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Phospholipid Distribution in Erythrocyte and Plasma of the Man, Cow, Pig and Rabbit[1]

Takashi Sato and Tatsuzo Fujii

Faculty of Pharmacy, Meijo University[2]

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The phospholipid class composition and the ratio of the amounts of two sphingomyelin fractions, supposed to have different fatty acid compositions, were determined by quantitative thin-layer chromatography on the erythrocytes and plasma of four representative mammalian species, man, pig, cow and rabbit.

A remarkable species difference was confirmed on the phospholipid class compositions between bovine erythrocyte and those of three other species. The phospholipid distribution in plasma was rather relatively similar each other in these species, with the predominant presence of choline phospholipids. The ratio of two sphingomyelin fractions varied appreciably from species to species. The bovine erythrocyte gave the highest value whereas the bovine plasma gave the lowest value of the F/S fractional ratio among these four species.

Phospholipid distribution in the erythrocytes of mammalian species has been studied by several investigators[3] but their results are not necessarily in good agreement, most probably due to the difference in the methods employed for the separation of individual phospholipids.

The present study was undertaken, as an approach to the elucidation of the species differences found in the structure and functions of mammalian erythrocyte membrane,[4] to confirm the characteristic distribution pattern of phospholipids in erythrocytes and also in the surrounding blood plasma of some representative mammalian species, by means of quantitative thin-layer chromatography (TLC) with excellent resolution. A special attention was paid to two sphingomyelin components resolved on the Silica Gel G thin-layer chromatogram which were recently found to have different fatty acid and sphingosine base composition,[5] because sphingomyelin is the most abundant phospholipid present in erythrocyte membrane. As the representative species, man, cow, pig and rabbit were selected.

Experimental

Preparation of Erythrocyte Suspension——Fresh blood, with an addition of trisodium citrate as anticoagulant, was separated into blood cells and plasma by centrifugation and the buffy leucocyte layer was

1) Part of the present report was presented at the Meeting of Tokai Branch, Pharmaceutical Society of Japan, Nagoya, September 1968 and at the 89th Annual Meeting of the Same Society, Nagoya, April 1969.

2) Location: *Yagoto Urayama 15, Tenpakucho, Showaku, Nagoya.*

3) *a*) J.C. Turner, A.M. Anderson and C.P. Grandal, *Biochim. Biophys. Acta*, **30**, 130 (1958); *b*) D.J. Hanahan, R.M. Watts and D. Pappajohn, *J. Lipid Res.*, **1**, 421 (1960); *c*) R.M.C. Dawson, N. Hemington and D.B. Lindsay, *Biochem. J.*, **77**, 226 (1960); *d*) J. de Gier and L.L.M. van Deenen, *Biochim. Biophys. Acta*, **49**, 286 (1961); *e*) P. Ways and D.J. Hanahan, *J. Lipid Res.*, **5**, 318 (1964); *f*) G.J. Nelson, *Biochim. Biophys. Acta*, **144**, 221 (1967).

4) L.L.M. van Deenen and J. de Gier, "The Red Blood Cells, A Comprehensive Treatise," ed. by C. Bishop and D.M. Surgenor, Academic Press, New York and London, 1964, p. 243.

5) *a*) P.D.S. Wood and S. Holton, *Proc. Soc. Exptl. Biol. Med.*, **115**, 990 (1964); *b*) H. Jatzkewitz, *Z. Physiol. Chem.*, **336**, 25 (1964); *c*) H. Pilz and H. Jatzkewitz, *J. Neurochem.*, **11**, 603 (1964); *d*) O. Minari, H. Tsubono, M. Akiyama and T. Sakagami, *J. Biochem.* (Tokyo), **62**, 618 (1967); *e*) C. Michalec and Z. Kolman, *J. Chromatog.*, **31**, 636 (1967); *f*) G. Soula, P. Valdiguie and L. Douste-Blazy, *Bull. Soc. Chim. Biol.*, **50**, 887 (1968).

removed off by aspiration. The red cells thus obtained was then washed twice with physiological saline and resuspended in saline to give a Hematocrit value of about 50%.

