# SOME LIPID CHARACTERISTICS OF RED CELL MEMBRANES OF VARIOUS ANIMAL SPECIES*

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SUMMARY

Determinations were carried out of some lipid classes of red cell ghosts of sheep, ox, pig, man, rabbit and rat. In the studied red cell ghosts only small differences appeared to exist in the amounts of total lipids, phosphatides and free cholesterol. On the other hand, determinations of the individual types of phosphatides revealed a most pronounced deviation in the quantitative proportions of the major phosphatides. In the given order of animals a significant increase of the lecithin percentage of the ghost phosphatides was observed. Possible relations between the specific lipid composition and the properties of the red cell membranes are discussed.

## INTRODUCTION

In an earlier paper attention was drawn to the question as to how far the lipid constituents contribute to the specific properties of the biological membranes[1]. A characteristic fatty acid composition was observed of lipids from red cell membranes of different species of mammals which suggested a possible relation between fatty acid composition and the permeability properties of the erythrocytes. In this respect differences in the proportions of the various lipid classes present in the membrane could also be of importance. In their pioneering studies PARPART AND DZIEMIAN[2] already tackled this problem and estimated a number of lipid components of the red cells of various animals. During the last few years the chemistry of lipids and particularly of phosphatides, was greatly enhanced by the application of chromatographical methods[3,4]. TURNER *et al.*[5,6] pointed out that the ubiquitously distributed lecithins are not abundant in the red cells of several ruminants.

To be able to elucidate whether besides variations in the fatty acid composition also other differences in the amounts of constituents from the membranous lipids are related with the distinction in permeability behaviour of red cells, additional information had to be acquired. Therefore analyses were carried out of several major lipid classes and next of some individual types of phosphatides in ghost from red cells of man, rat, rabbit, pig, ox and sheep.

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## METHODS

### *Extraction of lipids*

Erythrocytes were obtained by centrifuging freshly withdrawn defibrinated blood for 10 min at 2,500 × *g* and subsequently washing three times with equal volume of isotonic saline at room temperature. The buffy coat was removed cautiously. Ghosts were prepared by the $CO_2$ method as described earlier[1]. About 1 g of lyophilised ghosts was treated with 125 ml of dry ethanol–ether (3:1, v/v) for 18 h at room temperature. The extraction was repeated twice, but now for 1 h. The combined lipid extracts were evaporated under reduced pressure in a nitrogen atmosphere at a temperature below 35° and the residues were dried *in vacuo* over phosphorus pentoxyde. Contamination with non-lipid material of the extracts obtained in this way was low and no thorough-going purification was obtained by further solvent extraction or solvent partition.

### *Determination of main lipid classes*

The non-phosphorus lipids and the phosphatides were separated by means of silica chromatography according to BORGSTRÖM[7]. The fractionation of 100 mg of lipids was accomplished on silicic acid columns (8 g of silica Mallinckrodt A.R. 100 mesh, mixed with 4 g of Hyflo Super Cell and activated for 24 h at 120°) by eluating successively non-phospholipids and phosphatides with 400 ml of chloroform and 200 ml of methanol respectively. The solvents were removed and the residues dried *in vacuo*. The recovery of lipids was about 98 %. The phosphorus content of the fractions was ascertained according to FISKE AND SUBBAROW[8].

Cholesterol and cholesterol ester were estimated by means of the colorimetric procedure outlined by HANEL AND DAM[9]. Separation of cholesterol and cholesterol ester was achieved by chromatography of 5 mg of sample using columns containing 1.5 g of silica (equal parts Mallinckrodt silica A.R., 100 mesh, and Hyflo Super Cel). Cholesterol ester and free cholesterol were recovered by percolating the columns with successively 12 ml of chloroform–petroleumether (1:1, v/v) and 25 ml of ethylacetate–petroleumether[10] (1:1, v/v). Analysis of three mixtures of pure cholesterol and cholesterol palmitate in different ratios yielded 100.3 % (mean error: 0.7) and 95.8 % (mean error: 4.2) respectively of the theoretical values. The recovery of cholesterol added to six lipid samples of ghosts of various red cells was 97.5 % (mean error: 3.5).

