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Survival of rabbit and horse erythrocytes in vivo after changing the fatty acyl composition of their phosphatidylcholine

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The phospholipid composition and the distribution of phospholipids over the two leaflets of the membrane have been investigated for rabbit and horse erythrocyte membranes. Phosphatidylcholine (PC) comprises 39.4% and 41.3% of the total phospholipid complement of the rabbit and horse erythrocytes, respectively. In both membranes the distribution of this phospholipid is asymmetric: 70% of the PC is present in the outer layer of the rabbit membrane and 60% in that of the horse. The major species of this phospholipid class are the (1-palmitoyl-2-oleoyl)- and the (1-palmitoyl-2-linoleoyl)PC. The disaturated species, (1,2-dipalmitoyl)PC, is present in limited amounts only. Partial replacement of the native PC from intact erythrocytes was accomplished with a purified PC specific transfer protein from bovine liver. Replacement of the native PC species with (1-palmitoyl-2-oleoyl)PC up to 40% of the total PC complement had no effect on the osmotic fragility, the shape and the in vivo survival time of both erythrocyte species. Replacement of the native PC in both rabbit and horse erythrocytes with (1,2-dipalmitoyl)PC up to 20% gave rise to an increased osmotic fragility, a shape change from discocytic to echinocytic and a significant reduction in survival time measured after reinjection of the modified cells. At 30% replacement with (1,2-dipalmitoyl)PC the resulting spheroechinocytes appeared to be cleared from the circulation within 24 h after reinjection. The conclusion can be drawn that the repair mechanisms which may exist in vivo are insufficient to cope with the drastic changes in properties of the erythrocyte membrane which are induced by replacing more than 15% of the native PC by the dipalmitoyl species.

Introduction

Erythrocyte deformability is crucial for normal erythrocytic function. During its lifespan, the cell has to pass repeatedly passages in the microcirculation of which the internal diameter is considerably smaller than the diameter of the discoid cell in its resting state. It is widely accepted that impaired red cell deformability can have a deleterious effect on its survival in vivo [1]. Particularly in the spleen, where the cells have to pass through very small openings, 'non-functional' erythrocytes will be removed by the mononuclear-phagocytic system [2]. Erythrocyte deformability has been shown to be dependent on the viscosity of the intracellular content, geometry of the cell and viscoelastic properties of the membrane [3]. It was demonstrated previously, that retailoring of

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the native PC in the human and rat erythrocyte can have a dramatic effect on membrane integrity [4–6]. Replacement of native PC in part by (1,2-dipalmitoyl)PC leads to an echinocytic shape and a reduced deformability [7], whereas after replacement by (1-palmitoyl-2-oleoyl)PC no such abnormalities are observed. The question did arise, whether cells with a reduced deformability as a result of PC replacement would be removed faster from the circulation upon re-entering the bloodstream, or that repair processes are sufficiently active to restore the abnormalities. Little is known about these repair processes that occur in vivo to prevent destruction of altered, injured cells. Several reports show that cells, altered in vitro, undergo changes in vivo, permitting most or all of them to survive normally [8]. Mature erythrocytes have no or very limited synthetic capacity of macromolecules, such as phospholipids. Therefore, the exchange equilibrium that exists between the lipids in the cell membrane and those present in the serum, may be of great importance in this respect as it provides the possibility of lipid renewal in altered, injured cells. Erythrocytes from various mammals may greatly differ in size, shape and lipid composition. Both the shape and lipid composition of horse and rabbit erythrocytes, however, are comparable to those of human red cells, although their sizes are somewhat smaller [9,10]. This paper reports some general characteristics of the lipid bilayer of the red cell membrane of horse and rabbit as well as the effects that partial replacement of their native PC species by either (1,2-dipalmitoyl)PC or (1-palmitoyl-2-oleoyl)PC has on the survival time of these cells in vivo.

