Lipid Analyses and Fluidity Studies by Electron Spin Resonance of Red Cell Membranes in Hereditary High Red Cell Membrane Phosphatidylcholine Hemolytic Anemia

By Yoshihito Yawata, Takashi Sugihara, Mayumi Mori, Shigeru Nakashima, and Yoshinori Nozawa

Membrane lipid analyses and electron spin resonance (ESR) studies of membrane fluidity were carried out on the red cells of a Japanese patient with hereditary high red cell membrane phosphatidylcholine hemolytic anemia (HPCHA). Increased amounts of phosphatidylcholine (PC) and cholesterol were found in the membrane lipids of the affected patient, despite normal plasma lipids. The order parameter of cholesterol-free pure phospholipid liposomes prepared from this patient's red cells was decreased, apparently because of the increased PC. In contrast, the order parameter of the total red cell lipid liposomes (containing free cholesterol) was essentially normal. The overall fluidity of the intact red cells was determined by ESR with a spin probe, 5-SAL. Again, the order parameters were normal in the intact red cells of the patient with HPCHA. This suggests that the concomitant increase of membrane cholesterol and phosphatidylcholine serves to maintain normal membrane fluidity in the HPCHA red cells.

IN 1968, the first case of hereditary nonspherocytic hemolytic disease associated with altered phospholipid composition of the red cells (HPCHA) was described by Jaffé et al.[1] Since that time, several additional reports have been published.[2-8] This hemolytic disease is characterized by a mild hemolytic anemia with elevated phosphatidylcholine levels in the red cell membranes and normal plasma lipids. The mechanism of the hemolysis has not been described. Similarly, the reason that the accumulation of phosphatidylcholine is confined to the red cell membranes is not known, although the mechanism for that accumulation has been studied extensively by Shohet et al.[9]

Increased phosphatidylcholine affects the morphology and membrane functions of the red cells.[8-10] Membrane lipid fluidity is dependent on the type of cholesterol (free or esterified), the class of phospholipids, the molar ratio of cholesterol to phospholipids, the degree of saturation of fatty acids, the length of acyl chains, and the presence or absence of amphipathic compounds such as lysophosphatides.[11-13] Free cholesterol (FC) decreases the membrane lipid fluidity, while esterified cholesterol (EC) increases it. Phosphatidylcholine (PC) increases the membrane fluidity, and sphingomyelin (SM) and phosphatidylethanolamine (PE) decrease it. Short acyl chains and low saturation of fatty acids increase membrane fluidity, and longer acyl chains and high saturation of fatty acids decrease it. Lysophosphatides increase membrane fluidity. Compensation for an alteration in one or more of these variables to maintain normal membrane fluidity has been termed "homeoviscous adaptation"[14] by Sinensky. This phenomenon has been observed in *Escherichia coli*,[15] fungi (*Fusarium*),[16] *Tetrahymena*,[17] hibernating squirrels,[18,19] guinea pig lymphatic leukemia cells,[20] and human red cells with lecithin:cholesterol acyl transferase deficiency.[21] To consider the possibility of a similar "compensatory" mechanism in the red cell membranes of a patient with hereditary high red cell membrane phosphatidylcholine hemolytic anemia, we analyzed the red cell and plasma lipids and also determined the membrane fluidity in the intact cells as well as in liposomes prepared from all of the red cell lipids and liposomes prepared from only the red cell phospholipids.

MATERIALS AND METHODS

Case Study

The patient is a 68-year-old woman who was found to have a mild uncompensated hemolytic anemia at age 19. She had no known intercurrent illness or exposure to drugs, chemicals, or blood transfusions. She has had no episodes of acute, overt hemolysis and is able to perform normal household activities.[3]

She was hospitalized at age 65 for evaluation of her anemia and mild jaundice. Physical examination revealed no hepatosplenomegaly, lymphadenopathy, or chronic leg ulceration. The findings of her neurologic examination were normal.

