Erythrocyte osmotic fragility and blood indices in the one-humped camel (Camelus dromedarius): Correlations with age and sex

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SYMBOLS AND ABBREVIATIONS

RBC = Total Erythrocytic Count ($10^6/mm^3$)

WBC = Total Leucocytic Count ($10^3/mm^3$)

Hb = Hemoglobin (gm/dl)

PCV = Packed Cell Volume (%)

MCV = Mean Corpuscular Volume (μm^3)

MCH = Mean Corpuscular Hemoglobin (pg = 10^{-12} gm/ml)

MCHC = Mean Corpuscular Hemoglobin Concentration (%)

N = Neutrophil

L = Lymphocyte

M = Monocyte

E = Eosinophil

$p < 0.05$

* = Statistical interaction

± SE = Standard Error

H = Fractions of cells hemolyzed

X = Percent of sodium chloride concentration

X50 = Mean of erythrocyte fragility

β = Breadth of erythrocyte fragility distribution

INTRODUCTION

Order Artiodactyla.

Sub-order Tylopoda.

Family Camelidae

Six species in 3 genera (including 3 domesticated species).

Distribution SW Asia, Middle East, N Africa, Mongolia, Andes.

Members of the camel family, which are even toed ruminants, are among the principal large mammalian herbivores of arid habitats and they make a crucial contribution to man's existence and survival in desert environments. The domesticated one-humped dromedary of the Middle East, India, SW Asia and North Africa, and the two-humped Bactrian camel, which is still found wild in the Mongolian steppes, are well-known. Living members of the Camilidae include in South America four species: the "lamoids" or "cameloids". Of these, the vicuna and guanaco are wild and the llama and alpaca are domesticated.

Several hypotheses have been introduced as to its first domestication: was it before 2000 BC in central or southern Arabia, or was it as others believe as early as 4000 BC. Some place it between the 13th and 12th centuries BC. From Arabia it believed

that domesticated camels spread to Egypt and North Africa and later to East Africa and India (Macdonald, 1984).

The word "Dromedary" has been held by some to denote any one-humped camel. The French and Linnaeus (Leese, 1927) have given the Arabian species the scientific name of Camelus dromedarius. The Greek word "dromados," meaning "running" seems to be the origin of the word. Others, however, used the word exclusively in connection with the riding-camel of the one-humped species and not for the baggager or pack-animal.

From a study of the history and the distribution of the camel, it is plain that the camel can be acclimatized and made useful in arid countries with a hot summer, even though the winter may be fairly cold, However, camels do not do well in a damp climate.

There are about 17,000,000 camels in the world, mainly in the desert and semi-desert areas of northern Africa, Arabia and parts of Asia and the USSR. In these areas, the camel fulfils an economic need in terms of either transport or food production. Its unique powers of endurance in drought conditions and an ability to survive on poor fibrous vegetation, makes it well suited to such areas (Higgins, 1985). Camels play a special role in social customs and rituals. They are required as gifts for marriage and reparation for injury and murder. White camels are especially valued for their meat, wool and milk which amounts to approximately to 1.3 gallons (6 litres) per day for 9-18 months.

They walk 19 miles (30 km) a day at a leisurely pace to allow for feeding and resting. Camels can carry a load of 500lb - 600lb. for 25-30 miles a day for three days without drinking. Camels eat a wide variety of plants including thorns, dry vegetation, and salt bush that other mammals avoid (Macdonald, 1984). Since high roughage diets are often low in essential nitrogen, the camel must also be an efficient nitrogen conserver. This is accomplished by limiting nitrogen excretion in urine, and recycling nitrogen as urea into the rumen via the saliva. High ruminal levels of nitrogen also increases the rate of active fermentation. This urea recycling is more efficient when compared to the other domestic ruminants such as the cow, sheep or goat. Rumen contents are high in volatile fatty acids, but the absence of ruminal papillae slows their absorption. The relative proportion of volatile fatty acids is similar to that found in cattle and sheep on equivalent high-roughage, low-nitrogen diets. Dromedaries on free range will eat more they immediately need and can store substantial fat reserves, largely in the hump, to endure subsequent drought conditions (Higgins, 1985). The hump of the Arabian camel may contain as much as 100 lb of fat, each pound of which can yield 1.1 lb of water, or over 13 gallons for a 100 lb hump. To convert this, however, extra oxygen is needed and it has been calculated that the breathing needed to get this extra oxygen would itself lead to the loss of more than 13 gallons of water as vapor in the breath. The fat stored in the hump is broken down to supply energy, releasing water which is lost. The hump is thus really a reserve of energy (Burton and Burton, 1981).

Camels conserve water by producing dry feces and little urine. Their day-time body temperature rises by as much as 11-14.5ºF (6-8ºC) during hot weather to diminish the need for evaporative cooling by sweating, although sweat glands occur over most of their body for use when necessary. Their nostrils, which can be closed to keep out blowing sand and their nasal cavities, reduce water loss by moistening inhaled air and cooling exhaled air. A thick fur and underwool provide warmth during cold desert nights and some daytime insulation against the heat. Other adaptations that enable them to survive in deserts and other unfavorable environments include double rows of heavy protective eyelashes, haired ear openings, and keen senses of sight and smell (Macdonald, 1984).

Camels can endure long periods without water (up to 10 months if not working) and so can graze far from the oasis. They can exist on dry food for two weeks or more, depending on the temperature, because they tolerate a much greater depletion in body water than most other mammals. A camel may lose about 30% of its weight in body water without ill effects, as compared to about 12% in man. This remarkable water metabolism is largely accounted for by water retention in the rumen, excretion of concentrated urine through specialized kidneys and the ability to allow for a high body temperature rise before sweating. A low respiratory rate also helps to minimize evaporative losses (Higgins, 1985). When they drink, they can consume 30 gallons (136 litres) within a short time. The body fluids rapidly become diluted to an extent that could not be tolerated by other mammals. Absorbing large volumes of water into the blood in a

short space of time leads to hemolysis. This occurs in human red cells and the cells of most other mammals in saline solutions diluted to 0.5% (Yagil, 1985). Hemodilution is one of the causes of secondary death after large volumes of water are swallowed by a person floundering in a river or pool. It has been proven that no water has entered the lungs, but death was due to hemolysis following a large and rapid influx of water into the blood (Yagil, 1985).

The objectives of this study were to determine age-sex related changes in camel erythrocyte osmotic fragility and the dimension and indices of the cells which contribute to alterations in the cell's isotonic shape. Different characteristics of the red cell have also been included in the correlation and the results are herein reported.

LITERATURE REVIEW

The hematology of the camel has been extensively studied (Ponder, 1948; Durand and Kehouk, 1959; Bartels et al., 1963; Perk , 1963; Little et al., 1970; Ghosal et al., 1973; Sharma et al., 1973; Yagil et al., 1974 a,b,c; Ateeq, 1984; AlAli et al., 1988; Abdalla et al., 1988; Livne and Kuiper,1973; Knight et al., 1994).

The resistance of camels to hemodilution and hemoconcentration has stimulated interest in whether camel red cells undergo changes similar to those of ruminants, avian or human cells when they are placed in increasing and/or decreasing salt concentrations. Although a number of investigations have been published to introduce an answer to this question (Yagil, 1985 ; Mirgani, 1992 and ElMougy et al., 1993), yet, in view of the functional uniqueness of the erythrocytes of the camel, their study is of continual interest.