Lipid Extraction——Lipids were extracted from each erythrocyte suspension or plasma with chloroform–methanol (1:1 or 2:1 by volume) by the method of Ways and Hanahan[3e] and the extract was washed with 0.1M potassium chloride solution as described by Folch, *et al.*[6] The chloroform layer was separated from the aqueous layer and was evaporated *in vacuo* below 40°, and the residue was dissolved in chloroform–methanol (1:1, v/v). In order to know the amount of total lipids present, an aliquot of this extract was evaporated to dryness under nitrogen stream until constant weight of the residue was attained, and the residue was weighed. For determination of total phospholipids, phosphorus in the lipid extract was assayed by the method of Bartlett.[7]

Thin-Layer Chromatography——Thin-layer plate coated in a 0.5 mm thickness with Silica Gel G, H or HR (E. Merck AG, Darmstadt) which had previously been washed thoroughly with methanol and ether and then activated for 2 hours at 110°, was employed.

For the identification of phospholipid classes, two-dimensional chromatography with a 20×20 cm plate coated by Silica Gel H was used. A portion of lipid extract after proper concentration was spotted on the plate, developed first with chloroform–methanol–7M NH_4OH (90:45:11, v/v), dried by means of hair drier, and then developed with chloroform–methanol–acetic acid–water (90:40:12:2, v/v).[8] The phospholipid spots were detected by spraying the molybdenum reagent of Dittmer and Lester.[9] Ninhydrin reagent was also used for the identification of the phospholipids with free amino-group.

For one-dimensional separation followed by quantitative determination, the following systems were employed: Silica Gel G plate developed with chloroform–methanol–water (100:40:6, v/v), and Silica Gel HR plate developed with chloroform–methanol–acetic acid–water (60:30:8:4, v/v). The former system is favorable for the separation of the above-mentioned two sphingomyelin fractions[5] and the latter for the separation of phosphatidyl serine which is particularly abundant in erythrocyte.[10] An aliquot of each total lipid extract, containing about 20 μg of lipid phosphorus, was applied linearly to a 10×20 cm plate. After development, each phospholipid band was visualized by exposing the plate to iodine vapor and it was marked immediately. Each band was then scraped away by a small spatula into a centrifuge tube. The phospholipid in each fraction was extracted overnight with a 10 ml portion of a mixture of formic acid–methanol–chloroform (2:1:1, v/v), according to Burger, Fujii and Hanahan.[11] It was confirmed that the recovery of this extraction was almost quantitative.

Result and Discussion

Phospholipid Contents of Erythrocytes and Plasma

Total lipids and phospholipid contents of erythrocytes and plasma from four different mammalian species are shown in Table I. The amounts in mg of total lipids contained in 1 ml

Table I. Total Lipid and Phospholipid Contents in Erythrocytes and in Plasma

		Total lipids mg/ml cells or plasma	Phospholipid-P μg/ml cells or plasma	Phospholipids as phosphatidyl-choline mg/ml cells or plasma	Phospho-lipids % of total lipids
Erythrocyte	man	3.86	92.7	2.32	60.0
	pig	4.09	91.5	2.29	56.0
	cow	4.22	104.0	2.60	61.6
	rabbit	5.04	103.7	2.50	51.5
Plasma	man	3.29	38.6	0.965	29.3
	pig	1.93	16.4	0.411	21.3
	cow	2.44	27.2	0.679	27.8
	rabbit	1.62	19.1	0.478	29.5

Each figure is an average of the data obtained on 3 individual animals.

6) J. Folch, M. Lees and G.H.S. Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
7) G.R. Bartlett, *J. Biol. Chem.*, **234**, 466 (1959).
8) R.M. Broekhuyse, *Clin. Chim. Acta*, **23**, 457 (1969).
9) J.D. Dittmer and R.L. Lester, *J. Lipid Res.*, **5**, 126 (1964).
10) G. Gercken and U. Brockmann, "Stoffwechsel und Membranpermeabilitaet von Erythrocyten und Thrombocyten," ed. by E. Deutsch, E. Gerlach and K. Moser, Beorg Thieme Verlag, Stuttgart, 1968, p. 352.
11) S.P. Burger, T. Fujii and D.J. Hanahan, *Biochemistry*, **7**, 3682 (1968).

of packed erythrocytes are in the range of 3.9—5.0 and those of phospholipid (as phosphatidyl choline) are of 2.3—2.6. The phospholipid percentage of the total lipids in the respective erythrocyte is all in a narrow range of 52—62%.

