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Studies on Lipid of Red Cell Membrane in Patients with Iron Deficiency Anemia by the Use of Iatroscan

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ABSTRACT. Studies on red cell membrane lipid composition in patient with iron deficiency anemia (IDA) disclosed the possibility of abnormal metabolism of cellular membrane. Inversion of the SM/PC ratio (SM, sphingomyelin and PC, phosphatidyl choline, are the lipids of the outer lamella of erythrocyte membrane) might be responsible for the poikilocytosis seen in this anemia. In IDA patients, phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS), which are the lipids of inner lamella of the membrane decreased, but they returned to the normal range with increase in reticulocytes which were produced in accordance with the beneficial effect of the iron therapy.

Key words : Iron deficiency anemia — Chorea-acanthocytosis — Spur cell anemia — Red cell membrane lipid — Iatroscan — Poikilocytosis

Erythrocytes are incessantly exposed to the changing chemical composition of plasma,[1,2] which makes their environment. It has been known that red cells of abnormal morphology make their appearance in the peripheral blood of many patients with acquired diseases and their abnormality varies depending on the character of underlying disorders.[2,3] As for instance, in iron deficiency anemia, which is frequently encountered in Japan, characteristic microcytic and hypochromic red cells are seen and they make us aware of the presence of disordered metabolism of iron by their pathognomonic morphological changes of the peripheral blood smears. In fact this anemia responds to iron therapy quite well normal picture of red cell morphology is restored rapidly keeping pace with the improvement of iron metabolism.

The factor which plays an important role in bringing forth microcytic and hypochromic erythrocytes is, of course, the disorder of hemoglobin synthesis. However, disturbed metabolism of iron may be thought to affect the metabolism of red cell membrane harmfully. Therefore, we investigated the derangement of the composition of red cell membrane lipid which occurs in the patients under treatment of iron deficiency anemia with special reference to altered morphology of red cells and iron metabolism.

MATERIALS AND METHODS

—Materials—

Twenty-eight of normal adults and 38 of iron deficiency anemia patients who showed the peripheral blood picture of microcytic and hypochromic red cells

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with lowered erythrocyte indices (Hb 11.5 g/dl, MCV 83 fl, MCH 28 pg, saturation ratio of iron $< 15\%$) were studied. Some of them were chased throughout the course of their clinical improvement by iron therapy.

—Extraction of red cell membrane lipids—

1. Preparation and extraction: Three ml of anticoagulated venous blood were centrifuged to separate the layer of red cells from the plasma and buffy coat. The red cells were washed with physiological saline solution and centrifuged three times repeatedly to obtain the washed packed red cells. One ml

