5.0	5.2	12.0	5.2	8.1	8.7	14.5	5.4
36	45.0	46.1	31.5	46.1	54.7	54.8	35.2	54.1
38	33.8	32.8	30.9	33.3	31.4	31.3	30.8	29.8
40	13.7	13.5	17.1	13.1	8.4	7.8	13.1	8.8
42	2.2	2.1	7.1	2.0	1.2	1.0	3.9	1.4
44			1.1		tr.	tr.	0.6	0.1
46			0.1				tr.	

* Diglyceride acetates identified by total number of acyl carbons, including that of the acetic acid residue. Diglycerides identified by the carbon numbers of the corresponding acetates.

† As estimated by direct GLC of the total diglycerides (DG) or diglyceride acetates (DGAc) before and after hydrogenation.

‡ Random distribution determined as previously described (12).

§ Reconstituted composition estimates obtained by algebraic summation and normalization of data from argentation TLC.

was not accompanied by significant losses of the polyunsaturates due to autoxidation.

Fig. 1 shows the GLC patterns of the major diglyceride acetates recovered from the silver nitrate plates. The area percentages of the peaks, which represent the molecular weight distributions in each group of lecithins, are given in Tables 4 and 5 for subjects 1 and 2, respectively. It is seen that only a few major diglyceride acetates or lecithins occur in any one group of molecules having a given degree of unsaturation. This is true for both the plasma and the erythrocyte lecithins, and is in contrast to the random values, which usually cover a wider range of molecular weights (see Table 3).

The proportions of the different unsaturation groups in the total lecithin mixture, however, vary greatly. Thus, while the erythrocytes contain 5.1–6.7% fully saturated lecithins, the proportion of this material in the plasma lecithins is only about 0.3–0.7% of the total. The corresponding random values are, respectively, 3–4 and 50 times higher. The lecithins of the erythrocytes contained 33.6–35.5% monoene, while those of the plasma accounted for only 19.3–22.2% of the total in these two subjects. The differences between the dienoic lecithins of the plasma (37.8–38.9%) and the erythrocytes (31.0–35.1%) were smaller, but these values were again different from the corresponding random values, which were only one half of the experimental values. Of the polyene fractions, the tetraenes represented the largest percentage, which in the erythrocytes came close to the random estimates. In the plasma, however, the experimental value for the tetraenoic lecithins was considerably higher than the calculated result. The hexaenes occurred in nearly random proportions in both plasma and cells.

TABLE 4 MAJOR DIGLYCERIDE ACETATES DERIVED FROM THE LECITHINS OF THE PLASMA AND ERYTHROCYTES OF SUBJECT 1

Degree of Saturation*	Carbon Number	Plasma		Plasma		Erythrocytes		Erythrocytes	
		Exptl.†		Random‡		Exptl.		Random	
		moles %							
Saturates	32	10		3.8		5.2		0.9	
	34	70	0.3	47.4	18.5	70.4	6.7	51.2	22.3
	36	6		37.4		22.2		33.0	
	38	14		7.4		2.2		2.1	
Monoenes	32	0.1		0.2		tr.		tr.	
	34	1.8		8.0		1.3		2.5	
	36	79.8	22.2	62.7	16.9	77.1	33.6	71.2	18.7
	38	16.9		26.7		20.4		23.0	
	40	1.4		0.1		1.2		tr.	
Dienes	34	tr.		0.1		—		—	
	35	—		—		—		—	
	36	55.5	37.8	58.3	20.1	58.6	31.0	52.4	18.3
	38	40.6		36.8		40.2		40.1	
	40	3.9		1.8		—		—	
	42	—		—		1.2		0.8	
Trienes	35	—		—		—		—	
	36	9.5		5.4		6.6		2.4	
	38	55.5	9.8	78.4	10.7	75.0	3.8	82.0	7.9
	40	35.0		10.3		18.4		6.8	
	42	—		—		—		—	
Tetraenes	36	6.5		1.8		1.9		0.5	
	38	69.5		68.4		61.0		68.7	
	40	24.0	20.0	24.9	14.2	34.5	15.5	24.0	13.2
	42	—		—		2.6		1.9	
	44	—		—		tr.		0.3	
Pentaenes	36	—		—		tr.		tr.	
	38	48.7		10.5		25.5		7.1	
	39	—		—		—		—	
	40	35.9	3.9	61.5	6.6	63.4	3.6	81.5	6.7
	42	15.4		1.0		11.1		4.5	
Hexaenes	36	—		—		—		—	
	38	6.7		0.9		10.3		0.2	
	40	66.3	6.0	79.2	7.0	75.0	5.8	33.3	5.6
	42	27.0		9.3		14.7		10.0	

* Each acetate reported as a percentage of saturation class, and each saturation class as a percentage of total acetate mixture.

