BBA 3876

QUANTITATIVE STUDY OF ERYTHROCYTE–LYSOLECITHIN INTERACTION

CHAJA KLIBANSKY AND ANDRE DE VRIES

Rogoff Medical Research Institute, Department of Experimental Biology of the Tel Aviv University and the Labor Sickfund, Beilinson Hospital, Petah Tikva (Israel)

(Received July 4th, 1962)

SUMMARY

Quantitative studies were carried out on the attachment of lysolecithin to erythrocytes suspended in plasma enriched in lysolecithin by the action of snake-venom phospholipase A. The amount of lysolecithin attached to the erythrocyte was found dependent on its concentration in the medium. A correlation was established between the amount of lysolecithin attached to the erythrocyte and the erythrocyte shape. The amount of lysolecithin becoming attached to the erythrocyte in blood treated with phospholipase A is sufficient to induce sphering but too small to cause hemolysis. The attachment of lysolecithin to the erythrocyte and the corresponding change in the shape of the cell can be reversed by albumin. The attachment of lysolecithin to the erythrocyte does not cause quantitative changes in the phospholipids of the erythrocyte membrane, but in some cases brings about a loss of cholesterol.

INTRODUCTION

In previous studies it has been shown that the phospholipase A of *Vipera palestinae* venom, when added to human blood *in vitro*, causes non-hemolytic erythrocyte sphering[1] and, when injected into rabbits, causes erythrocyte sphering *in vivo* associated in some cases with shortening of the red cell life span[2]. This action *in vitro* and *in vivo* of the phospholipase A could be attributed to the formation of lysolecithin from plasma lecithin and subsequent attachment of the lysolecithin to the erythrocytes[3]. Whereas several studies have been carried out on the change in erythrocyte shape and the hemolysis produced by lysolecithin[4–7], no direct quantitation of the lysolecithin having become attached to the erythrocytes has been reported thus far. In the present study data will be presented on the correlation between the amount of lysolecithin attached to the erythrocyte and the ensuing change in its shape, and on the reversibility of lysolecithin attachment and erythrocyte shape change by albumin.

MATERIALS AND METHODS

Blood collection

Blood was drawn from normal human subjects by venipuncture using as anticoagulant potassium ammonium oxalate mixture, and centrifuged at room temperature at 800 × *g* for 3 min. The plasma was separated and used for the preparation of lysolecithin. The erythrocytes were kept at room temperature for about 30 min until use.

Preparation of lysolecithin-rich plasma

Plasma was treated by incubation with purified *Vipera palestinae* venom phospholipase A for 30 min at 37° with occasional agitation, using for each millilitre plasma 0.1 ml enzyme solution containing 20 μg protein according to the biuret reaction. *Vipera palestinae* venom was fractionated on a DEAE-cellulose column[8]. The phospholipase A containing fraction was heated at pH 5.5 in a boiling waterbath for 5 min and the precipitate discarded. The supernatant which contained the phospholipase migrated on paper electrophoresis as a single protein band.

Erythrocyte sphering

Lysolecithin-rich plasma was added to the packed erythrocytes in original hematocrit proportion and the suspension was kept at room temperature for various intervals of time. In addition to phasemicroscopic observation of the cell shape, rouleaux formation was examined in a 2 % polyvinylpyrolidone solution in buffered saline.

Estimation of percentage hemolysis

The amount of hemoglobin of the erythrocytes added to the lysolecithin-rich plasma was estimated by the acid hematin method. The hemoglobin liberated into the plasma from the sphered erythrocytes and that liberated by subsequent washings of the erythrocytes was determined by the benzidine reaction[9]. The percentage hemolysis was calculated after subtraction of the original plasma hemoglobin content from the total amount of hemoglobin found in the plasma and washings.

Extraction of lipids from the erythrocytes

The sphering action of the lysolecithin-rich plasma was arrested by diluting it seven-fold with saline (0.9 %); the sample was centrifuged for 3 min at 800 $\times$ g and the supernatant was drawn off. The erythrocytes were washed 4 times with saline and packed to about $8 \cdot 10^6$/mm^3, as determined with a Spencer counting chamber. Four duplicate counts were performed, two by each of two workers, each count in half a chamber, and the average taken. The last washing was evaporated to dryness *in vacuo* and checked for lysolecithin by the same procedure as used for the erythrocytes.

A 2-ml sample of the erythrocyte suspension was extracted with ethanol–ether according to ROWE[10] with the following modification: the final extractions were performed with absolute alcohol and then 96 % alcohol (equal volumes), since large amounts of lysolecithin are not satisfactorily extracted by absolute alcohol alone. The extract was then brought to a final volume of 2 ml in a measuring flask.

Red blood cell cholesterol

Cholesterol was determined in the alcoholic extract according to RAPPAPORT AND EICHHORN[11].

