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ALTERED COMPOSITION OF RED CELL MEMBRANE LIPIDS UNDER PATHOLOGICAL CONDITIONS

COMPARISON OF HEMATOLOGICAL DISEASES WITH HEPATOBILIARY TRACT DISORDERS

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Abstract

Altered lipid composition of erythrocyte membrane in diseases was studied by use of Iatroscan analysis with the blood samples of 22 normal subjects, 97 patients with hepatobiliary disorders (acute hepatitis 9, chronic hepatitis 29, hepatic cirrhosis 32, hepatic cancer 14 and obstructive jaundice 13), and 54 patients with hematological diseases (iron deficiency anemia 33, hereditary spherocytosis 10, paroxysmal nocturnal hemoglobinuria 2, hereditary elliptocytosis 3, autoimmune hemolytic anemia 2, spur cell anemia 2, and chorea-acanthocytosis 2).

Scrutiny of the results obtained disclosed that there was a salient contrast in the deviated pattern of erythrocyte membrane composition between the hepatobiliary disorders and the hematological diseases.

Namely, the erythrocyte membrane in hepatobiliary disorders are characterized by increased amount of total lipid and augmentation of free cholesterol which is distributed evenly between the inner and the outer laminas of the lipid double layer as well as rise in phosphatidyl choline which is mainly dispensed in outer lamina. As a result, lipids are contained in outer lamina more abundantly than in the inner lamina. (Increased resistance to hypo-osmolarity).

Conversely, in hematological diseases decrease in the total amount of red cell membrane lipid especially in phosphatidyl ethanolamine which is the main constituent of the inner lamina of lipid bilayer is noted. Diminution of lipids was more evident in the inner lamina than the outer lamina of the lipid bilayer (Decreased resistance to hypo-osmolarity).

On the basis of the result of this investigation some discussions were given about the possible relationship between the morphogenesis of poikilocytosis and the altered lipid composition of erythrocyte membrane.

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INTRODUCTION

Normal human erythrocytes secure its characteristic discoid shape by the peculiar conformation of its cellular membrane which is 75 Å thick and composed of a lipid bilayer coated with the net-work of contractile protein called spectrin inside, as well as studded with the villous projections of glycoproteins outside. These proteins comprise blood group substances, sialic acid and so forth, which bestow negative electric charge on the erythrocyte membrane. Erythrocytes leave its cellular membrane as a vacant sac which is designated stroma after complete leakage of their contents, the majority of which is hemoglobin. Erythrocytes undergo various stress that causes their deformation while they are flowing through the capillary system. They are deformed into any shape so that they may adapt to the external force, but restore their original discoid shape as soon as the strain is removed. This is the manifestation of the elasticity and the plasticity of erythrocytes, which depend on fluidity and viscosity of lipid bilayer and on the integrity of contractile spectrin net-work lining its inner surface.[1)]

Erythrocytes circulate continuously in the vascular system during the whole length of their life span of 120 days. Every time when they pass through the capillaries, they adapt themselves to the environmental changes, which include the mechanical forces arising from blood flow through a narrow lumen and the alterations of the chemical composition of blood ingredients that are the end-result of metabolism. These exert influence upon the red cell membrane, cause delicate deterioration of its properties, structural as well as chemical, and occasionally result in red cells of abnormal morphology. Target cells which appear in the peripheral blood of the patients with hepatobiliary disorders are one of the examples. Lipid analyses of red cell membrane were made by several investigators to elucidate the mechanism of genesis of red cells of such distorted morphology. Their studies disclosed that increase in both cholesterol and phosphatidyl choline (lecithin) played leading role in target cell formation.[2)] In hematological diseases poikilocytes of various types are encountered ; not only the target cells but also the erythrocytes of other varied shapes including spherocytes, ovalocytes, crenated cells, thorny cells (acanthocytes), and so forth are seen. The lipid analysis of cellular membranes of these poikilocytes has not yet been performed satisfactorily.[1,3,4)]

There are some reasons for the scarcity of such investigation : the conventional methods used for lipid analysis were cumbersome and unsuitable for routine clinical laboratory work which aimed to collect infomation from the test materials of patients with various diseases. It is about one or two years ago that a convenient procedure for the analysis of red cell membrane lipid by means of Iatroscan was established in our laboratory.[5)] Application of this method to the observation of patients has enabled us to compare the altered patterns of lipid composition of erythrocyte membrane in hepatobiliary disorders with those in hematological diseases.

The purpose of this paper is to present the data obtained in our experiment of lipid analysis of erythrocyte membrane by use of Iatroscan, together with the discussions about the factors causing alteration in red cell membrane lipid composition between the hepatobiliary disorders and the hematological diseases.

MATERIALS AND METHODS

One hundred and seventy-three subjects comprising three different groups of healthy and diseased people were examined for the lipid composition*[1] of erythrocyte membrane by Iatroscan analysis. The groups were the normal control (22 cases), the hepatobiliary disorders (97 cases) and the hematological diseases (54 cases).

Hepatobiliary disorders included acute hepatitis 9, chronic hepatitis 29, hepatic cirrhosis 32, hepatic cancer 14 and obstructive jaundice 13. Hematological diseases consisted of iron deficiency anemia 33, hereditary spherocytosis 10, paroxysmal nocturnal hemoglobinuria 2, hereditary elliptocytosis (with increased hemolysis 1 and without increased hemolysis 2), autoimmune hemolytic anemia 2, spur cell anemia 2 and acanthocytosis 2.