Materials and Methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, egg phosphatidate and cholesterol were purchased from Sigma (St. Louis, MO, U.S.A.). 1-Palmitoyl-2-[^{14}C]oleoyl-*sn*-glycero-3-phosphocholine, 1-palmitoyl-2-[^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine, sodium [^{51}Cr]chromate in saline, [^{111}In]indium oxine in saline and [^{3}H]glycerol trioleate were obtained from Amersham International (Cardiff, U.K.).

All other chemicals and solvents used were of PA grade.

Erythrocytes. Venous blood was collected from anaesthetized New Zealand white rabbits in heparinized tubes. The cells were pelleted by centrifugation for 5 min at 2500 × *g* and washed three times with a 10-fold excess of Hanks' buffer, pH 7.4 (refered to as buffer A throughout). The buffy coat was carefully removed by aspiration after each wash. Erythrocytes from horse (*Equus caballus*) were isolated in a similar way; the buffer, however, contained in this case 150 mM NaCl, 25 mM glucose, 1 mM EDTA, 10 mM Tris-HCl (pH 7.4), and 100 IU/ml penicillin and 100 μg/ml streptomycin (buffer B).

Preparation of vesicles. Vesicles were prepared from a lipid mixture containing either (1,2-dipalmitoyl)PC or (1-palmitoyl-2-oleoyl)PC mixed with an equimolar amount of cholesterol, 60 mol% of egg phosphatidate and trace amounts of [^{14}C]phosphatidylcholine identical to the PC species used for the bulk of the vesicles. A trace amount of [^{3}H]glycerol trioleate was also present, serving as non-exchangeable marker. The lipid mixture was dried from a chloroform/methanol solution (2 : 1, v/v) and vesicles were prepared as described before [4] in buffer A or buffer B when used to retailoring the native PC in rabbit or horse erythrocytes, respectively.

Preparation of transfer protein. The phosphatidylcholine specific transfer protein was purified from bovine liver according to Westerman et al. [11] and was stored at −20°C in 50% glycerol. Before use, glycerol was removed by overnight dialysis against buffer at 4°C. The volume of the resulting protein solution was reduced by concentration against flake poly(ethylene glycol) (Aquacide III, Calbiochem, San Diego, CA, U.S.A.) until a protein concentration of 150 μg/ml was reached.

Replacement of native PC. A 30–40% (v/v) suspension of cells was incubated at 37°C in a thermostated room, using a clinical blood rotator at 4 rpm in the presence of donor PC vesicles and 2 to 3 μM transfer protein as described before [4]. The ratio of vesicle PC to erythrocyte PC varied from 2 to 4. The buffer used was buffer A for the incubation of rabbit erythrocytes and buffer B for the incubation of horse erythrocytes. Aliquots were taken at timed intervals and added to 5 ml of buffer at 37°C. Cells were isolated by centrifu-

gation at 2500 × *g* and the residual vesicles and transfer protein were removed by two additional washes with an excess of buffer. Lipids were extracted according to the procedure of Rose and Oklander [12]. The extent of PC replacement was calculated from the specific radioactivity of the phosphatidylcholine fraction as described before [4]. These data were corrected for contamination of the erythrocytes with the lipid vesicles by measuring the amount of [^{3}H]glycerol trioleate present in the extracted lipids.

Osmotic fragility. The osmotic fragility was determined by measuring the change in turbidity of an erythrocyte suspension at 690 nm as function of salt concentration as described previously [4].

Cell shape. Samples of 10 μl of packed cells were fixed for 30 min at 20°C in 1 ml buffer containing 100 mM NaCl, 40 mM sodium citrate and 0.5% formaldehyde. Morphology was inspected by phase contrast light microscopy.