Other footnotes as in Table 3.

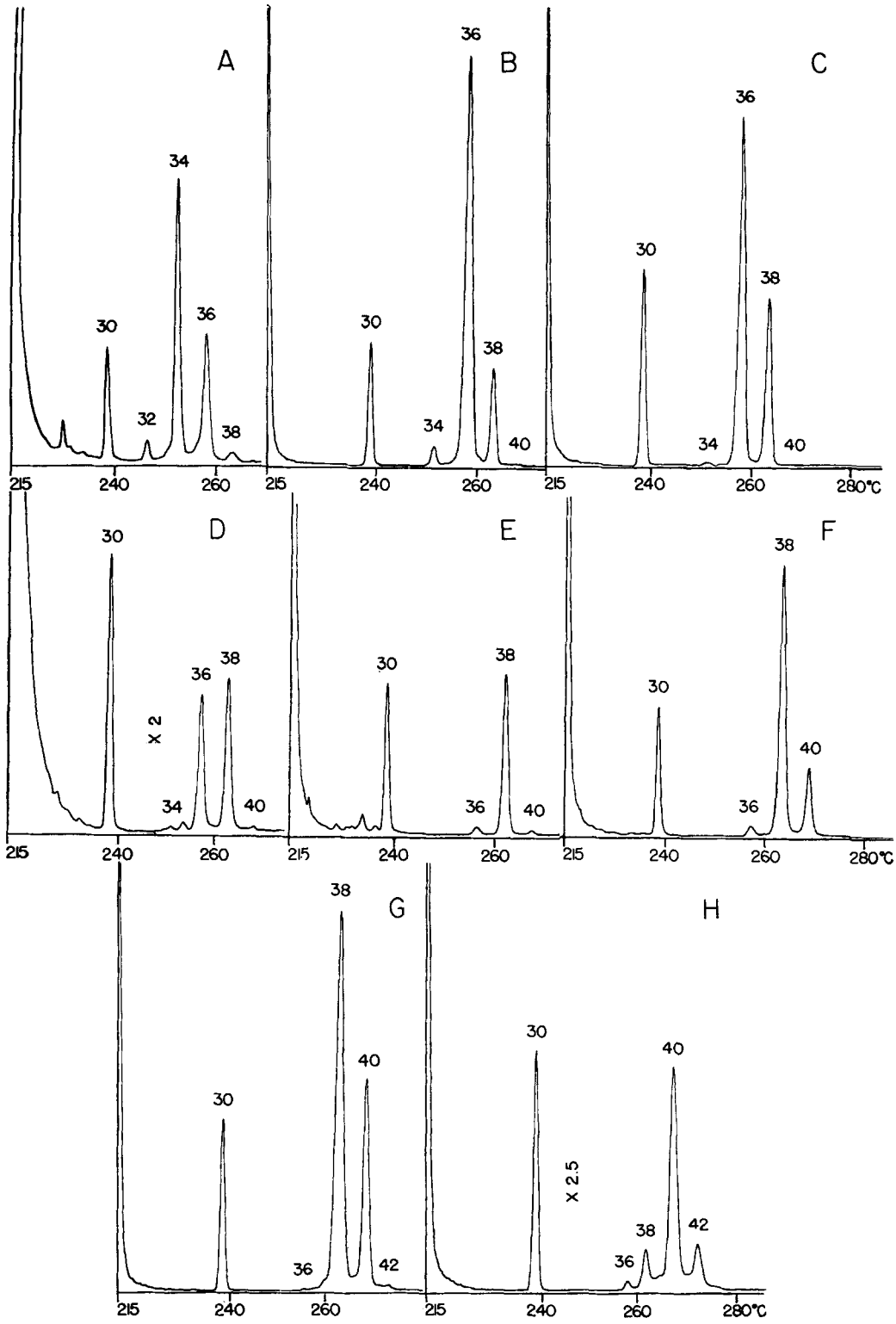

FIG. 1. GLC patterns of major diglyceride acetates of uniform degree of unsaturation from the erythrocyte lecithins of subject. 2. A, saturates; B, monoenes; C, dienes I; D, dienes II; E, trienes I; F, trienes II; G, tetraenes; H, pentaenes + hexaenes. Peaks identified by total number of acyl carbons in molecule. Temperature programs as shown; other chromatography conditions as given in the text.

