J.Cell.Mol.Med. Vol 4, No 4, 2000 pp. 270-276

Comparative studies of the protein composition of red blood cell membranes from eight mammalian species

H. Matei, L. Frentescu, Gh. Benga*

Department of Cell and Molecular Biology,
"Iuliu Hatieganu" University of Medicine and Pharmacy
Cluj-Napoca, Romania

Received: November 2, 2000; Accepted: December 5, 2000

Abstract

The polypeptide pattern of red blood cell (RBC) membranes from cow, sheep, horse, rabbit, guinea pig, rat, mouse, analyzed by polyacrylamide gel electrophoresis, was compared to human RBC counterpart. Some qualitative and quantitative differences were noted. Among the high molecular weight components the bands 2.1-2.3 appeared slightly decreased in rabbit and rat and increased in sheep RBC membranes. Band 3 appeared to have a higher molecular weight in the cow, guinea pig and mouse RBCs, and a lower molecular weight in the sheep RBCs. Band 4.1 from the RBC membranes of cow, sheep, rabbit and guinea pig was splitted into two sub-bands, while band 4.2 overlapped with band 4.1 in horse and guinea pig RBC membranes. There are marked differences in the number and position of bands in the 4.5 region, while band 4.9 is present in higher amounts in horse, rabbit and guinea pig RBC membranes. Band 6 (glyceraldehyde 3-phosphate dehydrogenase) was undetectable in horse, rat and mouse RBC membranes and was decreased in sheep, rabbit and guinea pig. There are also major differences in the region of band 7 and below ("post-7"). Band 8 was undetectable in horse, cow and guinea pig, and was in higher amounts in rat. A band corresponding to a molecular weight of about 22 kD in the "post-8" region was present only in guinea pig RBC membranes.

Keywords: red blood cell - membrane proteins - mammalian

Introduction

A primary function of red blood cells (RBCs) is the transport of oxygen from the lungs to other tissues and to return carbon dioxide from the tissues to the lungs, in processes mediated by the binding of these gases to haemoglobin. The confinement of the haemoglobin to the inside of the cell membrane ensures its retention in the vascular system [1]. This function, among others, identifies the plasma membrane of the RBC as being of major physiological importance. Much is known about the plasma

*Correspondence to: Dr. Gh. BENGA, PhD
Department of Cell and Molecular Biology,
"Iuliu Hatieganu" University of Medicine and Pharmacy
6 Pasteur St., 3400 Cluj-Napoca, Romania
Phone/Fax: 40-64-194373
E-mail: gheorgheb@hotmail.com

membrane of the human RBC than about any other eukaryotic membrane. There are a number of reasons for this: a) the availability of RBCs in large numbers (from blood banks, for example) uncontaminated by other cell types; b) the plasma membrane is the only membrane of RBCs and consequently it can be isolated without contamination by internal membranes; c) a number of diseases have been associated with defects in the structure of RBC membrane. Although the RBC membrane is the most-studied plasma membrane there are still some features that are not fully understood, for example its very high permeability for water (for reviews see Macey [2]; Benga [3]; Verkman and Mitra [4]). This has been explained by the presence in the RBC membrane of water channel proteins, called aquaporins. Our earlier labeling experiments with a radioactive chloromercuribenzensulfonate (a powerful inhibitor of water permeability) pointed to polypeptides migrating in the band 4.5 region on the electrophoregrams of RBC membrane proteins as accommodating water channels [5, 6]. Agre and coworkers [7] have shown that in this region migrates the glycosylated form of an integral membrane protein that was called CHIP 28, from channel-forming integral membrane protein of 28 kD, the first identified water channel protein, now called aquaporin 1 (AQP1). It has been characterized in detail (for reviews see Agre et al [8]) and its three dimensional structure at 3.8 Å resolution was recently described [9]. Despite these important advances in understanding the structural determinants of water permeation through aquaporins the physiological role of the high water permeability of RBC membrane is not fully understood. A classical approach to understanding structure-function relationships in biological systems is though the comparison among different selected species. Consequently, we have performed a programme of comparative biochemical and nuclear magnetic resonance investigations of water diffusional permeability of RBCs from laboratory, domestic and wild animals [10-20]. Taking into account all present knowledge regarding the role of proteins in mediation of water transport across the RBC membrane a detailed analysis of the protein composition of this membrane is warranted. In this paper we compare the polypeptide pattern of RBC membrane proteins of eight mammalian species for which the characteristics of water permeability were previously reported.