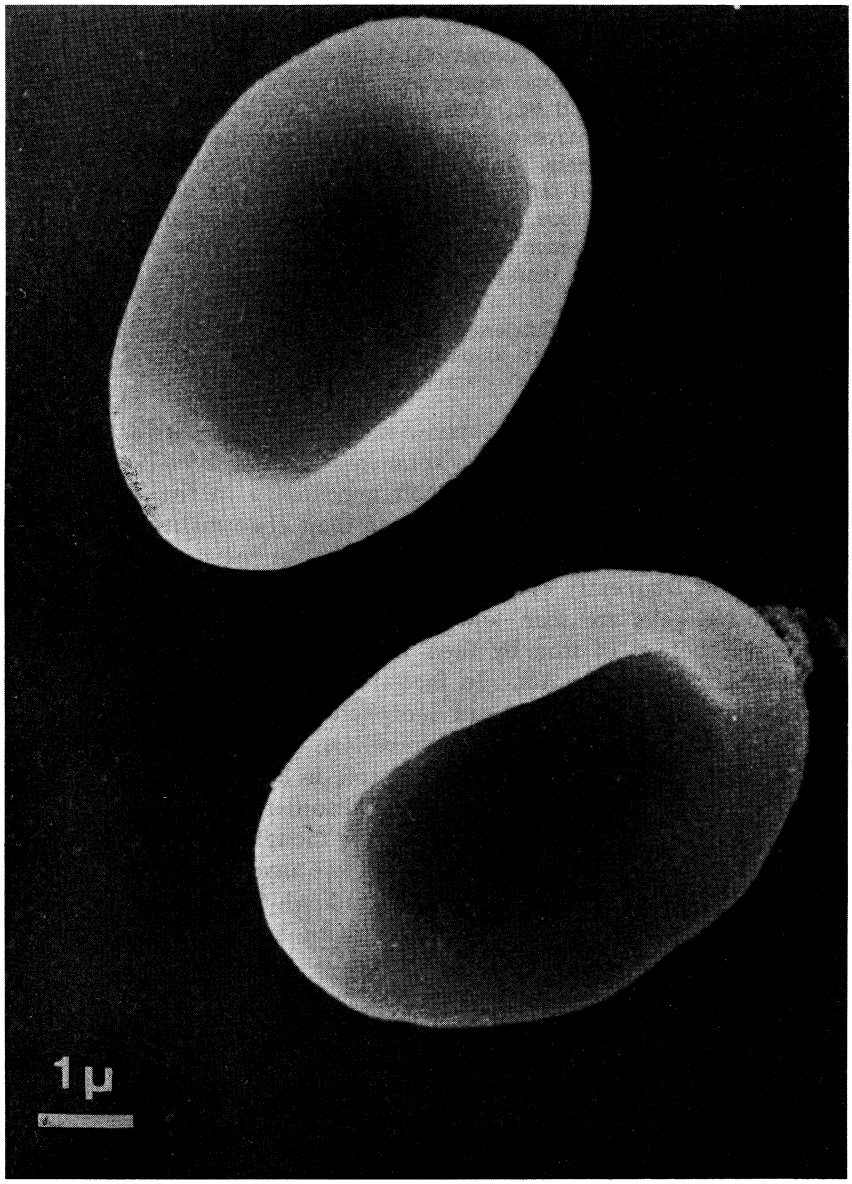

Fig. 1. Scanning electron micrograph of a thin, microcytic red cells in patient with iron deficiency anemia.

of H_2O was added to 0.9 ml of washed packed red cells to prepare hemolysate by hypotonic stress. Rose's method[4] was employed for extraction of lipid from the hemolysate. This yielded the upper layer containing physiological saline solution and the lower layer consisting of the extract of red cell membrane lipids. After removing the upper layer, the lower layer was filtered and freezed at $-20°C$ overnight. Seven ml of the extracted solution was put into a flask (25 ml in volume) and it was set on the rotary evaporator to evaporate the solvent. The residue of the lipid extract thus obtained was dissolved in 0.4 ml of Folch's solution (chloroform : methanol = 2 : 1) for analytical purpose.

2. Chromatographic separation and development of lipid extract: These procedure were performed by use of the thin-layer chromatography with flame ionization detection (Iatroscan method).[5]

RESULTS

1. In iron deficiency anemia (Fig. 1) the total lipid content (mg) of red cell membrane per unit amount (g) of hemoglobin contained in erythrocytes was about 18.8 mg/g Hb on the average, being apparently higher than in normal subjects (14.96 as average) as shown in Table 1 ($p<0.001$ compared with normals). However, when the content was expressed in terms of per 10^{10} red blood cells, it was 4.21 mg, being subnormal as compared with 4.49 mg in the normal ($p<0.01$).

2. SM/PC ratio of erythrocyte membrane lipid : The phospholipid ratio SM/PC (these lipids are chiefly distributed in the outer lamella of the lipid bilayer of red cell membrane) was over 1.0 in patients of iron deficiency anemia with distinct poikilocytosis (Table 2, Fig. 2). This ratio is 0.96 ± 0.10 in the normal subjects. Similarly, spur cell anemia (Table 2, Fig. 3), and chorea-acanthocytosis (Table 2, Fig. 4) which exhibit remarkable poikilocytosis gave the values over 1.0 for the SM/PC ratio.

