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Variability in the Lysolecithin Content of Human Erythrocyte Membranes[1)]

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It was confirmed that the lysolecithin contents of human erythrocyte membranes (stroma) can be varied remarkably depending on the plasma lysolecithin concentration *in vitro* and that simultaneously certain changes occur in the morphology of the whole cell as revealed by scanning electron microscopy, and also in the osmotic fragility of the membranes.

Incubation of erythrocytes in lysolecithin-rich plasma, prepared by the action of the plasma lecithin-cholesterol acyltransferase or of added phospholipase A of snake venom, resulted in remarkable increase in the lysolecithin content of the erythrocyte membranes and morphological changes in the erythrocytes from normal biconcave discs to crenated or spherical forms. The spherical cells, but not the crenated cells, showed increased osmotic fragility. After washing these lysolecithin-rich erythrocytes with serum albumin solution or with heated normal plasma, the erythrocyte lysolecithin content was restored almost to the normal level whereas the increased osmotic fragility still persisted.

Similar washes of the normal erythrocytes with albumin solution gave rise to about 43% decrease in the lysolecithin content, slight morphological change and also slight increase in the osmotic fragility. When these cells were incubated in heated normal plasma, the lysolecithin content was restored to the normal level, and at the same time both the slightly-altered shape and osmotic fragility were restored back to normal.

From these experimental findings, probable existence of two kinds of lysolecithin pools in human erythrocyte membranes and their roles in the maintenance of the membrane structure and functions were discussed.

It is an well-known fact that small but definite amount of lysolecithin (lysophosphatidylcholine) is present in normal human erythrocyte membranes.[3,4)] Among the phospholipids present in the erythrocyte membranes, lysolecithin is the one which could be most rapidly incorporated into the membranes from the surrounding medium.[5)] In fact, Klibansky and De Vries[6)] already reported that the lysolecithin content of human erythrocytes could be reversibly changed by incubating them with lysolecithin-rich plasma and by washing them with albumin solution. These authors further demonstrated that almost reversible morphological transformation of the erythrocyte occurred accompanying such an alteration in the lysolecithin content. There is also a report observing a stabilizing effect of low concentrations of lysolecithin in medium upon the erythrocyte membranes.[7)] All these experimental results suggest some important roles of this lysophospholipid in erythrocyte membrane structure.

The present study aims at systematic searches for the correlationships between the lysolecithin content of human erythrocyte membrane on one hand and the morphology as well as the osmotic fragility of the membranes on the other hand, employing the scanning electron microscopy which is powerful weapon of recent development for the clear-cut demonstration of morphological changes in the whole erythrocyte.

1) Part of the present report was presented at the 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April 1972.
2) Location: *Yagoto Urayama 15, Tenpakucho, Showaku, Nagoya.*
3) T. Sato and T. Fujii, *Chem. Pharm. Bull.* (Tokyo), **19**, 377 (1971).
4) P. Ways and D.J. Hanahan, *J. Lipid Res.*, **5**, 318 (1964).
5) T. Sakagami, O. Minari, and T. Orii, *Biochim. Biophys. Acta*, **98**, 356 (1965).
6) C. Klibansky and A. De Vries, *Biochim. Biophys. Acta*, **70**, 176 (1963).
7) P. Seeman, *Biochem. Pharmacol.*, **15**, 1767 (1966).

Experimental

Preparation of Washed Erythrocyte Suspension——Freshly-drawn blood, with heparin (0.05 mg of Na-salt equivalent to about 5 heparin units per ml blood) added as an anticoagulant, was centrifuged at $900 \times g$ for 15 minutes. The supernatant plasma was used for the preparation of lysolecithin-rich plasma. After removal of the buffy layer, the residual erythrocytes were washed three times with 0.15 M NaCl and resuspended in the saline.

Preparation of the Plasma Enriched in Lysolecithin——Human plasma alone was incubated at 37° for 24 hours in order that the naturally-occurring lecithin-cholesterol acyltransferase (LCATase)[8] acts on lecithin of the plasma lipoprotein and thus additional amount of lysolecithin be produced. In another experiment, plasma was incubated at 37° for one hour with purified snake venom phospholipase A added in a ratio of 0.1 ml enzyme solution, containing 58 μg protein, per 3 ml plasma.

