VETERINARSKI ARHIV 75 (2), 159-164, 2005

Relationship between microscopically measured radius and radius calculated from volume in buffalo erythrocytes

N. H. Mohan*, Lakshmi Kant Dubey, and Daya Shankar Singh

Department of Veterinary Physiology, College of Veterinary Science & A. H, NDUAT, Kumarganj, Faizabad, India

MOHAN, N. H., L. K. DUBEY, D. S. SINGH: Relationship between microscopically measured radius and radius calculated from volume in buffalo erythrocytes. Vet. arhiv 75, 159-164, 2005.

ABSTRACT

In the present study the relationship between microscopically measured radius, Rm, and radius calculated from the volume (assuming that the cell is spherical), Rc, is described. Mean Rc value was greater than the Rm value. Application of chi-square test showed that there is no variation between these two. The *t*-statistic was higher than the tabulated value. The correlation coefficient between Rm and Rc was 0. 7057. The regression equations for Rc upon Rm and Rm upon Rc was Rc = 0. 849Rm+0. 594 and Rm = 0. 5866Rc+0. 79865, respectively.

Key words: erythrocyte, buffalo, radius, volume, shape

Introduction

In a number of analytical studies the shape of mammalian erythrocyte is assumed as spherical (CAMERON and FULLERTON, 1990; MILLER and HENRIQUEZ, 1988; SKALAK et al., 1973) when actually it is anuclear, round and biconcave shaped. The dimension and shape of erythrocyte is directly related to the area available to gas exchange across cell membrane, making dimension-shape relationship important. Moreover, the diameter of erythrocytes measured in dry state (in smear) is smaller than in wet (natural) state (DACIE and LEWIS, 1975). The literature available regarding the degree of relationship between the actually biconcave red cell and assumption that it is spherical is sparse. Therefore, the

* Contact address:
Dr. N. H. Mohan, National Research Centre on Pig (ICAR), by Lane No 2 (North), Panjabari Road, Guwahati-781 022. Assam. India, Phone: +91 361 2333 359; E-mail: mnhiyer@yahoo.co.in

ISSN 0372-5480
Printed in Croatia

present study was undertaken with the object of comparing the radii, calculated from the volume of RBC, assuming that it is spherical, and the microscopically measured radii, and to analyse the relationship between these two.

Materials and methods

Blood was collected from 8 Murrah buffaloes aged between 2-5 years by jugular venepuncture in glass tubes containing ethylene diamine tetra acetic acid (1. 25 mg/ml of blood) as anticoagulant. Packed red cell volume (PCV) of blood samples was determined using Wintrobe tube and total red cell count (TRC) using a Neubaur counting chamber as per standard methods (DACIE and LEWIS, 1975). Mean corpuscular volume (MCV) was calculated using the formula.

$$MCV = \frac{PCV \times 10}{TRC \times 10^{-6}}$$

A total of 30 observations were made for the same number of blood samples obtained randomly from the buffaloes. Later, the radius of RBC was derived from MCV, assuming that the red cell is spherical. (Volume = ¾ π r^3). The radius was calculated as Rc = [¾ (MCV)] $^{1/3}$.

At the time of blood collection multiple smears were prepared in clean grease-free glass slides and the smears were stained using Leishman`s stain as per standard procedure (DACIE and LEWIS, 1975). Phosphate buffer (pH 6. 8) was used for the diluting the stain. From the smears, the radii of red blood cells were measured using a calibrated ocular micrometer. Average diameter of an erythrocyte was obtained from at least 20 observations per stained smear. Similarly, thirty values for radii were obtained for thirty blood samples.

The values of radii- calculated, Rc and microscopically measured, Rm were assumed to have some association. In order to obtain an idea of the relationship between these values they were subjected to statistical analysis (RANGASWAMY, 1995) as follows:

1. Chi-Square (χ^2) test- was conducted with a hypothesis of H_0: $R_m = R_c$ and H_1: $R_m \neq R_c$. The χ^2 statistic was calculated as $\chi^2 = \sum \{[R_m - R_c]^2 / R_c\}$, which follows a χ^2 distribution with 58 (n_1+n_2-2) degrees of freedom.