3. The ratio of [PC+SM] / [PE+PS] in red cell membrane lipid:

TABLE 1. Red cell membrane lipids in iron deficiency anemia.

		Composition of red cell membrane lipid					
			Outer leaflet		inner leaflet		
		FC	PC	SM	PE	PS	T.L.
NORMAL (28)	mg/g Hb	4.83±0.47	2.83±0.37	2.72±0.34	2.81±0.39	1.77±0.31	14.96±1.56
	mg/10^{10} RBC	1.45±0.14	0.85±0.11	0.81±0.10	0.84±0.11	0.53±0.09	4.49±0.47
	%	(32.8)	(18.9)	(18.2)	(18.8)	(11.8)	(100%)
IDA (33)	mg/g Hb	6.17±1.06	3.48±0.67	3.38±0.76	3.47±0.92	2.30±0.65	18.80±3.78***
	mg/10^{10} RBC	1.38±0.16	0.79±0.12*	0.75±0.13	0.77±0.16	0.52±0.12	4.21±0.59**
	%	(32.8)	(18.6)	(17.9)	(18.5)	(12.2)	(100%)

p values compared with normal: *$p<0.05$, **$p<0.01$, ***$p<0.001$, FC: free cholesterol, PC: phosphatidyl choline, SM: sphingomyelin, PE: phosphatidyl ethanolamine, PS: phosphatidyl serine, TL: total lipid, IDA: iron deficiency anemia. Relative percentage of each fraction to the total lipids contents are shown in parenthesis.

TABLE 2. Red cell membrane lipid composition in a patient with spur cell anemia, chorea-acanthocytosis and iron deficiency anemia. Poikilocytosis was evident.

	FC	PC	SM	PE	PS	T.L.	SW/PC
Spur cell anemia (M.K.)							
mg/g Hb	6.75	4.53	4.7	3.26	2.72	21.96	1.04
mg/10^{10} RBC	1.48	1.00	1.03	0.72	0.60	4.83	
%	(30.8)	(20.6)	(21.4)	(14.8)	(12.4)	(100)	
Chorea-acanthocytosis (S.K.)							
mg/g Hb	4.15	2.32	2.51	2.36	1.66	13.00	1.08
mg/10^{10} RBC	1.32	0.74	0.80	0.75	0.53	4.14	
%	(31.9)	(17.9)	(19.2)	(18.2)	(12.8)	(100)	
Iron deficiency anemia (N.M.)							
mg/g Hb	7.16	3.69	4.04	3.70	2.22	20.81	1.09
mg/10^{10} RBC	1.43	0.73	0.80	0.73	0.44	4.12	
%	(34.4)	(17.7)	(19.4)	(17.8)	(10.7)	(100)	

The [PC+SM]/[PE+PS] ratio in iron deficiency anemia (1.22 ± 0.18) was not significantly different from that of the normal subjects (1.18 ± 0.06).

4. Sequential changes of membrane lipid composition in patient with iron deficiency anemia under iron therapy: Figure 5 illustrates the clinical course of iron deficiency anemia under iron administration. Laboratory examination before the therapy revealed normal value of red cell count ($423\times10^4/\mu l$), and remarkably low hemoglobin concentration (8.0 g/dl) with a very low iron saturation ratio (serum iron 12 μg/dl, total iron binding capacity 412 μg/dl). Peripheral blood smear showed microcytic and hypochromic red cells together with distinctly poikilocytic cells.

Prior to the iron therapy, decrease in total lipid of red cell membrane and rise in the ratio of [PC+SM]/[PE+PS] due to diminished amount of inner component of membrane phospholipid (PE+PS) were observed. After iron administration hemoglobin concentration was increased and restoration of the ratio of [PC+SM]/[PE+PS] to the normal level was achieved in accordance with the improvement and alleviation of anemia as shown in Figure 5.