A marked reticulocytosis ($17.3 \times 10^4/\mu L$) with normocytic (MCV, 103 μm^3) slightly hyperchromic (MCH, 37.4 pg) anemia (RBC count, $262 \times 10^4/\mu L$, hemoglobin, 9.8 g/dL, hematocrit, 27.1%) was observed. The MCHC was 36.2%. There was slight anisocytosis and mild poikilocytosis with occasional target cells and stomatocytes. Bone marrow aspiration revealed normoblastic ery-

From the Division of Hematology, Department of Medicine, Kawasaki Medical School, Kurashiki, the Department of Medicine, Tokyo Metropolitan Geriatric Hospital, Tokyo and the Department of Biochemistry, Gifu University, Gifu, Japan.

Supported in part by a grant for the Idiopathic Disorders of Hematopoietic Organs from the Ministry of Health and Welfare, and by grant No. 59570524 from the Ministry of Education, the Japanese Government.

Submitted July 12, 1983; accepted June 4, 1984.

Address reprint requests to Dr Yoshihito Yawata, Division of Hematology, Department of Medicine, Kawasaki Medical School, Kurashiki, Japan 701-01.

0006-4971/84/6405-0029$03.00/0

throid hyperplasia. The WBC count and platelet count were normal.

The serum chemistry examination was normal except for a slight elevation of bilirubin (direct, 0.4 mg/dL; indirect, 0.8 mg/dL). The red cell life span (^{51}Cr $T_{1/2}$) was 16 days. Red cell osmotic fragility was decreased (minimum, 0.40%; maximum, 0.26%). The hemoglobin electrophoresis and the activities of glycolytic enzymes were normal. Family studies revealed two more affected individuals, as shown in Fig 1.

Extraction and Analysis of Lipids

Freshly drawn heparinized venous blood was centrifuged at 2,500 g for ten minutes to separate the red cells from the plasma.[22] The red cells were washed three times in isotonic saline (pH 7.4) by centrifugation, and the hematocrit was adjusted to 30%. The plasma was further centrifuged at 3,000 g for ten minutes to remove residual white cells and platelets.

Extraction of lipids from the plasma was performed by the method of Folch et al.[23] Red cell lipids were extracted using isopropyl alcohol and chloroform according to the method of Broekhuyse.[24] Phospholipid phosphorus was determined by the method of Bartlett.[25] The phospholipids were separated from cholesterol by thin-layer chromatography on Silica-Gel H (Merck) plates with petroleum ether-ethyl ether-acetic acid (70:30:1, vol/vol). The phospholipids stayed at the origin and were eluted with choloroform-methanol (1:1, vol/vol) and subsequently with chloroform-methanol (1:9, vol/vol). Recovery was over 99%, and the phospholipid composition of the cholesterol-free extract was the same as that of the whole lipid extract. The determination of individual phospholipid was carried out according to the method of Rouser et al.[26] For the analysis of the fatty acid composition of individual phospholipids, the corresponding spots on thin-layer chromatography plates were scraped and eluted with chloroform-methanol (1:1, vol/vol) and then with chloroform-methanol (1:9, vol/vol). Fatty acids of the phospholipids were converted to methyl esters by boron trifluoride,[27] and examined with a Gas Chromatograph GC-6A (Shimadzu, Japan) equipped with a digital integrator (Chromatopac E1A, Shimadzu). Gas chromatography was carried out using a glass column (200 cm × 3 mm) packed with 10% diethylene glycol succinate polyester.

Preparation of Liposomes

Liposomes were prepared according to the method of Kinsky.[28] Prior to preparation of cholesterol-depleted liposomes, total lipids from either controls or the patient's red cells were subjected to silicic acid column chromatography. The phospholipid fraction was separated from the neutral lipid fraction, principally cholesterol, by sequential elution using chloroform, chloroform-methanol (1:1, vol/vol) and chloroform-methanol (1:9, vol/vol). The separated phospholipids were utilized for preparing liposomes according to the same method as used for the total lipid (cholesterol-containing) liposomes.[28]

Spin Labeling Studies of Intact Red Cells and Extracted Lipids

Spin labeling studies were performed on liposomes of total lipids extracted from the red cells and on cholesterol-depleted pure phospholipid liposomes of the red cells and also on intact red cells.