Chromatographic separation and quantitative estimation of erythrocyte phospholipids

Chromatography was carried out on silicic acid impregnated paper according to REED *et al.*[12] using as solvent mixture diisobutylketone–*n*-dibutyl ether–acetic acid–water (20:20:20:3, v/v). The chromatogram was dried and stained with Rhodamine

G. 6 For photography Ponceau-red stain was applied according to HOOGHWINKEL AND VAN NIEKERK[13]. The spots were cut out and ashed with concentrated sulfuric acid — 70 % perchloric acid (3:2, v/v). The solutions obtained were diluted with water to 10 ml and the silicic acid precipitate removed by centrifugation. Phosphorus in the clear supernatants was determined according to BERENBLUM AND CHAIN[14]. The values were corrected for blank value of P in the paper. The recovery of total phosphorus from the various phospholipid spots ranged from 95 to 97 % of the phosphorus in the amount of extract applied. For lysolecithin determination the spots were cut out from two or more parallel strips of the same chromatographic run and combined in order to obtain a measurable colour intensity in the phosphorus reaction. The results obtained were expressed in $\mu g \times 10^{-9}$ phosphorus per cell, using the erythrocyte count performed before the lipid extraction.

Reversion of the lysolecithin induced spherocytosis

Reversion to the bidiscoid shape[15] was carried out using normal human serum albumin (Cutter Laboratories, Berkeley, Calif. (U.S.A.)), diluted to a 4.5 % solution in saline. Prior to addition of the albumin the spherocytes were washed 4 times with 15 ml of saline each time and then resuspended to about 7 000 000 cells/mm^3 following which an exact count was performed. 2 ml of this suspension was added to 5 ml of the albumin solution. This proportion was chosen to ensure efficient reversion. After 15 min of contact at room temperature, a sample was taken for micro-hematocrit measurement (International Micro-capillary centrifuge Model MB, International Equipment Company Boston, Mass.), generally giving a hematocrit of approx. 20 %. The mixture was centrifuged at 800 $\times$ g for 3 min and the packed cells were washed once with a large volume of saline and extracted, as described above, for lysolecithin determination. The volume of the albumin supernatant was calculated from the hematocrit value. An aliquot of the albumin supernatant was taken for lysolecithin determination after recentrifugation at 800 $\times$ g for 3 min. The aliquot was extracted with chloroform–methanol according to MARINETTI *et al.*[16], reextracted with absolute alcohol and subsequently with 96% alcohol (equal volumes) similarly to the procedure for the erythrocytes. Silicic acid paper chromatography was then carried out as described above and the lysolecithin phosphorus estimated quantitatively from the spot.

The ability of other blood proteins to reverse lysolecithin-induced erythrocyte sphering was also studied, using a 4.5 % saline solution of human fibrinogen (Fraction I), a 4.5 % saline solution of human γ-globulin (both from the Institute for plasma fractionation Jaffa) and human hemoglobin prepared by gradual osmotic hemolysis according to the procedure of DANON *et al.*[17] followed by Seitz filtration in order to remove the ghosts, concentration by evaporation to 4.5 %, and subsequent dialysis against isotonic NaCl solution.

RESULTS

Attachment of lysolecithin to erythrocytes associated with sphering

The erythrocyte sphering (and inability to form rouleaux) (Fig. 1b) caused by contact with lysolecithin-rich plasma is associated with attachment of lysolecithin to the cell. This is shown in the chromatogram of the erythrocyte lipids (Fig. 2) and by the erythrocyte lysolecithin values given in Table I. In these experiments unwashed