Phospholipase degradations. Cells were suspended in a buffer containing 0.9% NaCl, 10 mM $CaCl_2$, 0.25 mM $MgCl_2$ and 10 mM Tris-HCl (pH 7.5). Incubations with phospholipase A_2 from *Naja naja* and sphingomyelinase C from *Staphylococcus aureus* were carried out as described previously [13]. Following the incubations, cells were extracted [12] and phospholipids were analyzed by TLC [14] and phosphate determination [15].

Molecular species composition of PC. Diacylglycerols of PC were prepared as described previously [5] and converted to their trimethyl chlorosilane derivatives according to Myher and Kuksis [16]. The various molecular species were separated on a 3% Silar 5 CP column (Applied Science Laboratories Inc., State College, PA, U.S.A.) in a Packard gas chromatograph model 805 (Packard Instrument Co. Inc., Downers Grover, IL, U.S.A.), equipped with a dual glass U tube (180 × 0.3 cm) at 270°C.

Cholesterol/phospholipid ratio. Cholesterol and phospholipids in the lipid extract of cells were separated by TLC in silica gel (Merck 60 DC-Fertig Platten) using a solvent system of diethyl ether/hexane (85 : 15, by vol.). Cholesterol was measured according to the method of Zlatkis et al. [17] and phosphate according to Rouser [15].

^{51}Cr and ^{111}In labeling of erythrocytes. Erythrocytes were labeled with ^{51}Cr using standard procedures [18] by incubating 30 to 40% (v/v) cell suspensions in buffer at 37°C for 30 min in the presence of 1 to 2 μCi of Na $^{51}CrO_4$ per ml of packed cells. At the end of the incubation, an excess of ascorbic acid was added and the cells were pelleted by centrifugation for 5 min at 2500 × *g*, washed once with buffer and resuspended in buffer up to a 30 to 40% cell suspension. labeling of cells with ^{111}In was performed in a similar manner, omitting the ascorbic acid.

Lifetime determination. 3 ml of cell suspension were injected into a marginal ear-vein of each recipient rabbit and 10 to 20 ml into the vena jugularis of the horse. After 1 h and then at timed intervals of approx. 1 day, 2.5 ml blood samples were taken. The haemoglobin content was measured by determination of the absorbance at 408 nm and radioactivity was determined using a double-channel Philips PW 4800 Gamma counter. ^{51}Cr and ^{111}In were counted in a window from 280 to 360 keV and from 360 to 480 keV, respectively. When ^{51}Cr and ^{111}In were present in the same sample, radioactivity was determined in both channels. The cross-over of ^{51}Cr in the ^{111}In window was negligible and there was approx. 26.5% cross-over of ^{111}In in the ^{51}Cr window. The amounts of ^{51}Cr and ^{111}In present in the sample were calculated using a double-label programme in which corrections for spillover were made.

The measured radioactivity was corrected for physical decay and expressed relatively to the amount of haemoglobin in the sample.

Results

Phospholipid composition

The phospholipid complement of rabbit and horse erythrocyte membranes has been analyzed in detail. The overall phospholipid composition of the two membranes is shown in Table I. The results are in fair agreement with data published before [9,19]. PC and PE are the major constituents; sphingomyelin comprises about 15% of the total phospholipids and phosphatidylserine + phosphatidylinositol contents were found to be 8 and 15% of the total in rabbit and horse erythrocyte membranes, respectively. The cholesterol to phospholipid ratio is identical in both species (0.8

TABLE I

PHOSPHOLIPID COMPOSITION OF THE ERYTHROCYTE MEMBRANE

Data on phospholipid composition represent means ± S.D. ($n = 4$). Sph, sphingomyelin.

	Rabbit		Horse	
	Composition (%)	Degradation (%)	Composition (%)	Degradation (%)
PC	39.4 ± 0.5	70	41.3 ± 3.5	60
PE	33.6 ± 0.9	18	27.7 ± 3.4	36
Sph	16.6 ± 0.5	95	14.4 ± 1.1	100
PS + PI	8.2 ± 1.0	–	15.5 ± 1.1	–
Cholesterol / Phospholipid	0.8 ± 0.1		0.8 ± 0.1	

± 0.1 mol/mol). The PC present in both species has been characterized with respect to its fatty acid composition (Table II). The predominant species of this phospholipid class contain palmitate in combination with oleate or linoleate. The amount of disaturated PC species is low and comparable to the amount found in human erythrocytes [4].

