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# UNIQUE PROPERTIES OF THE CAMEL ERYTHROCYTE MEMBRANE

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## SUMMARY

In view of the exceptionally high osmotic stability of camel erythrocytes, several aspects of the membranes were examined.

1. Added linolenoyl sorbitol increased the osmotic stability of camel erythrocytes to only one-third of the increase of human erythrocytes similarly treated. Human, hamster, rat and sheep erythrocytes exhibited similar time courses of lysis by sonic irradiation, while camel erythrocytes were distinctly more stable. Unlike its effect on human erythrocytes, vinblastin did not impair the osmotic stability of camel erythrocytes.

2. Lipid composition of camel erythorcytes was analyzed. Lipid content per packed cell volume, phospholipid:cholesterol molar ratio, phospholipid composition as well as composition of the phospholipid fatty acids were all close to values reported for other species.

3. Ghosts prepared from camel erythrocytes had a protein:lipid ratio of 3.0, compared with a ratio of 1.25 in human erythrocyte ghosts, and showed a higher proportion of proline and arginine but lower proportion of glutamic acid.

4. It is proposed that the unique lipoprotein structure of the camel erythrocyte membrane may account for the unusual features of these erythrocytes.

## INTRODUCTION

The camel's tolerance to heat and dehydration is extraordinary[1,2]: it can lose water to the extent of about 30% of its body weight and still move about[3]. A dehydrated camel can regain its loss of water in a short time (over 10 l/min) and despite the large flow of water into the blood stream, the erythrocytes do not hemolyze[3,4]. Indeed, the osmotic fragility of camel's erythrocytes is outstandingly low and the cells are capable of swelling in hypotonic media to over twice their volume[5–7]. Even though the red blood cells of camel contain lecithin[8], they are resistant to the lytic effect of snake venom[9,10]. Erythrocytes of the camel are unique among mammalian types in being oval, rather than circular, discs[5,9].

All these characteristics indicate some unusual properties of the membranes of camel erythrocytes, but the available information concerning these membranes is limited. Thus, for example, the analysis of phospholipids is only qualitative[8]. The present communication examines several aspects of the camel erythrocyte

membrane and demonstrates that this membrane is indeed endowed with many unique properties.

## MATERIALS AND METHODS

An adult female camel (*Camelus dromedarius*, "Golda") of a local breed, was raised at the Negev Institute for Arid Zone Research, Beer Sheva, on a diet of grain concentrates and straw. The animal had ample supply of water when blood was collected. Heparinized blood samples from camel and adult humans and other species tested were washed three times with buffered 155 mM NaCl as described[11]. Osmotic potential of the erythrocyte suspension was determined as previously reported[12].

### *Fragility measurement*

For measurements of osmotic fragility, an aliquot of 20 $\mu$l of 30% erythrocyte suspension was pipetted into 3 ml of 2 mM sodium phosphate, pH 7.2, containing NaCl at various concentrations and, where indicated, linolenoyl sorbitol[12]. Immediately after addition of the cells, the content of each tube was thoroughly mixed. In case of slow hemolysis, the gradual mixing of the cells with the hypotonic medium was as previously described[13]. After 10 min at 20 °C the cells were centrifuged at $2000 \times g$ for 5 min and the percent hemolysis was determined by spectrophotometric measurement of the hemoglobin present in the supernatant.

The effect of vinblastin was tested by incubating erythrocyte suspensions (final concentration 24%) with vinblastin in phosphate-buffered (2 mM, pH 7.4) isotonic saline for 1 h at 20 °C. Aliquots of the incubated suspensions were tested for osmotic fragility as above.

Measurements of mechanical (sonic) fragility were performed in a flat bottom tube, of 13 mm diameter, containing 0.2% cell suspension in 3 ml of 155 mM NaCl, pH 7.2. A 100 W ultrasonic disintegrator (MSE, London) with an amplitude setting of 3 $\mu$m (peak to peak) was used. The titanium vibrator microprobe, with a tip diameter of 3 mm, was dipped halfway (12 mm) into the cell suspension. The irradiation lasted for varying time intervals at room temperature (23 °C). The temperature rose by 1.5 and 6 °C after 15 and 60 s of irradiation, respectively. The suspensions were centrifuged and analyzed for percent hemolysis as above.

