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Studies on Sphingomyelins in Human Erythrocytes and Plasma

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Introduction

In the previous papers[1,2] it has been reported that two spots of sphingophospholipids were detected on sphingomyelin region of Silica Gel thin-layer chromatograms of lipids from rat plasma, erythrocytes and liver. It has been also shown that in erythrocytes the incorporation of ^{32}P into the slow moving spot was remarkably higher than that into the fast moving one[2].

There have been some other reports noting two spots on thin-layer chromatograms for sphingomyelin from human plasma[3~5] and chylomicron[6], sheep, hog and steer sera, egg yolk[7] and ox brain[8]. Only a few investigations have been reported briefly on the nature of these lipids in human plasma[4,8] but not yet of those in erythrocytes.

In the present study these fast and slow moving sphingophospholipids (SPL-F and -S) separated from human erythrocytes and plasma were compared with each other and an attempt was made to elucidate the difference in their chemical structures.

Experimental

1. *Preparation of Fast and Slow Moving Sphingophospholipids (SPL-F and -S).*

Materials and Lipid Extraction Human ACD blood was supplied from the Blood Bank. Plasma was separated from blood cells by conventional procedure. Blood cells were suspended in physiological saline and erythrocytes were separated from plasma and leucocytes by repeated centrifugation at 220×g. Plasma and erythrocytes were extracted overnight with 25 volumes of chloroform-methanol (2 : 1, V/V) at room temperature respectively. The lipid extracts were washed by Folch's procedure[9].

Preparation of Phospholipids Stable to Mild Alkaline and Acid Hydrolysis The phospholipids separated by silicic acid column chromatography were first subjected to the mild alkaline hydrolysis by the procedure of Dawson[10]. The alkali-stable lipids were dissolved in HCl-chloroform-methanol (containing 0.7 mℓ. of conc. HCl in 20 mℓ. of chloroform-methanol 2 : 1 (V/V). less than 50 μg. of lipid phosphorus per mℓ.) and incubated for 30 minutes at 37°C. After incubation the reaction mixture was washed immediately by Folch's procedure[9] and the organic solvent layer was evaporated to dryness *in vacuo* at room temperature. The alkali-and acid-stable lipids thus obtained were redissolved in chloroform. Four grams of activated silicic acid* was packed in a column of 1 cm. diameter by slurrying with 3-4 volumes of methanol and the column

* Mallinckrodt (100 mesh), activated at 120°C for 12 hours.

was washed with chloroform sufficiently. Five mℓ. of the chloroform solution containing less than 20 mg. of alkali- and acid-stable lipids was applied onto the column and then eluted with the following solvents successively; 30 mℓ. of chloroform, 25 mℓ. of chloroform-methanol (C-M) (80 : 20, V/V), 25 mℓ. of C-M (60 : 40, V/V), 150 mℓ. of C-M (40 : 60, V/V), 25 mℓ. of C-M (20 : 80, V/V) and 50 mℓ. of methanol. Each fraction was examined by thin-layer chromatography and phosphorus determination. Sphingophospholipids were eluted in C-M (40 : 60, V/V) fraction (Fig. 1).

Fractionation of SPL-F and -S by Thin-Layer Chromatography (TLC.) Thin-layer plates (10×20 cm.) of 500 μ thickness were prepared with Silica Gel G* by the procedure of Stahl[11] and were activated for 2 hours at 120°C before use. The alkali- and acid-stable phospholipids (less than 250 μg. of lipids in 0.25 mℓ. of chloroform per plate) were applied on plates as a streak and developed with chloroform-methanol-water (65 : 25 : 4 or 75 : 25 : 0.5, V/V) at room temperature. For the detection of lipids purified Rhodamine B was used. Fifty mℓ. of 0.05% commercial Rhodamine B in C-M (80 : 20, V/V) was passed through a column (diameter 1 cm., activated silicic acid 4 g.) which had been washed with C-M (80 : 20, V/V). Most of the dye was eluted with additional 5 mℓ. of C-M (80 : 20, V/V). Although small amounts of the dye still remained on the column and could be eluted with methanol, use of this fraction, as well as use of commercial Rhodamine B without purification, caused a difficulty in subsequent removal of the dye from sphingophospholipids. After drying the plate at room temperature the Rhodamine B in C-M (80 : 20, V/V) solution was sprayed. The lipid regions were visualized under UV light (Fig. 2). Each band of SPL-F and -S was scraped off and the lipid was extracted three times by refluxing with 5 to 10 volumes of methanol respectively. SPL-F or -S were extracted together with the dye. The methanol extract

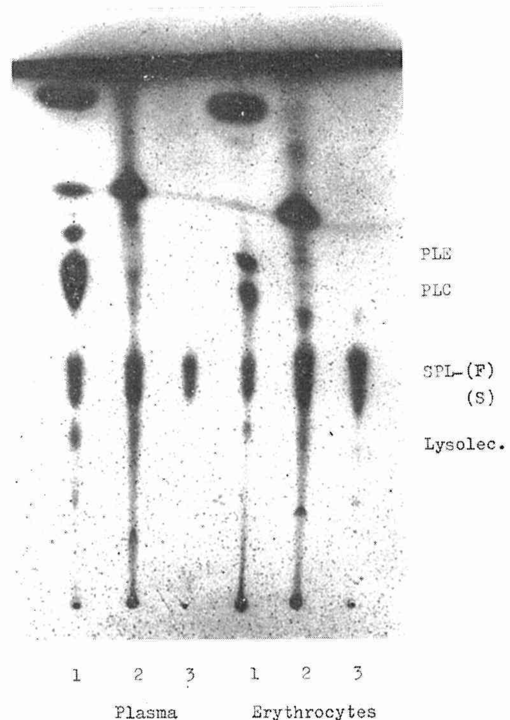


Fig. 1 Thin-layer chromatograms of alkali- and acid-stable lipids from plasma and erythrocytes.