Phospholipid localization

Using standard localization techniques [30] the distribution of the phospholipids over the two membrane layers has been determined. Phospholipase A_2 treatment, sphingomyelinase treatment as well as the incubation of red cells with a combination of the two enzymes revealed that 70% of the PC, 18% of the PE and 95% of the sphingomyelin are accessible for hydrolysis in intact rabbit erythrocytes. Similar values are found for horse erythrocytes: 60% of the PC, 36% of the PE and all of the sphingomyelin can be hydrolyzed by extracellular phospholipases (Table I). In both cell types phosphatidylserine is not accessible for the enzymatic attack. The accessibility of PC for phospholipases has been compared with that for a PC-specific transfer protein. The exchange profiles show that the PC is present in both cell types in two pools and that the amounts of exchangeable PC are identical to the hydrolyzable pools. On the basis of these experiments, a distribution of the major phospholipids over the two membrane layers could be calculated. Like in all erythrocytes investigated so far [30], the membranes are clearly asymmetric with respect to their phospholipid complement. The choline containing phospholipids are preferentially located in the outer membrane leaflet whereas the amino phospholipids prefer the interior layer.

TABLE II

MAJOR SPECIES OF PHOSPHATIDYLCHOLINE IN ERYTHROCYTE MEMBRANES

The fatty acids are denoted by their carbon atom number followed by the number of double bonds. The data are presented as percentages of the total amount of these major PC species and are the average of two (horse) and three (rabbit) determinations, respectively.

PC species	Rabbit	Horse
16:0, 16:0	12	14
16:0, 18:1	26	26
18:0, 18:1	7	2
16:0, 18:2	34	29
18:0, 18:2 / 16:0, 20:4	18	25
18:1, 18:2	3	4

Phospholipid modifications

Using the property of the PC-transfer protein from beef liver to stimulate a specific and one-for-one exchange of PC molecules between different membranes, we were able to replace the native PC molecules of the intact erythrocytes by well defined synthetic PC molecules. Before discussing the results of such replacement studies, it has to be emphasized that, in accordance with previous studies [5,6] control analyses showed that the PC replacement reaction did not result in any modification of the amounts of other membrane constituents. After the replacement the overall phospholipid composition, the phospholipid to cholesterol ratio, as well as the phospholipid to protein ratio, remained identical to the starting values; only the fatty acid composition of part of the PC was altered. In the present study we used 1-palmitoyl-2-oleoylphosphatidylcholine and 1,2-dipalmitoylphosphatidylcholine and investigated the effects that the replacement with these species had on osmotic fragility of the cells, their shape, and in particular their survival in vivo. Replacement with the (1-palmitoyl-2-oleoyl)PC proceeds

rapidly and about 40% of the native PC has been replaced in the present experiments by this species. As was found previously for human red cells as well, the replacement with (1,2-dipalmitoyl)PC levels off at considerably lower values, which has to be ascribed to a preference of the exchange protein for unsaturated species above the disaturated ones [29]. In order to increase the replacement levels, red cells have to be incubated repeatedly with fresh donor vesicle preparations [4]. Replacing extensive amounts of native PC (up to 40%) by (1-palmitoyl-2-oleoyl)PC in both red cell systems did not result in any change in osmotic fragility of the cells. This was to be expected since this mono-unsaturated PC species is a major native constituent of the PC complement of the two membranes and previous studies on human cells, which have a comparable PC composition, showed that extensive replacement of the PC in the outer leaflet could be achieved without consequences for erythrocyte fragility. On the other hand, an increase in the (1,2-dipalmitoyl)PC content resulted in a decrease in the resistance against osmotic stress (Fig. 1). A shift of the osmotic fragility curves towards higher tonicity values is observed upon an increase in the degree of saturation of the PC in the membrane of horse erythrocytes. Similar observations have been made with the red cells from rabbit. Concomitant with the disaturated PC enrichment and the increased osmotic fragility, a shape change was observed. The discoid shape is modified into an echinocytic appearance after replacement of about 20% of the native PC by the dipalmitoyl species. Additional replacement up to 35% resulted in the formation of spheroechinocytes. Similar observations have been made with human erythrocytes [5].