DISCUSSION

It has been well known by the investigations of various authors that there is close relationship between increased free cholesterol (FC) level of red cell membrane and target cell formation in hepatobiliary disorders.[2,3] On the other hand, disturbed metabolism of red cell membrane lipid has also been supposed about the cases of iron deficiency anemia that are frequently encountered in Japan. However its pathogenesis has not yet been revealed to date. This report aims to discuss the pathogenesis of the alteration of the membrane lipid in iron deficiency anemia.

The red cell membrane lipid content (per 10^{10} RBC) was decreased as shown in Table 1. It is supposed that two factors may be responsible for this decrease. One is the presence of hypochromia with microcytosis due to reduced heme synthesis and another is abnormal metabolism of red cell membrane lipid in

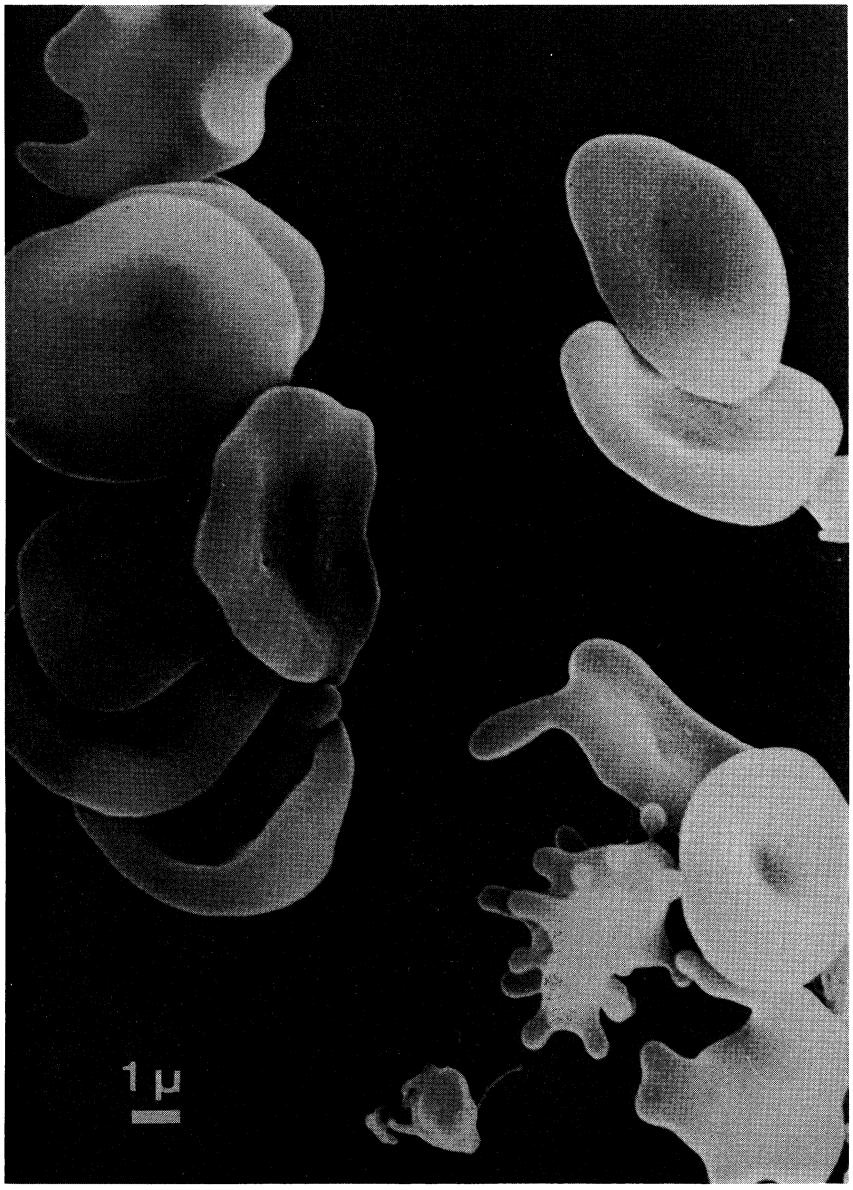

Fig. 2. Remarkable poikilocytosis in severe iron deficiency anemia.