A stearic acid spin probe, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (5-SAL), was purchased from Syva Co (Palo Alto, Calif) and utilized without further purification. In the preparation of spin-labeled lipid dispersions, special care was taken to prevent lipid denaturation by utilizing butylated hydroxytoluene (5 μg/mL) as an antioxidant. Extracted lipids were mixed with 1 mol/100 mL of 5-SAL in a test tube, and the solvent was evaporated under nitrogen. Two hundred microliters of 50 mmol/L Tris-buffered saline (pH 7.5) was added to a test tube with a few glass beads and vortexed with a thermomixer for one minute at 40 °C.[29] The spin-labeled lipid dispersions were taken into a glass capillary, and ESR spectra were recorded on a JEOL IX ESR Spectrometer (Japan Electron Optics Lab, Tokyo) at various temperatures. The determinations were performed in triplicate. The order parameter (S) and index of fluidity were calculated using the following formula:

$$S = \frac{T'// - T'\perp}{Tzz - \frac{1}{2}(Txx + Tyy)} \cdot \frac{a}{a'},$$

where Tzz, Txx and Tyy are the hyperfine principal values of the

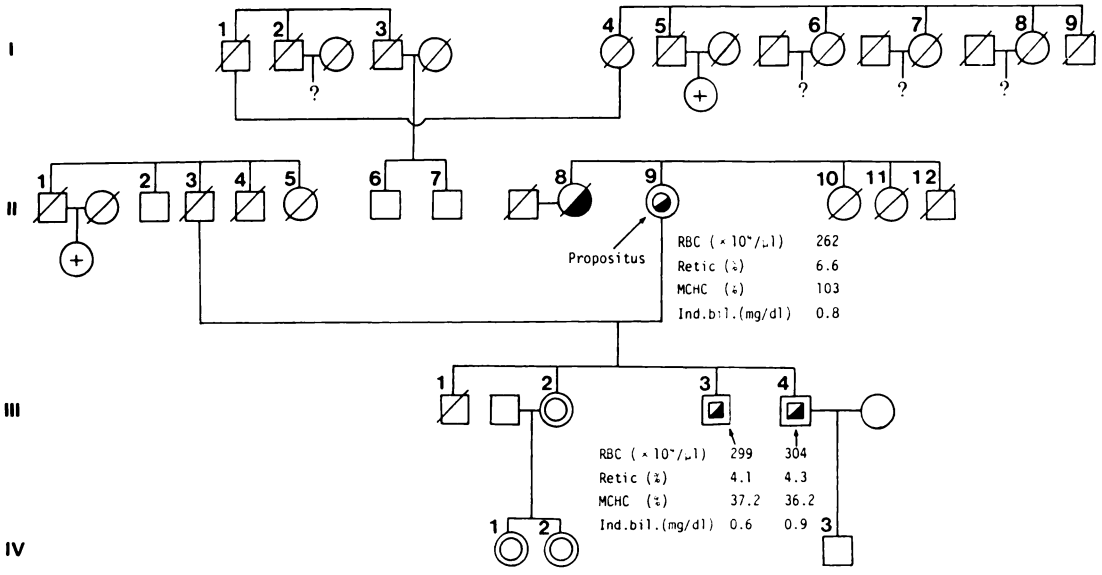

Fig 1. A pedigree of hereditary high red cell membrane phosphatidylcholine hemolytic anemia associated with increased sodium transport. ◻ ◎ , Examined, no anemia, no jaundice; ◪ ◉ , examined, anemia and jaundice; ⌀ , suspected on history; ◪ ⌀ , deceased.