Attachment and Detachment of Lysolecithin to and from the Erythrocyte Membranes——Washed erythrocytes were incubated at 37° for 30 minutes with lysolecithin-rich plasma prepared as above, in about 40% hematocrit proportion. An aliquot of the erythrocyte suspension was then centrifuged at $900 \times g$ for 15 minutes and washed twice with saline. The residual portion of the erythrocytes was further incubated at room temperature for one hour with 4.5% human serum albumin solution (Nutritional Biochemicals Corporation, Cleveland) in isotonic Tris-buffered saline (pH 7.4) or with heated normal plasma (with the LCATase inactivated), in approximately 30% hematocrit proportion. The mixture was then centrifuged to separate the supernatant albumin solution or plasma, and the erythrocytes were washed twice with saline.

In order to remove lysolecithin as far as possible from the intact erythrocyte membranes and then to make it rebound to such a treated erythrocyte, the following procedures were employed. Washed erythrocytes were incubated at room temperature for 30 minutes with 4.5% human serum albumin solution as described already and the albumin solution was separated by centrifugation. After this washing technique was repeated further twice, the residual erythrocytes were washed twice with saline. An aliquot of them was further incubated twice with heated normal plasma at room temperature for 30 minutes.

Lipid Extraction and Quantification of Phospholipid——Lipids were extracted from erythrocytes or from the medium (albumin solution or plasma) with chloroform–methanol mixture according to the method of Ways and Hanahan.[4] Quantitative estimation of phospholipid was performed by means of thin-layer chromatography (TLC) using Silica gel H or HR (E. Merck A.G., Darmstadt). Plates were developed in chloroform–methanol–acetic acid–water (60: 30: 8: 4, by volume). After development, phospholipids were visualized by exposure of the plate to iodine vapor. After the phospholipid bands were marked and the iodine vapor disappeared, each band was scraped away and transferred into a centrifuge tube. The extraction of phospholipid from the silica gel was carried out using a mixture of formic acid–methanol–chloroform, according to Burger, Fujii and Hanahan.[9] Phospholipid phosphorus was assayed by Bartlett's method.[10]

Scanning Electron Microscopic Observations——One volume of erythrocyte suspension in 50% hematocrit to be observed was added into 4 volumes of 0.9% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.4, for fixation.[11] After allowing to stand for one hour at room temperature, the mixture was centrifuged and the fixed cells precipitated were then washed three times with distilled water and resuspended in water. Small amount of this suspension was placed on a cover glass and dried at room temperature. After being coated with gold and carbon in a vacuum chamber, the specimen thus prepared were subjected to observations under scanning electron microscope, the JSM-S1 instrument of the Japan Electron Optic Laboratory Co. Ltd., with an accelerating voltage of 10 kV.

Test for Osmotic Fragility of Erythrocytes——To 4 ml of phosphate buffered-saline (pH 7.0) with varying NaCl concentration added 0.2 ml of washed erythrocyte suspension (5% hematocrit) and the mixture was incubated at 37° for 5 minutes. After centrifugation at $2000 \times g$ for 5 minutes, absorbance at 543 nm of the supernatant was determined as a measure for the extent of hemolysis and the percentage of the absorbance of the supernatant from complete hemolysate is expressed as the hemolysis percentage.

Result

Lysolecithin and Lecithin Contents of the Plasma Enriched in Lysolecithin

Results of phospholipid analyses on two kinds of plasma preparations obtained by enzymatic treatments to produce lysolecithin from the plasma lecithin, are cited in Table I.

8) J.A. Glomset, *J. Lipid Res.*, **9**, 155 (1968).
9) S.P. Burger, T. Fujii, and D.J. Hanahan, *Biochemistry*, **7**, 3682 (1968).
10) G.R. Bartlett, *J. Biol. Chem.*, **234**, 466 (1959).
11) *a*) J. Tokunaga, T. Fujita, and A. Hattori, *Arch. Histol. Jap.*, **31**, 21 (1969); *b*) F.M.M. Morel, R.F. Baker, and H. Wayland, *J. Cell Biol.*, **48**, 91 (1971).