1: Original lipids, 2: lipids stable to mild alkaline hydrolysis, 3: phospholipids stable to mild alkaline-acid hydrolysis (C-M (40 : 60 V/V) fraction of silicic acid column chromatography of mild alkaline-and acid- stable lipids).

PLE: phosphatidylethanolamine, PLC: phosphatidylcholine, SPL-F: fast moving sphingophospholipid, SPL-S: slow moving sphingophospholipid, Lysolec.: lysophosphatidylcholine.

Detected by phosphomolybdic acid spray. Developing solvent; chloroform-methanol-water 65 : 25 : 4 (V/V). See text for other details.

* Merck, A. G. Darmstadt, Germany.

was evaporated to dryness *in vacuo* below 50°C. The residue was dissolved in 5 mℓ. of C-M (80 : 20, V/V) (containing less than 20 mg. of lipids) and applied onto a column as described above. The column was eluted first with 20 mℓ. of C-M (80 : 20, V/V), next with 20 mℓ. of C-M (60 : 40, V/V) and then with 100 mℓ. of methanol. The dye was eluted in the C-M fractions and pure SPL-F or -S were well recovered in methanol fraction.

2. *Procedures for the Examination of Chemical Nature of SPL-F and -S.*

Methanolysis and Hydrolysis of SPL-F and -S. *Procedure* I : The methanolysis and the separation of the products were carried out by the procedure of Sweeley and Moscatelli[12]. SPL-F or -S (less than 15 mg. of lipids) were dissolved in 2 mℓ. of 2*N* HCl-methanol (conc. HCl-methanol 2 : 10 V/V) and refluxed for 8 hours. The reaction mixture was then fractionated quantitatively into fatty acid ester, long-chain base and water soluble hydrolysate fractions.

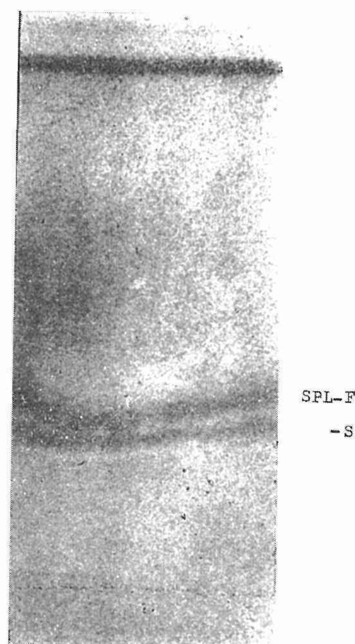


Fig. 2 Separation of SPL-F and -S by thin-layer chromatography.

Visualized by Rhodamine B spray and UV light. Developing solvent : chloroform-methanol-water 65 : 25 : 4 (V/V).

Procedure II : For the paper chromatographic examination of the water soluble hydrolysates the procedure of Rouser *et al.*[13] was used with a slight modification of hydrolysis condition. SPL-F or -S (less than 15 mg. of lipids) were hydrolyzed with 3 mℓ. of 4*N* HCl in a sealed tube at 120°C for 21 hours. After cooling the reaction mixture was extracted three times with 3 mℓ. of *n*-hexane. The aqueous layer was evaporated repeatedly to remove HCl. The residue was redissolved in water and subjected directely to the paper chromatography.

Gas-Liquid Chromatography (GLC) of Fatty Acid Methyl Esters The fatty acid ester fraction (Procedure I) was evaporated to dryness *in vacuo* below 50°C. The residue (less than 20 mg. of lipids) was dissolved in 5 mℓ. of hexane and applied onto a column (diameter : 1 cm.) packed with 2 g. non-activated silicic acid-Hyflo Super Cel* (1 : 1, W/W) by slurrying with chloroform. The column was eluted with 50 mℓ. of chloroform-hexane (10 : 90, V/V). The fatty acid methyl esters were eluted in the chloroform-hexane fraction and this fraction was evaporated to dryness. The residue was redissolved in chloroform and submitted to GLC.

GLC was performed with Hitachi gas chromatograph model KGL-2B equipped with flame ionization detector (H_2) and a Golay column BDS-45 (butanediol succinate, 0.5 mm. ×45 m.). The operating temperature was 195°C or 170°C and He was used as a carrier

* Johns-Manville, U. S. A.

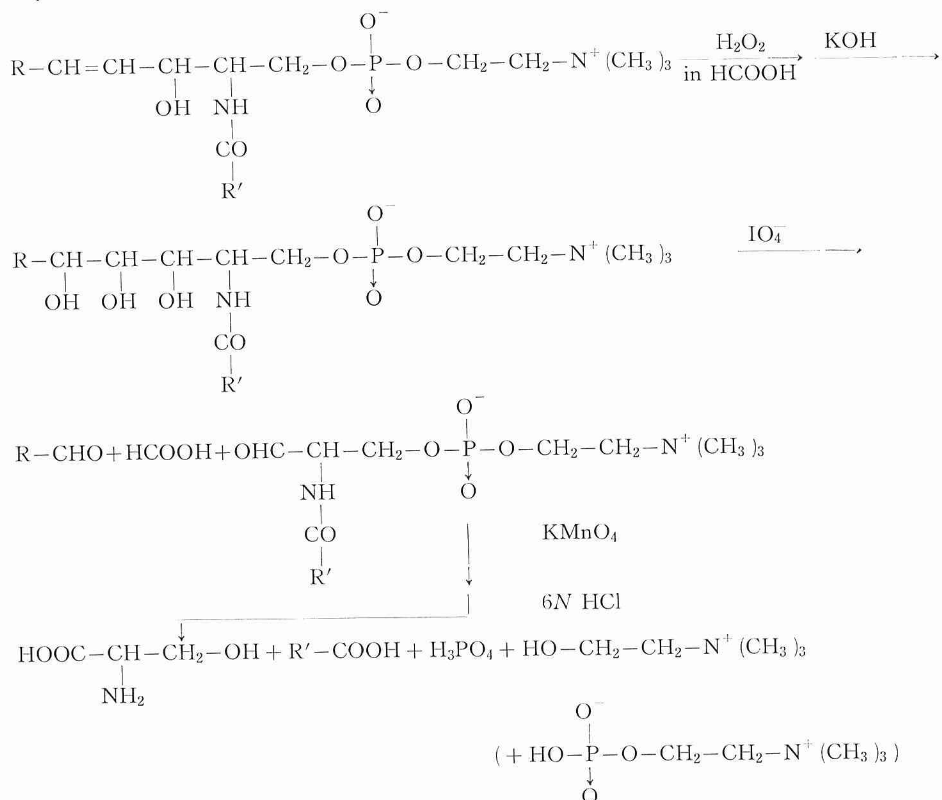
gas with a pressure of 0.5–0.6 kg/cm^2.