### *Qualitative paper chromatography of phosphatides*

Identification of the various types of phosphatides from ghosts of red cells was achieved by paper chromatography of intact phosphatides on silica impregnated paper[11] as well as by paper-chromatographic separation of the degradation products of the phosphatides. Mild alkaline hydrolysis was carried out according to DAWSON[12], acid hydrolysis was performed with 3.5 *N* HCl for 24 h at 100°. The methods used for the identification of the various hydrolysis products have been described[13,14].

### *Quantitative paper chromatography of phosphatides*

The individual phosphatides were separated on silica-impregnated paper with di-isobutylketone–acetic acid–water (40:25:5, v/v) as developer[11] (ascending technique). After localization of the phosphatides by means of Rhodamine 6 G staining and examination under u.v., the spots were cut out, eluated with 3 changes of 5 ml

of methanolic HCl (1 *N*) and after destruction the phosphorus content was estimated[8].

Recently BUNGENBERG DE JONG, HOOGHWINKEL *et al.*[15-18] developed a tricomplex staining procedure which allows a less elaborate quantitative determination of the major phosphatides separated on silica chromatograms. A number of analyses was carried out according to this method. The silica chromatograms were stained for 20 h in a bath of 0.005 % Ponceau red, 0.2 % uranyl nitrate and 0.01 *N* HCl. The papers containing the red-coloured spots of phosphatides were first dried carefully between filter paper and thereupon with the aid of a stream of warm air. The spots were cut out and the dye was eluated with 5 ml of 0.4 *N* HCl in *tert.*-butanol–water (1:1, v/v). The extinction of the dye solution was measured at 510 mμ. In agreement with HOOGHWINKEL[17-18], who reported a linear relation between eluate extinction and concentration of various natural phosphatides, we also found such a proportionality in the case of various lecithin and phosphatidylethanolamine preparations obtained by synthesis[19-22]. It appeared, and we agree with the above mentioned author, that determinations of blank values can be omitted if the stained spots are cut out narrowly. The recoveries of synthetic products as obtained with both procedures ranged from 87–99 %.

*Determinations of nitrogenous constituents*

To obtain additional information on the phosphatide composition a number of samples was analysed for choline, serine and ethanolamine content. Therefore about 30 mg of phosphatides recovered from the silica columns were subjected to hydrolysis with 6 ml of 4 *N* HCl for 40 h at 110°. After removal of the hydrochloric acid, choline estimations were performed in an adequate amount by means of the modification of the choline ennea jodide method, as outlined by KUSHNER[23]. Although, in our opinion, some objections can be raised against the principle of the method, a rigorous standardization of the procedure can yield reproduceable and accurate values. In determinations of the choline content of five different synthetic lecithins[19,20] we found 98.1 % (mean error: 2.1) of the calculated values. After addition of choline to six phosphatide samples 99.3 % (mean error: 3.9) was recovered.

Serine and ethanolamine were determined according to the principles described by LEVINE AND CHARGAFF[24]. Adequate samples of the hydrolysates were subjected to descending paper chromatography with isopropanol–acetic acid–water (3:1:1, v/v) as a developer. The separated spots of serine and ethanolamine were eluated and estimated by means of the ninhydrin reagent[25]. The applied procedure has some drawbacks for the estimation of ethanolamine because of possible contamination with ammonia. Experiments performed on reference substances revealed in 14 serine analyses 99.5 % (mean error: 5.0) and in 8 ethanolamine analyses 97.7 % (mean error: 12.2) of the theoretical values.

## RESULTS

*Major lipids*

Data on the content of total lipids and of some individual lipid classes of red cell ghosts are compiled in Table I. The amount of lipid material within each animal species is fairly constant and varies only to a small extent between the red cell ghosts of the studied animal species. This is also true for the phosphatide content as derived

from the lipid-phosphorus determinations and from the silica column chromatography. In the studied animal series the phosphatides amount to about 50–60 % of the total ghost lipids. The amount of phosphatides as determined gravimetrically after silica chromatography is about 10 % higher than the values calculated from the lipid-phosphorus determination carried out on the total lipid fractions. However, it appeared that the phosphorus content of the isolated ghost phosphatides did not exceed 3.5 %, whereas we usually reached a phosphorus content of 3.9 in other animal phosphatide preparations obtained with the same chromatographic technique. Hence the isolated phosphatide fractions of red cell ghosts appear to consist of about 10 % non-phosphorus lipids, which are assumed to belong mainly to the class of globosides[26,27]. From the data of YAMAKAWA[27], for instance, in the lipids of human ghost about 12 % of these glyco-lipids can be found.