The composition of red cell membrane lipids has been expressed in the 3 different ways, namely ① by the percent ratio of carbon atoms per each lipid component, ② by the net weight of relevant lipid per 1 g of hemoglobin, and ③ by the weight of individual lipid per 10^{10} red blood cells. The Iatroscan analysis gives the values of ① as the first hand estimations. Next, these values are converted into those of ② (mg/g Hb) and ③ (mg/10^{10} RBC) by checking up with hemoglobin concentration (Hb g/dl) and erythrocyte count (RBC 10^6 /μl), the measurement of which are performed simultaneously with the Iatroscan analysis with the same blood samples. The expression (mg/g Hb) provides us with a useful index for the amount of the individual lipids in relation to hemoglobin contained in an erythrocyte, whereas the expression (mg/10^{10} RBC) is suitable for the appraisal of changes of the amount of lipids in an erythrocyte in various diseases.

RESULTS

Analytical values of red cell membrane lipid in patients with hepatobiliary disorders and hematological diseases which were obtained in our study are shown in Table 1 (mg/g Hb) and Table 2 (mg/10^{10} RBC).

In Table 3 are listed the ratio of phospholipid compositions $\overline{PC+SM}/\overline{PE+PS}$ (phospholipids chiefly of the outer lamella per those mainly of the inner lamella of the lipid bilayer of RBC membrane), FC/PL (FC/$\overline{PE+PS+PC+SM}$) and SM/PC (phospholipid composition of outer lamella of the lipid bilayer).

Table 4 presents the erythrocyte membrane lipid composition (% ratio) in

*1 FC : free cholesterol, PE : phosphatidyl ethanolamine, PS : phosphatidyl serine, PC : phosphatidyl choline (lecithin), SM : sphingomyelin

patients with hepatobiliary and hematological diseases in comparison with that seen in normal subjects.

DISCUSSION

Scrutiny of Table 1 and Table 2, in which red cell membrane lipid compositions in hepatobiliary disorders and in hematological diseases are presented in comparison with that in normal subjects will lead us to the following interpretation.

TABLE 1. Erythrocyte membrane lipid composition (mg/gHb)

Diseases	n	Total	FC	PE	PS	PC	SM
Normal	22	14.89±1.43	4.80±0.43	2.85±0.32	1.77±0.33	2.78±0.33	2.67±0.29
Hepatitis, acute	9	14.58±2.67	5.55±0.88	2.06±0.55	1.41±0.44	3.41±0.96	2.13±0.55
Hepatitis, chronic	29	14.52±2.30	4.95±0.74	2.46±0.43	1.63±0.45	3.19±0.85	2.31±0.49
Hepatic cirrhosis	32	15.04±2.79	5.44±1.06	2.24±0.51	1.74±0.55	3.22±0.88	2.38±0.49
Hepatic cancer	14	14.54±1.96	5.33±0.68	2.22±0.35	1.55±0.44	3.38±1.09	2.09±0.50
Obstructive jaundice	13	19.03±2.95	7.09±1.00	2.15±0.48	1.57±0.57	6.11±1.20	2.10±0.47
Spur cell anemia	2	14.10±2.63	5.70±0.40	1.9 ±0.22	1.22±0.54	2.89±0.45	2.40±1.00
Iron deficiency anemia	33	18.80±3.78	6.17±1.06	3.47±0.92	2.30±0.65	3.48±0.67	3.38±0.76
Hereditary spherocytosis	10	12.56±1.67	4.54±0.42	2.06±0.60	1.36±0.34	2.40±0.48	2.20±0.33
Hereditary elliptocytosis(c)	1	12.10	4.11	1.87	1.30	2.79	2.03
Hereditary elliptocytosis(s)	2	11.47	3.93	2.10	1.42	2.02	2.00
Autoimmune hemolytic anemia	2	10.33	3.71	1.86	1.06	2.06	1.64
Paroxysmal nocturnal Hb-uria	2	13.03	4.56	1.89	1.51	2.71	2.36
Acanthrocytosis	2	12.28	4.06	2.20	1.57	2.17	2.28

(c) with increased hemolysis (s) without increased hemolysis

A. Hepatobiliary tract disorders are frequenty associated with increase in total lipid content of the erythrocyte membrane, which is mainly the result of accumulation of FC[6] and PC. Conversely, PE and SM are decreased. Increment of lipid content due to increase in FC and PC surpasses its decrement owing to decrease in PE and SM. This tendency is characteristically pronounced in obstructive jaundice. Total content of erythrocyte membrane lipids is not altered so evidently, being within the normal range in the majority of cases of acute and chronic hepatitis, because decrease in PE and PS mitigates against the lipid content raising effect of FC, which are actually accumulated in the erythrocyte membrane.