The usual fragility testing involves hemolysis of whole - blood samples over a range of osmotic pressures introduced by a series of different concentrations of NaCl solutions. From the response of the erythrocytes to a hypotonic insult, the test can be used to monitor the general body homeostasis. This was because the erythrocytes are influenced not only by the metabolic status of the cells but also by the composition of the plasma (Hussain and Voaden, 1985).

The erythrocyte fragility test has previously been introduced to measure erythrocyte tensile strength. This test is useful to detect clinically hypochromic congenital hemolytic anemia (Detragila et al., 1974).

Hemolysis curves (percent hemolysis vs NaCl concentration) are built to symbolize the cumulative frequency distribution of individual erythrocyte fragilities present in the sample. The NaCl concentration which produces 50% hemolysis is taken as a measure of the mean erythrocyte fragility (Detraglia et al., 1974).

The uniqueness of the camel erythrocytes among mammalian types lies in its elliptical, rather than circular shape (Turner et al., 1958; Perk et al., 1964; Schalm, 1965; and Yagil et al., 1983). This elliptical shape is of functional importance and gives members of the family Camelidae protection against the shearing pressure of excessive hydration and lysis after a period of dehydration. (Schmidt-Nielsen et al., 1956; Perk, 1963; Perk et al., 1964; Ponder, 1948; Schroter et al., 1990). The osmotic fragility of camel erythrocyte is markedly low and the cells are capable of swelling in hypotonic media to over twice their volume (Perk, 1963; Perk et al., 1964; Perk, 1966; Yagil, 1985; and El.Mougy et al., 1993). Yagil (1985) found that when camel erythrocytes were placed in saline solution ranging from 0.9 to 0.1 %, many camel red cells remained normal in 0.2% saline solution although their area had increased by 4%. On the other hand Perk (1966) and El.Mougy et al. (1993) found that total hemolysis occurred in saline solutions less than 0.2%.

Both an increase and a decrease in the cell volume was implicated to be a factor causing the blood cells more susceptible to hemolysis in most animal species (Perk et al., 1963; Yagil et al., 1974b). However, camel red blood cells are resistant to the effect of volume changes. Yagil et al. (1974 b)and El.Mougy et al. (1993) found that cell surface area decrease between 20 to 26% were still as osmotically stable as normal cells. For hemolysis to occur in camel erythroytes, an increase to over 196% of their original volume was recorded by Perk (1963) and Perk et al.(1964) compared to the 150% in the erythrocytes of the common domestic animals. In dehydration, the camel erythrocyte's surface area decreased by 26% while their number remained constant, this was accompanied by a drop in hemoglobin content. The reduced in cell surface area for gas exchange resulted in a drop in the blood PO_2 (Yagil , 1985).

The circulating camel red blood cells have a life span that ranges from 90 days in the winter to 120 days in summer. Many changes take place in enzyme activities, biophysical properties, and functional properties during the life span of the erythrocyte. Most of these changes occur shortly after the cell enters the circulation during the conversion from a reticulocyte, which still synthesizes protein, to a mature erythrocyte with no protein synthesis. There are additional changes which take place as the mature cell circulates, and some are responsible for the removal of the senescent cell from the circulation. Consequently, a blood sample contains a distribution of erythrocytes with different properties (Rifkind, et al., 1983). These cells are amenable to deformation, and as they are transported by blood, they maintain a perpendicular orientation to the

direction of the flow. Erythrocyte membranes are not stationary under fluid shear stress (Schmidt - Schonbein and Wells, 1969). Interestingly, these cells loose this property after fixation with glutaraldehyde (Agar and Board, 1983). Fixation of the erythrocyte membrane and hemoglobin by glutaraldehyde stops the hemoglobin movement and thus prevents orientation of cells in the stress field (Smith et al., 1979).

Strength of biological membranes and their stability are one of the major topics currently under investigation in biomembrane research. The camel erythrocyte membrane is of particular interest because of its unique properties. They are distinctly more stable to the lytic effects of sonic irradiation and vinblastin. The resistance of camel erythrocytes to vinblastin indicates differences in the membrane proteins. Recent published analysis showed that the lipid composition of the camel erythrocyte membrane is similar to that of other species, but that the protein / lipid ratio (3:1 in camel erythrocytes compared to 1.25:1 in human cells) is significantly higher in the camel (Eitan et al., 1976). These authors suggested that the resistance to osmotic lysis may be partially attributed to the increased protein content of the camel membranes. Ralston (1975) reported that the major proteins of the camel erythrocyte membrane are similar to those of the human and bovine species; but with a major difference in the major intrinsic membrane water- soluble protein "spectrin" which appears to be very tightly bound to the camel erythrocyte membrane. Concurrent with the total release of spectrin, the camel cells undergo a shape change from flat ellipsoids to spheres, suggesting an important shape - maintaining role for spectrin in the erythrocytes of this species.

An aging study of erythrocyte fragility in human was done to investigate changes which may happen in red cell membranes. Osmotically induced hemolysis study was performed on normal whole blood from young and old people by a modification of the method developed by Good in 1971 . The results suggested that the mean erythrocyte fragility and the distribution of fragilities (i.e., the distribution of types of erythrocytes) of a human blood sample vary as a function of age (Detraglia et al., 1974). In addition, human erythrocyte osmotic fragility varied with age of the donor and the turnover time of the cell population (Hussain and Voaden., 1985). The latter is reflected in heterogenecity of cell fragilities within individual samples. In the course of that study, sex - related differences were also detected.

The camel's erythrocytes demonstrate the presence of volume - dependent potassium transport. The camel erythrocyte does not have a Na^+ / K^+ -cotransport mechanisms as do RBCs in most species. Swelling of erythrocytes stimulates K^+ influx and the influx is chloride dependent. Hydrogen ion concentration also affects the K^+ influx in camel's erythrocytes. As a result, it is concluded that the K^+ transport in camel's erythrocytes has similarity to mammalian species (Gharaibeh and Rawashdeh, 1993). Swelling of young camel erythrocytes hypotonically stimulates ouabain - resistant potassium influx, a response that is lacking in old camel erythrocytes (Gharaibeh and Rawashdeh, 1993). There was a significant correlation between potassium influx in normo - and hypotonic media which might indicate that the anion - dependent transport system operates, to some extent, to regulate cell volume.

MATERIALS AND METHODS

The investigation was carried out in the Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine, King Saud University, AlQassem, Bureidah, Saudi Arabia.

Materials

Animals

One-humped camels (Camelus dromedarius) with body weights ranging from 200-700 kg were kept in a special farm on an open yard, with no available shade, and free pasture having free access to an open ground pasture which consisted of the natural vegetative shrubs of the area. Water was provided ad libitum. The camel's ages were determined by dentition marks and the records of the owners. The animals were divided into three age groups as follows:

Group A 22 immature camels (17 males and 5 females) 2-3 years old.

Group B 20 mature camels (9 males and 11 females) older than 5 years.

Group C 21 mature camels (6 males and 15 females) older than 10 years.

Chemicals

All chemicals used were reagent grade from Merck (Germany) .

Methods

Steady-state study

No chemotherapeutic agents were given for 12 months or year before the onset of the study because some drugs are known to induce macrocytosis, probably through a direct action on the bone marrow (de Gramont, 1985). Normochromic normocytic whole blood was classified by erythrocyte count, hemoglobin concentration, hematocrit corpuscular constants, and blood indices. All experiments were performed within 24-48 hours after the fresh blood was drawn.