The cholesterol analyses performed on the total lipid extracts indicated that the content of free cholesterol ranged from about 20–30 % in the different red cell ghosts and already showed the non-phosphorus lipids to consist of this component for about 75–90 %. This was confirmed by analysis of the so-called neutral lipid fraction, which was obtained by silica chromatography. These lipids of the red cell ghosts appeared to contain for example the following percentages of free cholesterol: sheep 93 %, rabbit 80 %, pig 84 %. The amount of triglycerides in red cell ghosts of various species is known to be rather low[28]. As expected the cholesterol content of the isolated

TABLE I

LIPID COMPOSITION OF RED CELL GHOSTS

Percentages are given of the amounts of total lipids extracted from lyophilised ghosts. The phosphatide percentages were calculated from the lipid-phosphorus determinations, maintaining a phosphorus content of 4 % of the ghost phosphatides. The standard errors of the means are placed between parentheses.

| *Animal* | *Number of analyses* | *Total lipid percentage* | *Number of analyses* | *Phosphorus percentage of total lipids* | *Phosphatide percentage of total lipids* |
|---|---|---|---|---|---|
| Sheep | 4 | 30 (1.0) | 4 | 2.40 (0.07) | 60 |
| Ox | 7 | 27.5 (1.0) | 5 | 2.23 (0.05) | 56 |
| Pig | 10 | 31 (0.5) | 6 | 2.10 (0.07) | 53 |
| Human | 6 | 25 (1.5) | 9 | 2.30 (0.02) | 58 |
| Rabbit | 8 | 28 (1.0) | 4 | 2.30 (0.06) | 58 |
| Rat | 3 | 22 (1.0) | 3 | 2.43 (0.09) | 61 |

Neutral lipids were isolated by silica chromatography of total lipids. Free cholesterol was determined in total lipid extracts.

| *Animal* | *Number of analyses* | *Neutral lipid percentage of total lipids** | *Number of analyses* | *Cholesterol percentage of total lipids* |
|---|---|---|---|---|
| Sheep | 3 | 28 (3) | 4 | 27 (4) |
| Ox | 3 | 33 (3) | 4 | 31.5 (2.5) |
| Pig | 5 | 27 (2) | 4 | 21.5 (2.5) |
| Human | 3 | 29 (2) | 5 | 23 (2.5) |
| Rabbit | 2 | 33 (1) | 4 | 21 (2) |
| Rat | 1 | 28 | 3 | 28 (3) |

* The phosphorus content of this fraction did not exceed 0.04 %.

phosphatides was negligible. The amount of esterified cholesterol did not exceed 1 % of the total lipids. The obtained values of these lipid classes of several of the studied red cell ghosts are in agreement with the available literature data[2,28,29]. A comparison with data gained from analyses of intact red cells cannot readily be made because of a loss of cholesterol during the preparation of ghosts[30].

*Phosphatides*

A more pronounced differentiation in the composition of the ghost lipids between the various animal species was encountered when the phosphatides were examined. Paper chromatography of the intact phosphatides and of their various degradation products prepared by acid and alkaline hydrolysis, demonstrated that nearly all studied red cell membranes contain phosphatidylethanolamine, sphingomyelin, phosphatidylcholine and small amounts of phosphatidylserine, inositolphosphatide and traces of a component behaving very similarly to cardiolipin. Furthermore, in several species plasmalogens and lyso-phosphatides were found. These qualitative experiments did demonstrate that within this animal series there are extreme differences between the proportions of the main types of phosphatides, especially with regard to the choline containing lipids.