In the cases of plasma, the absolute amounts of total lipids and phospholipids present in an unit volume vary appreciably. However, the phospholipid percentage of the total lipids falls in a rather narrow range of 21—30%, the values approximately half of those obtained on the erythrocytes.

Phospholipid Distribution in Erythrocytes

The two-dimensional TLC of the phospholipids extracted from human erythrocytes is schematically presented in Fig. 1. The phospholipid from the other three mammalian species also gave the similar patterns. Such a chromatogram was used for the identification of each fraction separated by one-dimensional TLC systems described below.

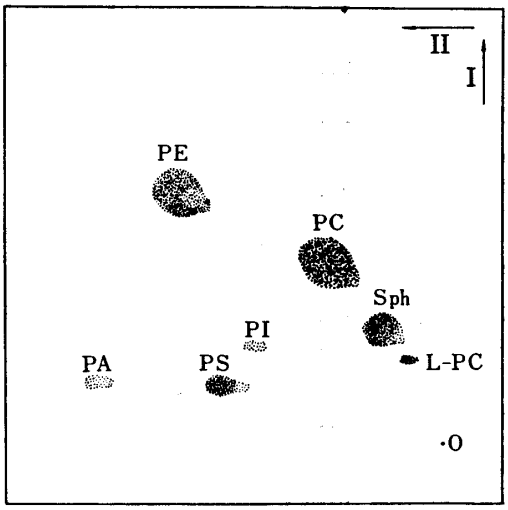
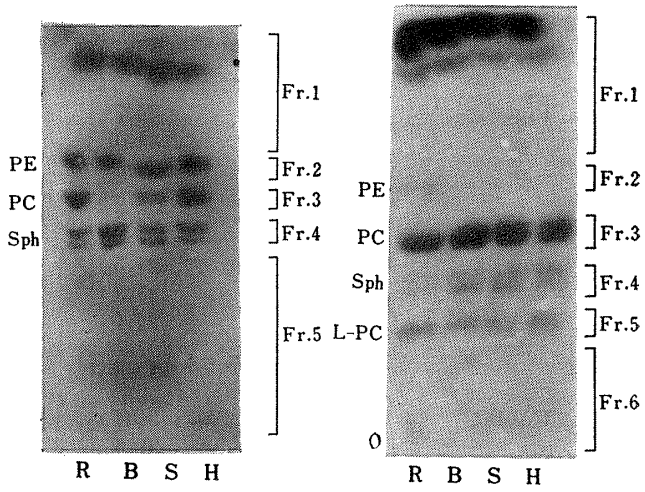


Fig. 1. Schematic Presentation of Two-Dimensional Thin-Layer Chromatogram of Human Erythrocyte Phospholipids

the first development: chloroform–methanol–7M NH_4OH (90:45:11, v/v)
the second development: chloroform–methanol–acetic acid–water (90:40:12:2, v/v)
phospholipid class:
PA; phosphatidic acid, PS; phosphatidyl serine, Sph; sphingomyelin, PE; phosphatidyl ethanolamine, PC; phosphatidyl choline, O; origin, PI; phosphatidyl inositol, L-PC; lysophosphatidyl choline



Erythrocyte **Plasma**

Fig. 2. Silica Gel G Thin-Layer Chromatograms of Erythrocyte and Plasma Phospholipids, developed with Chloroform–Methanol–Water (100:40:6, v/v)

detection: iodine vapor
animals: H man (human)
S pig (swine)
B cow (bovine)
R rabbit

phospholipid classes:
PE; phosphatidyl ethanolamine
PC; phoaphatidyl choline
Sph; sphingomyelin
L-PC; lysophosphatidyl choline