Survival studies

The results shown in Fig. 2 are derived from lifetime experiments on horse erythrocytes in the same individual. Virtually identical results were found for control cells and cells in which 40% of native PC was replaced by (1-palmitoyl-2-oleoyl)PC (Fig. 2, curves A). From the fact that ^{51}Cr radioactivity in the circulation decreases in a similar way, we conclude that no difference exists between the lifespan of normal horse erythrocytes and those in which native PC had been partly

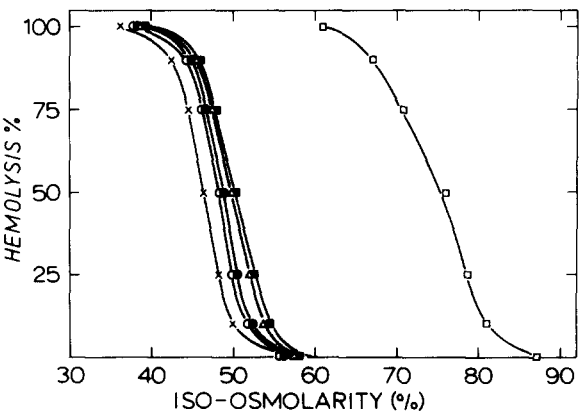

Fig. 1. Effect of replacement of native erythrocyte PC on osmotic fragility. The PC present in horse erythrocytes has been replaced by (1,2-dipalmitoyl)PC to different extents. Osmotic fragility curves were recorded after 0% (×); 5% (○); 10% (●); 15% (△); 20% (■) and 35% (□) replacement using a continuous dilution method [4]. The extent of hemolysis (expressed as % of total cells lysed) is plotted against the osmolarity (expressed as % of the isotonic value).

replaced by (1-palmitoyl-2-oleoyl)PC. Moreover, it indicates that the in vitro incubation of the cells in the presence of the PC transfer protein, in itself, does not affect their integrity. When native PC is replaced by (1,2-dipalmitoyl)PC, a significantly faster decrease in radioactivity is observed (Fig. 2,

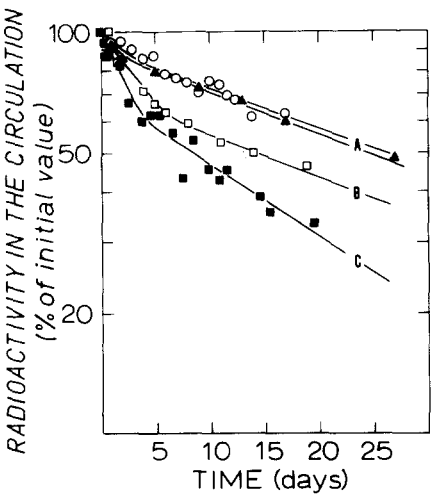

Fig. 2. Survival curve of horse erythrocytes based on ^{51}Cr recovery in the blood circulation. A: Control cells (○) and cells in which 40% of the native PC has been replaced by (1-palmitoyl-2-oleoyl)PC (▲). B and C: Cells in which 15% (□), resp., 25% (■) of the native PC has been replaced by (1,2-dipalmitoyl)PC.