### *Lipid extraction*

Lipid extraction of the washed erythrocytes with a chloroform–methanol mixture was as described[11]. Lipid extraction of the ghosts was similarly conducted.

### *Preparation of erythrocyte ghosts*

Ghosts of human and camel erythrocytes were prepared according to Dodge *et al.*[14], to obtain hemoglobin-free preparations. Erythrocyte suspensions were washed twice with 155 mM NaCl. The cells were then suspended in 155 mM sodium phosphate buffer, pH 7.6, and kept at 4 °C for 10 min. The preparation was kept at this temperature at all subsequent steps. After centrifugation, the packed cells were brought to 50% hematocrit with the isotonic sodium phosphate buffer and then diluted with 15 vol. of 10 mM sodium phosphate, pH 7.8, to lyse the cells.

The suspension was centrifuged for 20 min at 20 000 × *g*, the supernatant carefully removed and the pellet resuspended in 10 mM sodium phosphate and recentrifuged. The pellet was then suspended in 5 mM sodium phosphate, pH 7.8, the centrifugation repeated and the resultant pellet, which was essentially free of hemoglobin, was lyophilized and used for further analysis of the ghost preparation. Spectral measurements indicated that the ghost preparations contained less than 2% hemoglobin on protein basis.

Total lipids, phospholipids and cholesterol were determined before and after ghost preparation. In accordance with Dodge *et al.*[14] the lipid loss during ghost preparation was insignificant.

*Analytical procedures*

Determination of cholesterol[11,15] and of phospholipid content[11,16], thin-layer chromatographic analysis of phospholipids[11], and analysis of phospholipid fatty acids by gas–liquid chromatography[11,16], were conducted as cited. To aid in identification of fatty acid methyl esters, gas–liquid chromatograms were analyzed before and after hydrogenation. Protein determination of the ghost preparation was according to Lowry *et al.*[18]. For amino acid analysis, the ghost preparation obtained from 10 ml packed cells, were first extracted with chloroform–methanol to remove lipids and subsequently washed with 5 ml 0.1 M KCl, 5 ml of 0.05 M KCl and 5 ml of distilled water to remove water-soluble sugars. No protein was detected in these extracts. The protein residue was dried *in vacuo* and hydrolyzed in 6 M HCl in sealed tubes at 110 °C for 24 h. Amino acids were determined by chromatography with an amino acid analyzer (Beckman, Germany). Samples were analyzed twice, in duplicate, with identical results. Serine and threonine were both corrected for destruction. Tryptophan was detectable but its quantity was too small for calculation of percentage (Table IV).

## RESULTS

*Stability of camel erythrocytes*

In confirmation of previous reports[1,5–7], Fig. 1 shows that camel red blood cells exhibit unusually high osmotic stability. Furthermore, the protection against osmotic hemolysis afforded by added lipid to camel erythrocytes is much smaller than that commonly observed for other species[12,19]. Linolenoyl sorbitol, a synthetic lipid, was chosen for this experiment since it effectively stabilizes red blood cells in a wide range of hypotonic media (ref. 12 and unpublished observations). Fig. 1 demonstrates that addition of 3.3 $\mu$g/ml ($7.5 \cdot 10^{-6}$ M) of linolenoyl sorbitol causes a shift of 10 mM NaCl in the osmotic fragility curve for human erythrocytes, while the shift for camel erythrocytes amounts to only 3 mM NaCl. It can be seen from Fig. 2 that, for comparable protection, camel erythrocytes require 4 to 5 times as much added lipid as human erythrocytes.

The osmotic potential of camel erythrocytes was found to be 278 mosmoles. As this value is very similar to that of other species, the particular stability of camel erythrocytes is apparently related to membrane properties.

One such property could be an unusually slow hemolysis. In such a case it is anticipated that hemolysis conducted either rapidly or gradually should show

no appreciable difference. Yet, the osmotic fragility of camel erythrocytes was clearly lowered by slow hemolysis (Raz, A., and Livne, A., unpublished results), as already reported for other erythrocytes[13,20].

