THE ELECTRICAL CHARGE OF MAMMALIAN RED BLOOD CELLS

By HAROLD A. ABRAMSON AND LAURENCE S. MOYER*

(*From The Biological Laboratory, Cold Spring Harbor, Long Island*)

INTRODUCTION

Although the electrophoretic mobility of mammalian erythrocytes has been investigated to some extent, suitable estimation of their surface electrical charge has not as yet been presented. Recent developments in the theories of electrokinetic phenomena and in the theories of dilute solutions of electrolytes justify the calculation of the density of net surface charge from the available quantitative data on the electric mobility of red cells. In addition, through the work of Ponder, measurements of the surface areas of red cells permit a calculation of the effective net charge per red cell. The results of these calculations now show that the differences in electrokinetic potential (which is directly proportional to the electric mobility) calculated for the blood cells of a series of mammals bear no simple relationship to the net charge for the cells of each member of the series. These calculations thus throw new light on the differences in the physicochemical nature of the red cell under the experimental conditions employed and suggest further experimentation.

Theoretical

Since the electric mobility of red cells in salt solutions is independent of their orientation in the electric field (1) and since erythrocytes made spherical by traces of saponin have electric mobilities, within the limits of error, identical with the disc-shaped cells suspended in the same buffer, the electric mobilities of these microscopic particles can be treated by the theory derived for large particles (2). We can,

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* Sterling Fellow, Yale University, 1935–36.

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with von Smoluchowski, calculate the ζ-potential from the electric mobility, v,[1]

$$\zeta_E = \frac{4\pi\eta}{D} v, \tag{1}$$

(all units centimeter-gram-second and electrostatic units of charge), assuming that the viscosity, η, and dielectric constant, D, in the diffuse double layer do not assume values which are very different from those of the medium.[2] In any event, the measurements of v were carried out in the same medium so that any future corrections in the values of these constants would probably only change our results by a proportionality factor. From ζ_E, the net charge density, σ, on a surface may be calculated (2, 5, 6) in solutions containing any number of positive ions of the type, i, and negative ions of the type, j, by means of the generalized theory of Gouy, valid for large particles,

$$\sigma = \sqrt{\frac{NDkT}{2000\pi}} \sqrt{\Sigma c_i\left(e^{-z_i\frac{e\zeta}{kT}}-1\right) + \Sigma c_j\left(e^{+z_j\frac{e\zeta}{kT}}-1\right)}, \tag{2}$$

where N is Avogadro's number, k, the Boltzmann constant, e, the electronic charge, z, the valence, T, the absolute temperature, and c, the ionic concentrations in mols per liter existing in the body of the solution. σ has the same sign as ζ; all units are in centimeter-gram-second and electrostatic units of charge.

Inspection of this equation reveals that σ, under our conditions,

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[1] In general, it is desirable to calculate the ζ-potential rather than combine equations (1) and (2) (*vide infra*) because of the *possible* dependence of the electric mobility on the radius under other than the present conditions.

[2] "This assumption is not altogether unjustified for the following reasons:

1. Substitution of D and η of the solvent in the Onsager (3) conductance theory yields for concentrations up to about 0.05 N (simple salt solutions) satisfactory values for the limiting slopes and changes in mobility with concentration.

2. If the electric mobility of microscopically visible quartz particles covered with a film of adsorbed protein is studied in different concentrations of alcohol (4), it is possible to correlate the surface potential and surface charge calculated from these mobilities with the charge obtained by another (thermodynamic) method. As far as these results go, the characterization by the two parameters, viscosity and dielectric constant of the solvent, in the Helmholtz-Debye theory is correct within 10 per cent" (2).

will depend only on ζ, for all of the other terms are constants in a given solution of electrolyte. Simplifying equation (2) by collecting constants (except those here given by the concentration and valence) there is obtained:

$$\sigma = \alpha \sqrt{\Sigma c_i\left(e^{\frac{-z_i\zeta}{\beta}} -1\right) + \Sigma c_j\left(e^{\frac{+z_j\zeta}{\beta}} -1\right)},\qquad (3)$$

where $\alpha = 17{,}600$ and $\beta = 0.0256$ volt at 25°C. If ζ in volts is introduced into this equation with proper regard to its sign, the resultant value for σ will be in electrostatic units of charge. Since σ is the net charge per square centimeter, the effective net charge per cell may be calculated if the surface area of the cell is known.

RESULTS

The values of the charge were calculated by equation (3) from data obtained with various mammalian red cells (1) in isotonic (M/15) phosphate buffers at pH 7.4. In this case, the problem is complicated by the presence of three ionic types:[3] a single positive univalent type, i, and two negative types, j ($H_2PO_4^-$) and jj (HPO_4^{--}), of different valences. The method of calculation is illustrated as follows:

Let $v = -1.00\mu/\text{sec.}/\text{volt}/\text{cm.}$, then $\zeta = -0.0128$ volt, $z_i = 1$, $z_j = 1$, $z_{jj} = 2$, and $c_i = 0.120$, $c_j = 0.0133$, $c_{jj} = 0.0533$, so that,

$$\sigma = 17{,}600 \sqrt{0.120\left(e^{\frac{-1\times(-0.0128)}{0.0256}} -1\right) + 0.0533\left(e^{\frac{+2\times(-0.0128)}{0.0256}} -1\right) + 0.0133\left(e^{\frac{+1\times(-0.0128)}{0.0256}} -1\right)}.$$

Through the kindness of Ponder, we have been furnished with values for the surface areas (found by methods described in detail in his monograph (7)) of the various red cells investigated, with the exception of those for the sloth, where no data were available. It will be noted (Table I) that the net charge of the red cell does not vary in the same order, from species to species, as σ, the charge per unit area. Nor does there seem to be any clear relationship between net charge per cell and zoological classification.