TABLE I. Preparation of Lysolecithin-rich Plasma

Treatment	Lysolecithin content		Lecithin content	
	nmole/ml	% of total PL	nmole/ml	% of total PL
Control	190	8.9	1423	66.9
Incubated at 37°, 24 hr[a)]	490 (+300)	24.3	1054 (−369)	52.3
Incubated with phospholipase A, 37°, 60 min.	1434 (+1244)	73.9	116 (−1307)	6.0

PL=phospholipid
a) Subjected to the action of lecithin-cholesterol acyltransferase present in the plasma.
Figures in parenthesis indicate the differences between the experimental and control values

When normal plasma alone was incubated at 37° for 24 hours, the amount of lysolecithin was increased from 190 to 490 nmole/ml plasma by the action of LCATase originally present in the plasma. The increase in the lysolecithin content (300 nmole/ml) was almost balanced by the decrease in the lecithin content (369 nmole/ml). When normal plasma was incubated with added snake venom phospholipase A, more remarkable rise in lysolecithin content of plasma is noted, namely from 190 to 1434 nmole/ml. Lysolecithin in the plasma treated in such an way amounts as high as 74% of the total phospholipid, while lecithin occupied only 6% of it. In this case, too, the increased amount of lysolecithin (1244 nmole/ml) almost corresponds to the decreased amount of lecithin (1307 nmole/ml).

Changes in Lysolecithin Content of Erythrocyte Membranes after *in Vitro* Treatments

Table II shows almost reversible attachment to and detachment from the erythrocyte membranes of lysolecithin, depending on the lysolecithin concentration of the surrounding medium. When normal erythrocytes were incubated with the LCATase-treated plasma obtained as above, the amount in nmole of lysolecithin contained in ml of packed erythrocytes

TABLE II. Attachment and Detachment of Lysolecithin to and from Erythrocyte Membranes

Exp. No.	1st incubation of erythrocyte with	2nd incubation of erythrocyte with	Erythrocyte lysolecithin		Erythrocyte lecithin	
			Content nmole/ml cell	% of total PL	Content nmole/ml cell	% of total PL
Control	—	—	106	3.1	952	28.0
1	lysolecithin-rich plasma (LCATase-treated)	—	199 (+ 93)	6.0	953	28.8
2	lysolecithin-rich plasma (LCATase-treated)	normal plasma	109 (+ 3)	3.4	932	29.0
3	lysolecithin-rich plasma (phospholipase A treated)	—	525 (+419)	15.3	898	26.1
4	lysolecithin-rich plasma (phospholipase A treated)	albumin solution	148 (+ 42)	4.9	900	29.5
5	lysolecithin-rich plasma (phospholipase A treated)	normal plasma	123 (+ 17)	4.0	923	30.0
Control	—	—	105	2.4		
6	albumin solution	—	60 (− 45)	1.7		
7	albumin solution	normal plasma	104 (− 1)	2.5		

PL=phospholipid LCATase=lecithin-cholesterol acyltransferase
Figures in parenthesis indicate the differences between the experimental and control values

increased from 106 to 199, which correspond to 3.1 and 6.0% of the total membrane phospholipid, respectively (Exp. 1). When normal erythrocytes were incubated with the phospholipase A-treated plasma, increase in lysolecithin content was more remarkable (from 106 to 525 nmole/ml packed cells) (Exp. 3). The net increase in this case is therefore 419 as compared with 93 nmole/ml cells in the case of the above-mentioned LCATase treatment. In such erythrocyte with very high lysolecithin content, this lysophospholipid occupies as high as 15.3% of the total membrane phospholipid.

In order to remove the attached lysolecithin, these lysolecithin-rich erythrocytes were treated with 4.5% serum albumin solution or with normal plasma of which LCATase activity had been inactivated by heating at 56° for 30 minutes without causing denaturation of most of the plasma proteins. As is clear from Table II, most of lysolecithin having become attached to erythrocyte membranes was easily removed by either of these washing treatments (2nd incubation), and the lysolecithin content of the erythrocytes after such treatment reached to nearly normal or slightly higher-than-normal level (Exp. 2, 4, and 5). It was shown separately that repeated washes with simple saline did not cause any removal of lysolecithin from erythrocyte membranes.

TLC of the phospholipids in erythrocyte membranes before and after the above-indicated treatments is cited in Fig. 1. It is evident that lysolecithin removed from the lysolecithin-rich cells by albumin washes is recovered in the albumin solution.

