Composition of phospholipids and of phospholipid fatty acids of human plasma

GERALD B. PHILLIPS* and JAMES T. DODGE‡

Department of Medicine, College of Physicians and Surgeons, Columbia University, New York 10032, and The Roosevelt Hospital, New York 10019

ABSTRACT The composition of the phospholipids and of the total phospholipid fatty acids was determined in the plasma of 10 normal subjects. In addition the fatty acid composition of the plasma phosphatidyl ethanolamine, phosphatidyl serine, lecithin, sphingomyelin, and lysolecithin of 6 of the subjects was measured.

A wide array of fatty acids was found in the plasma total phospholipid similar to that found previously in red cell total phospholipid. The fatty acid composition in the plasma phospholipids of a given subject reflected that in his red cell phospholipids.

Each individual phospholipid displayed a distinctive fatty acid pattern, which was generally similar to that of the corresponding phospholipid of red cells, although some marked differences in individual fatty acid levels between the corresponding phospholipids of plasma and red cells were evident.

The high percentage of unsaturated fatty acids found in plasma lysolecithin suggests that this phospholipid did not arise entirely through the enzymatic cleavage of the β-fatty acid of lecithin.

KEY WORDS phospholipids · fatty acids · human plasma · phosphatidyl ethanolamine · phosphatidyl serine · lecithin · sphingomyelin · lysolecithin

ALTHOUGH the fatty acid composition of human plasma total phospholipid has been determined repeatedly (1–8), reports of a similar comprehensive analysis of the fatty acids of individual plasma phospholipids of man other than those of sphingomyelin (9–11) have been limited (10). The main difficulties in such an analysis have been (*a*) the incomplete separation of the individual phospholipids by silicic acid column chromatography, so that analysis of a segment of a phospholipid peak would not be representative of the whole peak (12, 13), while analysis of the whole peak would include contamination from adjacent peaks; (*b*) limited resolution of some of the fatty acid methyl esters by GLC; and (*c*) possible fatty acid autoxidation during extraction, storage, and analysis of the lipid sample. In the present study, the composition of the phospholipids and of the total phospholipid fatty acids was determined in the plasma of 10 normal subjects and the composition of the fatty acids in the plasma phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), lecithin, sphingomyelin, and lysolecithin was determined in 6 of the subjects. To overcome the difficulties enumerated, we isolated the individual phospholipids by TLC on silicic acid, which allowed apparently complete separation; analyzed the fatty acids with improved GLC methods, which permitted a more comprehensive analysis; and used the antioxidant 2,6-di-*tert*-butyl-*p*-cresol (BHT) during lipid extraction, storage, and analysis to prevent autoxidation.

MATERIALS AND METHODS

Extraction of Plasma

20 ml of venous blood was obtained from each of 7 male and 3 female, apparently healthy, fasting subjects between 20 and 40 yr of age and anticoagulated with 0.15 ml of a solution containing 30 g EDTA per 100 ml

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; PE, phosphatidyl ethanolamine; PS, phosphatidyl serine; PI, phosphatidyl inositol; BHT, 2,6-di-*tert*-butyl-*p*-cresol. In the abbreviation of the fatty acids, the first two digits state the number of carbon atoms, the third digit states the number of double bonds, and the digit after the omega states the number of carbon atoms from the methyl end of the acyl chain to the middle of the terminal double bond.

* Recipient of Lederle Medical Faculty Award. Present address: The Roosevelt Hospital, 428 W. 59th Street, New York 10019.

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(Vacutainer, Becton-Dickinson & Co., Rutherford, N.J.). A brief dietary history revealed that none of the subjects was on a special diet except one who intentionally consumed larger amounts of unsaturated fat than the others. The blood was chilled immediately at 0°C and then centrifuged at 4°C; the plasma was removed, kept at 4°C, and extracted within 2 hr after withdrawal of the blood. The red cells were processed and analyzed for phospholipids and total and individual phospholipid fatty acids; the results of the red cell analyses are reported separately (14). The plasma samples of five of the subjects were extracted essentially as described previously (15), ie., 1 volume of plasma was added to 15 volumes of methanol–chloroform 1:1 and mixed; the mixture was allowed to stand for 15 min with occasional shaking, filtered to remove the precipitate, emulsified with an equal volume of distilled water, centrifuged to remove the water–methanol layer, and taken to dryness in a rotary vacuum evaporator at 40°C (Method 1). The other five plasma samples were extracted in a manner similar to that used for the red cells (14), as follows: 1 volume of plasma was added to 7 volumes of methanol and mixed and the mixture was allowed to stand for 30 min with occasional shaking; 7 volumes of chloroform was then added and mixed and the mixture was allowed to stand for 20 min with occasional shaking before being filtered; 7 volumes of chloroform was added to the filtrate and mixed, and the mixture was emulsified with 0.2 of its total volume of 0.05 M KCl (16) and centrifuged; the upper layer was then removed and the bottom layer taken to dryness in a rotary vacuum evaporator at 40°C (Method 2). The antioxidant BHT (Ionol CP Shell Chemical Corp, New York) was present in the methanol used for extraction at a concentration of 5 mg/100 ml. The extracts were stored at −22°C in chloroform containing 5 mg of BHT per 100 ml.