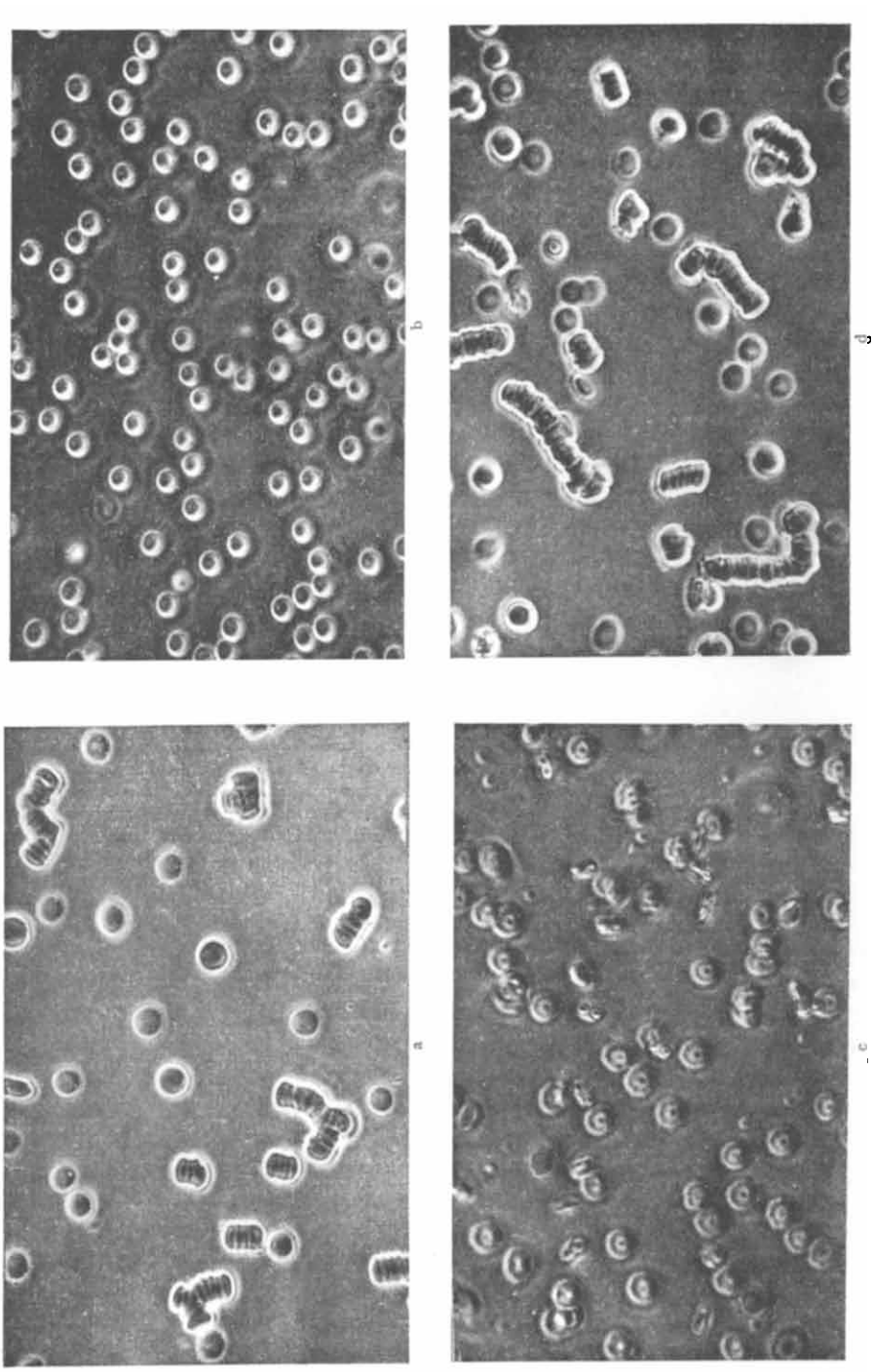

Fig. 1. *Erythrocyte cell sphering induced by lysolecithin-rich plasma and reversion to bidiscoid by albumin. All media contained 2% polyvinylpyrolidone.* 1 a, normal bidiscoids in saline, rouleaux formation; 1 b, spheres after contact with lysolecithin-rich plasma; 1 c, partial reversion by 4.5% albumin solution – deformed cells, cup forms; 1 d, complete reversion to bidiscoid by resuspension in 4.5% albumin solution, rouleaux formation.

packed erythrocytes had been added in the original hematocrit proportion to lysolecithin-rich(phospholipase A-treated) plasma, and left in contact during 15–60 min at room temperature. Lysolecithin analysis of the sphered erythrocytes was carried out after 4 saline washings, as described in the chapter METHODS. Evidence that saline

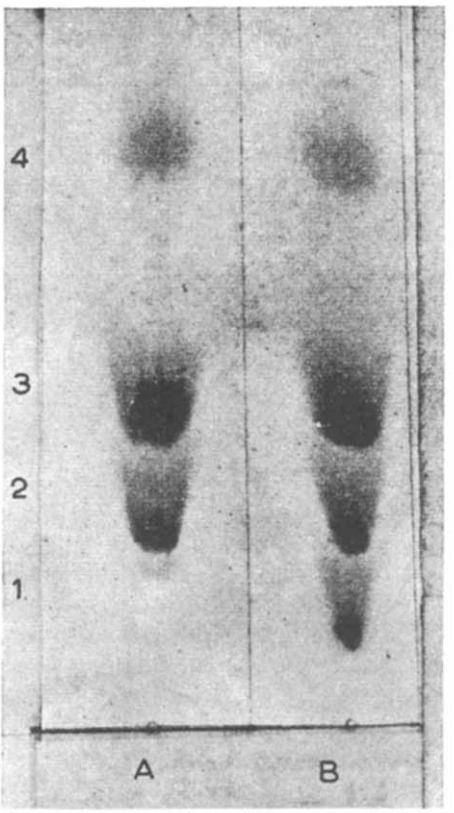

Fig. 2. Chromatogram of phospholipids from erythrocytes before and after contact with lysolecithin-rich plasma. Chromatograms were stained with Ponceau-red. A, Normal bidiscoid cells; B, spherocytic cells after contact with lysolecithin-rich plasma. Identification of components: spot 1, lysolecithin; spot 2, sphingomyelin; spot 3, lecithin; spot 4, phosphatidyl ethanol amine (inositol phosphatide and phosphatidyl serine are not stained by Ponceau-red).