Consecutive pursuit of the change in the erythrocyte membrane lipid

TABLE 2. Erythrocyte membrane lipid composition (mg/10^{10} RBC)

Diseases	n	Total	FC	PE	PS	PC	SM
Normal	22	4.47±0.43	1.44±0.13	0.86±0.10	0.53±0.09	0.83±0.09	0.80±0.08
Hepatitis, acute	9	4.40±0.83	1.68±0.28	0.62±0.17	0 43±0.14	1.03±0.29	0.64±0.16
Hepatitis, chronic	29	4.57±0.70	1.56±0.23	0.77±0.13	0.51±0.13	1.00±0.27	0.72±0.15
Hepatic cirrhosis	32	4.89±0.80	1.76±0.33	0.73±0.15	0.56±0.17	1.07±0.26	0.77±0.13
Hepatic cancer	14	4.86±0.71	1.78±0.29	0.74±0.14	0.51±0.15	1.13±0.35	0.69±0.15
Obstructive jaundice	13	6.41±1.21	2.38±0.41	0.76±0.19	0.53±0.21	2.05±0.45	0.70±0.18
Spur cell anemia	2	4.72	1.9 ±1.00	0.64±0.06	0.41±0.18	0.97±0.13	0.80±0.33
Iron deficiency anemia	33	4.21±0.10	1.38±0.17	0.77±0.16	0.52±0.12	0.79±0.13	0.75±0.13
Hereditary spherocytosis	10	4.13±0.78	1.46±0.21	0.67±0.19	0.43±0.08	0.80±0.20	0.77±0.18
Hereditary elliptocytosis(c)	1	3.88	1.32	0.60	0.42	0.89	0.65
Hereditary elliptocytosis(s)	2	3.35	1.15	0.61	0.42	0.59	0.58
Autoimmune hemolytic anemia	2	3.73	1.34	0.66	0.38	0.75	0.59
Paroxysmal nocturnal Hb-uria	2	4.18	1.45	0.62	0.49	0.87	0.76
Acanthrocytosis	2	3.51±0.16	1.29±0.05	0.70±0.08	0.50±0.05	0.69±0.08	0.72±0.11

(c) with increased hemolysis (s) without increased hemolysis

composition with vicissitude of clinical symptoms and signs in patients with acute hepatitis, from onset to recovery (Figure 1), disclosed the following sequence of events : (1) early stage (within second and third week after onset) —— decrease in PE and SM together with increase in FC, (2) middle stage (the third week) —— fall of PE and SM and rise in FC content are seen persistently, but diminution of PE becomes less evident, (3) recovery stage (the sixth week from onset) —— FC is restored to normal range, but SM still remains on the subnormal level, and (4) complete recovery (the tenth week) —— normal lipid composition of erythrocyte membrane is restored.[7]

Deviations from the normal lipid composition of red cell membrane in hepatobiliary diseases show the most prominent aspect in obstructive jaundice. At least, two mechanisms are considered for the causation of these erythrocyte membrane lipid changes.[8,9,10]

1) Reduced activity of LCAT (lecithin-cholesterol acyltransferase : an enzyme which esterifies FC by transfer of acyl radical from PC and decrease in plasma HDL (α-lipoprotein) which comprises LCAT as a component.

FC of erythrocyte membrane is in active exchange with plasma LDL (β-lipoprotein : rich in FC) which surrounds erythrocyte. In the HDL particles suspended in plasma, LCAT regulates the amount of FC through its conversion into esterified cholesterol by LCAT so that exuberance of FC may not move from HDL to LDL. Both LCAT and HDL are the products of the liver and

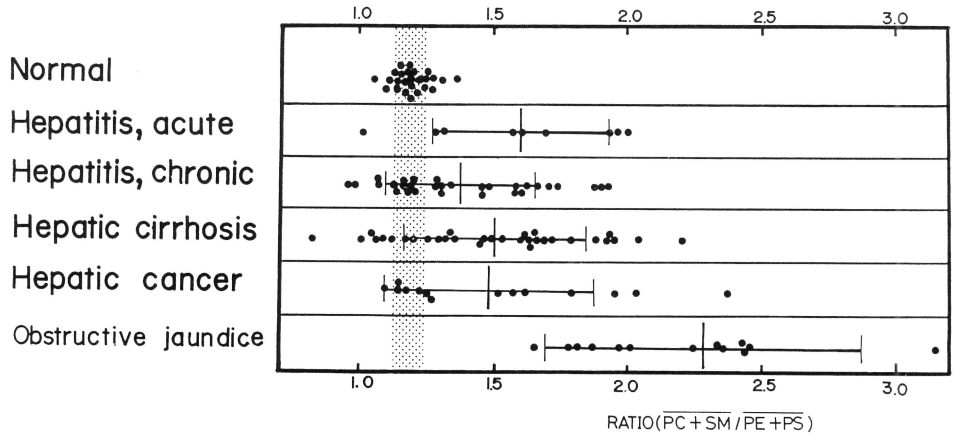

Fig. 1. Phospholipid composition ratio $\overline{PC+SM}$ (outer lamella)/$\overline{PE+PS}$ (inner lamella) of erythrocyte membrane in hepatobiliary diseases

they are supplied to plasma. Therefore, damage to hepatic cells in hepatobiliary disorders bring about decreased supply of HDL and LDL to plasma. If obstruction to biliary tract occurs concomitantly, regurgitation and seepage of bile from the bile canaliculi result in accumulation of bile salts, which inhibit LCAT activity, in blood. Thus, FC is increasd excessively in plasma and passes into the erythrocyte membrane from the plasma lipoprotein.[8,9]

2) LPX[11]

In patients with biliary obstruction, both FC and PC, which are to be excreted from liver cell into bile, accumulate in blood on account of bile reflux, and these combine with a minor ingredient of protein in plasma to make up molecules of a new lipoprotein called LPX. The lipid composition of LPX is FC, 30 percent, and PC, 70 percent. It is worthy of mentioning that no esterified cholesterol is contained in LPX. The molecules of LPX which are abundant in FC and PC come into contact with erythrocytes, and adhere to their outer surface. LPX molecules transfer their own FC and PC to the erythrocytes during their time of adherence, thus raising the contents of FC and PC of erythrocyte membrane.[11,12] Increase in FC and PC in the erythrocyte membrane in biliary obstruction is therefore understood as a result of the combined effect of these metabolic disturbances occurring in this disorder.