Thin-layer Chromatography and Determinations

To determine the concentration of the individual neutral lipids, we chromatographed an aliquot of lipid extract on a 0.5 mm layer of Silica Gel HR (Brinkmann Instruments, Inc., Westbury, N.Y.) with hexane–diethyl ether–glacial acetic acid 80:20:1 containing 50 mg of BHT per 100 ml to prevent autoxidation during chromatography (17). The plate was sprayed with 2,7-dichlorofluorescein and the cholesterol, cholesteryl ester, and triglyceride spots, which were completely separated, were each scraped off and eluted three times with 2 ml aliquots of methanol–chloroform 1:2. Cholesterol and cholesteryl ester concentrations were determined in duplicate by the method of Abell, Levy, Brodie, and Kendall (18), which was modified in that the cholesteryl ester was exposed to alcoholic KOH at 75°C for 60 min. Triglyceride concentration was estimated in duplicate by the ester bond method of Rapport and Alonzo (19).

Total plasma lipid phosphorus was determined in triplicate by an adaptation (14) of Marinetti's modification of Bartlett's method (20, 21).

The phospholipid composition was determined in triplicate by TLC of aliquots of the lipid extract on Silica Gel HR, 0.5 mm thick, in chloroform–methanol–glacial acetic acid–water 25:15:4:2 (22) containing 50 mg of BHT per 100 ml; the TLC plates were prepared with water ("neutral plates"). The scraped-off spots were digested directly for phosphorus determination as described previously (14).

Preparation and GLC of Methyl Esters

To prepare the methyl esters of the total and individual plasma phospholipids, we separated these fractions by TLC and converted the fatty acids of each fraction to methyl esters with boron trifluoride–methanol reagent (Applied Science Laboratories Inc., State College, Pa.) using the method of Morrison and Smith (23) exactly as described previously (14).

The total phospholipid methyl ester preparations from the five plasma samples that were extracted by Method 1 were refined further by TLC on Silica Gel HR, 0.5 mm thick, with toluene containing 50 mg of BHT per 100 ml as the developing solvent. The methyl ester spot was eluted with chloroform containing BHT, 5 mg/100 ml, and analyzed by GLC. Because of their low concentration, the dimethyl acetals were not analyzed by GLC.

Analysis of the fatty acid methyl esters by GLC was carried out on the total plasma phospholipid of all 10 subjects, on the PE, PS, lecithin, and sphingomyelin of 6 subjects (5 extracted by Method 2), and on the lysolecithin of 5 subjects (4 extracted by Method 2). The total phospholipid fatty acid methyl esters were analyzed both before and after resolution by TLC in toluene in one subject (extracted by Method 1) and on extracts obtained both by Methods 1 and 2 in another subject.

GLC was performed with a Barber-Colman instrument, model 5000, equipped with paired 8-ft columns of 8% EGSS-X (an ethylene glycol succinate polyester combined with a silicone) on Gas-Chrom P, 100–120 mesh (Applied Science Laboratories Inc.) and dual flame ionization detectors. The methods for GLC and identification and quantitation of peaks have been described in detail (14). Palmitoleic acid was not quantified because of interference by a BHT artifact. Since several peaks contained mixtures of fatty acids, values are expressed as weight rather than moles per cent. The analysis of fatty acid methyl ester standards agreed with standard composition data with a relative error of less than 2% for major components (>10% of total mixture) and less than 3.5% for minor components (<10% of total mixture).

A description of the solvents used has been reported (14).

RESULTS

The composition of the individual plasma phospholipids and neutral lipids of the 10 subjects is shown in Table 1. Since the plasma samples of 5 of the subjects were extracted by Method 1 and 5 by Method 2, the values for the two groups are reported both separately and in combination.