Table 1. Plasma Lipids of the Patient With Hereditary High Red Cell Membrane Phosphatidylcholine Hemolytic Anemia

	Patient (n = 7)	Normal (n = 20)
Cholesterol (mg/dL)	191 ± 25	193 ± 31
Free fatty acids (mg/dL)	470 ± 58	450 ± 150
Triglyceride (mg/dL)	88 ± 31	95 ± 65
Phospholipids (mg/dL)	152 ± 32	180 ± 20
β-Lipoprotein (mg/dL)	405 ± 74	350 ± 150
Lipoprotein fraction	Normal	
Phospholipids (%)		
Lysophosphatidylcholine	3.8 ± 2.1	5.5 ± 1.8
Sphingomyelin	15.9 ± 2.0	16.5 ± 2.3
Phosphatidylcholine	72.5 ± 11.1	64.9 ± 12.7
Phosphatidylserine + phosphatidylinositol	2.0 ± 1.5	1.7 ± 1.3
Phosphatidylethanolamine	4.4 ± 4.3	4.4 ± 3.8

In duplicate determinations (Mean value ± 1 SD).

nitroxide radical, and a/a′ is a correction coefficient for the change of polarity in an aqueous solution.[30]

The order parameter measures the mean value of $(3\cos^2\theta - 1)/2$ where θ is the angle between the principal z axis of the nitroxide moiety and the z′ axis in the effective axis system. The smaller S becomes, the greater the spread in θ or the amplitude of rapid anisotropic motion. In the present study, we used the order parameter of fatty acid spin label incorporated into the membrane as a measure of membrane lipid fluidity.

RESULTS

Plasma Lipid Composition

The total lipid content, cholesterol, free fatty acids, triglyceride, phospholipids, and β-lipoprotein in plasma were completely normal in this patient (Table 1). No abnormal lipoprotein fraction was detected.

Red Cell Membrane Lipid Composition

Phosphatidylcholine (PC) and sphingomyelin (SM) levels were increased significantly in both absolute and relative amounts in comparison with control red cells ($P < .01$). It should be noted that the ratio of SM to PC (SM/PC) was markedly reduced (0.63 ± 0.03), compared with normal controls (0.90 ± 0.07). Phosphatidylethanolamine (PE) was increased in percentage, although there was no significant increase in the absolute amount of PE. Phosphatidylserine (PS) and phosphatidylinositol (PI) levels were increased in absolute amounts but not in percentages of total lipids. The ratio of free cholesterol to phospholipids was slightly elevated in the HPCHA red cells, compared with controls (Table 2).

Fatty Acid Composition of the Phospholipids

Red cell phospholipids were somewhat less unsaturated than normal controls (Table 3). The principal changes seen were moderate reductions of arachidonic and linoleic acids and increases of stearic acid in the patient's red cells.

Analysis of Electron Spin Resonance Spectra of Spin-Labeled Phospholipids Extracted From the Patient's Red Cells

Electron spin resonance studies on membrane lipid fluidity were carried out over a wide range of temperatures (15 °C to approximately 47 °C). The order parameter (S) was calculated from the ESR spectra in the intact red cells, in the total lipid (cholesterol-containing) liposomes, and in the pure phospholipid (cholesterol-depleted) liposomes. Results at representative temperatures (37 °C, 43 °C, and 31 °C) are shown in Table 4.

In the liposomes of the pure phospholipids of the patient's red cells, the order parameters were lower than those from normal controls ($P < .01 \sim .02$). Thus, pure phospholipid liposomes in the HPCHA red cells showed appreciably increased fluidity. In contrast, in the liposomes of total lipids containing free cholesterol prepared from the patient's red cells, the order parameters were slightly lower than those from

Table 2. Red Cell Membrane Lipids in the Patient With Hereditary High Red Cell Membrane Phosphatidylcholine Hemolytic Anemia