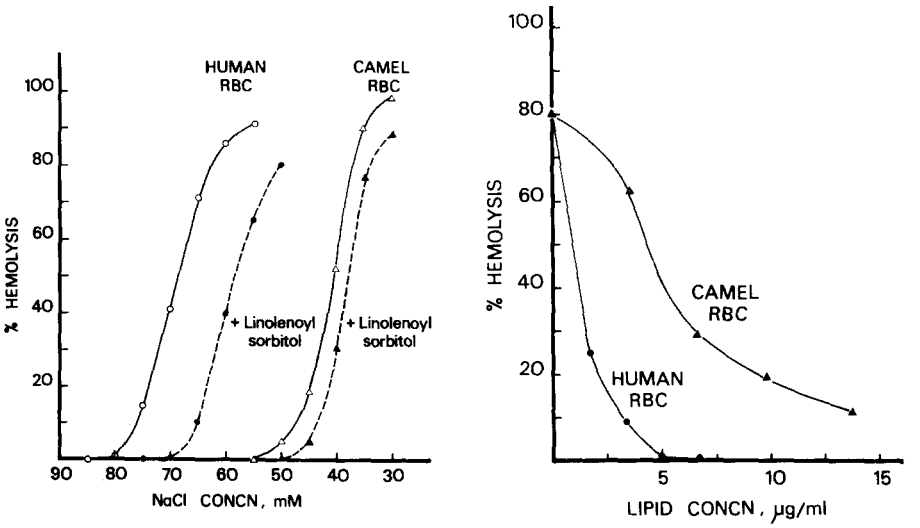


Fig. 1. Osmotic fragility of human and camel red blood cells (RBC). The effect of the addition of 3.3 µg/ml linolenoyl sorbitol preparation is presented by dashed lines. The data presented in the figure were obtained in five different experiments. The variation in fragility curves for both human and camel erythrocytes amounted to less than 1 mM.

Fig. 2. Effect of added lipid (linolenoyl sorbitol preparation) on osmotic stability of human and camel red blood cells (RBC). NaCl concentration: 63 mM for human cells and 36 mM for camel cells.

The unique stability of camel erythrocytes is also expressed following ultrasonic irradiation. Fig. 3 shows that the time course of sonic hemolysis of either human, sheep, hamster or rat erythrocytes is essentially identical. In contrast, camel erythrocytes had to be irradiated for about three times as long a period to obtain comparable hemolysis. This distinct difference is apparently not related to the erythrocyte size, since the erythrocytes of the four species with similar resistance do differ in size. Furthermore, the ratio of area to volume in the camel red cell is similar to that in the sheep red cell[5].

The differential effect of vinblastin, which is known to precipitate microfilament proteins[21], further illustrates the unusual properties of the camel erythrocyte membrane (Fig. 4). The osmotic fragility of human erythrocytes is markedly increased by 1 mM vinblastin, as already reported[22]. However, vinblastin, even at a 2-fold higher concentration, did not impair the osmotic stability of camel erythrocytes.

*Lipid composition of camel erythrocytes*

The total lipid content of camel erythrocytes (Table I), on the basis of packed cell volume or per cell, is similar to that found for other species[23]. The molar ratio

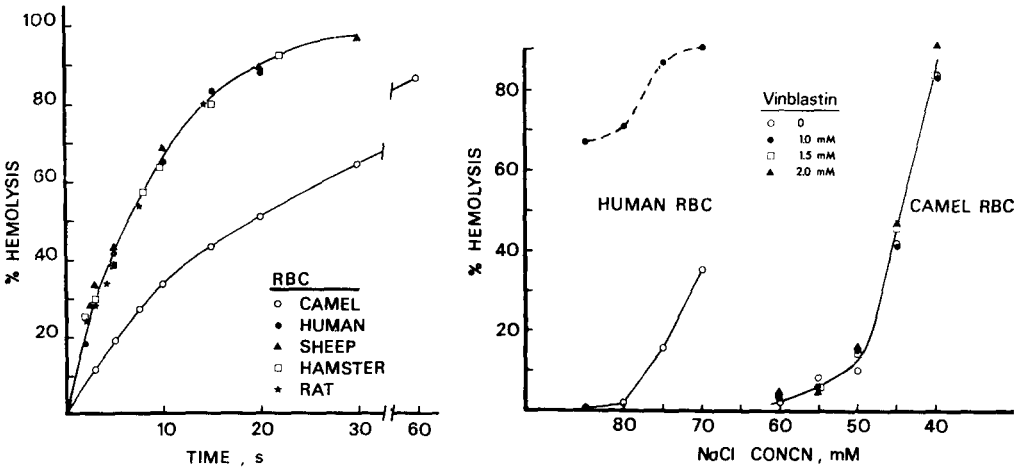


Fig. 3. Effect of ultrasonic irradiation on the hemolysis of erythrocytes of various animals. RBC, red blood cell.