TABLE 5 MAJOR DIGLYCERIDE ACETATES DERIVED FROM THE LECITHINS OF THE PLASMA AND ERYTHROCYTES OF SUBJECT 2

Degree of Saturation*	Carbon Number	Plasma				Erythrocytes			
		Exptl.†		Random‡		Exptl.		Random	
		moles %							
Saturates	32	4.9		1.2		6.8		1.6	
	34	72.0	0.7	49.2	22.4	74.1	5.1	57.3	20.9
	36	21.9		38.6		19.1		32.4	
	38	1.2		7.5		—		—	
Monoenes	32	—		—		—		—	
	34	2.9		5.9		2.6		5.7	
	36	87.4	19.3	64.3	12.8	83.1	35.5	70.5	19.3
	38	9.7		27.4		14.3		21.2	
	40	—		—		—		—	
Dienes	34	—		—		1.9		0.8	
	35	—		—		0.1		1.1	
	36	64.7	38.9	65.4	19.8	67.0	35.1	60.8	21.7
	38	33.3		31.8		30.0		35.3	
	40	2.0		0.5		1.0		1.3	
	42	—		—		—		—	
Trienes	35	0.6		1.7		—		—	
	36	2.0		4.5		5.1		6.6	
	38	69.6	9.1	74.8	8.8	78.0	5.3	81.8	9.5
	40	27.8		15.6		16.5		8.0	
	42	—		—		0.4		tr.	
Tetraenes	36	0.5		0.6		1.0		0.7	
	38	66.2		69.5		71.0		72.9	
	40	32.2	23.8	24.4	16.3	27.5	12.9	21.5	11.7
	42	1.1		2.2		0.5		tr.	
	44	—		—		—		—	
Pentaenes	36	—		—		2.0		0.1	
	38	22.0		10.5		24.2		12.1	
	39	7.0	2.9	0.1	6.0	2.5	2.6	0.1	5.3
	40	61.7		76.3		51.6		79.3	
	42	9.3		7.0		19.7		7.9	
Hexaenes	36	—		—		1.4		tr.	
	38	0.6		0.5		2.8		0.6	
	40	74.7	5.3	82.3	7.5	72.1	3.5	84.6	4.6
	42	24.7		13.5		23.7		12.4	

* Each acetate reported as a percentage of saturation class, and each saturation class as a percentage of total acetate mixture.
Other footnotes as in Table 3.

Molecular Species

Table 6 lists the major individual lecithins of the plasma and red blood cells of two men. The molecular species have been specified as explained (9). The estimates were derived by reconstitution of the over-all molecular weight distribution (Tables 4 and 5) and the fatty acid composition (Tables 7 and 8).

On the basis of the data given in Tables 1, 4, and 5 it can now be seen that the saturated lecithins of plasma are largely made up of dipalmitoyl lecithin with lesser amounts of material containing palmitic and either myristic or stearic acids. The choice is similarly restricted for the saturated lecithins of the erythrocytes, as judged from the data in Tables 2, 4, and 5. The random values for the plasma and erythrocyte lecithins attribute the major proportion of the saturates to the stearoyl palmitoyl lecithin. The major monoenes in both plasma and cells were (16:0 18:1) and (18:0 18:1) lecithins, as predicted by the random calculation. The palmitoyl derivative, however, occurred in nearly double the amount predicted by randomization.

The major dienes in both plasma and cells were (16:0 18:2) and (18:0 18:2), occurring in about twice the random proportions. In the erythrocytes, the minor (18:1 18:1) component (2–3% of total in the two subjects) approached the random value (4% of total). The trienes in the plasma lecithins were present in nearly random amounts, while those in the erythrocytes occurred in one half the predicted values. The tetraenes were largely made up of (16:0 20:4) and (18:0 20:4), although random calculations predicted significant amounts of (18:2 18:2) lecithins also. The pentaenes and hexaenes occurred in about half the random values in both tissues of these subjects. Among the latter, the (16:0 22:6) and the (18:0