The cellular membrane of erythrocyte is formed by a bilayer system of lipids, consisting of an inner lamella abundant in PE and PS, and an outer lamella rich in PC and SM.[1] FC distributes evenly in both lamella. Of these lipids PC bestows increased elasticity and flexibility on the cellular membrane, while FC and SM confer augmented viscosity and rigidity to the membrane, thus rendering the erythrocyte less deformable by mechanical stress and increasingly resistant to hypo-osmolar stress and hemolysis. Investigation on the relation between the resistance of erythrocytes to the rectilinearly increasing

hypo-osmolarity as examined by coil-planet centrifugation technique and the FC content of the erythrocyte membrane as analyzed by Iatroscan technique demonstrated that the hemolysis end point (HEP) shifted to the hypo-osmolar side with increase in FC content of the erythrocyte membrane.[8] When examined by the coil planet centrifugation method the hemolysis band appearing in the coil is dislocated as a whole (both the hemolysis starting point and the hemolysis end point) from the normal range to the hypo-osmolar side (to the right) with transformation of the shape of its hemolysis curve from the normal pattern (L type : hemolysis curve has its peak or maximum point, to the left) to an abnormally skewed pattern (R type : hemolysis maximum point is deviated to the right, that is to the hypo-osmolar side), with a dextro-positioned prominence. This finding is a manifestation of increased resistance of erythrocyte membrane against the hypo-osmolar stress.[13,14]

Increased content of FC in red cell membrane of the patients with hepatobiliary disorders which is seen in Table 1 and 2 is consistent with the findings (increased resistance to hypo-osmolarity) observed by coil planet centrifugation.

Table 3 and Figure 1 are the summary of the results obtained from the examination of the ratio of phospholipid compositions of the lipid bilayer system of erythrocyte membrane, that is $\overline{PC+SM}$ (chief component of outer lamella) per $\overline{PE+PS}$ (chief component of inner lamella). At a glance of this table it is apparent that $\overline{PC+SM}$ per $\overline{PE+PS}$ is obviously elevated in hepatobiliary disorders.*[2] Excess amount of outer lamella lipids over the inner lamella lipids is characteristic of this kind of diseases. So far as this ratio remains around

TABLE 3. Erythrocyte membrane lipid ratios

Diseases	n	$\overline{PC+SM}/\overline{PE+PS}$	FC/PL	SM/PC
Normal	22	1.18	0.48	0.98
Hepatitis, acute	9	1.59	0.62	0.64
Hepatitis, chronic	29	1.37	0.52	0.75
Hepatic cirrhosis	32	1.50	0.56	0.75
Hepatic cancer	14	1.48	0.59	0.65
Obstructive jaundice	13	2.28	0.60	0.35
Spur cell anemia	2	1.69	0.68	0.81
Iron deficiency anemia	33	1.22	0.49	0.96
Hereditary spherocytosis	10	1.42	0.55	0.98
Hereditary elliptocytosis(c)	1	1.52	0.51	0.73
Hereditary elliptocytosis(s)	2	1.13	0.52	0.99
Autoimmune hemolytic anemia	2	1.27	0.56	0.80
Paroxysmal nocturnal Hb-uria	2	1.51	0.54	0.87
Acanthrocytosis	2	1.19	0.49	1.05

(c) with increased hemolysis (s) without increased hemolysis

1.5 with mild (less than 1.3 times of normal) increase in FC (which makes erythrocyte rigid), and the surplus lipids of the outer lamella to inner lamella distribute evenly through the cell surface, erythrocyte morphology is kept normal in shape. However, it is speculated that, if the excessiveness of the outer lamella lipids over the inner lamella lipids is unevenly distributed on various portion of the cellular membrane, thorny projections (spur cell or echinocyte) will arise, corresponding to the loci where FC and other lipids (PC and SM) are accumulated.