curves B and C), indicating that these modified cells have a shorter lifespan than normal erythrocytes. The curve recorded for the decrease in radioactivity seems to consist of two components, indicating that part of the cell population is selected faster from the circulation. A further reduction of the lifespan in vivo of horse erythrocytes is observed when the replacement of the native PC by (1,2-dipalmitoyl)PC is increased (Fig. 2, curves B and C). Moreover, in experiments in which approx. 30% of native PC had been replaced by (1,2-dipalmitoyl)PC, only very low levels of ^{51}Cr could be found in blood samples drawn after 24 h. This indicates that most of these cells had been taken out of the circulation within one day. In Fig. 3, ^{51}Cr lifetime studies are shown in three rabbits of identical breed and age. Also from these results, it can be concluded that replacement of native PC by (1-palmitoyl-2-oleoyl)PC has no effect on the lifespan of the erythrocytes (Fig. 3, curve A), whereas replacement of native PC by (1,2-dipalmitoyl)PC leads to a decrease in the overall lifetime of those cells (Fig. 3, curve B). Again, similar to the observations with horse erythrocytes, curve B in Fig. 3 indicates that a heterogeneity in cell population towards selection from the circulation exists. In order to exclude the possibility that the lifetime of red cells may differ

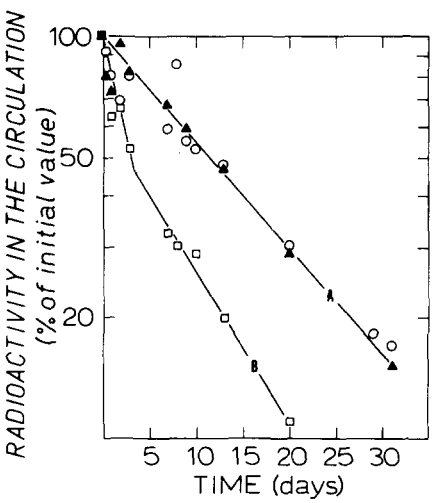

Fig. 3. Survival curves of rabbit erythrocytes based on ^{51}Cr recovery in the circulation. A: Control cells (○) and cells in which 40% of the native PC has been replaced by (1-palmitoyl-2-oleoyl)PC (▲). B: Cells in which 15% of native PC has been replaced by (1,2-dipalmitoyl)PC (□).

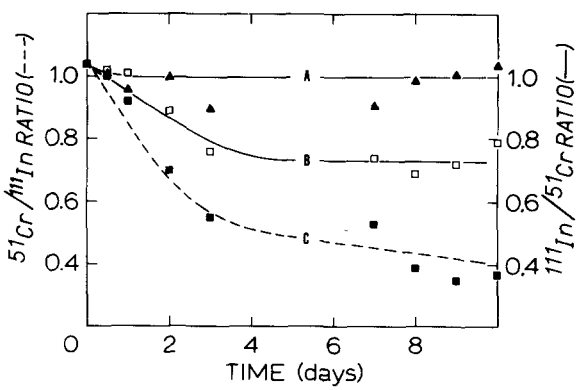

Fig. 4. Survival curves of subpopulations of erythrocytes in rabbit based on the relative amounts of ^{51}Cr and ^{111}In radioactivity in the circulation. (▲): $^{111}In/^{51}Cr$ ratio in the circulation. Cells in which native PC has been replaced by (1-palmitoyl-2-oleoyl)PC to an extent of 40% were labeled with ^{111}In, control cells with ^{51}Cr. (□): $^{111}In/^{51}Cr$ ratio in the circulation. Cells in which 15% of native PC has been replaced by (1,2-dipalmitoyl)PC were labeled with ^{111}In, control cells with ^{51}Cr. (■): $^{51}Cr/^{111}In$ ratio in the circulation. Cells in which 25% of native PC has been replaced by (1,2-dipalmitoyl)PC were labeled with ^{51}Cr, control cells with ^{111}In.