TABLE 6 MAJOR LECITHINS OF THE PLASMA AND ERYTHROCYTES OF MAN*

Chemical Classes†	Fatty Acids 1-‡	Fatty Acids 2-§	Subject 1 Plasma		Subject 1 Erythrocytes		Subject 2 Plasma		Subject 2 Erythrocytes	
			moles %							
Saturates	16:0	14:0	10.0		5.2		4.9		6.8	
	18:0	14:0	—		—		—		4.6	
	16:0	16:0	70.0	0.3	70.4	6.7	72.0	0.7	69.5	5.1
	18:0	16:0	6.0		22.2		21.9		19.1	
	20:0	16:0	—		2.2		—		—	
	18:0	18:0	14.0		—		1.2		—	
Monoenes	14:0	16:1	0.1		tr.		—		—	
	14:0	18:1	0.5		—		1.3		0.5	
	16:0	16:1	1.3		1.3		1.6		2.1	
	16:0	18:1	76.5		77.1		85.7		80.7	
	16:0	20:1	3.3	22.2	—	33.6	tr.	19.3	1.0	35.5
	18:0	16:1	3.3		tr.		1.7		2.4	
	18:0	18:1	12.8		19.4		9.7		13.3	
	18:0	20:1	1.4		—		—		—	
	20:0	16:1	0.8		1.0		—		—	
	22:0	16:1	—		1.2		—		—	
Dienes	14:0	18:2	—		—		—		0.9	
	14:0	20:2	—		—		0.2		2.2	
	15:0	18:2	—		—		—		0.1	
	16:0	18:2	54.2		58.6		62.6		61.7	
	16:0	20:2	—		1.3		—		2.7	
	16:0	22:2	1.0		—		—		—	
	18:0	18:2	35.3	37.8	29.9	31.0	30.1	38.9	22.4	35.1
	18:0	20:2	1.4		—		2.0		0.5	
	18:0	22:2	—		1.2		—		—	
	22:0	16:2	—		—		—		0.5	
	16:1	16:1	tr.		—		tr.		1.0	
	16:1	18:1	1.3		tr.		1.9		3.1	
	18:1	18:1	5.3		9.0		3.2		4.9	
	18:1	20:1	1.5		—		—		tr.	
Trienes	14:0	20:3	—		—		0.1		0.7	
	16:0	20:3	33.0		38.9		42.3		52.1	
	16:0	22:3	15.0		—		10.1		—	
	18:0	20:3	20.0		10.5		17.1		15.1	
	22:0	18:3	—		—		—		0.4	
	15:1	18:2	—	9.8	—	3.8	0.6	9.1	—	5.3
	16:1	18:3	9.5		6.6		1.9		4.4	
	16:1	22:2	—		—		0.3		—	
	18:1	18:2	22.5		36.1		27.3		25.9	
	18:1	20:2	—		7.9		—		0.7	
	20:1	18:2	—		—		0.3		0.7	
Tetraenes	14:0	20:4	6.5		1.9		0.5		1.0	
	16:0	20:4	65.0		55.5		63.8		66.0	
	16:0	22:4	—		2.6		2.1		—	
	18:0	20:4	17.5		27.1		28.1		25.5	
	18:0	22:4	—		1.3		0.3		—	
	20:0	20:4	—	20.0	1.3	15.5	0.3	23.8	0.5	12.9
	16:1	20:3	—		1.6		1.0		1.0	
	16:1	22:3	—		—		0.5		—	
	18:1	20:3	6.5		4.8		1.5		2.0	
	18:1	22:3	—		—		0.5		tr.	
	18:2	18:2	4.5		3.9		1.4		4.0	

TABLE 6 *continued*

Fatty acids designated by chain length: number of double bonds.

* Each acetate reported as a percentage of saturation class, and each saturation class as a percentage of total acetate mixture.

‡ Fatty acids from the lysolecithins liberated by phospholipase A.

§ Free fatty acids liberated by phospholipase A.