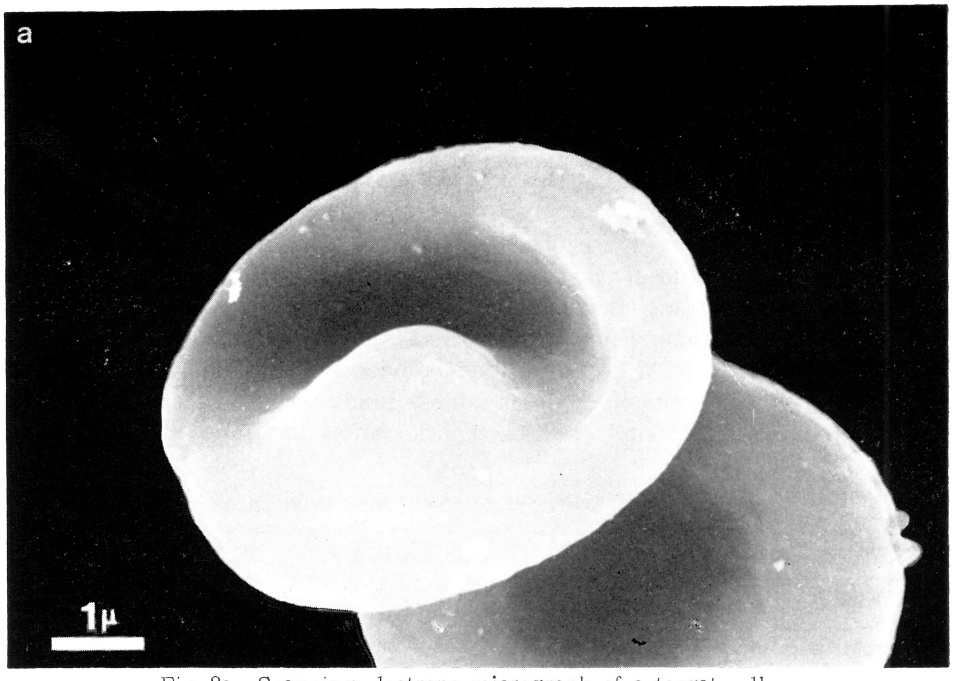

Fig. 2a. Scanning electrone micrograph of a target cell

In case that the ratio of $\overline{PC+SM}/\overline{PE+PS}$ is larger than 2.0, and the lipids spread evenly throughout the membrane, red cells transform morphologically from discoid shape to bowl-like shape, giving rise to target cells which are Wright-stained as a disc just like a ring with densely colored central spot (Figure 2). The FC content of red cell membrane increases two times as high as the normal, in obstructive jaundice. However, simultaneous rise of $\overline{PC+SM}/\overline{PE+PS}$ to the level 2.3 fold as high as the normal cancels the projection-producing effect of increased FC content by the fluidity-maintaining effect of increased PC content.[14] Thus target cells seen in biliary obstruction are not associated with projections. In spur cell anemia, the ratio, $\overline{PC+SM}$ to $\overline{PE+PS}$,

and FC content are not so much increased as in obstructive jaundice, but remain around the levels which are encountered in hepatic cirrhosis and hepatoma. Therefore, it is supposed that uneven distribution of FC on the surface of erythrocyte membrane may be the principal causal factor of spur formation.

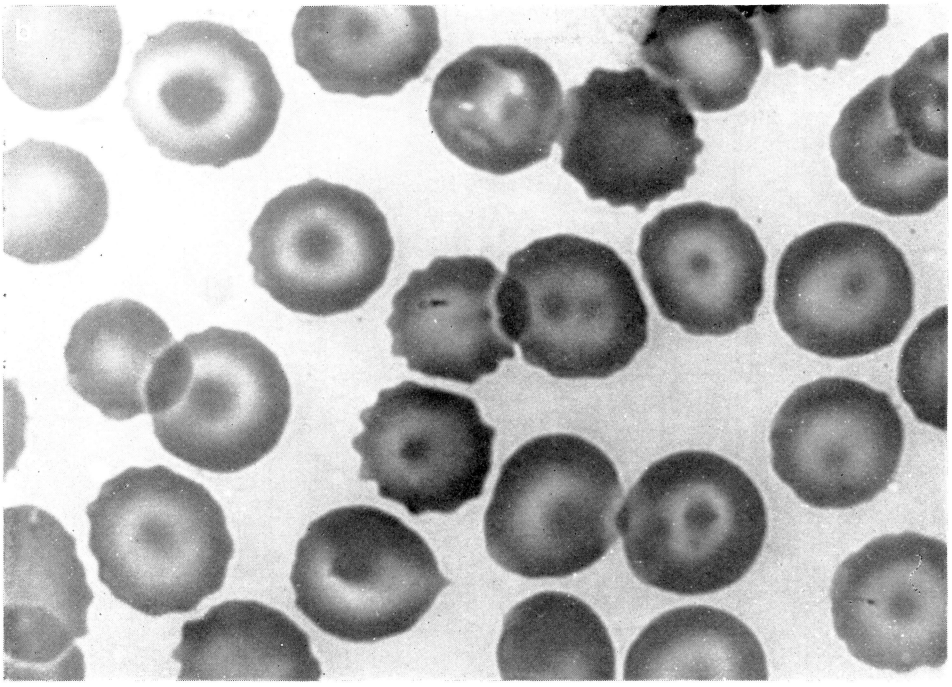

Fig. 2b. Target cells on peripheral blood smear

The lipid composition of the erythrocyte membrane of spur cell anemia is presented in Table 3, which shows that there is marked increase in the FC to PL (PL=PE+PS+PC+SM) ratio to the level 1.4 times as high as the normal, surpassing that of obstructive jaundice (1.25 times of normal). This deviated lipid composition is responsible for the formation of projection of cellular membrane in erythrocytes of spur cell anemia which are rich in cholesterol. Conversely, in biliary obstruction SM which is a rigidity-promoting lipid of the erythrocyte membrane and cooperates with cholesterol in producing projections is decreased to 1/2.8 level of the normal, being unable to sustain the positive effect of cholesterol for producing projections. Absence of spur cells in spite of numerous target cells in the peripheral blood of the patients with biliary obstruction will be understood as a result of decrease in SM.