The fatty acid composition of the plasma total phospholipid from the 10 subjects is shown in Table 2. As the polarity of the GLC column changed with time (14), the single peaks containing 20:3ω6 + 22:0 and 22:4ω6 + 24:1ω9, respectively, split so that the individual components could be estimated in the samples of 6 subjects. Since half of the samples were extracted by Method 1 and half by Method 2, the plasma of 1 subject was extracted by each of the two methods and the phospholipid fatty acids were analyzed. No significant difference between the two samples in any fatty acid comprising over 1% of the total fatty acid was observed. Similarly, since the phospholipid fatty acid methyl esters were isolated by TLC in the samples extracted by Method 1, those from one of these plasma extracts were analyzed by GLC both before and after TLC. Again no significant difference between the two samples in any fatty acid comprising over 1% of the total fatty acid was noted. That the elimination of the dimethyl acetals by TLC produced no discernible difference in values can be explained by the relatively low concentration of plasmalogens in normal human serum (15, 24). Table 2 shows that the plasma phospholipid contains a wide spectrum of fatty acids. Four of the fatty acids (16:0 18:0, 18:1ω9, 18:2ω6), however, made up about 70% of the total, while most of the other individual acids made up less than 1% of the total.

The fatty acid composition of PE, PS, lecithin, sphingomyelin, and lysolecithin in the plasma of 6 of the subjects (5 extracted by Method 2, 1 by Method 1) is shown in Table 3. The fatty acid patterns of the sample extracted by Method 1 were similar to the others except for a considerably higher level of 22:4ω6 and 22:6ω3 in the lysolecithin. Small quantities of lysolecithin that could be demonstrated in the washings of the extracts could have accounted for these differences. The lower concentrations of PE, PS, and lysolecithin required that the GLC be carried out at a higher sensitivity, making quantification, especially of the minor fatty acid methyl ester peaks, less accurate than with lecithin and sphingomyelin. This increased difficulty in quantification may account for the wider range of values between subjects observed with PE, PS, and lysolecithin. Each

TABLE 1 PHOSPHOLIPID AND NEUTRAL LIPID COMPOSITION OF PLASMA FROM 10 NORMAL SUBJECTS

Determination	Results: Extraction Method 1*	Extraction Method 2*	Total†
		μmoles/ml	
Free cholesterol	1.62	1.25	1.44 ± 0.31
Cholesteryl ester	3.78	3.04	3.42 ± 0.66
Triglyceride	1.19	0.54	0.87 ± 0.52
Lipid phosphorus	3.04	2.51	2.78 ± 0.50
Phospholipid distribution		*moles/100 moles of phosphorus recovered*	
Phosphatidyl ethanolamine	3.9	3.4	3.6 ± 0.8
Phosphatidyl serine	2.3	1.5	1.9 ± 0.7
Lecithin	72.5	68.3	70.4 ± 3.0
Sphingomyelin	16.1	18.7	17.4 ± 2.2
Lysolecithin	4.9	7.5	6.2 ± 2.3
Origin of TLC plate	0.4	0.6	0.5 ± 0.6
Recovery of phosphorus after TLC (%)	94.5	96.4	95.4 ± 5.5

* Mean of values from 5 of the subjects.
† Mean ±SD of values from the 10 subjects, 5 of whose plasmas were extracted by Method 1 and 5 by Method 2.

phospholipid had a distinctive fatty acid pattern with prominent distinguishing features. For example, the predominant unsaturated fatty acid in PE and PS was 20:4ω6, while in lecithin and lysolecithin it was 18:2ω6, A characteristic high level of 18:0 was present in PS and of 16:0 in lysolecithin. In general, the fatty acids were most unsaturated in PE and PS and by far the most saturated in sphingomyelin. About 95% of the sphingomyelin fatty acids were saturated or had only one double bond.

The small amount of phosphatidyl inositol (PI) present in human plasma (15) may have been scraped from the TLC plate either with the PS or lecithin and thereby have affected the distribution of these phospholipids or their fatty acids.