Materials and methods

Samples of blood were collected into heparinized tubes and used in the same day. The RBCs were isolated by centrifugation and washed three times in 150 mM NaCl, 5.5 mM glucose, 5 mM Hepes [4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid], pH 7.4. The washed cells were hemolysed in 20 vol. of 5 mM phosphate buffer, pH 8.0, (5P8) and the membranes ("ghosts") were collected by centrifugation at 13150 g for 15 min. through 5P8 at 2-4ºC. The membranes were washed free of haemoglobin by repeated centrifugation through 5P8 until the pellet was white or pale pink in color. The pellet was suspended in 5P8 at a protein concentration of 1 mg/ml. The protein amount was determined by the procedure of Lowry et al [21]. Membrane peptides were separated using sodium dodecylsulfate (SDS) polyacrylamide system described by Laemmli [22] using a Bio-Rad PROTEAN II xi Cell (Bio-Rad Laboratories, Hercules, California, USA). The slab gel consisted of a running gel of 12% acrylamide and 5% staking gel. The acrylamide-to-bisacrylamide ratio was maintained at 36.5:1 in both gels. Samples of 30 ìl/30 ìg protein were applied and the electrophoresis was carried out at 15 mA (40V) for 1.5 hr and at 20 mA (100V) until the dye reached the bottom of the running gel (about 15 hours) in the running buffer (25mM Tris, 190 mM Glycine, 0.1% SDS). The gels were fixed for 1 hr in 45% (vol./vol.) methanol/10% (vol./vol.) acetic acid and then stained 15 min in the solution containing 0.07% (wt./vol.) Coomassie brilliant blue R-250. Destaining was performed with 10% (vol./vol.) acetic acid. The relative amounts of membrane polypeptides, were calculated from densitometric scans obtained using a Bio-Rad GS 700 Imaging Densitometer (Bio-Rad Laboratories, Hercules, California, USA), assisted by a PC with Analyst 1 software.

The protein fractions separated were labelled according to the nomenclature proposed by Fairbanks et al. [23].

Band number	Human	Horse	Cow	Sheep	Rabbit	Guinea pig	Rat	Mouse
1 + 2	26.6±0.6	20.9±1.6*	22.9±1.0	22.3±1.6	18.6±0.5	21.9±1.4	23.0±0.5	24.1±1.6
2.1 + 2.3	4.5±0.2	7.5±0.9	4.9±1.7	11.3±0.2*	2.1±0.2*	4.6±0.5	2.4±0.3*	4.2±0.6
3	29.7±1	21.5±0.6*	27.8±0.7	23.4±0.3*	23.8±0.8*	26.0±0.1	25.3±1.8	25.4±0.6
4.1	6.1±0.9	9.6±0.3	5.3±0.6	5.6±0.6	6.6±0.3	12.4±06	7.8±0.4*	5.6±0.5
4.5	6.1±0.5	10.9±0.6*	8.2±0.7	6.0±0.4	9.0±1.7	8.3±1.8	10.2±1.4*	6.9±0.3
4.9	1.3±0.2	3.8±0.3*	1.2±0.3	1.8±0.2	5.1±0.7*	5.0±0.5*	2.1±0.7	1.5±0.3
5	8.3±0.8	6.9±0.4	6.6±0.3	7.1±0.3	6.5±0.3	6.2±0.5	5.9±0.9	5.7±0.3
Post 5	-	5.1±0.6	-	-	-	-	-	-
6	4.4±0.3	-	5.0±0.3	1.9±0.2*	2.7±0.1*	3.2±0.4*	-	-
6.9	0.7±0.1	0.9±0.2	1.8±0.3	2.0±1.2	0.9±0.2	0.4±0.1	-	0.3±0.02
7	2.3±0.3	4.3±0.7	2.6±0.3	2.7±0.3	1.9±0.2	3.3±0.1	1.2±0.5	1.0±0.04*
Post 7	-	4.1±0.2	-	-	4.0±1.1	1.8±0.5	4.9±1.2	6.4±1.1
8	0.9±0.3	-	0.9±0.6	1.9±0.1	1.2±0.92	-	4.2±0.4*	2.8±0.5
Post 8	-	0.7±0.02	0.4±0.2	0.3±0.3	0.2±0.1	3.4±0.6	0.2±0.2	2.4±0.1
9	-	0.6±0.03	-	-	-	0.4±0.2	0.2±0.01	-
Front	0.5±0.3	2.5±0.1	3.4±0.2	3.6±0.4	5.3±0.9	2.2±0.3	3.7±0.1	5.3±0.7