2. F-test. The test statistic was calculated as $F = S_1^2/S_2^2$ to assess homogeneity of the samples. S_1^2 and S_2^2 stand for variances of Rm and Rc, respectively.

3. t-test. To see whether Rm and Rc differed significantly from one another t-test was applied assuming equal variances. The t statistic was calculated as $(\bar{R}m - \bar{R}c)/[S^2 (1/n_1+1/n_2)]$, with 58 degrees of freedom, whereas S^2 is the pooled variance.

Besides these tests, correlation and regression studies were made using $\bar{R}m$ and $\bar{R}c$ data.

Results and discussion

Rm and Rc data is presented in Table 1 and Fig. . 1. Mean Rm and Rc was 2. 246 ± 0. 0191 and 2. 502 ± 0. 023, respectively. The calculated radius was greater than the radius measured directly from micrometry. This may be due to the assumption that erythrocyte is spherical when it is actually biconcave disc shaped.

The calculated χ^2 statistic was smaller than the Table value of χ^2_{58} at 5% significance level. The acceptance of null hypothesis shows that there is no significant variation between Rm and Rc. The variance of Rm and Rc was 0. 0109 and 0. 0157, respectively. The F value calculated (0. 69) was lower than that of Table value (1. 8409), indicating that the data is homogeneous.

The calculated t-statistic (8. 58) was greater than the tabulated value (α = 0. 05, df = 58) indicating a difference between two. In a practical sense the difference should exist.

Further, the application of correlation technique showed a positive correlation (0. 7057) between Rm and Rc. On the basis of this the regression equation on Rm upon Rc was given as Rm = 0. 5866Rc+0. 79865. Similarly, the regression equation of Rc upon Rm was calculated to be Rc = 0. 849Rm+0. 5984.

The erythrocyte has no internal structure and therefore its shape and size is determined by the physical properties of the membrane and cell volume (DEULING and HELFRICH, 1976; WAUGH and HOUCHMUTH, 1995). One reason for the difference between Rc and Rm might be due to difference in the pH of the suspension media (plasma) or the staining solution/diluent. The shape and size of the erythrocyte may vary due to a change in the

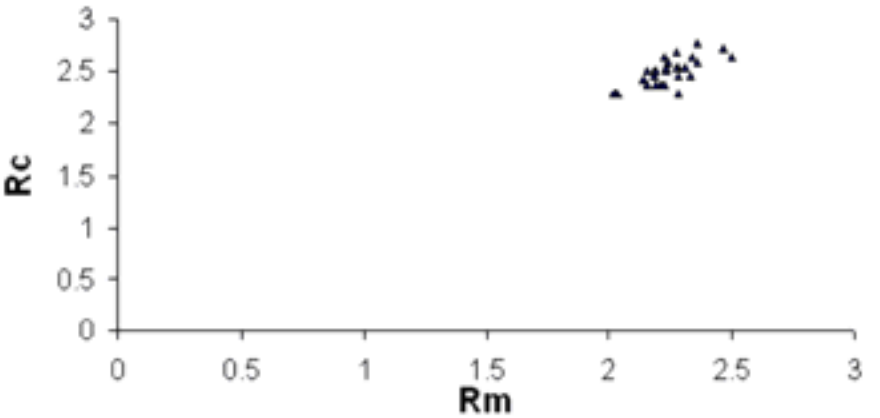

Fig. 1. Scatter diagram showing the relationship between calculated radius (Rc) and microscopically measured radius (Rm) in buffalo erythrocytes

Table 1. Radius calculated from volume (Rc) and microscopically measured radius (Rm) in buffalo erythrocytes

Sample no	Radius measured from smear (R_m)	Radius calculated from volume, assuming erythrocyte as spherical (R_c)
1	2.27	2.68
2	2.28	2.28
3	2.14	2.412
4	2.33	2.46
5	2.06	2.28
6	2.28	2.54
7	2.5	2.63
8	2.46	2.719
9	2.15	2.5
10	2.02	2.28
11	2.28	2.46
12	2.19	2.37
13	2.24	2.588
14	2.31	2.54
15	2.34	2.63
16	2.36	2.588
17	2.18	2.5
18	2.23	2.5
19	2.21	2.368
20	2.18	2.45
21	2.36	2.588
22	2.23	2.544
23	2.15	2.37
24	2.18	2.456
25	2.22	2.378
26	2.36	2.763
27	2.18	2.5
28	2.19	2.5
29	2.27	2.544
30	2.22	2.632
Mean ± S.E	2.246 ± 0.0191	2.502 ± 0.023

difference between the outer and inner monolayer areas (ΔA) of the membrane bilayer (EVANS, 1974), caused by change in pH (SHEETZ and SINGER, 1974).