A series of hemolysis solutions ranging from 1.0 - 9.0 g / l NaCl (Merck) were prepared by volumetric dilution of a Molar NaCl solution. Whole blood samples were drawn from a group of healthy animals between 10 - 12 AM during the month of May 1994. The ambient temperature was 43ºC and the humidity percent was 15%. All animals were apparently physiologically normal at the time of the study; there were no red cell disorders and they were not anemic (range of the Hb in the different groups was 12.2 ± 0.4 - 14.5 ± 0.4 g / dl). The following hematological determinants were made : (1) Total erythrocyte (RBC) and total white cell (WBC) counts were enumertated using the Newbauer hemocytometer; (2) hemoglobin (Hb) concentration by the cyanomethaemoglobin method (Tietz, 1984); (3) hematocrit (PCV) using the Clay

Adams microhematocrit centrifuge. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)were calculated from the values of RBCs, PCV and Hb as described by Dacie and Lewis (1970). Differential leucocytic counts were estimated in smears stained by Leishman (Schalm, 1965).

Osmotic fragility

The method used was that described by Kappy et al., (1981). Ten to 15 ml of blood were collected into clean tubes containing heparin as an anticoagulant. The heparinized blood was obtained by jugular vein puncture. The erythrocytes were separated from the plasma by centrifugation (1200 g for 10 min.) at room temperature (23-25°C). The cells were then washed twice by suspending them in isotonic saline solution (0.9% NaCl) and recentrifuged (4000 g for 10 min.). This procedure provided erythrocytes free of plasma and buffy coat.

In the present study, the osmotic fragility was determined with hypotonic saline, (pH 7.2) prepared from double glass distilled water which had been boiled to remove the dissolved carbon dioxide (no buffer was added). For the osmotic fragility determinations, 0.02 ml washed RBC suspension at 5% hematocrit was added to a series of duplicate test tubes containing 3.0 ml decreasing concentrations of saline (1.0 - 0.1% NaCl) in 0.05% decrements. The suspensions were gently mixed several times by inversion and then left at room temperature for 30 min. The tubes were remixed by inversion, and were

inversion, and were centrifuged at 2000 rpm for 10 min. The optical density (OD) of the supernatants (hemolysates) was measured at 543 nm (hemoglobin) on a B&L Spectronic 20 using the supernatant from the 1.0% saline tube as a blank. Percent lysis was calculated as follows (Detraglia et al., 1974):

$$\% \text{ lysis} = \frac{\text{OD543} \times 100}{\text{OD543 at 0.0\% saline (100\% lysis)}} \qquad (1)$$

$$H = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \qquad \text{(hemolysis fraction)}$$

The concentration of salt that resulted in the lysis of 50% of a given RBC preparation was designated as the Osmotic Fragility50 (OF50) of the preparation.

Hemolysis curves (hemolysis fraction vs NaCl concentration) were built from the experimental hemolysis data by using the equation (Detraglia et al., 1974):

$$H = \frac{1}{e^{\beta (x - x50)} + 1} \qquad (2)$$

where H is the fraction of erythrocytes hemolyzed, X is the percent of NaCl concentration, X50 is the mean erythrocyte fragility (percent NaCl per L), and β is a measure of the breadth of the erythrocyte fragility distribution.

In a linear form, equation 2 can be written as follows (Detraglia et al., 1974):

$$\ln\left(\frac{1-H}{H}\right) = \beta X - \beta X_{50} \tag{3}$$

the equation was analyzed by computer using a least squares formulation. Substituting ln(1- H/H) for Y in the following formulas,

$$\beta = \frac{N\sum XY - \sum X \sum Y}{N\sum X^2 - (X)^2} \tag{4}$$

and

$$X_{50} = \left[\frac{\sum X^2 \sum Y - \sum X \sum XY}{N\sum X^2 - \left(\sum X\right)^2}\right] * \left[\frac{-1}{\beta}\right] \tag{5}$$

where N is the number of experimental points (X, Y). Results are expressed as the mean ± SEM. The distribution function is obtained (Detraglia et al., 1974):

$$\frac{dH}{dX} = \frac{-\beta e^{\beta (x - x50)}}{[e^{\beta (x - x50)} + 1]^2} \qquad (6)$$

Statistical analysis

Analysis of variance (completely randomized design - CRD) was used between age groups per sex. Moreover, the correlation coefficients were estimated between percent of hemolysis, RBC, PCV, MCV, Hb, MCH and MCHC (Steele and Torrie, 1980).

RESULTS

Hematological Data on Whole Blood From The Camel

The mean blood parameters for the 63 camels in this study are shown in Tables (1 and 2) and are identical with expected normal values. It can be seen that there are significant correlations between age, sex and some of the erythrocyte values (Table 1). It is worth noticing that the values of the MCHC did not change with sex or age suggesting a constant amount of water per cell.

Erythrocyte Osmotic Fragility In Camels

In the camel fragiligrams, the degree of RBC hemolysis (ordinate) was recorded as a function of the gradually decreasing saline concentration (abscissa). The resulting "concentration - hemolysis " curves were all sigmoid in pattern, a form typical of a continuous distribution of characteristics in heterogenous populations. Marked differences were found in the steepness of the sigmoid curves i.e., the degree of heterogeneity of the RBC population. The salt concentration at which hemolysis started and was completed varied considerably among the camels in accordance to their individuality, sex and age.

Table 1. Camel Osmotic fragility(X-50), breadth of fragility distribution and blood parameters.

Blood Picture		RBC	PCV	MCV	Hb	MCH	MCHC	X-50	β
		$10^6/mm^3$	%	fl	g/dl	pg	g /dl		
Age	Sex								
	Male	8.41	28.58	33.89	12.2	14.96	44.24	0.25	17.64
36 Months	n =17	± 0.29	± 0.97	± 1.45	± 0.38	± 0.61	± 0.75	± 0.06	± 4.17
n =22	Female	9.14	29.33	33.5	13.36*a	15.05	47.05	0.33	22.79
	n =5	± 0.36	± 1.78	± 2.9	± 0.79	± 0.43	± 6.40	± 0.02	± 2.34
	Male	9.52*b	31.18	32.64	14.51*b	15.27	48.77	0.31	29.91
90 Months	n =9	± 0.16	± 2.40	± 2.27	± 0.39	± 0.40	± 4.07	± 0.03	± 3.01
n =20	Female	9.28*a	32.69*b	35.33	14.53*b	15.71	44.52	0.27	24.05
	n =11	± 0.21	± 1.03	± 1.07	± 0.40	± 0.43	± 0.72	± 0.07	± 3.22
	Male	9.53*ab	26.84	28.24	12.88	13.56	47.9	0.36	21.29
120 Months	n =6	± 0.27	± 1.17	± 1.26	± 0.73	± 0.81	± 1.26	± 0.03	± 1.96
n =21	Female	8.76	27.3	32.76	13.25	15.51	47.54	0.36	22.91
	n =15	± 0.39	± 0.96	± 1.68	± 0.44	± 0.80	± 0.84	± 0.03	± 2.60

See page (v) for abbreviations.

*a= Significant ($p<0.05$) difference between males and females within each group.

*b= Significant ($p<0.05$) difference between males or females in all groups.

Table 2. Camel leucocytic counts at 36, 90 and 120 months.

Age	36 Months		90 Months		120 Months	
Sex / Leucocytic counts	n = 17 Male	n = 5 Female	n = 9 Male	n = 11 Female	n = 6 Male	n = 15 Female
WBC $X10^3/mm^3$	10.19 ± 0.69	9.80 ± 0.51	9.94 ± 0.53	11.53*a ± 0.27	8.62 ± 0.81	15.13*ab ± 0.83
N %	46.06 ± 4.58	36.00 ± 5.83	37.56 ± 4.09	33.46 ± 2.75	35.00 ± 6.38	38.2 ± 3.15
L %	37.77 ± 4.40	49.20 ± 7.14	41.44 ± 3.17	50.55*a ± 3.13	50.33*a ± 6.62	42.6 ± 2.89
M %	11.88 ± 1.35	8.80 ± 2.15	13.56 ± 1.72	11.27 ± 1.77	9.67 ± 2.39	13.47 ± 1.25
E %	4.29 ± 0.90	6.00 ± 1.26	7.44 ± 3.09	4.72 ± 0.98	5.00 ± 0.68	5.73 ± 1.09

See page (v) for abbreviations.