from one individual to another, and the effect this might have on our results, we used a $^{51}Cr/^{111}In$ double-label technique. This technique is in essence identical to the one used for platelet survival studies [20]. Cells, taken from the animal, were split in two portions. One portion was used to retailor native PC while the other part was used as a control. After the replacement incubation, one sub-population was labeled with ^{51}Cr and the other with ^{111}In. The two sub-populations were mixed and injected in the animal. In Fig. 4 the relative amount of label in modified cells compared to control cells is given. It can be concluded from curve A in Fig. 4, that no clear difference is found in the lifetime of ^{111}In-labeled cells in which native PC had been replaced to an extent of 40% by (1-palmitoyl-2-oleoyl)PC when compared to that of ^{51}Cr-labeled control cells. ^{111}In-labeled cells in which 15% of native PC had been replaced by (1,2-dipalmitoyl)PC have a shortened lifetime relative to ^{51}Cr-labeled control cells (Fig. 4, curve B). This difference was even more pronounced when 25% of the native PC had been replaced by (1,2-dipalmitoyl)PC and survival of these ^{51}Cr-labeled cells was compared with that of ^{111}In-labeled control cells (Fig. 4, curve C).

Discussion

The PC-specific transfer protein from bovine liver has been used to study the localization and properties of PC in erythrocytes and other membranes. In addition the protein has been applied to replace PC molecules and to modify this way in a highly specific manner the lipid bilayer in order to determine how PC structure can influence the overall structure and function of a membrane. In previous reports it was shown that replacement of PC in intact human erythrocytes, resulting in a change in the degree of (un)saturation of this lipid in the membrane, can lead to increased osmotic fragility, modified K^+ permeability, hemolysis and drastic changes in red cell shape [4,5]. As it is known that defect erythrocytes are removed in vivo in an efficient way, the question had to be asked if the cells, modified in their PC composition and reinjected into the bloodstream, were recognized as being abnormal and removed, or if repair mechanism(s) were able to prevent this. To answer this question, experiments were carried out in two animal species, the erythrocytes of which had to be characterized first with respect to their phospholipid and in particular their PC composition. The results are completely in line with previously published data and with the characteristics of membranes from other red cell species.

The phosphatidylcholine transfer protein from bovine liver has been used to partly replace native PC from intact horse and rabbit erythrocytes by (1,2-dipalmitoyl)PC and (1-palmitoyl-2-oleoyl)PC. It is important to note that this procedure primarily effects the PC in the outer monolayer of the membrane and none of the other membrane constituents [5,6]. Cholesterol depletion is prevented by the presence of cholesterol in the donor vesicle systems and release of lipid vesicles from the cells, as observed during incubations with (1,2-dimyristoyl)PC vesicles [31], does not occur in case (1,2-dipalmitoyl)PC is used. Replacement of up to 40% of the native PC by (1-palmitoyl-2-oleoyl)PC did not lead to changes in the cell membrane integrity as was judged from the morphology or osmotic fragility of the cells. On the other hand, replacement of native PC by (1,2-dipalmitoyl)PC did lead to echinocytic cells with an increased sensitivity to osmotic stress. It can therefore be concluded, as was previously also found in studies on erythrocytes from rat [6] and human [4,5], that erythrocytes from rabbit and horse only tolerate a limited change in the fatty acyl composition of their PC.