TABLE 6 *concluded*

Chemical Classes†	Fatty Acids 1-‡	Fatty Acids 2-§	Subject 1 Plasma		Subject 1 Erythrocytes		Subject 2 Plasma		Subject 2 Erythrocytes	
			moles %							
Pentaenes	14:0	20:5	—		—		—		2.0	
	14:0	22:5	12.8		—		—		2.0	
	16:0	20:5	30.9		25.5		22.0		18.7	
	16:0	22:5	10.6		13.9		4.7		12.5	
	18:0	20:5	10.3		12.5		2.3		4.1	
	18:0	22:5	—		tr.		2.3		4.1	
	15:1	20:4	—		—		7.0		—	
	16:1	20:4	5.0		tr.		tr.		3.5	
	16:1	22:4	—	3.9	—	3.6	1.2	2.9	0.6	2.6
	18:1	20:4	15.0		34.2		51.2		30.3	
	18:1	22:4	15.4		tr.		4.7		4.1	
	18:2	20:3	—		2.8		2.3		4.1	
	18:2	22:3	—		11.1		—		9.9	
	20:1	20:4	—		—		2.3		0.6	
	20:1	22:4	—		—		—		0.6	
	20:2	20:3	—		—		—		1.0	
	20:2	22:3	—		—		—		1.9	
Hexaenes	12:0	22:6	tr.		tr.		tr.		1.4	
	14:0	22:6	6.7		10.3		0.6		2.1	
	16:0	22:6	62.3		47.4		65.3		59.6	
	18:0	22:6	27.0		9.5		20.9		14.2	
	16:1	20:5	—		—		tr.		0.7	
	16:1	22:5	—	6.0	4.3	5.8	0.6	5.3	1.1	3.5
	18:1	20:5	—		6.1		2.5		1.4	
	18:1	22:5	—		—		1.3		2.8	
	18:2	20:4	4.0		17.2		6.3		10.0	
	18:2	22:4	—		5.2		2.5		5.7	
	20:2	20:4	—		—		—		1.0	

22:6) components were present in the expected proportions, but significant amounts of (18:2 20:4) lecithin were also present.

Table 9 compares the average composition of the main molecular species of the erythrocyte and plasma lecithins of man, determined in this study, with those obtained for the blood of man and rabbit by Van Golde et al. (8) and of rat by Kuksis, Marai, Breckenridge, Gornall, and Stachnyk (12). It is seen that there are practically no qualitative differences, except in the pentaenes and in the hexaenes, which Van Golde et al. failed to detect. The major classes of plasma lecithins of man as determined by us and by Van Golde et al. compared as follows: dienes (38.5% vs. 41.7%), monoenes (20.5% vs. 20.3%), trienes (9.5% vs. 16.7%), tetraenes (22.0% vs. 14.7%), and saturates (0.5% vs. 3.6%). The discrepancies were somewhat larger for the erythrocyte lecithins of man examined in these studies: dienes (32.5% vs. 36.5%), monoenes (34.5% vs. 22.0%), trienes (4.5% vs. 15.1%), tetraenes (14.0% vs. 10.0%), and saturates (6.0% vs. 14.2%). This difference from the results of Van Golde et al. in the proportions of the lecithin classes could be due to dietary differences. Within each class of unsaturation, the unsaturated acids were paired with the saturated acids approximately in the ratio in which the latter occurred in the total fatty acid mixture.

Quantitatively, some of the molecular species are much more important in the rat than in man or rabbit. Thus the rat contains 25% saturated lecithin in its erythrocytes, while those of man and rabbit have only 6–14.2%. The monoenes and dienes are present in the rat erythrocytes in proportionally smaller amounts, while the hexaenes are twice as high in the rat as in man, with the tetraenes accounting for about equal proportions of the total. On the basis of the present study, the dienoic and tetraenoic lecithins of plasma occur in about the same proportions in both man and rat, while the proportions of hexaenoic lecithins are three times as high in rat plasma as in human plasma. The percentage of lecithin monoenes in rat plasma is about half that in the plasma of man.

It can be said that for each mammalian species the lecithin of the erythrocyte membrane is qualitatively similar to that of the surrounding plasma, but that the two lecithin pools do not undergo complete equilibration. On the basis of studies of ^{32}P turnover, Reed, Murphy, and Roberts (19) have recently concluded that in man and dog only about 60% of erythrocyte lecithin is exchangeable. It will be of interest to identify the types of the lecithin species that participate in the acyl exchanges and those which undergo a total substitution, if such a differentiation can be made.