DISCUSSION

Although the fatty acid composition of human plasma total phospholipid has been analyzed previously (1–8), the present study includes evidence for and measurements of a larger number of fatty acids than has hitherto been reported. As a result of the diluting effect of the additional fatty acids quantified, the relative amounts of the major fatty acids are generally lower than in the previous studies. When only the major fatty acids are considered, the relative amounts found in the present study are similar to those reported previously (1–8). An exception is arachidonic acid, the relative amount of which is higher, and considerably so in some cases, than

TABLE 2 FATTY ACID COMPOSITION* OF TOTAL PHOSPHOLIPIDS FROM PLASMA OF 10 NORMAL SUBJECTS

Components†	Distribution
	g/100 g of total fatty acid
16:0	26.0 ± 1.4
17:0	0.58 ± 0.16
18:0	13.5 ± 0.9
18:1ω9	11.4 ± 1.8
19:0	—‡
18:2ω6§	19.7 ± 2.2
20:0	0.74 ± 0.19
20:1ω9 + 18:3ω3	0.45 ± 0.13
20:2ω9 + 20:2ω6 + 21:0	0.84 ± 0.14
20:3ω9	0.60 ± 0.21
20:3ω6 + 22:0‖	4.5 ± 0.9
20:4ω6 + 22:1ω9	10.7 ± 1.6
23:0	0.93 ± 0.19
20:5ω3 + 22:2ω6§	0.67 ± 0.29
24:0	1.5 ± 0.5
22:4ω6 + 24:1ω9‖	2.7 ± 0.4
22:5ω6	0.40 ± 0.16
24:2ω9 + 24:2ω6	0.21 ± 0.09
22:5ω3	0.83 ± 0.22
26:0 + 22:6ω3	3.6 ± 0.6
26:1ω9 + 24:4ω6	0.20 ± 0.09

* Values are shown as mean ± SD.
† This shorthand designation is explained in the *Abbreviations* (p. 676).
‡ This peak was too small for quantification at the sensitivities used in this part of the analysis but was estimated to be <0.5% of the total.
§ May contain the ω9 component.
‖ In 6 of the subjects, the components separated enough to be estimated individually and the values are as follows: 20:3ω6, 3.1 ± 1.0; 22:0, 1.6 ± 0.3; 22:4ω6, 0.26 ± 0.07; and 24:1ω9, 2.2 ± 0.4.

in most of the previous studies (1–6). The inclusion of a small amount of 22:1ω9 in the arachidonic acid peak in the present study does not adequately explain this discrepancy; a more likely explanation is that the arachidonic acid level in certain previous studies may have been lowered artifactually by autoxidation, to which this fatty acid is particularly susceptible (25–27). Autoxidation may have been prevented in the present study through the use of the antioxidant BHT.

We observed a wide array of fatty acids in plasma phospholipid similar to that reported separately for the phospholipid of the red cells of these same subjects (14). As with the red cells, the linoleic acid level had the largest absolute standard deviation, and the subject with the largest estimated unsaturated fat intake had the highest level. The profound influence of dietary fat intake on plasma phospholipid fatty acid levels was clearly demonstrated by Ahrens et al. (2). Although the fatty acid composition of the total phospholipid of plasma of the subjects in the present study differed considerably from that of red cells, the plasma levels of individual fatty acids reflected those in red cells. This point is illustrated in Table 4, which shows the correlation coefficients for major fatty acids between the plasma and red cells of these subjects; these coefficients were calculated by relating the level of a given fatty acid in the plasma and red cells of the same subject for the series of 10 subjects. Such correlation is easily explained by the dem-