By dividing the net charge by the electronic charge (4.77×10^{-10} e.s.u.), the number of effective electrons at the surface has been calcu-

[3] The concentrations of the H^+ and PO_4^{---} ions are here neglected.

lated (Table I, Column 7). For example, in the case of man, there are fifteen million electrons on each red cell, the highest value among these mammals. One might say that this corresponds to the "valence" of each cell. A similar computation of the net charge has been made for the typhoid bacillus (8). By assuming that each effective electronic charge occupies an ionic area of, say, 1×10^{-15} cm.^{2}, the percentage of the surface occupied by these charges may be roughly estimated (Table I, Column 8). The values never rise far above 1 per cent, which agrees in magnitude with data obtained on other surfaces (2).

TABLE I

Animal	Mobil-ity	ζ	σ	Area	Net charge	Number of elec-trons	Area occu-pied
	$\mu/sec.$	$volts$	$e.s.u.$	$cm.^2 \times 10^6$	$e.s.u. \times 10^2$	$\times 10^{-4}$	$per cent$
Rabbit	0.55	0.00704	1890	1.10	2.08	4.37	0.40
Sloth	0.97	0.0124	3330	—	—	—	—
Pig	0.98	0.0125	3360	0.95	3.19	6.70	0.70
Opossum	1.07	0.0137	3680	1.56	5.74	12.0	0.77
Guinea pig	1.11	0.0142	3780	1.15	4.35	9.14	0.80
Man	1.31	0.0168	4500	1.63	7.34	15.4	0.94
Rhesus monkey	1.33	0.0170	4570	1.37	6.26	13.2	0.96
Cat	1.39	0.0178	4780	0.80	3.82	8.03	1.00
Mouse	1.40	0.0179	4800	0.96	4.61	9.70	1.01
Rat	1.45	0.0186	4980	1.02	5.08	10.7	1.05
Dog	1.65	0.0211	5660	1.22	6.90	14.5	1.19

DISCUSSION

In general, for small values of v, the Debye approximation,

$$\sigma = \frac{D}{4\pi} \zeta \kappa ,$$
(4)

may be employed. It was not clear to the writers whether this equation would give values in M/15 phosphate buffer different from those obtained with equation (3). In Fig. 1 are plotted $\sigma - v$ curves for this phosphate buffer, calculated both by means of the exact formula (Equation 2) and by the approximate formula given by equation (4). Note that in the range of v (up to 1.65 μ per sec.) encountered in this
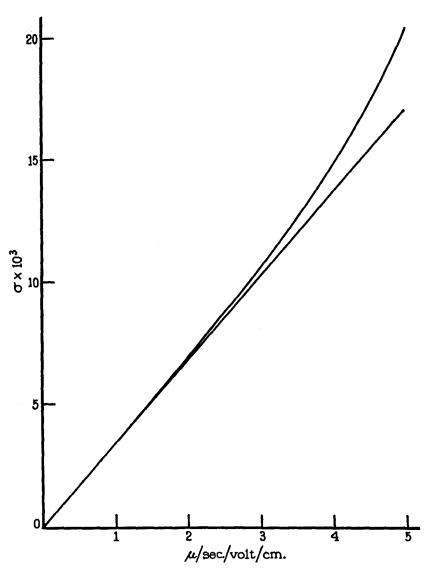
FIG. 1. The straight line has been calculated according to the Debye approximation and the curved one by means of equation (2) for M/15 phosphate buffer.

investigation, values of σ calculated by the two methods agree within the limits of error. Important divergences occur above 2 μ per sec.

The changes in the surface chemistry of the red cell due to alteration of the suspending medium may be investigated with profit by means of the method utilized to calculate the charge.   Thus, mammalian red cells increase their electric mobilities in isotonic glucose buffered slightly by phosphate (1).   Advantage could be taken of this effect to determine if the net charge is affected or if it is only the ζ-potential which varies.   Specific ion or molecular effects could be more closely followed.   The same procedure is, of course, applicable to other types of cells.

In a short series of experiments on the electrophoretic mobilities of red cells in twelve cases of varying types of anemia (1), it was found that both the macrocytes and microcytes when suspended in the same phosphate buffer have mobilities, with few exceptions, which are identical, within the limits of error, with the mobility of erythrocytes from a normal individual.   Theory demands that large particles which exhibit identical mobilities in solutions of the same ionic concentration must in each case have an equal number of charges per unit area (2).   Obviously if σ is nearly the same for both normal cells and the cells of abnormal size found in the anemias, the net charge per cell must be markedly different, for the two types of cells have very different surface areas.   Hence some mechanism seems to exist, capable of stabilizing the charge per unit area, within limits, while the surface undergoes comparatively marked changes in area and shape. The conditions which must be satisfied to establish the identity of two surfaces have been discussed before (9).

SUMMARY

From data on the surface area and electrical mobilities of mammalian red blood cells in $M/15$ phosphate buffer at pH 7.4, it has been possible, with the help of the Gouy and von Smoluchowski theories, to calculate the net surface charge per cell as well as the charge per unit area.   It was found that a single mammalian red cell has a net surface charge ranging from four to fifteen million electrons, depending on the species.   No clear relationship between zoological classification and surface charge is apparent.   It is suggested that a mechanism exists which is capable of keeping the surface density of net charge constant when comparatively large changes in surface area occur in the anemias.

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