washings do not remove the attached lysolecithin from the red blood cell surface will be presented further on. Under these conditions the average amount of lysolecithin having become attached to the red cell in the various experiments carried out with blood from different subjects, ranged from 0.78 to 1.35 $\mu g \times 10^{-9}$ P per cell. As can be seen in Table I, the amount of lysolecithin found in the erythrocyte lipid extract already after 1-min contact of the erythrocytes with the lysolecithin-rich plasma, did not increase with more prolonged periods of contact. Thus, the attachment of lysolecithin to the erythrocytes was practically instantaneous. The lysolecithin-induced erythrocyte sphering was not associated with changes in the other erythrocyte phospholipids, as demonstrated by a representative experiment in Table II. The small differences found in the various erythrocyte phospholipid components after the red cells had become sphered were probably due to technical inadequacy inherent in the separation of the adjacent spots on the chromatogram. The lysolecithin spot, on the other hand, is usually far behind the other spots, so that the lysolecithin separation is much more satisfactory. Moreover, for lysolecithin determination, only chromatograms with satisfactory separation of the lysolecithin spot were used.

The attachment of lysolecithin to the erythrocyte associated with the sphering was in some experiments accompanied by a loss of cholesterol from the cell, as seen in Table I. Loss of cholesterol, however, was not a constant phenomenon and in some experiments, even after the erythrocytes had been in contact with lysolecithin-rich plasma for a period of 1 h, their cholesterol content had not changed.

Hemolysis was negligible in all these experiments. During the contact of the erythrocytes with the lysolecithin-rich plasma, hemoglobin was released into the plasma to amounts not exceeding 0.16 and 0.5 % of the total erythrocyte hemoglobin after 1 and 60 min of contact, respectively. The repeated washings liberated from the erythrocytes not more than 0.5 % of the hemoglobin, the hemolysis occurring during the complete procedure therefore not exceeding 1 %. The loss of erythrocyte cholesterol occurring in some experiments was not correlated with variations in the degree of hemolysis.

Detachment of lysolecithin from sphered erythrocytes by albumin

Suspension of lysolecithin-induced spherocytes in 4.5 % albumin solution resulted in the loss of the spheric shape. The cells did not regain, however, the original bidiscoid form but were deformed, often cup shaped and were not able to form rouleaux (Fig. 1c). It may be seen in the chromatogram (Fig. 3) and in Table III, (Expts. 1 and 2) that suspension of the lysolecithin-induced spherocytes in 4.5 % albumin solution caused the bulk of the attached lysolecithin to move from the erythrocytes into the albumin solution. Corresponding to the impression gained from

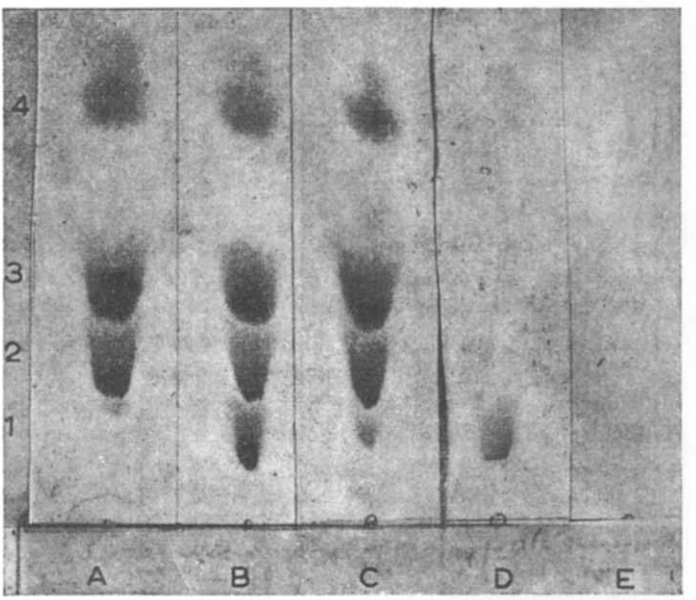

Fig. 3. Chromatogram of phospholipids from erythrocytes before and after contact with lysolecithin-rich plasma and after subsequent contact with albumin solution. Chromatograms were stained with Ponceau-red. A, normal bidiscoid cells; B, spherocytic cells after contact with lysolecithin-rich plasma; C, deformed cells and cups after subsequent contact with 4.5 % albumin solution and one saline washing; D, lysolecithin in albumin solution; E, control albumin solution. Identification of components as in Fig. 2.