	Patient (n = 4)	Normal (n = 10)
Free cholesterol (FC)	1,765 ± 96*	1,202 ± 103
Total phospholipids (PL)	3,440 ± 102*	2,604 ± 241
Lysophosphatidylcholine (L-PC)	72 ± 12 (2.1 ± 0.3%)	34 ± 18 (1.3 ± 0.7%)
Phosphatidylcholine (PC)	1,242 ± 59* (36.1 ± 1.7%)*	747 ± 73 (28.7 ± 2.8%)
Sphingomyelin (SM)	777 ± 28* (22.6 ± 0.8%)*	674 ± 49 (25.9 ± 1.9%)
Phosphatidylethanolamine (PE)	843 ± 35 (24.5 ± 1.0%)*	805 ± 42 (30.9 ± 1.6%)
Phosphatidylserine (PS) + Phosphatidylinositol (PI)	468 ± 17* (13.6 ± 0.5%)	344 ± 34 (13.2 ± 1.3%)
PC + SM + L-PC/PE + PS + PI	1.59 ± 0.02*	1.27 ± 0.04
FC/PL ratio	1.00 ± 0.02*	0.90 ± 0.04
SM/PC ratio	0.63 ± 0.03*	0.90 ± 0.07

The numbers represent $\mu g/10^{10}$ RBCs.

The numbers in parentheses represent the percentage of phospholipids (mean value ± 1 SD).

*$P < .01$, significant difference.

Table 3. Fatty Acid Composition of Red Cell Lipids of the Patient With Hereditary High Red Cell Membrane Phosphatidylcholine Hemolytic Anemia

	Total Lipids		Phosphatidylcholine (PC)		Sphingomyelin (SM)		Phosphatidyl-ethanolamine (PE)		Phosphatidylserine (PS)	
	HPCHA (n = 3)	Normal (n = 7)	HPCHA (n = 3)	Normal (n = 7)	HPCHA (n = 3)	Normal (n = 7)	HPCHA (n = 3)	Normal (n = 7)	HPCHA (n = 3)	Normal (n = 7)
DMA	1.4	0.5 ± 0.4	—	—	—	—	5.4	4.9 ± 0.4	—	—
16:0	21.8	21.4 ± 1.2	36.7	35.4 ± 1.7	30.1*	25.1 ± 1.9	20.1*	17.4 ± 2.0	3.7	3.5 ± 0.7
DMA	1.2	2.0 ± 0.5	—	—	—	—	5.8	7.3 ± 1.1	—	—
18:0	15.7*	14.5 ± 0.4	13.3*	12.4 ± 0.5	6.2*	7.0 ± 0.5	10.7*	8.7 ± 0.2	49.3	47.8 ± 2.8
18:1	15.1*	13.3 ± 0.9	23.3*	17.0 ± 0.7	1.8	2.6 ± 1.1	17.0	17.1 ± 0.7	5.7*	7.9 ± 1.0
18:2	8.0*	10.8 ± 1.1	11.7*	21.9 ± 1.3	1.6	2.7 ± 1.3	3.8*	6.4 ± 1.2	1.7	2.6 ± 0.6
20:0	—	—	—	—	1.6	1.8 ± 0.4	—	—	—	—
20:3	0.9	1.2 ± 0.2	1.2	1.4 ± 0.4	—	—	—	—	1.4	1.6 ± 0.2
20:4	9.9*	12.4 ± 0.9	4.0	4.4 ± 0.5	—	—	14.0*	16.2 ± 0.9	15.9*	18.0 ± 1.0
20:5	1.8	1.4 ± 0.4	1.8	1.6 ± 0.4	—	—	2.7	2.9 ± 0.8	—	—
22:0	—	—	—	—	8.0	7.4 ± 0.7	—	—	—	—
22:4	—	—	—	—	—	—	3.9	3.6 ± 0.4	2.2	2.1 ± 0.4
22:5	3.2	2.8 ± 0.2	—	—	2.5*	4.1 ± 0.7	4.4*	3.5 ± 0.6	3.8*	2.8 ± 0.3
22:6	8.3	7.4 ± 0.9	3.9*	2.6 ± 0.5	—	—	9.0	8.9 ± 1.0	13.0*	10.6 ± 0.9
24:0	4.2	4.9 ± 0.6	—	—	20.1	19.5 ± 0.9	—	—	—	—
24:1	3.9	4.1 ± 0.7	—	—	24.7	26.4 ± 2.9	—	—	—	—
Unsaturation (%)	54.2	55.2 ± 0.9	48.7*	51.3 ± 1.1	34.7*	37.9 ± 1.6	56.9*	60.6 ± 1.3	45.6	47.7 ± 2.3
Unsaturation index	159.7	165.9 ± 5.9	106.0*	114.9 ± 6.7	53.3*	63.9 ± 3.9	198.7	204.9 ± 13.1	196.0	192.1 ± 6.8