Results

We have compared the membrane polypeptide electrophoretic pattern of several mammalian RBCs with their human counterpart (Fig. 1 and Table 1). Although the general pattern of membrane proteins is very similar in all mammalian RBCs there are some qualitative and quantitative differences.

In regard with bands 1 and 2 (spectrins) the resolution of high molecular weight polypeptides is not highly accurate in the 12% acrylamide, so that the apparent decreased amount of spectrins in the non-human RBC membranes must be considered cautiously.

The bands 2.1-2.3 appeared decreased in rabbit and rat and slightly decreased in mouse RBC membranes.

There are obvious differences in the position along the gel and the diffuseness of band 3 (the anion exchanger); these reflect differences in the molecular weight and glycosylation of this major protein. Band 3 appeared to have a higher molecular weight (around 100 kD) in the cow, guinea pig and mouse RBCs and a lower molecular weight (around 90 kD) in the sheep RBC.

Bands 4.1 showed a splitting into two sub-bands in the RBC of cow, sheep, rabbit and guinea pig, while band 4.2 had a lower mobility in horse and guinea pig RBC membranes, so that sometimes it overlapped with band 4.1.

There are marked differences in the number and position of bands in the 4.5 region, while band 4.9 is present in higher amounts in horse, rabbit and guinea pig RBC membranes. Band 6 (glyceraldehyde 3-phosphate dehydrogenase) was undetectable in horse, rat and mouse RBC membranes and was decreased in sheep, rabbit and guinea pig. There are also major differences in the region of band 7 and below ("post-7"). Band 8 was undetectable in horse, cow and guinea pig, and was in higher amounts in rat. A band corresponding to a molecular weight of about 22 kD in the "post-8" region was present only in guinea pig RBC membranes.