TABLE 7 FATTY ACID COMPOSITION OF THE DIGLYCERIDE ACETATES OF THE BLOOD LECITHINS OF SUBJECT 1*

Fatty Acid	Saturates		Monoenes		Dienes		Trienes	
	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes
				moles %				
14:0	5.0	2.6	0.3	tr.				
16:0	78.0	85.2	40.6	39.2	27.6	30.0	24.0	19.4
16:1			2.7	1.7	0.7	tr.	4.7	3.3
18:0	17.0	11.1	8.7	9.7	18.4	15.5	10.0	5.3
18:1			45.0	48.3	6.1	9.0	11.3	22.0
18:2					45.2	44.3	16.0	21.4
20:0		1.1	0.4	0.5				
20:1			2.3		0.8			
20:2					0.7	0.6		3.9
20:3							26.5	24.7
22:0				0.6				
22:2					0.5	0.6		
22:3							7.5	

Fatty Acid	Tetraenes		Pentaenes		Hexaenes	
	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes
			moles %			
14:0	3.2	1.0	6.4	tr.	3.3	5.1
16:0	32.5	29.0	20.7	19.7	31.2	23.7
16:1	tr.	0.8	2.5	tr.		2.1
18:0	8.8	14.2	5.2	6.3	13.5	4.6
18:1	3.3	2.4	15.2	17.1		3.2
18:2	4.5	3.9		7.0	2.0	11.2
20:0		0.6				
20:3	3.2	3.2		1.4		
20:4	44.5	42.9	10.0	17.1	2.0	8.6
20:5			20.6	19.0		3.2
22:3				5.5		
22:4		2.0	7.7			2.6
22:5			11.7	6.9		2.1
22:6					48.0	33.6

* Diglyceride acetates resolved by TLC on silver nitrate-treated plates. Each fatty acid reported as percentage of saturation class.

TABLE 8 FATTY ACID COMPOSITION OF THE DIGLYCERIDE ACETATES OF THE BLOOD LECITHINS OF SUBJECT 2*

Fatty Acid	Saturates		Monoenes		Dienes		Trienes	
	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes
					moles %			
14:0	2.4	5.7	0.6	0.3	0.1	1.1	tr.	0.4
15:0						0.1		
15:1							0.3	
16:0	85.4	82.4	43.6	41.9	31.3	32.2	26.2	26.0
16:1			1.7	2.3	1.0	2.1	1.1	2.2
16:2						0.3		
18:0	12.2	11.9	5.7	7.8	16.0	11.5	8.6	7.6
18:1			48.4	47.2	4.2	6.4	13.6	13.3
18:2					46.3	43.3	15.0	15.5
18:3								0.2
20:0							0.2	
20:1			tr.	0.5				0.3
20:2					1.1	2.7		0.3
20:3							29.8	34.0
22:0						0.3		0.2
22:2							0.1	
22:3							5.1	

TABLE 8 *continued*

TABLE 8 *concluded*

Fatty Acid	Tetraenes		Pentaenes		Hexaenes	
	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes
			moles %			
12:0						0.7
14:0	0.2	0.5		2.0	0.3	1.0
15:1			3.5			
16:0	33.0	33.0	13.4	15.7	32.6	29.8
16:1	0.7	0.5	0.6	2.0	0.3	0.9
18:0	14.2	12.8	2.3	4.1	10.4	7.1
18:1	1.0	1.0	28.0	17.2	1.9	2.2
18:2	1.4	4.0	1.2	7.0	4.4	7.8
20:0	0.1	0.2				
20:1			1.1	0.6		tr.
20:2				1.5		0.5
20:3	1.3	1.5	1.2	2.5		
20:4	46.3	46.5	26.7	17.2	3.2	5.5
20:5			12.1	12.4	1.2	1.1
22:3	0.5			5.9		
22:4	1.3		6.4	2.6	1.3	2.8
22:5			3.5	9.3	1.0	2.0
22:6					43.4	38.6

* Diglyceride acetates resolved by TLC on silver nitrate-treated plates. Each fatty acid reported as a percentage of saturation class.