A close correlation of the appearance and disappearance of target cells with the alterations of red cell membrane lipid composition in obstructive

*2 PE, PS and PC are convertible to each other in the following way : PS⇄PE→PC.

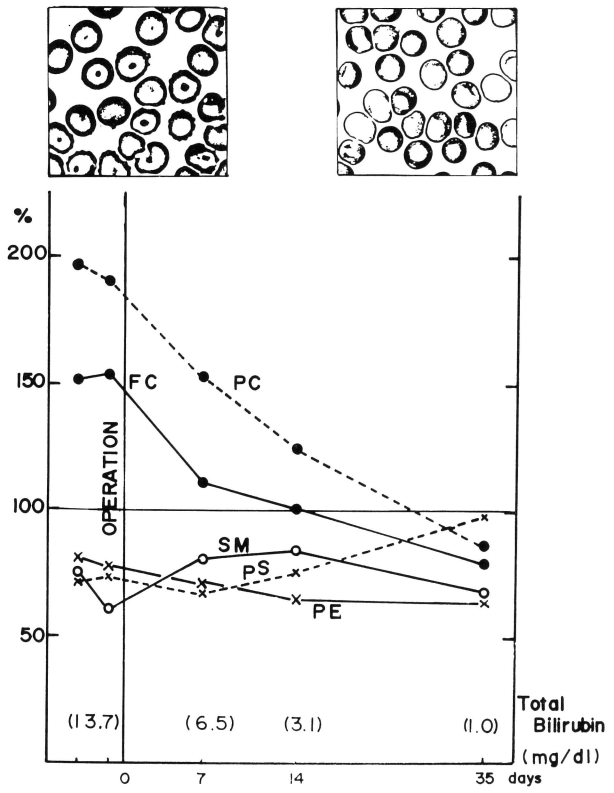

Fig. 3. Change in the lipid composition of erythrocyte membrane as collated with the transformation of erythrocyte morphology (from target cell to discoid cell) in obstructive jaundice

jaundice (cholecystectomy was performed) is clearly seen in Figure 3. A significant increase in FC (which makes lipid bilayer system thicker and increasingly viscous) in conjunction with more marked increase in PC than in FC (which makes outer lipid lamella of erythrocyte membrane more fluid) is apparently seen in this figure. Therefore, it is germane to presume that these increment of FC and PC contributed the transformation of discoid cell to target cell. (It is supposed that decreased content of inner lamella of lipid bilayer which comprises PE and PS, especially the diminution of PS content, may exercise influence on erythrocyte membrane in favor of formation of target cell when accumulation of FC and PC is coexistent.)

B. Hematological diseases

Generally, there is a tendency toward reduction of the total lipid content of red cell membrane in hematological diseases (Table 1). Expression of total lipid content in terms of the amount of lipid per weight of hemoglobin contained

in an erythrocyte, such as mg lipid/g Hb, is occasionally unsuitable for the appraisal of fluctuation of erythrocyte membrane lipid, because in microcytic hypochromic anemia hemoglobin contained in a cell frequently drop unproportionately steeply as compared with the decrease in the number of cells, thus masking the diminution of the total lipid content of the cellular membrane obscure even though it is of significant level. Therefore, total lipid content of cellular membrane per cell, mg lipid/10^{10} RBC, is preferred to mg lipid/gHb when pathological change in the lipids of erythrocyte membrane is discussed about anemias, particularly about microcytic hypochromic anemias. When the lipid content of erythrocyte membrane is viewed in this way (Table 2) with respect to a certain specified number of cells (mg lipid/10^{10} RBC) in hematological diseases, it is unanimously subnormal in all of them. (Spur cell anemia is an exception. This disease is characterized by remarkable increase in FC content of a erythrocyte membrane and it is frequently associated with hepatobiliary disorders and it occurs as a result from abnormal metabolism of lipoprotein and cellular membrane.)

The fall of total lipid content of red cell membrane in hematological diseases mirrors the summation of the unanimous diminution of five lipid constituents of the membrane, particularly of PE and PS of the inner lamella. Decrease in PE is more evident.

The reduction of the surface area of the red cell mambrane will be responsible for the diminution of the total lipids of the cellular membrane in iron deficiency anemia, because the red cells in this disease is characterized by microcytosis. The lipid components are all alike subnormal in content as seen Table 4. However, it is worthy of mentioning that the lipid composition expressed by FC : PE : PS : PC : SM is within the normal range.

Figure 4 illustrates how the rise in the ratio of $\overline{PC+SM}/\overline{PE+PS}$ and the decrease in total lipid content of erythrocyte membrane in a typical case of iron deficiency anemia are restored to the normal level steadily with the increase in hemoglobin concentration which is brought about by adequate iron medication. It is interesting that there is a remarkable diminution of the content of $\overline{PE+PS}$ (the phospholipid of inner lamella of lipid double layer of erythrocyte membrane) in comparison with the $\overline{PC+SM}$ content at the period when hemoglobin concentration is very low (8 g/dl).