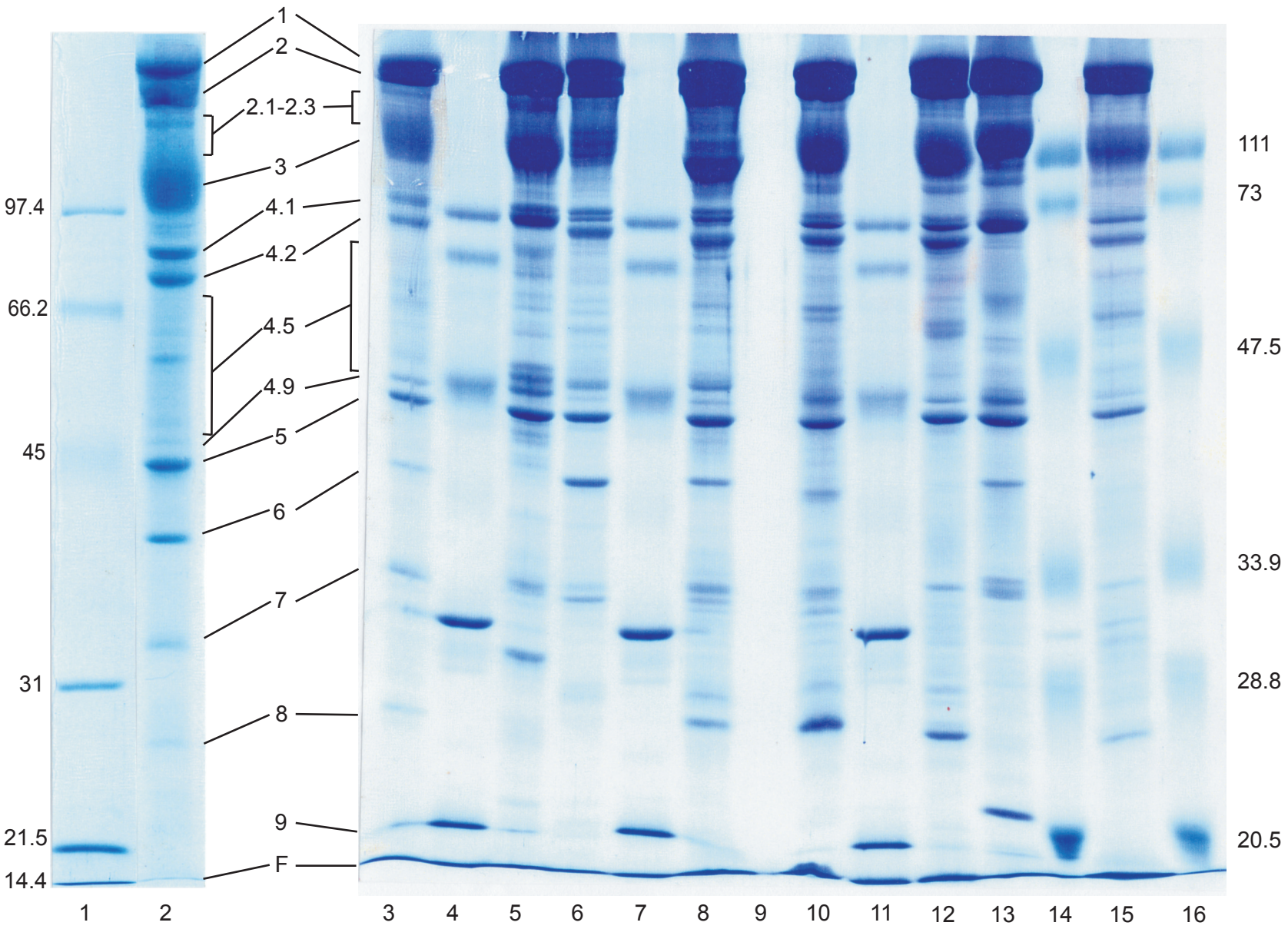
Discussion

Since the introduction of polyacrylamide gel electrophoresis in the presence of dodecyl sulfate, the proteins of human RBC membrane have been extensively studied, the functions of many of them elucidated, and the organization of the membrane cytoskeleton and its atachement to the membrane revealed. In contrast, knowledge of the composition and structure of RBCs from other species is less extensive. Lenard [24] performed some comparative experiments with several different species, but at the time their work was made, caracterization of the human RBCs membrane proteins was incomplete and resolution of the different proteins had not reached the stage currently possible.

Ballas [25] described the comparative distributions of glyceraldehyde 3-phosphate dehydrogenase activity and the relative amounts of membrane proteins in RBCs of guinea pig, rabit, rat and mouse. However, he has used 4% polyacrylamide and consequently obtained only a good resolution of high molecular weight polypeptides from the RBC membrane.

We have used 12 % polyacrylamide in order to get a good resolution of the medium and low molecular weight region of the electroforegram, since aquaporins migrate in this region. It is for the first time that analyses of RBC membrane proteins from eight mammalian species are performed simultaneously in one laboratory. Moreover, a powerful system for densitometric analysis of electrophoregrams was used. The results are in good agreement with previous reports from different laboratories, including our own [10, 17, 24, 25]. However, it should be emphasized that more marked differences in the protein electrophoretic pattern were noted in the regions of bands 4.5 and below band 7 where migrate the glycosylated and nonglycosylated forms of the aquaporin 1, respectively. This suggest that the differences in the water permeability of the RBCs from various species are due to differences in the protein components