The present study indicates that even though there a difference exists between the radius calculated, assuming erythrocyte to be spherical, and microscopically measured radius, a high positive correlation exists between these two. Thus, one can be used to predict the value of other. PRICE-JONES (1933) has reported that red blood cells shrink on drying, their diameters then being 8-16% less than when cells are suspended in plasma. This may one of the reasons for the difference between these two values. The authors feel that this study will help in developing a simple mathematical model for studying structure-function relationship in erythrocytes.

Acknowledgements

The authors wish to express their thanks to The Dean, College of Veterinary Science &A. H, and to the Professor and Head of the Department of Agricultural Statistics, College of Agriculture, N. D. University of Agriculture and Technology, Faizabad, India for providing the necessary facilities for conducting the research work.

References

CAMERON, I. L., G. D. FULLERTON (1990): A model to explain the osmotic pressure behaviour of haemoglobin and serum albumin. Biochem. Cell. Biol. 68, 894-898.

DACIE, J. V., S. M. LEWIS (1975): Practical Hematology. 5th ed. ELBS/Churchill Livingstone, Edinburgh, pp. 44-49.

DEULING, H. J., W. HELFRICH (1976): The curvature elasticity of fluid membranes. J. de Physique. 37, 1335-13345.

EVANS, E. A. (1974): Bending resistance and chemically induced moments in membrane bilayers. Bioph. J. 14. 923-931.

MILLER, C. E., C. S. HENRIQUEZ (1988): Three-dimensional finite element solution for biopotentials: Erythrocyte in an applied field. IEEE Trans. Biomed. Eng. BME-35, 856-866.

PRICE-JONES, C. (1933): Red cell diameter. Oxford University Press, Oxford. (Cited by DACIE, J. V., S. M. LEWIS (1975): Practical Hematology. 5th ed. ELBS/Churchill Livingstone, Edinburgh.

RANGASWAMY, R. (1995): A Text Book of Agricultural Statistics. New Age International Publishers, New Delhi. 91-93, 100-101, 114-116 and 142-175.

SKALAK, R., A. TOZEREN., R. P. ZARDA., S. CHIEN (1973): Strain energy function of red blood cell membranes. Biophys. J. 13, 245-264

SHEETZ, M. P., S. J. SINGER (1974): Biological membranes as bilayer complexes. A mechanism of drug-erythrocyte interaction. Proc. Nat. Acad. Sci. U. S. A. 71, 4457-4461.

WAUGH, R. E., R. M. HOUCHMUTH (1995): Mechanics and deformability of hemocytes. In: The Biomedical Engineering Hand Book. (Bronzin, J. D., Ed.), CRC Press, Broca Raton, 474-486.

Received: 15 May 2003
Accepted: 1 March 2005

MOHAN, N. H., L. K. DUBEY, D. S. SINGH: Odnos između mikroskopski izmjerenog i na osnovi volumena izračunatog polumjera eritrocita u bivola. Vet. arhiv 75, 159-164, 2005.

SAŽETAK

Opisan je odnos između mikroskopski izmjerenog polumjera eritrocita (Rm) i polumjera izračunatog na osnovi volumena (Rc) pod pretpostavkom da su stanice okrugle. Srednja vrijednost Rc bila je veća od Rm srednje vrijednosti. Pomoću hi-kvadrat testa nisu ustanovljene varijacije u spomenutim značajkama. Statistički je t-vrijednost bila veća od izračunatih vrijednosti. Korelacijski koeficient između Rm i Rc iznosio je 0,7057. Regresijske jednadžbe za Rc prema Rm i Rm prema Rc iznosile su Rc = 0,849 Rm + 0,594 i Rm = 0,5866 Rc + 0,79865.

Ključne riječi: eritrociti, bivol, polumjer, volumen, oblik