TABLE I

ATTACHMENT OF LYSOLECITHIN TO ERYTHROCYTES SPHERED BY CONTACT WITH LYSOLECITHIN-RICH PLASMA

Expt. No.	Time of contact with lysolecithin-rich plasma (min)	Per cell: Before contact (bidiscoid) — Lysolecithin P (μg × 10⁻⁹)	Per cell: Before contact (bidiscoid) — Cholesterol (μg 10⁻⁷)	Per cell: After contact (spheric) — Lysolecithin P (μg 10⁻⁹)	Per cell: After contact (spheric) — Cholesterol (μg 10⁻⁷)
1	15	0.02	1.27	1.24	0.88
2	15	0.16	1.26	1.48	1.16
3	15	0.38	1.32	1.64	1.01
4	15	0.15	1.20	1.50	1.02
5	1	0	1.03	1.22	1.04
	30			1.11	1.02
6	1	0.087	0.99	1.40	1.06
	30			1.31	0.94
7	1	0	1.60	0.87	1.72
	30			0.78	1.62
	60			0.89	1.70

TABLE II

PHOSPHOLIPIDS OF ERYTHROCYTES SPHERED BY CONTACT WITH LYSOLECITHIN-RICH PLASMA

	Phosphorus per cell (μg 10⁻⁹) — Before contact (bidiscoid)	Phosphorus per cell (μg 10⁻⁹) — After contact (spheric)
Lysolecithin	0.014	1.24
Inositol phosphatide	0.46	0.52
Sphingomyelin	1.80	2.15
Lecithin	2.65	2.48
Phosphatidyl serine	0.80	1.05
Phosphatidyl ethanolamine	1.67	1.86

TABLE III

DETACHMENT OF LYSOLECITHIN FROM SPHERED ERYTHROCYTES BY ALBUMIN (4.5%)

Expt. No.	Per cell: Before contact with lysolecithin — Shape	Per cell: Before contact with lysolecithin — Lysolecithin P (μg 10⁻⁹)	Per cell: After contact* with lysolecithin — Shape	Per cell: After contact* with lysolecithin — Lysolecithin P (μg 10⁻⁹)	Per cell: After contact* with albumin — Shape	Per cell: After contact* with albumin — Lysolecithin P (μg 10⁻⁹)	Per cell: Lysolecithin found in albumin solution** — Lysolecithin P (μg 10⁻⁹)	Per cell: After subsequent saline washings — Shape	Per cell: After subsequent saline washings — Lysolecithin P (μg × 10⁻⁹)	Per cell: After subsequent contact with albumin* — Shape	Per cell: After subsequent contact with albumin* — Lysolecithin P (μg 10⁻⁹)
1	Bidiscoid	0.02	Sphere	1.24	Deformed	0.33	0.90				
2	Bidiscoid	0.16	Sphere	1.48	Deformed	0.37	1.02				
3	Bidiscoid	0.15	Sphere	1.50	Deformed	0.51		Deformed	0.57	Bidiscoid	0.18

* Contact: 15 min at room temperature.
** Expressed as amount detached from one cell: total amount in albumin solution divided by number of cells.

the chromatogram, the data in Table III show that, following a single contact with the albumin, the lysolecithin content of the erythrocyte lipid extract did not completely return to the value of the original bidiscoids. It seems probable that the incomplete reversion of the erythrocyte shape to normal bidiscoid is correlated with the small amount of lysolecithin left attached to the cell. Indeed, resuspension of the albumin-treated cells after two saline washings in a new albumin solution, caused all cells to regain the normal bidiscoid shape with ability to rouleaux formation (Fig. 1d) and, at the same time, to lose the still attached lysolecithin (Fig. 4 and Table III). It should be noted that two saline washings performed following the first contact with albumin were not able to remove this small amount of lysolecithin from the erythrocyte. It may be inferred from these results that the amounts of lysolecithin found on the erythrocytes given in Table I, although determined after saline washings, represent the actual amounts of lysolecithin present on the cells when being suspended in the lysolecithin-rich plasma.

The state in which the lysolecithin is present in the albumin solution after detachment from the sphered erythrocytes, is not known. The lysolecithin did not dialyse from the albumin–lysolecithin solution through a cellophane membrane (Dialyzer Tubing Cellulose, Arthur H. Thomas Company, Philadelphia (U.S.A.)) against buffered saline at pH 7.2 and 8.6, as demonstrated by unchanged lysolecithin content in the dialysis bag. Paper–electrophoresis of the albumin–lysolecithin solution carried out on Whatman paper No. 1 with Michaelis buffer of ionic strength 0.1 at pH 7.2 and 8.6, and stained for protein with naphthalene black and for lipoprotein with Oil red O,