Table 1 Distribution of proteins among the electrophoretic bands pattern of the different species red blood cell membranes. The samples were prepared and analysed as described in "Materials and methods". The results are expressed as % protein in the bands numbered essentially according to Fairbanks et al.(1971). The results are expressed as means ± S.D. for 3-5 determinations. * $p<0.05$


1
2
2.1-2.3
3
4.1
4.2
4.5
4.9
5
6
7
8
9
F
97.4
66.2
45
31
21.5
14.4
111
73
47.5
33.9
28.8
20.5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

involved in water diffusion. Further studies of this topic appear very important in order to clarify the physiological significance of the high water permeability of red blood cells and to explain the differences of process among various species.

Acknowledgments

We thank Ms. Romana Vulturar, Mr. Adrian Florea and Mr. Tudor Ponoran (Department of Cell and Molecular Biology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania) for assistance with experimental and secretarial work. We also thank the National Council for Higher Education Scientific Research of Romania (grant 219) for financial support.

References

1. **Guyton A.C.,** Human physiology and mechanisms of diseases. 4th edition, W.B. Saunders, Philadelphia, pp. 37, 1987
2. **Macey R.I.,** Transport of water and urea in red blood cells, *Am. J. Physiol.,* **246**:195, 1984
3. **Benga Gh.,** Water transport in red blood cell membranes, *Prog. Biophys. Mol.,* **51**:193, 1988
4. **Verkman A.S., Mitra A.K.,** Structure and function of aquaporin water channels, *Am. J. Physiol.,* **278**:13, 2000
5. **Benga Gh., Popescu O., Borza V., Pop V.I., Muresan A., Mocsy I., Brain A., Wriggleswoth J.**, Water permeability of human erythrocytes. Identification of membrane proteins involved in water transport, *Eur. J. Cell. Biol.,* **41**:252, 1986
6. **Benga Gh., Popescu O., Pop V.I., Holmes R.P.,** Chloromercuribenzensulfonate binding by membranes proteins and the inhibiton of water transport in human erythrocytes, *Biochem.,* **25**:1535, 1986
7. **Preston G.M., Caroll T.P., Guggino W.B., Agre P.,** Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein, *Science,* **256**:385, 1992
8. **Agre P., Preston G.M., Smith B.L. et al.,** Aquaporin CHIP: the archetypal molecular water channel, *Am. J. Physiol.,* **265**:F463, 1993
9. **Murata K., Mitsuoka K., Hirai T., Walz T., Agre P., Heymann J.B., Engel A., Fujiyoshi Y.,** Structural determinants of water permeation through aquaporin-1, *Nature,* **407**:599, 2000
10. **Benga Gh., Borza T., Popescu O., Porutiu D., Matei H.,** Comparative nuclear magnetic resonance studies of red blood cells from sheep and cow, *Comp. Biochem. Physiol.,* **104**:589, 1993
11. **Benga Gh., Chapman B.E., Gallagher C.H., Agar N.S., Kuchel P.W.**, NMR studies of diffusional water permeabiliy from eight species of marsupial, *Comp. Biochem. Physiol.,* **106**:515, 1993
12. **Benga Gh., Chapman B.E., Gallagher C.H., Cooper D., Kuchel P.W.,** NMR studies of diffusional water permeability of red blood cells from Macropodid marsupials (Kangaroos and Wallabies), *Comp. Biochem. Physiol.,* **104**:799, 1993
13. **Benga Gh., Matei H., Borza T., Porutiu D., Lupse C.,** Comparative nuclear magnetic resonance studies on water diffusional permeability of red blood cells from mice and rats, *Comp. Biochem. Physiol.,* **104**: 491, 1993
14. **Benga G., Matei H., Borza T., Porutiu D., Lupse C.**, Comparative nuclear magnetic resonance studies of diffusional water permeability of red blood cells from different species. V--Rabbit (Oryctolagus cuniculus), *Comp Biochem Physiol B.,* **106**:281-5, 1993