Fig. 4. Osmotic fragility of human and camel erythrocytes following 1 h incubation with vinblastin. Similar results were also obtained after 2 h incubation. RBC, red blood cell.

TABLE I

TOTAL LIPIDS, PHOSPHOLIPIDS AND CHOLESTEROL OF CAMEL ERYTHROCYTES

| | *Units* | *Mean ± S.E.* |
|---|---|---|
| Total lipids | mg/ml packed cells | 6.05 ± 0.60 |
| | mg × $10^{-10}$/cell | 1.97 ± 0.21 |
| Phospholipids | $\mu$moles × $10^{-10}$/cell | 1.53 ± 0.08 |
| Cholesterol | $\mu$moles × $10^{-10}$/cell | 1.50 ± 0.08 |
| Phospholipids:cholesterol | molar ratio | 1.02 |

TABLE II

PHOSPHOLIPID COMPOSITION OF CAMEL ERYTHROCYTES

| | *% ± S.E.* |
|---|---|
| Sphingomyelin | 40.5 ± 1.9 |
| Phosphatidylserine | 22.4 ± 1.5 |
| Phosphatidylethanolamine | 21.9 ± 1.6 |
| Phosphatidylcholine | 11.8 ± 0.8 |
| Unidentified * | 3.4 |

* Ninhydrin negative, $R_F$ in thin-layer chromatography: 0.56 with the solvent mixture chloroform–methanol–ammonia (65:34:4, by vol.) and 0.88 with the solvent mixture chloroform–acetone–methanol–acetic acid–water (10:4:2:2:1, by vol.).

of phospholipid:cholesterol for camel erythrocytes is 1 as is the value found for other erythrocyte membranes.

Phospholipid composition of camel erythrocytes is presented in Table II. Sphingomyelin is the major phospholipid and together with phosphatidylcholine it comprises more than 50% of the total phospholipids. This observation is in line with the conclusion[24] that the sum of the choline-containing lipids is always in the range of 40 to 60%. It is noteworthy that the proportion of the acidic phosphatidylserine (Table II) is among the highest values reported.

The composition of the phospholipid fatty acids was of particular interest, in view of the possible role of the acyl groups in determining osmotic stability of erythrocytes[11,17]. However, Table III shows that the phospholipid fatty acid composition is within the range of acyl group distribution already reported[24–26]. Each of the phospholipids shows a distinctive and characteristic fatty acid pattern as pointed out for other red cells[24–26]. Furthermore, the choline phospholipids contain relatively more saturated fatty acids than the acidic phospholipids. Lignoceric (24:0) and nervonic (24:1) acids are present almost exclusively in the sphingomyelin as reported for several species[24–29].

*Protein content and amino acid composition of camel erythrocyte ghosts*

Ghosts of human erythrocytes have been studied to a great extent. Therefore, a comparison was made between the ghosts of human and camel erythrocytes which were prepared under identical conditions. The protein content per mg lipid (w/w) in human erythrocyte ghosts was 1.25, a value close to that reported[30]. However, the protein:lipid ratio of camel erythrocyte ghosts (3.0, w/w) was distinctly higher. Values for protein: lipid ratios for ghosts are probably minimal, as membrane

TABLE III

FATTY ACID COMPOSITION OF CAMEL ERYTHROCYTE PHOSPHOLIPIDS

| *Fatty acid* | *Percentage of total fatty acids* | | | |
|---|---|---|---|---|
| | *Phosphatidyl-choline* | *Sphingomyelin* | *Phosphatidyl-serine* | *Phosphatidyl-ethanolamine* |
| 16:0 | 25.6 | 15.1 | 2.4 | 7.4 |
| 18:0 | 24.0 | 20.1 | 31.0 | 12.4 |
| 18:1 | 15.0 | 11.0 | 49.0 | 46.3 |
| 18:2 | 25.0 | 3.2 | 15.4 | 28.7 |
| 20:0 | 0.8 | 1.1 | 0.5 | 1.0 |
| 20:1 | 0.6 | 0.6 | — | — |
| 20:2 | 0.7 | 1.0 | — | — |
| 20:3 | 0.7 | — | — | 0.6 |
| 20:4 | 5.1 | 6.2 | 0.6 | 3.1 |
| 22:0 | — | 2.2 | — | — |
| 22:2 | — | 4.1 | — | — |
| 22:5 | — | — | 0.5 | — |
| 22:6 | — | 2.1 | 0.3 | — |
| 24:0 | 2.5 | 24.6 | 0.3 | 0.5 |
| 24:1 | — | 8.7 | — | — |

proteins may be lost during the preparation of ghosts at low ionic strength, while stromal lipids are retained[14].