The Silica Gel G TLC of the phospholipids from the erythrocytes of man, pig, cow and rabbit, with a solvent system of chloroform–methanol–water (100:40;6, v/v), is shown in Fig 2. With such a method, the three major phospholipid classes of the erythrocytes, namely, phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and sphingomyelin (Sph) are clearly separated each other. Resolution of sphingomyelin into two bands (F and S) will be referred later. It had previously been demonstrated that, of the minor phospholipid components, phosphatidyl serine (PS), phosphatidyl inositol (PI) and lysophosphatidyl choline (Lyso-PC) are located on the region between the original line and the sphingomyelin band, and also that phosphatidic acid (PA) is present above the phosphatidyl ethanolamine band. Therefore, the TLC was separated into five fractions as indicated in the Fig. 2. The gel band corresponding

to each fraction was scraped off, the phospholipid (s) was extracted from the gel, and the phospholipid phosphorus was determined as described already. The results, showing the phospholipid class composition of the erythrocyte of each species, are presented in Table II. The plasma phospholipids in Fig. 2 will be referred later.

Table II. Phospholipid Class Composition of Erythrocyte revealed by Silica Gel G Thin-Layer Chromatography with a Solvent System of Chloroform–Methanol–Water (100:40:6) (Percentage of the Total Phospholipid)

	Fr. 1 Phosphatidic acid	Fr. 2 Phosphatidyl ethanolamine	Fr. 3 Phosphatidyl choline	Fr. 4 Sphingomyelin	Fr. 5 Lysophosphatidyl choline, phosphatidyl serine, phosphatidyl inositol
Man	3.4[a)]	26.2	30.2	28.3	11.9
	2.0	27.3	27.4	31.6	11.7
	2.8	26.3	32.2	27.2	11.4
	2.7[b)]	26.6	30.0	29.0	11.7
Pig	0.5	34.7	21.9	21.4	21.5
	1.5	34.0	25.8	26.1	12.6
	2.4	29.6	24.9	25.6	17.5
	1.5	32.8	24.2	24.3	17.2
Cow	1.3	27.5	2.7	49.8	18.7
	1.5	27.3	7.3	45.8	18.1
	1.8	24.1	5.2	48.2	20.7
	1.5	26.3	5.1	47.9	19.2
Rabbit	1.8	30.4	32.4	19.8	15.6
	1.7	29.6	29.7	22.1	16.9
	1.3	29.4	30.9	21.7	16.7
	1.6	29.8	31.0	21.2	16.4

a) Each of three figures above horizontal line is an average of the data on three thin-layer plates to which the lipid fraction from individual animal was applied.
b) The figure under the line is the average of the values on three individual animals of the respective species.

Each of three figures on respective species was derived from the experiment with three thin-layer plates to which the lipid fraction from individual animal had been applied. The figure under the horizontal line is the average of these values on individual animals.

The erythrocyte phospholipids were subjected to an another TLC system with acidic solvent mixture as described in Fig. 3. It is clear from the photo that in this sysyem phosphatidyl serine, relatively rich in erythrocyte phospholipids, is well separated. It was confirmed that phosphatidyl inositol, though not clearly seen in the chromatogram, is situated between the phosphatidyl serine and phosphatidyl choline bands. With this solvent system, it is noted that sphingomyelin is not resolved into two fractions in contrast with the case by neutral solvent system already reported above.

The phospholipid class composition of erythrocytes as revealed by such a Silica Gel HR TLC with a solvent system of chloroform–methanol–acetic acid–water (60:30:8:4, v/v) is presented in Table III. The methods employed and the mode of the expression are just the same as the preceding table (Table II).

The average value of the phospholipid class composition for the respective mammalian species, obtained by the above-mentioned two TLC systems, is shown in Table IV. It is

noteworthy that the datad obtaine by two separate TLC systems agree well, at least as far as the three major phospholipid classes be concerned.

The results clearly indicate striking species variations in choline phospholipid distribution, similar to those reported previously.[3] In bovine erythrocyte, the phosphatidyl choline is extremely low in comparison with the other species whereas sphingomyelin is remarkably high. Consequently, the total percentage of the choline phospholipids in bovine erythrocyte phospholipids remains to be nearly comparable to the other animal species (50—54%). According to Turner,[3a] Hanahan, *et al.*,[3b] and Nelson,[3f] phosphatidyl choline is completely absent in the cow erythrocyte. In contrast, de Gier and van Deenen[3d] obtained a value of 7% for this phospholipid in bovine red blood cells by means of paper chromatography. The results obtained by the present authors are in agreement with the data reported by the last-mentioned authors. Such discrepancy among the results reported by different investigators might possibly be due to differences in nutritional condition of the animals employed or due to physiological difference of different animal strains of the same species. In fact, one of the present authors (Fujii) has an actual experience that, working in Dr. Hanahan's laboratory in the United States, he could not detect any phosphatidyl choline on the TLC of bovine erythrocyte phospholipids, performed by the identical technique as used in the present work.