TLC of Long-Chain Bases The long chain base fraction (procedure I) was evaporated to dryness *in vacuo* below 50°C. The residue (less than 10 mg. of lipids) was dissolved in 5 mℓ. of chloroform and applied onto a column (diameter 1 cm., activated silicic acid 4 g., washed with chloroform) as described before. The elution was carried out first with 20 mℓ. of chloroform and then with 40 mℓ. of C–M (20 : 80, V/V). The long chain bases were eluted in C–M fraction.

This fraction was thin-layer-chromatographed with the plates of 250 μ thickness of Silica Gel G activated at 120°C for 2 hours before use. Chloroform-methanol-2*N* aq. ammonia (40 : 10 : 1, V/V) according to Sambasivarao and McCluer[14] was used as a developing solvent. The spots were detected by ninhydrin spray.

Paper Chromatography of Water Soluble Hydrolysates The water soluble hydrolysates (procedure II) were paper-chromatographed by ascending technique with the following developing solvents; phenol saturated with water, 95% ethanol-conc. ammonia (95 : 5, V/V) or *n*-butanol-glacial acetic acid-water (100 : 30 : 85, V/V)[15]. The spots were detected by the same reagents as described below for qualitative test on TLC.

Performic Acid-HIO$_4$ Oxidative Cleavage of SPL–F and –S SPL–F or –S (15 mg.) were directly subjected to the performic acid-HIO$_4$ oxidative cleavage followed by KMnO$_4$ oxidation and acid hydrolysis (Scheme 1). The procedure used was based on



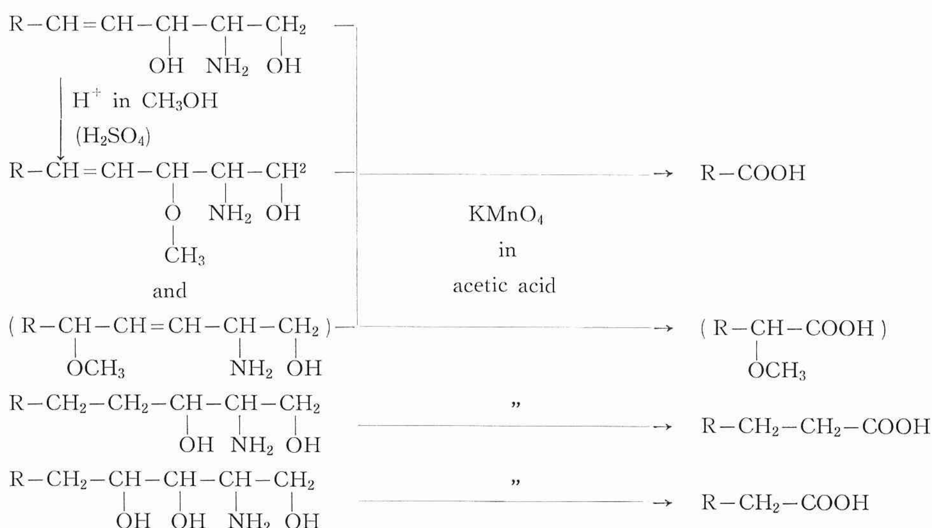
Scheme 1. Performic acid-HIO$_4$ oxidative cleavage.

that of Marinetti *et al*[16]. The fraction of hydroxyamino acid produced by this oxidation was separated and examined by the following paper chromatography.

Paper Chromatography of Hydroxyamino Acids For desalting the hydroxyamino acid fraction was first passed through a column of Amberlite IR-120 (H^+) and amino acids were eluted from the column with 1*N* aq. ammonia. The alkali eluate was evaporated to dryness *in vacuo* to remove excess ammonia. The residue was redissolved in water and then passed through a column of Amberlite IR A-410 (OH^-). Amino acids were eluted from the column with 1*N* HCl. The acid eluate was evaporated repeatedly to dryness *in vacuo* at 40°C to remove excess HCl. The residue was applied on paper after redissolving it in a small volume of water.

Paper chromatography was carried out by ascending technique with the following solvent systems; phenol-water (7 : 3, V/V), *n*-butanol saturated with water and collidine saturated with water. The spots were detected by ninhydrin spray.

GLC of $KMnO_4$ Oxidation Products of Long-Chain Bases The long-chain bases were prepared from plasma and erythrocyte sphingomyelin fractions by the modified methanolysis procedure by Gaver and Sweeley[17] in which a minimum formation of 3-0-methyl derivatives took place. The bases were purified by the silicic acid chromatography and then subjected to $KMnO_4$ oxidative cleavage in acetic acid according to the procedure of James and Webb[18] (Scheme 2). The fatty acids produced by the oxidation were methylated by reflux with dry 3*N* HCl-methanol (2 mg. of lipids/mℓ.) for 3 hours and were subjected to GLC.