Fig.1 Electrophoretic pattern of membrane polypeptides from red blood cell membranes of eight mammalian species. The samples were prepared and analysed as described in Materials and methods. The molecular weight of markers are given in kdaltons. The nomenclature devised by Fairbanks et al. [23] is used to identify membrane proteins, and F represents the migration of the tracking dye. Lanes 1, 4, 7, 11: molecular weight markers (from Bio-Rad: Phosphorylase B: 97.4 kD; Bovine serum albumin: 66.2 kD; Ovalbumin: 45 kD; Carbonic anhydrase: 31 kD; Soybean trypsin inhibitor: 21.5 kD and Lysozyme: 14.4 kD). Lanes 14, 16: prestained molecular weight markers (from Bio-Rad: Phosphorylase B: 111 kD; Bovine serum albumin: 73 kD; Carbonic anhydrase: 33.9 kD; Soybean trypsin inhibitor: 28.8 kD and Lysozyme: 20.5 kD). Lanes 2, 4: man, lane 5: horse, lane 6: cow, lane 8: sheep, lane 10: rabbit, lane 12: rat, lane 13: guinea pig, lane 15: mouse.

15. **Benga Gh., Chapman B.E., Hinds L., Kuchel P.W.,** Comparative NMR studies of diffusional water permeability of erythrocytes from some animals introduced to Australia: rat, rabbit and sheep, *Comp. Haematol. Int.*, **4**:232, 1994
16. **Benga Gh., Ralston G.B., Borza T., Chapman B.E., Gallagher C.H., Kuchel P.W.,** Diffusional water permeability of red blood cells from Echidna (*Tachyloglossus aculeatus*), *Comp. Biochem. Physiol.*, **107**:45, 1994
17. **Benga Gh., Borza T., Matei H., Hodor P., Frentescu L., Ghiran I., Lupse C.,** Comparative nuclear magnetic resonance of water permeability of red blood cells from different species. VIII. Adult and fetal guineea pig (*Cavia porcellus*), *Comp. Haematol. Int.,* **5**:106, 1995
18. **Benga Gh., Matei H., Chapman B.E., Bulliman B.T., Gallagher C.H., Agar N.S., Kuchel P.W.,** Comparative nuclear magnetic resonance studies of diffusional water permeability of red blood cells from different species. IX. Australian feral chicken and domestic chicken (*Gallus domesticus*), *Comp. Haematol. Int.,* **6**:92, 1996
19. **Benga, Gh., Grieve, S.M., Chapman, B.E., Gallagher, C.H., Kuchel, P.W.,** Comparative NMR studies of diffusional water permeability of red blood cells from different species. X. Camel (*Camelus dromedarius*) and alpaca (*Lama Pacos*), *Comp. Haematol. Int.,* **9**:43, 1999
20. **Benga Gh., Kuchel P.W., Chapman B.E., Cox G.C., Ghiran I., Gallagher C.H.,** Comparative cell shape and diffusional water permeability of red blood cells from Indian elefant (*Elephas Maximus*) and man (*Homo sapiens*), *Comp. Haematol. Int.,* **10**:1, 2000
21. **Lowry O.H., Rosebrough N.V, Farr A.L., Randall R.J.,** Protein measurement with the Folin phenol reagent, *J. Biol. Chem.,* **193**:265, 1951
22. **Laemmli U.K.,** Cleavage of structural proteins during the assembly of the head of bacteriophage T_4, *Nature,* **227**:680, 1970
23. **Fairbanks G., Steck T.L., Wallach D.F.H.,** Electrophoretic analysis of the major polypeptides of the human erytrocyte membrane, *Biochemistry,* **10**:2606, 1971
24. **Lenard B.,** Protein components of erythrocyte membranes from different animal species, *Biochemistry,* **9**:5037, 1970
25. **Ballas S.K.,** Comparative distribution of glyceraldehyde 3-phosphate dehydrogenase activity in human, guinea-pig, rabbit and mouse erythrocytes, *Comp. Biochem. Physiol.,* **87**:837, 1987