*a = Significant ($P< 0.05$) difference betweeb males and females within each group.

*b = Significant ($P< 0.05$) difference betweeb males or females in all groups.

Variation with age

Typical hemolysis curves for male camels are shown in Fig. 1 and for the females in Fig. 2 for the ages of 36, 90 and 120 months. The hemolysis curves are sigmoidal and represent cumulative frequency distributions of individual erythrocyte fragilities present in the sample. Results of the hemolysis experiments on the blood of male and female camels are given in Table 1 along with their mean clinical parameters. The results indicate that both the mean fragility (X-50) and the breadth of the fragility distribution (β) vary with age. The values of X-50 are 0.25, 0.31 and 0.35 NaCl% for male camels aged 36, 90 and 120 months, respectively. At the same time, the β parameters of the distribution breadth had a peak at the age of 90 months (29.91). The females exhibited the same peak at the same age with (24.05). The females' values for their X-50 were 0.33, 0.27 and 0.35 NaCl% for female cameles aged 36, 90 and 120 months, respectively. The value X-50 controls the position of the distribution maximum, and as β decreases, the distribution broadens. The composite hemolysis distribution curves are shown graphically in Figs. 3 and 4 for the male and female camels.

Variation with sex

In all, 32 male and 31 female camels were studied. The fragility curves of male (Fig. 1) and female (Fig. 2) camels reveal that the mean osmotic fragility (X-50) for the total population was higher in the females during the ages of 36 and 120 months (0.33% and 0.35% NaCl, respectively). At the age of 90 months, the X-50 was lower in the

female (0.27% NaCl) than in the male (0.31% NaCl). The mean values for the X-50 and β -parameters and their changes with sex are indicated in (Table 1).

The correlation between the camel erythrocyte fragility and RBC, PCV, MCV, Hb, MCH and MCHC

Osmotic fragility indicated a negative correlation with the RBCs in the female (-0.86), PCV (male -0.32, and female - 0.99), MCV (male -0.99, and female -0.925), Hb in the female only (-0.98) and MCH in the female only (-0.540). While a positive correlation was recorded with the RBC in the male (+0.91), Hb in the male (+0.36), MCH in the male (1.00) and in the MCHC in both male (+0.81) and female(+0.99). (Table 3).

Table 3. Correlation between camel erythrocyte fragility with blood parameters.

Blood parameters	Male	Female
RBCs	+ 0.91	- 0.86
Hb	+ 0.36	- 0.98
PCV	- 0.32	- 0.99
MCV	- 0.99	- 0.93
MCH	+ 1.00	- 0.54
MCHC	+ 0.81	+ 0.99

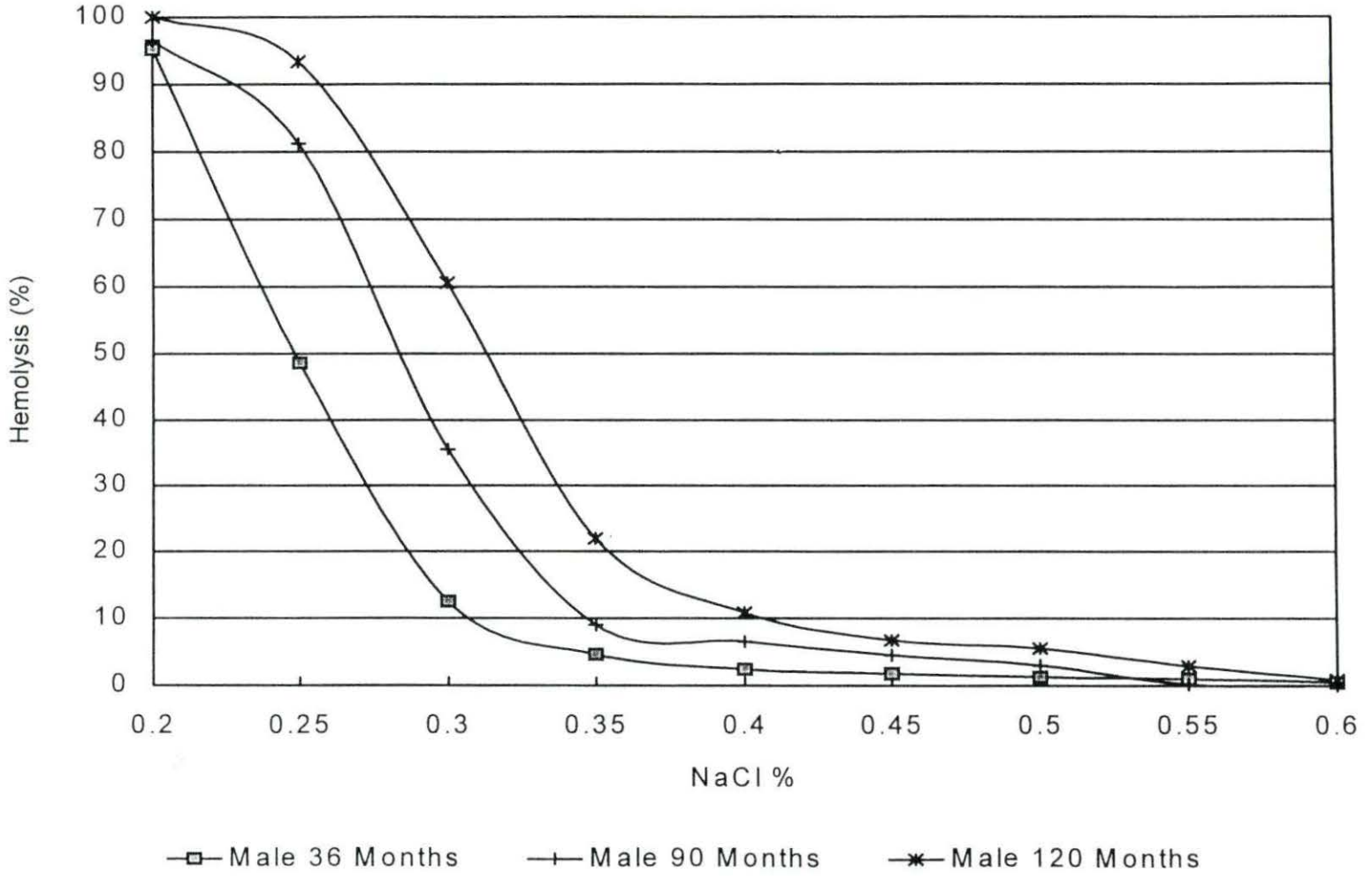

Figure 1. Fragility curves of male camels aged 36, 90 and 120 months.