Examination of Table II, which presents the data of the paper-chromatographic analysis, indicates that actually differences exist in the quantitative proportions of the individual types of phosphatides between the various species. The ghosts of red cells of sheep and ox showed a high sphingomyelin and a very low lecithin content, while in the sequence of pig, man, rabbit and rat the lecithin percentage increases considerably. Consequently the increase of lecithin is accompanied by a decrease of the content of the other major phosphatides, viz. of sphingomyelin and of cephalins.

As has already been mentioned the determination of some minor phosphatides was omitted, the recoveries of the phosphorus content range from about 87 % to 100 %, thus indicating that the most abundant phosphatides are included. Furthermore, the data obtained by means of phosphorus determination and with the tricomplex staining procedure are in very good agreement, showing once more the suitability of the latter method. The values of sphingomyelin and lyso-phosphatides are taken together in Table II, since the separation of both classes could not be effected in all cases. In a number of analyses a separation was achieved between sphingomyelin and lyso-lecithins, which latter component in several species amounted to about 3 % of ghost phosphatides. The data obtained in this way suggested that the variation in the lecithin content of the red cell ghost between the various species is particularly counteracted by a shift of the sphingomyelin content, such that the total amount of choline containing phosphatides is nearly equal in the studied membranes. Confirmatory evidence was obtained from choline determinations which actually show only a limited distinction in the content of choline phosphatides of the membranes under consideration (Table III). There is also some variation in the cephalin content (Tables II and III) in the ghost lipids of the studied species. Taking into account the fundamental differences between the methods used, the data of Tables II and III are in reasonable agreement.

A detailed comparison of the data given in both tables shows, that the amount of lecithin and sphingomyelin, including lyso-phosphatides taken together from Table II, in most cases dominates the percentage of choline phosphatides calculated

from the choline analyses (Table III), whereas the total amount of ethanolamine and serine phosphatides (Table III) exceeds that of the paper-chromatographically estimated cephalins (Table II). These differences however are only ostensible, since lysocephalins are located on the paper chromatograms in the sphingomyelin region and

TABLE II

PHOSPHATIDE COMPOSITION OF RED CELL GHOSTS

Proportions of the individual phosphatides are given as percentages of the total phosphatides, omitting the content of some minor phosphatides. Values were obtained by paperchromatographical analyses. Data provided with phosphorus recoveries were obtained by determinations of the phosphorus content of the separated components. All other data were estimated by means of the tri-complex staining method[17,18]. The standard errors of the means are placed between parentheses*.

| *Animal* | *Number of animals* | *Per cent lecithin* | *Per cent sphingomyelin + lysophosphatides* | *Per cent cephalins* | *Per cent phosphorus recovery* |
|---|---|---|---|---|---|
| Sheep | 3 | 0 | 65.5 | 34.5 | 92 |
| | 2 | 0 | 64 | 36 | 88 |
| | 1 | 1.5 | 65 | 33.5 | — |
| | 2 | 0 | 63 | 37 | — |
| | 1 | 2 | 61 | 37 | — |
| Mean | | 1 (1) | 63 (1) | 36 (1) | |
| Ox | 2 | 11 | 53 | 35 | 100 |
| | 3 | 11.5 | 67 | 21.5 | 93 |
| | 1 | 8 | 58.5 | 33 | 87 |
| | 1 | 0 | 63 | 37 | — |
| | 2 | 5 | 56 | 39 | — |
| | 1 | 2 | 68 | 30 | — |
| | 1 | 9 | 66 | 25 | — |
| Mean | | 7 (2) | 61 (2.5) | 32 (2.5) | |
| Pig | 2 | 25.5 | 30.5 | 44 | 95 |
| | 1 | 30 | 42 | 28 | 99 |
| | 2 | 29 | 42 | 29 | — |
| | 1 | 30 | 35 | 35 | — |
| | 1 | 29 | 33 | 38 | — |
| Mean | | 29 (1) | 36 (2.5) | 35 (3) | |
| Human | 3 | 41 | 42 | 17 | 98 |
| | 1 | 41 | 45 | 18 | 92 |
| | 1 | 35 | 36 | 29 | — |
| | 2 | 42 | 35 | 23 | — |
| | 1 | 36 | 33 | 31 | — |
| Mean | | 39 (1.5) | 37 (2.5) | 24 (3) | |
| Rabbit | 7 | 51.5 | 20.5 | 28 | 89 |
| | 8 | 42 | 30 | 28 | 100 |
| | 8 | 47 | 28 | 25 | — |
| | 6 | 35 | 38 | 27 | — |
| Mean | | 44 (3.5) | 29 (3.5) | 27 (1) | |
| Rat | 12 | 58 | 27 | 15 | 96 |
| | 18 | 51 | 29 | 20 | — |
| | 18 | 59 | 24 | 17 | — |
| Mean | | 56 (2.5) | 26 (1.5) | 18 (2) | |