TABLE 3 FATTY ACID COMPOSITION* OF THE INDIVIDUAL PHOSPHOLIPIDS OF PLASMA FROM 6 NORMAL SUBJECTS

Components†	Fatty Acid Distribution (g/100 g of Fatty Acid)									
	Phosphatidyl Ethanolamine		Phosphatidyl Serine		Lecithin		Sphingomyelin		Lysolecithin	
	Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*
16:0	8.0	5.5 –10.6	7.8	6.3 – 9.0	26.1	24.1 –27.4	26.2	23.4 –29.4	39.5	35.1 –42.9
17:0	0.54	0.34– 0.83	0.66	0.40– 1.1	0.53	0.40– 0.67	0.64	0.44– 0.92	1.0	0.62– 1.3
18:0	15.4	13.1 –18.1	32.6	30.1 –34.6	13.6	12.4 –15.2	8.2	6.3 –10.0	15.2	12.6 –17.6
18:1ω9	7.9	4.5 –13.5	11.6	7.4 –16.2	12.9	10.6 –15.9	1.8	1.2 – 2.3	13.5	10.5 –16.7
18:2ω6‡	15.8	9.5 –23.1	8.6	6.9 –11.4	24.7	21.2 –27.6	1.1	0.59– 1.8	21.1	15.9 –28.3
20:0	1.3	0.45– 2.2	0.89	0.26– 1.4	0.14	0.09– 0.22	4.1	3.8 – 4.4	0.33	0.05– 0.06
20:1ω9 + 18:3ω3	0.76	0.36– 0.99	0.84	0.34– 1.3	0.45	0.26– 0.64	0.46	0.16– 0.75	0.31	0.08– 0.58
20:2ω9 + 20:2ω6 + 21:0	1.2	0.23– 2.0	1.6	0.52– 2.1	0.67	0.60– 0.80	0.71	0.26– 1.0	0.62	0.41– 0.94
20:3ω9	0.58	0.26– 1.0	0.98	0.57– 1.3	0.46	0.36– 0.60	tr.		0.29	0.06– 0.47
20:3ω6	1.7	0.95– 2.5	3.2	2.3 – 4.2	3.0	2.2 – 4.6			1.1	0.60– 1.5
22:0							14.5	11.1 –20.0		
20:4ω6 + 22:1ω9	26.3§	20.9 –32.6	25.1§	19.9 –30.5	10.9§	8.3 –14.0	1.6‖	1.1 – 2.4	3.4§	1.9 – 4.4
23:0	0.22	0.05– 0.44	0.27	0.14– 0.43	0.15	0.07– 0.19	7.0	5.4 – 8.7	0.30	0.07– 0.66
20:5ω3 + 22:2ω6‡	1.4	0.98– 2.2	0.20	0.13– 0.29	0.52	0.34– 0.76	0.80	0.62– 1.0	0.20	tr. – 0.36
24:0	0.28	0.09– 0.42	0.52	0.44– 0.64	0.26	0.08– 0.57	11.5	7.9 –15.1	0.10	tr. – 0.25
22:4ω6	1.9	1.4 – 2.3			0.48	0.27– 0.61			0.95	0.13– 2.8
24:1ω9	tr.		1.2	0.86– 1.6			18.3	12.3 –23.2	0.23	tr. – 0.53
22:5ω6	1.1	0.15– 1.7	0.41	0.23– 0.66	0.34	0.26– 0.56	0.67	tr. – 0.92	0.07	tr. – 0.21
24:2ω9 + 24:2ω6	tr.		tr.		tr.		2.0	1.2 – 3.6	tr.	
22:5ω3	2.8	2.1 – 3.9	0.81	0.49– 1.1	0.74	0.53– 1.0	T		0.14	tr. – 0.37
26:0							0.34	tr. – 0.77		
22:6ω3	12.5	8.3 –16.2	2.3	1.8 – 3.3	4.1	3.0 – 5.9			1.4	0.75– 3.2
26:1ω9 + 24:4ω6	0.42	tr. – 0.54	0.48	0.32– 0.91	tr.		tr.		0.22	tr. – 0.56

* Five of the plasma samples were extracted by Method 2, one by Method 1.
† See †, Table 2. tr., ≦ 0.05% of total. Blank spaces: component not detected.
‡ May contain the ω9 component.
§ Appears to be almost entirely 20:4ω6.
‖ Appears to be largely 22:1ω9.

TABLE 4 RELATIONSHIP BETWEEN PLASMA AND RED CELL FATTY ACIDS IN 10 NORMAL SUBJECTS

Fatty Acid	Correlation Coefficients*
16:0	+0.42
18:0	+0.80
18:1ω9	+0.51
18:2ω6	+0.73
20:4ω6	+0.66
24:0	−0.50
22:6ω3	+0.85

* The SEM for a series of 10 is 0.33.

onstrated ready exchange of phospholipids between plasma and red cells (28, 29). The rapid response of red cell phospholipid fatty acid levels to dietary triglyceride manipulation (30–33) further supports the concepts of exchange and of a parallel relationship between levels of individual fatty acids in plasma and red cells. The possible negative correlation for 24:0 (Table 4), which is found almost exclusively in the sphingomyelin, is unexplained, as sphingomyelin has been reported to exchange between plasma and red cells as rapidly as lecithin (29).

The fatty acid distribution of each individual phospholipid showed a characteristic pattern. For example, the main unsaturated fatty acid of PE and PS was arachidonic, while in lecithin and lysolecithin, it was linoleic; the main saturated fatty acid of PE and PS was stearic, which comprised about one-third of the fatty acid of PS, while in lecithin, sphingomyelin, and lysolecithin, it was palmitic, which made up about two-fifths of the fatty acid of lysolecithin. Sphingomyelin was in sharp contrast to the glycerophospholipids because it was highly saturated and because it contained considerable amounts of fatty acids, such as 22:0, 23:0, 24:0, and 24:1, which were present in only minor quantities if at all in the other phospholipids.