TABLE 9 COMPARISON OF THE MAJOR LECITHINS OF THE BLOOD OF MAN, RAT, AND RABBIT

Fatty Acids		Man				Rat†		Rabbit*	
		Plasma		Cells					
1-	2-	V. Golde*	Present Study	V. Golde*	Present Study	Plasma	Cells	Plasma	Cells
					moles %				
16:0	16:0	2.9	0.4	9.5	4.2	0.8	20.2	2.5	10.3
18:0	16:0	0.7	0.1	3.7	1.3	0.1	4.3	1.7	3.0
16:0	16:1	1.4		1.8				1.0	1.0
16:0	18:1	13.0	16.6	14.0	27.2	5.5	14.5	14.4	19.5
18:0	16:1	1.4		1.8				1	1
18:0	18:1	4.5	2.3	4.4	5.5	2.3	4.4	5.5	3.4
18:1	18:1		1.5	4.8	2.3	1.5	0.8		
16:0	18:2	29.3	22.3	20.4	19.5	19.4	12.4	23.5	24.0
18:0	18:2	12.4	12.7	11.3	8.5	16.0	5.6	24.3	17.8
18:1	18:2	10.5	2.4	9.3	1.4	2.5	0.7	11.0	8.2
16:0	18:3	0.5		1.0				2.5	2.8
18:0	18:3	0.5		1.0				4.0	
16:0	20:3	3.5	3.6	3.5	2.1	0.7	0.4	0.6	4.1
18:0	20:3	1.7	1.8	1.3	0.5	0.5	0.3	0.6	
16:0	20:4	9.2	14.1	6.6	8.5	5.7	5.4	2.4	2.0
18:0	20:4	5.5	5.1	3.4	3.6	13.3	7.5	2.0	1.4
18:1	20:3		0.9		0.4	0.7	0.8		
18:2	18:2		0.7		0.6	1.8	2.7		
16:0	20:5		1.0		0.8	0.8	0.5		
18:1	20:4		1.1		1.1	0.4	0.8		
16:0	22:6		3.5		2.7	6.9	4.0		
18:0	22:6		1.3		0.6	4.8	2.3		
18:2	20:4		0.3		0.7	5.5	3.0		

Values are estimated best fit only, not absolute identities or proportions.
* Modified from Van Golde et al. (8).
† Modified from Kuksis et al. (12).

These studies were supported by grants from the Ontario Heart Foundation, Toronto, Ontario, and the Medical Research Council of Canada.

Manuscript received 19 February 1968; accepted 1 October 1968.

References

1. Marai, L., and A. Kuksis. 1967. *Proc. Can. Fed. Biol. Soc.* **10:** 133.
2. Dawson, R. M. C. 1966. *In* Essays in Biochemistry. P. N. Campbell and G. D. Greville, editors. Academic Press, Inc., New York. **2:** 69.
3. Van Deenen, L. L. M., and J. De Gier. 1964. *In* The Red Blood Cell. C. Bishop and D. M. Surgenor, editors. Academic Press, Inc., New York. 243.
4. Lovelock, J. E., A. T. James, and C. E. Rowe. 1960. *Biochem. J.* **74:** 137.
5. Waku, K., and W. E. M. Lands. 1968. *J. Lipid Res.* **9:** 12.
6. Switzer, S., and H. A. Eder. 1965. *J. Lipid Res.* **6:** 506.
7. Mulder, E., and L. L. M. Van Deenen. 1965. *Biochim. Biophys. Acta.* **106:** 348.
8. Van Golde, L. M. G., V. Tomasi, and L. L. M. Van Deenen. 1967. *Chem. Phys. Lipids.* **1:** 282.
9. Kuksis, A., W. C. Breckenridge, L. Marai, and O. Stachnyk. 1969. *J. Lipid Res.* **10:** 25.
10. Hill, J. G., A. Kuksis, and J. M. R. Beveridge. 1964. *J. Amer. Oil Chem. Soc.* **41:** 393.
11. Skipski, V. P., R. F. Peterson, and M. Barclay. 1964. *Biochem. J.* **90:** 374.
12. Kuksis, A., L. Marai, W. C. Breckenridge, D. A. Gornall, and O. Stachnyk. 1968. *Can. J. Physiol. Pharmacol.* **46:** 511.
13. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. *J. Biol. Chem.* **266:** 497.
14. Dodge, J. T., and G. B. Phillips. 1967. *J. Lipid Res.* **8:** 667.
15. Phillips, G. B., and J. T. Dodge. 1967. *J. Lipid Res.* **8:** 676.
16. Hill, J. G., A. Kuksis, and J. M. R. Beveridge. 1965. *J. Amer. Oil Chem. Soc.* **42:** 137.
17. Williams, J. H., M. Kuchmak, and R. F. Witter. 1966. *Lipids.* **1:** 89.
18. Williams, J. H., M. Kuchmak, and R. F. Witter. 1966. *Lipids.* **1:** 391.
19. Reed, C. F., M. Murphy, and G. Roberts. 1968. *J. Clin. Invest.* **47:** 749.