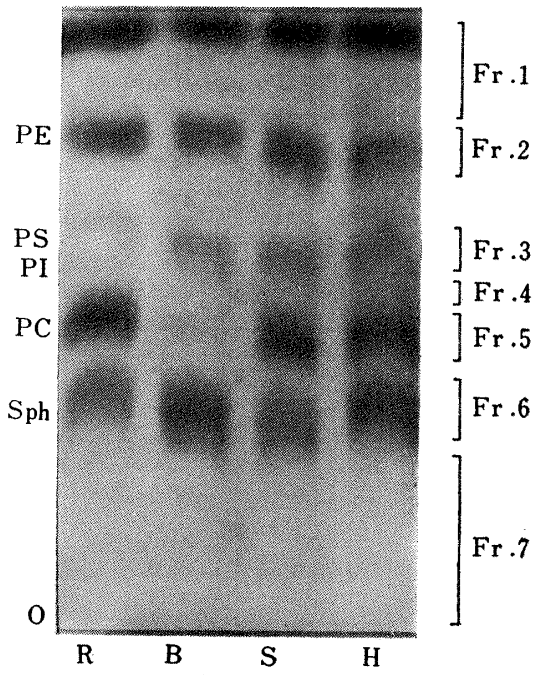


Fig. 3. Silica Gel HR Thin-Layer Chromatogram of Erythrocyte Phospholipids, developed with Chloroform–Methanol–Acetic Acid–Water (60:30:8:4, v/v)

detection: iodine vapor
animals: H man (human)
S pig (swine)
B cow (bovine)
R rabbit
phospholipid classes:
PE phosphatidyl ethanolamine
PS phosphatidyl serine
PC phosphatidyl choline
Sph sphingomyelin
PI phosphatidyl inositol

The percentage of phosphatidyl ethanolamine, another principal phospholipid in the erythrocytes, does not differ considerably in different species examined, ranging from 25 to 33% of the total phospholipids. The red blood cells of man, pig and cow were found to contain 12 to 13% of phosphatidyl serine, while the rabbit erythrocyte contains slightly lower percentage of this acidic phospholipid (8%). The class compositions of the minor phospholipids, including phosphatidic acid, phosphatidyl inositol and lysophosphatidyl choline, vary considerably and lay in a range of 1.5—3.5%, 1.8—2.7%, and 2.6—5.4%, respectively. It does not necessarily means, however, that there exists definite species difference in the distribution of these phospholipids, because technical errors resulting from handling of so minute a quantity of phospholipid on TLC be considerably great. The contents of these minor phospholipids remain to be re-examined with some other systems of TLC.

Plasma Phospholipid Distribution

The TLC of plasma phospholipids from each animal species is presented in Fig. 2. Because lysophosphatidyl choline is generally present in blood plasma in a significant quantity, it was separated as an independent fraction. Thus, the phospholipids were separated into six

Table III. Phospholipid Class Composition of Erythrocyte as Revealed by Silica Gel HR Thin-Layer Chromatography with a Solvent System of Chloroform–Methanol–Acetic Acid–Water (60:30:8:4) (Percentage of the Total Phospholipid)

	Fr. 1 Phosphatidic acid	Fr. 2 Phosphatidyl ethanolamine	Fr. 3 Phosphatidyl serine	Fr. 4 Phosphatidyl inositol	Fr. 5 Phosphatidyl choline	Fr. 6 Sphingomyelin	Fr. 7 Lysophosphatidyl choline
Man	3.0[a]	27.5	10.0	1.7	28.2	26.1	3.7
	1.9	18.4	12.0	1.9	29.0	29.7	7.2
	1.3	28.2	13.4	1.8	26.6	23.5	5.2
	2.1[b]	24.7	11.8	1.8	27.9	26.4	5.4
Pig	1.8	29.3	13.3	1.3	27.9	23.0	3.4
	1.7	29.5	13.0	2.5	27.6	21.4	4.3
	1.8	29.4	13.2	1.9	27.8	22.2	3.9
Cow	3.2	28.1	11.9	2.0	6.3	45.5	3.1
	2.8	28.9	11.6	3.1	6.7	44.8	2.1
	3.0	28.5	11.8	2.6	6.5	45.2	2.6
Rabbit	2.2	31.0	7.6	3.4	35.0	15.5	5.3
	3.6	32.0	7.3	2.8	33.5	16.9	3.9
	4.7	33.1	8.9	2.0	28.0	20.7	2.7
	3.5	32.0	7.9	2.7	32.2	17.7	4.0