iron deficiency. Yoshimoto *et al.*[6] pointed out lowered sodium transport in the IDA patients. No improvement of sodium transport was achieved by the exchange of the IDA cytosol fractions for the normal RBC cytosol fractions. These observations suggest that abnormalities in red cell membrane lipid fractions are chiefly related to the formation of microcytosis. Table 2 summarizes the study on the role played by sphingomyelin (SM) and phosphatidyl choline (PC) which are chief phospholipid components of the outer lamella.

Three cases (spur cell anemia, chorea-acanthocytosis and IDA) associated

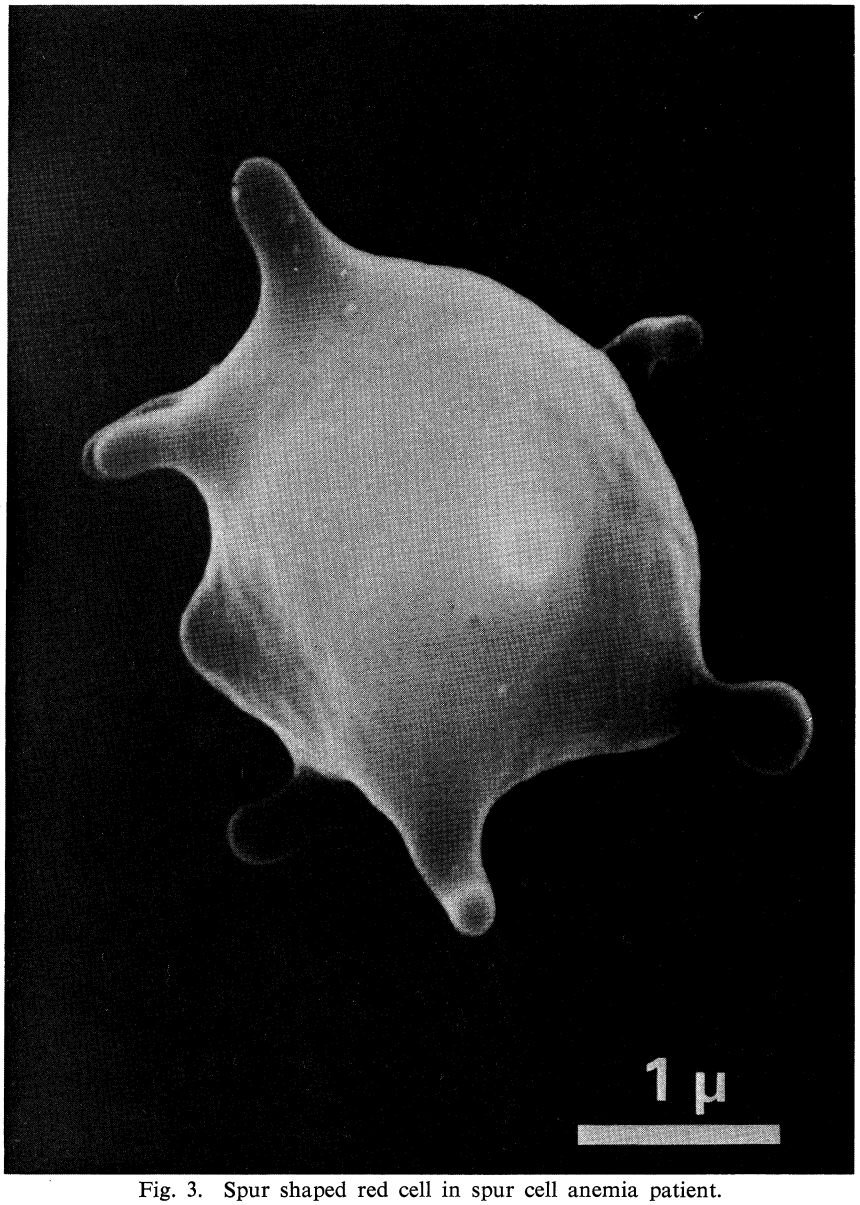

Fig. 3. Spur shaped red cell in spur cell anemia patient.