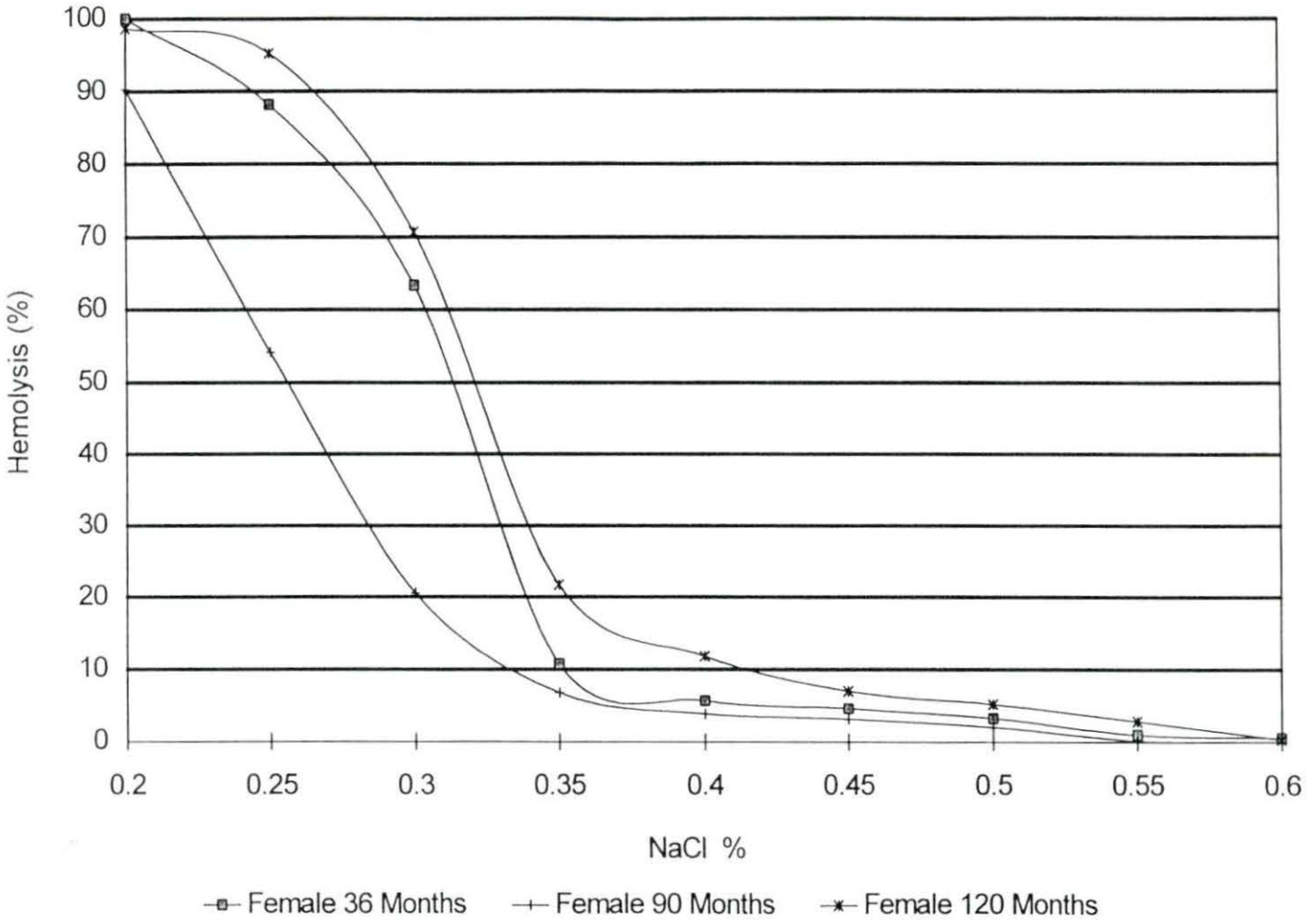

Figure 2. Fragility curves of female camels aged 36, 90 and 120 months.

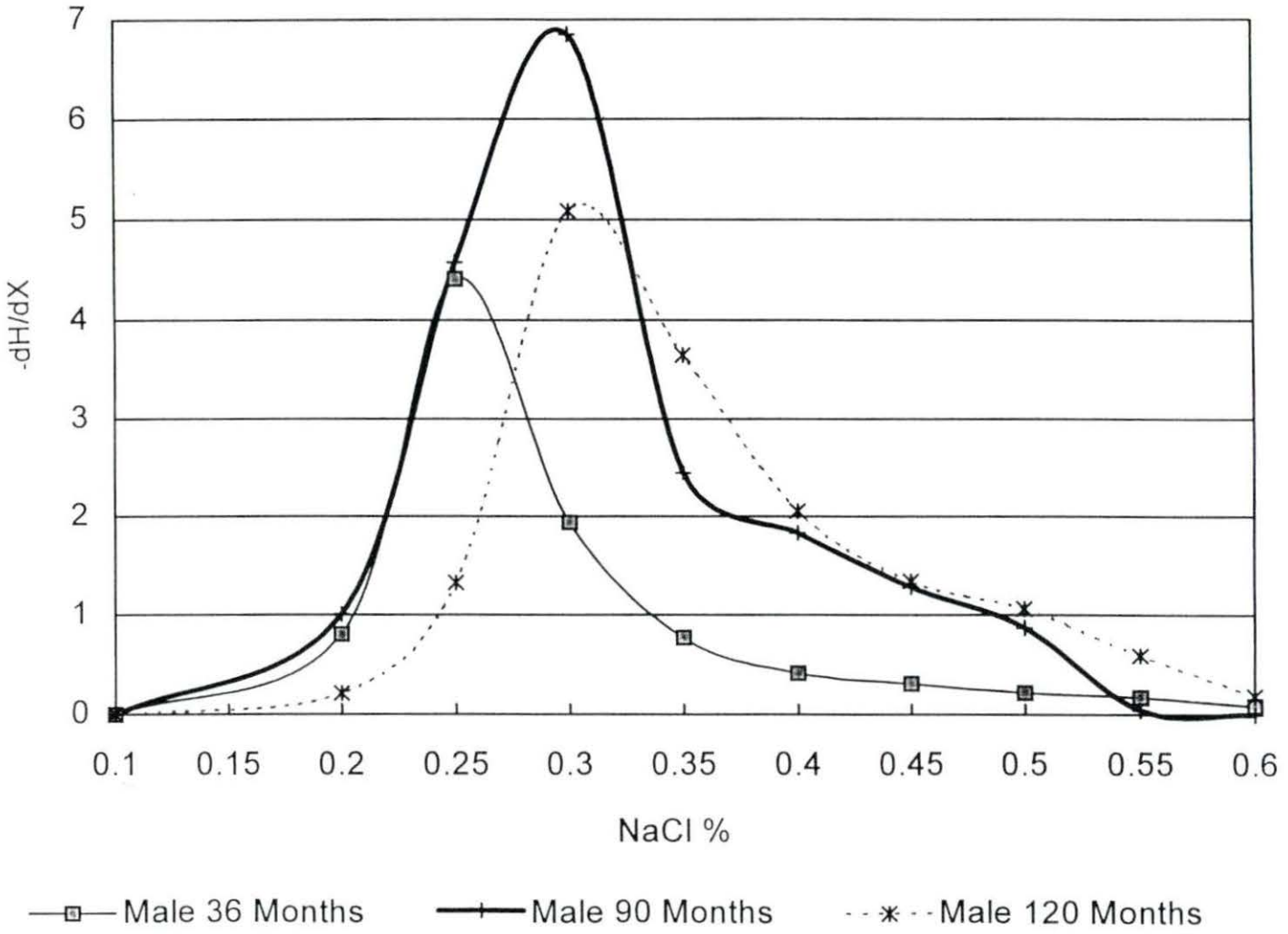

Figure 3. Distribution analysis by equations 3 and 6 of the average curves shown in Fig.1.