a) Each of 2 or 3 figures above horizontal line is an average of the data on thin-layer plates to which the lipid fraction from individual animal was applied.
b) The figure under the line is the average of the values on 2 or 3 individual animals.

Table IV. The Summary of the Erythrocyte Phospholipid Class Composition (Percentage of the Total Phospholipid)

		Sphingomyelin	Phosphatidyl choline	Lysophosphatidyl choline	Phosphatidyl ethanolamine	Phosphatidyl serine	Phosphatidyl inositol	Phosphatidic acid
Man	I[a]	29.0	30.0		26.6			2.7
	II[b]	26.4	27.9	5.4	24.7	11.8	1.8	2.1
Pig	I	24.3	24.2		32.8			1.5
	II	22.2	27.8	3.9	29.4	13.2	1.9	1.8
Cow	I	47.9	5.1		26.3			1.5
	II	45.2	6.5	2.6	28.5	11.8	2.6	3.0
Rabbit	I	21.2	31.0		29.8			1.6
	II	17.7	32.2	4.0	32.0	7.9	2.7	3.5

a) developed with the solvent system I: chloroform–methanol–water (100:40:6, v/v)
b) developed with the solvent system II: chloroform–methanol–acetic acid-water (60:30:8:4, v/v)

fractions as shown in Fig. 2. The phospholipid class composition, revealed by phosphorus determination in each fraction, is shown in Table V. In contrast with the erythrocyte phospholipids, no striking species difference in the class composition is notable in the case of plasma phospholipids. In any of the species examined, the choline phospholipids (phosphatidyl choline, lysophosphatidyl choline and sphingomyelin) are predominantly present, amounting up to 92—97% of the total phospholipid in man, pig and cow. With the rabbit plasma phospholipid, they occupies 88% of the total phospholipid, the value slightly lower than in

TABLE V. Phospholipid Class Composition of Blood Plasma revealed by Silica Gel G Thin-Layer Chromatography with a Solvent System of Chloroform–Methanol–Water (100:40:6) (Percentage of the Total Phospholipid)

	Fr. 1 Phosphatidic acid	Fr. 2 Phosphatidyl ethanolamine	Fr. 3 Phosphatidyl choline	Fr. 4 Sphingomyelin	Fr. 5 Lysophosphatidyl choline	Fr. 6 Phosphatidyl serine, phosphatidyl inositol
Man	0.7[a]	2.7	60.8	16.5	19.1	0.2
	0.8	3.2	56.7	21.8	16.8	0.7
	0.6	2.7	60.0	22.7	13.6	0.4
	0.7[b]	2.9	59.2	20.3	16.5	0.4
Pig	1.2	4.7	48.7	29.8	12.7	2.9
	1.1	4.2	60.5	17.8	13.5	3.5
	1.3	3.6	46.2	33.2	14.0	1.7
	1.2	4.2	51.8	26.7	13.4	2.7
Cow	0.7	1.9	70.8	19.1	6.6	0.9
	0.7	1.8	48.0	32.3	13.5	3.7
	1.1	2.0	64.7	18.8	11.5	1.0
	0.8	1.9	61.2	23.4	10.5	2.2
Rabbit	0.4	10.1	54.5	11.2	21.6	2.2
	1.1	8.9	52.9	12.5	22.4	2.2
	0.9	9.6	57.0	11.7	19.0	1.9
	2.1	8.7	56.5	14.1	16.6	1.8
	1.1	9.3	55.3	12.4	19.9	2.0

a) Each of 3 or 4 figures above horizontal line is an average of the data on three thin-layer plates to which the lipid fraction from individual animal was applied.
b) The figure under the line is the average of the values on 3 or 4 individual animals.

the above-mentioned three mammalian species. On the other hand, the rabbit plasma contains more phosphatidyl ethanolamine (9.3%) than the other species (2—4%).