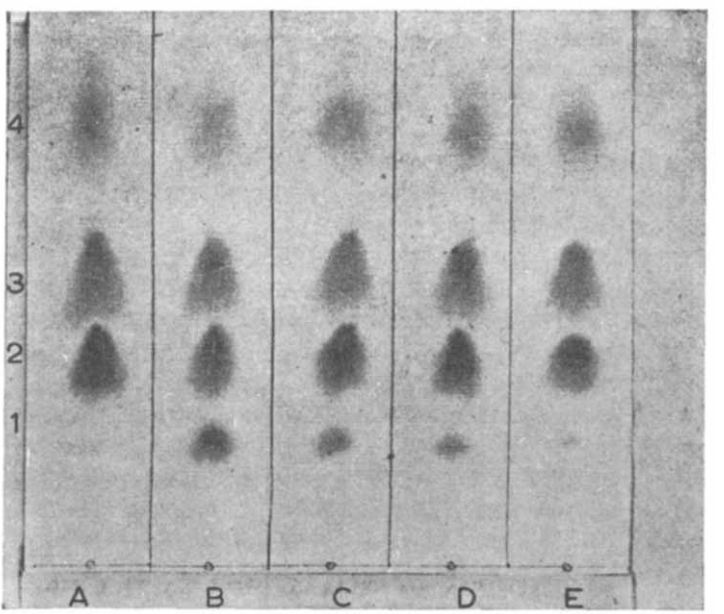

Fig. 4. Chromatogram of phospholipids from erythrocytes after contact with lysolecithin-rich plasma and subsequent repeated albumin treatment. Chromatograms were stained with Ponceau-red. A, Normal bidiscoid cells; B, spherocytic cells after contact with lysolecithin-rich plasma; C, deformed cells and cups after subsequent contact with 4.5% albumin solution and one saline washing; D, bidiscoids and cups after subsequent saline washing; E, bidiscoids after renewed suspension in 4.5% albumin solution. Identification of spots as in Fig. 2.

respectively, did not reveal lipid at the location of the albumin. Neither was lysolecithin revealed at the albumin spot when the electrophoresis was carried out after seven-fold concentration of the albumin–lysolecithin solution under a fan at room temperature.

Human fibrinogen, γ-globulin and hemoglobin were not able to abolish the spheric form of the erythrocytes induced by contact with lysolecithin-rich plasma, and quantitative determination of erythrocyte lysolecithin following contact of the sphered cell with hemoglobin did not show any decrease.

Dependence of amount of lysolecithin attached to the erythrocyte on the lysolecithin concentration in the medium

The effect of lysolecithin concentration in the medium on the attachment of lysolecithin to the erythrocyte was investigated. Dilution of phospholipase-treated plasma (lysolecithin-rich) with untreated plasma would entail further production of lysolecithin by phospholipase from the additional lecithin. Dilution of phospholipase-treated plasma with saline would have changed in addition to lysolecithin concentration also the protein concentration. In view of the ability of albumin to remove lysolecithin from the erythrocyte surface, we chose conditions of constant albumin concentration by diluting phospholipase-treated plasma with 4.5 % human albumin.

The dependence of the quantity of lysolecithin becoming attached to erythrocytes on the lysolecithin concentration in lysolecithin-rich plasma–albumin mixtures is shown in Fig. 5 and Table IV. In this experiment the concentration of the erythrocytes in the medium was kept constant at original hematocrit value. It can be seen that with

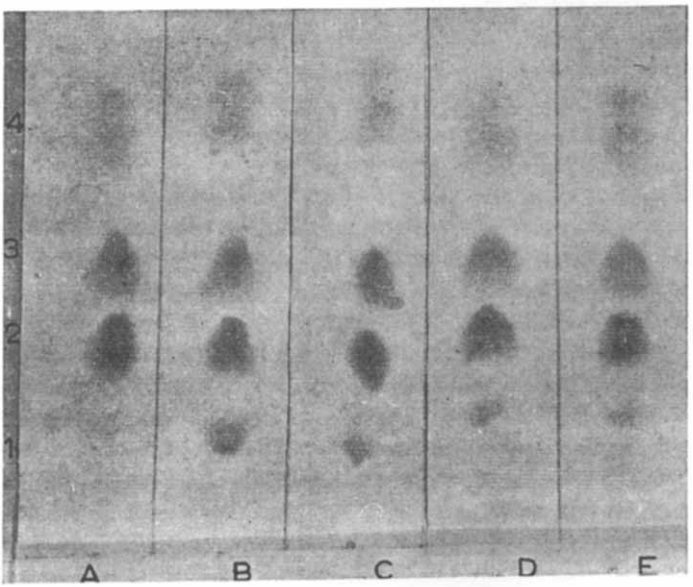

Fig. 5. Dependence of amount of lysolecithin becoming attached to the erythrocytes on the concentration of lysolecithin in the medium (mixture of phospholipase-treated plasma and human albumin). Chromatograms were stained with Ponceau-red. A, normal bidiscoid cells; B, C, D and E, decreasing quantities of lysolecithin attached to the erythrocytes suspended in media of decreasing lysolecithin concentration, corresponding to the values in Table IV. Identification of spots as in Fig. 2.