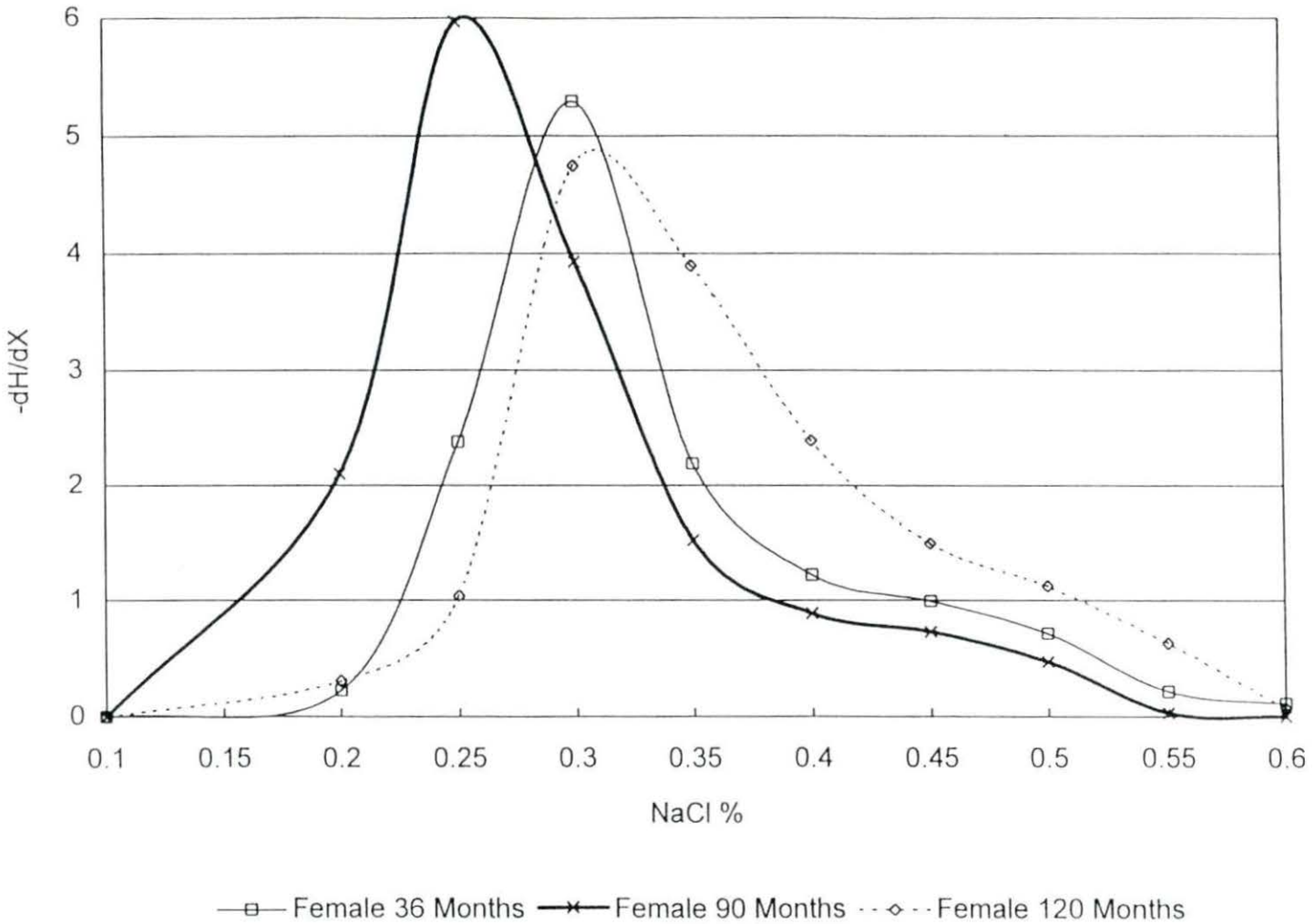

Figure 4. Distribution analysis by equations 3 and 6 of the average curves shown in Fig. 2.

DISCUSSION

Age, sex, nutrition and disease may affect the resistance of erythrocytes to osmotic hemolysis. These factors have little attention in veterinary hematology, although abundant work was reported in human (Perk et al., 1963).

Osmotic fragility studies are a sensitive method for the detection of abnormalities in the relationship of red cell volume to cell surface area. Hence, they can be used to identify changes in the isotonic shape of red cells, from which flow properties dependent on shape can be established (Bowdler et al., 1981). An abnormal reduction in cell surface area with increasing cell density, Rh null syndrome, explains the increased osmotic fragility of whole blood (Ballas et al., 1984).

The unique stability of camel erythrocytes was established under osmotic and mechanical stresses of sonic irradiation. The osmotic stability relates to the capability of the membrane to expand in area , whereas the mechanical stability refers to the applied external forces. The reason of this unusual stability of the erythrocyte membrane is unknown (Livne and Kuiper, 1973). Camel erythrocytes, which are ellipsoidal, do not deform but orient in the stress field . The failure of camel erythrocytes to deform is unusual compared to erythrocytes from other mammals. Their shape (flat, thin elliptocytes) apparently allows them to traverse the circulatory system without the usual problems of reduced deformability. Although the exact reason for their rigidity is unknown, they do have a high protein - to - lipid ratio in the membrane. The membrane

rigidity may be functionally related to their unusual osmotic resistance. As previously mentioned, camels are able to withstand long periods of dehydration, then drink large quantities of water in a short time without ill effects. None of the species with deformable erythrocytes can tolerate such a rapid rehydration without hemolysis (Smith et al., 1979).

Camel erythrocytes are the most resistant to hypotonic saline solution when compared to erythrocytes from other ruminants. This finding lends support to the previously reported data (Soliman and El Amrousi, 1966a) and the hypothesis of Ponder (1935) who maintains that when animals are listed in order of their red cell volume, they are arranged in order of the degree of hypotonicity which just caused hemolysis i.e., the larger the corpuscle, the greater is its resistance to hemolysis.

Calculations of the mean thickness of erythrocytes of various animal species have revealed that the camel and the llama have relatively thin erythrocyte wall. Erythrocytes of other animals (dogs, cats, cows, horses, sheep and goats) appear comparatively thick when viewed with the scanning electron microscope. The thinness of camel and llama erythrocytes becomes obvious upon examination of blood in wet mounts, but is not readily apparent in air - dried stained blood films. Scanning electron microscopy of glutaraldehyde of a fixed camel and llama erythrocytes provided convincing evidence of this species differences. This morphological characteristic may provide erythrocytes with an unusual capacity to withstand abrupt decrease in blood osmolarity since camel erythrocytes have been found to double their volume in

hypotonic solutions before lysing. It is known that "thin" erythrocytes are osmotically resistant, while "thick" erythrocytes are relatively fragile (Jain and keeton, 1974).

Resistance to lysis was also partially attributed to a high water binding capacity of the erythrocytic components. Approximately 50% more oxygen can be taken up per unit volume of red cells in the Camelidae than in other mammals. This characteristic was related to a high hemoglobin concentration in the erythrocytes (Rifkind et al., 1983). The mean red cell hemoglobin concentration has been implicated as a factor affecting response to hypotonicity (Hussan and Voaden, 1984).