* The mean values have been calculated, not taking into account the number of animals of each experiment, since standard deviation of analyses of one sample of blood were of the same range as those performed on different samples.

are determined together with the latter component (Table II). The presence of small amounts of lyso-cephalins besides lyso-lecithins in ghost lipids was also demonstrated by a positive ninhydrin reaction of this sphingomyelin lyso-phosphatides spot.

*Aging effect*

In connection with the foregoing it is noteworthy to mention an intriguing phenomenon encountered in the analyses of ghost phosphatides. Storage of dry lipid samples of red cell ghost *in vacuo* over phosphorus pentoxyde in the dark at 5° gave, after about a week, a considerable change in the percentages of cephalins. This is demonstrated by the following experiment. Two ghost lipid samples (human) directly analysed paper-chromatographically, revealed as mean values: lecithin, 35.5 % (± 0.5), sphingomyelin including lyso-phosphatides, 33 % (± 3), cephalins, 31.5 % (± 1). After storage under the described conditions for 10 days the analysis revealed: lecithin, 38 % (± 1), sphingomyelin including lyso-phosphatides, 51 % (± 1), cephalins 11 % (± 1).

Apparently, these is a conversion of a great part of phosphatidylethanolamine into a component that is paper-chromatographically very like lyso-phosphatidylethanolamine. The same phenomenon was also observed in preparations of ghost lipids of other animal species, although, as far as our experience goes, the phenomenon is less pronounced in the case of several animals. It must be emphasized that the data presented in Table II concern analyses made as quickly as possible.

We assume that part of the phosphatidylethanolamine of red cell ghost consists of a component, probably of the plasmalogen type, having a labile attached paraffin chain. In this respect it is of interest to note that HANAHAN *et al.*[31] very recently observed that when human blood was collected in heparin, phosphatidylethanolamine of red cells tended to form lyso-derivatives. Further evidence for this conversion of the ethanolamine phosphatide into a lyso compound was obtained by paper-chromatographical comparison with a defined lyso-phosphatidylethanolamine (Fig. 1). Therefore γ-stearoyl-β-oleoyl-L-α-phosphatidylethanolamine obtained by total synthesis* was degradated by phospholipase A. Just like in the case of the corresponding "mixed-acid" lecithin[21,22] only one molar equivalent of oleic acid was liberated,

TABLE III

PROPORTIONS OF SOME TYPES OF PHOSPHATIDES OF RED CELL GHOSTS

Percentages are given of the individual phosphatides as derived from choline, ethanolamine and serine analyses, carried out in hydrolysates of isolated phosphatides. The standard errors of the means are placed between parentheses.

| *Animal* | *Number of analyses* | *Per cent choline phosphatides* | *Per cent ethanolamine phosphatides* | *Per cent serine phosphatides* |
|---|---|---|---|---|
| Sheep | 2 | 55 (3) | 44 (2) | 1 (1) |
| Ox | 2 | 59 (3) | 34 (2) | 7 (1) |
| Pig | 5 | 57 (2) | 40 (3) | 3 (2) |
| Human | 3 | 65 (7) | 32 (9) | 3 (2) |
| Rabbit | 2 | 59 (1) | 39 (2) | 2 (1) |
| Rat | 2 | 70 (5) | 29 (5) | 1 (1) |

* G. H. DE HAAS, to be published shortly.

yielding the γ-lyso-phosphatidylethanolamine. This latter component indeed coincided on the silica paper chromatograms with the ninhydrin-positive lyso-compound of the aged ghost samples.