TABLE IV

DEPENDENCE OF AMOUNT OF LYSOLECITHIN ATTACHED TO ERYTHROCYTES ON THE CONCENTRATION OF LYSOLECITHIN IN MIXTURES OF LYSOLECITHIN-RICH PLASMA AND 4.5% ALBUMIN

Medium			*Lysolecithin attached per cell*		*Erythrocyte shape*
Plasma – albumin parts (v/v)		*Lysolecithin P (mg %)*	*P (μg 10^{-9})*	*Corresponding to chromatogram in Fig. 5*	
1	0	4.7	1.05	B	Spheres
1	2	1.57	0.57	C	Mostly spheres, few crenated spheres
1	5	0.78	0.40	D	Mostly crenated spheres
1	9	0.47	0.32	E	Crenated bidiscoids

decreasing lysolecithin concentration in the medium the quantity of lysolecithin attached to the cells decreased and correspondingly the erythrocyte shape changed from spheric to crenated bidiscoid.

DISCUSSION

The quantitative study of lysolecithin attachment to the erythrocyte showed that the change in the erythrocyte shape depends on the amount of lysolecithin attached. Complete sphering occurred when the amount of lysolecithin attached was higher than about $2 \cdot 10^{-11}$ μmoles/cell. The erythrocyte gained the aspect of a crenated sphere at about $1.3 \cdot 10^{-11}$ μmoles of lysolecithin attached, and of a crenated bidiscoid at about 10^{-11} μmole. At none of these different erythrocyte shapes were the cells able to form rouleaux. At the amount of $0.6 \cdot 10^{-11}$ μmole, the cell maintained its normal bidiscoid shape, as observed by ordinary light- and phase-microscopy, and the ability of the erythrocytes to form rouleaux was preserved.

According to GORTER AND HERMANS[18] the minimal amount of lysolecithin required to produce complete hemolysis, is just sufficient to form a monolayer on the erythrocyte surface. Assuming an erythrocyte surface of 163 μ^2 (see ref. 4) and a surface area per molecule of lysolecithin of 108 Å^2 (see ref. 19) complete lysis then would require adsorption of $2 \cdot 10^{-13}$ μmoles lysolecithin/cell. Accordingly, complete sphering in our experiments occurred when only about one tenth of the surface would be covered.

The present results show that attachment to the erythrocyte of lysolecithin in an amount twice that required for sphering, does not produce hemolysis. These results explain why the sphered erythrocytes in normal human blood treated with venom phosphatidase do not lyse[1] since the amount of lysolecithin adsorbed per cell under these conditions does not exceed $2.6 \cdot 10^{-11}$ μmoles. When adding erythrocytes to venom-treated plasma in 40% hematocrit proportion, it was found that of the 1 μmole lysolecithin present in 1 ml of the system, only 0.2 μmole ($5 \cdot 10^9 \cdot 1.2 \cdot 10^{-9}$ μg) *i.e.* only 20% of the lysolecithin became attached to the erythrocytes.

The nature of the binding of lysolecithin to the erythrocytes surface is not known. GORTER AND HERMANS[18], on the basis of experiments confirmed by COLLIER[20] which showed that a fixed amount of lysolecithin hemolyzes a fixed number of cells, suggested

that lysolecithin adsorbed to the erythrocyte is not released following hemolysis. This indicates a firm binding of the lysolecithin to the erythrocyte surface, consistent with our observation that attached lysolecithin is not removed from the erythrocyte by saline washing. On the other hand, the studies of COLLIER[20] on rabbit erythrocytes, treated with "lysolipid" obtained by the action of rattle snake venom on egg yolk, suggest an equilibrium between the lysin dissolved in the medium and that adsorbed on the cells. That the attachment of lysolecithin to the erythrocytes is indeed reversible, is evident from the observed reversal of lysolecithin-induced sphering by albumin, associated with complete removal of the attached lysolecithin from the cell. Moreover, by using a system containing constant erythrocyte and albumin concentrations, the amount of lysolecithin attached to the erythrocyte was shown to be dependent on the lysolecithin concentration in the medium.

COLLIER[21] (cited[20]) proposed that the action of lysolecithin on the erythrocyte is due to its forming a complex with cholesterol in the membrane. COLLIER AND CHEN[21] pointed out that the amount of lysolecithin required for complete lysis of rabbit erythrocytes is roughly equivalent, on a molar basis, to the cholesterol content of the cell. Our observations show that, if indeed, the attached lysolecithin is bound to the cholesterol of the cell membrane, this reaction is reversible, since, after removal of the attached lysolecithin from the sphered cell by albumin, the normal cholesterol content of the cell is maintained in many experiments.