Evans et al. (1970) presented data on the relationships between three groups of sheep (Delta type Merino sheep) and the camel data presented by Little et al. (1970) and / or Bartels et al. (1963). It seemed that a true δ - type sheep (which has a [$Na^+ + K^+$ pump] in the erythrocyte-wall very similar to that of the camel) might also possess some of the other unusual characteristics of camel red blood cells, that is, a very low median corpuscular fragility and a high mean corpuscular hemoglobin concentration and cell dry matter percentage. A suggestion was made by Evans et al. (1970) that the mean corpuscular volume differences are the result of differences in the spherocity of the cells, with the d - like erythrocytes being the less spherical. This, in the absence of any information regarding the cell membrane, might explain the large differences in median corpuscular fragility found between these groups of animals in which the more spherical cells were the first to rupture. In a comparison of the osmotic fragilities of camel and cattle erythrocytes, it was assumed that the membrane of the camel erythrocytes is less

elastic than that of cattle, and that the stressing of the membrane in the camel causes less "counter stress" than one would expect in a more elastic membrane.

The red cell count and the mean red cell hemoglobin concentration were significantly lower in women than in man . However, hemoglobin concentrations tend to fall in older men, and rise in older women. No age - related change in hematocrit values were detected (Hussain and Voaden, 1984). Body aging is accompanied by a more marked aging of the blood cells during their circulation in the blood. Factor analysis of the osmotic erythrocyte resistance indicates that osmotic fragility increases with age (Abe et al., 1984; and Voitenko, 1984).

An increase in X-50 and a decrease in β -values have been noted previously in studies in all male and mixed human populations. It was observed that the shift to higher mean osmotic fragilities with age arises from a small but significant reduction in surface area to cell volumes. Moreover, it was suggested that a higher proportion of older cells in the circulation might be a contributing factor. The increased osmotic resistance is mainly due to older cells which have a more favorable mean surface area to mean cell volume (MSA / MCV) since older cells in individual samples show an increased range of fragilities (Hussain and Voaden, 1984). Unlike erythrocytes from elderly humans, red blood cells from old mice are not more sensitive than are cells from young animals to lysis in hypotonic solutions, probably because the mean corpuscular volume decreases rather than increases with age in this species (Tyan, 1982).

The present study expands the evidence for an age - related increase in red cell osmotic fragility and the changes in cell dimensions which are responsible. The effect of age is to some extent masked by another modifier of comparable magnitude, namely the mean corpuscular hemoglobin concentration (MCHC), which is not itself an age - related variable.(Newman and Gitlow, 1943; Olbrich, 1948; Detraglia et al., 1974; and Bowdler et al., 1981)

The negative correlation between osmotic fragility and MCHC in normal subjects does not appear to have been recognized previously, although it is inherent to Ponder's classical formulation of the osmometric behavior of red cells. Since the change in volume resulting from water influx is proportional to the fractional volume of the intracellular water compartment, cells with a smaller water compartment (and higher MCHC) will vary proportionately less in volume when subject to osmotic stress. Provided that there is no systematic variation of surface area with MCHC, this leads to a negative correlation between osmotic fragility and MCHC. There was no correlation between MCHC and the surface area of the red cell (Bowdler et al.,1981). Hence, there is no compensatory decrease in surface area with a reduction in the compartment volume of water. The increased osmotic fragility of red cells with age cannot, therefore, be ascribed to an increase in intracellular water.

Partial hemolysis as a method to separate cells according to cellular age was questioned by Rifkind et al.(1983). It can achieve only a partial age separation with a higher concentration of the old cells included in the most fragile cells than in the least

fragile cells. These results were in contradiction to what was presumed to be a direct relationship between cellular age and osmotic fragility.

In these studies, a rather dramatic increase in fragility during the maturating of reticulocytes was observed. Based on earlier reports which showed that most of the reticulocytes were less fragile than mature cells, it was assumed that the fragility distribution reflects the distribution of cellular ages. Researchers observed this decrease in fragility using rabbit blood enriched with reticulocytes by repeated bleeding, and using centrifugation to separate a reticulocyte - rich fraction. This finding was also confirmed by using radioactive labels and comparing the distribution of radioactivity among fractions of different fragility shortly after labeling. However, attempts were made to compare changes in fragility during aging of the mature cell, no significant age change was detected. Rifkind et al.(1983) were consistent with these results that only small changes are observed in the midpoint. The importance of their findings was that there was a clear increase in the broadness of the osmotic fragility distribution (see Figs. 1 and 3).

Before 1959, there had been no studies of the changes in the distribution of fragilities during aging of the mature erythrocytes. However, in one of the primary studies which were directed mainly to the changes in the distribution of fragilities of young cells, data were collected up to 60 days after labeling with sulfur - 35 (S^{35}). A close look at their data collected at longer times indicated that the radioactivity which was found in cells with an intermediate fragility at day 10 subsequently spreaded out to

a wider range in fragility, extending to both lower and higher fragilities than the 10-day peak at longer times. These results agreed with the results of Rifkind et al (1983) that older cells had a broader fragility distribution than the younger mature cells.

An increase in the total broadness of the osmotic fragility curve was observed in other cases. However, in most of these cases including blood of newborns, stored blood, and certain diseased states the broadness was accompanied with a clearly discernible heterogeneity of the cell population.

The increase in the broadness of an apparent single population distribution as seen in the results of Rifkind et al. (1983) for cellular aging, was only reported for aging of the entire organism. However, even in that case the entire distribution became more fragile and no cells appeared to become less fragile. These cellular aging results, however, clearly indicated that the broader distribution of old, more dense, cells contained cells which were both more fragile and less fragile than the cells in the original, i.e., young, distribution of cells.

Increased osmotic resistance is found in diabetic erythrocytes (Ghigo et al., 1983), in cases of human and mice beta- thalassemia (Popp et al., 1985), while osmotic fragility increases in hereditary spherocytosis (Reinhart et al, 1994), in lead workers (Ronnevi et al., 1982) an observation of interest as a possible manifestation of a generalized membrane defect and where a close relationship was observed between osmotic fragility and hematocrit, osmotic fragility and hemoglobin and osmotic fragility and mean corpuscular hemoglobin (Karia et al., 1982),Huntington disease (McCormack

et al., 1982),Gilbert syndrome (Preisig et al., 1982), an increase in the Na^{+}-K^{+} pump rate of the red cells (Mutoh et al., 1983). There is extensive evidence that uremia affects the fragility and deformability of red blood cells (Peuchant et al., 1988; and Langsdorf and Zydany, 1993). The life span of the RBCs in uremics is shorter than normal and an enrichment of circulating RBCs by young cells occurs in uremia patients. The median osmotic fragility (MOF) of the young cells was lower in uremic than the MOF of old cells (Malachi et al., 1986). Parathyroid hormone was suggested to be one of the factors responsible for raising the osmotic fragility of red blood cells (Malachi et al., 1986). In rats, a mutant strain, Nagase an albuminemia rats (NAR) had an increased tendency for blood hemolysis and increased potassium efflux in erythrocytes. This potassium efflux was prevented in the presence of rat albumin. These findings suggest that a deficiency of serum albumin may increase the permeability of potassium in the erythrocyte membrane of NAR (Sugiyama et al., 1984). The effects of this variable on the fragility curve, which can be observed in various diseased states, were essentailly the basis for the suggested clinical use of osmotic fragility tests (Rifkind et al., 1983).

The increase in the broadness of the fragility distribution may be explained by changes which occur during cellular aging. The decrease in membrane surface area that results in smaller spherical red cells increases the osmotic fragility, while the decreased K^{+} concentration produces a decrease in the osmotic fragility. Leakage of K^{+} has been implicated in the decreased osmotic fragility associated with the accumulation of Ca^{++} (Rifkind et al., 1983). The decrease in the midpoint of the fragility curve obtained when

gradual hemolysis is compared with rapid hemolysis due to the prelytic leakage of K^+ when gradual hemolysis occurs.