Scheme 2. $KMnO_4$ oxidation of long-chain bases.

Other Methods Infrared (IR) spectra were recorded by means of Hitachi double beam infrared spectrophotometer Model EPI S-2 with NaCl prism using cells of 0.5 mm. or 0.1 mm. thickness. Chloroform was used as a solvent.

For the qualitative test Silica Gel G thin-layer plates of 250 μ thickness were used. The detection of spots was carried out by spraying the following reagents; 5% phospho-

molybdic acid in 95% ethanol for lipids, Hanes-Isherwood's reagent[19] and UV light for phosphorus, Dragendorff's reagent[20] for quaternary ammonium compounds, 0.2% ninhydrin in *n*-butanol-10% acetic acid (95 : 5, V/V)[20] for free amino group, 10% diphenylamine in 95% ethanol-conc. HCl-acetic acid (20 : 100 : 80, V/V)[20] for glycolipids and Schiff's reagent[21] for fatty aldehydes.

For the estimation of phosphorus Bartlett's method[22] was used and nitrogen was determined by the method of a direct nesslerization of the Kjeldahl digests with KCN-Nessler reagent according to Minari and Zilversmit[23].

Results and Discussion

In this experiment one liter of human blood was used and the amounts of lipid phosphorus found at each step of the separation procedures were as follows; 31.42 mg. and 49.62 mg. in original extracts of plasma and erythrocytes, 5.81 mg. and 14.25 mg. in alkali-stable lipid fractions and 4.29 mg. and 11.26 mg. in alkali-and acid-stable phospholipid fractions, respectively. About 90.5% of alkali- and acid-stable phospholipids consisted of these sphingophospholipids and the ratios of SPL-F to SPL-S by lipid phosphorus were 2.6 in plasma and 3.2 in erythrocytes. The recovery of lipid phosphorus from the thin-layer plates was 90.2–96.5%.

By this thin-layer chromatographic procedure SPL-F and -S were well separated

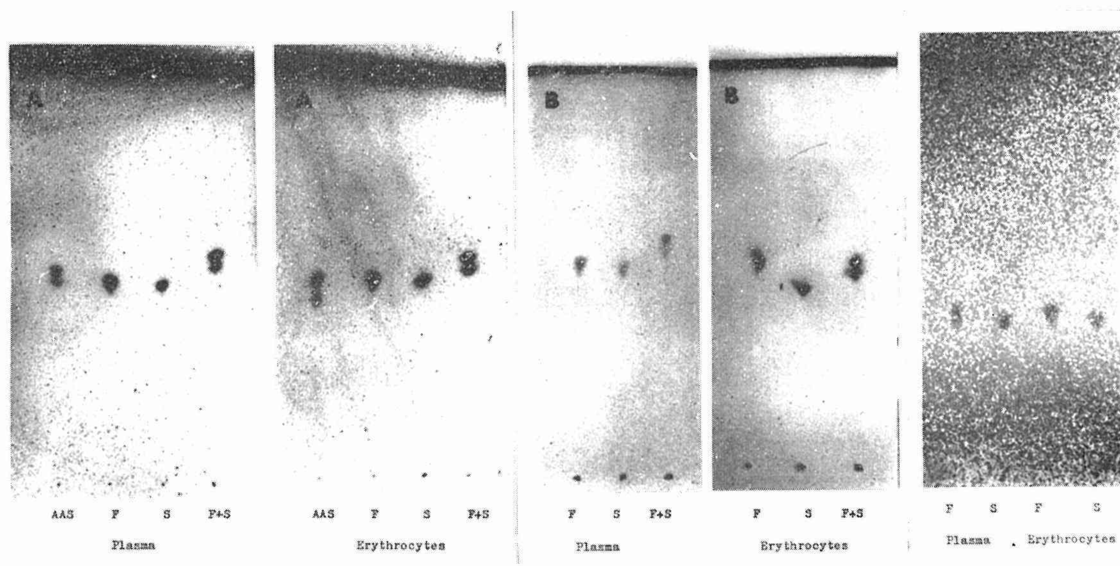


Fig. 3 Thin-layer chromatograms of plasma and erythrocyte SPL-F and -S.

AAS: lipids stable to the mild alkaline-acid hydrolysis (same as 3 in Fig. 1). F: fast moving sphingophospholipid (SPL-F). S: slow moving sphingophospholipid (SPL-S). F+S: a mixture of the separated SPL-F and -S.

Developing solvent: chloroform-methanol-water 65 : 25 : 4 (V/V).

Detected by phosphomolybdic acid spray for lipid (A), Hanes-Isherwood's reagent spray and UV light for phosphorus (B) and Dragendorff's reagent spray for quaternary ammonium group (C).

from each other without any contamination as shown in Fig. 2 and 3. Attempts to fractionate these two lipids by silicic acid or Silica Gel G column chromatography were also made without success. When iodine vapour and fluorescein derivatives were used for the visualization of the lipids on thin-layer plates, they could not be easily removed from these sphingophospholipids by the subsequent column chromatographic treatment as in the use of commercial Rhodamine B without purification.

Infrared spectra of SPL-F and -S from erythrocytes and plasma are shown in Fig. 4. The patterns are very similar to each other and show obviously that these substances contain free hydroxyl group (3430 cm^{-1}), acylated amino group (1650 cm^{-1}, 1560 cm^{-1}), phosphate ester bond (1240–1220 cm^{-1}, 1090 cm^{-1}, 1055 cm^{-1}), *trans* C to C double bond (980 cm^{-1}) and no ester carbonyl group. Each of the patterns is in good agreement with that of sphingomyelin. Comparing the patterns with each other, however, it seems that the absorptions of phosphate ester bond (1240–1220 cm^{-1}) of SPL-S from both erythrocytes and plasma are somewhat weaker than that of SPL-F.