Another possibility is an interaction between the adsorbed lysolecithin and the lecithin of the erythrocyte membrane. SAUNDERS[22] and THOMAS AND SAUNDERS[23] investigated the interaction between lysolecithin and lecithin resulting in the formation of viscous sols. Their results indicated that the shape of the lysolecithin–lecithin micelle depends on the relative concentrations of these two components in the complex. According to ROBINSON AND SAUNDERS[24] lysolecithin in watery solution is present in a symmetrical micellar shape, whereas, according to ROBINSON[25], lecithin disperses in water to form large disc-like micelles. Increasing the lecithin proportion in the lysolecithin–lecithin complex would favour the disc-like model[25], whereas increasing the lysolecithin proportion would further the spherical shape[23].

SAUNDERS[22] proposed the theory that in the cell membrane lecithin is stabilized by lysolecithin at a concentration which is not lytic. It is interesting to speculate whether the disc–sphere transformation of the erythrocyte induced by lysolecithin is due to interaction between the latter and the lecithin of the membrane, thus decreasing the lecithin proportion in the lysolecithin–lecithin complex.

Finally, the possibility must be considered that the lysolecithin interacts with non-lipid constituents of the erythrocyte membrane. Hemoglobin, probably, is not involved in the lysolecithin binding, in view of the inability of an osmotic hemolysate to reverse lysolecithin-induced disc–sphere transformation and to remove attached lysolecithin from the erythrocyte surface.

ACKNOWLEDGEMENTS

This work was supported by a research grant (E-3171) from the National Institute of Health, Bethesda, Md. (U.S.A.).

The authors are indebted to Mr. Z. OSIMI and Mr. J. SHILOACH for technical assistance and to Mrs. K. NORTON for the photography.

REFERENCES

1 Y. AVI-DOR, E. CONDREA AND A. DE VRIES, *Rev. franc. Études Clin. et Biol.*, 5 (1960) 819.
2 A. DE VRIES, CH. KIRSCHMANN, CH. KLIBANSKY, E. CONDREA AND S. GITTER, *Toxicon*, 1 (1962) 19.
3 CH. KLIBANSKY, E. CONDREA AND A. DE VRIES, *Am. J. Physiol.*, 203 (1962) 114.
4 E. PONDER, *Hemolysis and Related Phenomena*, Grune and Straton, New York, 1948.
5 S. GITTER, S. KOCHWA, D. DANON AND A. DE VRIES, *Arch. intern. Pharmacodynamie*, 118 (1959) 350.
6 K. W. WILBUR AND H. B. COLLIER, *J. Cellular Comp. Physiol.*, 22 (1943) 233.
7 M. E. GREIG AND A. J. GIBBONS, *Arch. Biochem. Biophys.*, 61 (1956) 335.
8 S. KOCHWA, CH. PERLMUTTER, S. GITTER, J. RECHNIC AND A. DE VRIES, *Am. J. Trop. Med. Hyg.*, 9 (1960) 374.
9 C. CREDITOR, *J. Lab. Clin. Med.*, 41 (1953) 307.
10 E. C. ROWE, *Biochem. J.*, 73 (1959) 438.
11 F. RAPPOPORT AND F. EICHHORN, *Clin. Chim. Acta*, 5 (1960) 161.
12 C. F. REED, S. N. SWISHER, G. V. MARINETTI AND E. G. EDEN, *J. Lab. Clin. Med.*, 56 (1960) 281.
13 G. J. M. HOOGHWINKEL AND H. P. G. A. VAN NIEKERK, *Koninkl. Ned. Akad. Wetenschap. Proc. Ser. B*, 63 (1960) 258.
14 I. BERENBLUM AND E. CHAIN, *Biochem. J.*, 32 (1938) 295.
15 A. DE VRIES, E. CONDREA AND S. GITTER, *Proceedings of the Staff Meetings of the Beilinson Hospital, Israel No. 10*, 1961.
16 G. V. MARINETTI, M. ALBRECHT, T. FORD AND E. STOTZ, *Biochim. Biophys. Acta*, 36 (1959) 4.
17 D. DANON, A. NEVO AND Y. MARIKOWSKY, *Bull. Research Council Israel Sect. E*, 6 (1956) 36.
18 E. GORTER AND J. J. HERMANS, *Rev. trav. chim.*, 62 (1943) 681.
19 A. HUGHES, *Biochem. J.*, 29 (1935) 430; N. ROBINSON, *J. Pharm. Pharmacol.*, 13 (1961) 321.
20 H. B. COLLIER, *J. Gen. Physiol.*, 35 (1952) 617.
21 H. B. COLLIER AND H. L. CHEN, *Can. J. Research E*, 28 (1950) 289.
22 L. SAUNDERS, *J. Pharm. Pharmacol.*, 9 (1957) 834.
23 I. L. THOMAS AND L. SAUNDERS, *J. Pharm. Pharmacol.*, Suppl., 10 (1958) 182 T.
24 N. ROBINSON AND L. SAUNDERS, *J. Pharm. Pharmacol.*, Suppl., 11 (1959) 115 T.
25 N. ROBINSON, *Trans. Faraday Soc.*, 56 (1960) 1260.