A swelling - stimulated K^+ transport exists in duck red cells, rabbit and sheep red cells. Gharaibeh and Rawashdeh (1993) suggested that this system occurs in camel erythrocytes and is inhibited by aging. The correlation between the basal and swelling - stimulated influxes in camel red blood cells is in good agreement with a similar finding reported in rabbit red blood cells, this showed that the anion - dependent transport system operated to some extent in cells of normal volume. In other words, the tonic activity of this system may affect the physiological regulation of cell volume, or if cells swell from normal volume (set point) the volume could be corrected by changes in the anion - dependent K^+ efflux.

The conclusion that swelling - activated KCl transport has a major role in cell volume regulation in old red blood cells may be premature because older camel red cells do not have a swelling - activated K^+ flux. The principal additional factor which is known to change the osmotic fragility is the shape of the cell. When fragility increases, the surface area to volume ratio declines. The individual dimensions of the cells do not vary significantly with the age of the donor, and it seems that the ratio, and not the individual dimensions, is important.

The sphericity index (SI) was introduced by Bowdler et al., (1981) to compare the shapes of red cells within a spectrum from zero (a laminar disc) to unity (a true sphere). He postulated a notional cell with a modal fragility and mean volume for the

population of its sample. The SI was shown by Bowdler et al. (1981) to have a positive correlation with age, which confirms that the age - related increase in osmotic fragility is due to isometric sphering with a relative diminution in surface area.

A decrease in the tensile strength was inferred from the increase in the osmotic fragility in cells from a group of elderly donors (Bowdler et al., 1981). However, the contribution of the tensile properties of the membrane to osmotic fragility is controversial, and there is no evidence that they constitute a critical variable. The data of Bowdler et al. (1981) indicated that the age - related tendency arises from a small, but significant reduction in surface area relative to cell volume. The magnitude of the diminution in surface area required to produce the observed changes in osmotic fragility can be computed and was shown to be small. For example, the expected difference in the osmolality for 50% hemolysis across a 50 - year age span is approximately +4.5 mosm/L. The difference in surface area for two cells with an isotonic volume of 91 μm^3 and $X_{0.05}$ of 120 and 124.5 mosm/L, respectively, is 2.8μm^3,which is a difference of approximately 2% in surface area.

The minimal cylindrical diameter (Dmm) was utilized as a measure of the maximum deformation required of individual cells for traversing small channels by adopting a cylindrical form. Since the surface area of red cells is linearly proportional to volume, and not to geometrical factors alone, there is little variation in Dmm within a normal red cell sample. The Dmm shows no correlation with age. This indicates that within the age range of the normal subjects studied, the change in shape with age is not

enough to impair the perfusion of normal capillaries. This, however, does not exclude the possibility that the change in shape may compound the effects of micro vascular abnormalities in pathological circumstances, and such circumstances need to be addressed.

The spleen and lymphoid tissues involute progressively with age and this may reduce the efficiency with which the aging red cell is removed from the circulation. Since the retention of older cells in the circulation decreases the stimulus to produce new cells, this mechanism would cause an increase in both the mean osmotic fragility of the population and the range of fragilities represented within the population.

Male chicken erythrocytes are more susceptible to osmotic lysis than those from female birds. Administration of diethylstilbestrol to immature male chickens increases osmotic resistance. In contrast, a decrease osmotic resistance occurs in dogs during mid - estrous. It is possible, therefore, that one or more of the sex hormones play a fairly direct role in determining erythrocyte fragility. However, specific post - menopausal differences in the female population was not detectable (Hussain and Voaden, 1984).

The shape of the camel erythrocyte membrane, unlike that of the human or bovine, is stable in whole erythrocytes as well as in the isolated ghosts. Metabolic depletion, treatment with calcium and heating to 60°C, all induce disc-sphere changes or fragmentation of human erythrocytes and ghosts, but appear to be without effect on camel erythrocytes (Ralston,1975). This remarkable stability is consistent with the resistance of camel erythrocytes to osmotic lysis as shown in the present study.

Microtubules have been reported to be involved in shape maintenance of avian erythrocytes. However, it is unlikely that they are responsible for the stability of camel erythrocytes, since the potent microtubule disrupting agents, colchicine and vinblastin, did not change the shape of camel red cells and ghosts at concentrations sufficiently high to disrupt the micro tubules of the avian cells. At the same time, colchicine and vinblastine did not cause the transition to spherocytes in camel red cells that had been observed in human red cells treated with these agents.

In addition to the stable shape of the camel erythrocyte membrane, the water - soluble protein, spectrin appears to be more tenaciously bound to the camel erythrocyte membrane than it is in other species (Ralston, 1975). Although spectrin may play a major role in the maintenance of membrane shape in the camel erythrocyte, other proteins of the membrane may be important. The failure of the camel ghosts to fragment after spectrin release indicates that, even in the absence of spectrin, the membrane shows greater stability than those of the bovine and human species.

Spectrin and the integral proteins of the membrane are closely associated in the human erythrocyte membrane. The presence of spectrin on the membrane prevents the lateral movement of integral proteins in the membrane. Therefore the glycoproteins, component 3 and spectrin may all interact to maintain the integrity of the membrane. Removal of spectrin may permit the lateral movement of integral proteins, and disrupt the shape of the membrane and its integrity resulting in fragmentation of human and bovine membranes. In camels, however, differences in the integral proteins and their

greater abundance relative to the lipid content of the membrane may account for this resistance to disruption.

The similarity of human, bovine and camel spectrin, and the marked differences between the glycoproteins and between the component 3 proteins, suggest that the major difference lies not in spectrin, but in the integral proteins. Bands 1 , 2, and 5 of erythrocyte membrane create a network of micro filaments adherent to the cytoplasmic surface of the erythrocyte membrane, offering the membrane a skeletal support (Eitan et al., 1976) which could be designated as the "peripheral skeleton". Eitan et al. (1976) proposed that the camel's system is distinguished by an additional, more prominent network, termed tentatively "integral skeleton". This network is stabilized by protein - protein interactions of closely distributed integral proteins and possibly also by interaction with lipids. The unusual ratio of solvents required for effective extraction of lipids from camel erythrocytes may reflect unusual lipid - protein interaction, since the lipid composition of camel erythrocytes is essentially similar to that of other species.

Camel erythrocytes are characterized by being uniquely stable to osmotic changes and resistant to the lytic effect of sonication. The stability of the camel erythrocyte membrane and the glutaraldehyde-treated membrane of human erythrocytes is similar. However, cross-linking by glutaraldehyde is irreversible and involves covalent bonds, while the interactions stabilizing the "integral skeleton" are essentially reversible and apparently dynamic. This allows the camel cells to swell in hypotonic media, albeit differently from erythrocytes of other species.

Our findings suggest that the red cell mechanical resistance of camels increases with age and this agrees with the recent observations of Dudaev and Al Mubarak (1990).

Magnani et al. (1988) compared the hematological parameters of young and old mice. They suggested that old animals possess a chronologically younger population of erythrocytes than do young animals. The erythrocyte's osmotic fragility indicate that circulating erythrocytes in old animals constitute an heterogeneous cell population whose properties cannot be explained on the basis of a chronologically younger erythrocyte population. Erythrocytes in aged, senescence - accelerated mice were less fragile than those with normal aging characteristics.

In summary our findings suggest that the red cell mechanical resistance of camels increases with age in males and females, at the same time, we found out that the female's erythrocytes were more resistant to osmotic fragility than male's erythrocytes. The exceptional structural configuration of the lipoprotein and phospholipids in the camel erythrocytes has yet to be further defined and characterized. It may account for the specific particular osmotic stability of the camel erythrocytes.

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