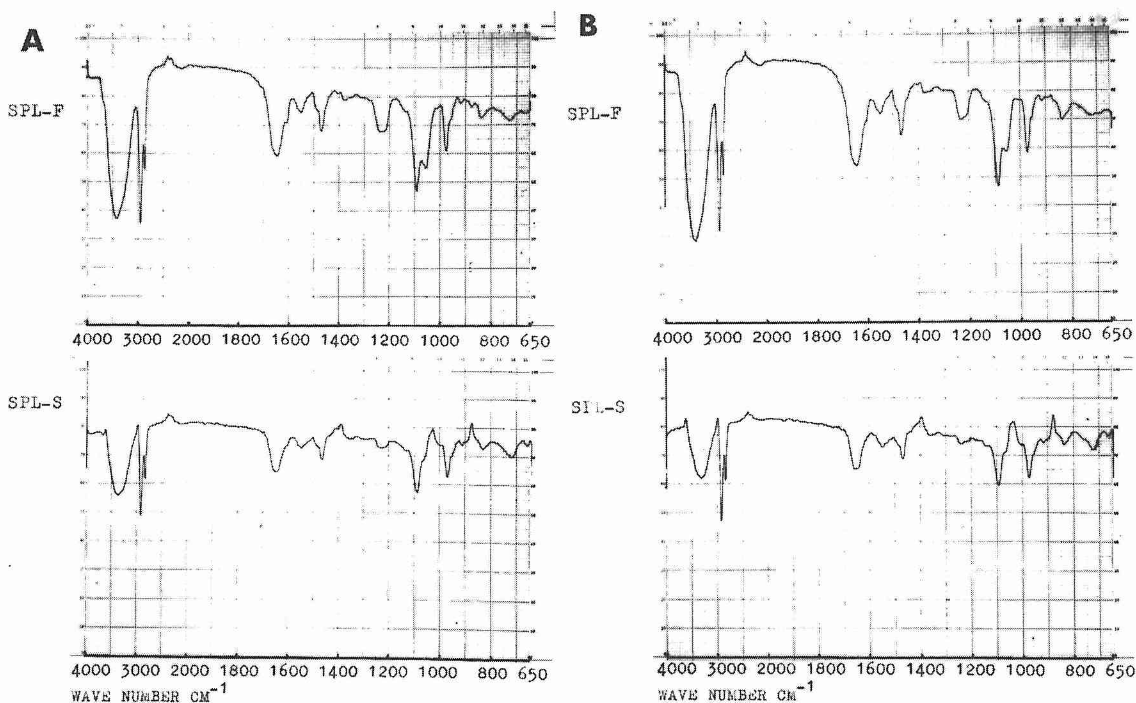


Fig. 4 Infrared spectra of SPL-F and -S from plasma and erythrocytes.
A : plasma, B : erythrocytes. NaCl prism. Cell thickness : 0.5 mm.
Solvent : chloroform.

As shown in Table 1, the results of qualitative tests on functional groups of SPL-F were exactly the same with those of SPL-S and so were those of their hydrolysis products. Molar ratios of nitrogen to phosphorus in SPL-F, -S and their hydrolysis products are shown in Table 2. All of the original samples gave positive Dragendorff's

test and a negative ninhydrin test and N/P ratios of these samples were about 2. After hydrolysis aqueous layer gave also a positive Dragendorff's test and a negative ninhydrin test, but N/P ratios decreased to about 1, whereas organic layer showed a positive ninhydrin test and negative for phosphorus. The tests for sugar and aldehyde were always negative. These results are very similar to those on sphingomyelin.

Table 1 *Qualitative Analyses of Plasma and Erythrocyte SPL-F and -S and of Their Hydrolysates*

	Original		Hydrolysate: Aqueous layer		Hydrolysate: Long-chain base fraction		Hydrolysate: Fatty acid (methyl ester) fraction		Detection method used
	F	S	F	S	F	S	F	S	
Phosphorus	+	+	+	+	−	−	/	/	Hanes-Isherwood's reagent and UV light.
Free amino group	−	−	±	±	+	+	/	/	Ninhydrin.
Quaternary ammonium group	+	+	+	+	−	−	/	/	Dragendorff's reagent
Suger	−	−	−	−	/	/	/	/	Diphenylamine reagent
Fatty aldehyde	−	−	/	/	/	/	−	−	Schiff's reagent

Plasma and erythrocyte SPL-F and -S showed all the same results. Hydrolysis was carried out by refluxing with 2*N* HCl-methanol (conc. HCl-methanol 2 : 10, V/V) for 8 hours.

Table 2 *Nitrogen/Phosphorus Molar Ratios of Plasma and Erythrocyte SPL-F and -S and of Their Water Soluble Hydrolysates*

	Plasma		Erythrocytes	
	Original	Aqueous layer of hydrolysate	Original	Aqueous layer of hydrolysate
SPL-F	2.14	1.06	2.06	1.12
SPL-S	2.08	0.80	1.78	1.01

Hydrolysis was carried out by refluxing with 2*N* HCl-methanol (conc, HCl-methanol 2 : 10, V/V) for 8 hours.

Table 3 *Recoveries of Phosphorus in Aqueous Layer of Hydrolysates of Plasma and Erythrocyte SPL-F and -S*

	Plasma			Erythrocytes		
	Original (μg.)	Aqueous layer of hydrolysate (μg.)	Recovery (%)	Original (μg.)	Aqueous layer of hydrolysate (μg.)	Recovery (%)
SPL-F	507	498	98.2	435	399	91.7
SPL-S	184	168	91.3	130	121	92.5

The samples are the same as in Table 2.