The numbers represent the percentage of phospholipid.
*$P < .01$, significant difference.

normal controls. The differences, however, were not statistically significant ($P > .5$).

In the intact red cells, the order parameters of ESR spectra in the HPCHA patient also appeared to be lower than those in normal controls. The differences, however, were minimal and not statistically significant ($P > .05$).

DISCUSSION

Phospholipids and cholesterol are the major lipids in human red cell membranes. The interactions between phospholipids and cholesterol impose a degree of immobility on the portion of the lipid molecules for some distance in the lipid bilayer of the red cell membranes.[31]

Table 4. Order Parameters in Electron Spin Resonance (ESR) for Intact Red Cells, Total Lipid Liposomes Containing Free Cholesterol, and Phospholipid (Free Cholesterol-Depleted) Liposomes in Red Cells of a Patient With Hereditary High Red Cell Membrane Phosphatidylcholine Hemolytic Anemia

		Order Parameter (S)					
Temperature	Sample	Normal (n = 8)	p	HPCHA (n = 5)	Δ	P	
37 °C	Intact RBCs	0.660 ± 0.005	NS	0.657 ± 0.006	0.003 ± 0.002	NS	*
	Total lipid (FC+) liposomes	0.648 ± 0.003	NS	0.643 ± 0.004	0.005 ± 0.002	*	
	Phospholipid (FC−) liposomes	0.606 ± 0.003	†	0.593 ± 0.005	0.013 ± 0.003		
43 °C	Intact RBCs	0.641 ± 0.004	NS	0.636 ± 0.006	0.005 ± 0.003	NS	*
	Total lipid (FC+) liposomes	0.616 ± 0.004	†	0.609 ± 0.005	0.007 ± 0.002	†	
	Phospholipid (FC−) liposomes	0.581 ± 0.003	*	0.565 ± 0.005	0.016 ± 0.002		
31 °C	Intact RBCs	0.690 ± 0.006	NS	0.682 ± 0.007	0.008 ± 0.002	NS	*
	Total lipid (FC+) liposomes	0.681 ± 0.006	NS	0.672 ± 0.004	0.009 ± 0.002	†	
	Phospholipid (FC−) liposomes	0.634 ± 0.005	*	0.617 ± 0.005	0.017 ± 0.003		

FC, free cholesterol; HPCHA, hereditary high red cell membrane phosphatidylcholine hemolytic anemia.
Significant, $P < .01$ (*), $P < .02$ (†); NS, not significant.
Mean value ± SD.
Δ, Differences of order parameters between normal and HPCHA.

The addition of cholesterol to pure phospholipid membranes decreases fluidity and increases the order parameters measured by ESR. Fludity in red cell membranes studied with hydrophobic probes (such as SAL) can be detected by electron spin resonance. Membrane fluidity is influenced by (1) the amount of free cholesterol; (2) the nature of the phospholipids; (3) the degree of fatty acid saturation; and (4) the length of acyl chains.

A "compensatory" mechanism for abnormal membrane fluidity has recently been observed in general biology[14-20] and also in one example of a hemolytic condition in human red cells of lecithin: cholesterol acyl transferase deficiency.[21,32]

In the present study, the possibility of a similar mechanism was investigated in the red cells of a patient with hereditary high red cell membrane phosphatidylcholine hemolytic anemia, in which phosphatidylcholine is increased only in the red cell membranes.