Possibility that whether or not intact membrane lysolecithin could be removed by the similar washing procedures as above, was then investigated. Exp. 6 of Table II shows that, of the original 105 nmole of lysolecithin per ml of packed cells, 45 nmole (43%) could be removed by incubation with albumin solution. It was also demonstrated that the corresponding amount was recovered in the chloroform-methanol extract of the washings which contained no other phospholipid as revealed by the TLC.

Such erythrocyte preparation with reduced lysolecithin content was further incubated with heated normal plasma (Exp. 7, Table II). After this second incubation, the lysolecithin content was restored almost to the normal level.

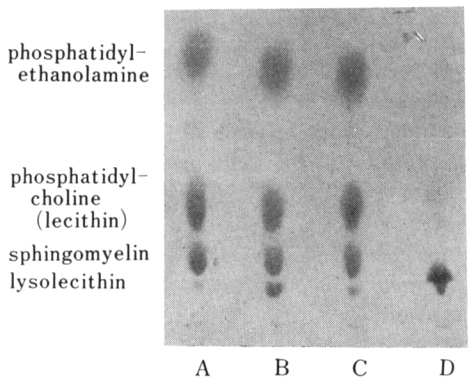


Fig. 1. Reversible Binding of Lysolecithin to Erythrocyte Membrane

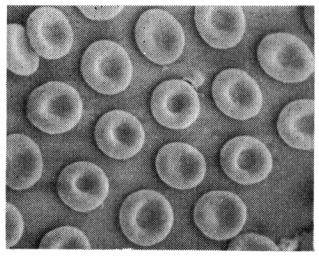
A: control
B: Exp. 3, Table II
C: Exp. 4, Table II
D: albumin solution of Exp. 4, Table II
developing solvent: $CHCl_3$–MeOH–H_2O (65:25:4, by volume)
detection: Dittmer Reagent

Thus, it was disclosed that attachment of an additional amount of lysolecithin to erythrocyte membranes is almost reversible, probably due to weak affinity of this lysophospholipid to the membrane binding sites. It is to be noted that, as is clear from Table II, no significant change in lecithin content of erythrocytes was observed after any of the above-mentioned treatments, indicating that exchange between plasma lysolecithin and erythrocyte membrane lecithin or between membrane lysolecithin and plasma lecithin does not occur in the case of lysolecithin attachment to and detachment from erythrocyte membranes.

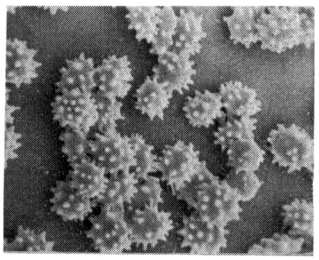
Morphological Changes of Erythrocytes Accompanying the Changes in Lysolecithin Contents of Erythrocyte Membranes

Changes in erythrocyte morphology accompanying the attachment to and removal from the treated erythrocyte membranes are shown in the scanning electron micrographs in Fig. 2.

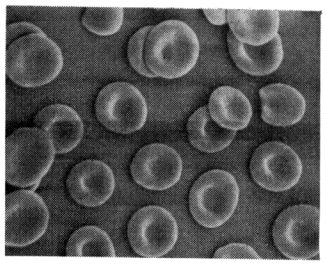
As seen in Fig. 2A, normal erythrocytes, containing 106 nmole lysolecithin per ml of packed cells, appeared as smooth-surfaced, biconcave discs. After incubation with lysolecithin-



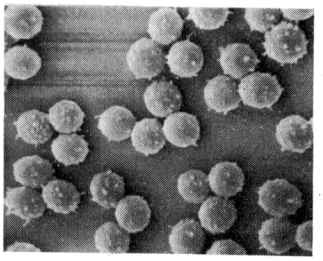
A) control (lysolecithin 106) [a)]



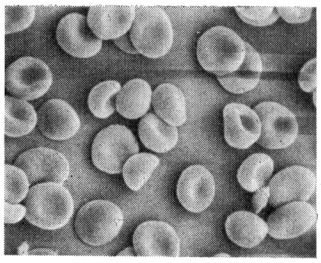
B) Exp. 1, Table II (lysolecithin 199)