As shown in Table 3 the recoveries of phosphorus in the aqueous layer after hydrolysis by refluxing with 2*N*-HCl in methanol for 8 hours indicate that almost all of phosphorus components were split off from the long chain base under the conditions used. As shown in Fig. 5, the paper chromatograms of water soluble hydrolysate

obtained by $4N$ HCl at 120°C for 21 hours gave only a single spot of phosphoric acid and of choline respectively and no spot positive in both tests for phosphorus and quaternary ammonium group. No spot positive either to ninhydrin test or to diphenylamine test for sugar could be found. In order to examine Dragendorff's test positive substance in the hydrolysates, paper chromatographic studies were carried out with the solvent system for quaternary ammonium bases according to Bregoff *et al*[15]. The base was identified as choline.

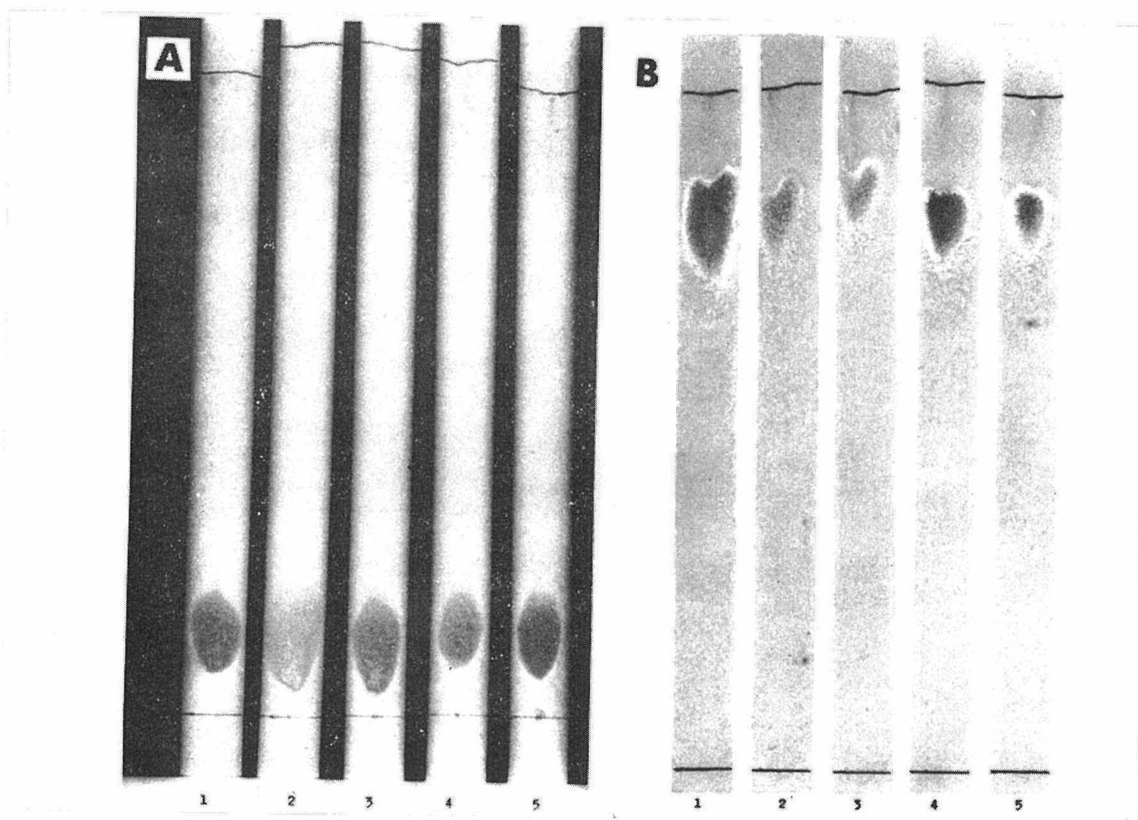


Fig. 5 Paper chromatograms of the water soluble hydrolysates of plasma and erythrocyte SPL-F and -S.

Hydrolysis was carried out with $4N$ HCl at 120°C for 21 hours. Developing solvent: phenol saturated with water.

A: Detcted by Hanes-Isherwood's reagent spray and UV light for phosphorus. ortho-phosphoric acid (1), water soluble hydrolysate of plasma SPL-F (2) and -S (3), and of erythrocyte SPL-F (4) and -S (5).

B: Detected by Dragendorff's reagent spray for quaternary ammonium group. Choline chloride (1). (2)-(5) are the same as in A.

Rouser *et al.*[13] found a sphingolipid containing phosphonic acid group from sea anemone, which liberated 2-aminoethylphosphonic acid by the hydrolysis with $2N$ HCl for 21 hours at 100°C and was then formulated as ceramide aminoethylphosphonate. Kittredge *et al.*[24] also obtained 2-aminoethylphosphonic acid from sea anemone lipids by

the hydrolysis with $4N$ HCl for 24 hours at 100°C. They reported that any liberation of phosphate could not be observed upon hydrolysis with $8N$ HCl at 150°C for 48 hours. On the other hand, the presence of trimethylammonium ethylphosphonate as a lipid component, however, has not been reported yet.

In the present results, both SPL-F and -S were relatively easily hydrolyzed to liberate free phosphoric acid and choline. Consequently, it might be considered that linkages of phosphorus component to choline or long-chain base in SPL-F and -S are both phosphate ester bond.

The gas-liquid chromatographic patterns of the fatty acid fractions, which are shown in Fig. 6, indicate that, in erythrocytes as well as in plasma, fatty acids higher than C_{20} are strikingly concentrated in SPL-F and the major fatty acids in SPL-S are palmitic acid and stearic acid while SPL-F also contains these acids. These findings are in good agreement with the observation on the less polar and more polar component in human plasma sphingomyelin by Woods and Holton[5].