In case of human erythrocytes, the amino acid composition of the ghost protein (Table IV) was similar to that of previous reports[30,31]. The corresponding

TABLE IV

AMINO ACID COMPOSITION OF HUMAN AND CAMEL ERYTHROCYTE MEMBRANE PROTEINS

+, detectable.

| *Amino acid* | *Residues per 100 residues* | |
|---|---|---|
| | *Human* | *Camel* |
| Lysine | 5.5 | 5.7 |
| Histidine | 2.9 | 2.7 |
| Arginine | 5.0 | 6.0 |
| Aspartic acid | 8.1 | 7.9 |
| Threonine | 5.4 | 4.9 |
| Serine | 6.1 | 6.4 |
| Glutamic acid | 13.0 | 12.3 |
| Proline | 5.3 | 6.8 |
| Glycine | 6.5 | 6.4 |
| Alanine | 8.4 | 7.7 |
| Cystine (half) | 0.7 | 0.7 |
| Valine | 6.8 | 6.8 |
| Methionine | 2.6 | 2.0 |
| Isoleucine | 4.3 | 4.4 |
| Leucine | 11.8 | 12.2 |
| Tyrosine | 2.7 | 2.6 |
| Phenylalanine | 5.0 | 4.5 |
| Tryptophan | + | + |

composition of the camel erythrocyte ghosts is more basic in view of a higher proportion of arginine and a lower proportion of glutamic acid. Furthermore, a greater abundance of proline is observed in the camel ghost preparation.

## DISCUSSION

The unique stability of camel erythrocytes has been established under osmotic stress (Fig. 1) as well as under mechanical stress of sonic irradiation (Fig. 3). While the osmotic stability relates to the capability of the membrane to expand in area[20], the mechanical stability refers to forces applied to the cell externally. What constitutes this unusual stability of the erythrocyte membrane? As lipids and proteins are the major membrane components, the role of each of these constituents should be examined. The stability of camel erythrocytes is apparently not related to some unusual characteristics in the lipid profile as the analyses (Tables I–III) did not show any outstanding feature of the lipid components. Therefore, attention must be focused on the protein moiety and its interaction with lipids.

The resistance of camel erythrocytes to vinblastin (Fig. 4) indicates differences in the membrane proteins, in comparison with human erythrocytes. However, the interpretation of this resistance is at present equivocal, as several alternative explanations are still open: (a) vinblastin does not reach the site of its effect (presumably at the inner face of the membrane[32]); (b) camel erythrocytes do not contain microfilamentous proteins; and (c) camel erythrocytes are particularly rich in spectrin, or in spectrin-like proteins. These proteins may have to be titrated with a particularly high dose of vinblastin to induce osmotic fragility.

Amino acid distribution of a composite membrane protein is not a sensitive measure to delineate differences between membrane proteins, particularly since different membranes may have similar composition[31]. Yet, distinct differences in amino acid composition, as shown in Table IV, may point to some profound structural features of the camel erythrocytes. In comparison with human erythrocytes, a stronger electrostatic attraction between lipids and proteins is indicated by the acidic nature of the phospholipids (Table II) and the more basic nature of the stromal proteins (Table IV). The unusually high protein:lipid ratio of the camel erythrocyte ghosts is in line with a strong protein–lipid interaction.

The elevated proportion of proline in the camel erythrocyte stroma may have a pronounced impact on the membrane conformation. Indeed, proline is known to alter protein conformation drastically[33], and collagen, which is rich in proline, is a particular expression of this phenomenon. The exceptional structural configuration of the lipoprotein in the camel erythrocyte has yet be to further defined and characterized. It may account for the specific features of the camel erythrocytes: (a) unique oval shape[5,9]; (b) particular stability; (c) low affinity for added lipid (Figs 1 and 2); and (d) low degree of extractability of phospholipids by ether–methanol mixtures[9].

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