Extreme discrepancies are known to exist between the data reported by various authors on the phosphatide composition of red cells[28]. Moreover, most data available cover analyses made on intact red cells, whereas the present paper deals with analyses made on ghosts. Although admittedly, the difference of phosphatide content of intact cells and their ghosts is rather small[31-34], a shift in the proportion cannot be precluded definitely. Our data on the human red cell ghost are in agreement with the values reported by FORMIJNE *et al.*[35] on intact red cells. The observation and statements of TURNER *et al.*[5,6] with respect to the differences between red cell phosphatides of

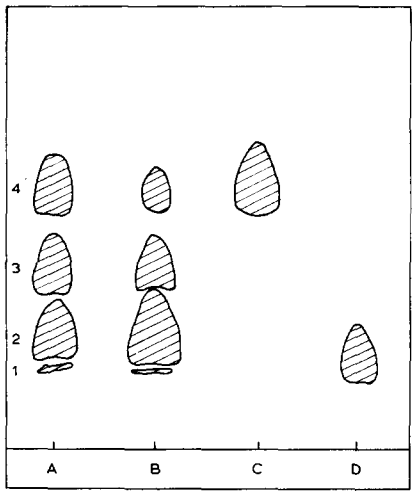


Fig. 1. Paper-chromatographical demonstration of the conversion of cephalin into lyso-compound in lipids of human red cell ghost. Paper chromatogram developed on silica-impregnated paper (see text); lyso-lecithin, 1; sphingomyelin and lyso-cephalins, 2; lecithin, 3; cephalins, 4. Phosphatides of human red cell ghost, normal, A; like A, but lipid sample was stored for 10 days as described in text, B; synthetic cephalin: γ-stearoyl-β-oleoyl-L-α-glycerylphosphorylethanolamine, C; lyso-cephalin: γ-stearoyl-L-α-glycerylphosphorylethanolamine, obtained from synthetic cephalin by phospholipase A degradation, D.

several ruminants and non-ruminants can be confirmed. Moreover, our analytical values of human and bovine ghosts are in excellent agreement with those of a very recent and detailed study on the lipids of these red cells of HANAHAN *et al.*[31], which we learned about when this manuscript was in preparation.

## DISCUSSION

For the main purpose of the present study, to acquire further information on possible relations between lipid composition and permeability properties of red cells it seems that, in particular, the noticed variations within the phosphatide moiety are of interest. Although also some differences may occur in the region of the minor phosphatides, a most intriguing variation appears to exist in the ratio of sphingomyelin, lecithin and cephalins between the various red cell membranes. In Fig. 2 are shown

data on the lecithin content of red cell membranes taken from Table II, and data on the hemolysis times of the corresponding red cells in isotonic solutions of glycol as measured by JACOBS *et al.*[36]. These hemolysis times are assumed to be related in reversed ratio to the permeation velocities. Although on some points there is a small breakdown, there appears to be a clear resemblance between the variations of both membranous characteristics of the red cells in the animal species under consideration.

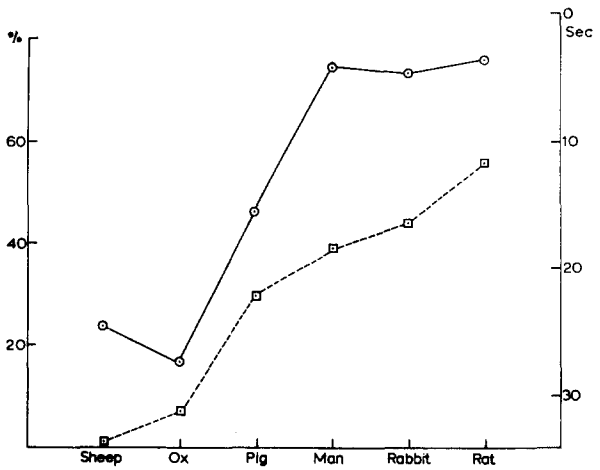


Fig. 2. Comparison of lecithin content of red cell ghosts with the permeability for glycol of the corresponding red cells. Mean percentages of lecithin from Table II (left hand ordinate), lower curve. Hemolysis times of erythrocytes in isotonic solution of glycol[36] (right hand ordinate), upper curve.