The Content of Two Sphingomyelin Fractions

As already demonstrated in Fig. 2, sphingomyelin of erythrocyte and plasma is similarly resolved into two distinct bands when developed with a solvent mixture of chloroform–methanol–water (100:40:6). These two components, named as F (fast) and S (slow), are known to be mainly composed of C_{20}—C_{24} and C_{16-18} fatty acids, respectively.[5] Each of these bands was scraped off and the amount of each component was determined by the phosphorus determination. The ratio of the amount of F and S (F/S ratio) was obtained on the individual animal and the average value for the respective species was calculated, as reported in Table VI. From such an average value of F/S ratio, the percentage of each component of the total sphingomyelin is derived.

With the erythrocyte sphingomyelin, it is worth of interest to note that the bovine erythrocyte sphongomyelin has the highest F/S ratio (2.35) in comparison with the three other species (1.3—1.9). Namely in bovine erythrocyte sphingomyelin, about 70% are occupied by the F component, whereas in man, pig and cow erythrocytes, the F component amounts up to 56—65% of the total sphingomyelin. Thus, it was disclosed that the bovine red blood cell contains not only higher percentage of sphingomyelin than the other mammalian species, but also contains higher percentage of the F component of sphingomyelin which is rich in higher fatty acids with C_{20}—C_{24}.

Table VI. Percentage of Two Sphingomyelin Fractions in Erythrocyte and Plasma

		F/S ratio		Percentage of F and S fractions	
		Data on individual animals[a]	Average	Sphingomyelin F	Sphingomyelin S
Erythrocyte	man	1.70 1.91 1.94	1.85	64.9	35.1
	pig	1.43 1.27 1.10	1.27	55.9	44.1
	cow	2.32 2.50 2.22	2.35	70.1	29.9
	rabbit	1.65 1.03 1.52	1.40	58.3	41.7
Plasma	man	1.48 1.52 1.53	1.51	60.2	39.8
	pig	1.04 0.66 0.82	0.84	45.7	54.3
	cow	0.56 0.66 0.55	0.59	37.1	62.9
	rabbit	0.71 0.84 0.62 0.67	0.71	41.5	58.5

a) Each figure represents an average of the data from three thin-layer plates to which the lipid fraction from individual animal was applied.

It has been known already that the general properties of bovine erythrocyte membrane quite are different from those of many other animal species.[4] For example, the bovine red blood cell is rich in sodium ion and poor in potassium ion, in contrast with other mammalian erythrocytes and tissue cells. Burger, Fujii and Hanahan[11] obtained the further evidence on the structural differences between the bovine and human erythrocyte membrane that the bovine erythrocyte membrane is far more labile against hypotonic treatment than human cells and easily releases acetylcholinesterase lipoprotein which is located on the outer surface of the membrane. Whether or not such characteristic properties of bovine red blood cell membrane originate from the differences in its chemical composition and set-up of the membrane components remains to be solved, but the experimental evidences obtained in this work, together with those obtained up to now, suggest strongly such a possibility.

As for the plasma sphingomyelin, the F/S ratio in each species is significantly lower than the value obtained on the erythrocyte sphingomyelin on the respective species. It is interesting to note that the bovine plasma gave the lowest value of the F/S ratio (0.6) among the four mammalian species examined. Sphingomyelin extracted from human erythrocyte gave the highest value (1.5) and that from pig and rabbit erythrocyte the intermediate value (0.7—0.8). In this point, too, the species difference is found to be remarkable.

The values of the F/S ratio obtained by the present authors on human erythrocyte and plasma do not agree well with the values reported by the previous investigators. Minari, *et al.*[5d] reported the value of 3.2 for human erythrocyte and 2.6 for the plasma. Wood and Holton[5d] stated the value of 1.15 for human plasma sphingomyelin. The discrepancy among the values obtained by different investigators might be due to the incomplete resolution of these components on TLC. More appropriate method for the separation of these two sphingomyelin components was searched for by the present authors without success.