with distinct poikilocytosis were examined for the red cell membrane lipid. In all of them inversion of SM/PC ratio (over 1.0) was seen. SM is thought to make red cell membrane lipid and the inversion of SM/PC ratio may affect the red cell morphology to some extent.

Figure 5 is a clinical course of IDA under iron therapy. It is conceived that iron deficiency state causes the abnormal metabolism of membrane lipid of RBC. In fact, improvement in anemia normalizes the lipid composition of the membrane of RBC. Since lipid is not synthesized anew in mature red cell, it is hardly plausible that microcytic and hypochromic red cells which were

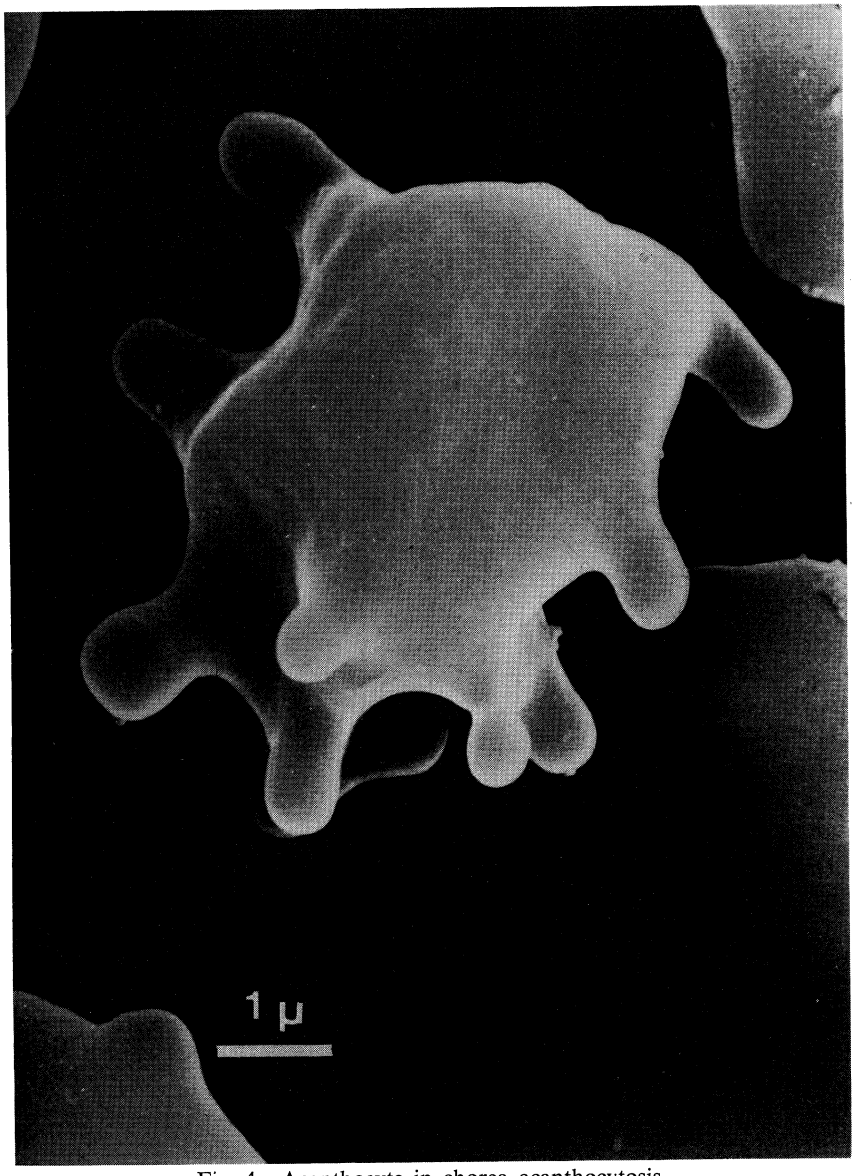

Fig. 4. Acanthocyte in chorea-acanthocytosis.