The fluidity of pure phospholipid liposomes prepared from the patient's red cells was increased significantly ($P < .02$), chiefly because of the marked increase of phosphatidylcholine, which is intrinsic to the disorder. Change in the phospholipids, such as in the class of phospholipids, in fatty acid composition, in saturation/unsaturation, and in carbon number of acyl chains, appeared to compensate only minimally for the marked increase in the fluidity caused by the increased phosphatidylcholine.

In contrast, the cholesterol-containing "total liposomes" showed virtually normal fluidity, apparently because of the presence of free cholesterol, which made the membranes less fluid. Thus, the increased membrane fluidity induced by the increased PC was essentially corrected by the increased cholesterol.

Fluidity was also examined in the intact red cells of the HPCHA patient. Nearly normal membrane fluidity was found in the whole cells. Thus, the overall membrane fluidity was maintained nearly normally in the intact red cells by the increased cholesterol, even with the increased phosphatidylcholine. This suggests that in the intact cells, also, the elevated cholesterol seems to compensate for fluidity abnormalities that might otherwise occur because of the elevated PC.

ACKNOWLEDGMENT

We sincerely thank Mrs B. Fuller for her help in preparing the manuscript.

REFERENCES

1. Jaffé ER, Gottfried EL: Hereditary nonspherocytic hemolytic disease associated with an altered phospholipid composition of the erythrocytes. J Clin Invest 47:1375, 1968

2. Takaku F, Maezawa M: Pathophysiology of red cell lipids. Acta Haematol Jpn 39:589, 1976

3. Yawata Y, Takemoto Y, Yoshimoto M, Miyashima K, Koresawa S, Mori M, Miwa T, Murai Y: The Japanese family of congenital hemolytic anemia with high red cell membrane phosphatidyl choline and increased sodium transport. Acta Haematol Jpn 45:672, 1982

4. Uchida T, Kimura H, Sakai K, Igarashi T, Matsuda S, Kariyone S, Maezawa M, Takaku F: A case of high phosphatidyl choline hemolytic anemia (in Japanese). Jpn J Clin Hematol 23:193, 1982

5. Karasawa M, Sawamura M, Okamoto K, Iwata N, Omine M, Maekawa T, Sugihara T, Yoshimoto M, Yawata Y: A case of congenital non-spherocytic hemolytic anemia associated with high red cell membrane phosphatidyl choline and enhanced Na^+ transport (in Japanese). Jpn J Clin Hematol 23:1816, 1982

6. Takemori H, Haneda Y, Kimura A, Chiba Y, Yoshida Y, Sugihara T, Shimoda M, Miyashima K, Yawata Y: A case of high red cell membrane phosphatidyl choline hemolytic anemia with low plasma cholesterol (in Japanese). Jpn J Clin Hematol 24 (suppl):286a, 1983

7. Fujiwara F, Konishi S, Imashuku S, Miyashima K, Sugihara T, Yawata Y: A case of congenital high red cell membrane phosphatidyl choline hemolytic anemia with stomatocytosis and increased sodium influx (in Japanese). Jpn J Clin Hematol (in press)

8. Yawata Y: Recent progress in research on red cell membrane disorders in Japan: Pathogenesis of microspherocytosis in hereditary spherocytosis, sodium transport abnormalities in stomatocytosis, and homeoadaptive compensatory mechanism for membrane fluidity in red cell membrane lipid abnormalities. Acta Haematol Jpn 46:1426, 1983

9. Shohet SB, Livermore BM, Nathan DG, Jaffé ER: Hereditary hemolytic anemia associated with abnormal membrane lipids: Mechanism of accumulation of phosphatidyl choline. Blood 38:445, 1971