Similar tendency toward the reduction of total lipid content of red cell membrane is seen in hereditary spherocytosis. Unlike in iron deficiency anemia the diminution is seizable by appraisal of the lipid content in terms of mg lipid/g Hb as well as in terms of mg lipid/10^{10} RBC. Other diseases, such as hereditary elliptocytosis, autoimmune hemolytic anemia, paroxysmal nocturnal hemoglobinuria and acanthocytosis, also show decrease in total lipid of cellular membrane (mg/g Hb and mg/10^{10} RBC) in spite of their dissimilarity in erythrocyte morphology and manifestation of hemolysis. Scrutiny of Table 4 will make us recognize the absolute as well as relative diminution of the inner lamella lipids ($\overline{PE+PS}$) in these diseases, and this exhibits a contrast to the

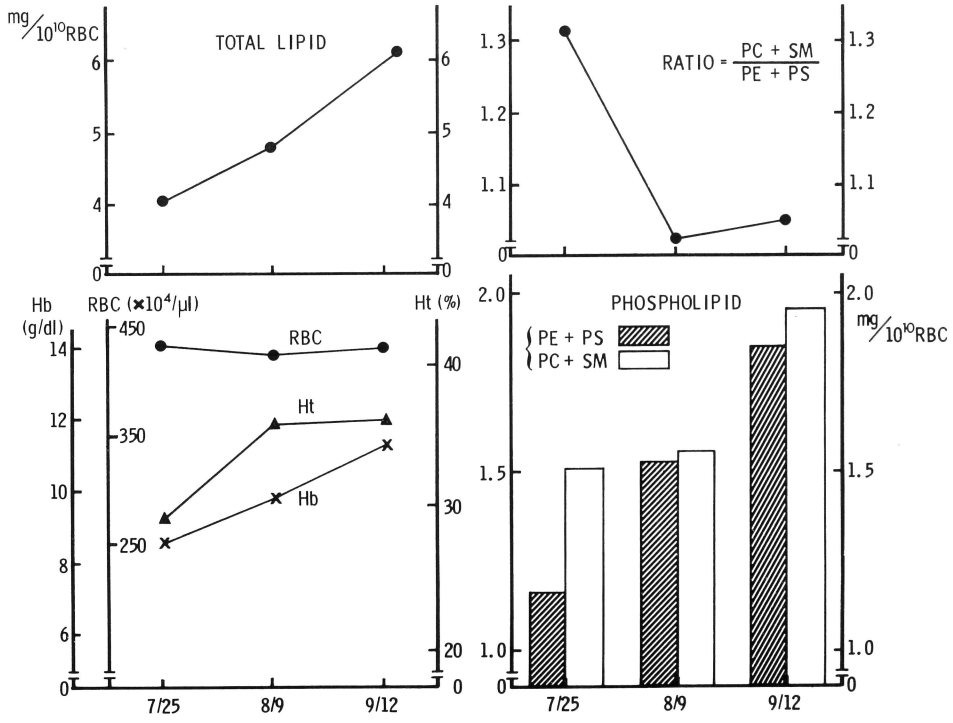

Fig. 4. Restoration of normal lipid composition ($\overline{PC+SM}/\overline{PE+PS}$) of erythrocyte membrane with improvement in hematological tests (Hb, Ht and RBC) in iron deficiency anemia

outer lamella lipids ($\overline{PC+SM}$), which are decreased absolutely, but are apt to increase relatively. The tendency toward relative increase in the outer lamella lipids is especially evident in anemias associated with increased hemolysis and reticulocytosis (autoimmune hemolytic anemia, paroxysmal nocturnal hemoglobinuria, and hereditary elliptocytosis with increased hemolysis). In hereditary spherocytosis there is a significant rise in FC (percent) in the face of almost normal SM (percent) in the membrane lipid composition. However, in acanthocytosis, SM (percent) is elevated while FC (percent) is subnormal. Hereditary spherocytosis and acanthocytosis make contrast with each other in this respect.

The results of our observation which has been presented above, are not significant enough to support the view that the altered composition of membrane lipid bilayer system (decreased lipid content of inner lamella associated with slight increase in the outer lamella lipids which is insufficient to compensate for the loss of the inner lamella lipids) is the foremost cause for the production of spherocytes, elliptocytes or thorny erythrocytes in these hematological diseases. Contrariwise, it is thought that the distorted lipid composition of erythrocyte

TABLE 4. Erythrocyte membrane lipid composition (% ratio)

Diseases	n	Total	FC	PE	PS	PC	SM
Normal	22	100	31.7±3.8	19.90±2.7	12.1±2.2	19.3±2.6	17.0±2.2
Hepatitis, acute	9	100	39.4±3.3	13.0 ±1.7	12.7±3.2	20.6±1.9	14.3±2.5
Hepatitis, chronic	29	100	34.6±4.6	17.0 ±2.3	12.0±3.0	21.2±4.6	15.2±3.4
Hepatic cirrhosis	32	100	36.7±6.8	14.0 ±3.2	12.2±3.9	21.5±3.4	15.2±1.8
Hepatic cancer	14	100	38.0±6.3	14.0 ±2.1	10.5±3.0	24.7±5.7	12.8±2.7
Obstructive jaundice	13	100	38.7±5.7	12.5 ±3.0	7.3±0.7	30.5±1.7	11.0±2.0
Spur cell anemia	2	100	40.4	13.5	8.6	20.5	17.0
Iron deficiency anemia	33	100	33.4±1.8	17.6 ±2.3	12.0±3.0	19.50±1.9	17.5±1.6
Hereditary spherocytosis	10	100	37.2±3.9	16.0 ±2.4	10.6±1.9	18.80±1.7	17.4±1.9
Hereditary elliptocytosis(c)	1	100	33.5	17.1	12.4	18.3	18.7
Hereditary elliptocytosis(s)	2	100	35.2	19.7	12.4	17.0	15.7
Autoimmune hemolytic anemia	2	100	33.5	20.0	10.0	20.1	16.4
Paroxysmal nocturnal Hb-uria	2	100	35.2±4.5	14.5 ±3.3	11.6±0.1	20.7 ±1.7	18.0±0.5
Acanthocytosis	2	100	33.0	17.9	12.8	17.7	18.6