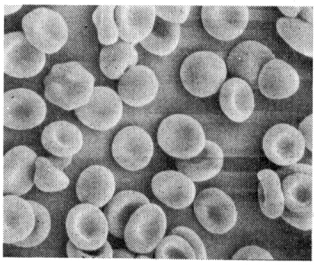
C) Exp. 2, Table II (lysolecithin 109)



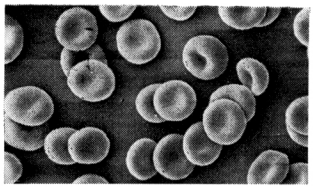
D) Exp. 3, Table II (lysolecithin 525)



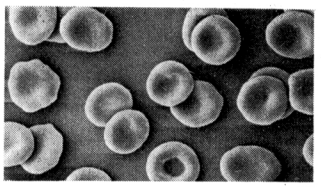
E) Exp. 4, Table II (lysolecithin 148)



F) Exp. 5, Table II (lysolecithin 123)



G) Exp. 6, Table II (lysolecithin 60)



H) Exp. 7, Table II (lysolecithin 104)

Fig. 2. Changes in Erythrocyte Morphology depending on the Membrane Lysolecithin Content as revealed by Scanning Electron Microscopy

a) Figures in parenthesis indicate the lysolecithin content of the erythrocytes in n mol per ml of the packed cells

rich plasma prepared by LCATase action, the erythrocytes now having 199 nmole lysolecithin per ml, are transformed into crenated spheres (Fig. 2B). By washing them with normal plasma, most of these crenated forms were reversed to those with smooth-surfaced discoid forms of which lysolecithin content becomes almost normal as shown already in Exp. 2 of Table II. A few of the erythrocytes, however, do not appear to be returned to their original forms but to be of slightly flattened discs (Fig. 2C).

Fig. 2D shows the cells exposed to lysolecithin-rich plasma produced by the action of snake venom phospholipase A added to normal plasma. The erythrocytes, having 525 n mol

of lysolecithin per ml of packed cells, are all of spherical shape with short projections which suggest that they are in the course of transformation from crenated to spherical forms. When these spherical erythrocytes were washed with albumin solution or with heated normal plasma (Fig. 2E, F), the majority of the cell population seems to be returned to almost normal shape, whereas a small portion of them remained in cup-like swollen shape or flattened discs, probably in accordance with the incomplete removal of erythrocyte membrane lysolecithin additionally attached, as shown in Exp. 4 and 5 of Table II.

When the erythrocytes were depleted of about 43% of lysolecithin originally present by means of incubation with albumin solution (Exp. 6, Table II), they appear to be of slightly irregular shape as revealed by scanning electron micrograph shown in Fig. 2G. Some of these cells appear as thickened and swollen forms. After they regained almost normal content of lysolecithin by incubation with heated normal plasma (Exp. 7, Table II), their shape were restored to almost normal appearance, as shown in Fig. 2H.

Changes in Osmotic Fragility of Erythrocyte Membranes Accompanying the Changes in Lysolecithin Contents

As indicated in Fig. 3, practically no change in the osmotic fragility of erythrocytes was observed with the cells of crenated form containing slightly increased content of lysolecithin, when they were incubated with the plasma with moderately increased lysolecithin concentration (Exp. 1, Table II). On the other hand, definite increase in osmotic fragility was detected on erythrocyte which had been incubated in phospholipase A-treated plasma with remarkably high lysolecithin concentration (Exp. 3, Table II). Thus, these spherical erythrocytes with raised lysolecithin content of the membranes become osmotically fragile but not too much as to cause hemolysis in an isotonic medium.

It is interesting to note that after removal of most of the additionally-bound lysolecithin from erythrocyte membrane by washing them with albumin solution or with normal plasma, the raised osmotic fragility did not tend to be reduced toward normal, in spite of the fact that the erythrocyte morphology almost returned to normal.