The percentages of six or seven fatty acids have been estimated previously in plasma lecithin (34–36), sphingomyelin (34, 35), lysolecithin (37), and PE (36). The values obtained in these studies show considerable disagreement with each other as well as with those found in the present study. These differences could have been due to less effective methods of phospholipid and fatty acid separation and the analysis (apparently) of only one or two samples in previous studies. More extensive studies of the fatty acid composition of plasma sphingomyelin have been carried out by Sweeley (9) and Svennerholm, Ställberg-Stenhagen, and Svennerholm (11). The values these investigators found are in general agreement with those we found with a few outstanding exceptions, such as a value of 41.7% for 16:0 in the study of Sweeley (9) and 4.9% and 35.3% for 24:0 and 24:1, respectively, in the study of Svennerholm et al. (11). Williams, Kuchmak, and Witter (10) have recently reported detailed analyses of the fatty acid distribution of the major as well as minor phospholipids of a sample of pooled human serum. The fatty acid compositions of lecithin and PE these investigators found were similar to those observed in the present study, but those of sphingomyelin and lysolecithin showed conspicuous differences. Examples of such differences were their values of 42.5% 16:0 and 8.3% for 24:1ω9 in sphingomyelin and 12.3% for 18:2 in lysolecithin. One possible explanation for the differences from our results is that the individual phospholipids analyzed by Williams et al. (10) apparently were purified fractions which might not have been representative of the whole phospholipid species. The fatty acid distribution of PS observed in the present study was considerably different from the PS but very similar to the PI pattern reported by Williams et al. (10). These two phospholipids are difficult to separate both by the column and thin-layer chromatographic methods used in these studies. Support for the pattern described in the present study as being that of PS is provided by the similarity of the pattern found for the PS of red cells (14), where PS comprises about 15% and PI less than 1% of the total phospholipid (14, 38).

About 56% of the fatty acids of the plasma lysolecithin were saturated, in contrast to the value of 76.5% found by Gjone, Berry, and Turner (39), 76% by Tattrie (37), and 64.3% by Williams et al. (10). Since the fatty acids in the α-position of lecithin appear to be mainly saturated and those in the β-position unsaturated (40, 41), this degree of unsaturation in lysolecithin indicates that it did not arise entirely from enzymatic cleavage of the β-fatty acid of lecithin, as might occur by the action of a phospholipase A (41) or the plasma cholesterol esterifying system (42, 43).

The fatty acid patterns of the individual phospholipids of plasma and red cells showed striking similarities, and the difference in fatty acid distribution between the total phospholipid of plasma and red cells can be attributed at least in part to different phospholipid distributions. For example, the linoleic acid of plasma and red cell phospholipid resided predominantly in the lecithin and the ratio of linoleic acid in plasma phospholipid to that in red cell phospholipid was approximately the same as the respective lecithin ratios. Nevertheless, impressive differences for certain fatty acids between the same phospholipid species of plasma and red cells were evident; for example, the relative amount of 24:0 in red cell sphingomyelin was about double that in plasma sphingomyelin, an observation which may be related to the lack of positive correlation noted between the level of plasma and red cell 24:0 (Table 4). Possible explanations for these differences, taking into account the demonstrated exchange of phospholipids between plasma and red cells (28, 29), are (*a*) that molecules of a given phospholipid that differ in fatty acid composition have different binding affinities for the

red cell membranes and the plasma lipoproteins, and (*b*) that only a fraction of the phospholipids of the red cell and plasma lipoprotein exchanges. The higher standard deviations for the phospholipid fatty acid levels of plasma as compared to red cells suggests that the phospholipid fatty acid levels of plasma are more responsive to alteration of diet than are those of red cells.

The composition of the neutral lipids and individual phospholipids found in the present study with silicic acid TLC was similar to that found previously in this laboratory with silicic acid column chromatography (44). The higher value for PS found in the present study may have been due to inclusion of PI with the PS, possible contamination with red cells, or the technical error involved in analysis of such a minor component. The appearance of a spot with the R_f of PS on TLC and the similarity between the fatty acid distribution of this spot and that of red cell PS suggest that some PS is present in human plasma.

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