(c) with increased hemolysis (s) without increased hemolysis

membrane bilayer system is the consequence of such kinds of poikilocytosis.

Measurement of osmotic resistance of erythrocytes by means of coil planet centrifugation discloses a slight tendency toward right shift of hemolysis curve, suggestive of decreased hypo-osmolar fragility, in iron deficiency anemia.[16] The result is quite different in other anemias. In autoimmune hemolyic anemia the hemolysis end point (HEP) lies within the normal range, but hemolysis starting point shifts to the hyper-osmolar side, mirroring the presence of a population of erythrocytes which are fragile to the hypo-osmolar stress. Hereditary spherocytosis is characterized by the distinct left shift of both HEP and HSP to the hyper-osmolar side owing to the pathognomonical increase in osmotic fragility.[16] The increased osmotic fragility of erythrocytes in this disease may be explained by the decrease in lipid content of erythrocyte membrane particularly of its inner lamella.

As was mentioned in the section of the analytical results of red cell membrane lipid there is a striking contrast in the pattern of alteration between the hepatobiliary diseases and the hematological disorders.

(A). In hepatobiliary disorders, increased amount of total lipids in the erythrocyte membrane, especially the accumulation of FC which is distributed evenly between the inner and outer layer of the lipid bilayer and the rise in PC are the characteristic findings, which result in the excessive growth of

the outer lamella over the inner lamella as well as the increased resistance of erythrocyte membrane to hypo-osmolarity.

(B). Hematological diseases are distinguished from the hepatobiliary diseases by the decrease in the total amount of lipids in red cell membrane, particularly in PE, which is the main constituent of the inner lamella of lipid bilayer. This brings about the diminution of lipid content of the inner lamella below the outer lamella, and decreased resistance to hypo-osmolarity or increase in osmotic fragility of erythrocyes.

Contemplation on the causal factor of the appearance of these contrary features of the alteration of lipid composition of erythrocyte membrane in hepatobiliary and hematological disorders will lead us to the following speculation.

1° The liver is not a hematopoietic organ which guides the final organization and composition of the lipid double layer of erythrocyte membrane at the initial stage of erythropoiesis. Instead, the liver is the central organ of metabolism, and supplies the blood plasma with lipoproteins, which contain various lipids (especially FC and PC) in varied proportions. These lipoproteins surround and come in contact with the surface of the erythrocytes which are suspended in the blood plasma. Thus an exchange of lipids between the lipoprotein and the cellular membrane of erythrocytes takes place until equilibrium is established between them. As for instance, the altered lipid composition of red cell membrane in hepatobiliary disorders is the end-result of such a lipid exchange between the erythrocytes which are suspended in the blood and the blood plasma, the lipid composition of which has been changed pathologically by the abnormal inflow of lipoproteins from the diseased liver and the obstructed biliary tract while the stream of floating erythrocytes is flowing through the vascular system of this organ. The alterations of lipid composition of red cell membrane in hepatobiliary disorders which is represented by the increase in FC and PC are the final aspect of modification of mature erythrocytes in the pathological milieu of the blood plasma of the patients with hepatobiliary diseases, which is rich in lipoproteins. The pathological lipid composition of the erythrocyte membrane in this case is not related to the abnormal erythropoiesis but to the effect secondary to the distorted metabolism in the hepatobiliary diseases.

2° Erythrocytes are produced in the bone marrow which is the only one hematopoietic organ in adults. The characters of red cells, namely the organization of their cellular membrane and the composition of the lipid bilayer, are determined congenitally by the relevant genes inscribed on the DNAs of the chromosomes of early rubricytic cells in the bone marrow tissue. It seems that abnormally pre-determined characters of red cell membrane are apt to manifest in the inner lamella (decreased content in PE) rather than in the outer lamella, and result in decrease in total lipid content of the whole cellular membrane. Hereditary spherocytosis is one of the examples of such abnormalities. These abnormalities are accordingly classified as the primary effect of hematopoietic organ which has been deranged either congenitally or acquired.

After erythrocytes have been sent into the blood stream from the bone marrow which is their native place, they may be exposed to a milieu (blood plasma) in which substances disastrous to their cellular membrane (immune bodies and complement) are existing. These substances may enhance the diminution of lipids (PE) in the inner lamella of the membrane, leading to their fragility to the external stress. This will be the case of autoimmune hemolytic anemia. In case that hematological diseases are complicated by the association of hepatobiliary disturbances the abnormality of the lipid composition of the red cell membrane may undergo further modification by the secondary effect of diseased hepatobiliary system. Spur cell anemia is thought to be one of the examples of this sort.

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