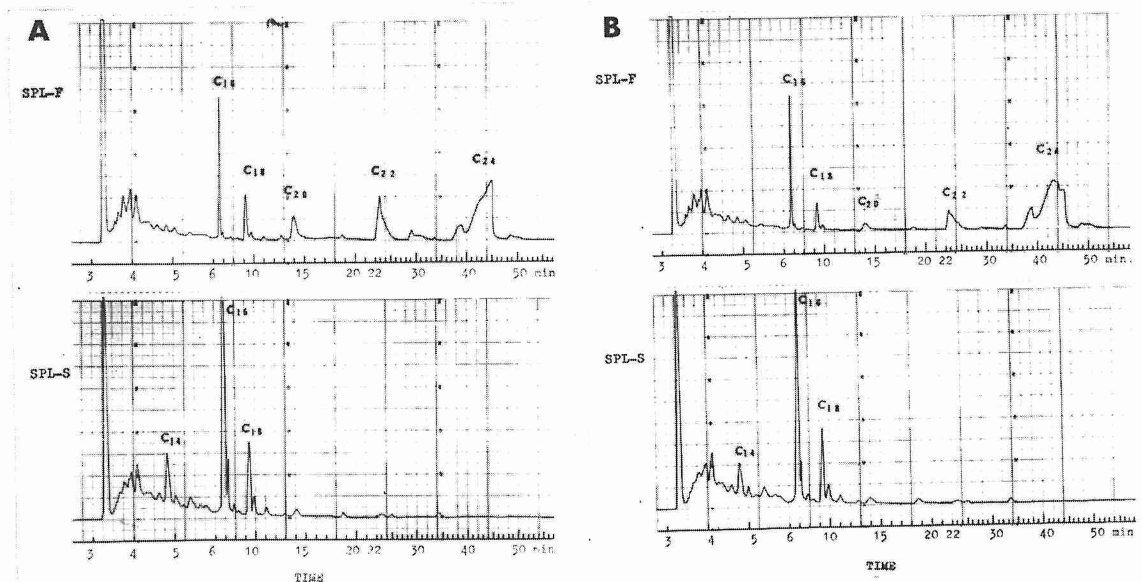


Fig. 6 Gas-liquid chromatographic analyses of fatty acids of SPL-F and -S from plasma and erythrocytes.

A: plasma, B: erythrocytes. Golay column BDS-45 (butanediol succinate, 0.5 mm.×45 m.). Detector: flame ionization detector (H_2), Carrier gas: He, 0.5 kg/cm², Flow rate: 5 mℓ/min. Operation temperature: 195°C.

When the long-chain base fractions obtained from SPL-F and -S by the hydrolysis method of Sweeley and Moscatelli[12] and purified by the silicic acid column chromatography were thin-layer-chromatographed by the procedure of Sambasivarao and McCluer[14], any appreciable difference could not be found among the bases from SPL-F and -S ui erythrocytes and plasma as shown in Fig. 7. The patterns were very similar to that of sphingosine bases reported[14].

At the present time, eighteen long-chain bases are known as constituents of various sphingolipids, that is, $C_{16}\sim C_{20}$-sphingosines, $C_{16}\sim C_{20}$-dihydrosphingosines, $C_{16}\sim C_{18}$-dehydrosphingosines, $C_{17}\sim C_{20}$-phytosphingosines and C_{18}-dehydrophytosphingosine[25]. Sambasivarao and McCluer[26] found C_{20}-sphingosine in brain gangliosides form human and various animals and, according to Stanacev and Chargaff[27], ox brain mucolipids contained C_{18} sphingosine and C_{20}-sphingosine in almost equal quantities and much smaller amounts of their dihydro derivatives also. Karlsson and Holm[25] found C_{19}-sphingosine derivative in a cerebrin from the yeast, *Torulopsis utilis*. In human plasma sphingomyelins, C_{16} and C_{17}-sphingosine were found by Karlsson[28,29]. On the other hand, Weiss[30] reported that a considerable amount of 1-hydroxy-2-amino-5-methoxy-3-octadecene was formed by an allylic rearrangement during preparation of sphingosine from sphingolipids by methanolic sulfuric acid hydrolysis.

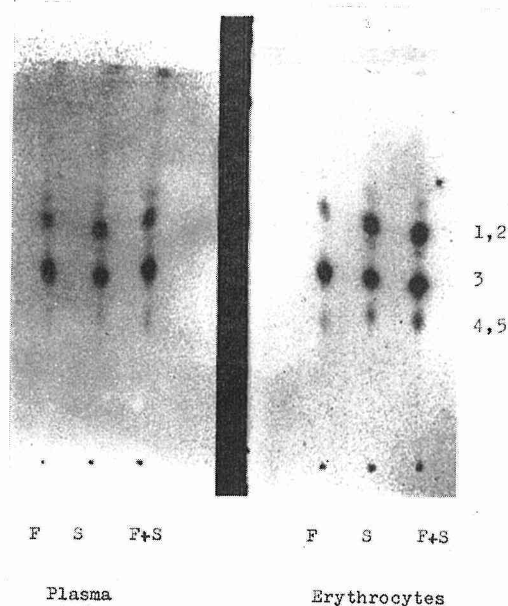


Fig. 7 Thin-layer chromatograms of the long-chain bases of plasma and erythrocyte SPL-F and -S.

1, *erythro*-3-0-methylsphingosine. 2, *threo*-3-0-methylsphingosine. 3, *erythro*-sphingosine. 4, *threo*-sphingosine. 5, dihydrosphingosine. Detected by ninhydrin spray. Developing solvent; chloroform-methanol-2*N* aq. ammonia 40 : 10 : 1 (V/V).