A critical and physiologically significant test on the overall membrane integrity is the survival of cells in vivo. The random labeling of erythrocytes using ^{51}Cr should, by measuring the fall in specific radioactivity of circulating cells, give direct information on the mean lifespan of these erythrocytes in vivo. However, several pitfalls [21] may obscure an exact determination of cell lifespan from the apparent lifetime of ^{51}Cr in the circulation. In spite of all its shortcomings, the ^{51}Cr method is widely used because of its technically simple experimental approach. The results can, although they do not give an exact value of the lifespan in vivo, be used in a relative sense. In our experimental set-up we compared ^{51}Cr lifetime studies of various cells in one individual (horse) or three individuals (rabbit), comparable with respect to breed and age. We assumed that the decrease of specific radioactivity of the cells in the circulation, is a reflection of the lifespan in vivo. In addition, we used ^{51}Cr- and ^{111}In-labeled sub-populations of erythrocytes to investigate simultaneously the survival of modified and non-modified cells in the same animal. The use of ^{111}In as a radionuclide for platelet, neutrophil and lymphocyte labeling has shown that this component as ^{111}In oxine diffuses passively through the cell membrane and transfers its radioactivity to cytoplasmic components [22]. Comparative studies using ^{113m}In, ^{51}Cr or ^{99}Tb on red cell volume and splenic red cell pool, gave identical results [23] and showed that the elution time of the ^{113m}In isotope from red cells is also very low. Double-label studies using both ^{51}Cr and ^{111}In were shown to be successful in studies on platelet survival [20].

As shown in Figs. 2–4, both the overall survival time of the ^{51}Cr as well as that of the ^{111}In radionuclide is considerably shorter in the circulation when these labels are incorporated in cells in which native PC has been partly replaced by (1,2-dipalmitoyl)PC. No significant difference is found between control cells and cells in which native PC has been partly replaced by (1-palmitoyl-2-oleoyl)PC. Although an increased elution of label

from the (1,2-dipalmitoyl)PC-enriched cells cannot rigorously be excluded, it seems likely that our results indicate an accelerated destruction of these cells. The precise mechanism of destruction, limiting the lifespan of erythrocytes, is unclear. Abnormal cells with a mild injury are removed from the circulation by the sensitive sequestering system of the spleen. Cells with a more severe injury are destructed in both spleen and liver and cells which have grossly lost their structural integrity are also destroyed as they circulate, with release of their intracellular content. Several studies point out that the spleen filter is able to detect membrane injury too subtle to be observed in vitro [2]. An important parameter that influences the viability of erythrocytes has been attributed to their ability to deform under mechanical stress [1]. It has been observed that human cells in which native PC was partly replaced by (1,2-dipalmitoyl)PC have a decreased deformability, which appeared to be most pronounced with respect to their filterability [7]. It is therefore most likely that, especially during sequestration in the spleen, these cells will be recognized as abnormal ones. This will lead to either a 'repair' of the abnormality or an accelerated destruction of these cells. Relatively little is known about the repair processes that occur in vivo and which prevent an early destruction of injured cells. Cells in which the lipid component of the membrane has been altered in vitro by cholesterol depletion [24], undergo changes in vivo, permitting most, if not all, of them to survive normally. This is probably achieved by rapid exchange equilibrium with plasma. No de novo synthesis of fatty acids is found in mature erythrocytes [25]. Therefore, the equilibrium of membrane lipids with those in plasma may be the most important way for the cell to modify the acyl chain composition of its phospholipids. This exchange is primarily confined to cholesterol [26] and phosphatidylcholine [27]. The exchange rate of PC via this pathway is considerably slower than of cholesterol [26]. Nevertheless, approximately one-fourth of red cell PC will be exchanged this way within 24 hours [27]. It should, however, be realized that not all species are equally rapidly available to take part in this exchange process. It has been shown that the fatty acyl composition of the red cell can be modulated by plasma, but will not become identical to it [28]. The species specificity in this exchange reaction is also found in vitro where it was shown that unsaturated species leave the membrane at a higher rate than the disaturated (1,2-dipalmitoyl)PC [29]. It will be obvious from this study that replacement of native PC in the red cell membrane by (1,2-dipalmitoyl)PC induces abnormalities in the cell, which are recognized by the in vivo circulation system and will result in an accelerated destruction. The in vivo repair mechanism appears to be not fast enough to permit a normal survival of these cells in the circulation, probably due to the low rate at which the (1,2-dipalmitoyl)PC can be exchanged with the plasma.

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