10. Shohet SB, Nathan DG, Livermore BM, Feig SA, Jaffé ER: Hereditary hemolytic anemia associated with abnormal membrane lipid. II. Ion permeability and transport abnormalities. Blood 42:1, 1973

11. Cooper RA: Abnormalities of cell-membrane fluidity in the pathogenesis of disease. N Engl J Med 297:371, 1977

12. Giraud F, Claret M, Garay R: Interactions of cholesterol with the Na pump in red blood cells. Nature 264:646, 1976

13. Cooper RA, Durocher JR, Leslie MH: Decreased fluidity of red cell membrane lipids in abetalipoproteinemia. J Clin Invest 60:115, 1977

14. Sinensky M: Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in Escherichia coli. Proc Natl Acad Sci USA 71:522, 1974

15. Marr AG, Ingraham JL: Effect of temperature on the composition of fatty acids in Escherichia coli. J Bacteriol 84:1260, 1962

16. Miller RW, De La Roche IA: Properties of spin labelled membranes of Fusarium oxysporum f. sp. lycopersici. Biochim Biophys Acta 443:64, 1976

17. Fukushima H, Martin CE, Iida H, Kitajima Y, Thompson GA Jr, Nozawa Y: Changes in membrane lipid composition during temperature adaptation by a thermotolerant strain of Tetrahymena pyriformis. Biochim Biophys Acta 431:165, 1976

18. Raison JK, Lyons JM: Hibernation: Alteration of mitochondrial membranes as a requisite for metabolism at low temperature. Proc Natl Acad Sci USA 68:2092, 1971

19. Keith AD, Aloia RC, Lyons J, Snipes W, Pengelley ET: Spin label evidence for the role of lysoglycerophosphatides in cellular membranes of hibernating mammals. Biochim Biophys Acta 394:204, 1975

20. Philippot JR, Cooper AG, Wallach DFH: 25-Hydroxycholecalciferol and 1,25-dihydroxycholecalciferol are potent inhibitors of cholesterol biosynthesis by normal and leukemic (L_2C) guinea pig lymphocytes. Biochem Biophys Res Commun 72:1035, 1976

21. Yawata Y, Miyashima K, Sugihara T, Murayama N, Hosoda S, Nakashima S, Iida H, Nozawa Y: Self-adaptive modification of red cell membrane lipids in lecithin:colesterol acyl transferase deficiency. Lipid analysis and spin labeling. Biochim Biophys Acta 769:440, 1984

22. Yawata Y, Jacob HS: Abnormal red cell metabolism in patients with chronic uremia: Nature of the defect and its persistence despite adequate hemodialysis. Blood 45:231, 1975

23. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497, 1957.

24. Broekhuyse RM: Improved lipid extraction of erythrocytes. Clin Chim Acta 51:341, 1974

25. Bartlett GR: Phosphorus assay in column chromatography. J Biol Chem 234:466, 1959

26. Siakotos AN, Rouser G: Quantitative thin-layer chromatography of lipids by photographic densitometry. Anal Biochem 14:162, 1966

27. Morrison WR, Smith LM: Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 5:600, 1964

28. Kinsky SC: Preparation of liposomes and a spectrophotometric assay for release of trapped glucose marker. Methods Enzymol 32:501, 1974

29. Ohki K, Kasai R, Nozawa Y: Correlation between fluidity and fatty acid composition of phospholipid species in tetrahymena pyriformis during temperature acclimation. Biochim Biophys Acta 558:273, 1979

30. Butterfield DA, Purdy MJ, Markesbery WR: Electron spin resonance, hematological, and deformability studies of erythrocytes from patients with Huntington's disease. Biochim Biophys Acta 551:452, 1979

31. Evans EA, Hochmuth RM: Membrane viscoelasticity. Biophys J 16:1, 1976

32. Godin DV, Herring FG: Spin label studies of erythrocytes with abnormal lipid composition: Comparison of red cells in a herditary hemolytic syndrome and lecithin:cholesterol acyltransferase deficiency. J Supramol Struct Cellular Biochem 15:213, 1981