Although significant difference between free long chain bases obtained from SPL-F and -S could not be found by TLC, further studies were performed to confirm the positions of free and phosphorylcholine bound hydroxyl groups, acylated amino group and C to C double bond. Then SPL-F or -S were subjected to performic acid-HIO_4 oxidative cleavage followed by permanganate oxidation and acid hydrolysis. The hydroxyamino acid produced by the oxidation was then examined by paper chromatography. As shown in Fig. 8, the paper chromatograms of all samples gave a major spot which moved identically with an authentic serine. These results support obviously that in plasma and also in erythrocytes almost all the long-chain bases of both SPL-F and -S have a structure of 1, 3-dihydroxy-2-amino-4-ene of which hydroxyl group at C_1 binds to phosphorylcholine, hydroxyl group at C_3 is free and amino group is acylated.

Although phytosphingosine and $C_{16}\sim C_{20}$-sphingosines are able to give serine by this oxidation procedure, the thin-layer chromatograms of the long-chain bases from both SPL-F and -S did not show a spot corresponding to phytosphingosine.

For the examination on the carbon number and branching of long-chain bases and determination of number and site of C to C double bond, the long-chain bases were subjected to $KMnO_4$ oxidative cleavage followed by GLC. As shown in Fig. 9, the

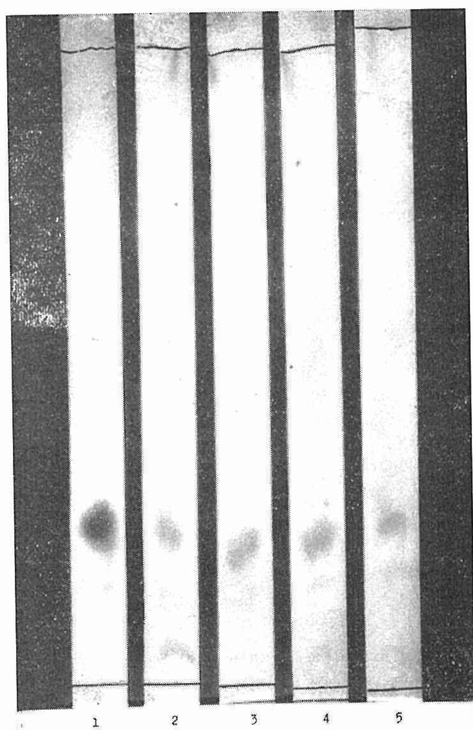


Fig. 8 Paper chromatograms of hydroxy-amino acids obtained by performic acid-HIO_4 oxidative cleavage of plasma and erythrocyte SPL-F and -S followed by $KMnO_4$ oxidation and HCl hydrolysis.

DL-serine hydrochloride (1), hydroxy-amino acid fractions derived from plasma SPL-F (2) and -S (3), and from erythrocytes SPL-F (4) and -S (5). Detected by ninhydrin spray. Developing solvent; phenol-water 7:3 (V/V). See text for other details.

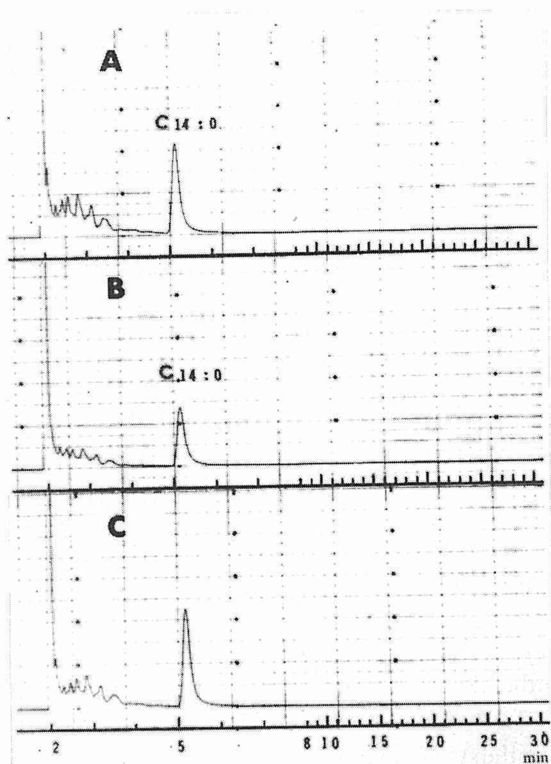


Fig. 9 Gas-liquid chromatographic analyses of fatty acids in $KMnO_4$ oxidation products of long-chain bases of plasma and erythrocyte sphingomyelins.

A: erythrocytes, B: plasma, C: myristic acid which was subjected to $KMnO_4$ oxidation under the same conditions as A and B.

Column, detector, carrier gas and other conditions are the same as in Fig. 6. except operation temperature of 170°C.

gas-liquid chromatographic patterns indicated that main long-chain fatty acid produced by the oxidation was only myristic acid. These results support, therefore, that main long-chain base of both SPL-F and -S has a straight chain structure with C_{18}, in which any more C to C double bond other than at C_4 or any more hydroxyl group other than at C_1 and C_3 are not present. The presence of C_{16} and C_{17}-sphingosine bases as reported by Karlsson[28,29] could not be clarified in this experiment.

Summary

Two sphingophospholipids, that is, the fast moving and the slow moving on TLC, were separated from each other by Silica Gel G TLC of the alkali- and acid-stable

phospholipids isolated from human erythrocytes and plasma. The examination and the comparison of the chemical structures of thus separated lipids were studied by IR analysis and by paper chromatography, TLC and GLC of the products from acid hydrolysis, performic acid-HIO_4 cleavage and $KMnO_4$ oxidation. From the results both substances were found to be sphingomyelins of ordinary type. A distinguished difference between two lipids was in the composition of fatty acid component. The GLC of fatty acids indicated that, in erythrocytes as well as in plasma, fatty acids higher than C_{20} predominated and concentrated in the fast moving sphingomyelin, whereas major fatty acids in the slow moving one were palmitic acid and stearic acid. The ratios of phosphorus amounts of the fast moving sphingomyelin to the slow moving one were 2.6 in plasma and 3.2 in erythrocytes respectively.

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