once produced as such become normocytic and normochromic erythrocyte by iron therapy. Increased production of normal mature red cells by iron medication is the major cause of restoration of normal red cell membrane lipid composition.

Next, the increase in reticulocytes following the iron administration is an important factor for the improvement of [PC+SM]/[PE+PS] ratio. Reticulocytes are normal in the lipid contents of their membrane so far as phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS) are concerned, but aged erythrocytes and pathological red cells are low in PE and PS contents of the

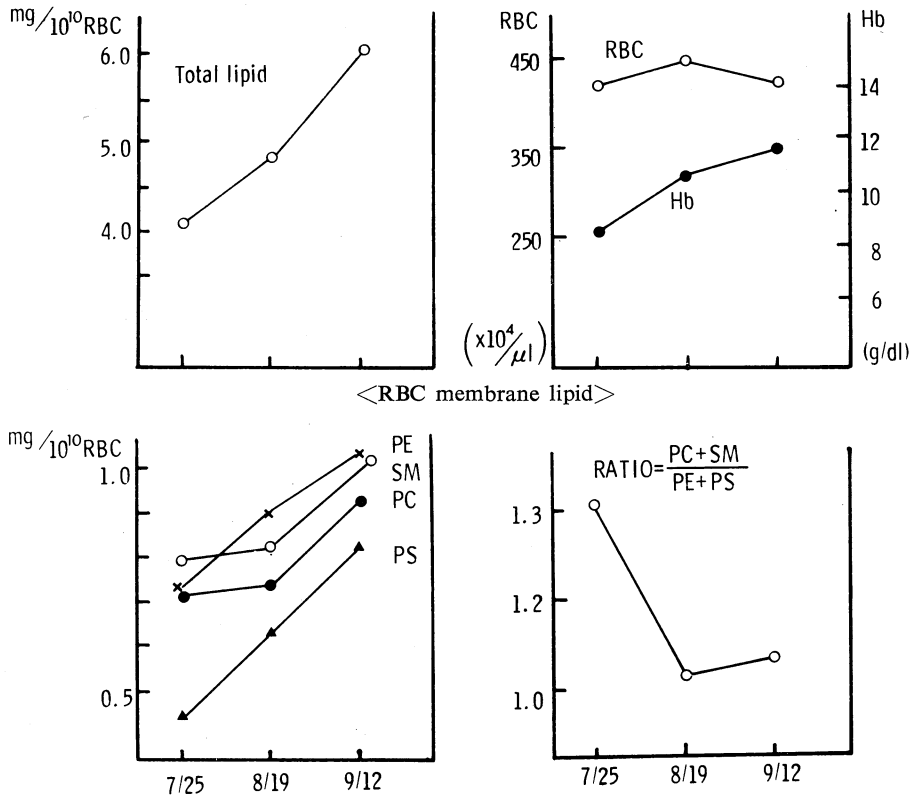

Fig. 5. Restoration of phospholipid composition in accordance with improvement of hematological parameters (RBC, Hb) in iron deficiency anemia by iron administration.

membrane. PE and PS, which are mainly distributed in the inner lamella, are synthesized from PC and SM by adenosine triphosphate dependent acylation. Therefore, it may be speculated that pathological red cells with disordered iron metabolism is susceptible to abnormal lipid synthesis, especially in the inner lamella lipid component.

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