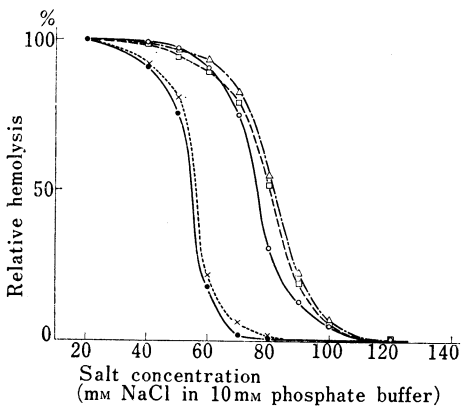


Fig. 3. Changes in Osmotic Fragility of Erythrocytes as the Result of Additional Attachment of Lysolecithin to the Membrane

—●—: control (lysolecithin 106)[a)]
·····×·····: Exp. 1, Table II (lysolecithin 199)
—○—: Exp. 3, Table II (lysolecithin 525)
– –□– –: Exp. 4, Table II (lysolecithin 148)
·–·–△–·–·: Exp. 5, Table II (lysolecithin 123)

a) Figures in parenthesis indicate the lysolecithin content of the erythrocytes in nmole per ml of the packed cells

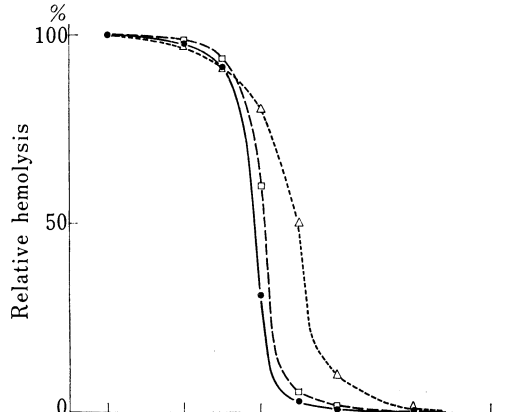


Fig. 4. Changes in Osmotic Fragility of Eythrocytes as the Result of Removal of Lysolecithin from the Intact Erythrocyte Membrane

—●—: control (lysolecithin 105)[a)]
–––△–––: Exp. 6, Table II (lysolecithin 60)
– –□– –: Exp. 7, Table II (lysolecithin 104)

a) Figures in parenthesis indicate the lysolecithin content of the erythrocytes in nmole per ml of the packed cells

When the intact erythrocytes were treated repeatedly with albumin solution and their lysolecithin content was markedly reduced (Exp. 6, Table II), they became slightly more fragile in their osmotic resistance than the intact cells, as shown in Fig. 4. Such increase in osmotic fragility, however, is restored to almost normal level when they regained the normal amount of lysolecithin by the incubation in heated normal plasma (Exp. 7, Table II). Therefore, in this case, the change in the osmotic fragility of the erythrocyte is rather reversible, contrary to the situation observed in the above-mentioned case of the additional attachment and removal of very large amount of lysolecithin to and from the erythrocyte membranes.

Discussion

It is clear from the experimental results obtained in the present study that lysolecithin content of human erythrocytes is dependent on the lysolecithin concentration of the blood plasma. In normal erythrocytes, about 100 nmole of lysolecithin per ml of the packed cells are present, though it is localized only in the membrane part of the cells, while in normal plasma about 200 nmole of lysolecithin are contained per ml. As far as this concentration gradient is maintained *in vivo*, the erythrocyte lysolecithin content seems to remain almost constant. When we incubated normal erythrocytes *in vitro* in a lysolecithin-enriched plasma preparation, containing about 500 or 1400 nmole lysolecithin per ml, we obtained the resulting erythrocyte lysolecithin content of about 200 or 530 nmole per ml of packed cells, respectively. It means that the amount of lysolecithin contained per unit volume of the erythrocytes is equivalent to roughly 40% of the amount per the same volume of the plasma. The lysolecithin taken up from the plasma is, of course, found in the membranes. Such incorporation of lysolecithin from plasma into erythrocyte membrane appears to be due to simple diffusion process, in view of the fact that the incorporation is a very rapid process[5] and also that no exchanged removal of the other membrane phospholipid occurs, as demonstrated in the present investigation.

It was confirmed by the present study that the lysolecithin additionally bound to erythrocyte membranes could be removed almost completely by washing the erythrocyte with serum albumin solution or with normal heated plasma, although washing them with saline alone failed to remove any significant amount of this lysophospholipid. Furthermore, it was revealed by an experiment with intact erythrocytes that a part of lysolecithin originally present in the normal erythrocyte membranes also can be removed by the similar wash procedures with albumin solution. Therefore, this portion (about 40%) of the membrane lysolecithin seems to be bound to the membrane structure considerably more loosely than the rest of this lysophospholipid, and it is also true with the lysolecithin molecules which are additionally attached to the membranes by *in vitro* incubation with lysolecithin-rich medium. These fact suggest the presence of two kinds of lysolecithin pools in the erythrocyte membranes, as pointed out already by Wittels.[12] The functional differences between these two kinds of lysolecithin pools, as the essential membrane components, remain to be solved by the future studies.