Statistical analysis of both magnitudes revealed a correlation coefficient $r = 0.952$ and a degree of significance $p < 0.01$. Since the permeability process of red cells is a very complex phenomenon[36], definitive conclusion cannot be drawn from one comparison, but there are also notable coincidences to be noted between permeability for several other components *e.g.* glycerol[36] and glucose[37] and the lecithin or sphingomyelin content of a number of red cell membranes. These correlations suggest that, besides the variation in fatty acid constituents, also the proportions of various types of phosphatides abundant in the membranes may contribute to the distinction of the membranous properties *e.g.* permeability of the red cells of the various animal species.

The question arose as to what extent the differences in the fatty acid patterns and the differences in the phosphatide composition of the various red cell membranes are related. In the sequence of animals: rat, man, rabbit, pig, horse, ox and sheep notable variations were observed in the fatty acid patterns of the alkaline saponifiable lipids of red cell membranes *e.g.* a decrease of the ratio palmitic acid–oleic acid[1]. Extension of the gas-chromatographical analyses showed that also the arachidonic acid content of ghost lipids varies in the given order of animals[38]. The fatty acid data of four animal species appeared to agree with data reported by JAMES AND LOVELOCK[39], as we have learned only recently[28].

Evidently the variations in the phosphatide composition dealt with in the present paper, and the variations in the fatty acid composition of the red cell membranes differ in nearly the same order of animals. Although we have not determined

so far the fatty acid patterns of all individual types of phosphatides of red cell ghosts from all the animal species under consideration, it appeared that the cephalins generally have a relatively high content of several unsaturated fatty acids, while the choline phosphatides, and in particular sphingomyelin, contain a high percentage of saturated fatty acids. However, also within one individual class of phosphatides the fatty acid composition may differ from animal species to species as we found to be the case with the percentage of some unsaturated fatty acids present in the cephalins of the different red cell ghosts. Recent investigations of HANAHAN *et al.*[31] also demonstrated that in human and bovine red cells the various types of phosphatides have remarkably characteristic fatty acid patterns. These results stimulate a further extension of the analysis of the red cell ghosts to obtain complete information of all types of fatty acids and other apolar chains present in the distinct phosphatides of the animal series under investigation. On account of the data now available the specific fatty acid and phosphatide compositions of the red cell membranes are assumed to be related such that within each individual membrane fatty acids and alcohol-nitrogenous constituents are linked together in a number of preferential combinations. Both the apolar and polar moiety of the membranous phosphatides may contribute to the distinction of the "fine-architecture" between the homologue membranes, as a consequence of which differences unfold in the membranous properties *e.g.* in permeability and in the stability against hemolytic agents. As regards the problem in which way the observed chemical differences of the membrane components bring about differences in the membranous structures, studies on monolayers of various defined phosphatides containing very different fatty acids, inform that the nature of the fatty acids or apolar moiety mainly determines the interfacial behaviour of these components. On the other hand, the chemical composition of the polar moiety seems very important with regard to the various possibilities for the interaction or associations between phosphatides and other membrane components. In this respect it is worth noting, that the proportions of "loosely-bound" and "strongly-bound" phosphatides, as defined by PARPART AND BALLENTINE[34], vary also to some extent between the different red cell membranes[38,40]. Furthermore, these two classes, which are attached in a different way or degree to other membranous components appear to deviate greatly in their phosphatide composition.

Besides the notable difference in the major phosphatides of the red cell membranes also certain variations may exist in the quantities of phosphatides present only in small amounts. Since these minor components often show a dynamic character, as measured from the degree of isotopic incorporations, an active part of these components in the transport mechanism across the membranes cannot be precluded[41–44]. Also in this respect a determination of the structure of the labile ethanolamine-phosphatide of ghost lipids is wanted and seems of interest in connection with the suggestions of COLLINS[45] on phosphatides containing triester groups.

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