Our experimental results reported above clearly indicate the dependence of the erythrocyte membrane morphology on the lysolecithin content of the membranes. Namely, when relatively small amount of lysolecithin is incorporated from the medium into the erythrocyte membranes, conversion in the shape from normal biconcave disc to crenated sphere takes place. These crenated erythrocytes, however, are further deformed to be of almost smooth sphere with very short projections (indentations) after further increase in the membrane lysolecithin content experimentally induced *in vitro*. Repeated washes of the erythrocyte with albumin solution or with heated normal plasma resulted in restoration of the normal biconcave disc shape with most of the erythrocyte population undergone the above-mentioned mor-

12) B. Wittels, *Biochim. Biophys. Acta*, **210**, 74 (1970).

phological changes, though a small part of them still have somewhat abnormal shapes (cup-like or flattened shape). Such an almost reversible morphological changes in the erythrocyte membranes remind us the similar changes already observed by Nakao, *et al.*,[13,14] which appear as the result of a change in adenosine triphosphate (ATP) level inside the erythrocyte. According to these authors, the erythrocytes depleted of the intracellular ATP by long-term storage at 4° or by inhibition of the glycolytic activity in the presence of fluoride, became to be crenated or smooth spheres. But after incubation of these erythrocytes with adenine, inosine, phosphate and glucose, they regained the original biconcave disc shape with regeneration of intracellular ATP. It is interesting to know that very similar morphological changes occur almost reversibly as a result of a change in the content of a membrane lysophospholipid component and also as a result of a change in ATP concentration in the intracellular fluid. The mechanism underlying these phenomena deserves further investigations.

When the lysolecithin content of the intact erythrocyte was reduced by incubating them with albumin solution, they appear as slightly modified shape. They, however, regained the almost normal morphology after regaining the normal lysolecithin content by incubation in heated normal plasma.

As representing the possible changes occurring in the membrane structure of the erythrocytes with increased lysolecithin content, marked increase in the osmotic fragility was demonstrated with the spherical cells. On the crenated cells, however, no apparent change in the osmotic fragility was recognized. Thus, it is presumed that this kind of change in membrane properties is preceded by the morphological changes. Furthermore, it is worth to note that such a change in osmotic fragility is never be reversed by the removal of attached lysolecithin, in contrast with the morphological changes which can almost reversibly corrected. Therefore, it may be true that the very large amount of lysolecithin additionally bound to erythrocyte membrane, in addition to the natural amount of this phospholipid in the intact membrane, brought about irreversible damage in a part of the membrane structure which is responsible for the maintenance of the normal osmotic resistance, although the damage is not so profound or widespread as to prevent the cells now taking the form of spherocyte to transform back to the normal shape. It is worth to note in this respect that a similar increase in the osmotic fragility, observed in the erythrocyte with lower-than-normal lysolecithin content, was reversed to an appreciable extent by the re-addition of the lost amount of lysolecithin to the membrane by incubation of the cells with heated normal plasma.

Thus, it now becomes apparent that lysolecithin in erythrocyte membrane may play a critical role in the determination of the osmotic resistance of the membrane as well as of the shape of the whole cell. Human erythrocyte is reported to contain normally about 2—5% of lysolecithin per total phospholipid.[3,15] With the lysolecithin content near normal range, human erythrocyte maintains its normal shape (biconcave disc) and normal membrane properties. With the content of lysolecithin slightly higher or lower than the normal range (for example, 6.0 or 1.7% of the total phospholipid), the erythrocyte changes its shape alone or both shape and osmotic resistance, reversibly. With remarkably increased lysolecithin content above certain limit (for example, 15%), however, the cells undergo irreversible alteration in its osmotic resistance, probably due to big distortion in the membrane structure brought about by the surface-active action of this lysophospholipid, in addition to advanced degree of morphological alteration (to form spherocyte) which can still be reversibly restored.

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