DIELECTRIC PROPERTIES AND ION MOBILITY IN ERYTHROCYTES

H. PAULY *and* H. P. SCHWAN

From the Department of Biomedical Engineering, The Moore School of Electrical Engineering, University of Pennsylvania, Philadelphia

ABSTRACT The impedance of erythrocytes of man, cattle, sheep, dog, cat, rabbit, and chicken was measured in the range from 0.5 to 250 Mc. The dielectric constant of the red cell interior is 50 at 250 Mc, varies but little with species, and can readily be accounted for by the cells' hemoglobin content. The electrical conductivity of the red cell interior was determined between 70 and 100 Mc. The values differ from species to species within the rather limited range from 4.4 to 5.3 mmho/cm. Removal of the cell membranes does not affect the conductivity. Hence, the cell interior behaves, from an electrical point of view, like a highly concentrated hemoglobin solution. A theoretical value for the electrical conductivity of erythrocyte interiors, which is calculated on the basis of the salt content of the cell, ion mobility, and the volume concentration of the hemoglobin, is roughly twice as large as the measured value. This discrepancy is typical not only of the red blood cell. Pertinent measurements show that it is probably caused by hydrodynamic and possibly by electrostatic effects also, which lower the mobility of the ions. From the lower electrical mobility it appears that a lowered diffusion constant of the electrolytes and nonelectrolytes within the cell is indicated.

INTRODUCTION

The electrical conductivity of an electrolyte is determined by the concentration and mobility of its ions. Therefore, the electrical conductivity of the cell interior should provide information about the state of the ions within the cell, i.e., whether they are bound or free moving.

One of the most thoroughly investigated cells is the red blood cell of mammals. This cell exhibits a simple architecture, and its composition of minerals, proteins, and lipids is well known. Höber (1) first attempted to measure the internal conductance of erythrocytes and found a value of 1.6 mmho/cm to 6.6 mmho/cm at 25°C. Measurements of Fricke and Morse (2) yielded an internal resistance of bovine and sheep erythrocytes corresponding to a conductivity of 3.2 mmho/cm. This value was obtained by extrapolating impedance measurements which were conducted between 1 kc and 4.5 Mc to higher frequencies. Later, Fricke and Curtis

(3) published values for sheep, rabbit, and chicken of 7.2 mmho/cm at 20°C. The deviation from previously published values was due to improved extrapolation to such high frequencies that the cell membrane could be expected to be short-circuited.

During the 1940's, microwave techniques were developed which permitted the direct determination of impedances at frequencies up to more than 10,000 Mc. At these high frequencies, i.e. above 100 Mc, the conductivity of the internal phase could be measured more directly, since the highly insulating cell membrane would be effectively short-circuited (4). Thus, values were obtained by Rajewsky and Schwan at 100 to 1000 Mc (5) and by Cook at even higher frequencies (6) with human erythrocytes. The authors found conductivities of 9 to 10 mmho/cm at 25°C.

The following investigation attempts to clarify the discrepancies between the various values quoted above and to understand the conductance values in terms of protoplasmic composition.

METHODS

Sample Preparation. Blood was obtained by venous puncture. Clotting was prevented by the addition of 1 volume per cent of heparin solution (5000 USP units/cm^3). We observed no difference in the internal conductance or dielectric constant of erythrocytes in normal, heparinized, and citrated blood. The blood sample was either measured directly or centrifuged on order to obtain packed red blood corpuscles. We used a Servall centrifuge SS-4 (Ivan Sorvall, Norwich, Conn.) for 3 min at 10,000 RPM (12,000 *g*). The clear serum was used for conductivity measurements. The sediments of several tubes were collected after removal of the leukocytes, poured together, and tightly packed by centrifugation at 17,000 RPM (34,000 *g*) for 20 min. Samples of the packed sediment were investigated without further treatment. Other samples were treated with saponin, toluene, or distilled water.

The volume fraction occupied by the erythrocytes was determined with an International hematocrit centrifuge (International Equipment Co., Boston, Mass.). The cell sediment can be assumed to be tightly packed to about 5% (7). Small amounts of trapped serum below 10% do not affect the determination of the electrical properties of the red blood corpuscle interior. This may be readily seen from a pertinent discussion of equation (1).

Impedance Bridge and Sample Holder. Sample impedances were measured with the "RX-Meter, Type 250-A" of the Boonton Radio Corporation (Rockaway Township, New Jersey) which permits readings from 0.5 to 250 Mc. The sample holder (Fig. 1) is made from lucite. The platinum electrodes, sandblasted to reduce surface polarization, are 8 mm long and 2 mm in diameter. Most of the measurements were made at frequencies higher than 50 Mc, where electrode polarization was found not to cause errors. Usually we filled the sample holder with a 4 ml sample which was more than that needed to cover the electrodes adequately. The temperature was measured with a thermocouple immediately before and after measurements. Experimental procedures concerning measurements, the determination of the cell constants for the dielectric constant ε and the conductivity κ, the correction for series inductances of the binding post of the RX-Meter, and the sample cell have been presented before (8, 9).

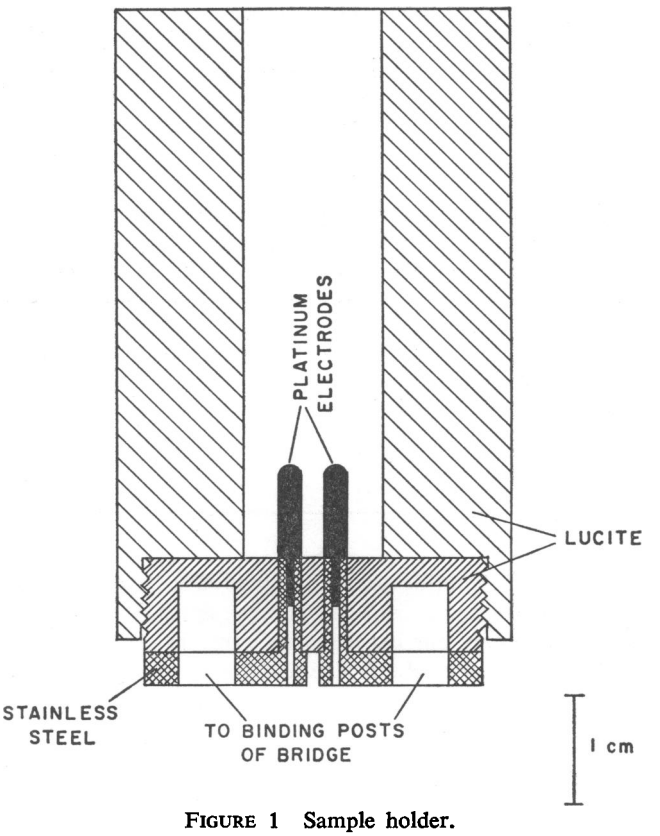

FIGURE 1 Sample holder.

Evaluation of the Data. For whole blood and resuspended erythrocytes the internal conductivity κ_c was calculated from the volume fraction p taken by the erythrocytes, the conductivity κ measured at high frequencies, and the conductivity κ_s of the serum. Fricke's equation

$$\frac{\kappa - \kappa_s}{\kappa + x\kappa_s} = p\frac{\kappa_c - \kappa_s}{\kappa_c + x\kappa_s} \tag{1}$$

was used where x is a "form factor" which has been tabulated by Fricke for various cell shapes and κ_c/κ_s ratios (10). With an axial ratio of the equivalent oblate spheroid of 1:4 and with $\kappa_c/\kappa_s = 0.4$, a form factor $x = 1.55$ applies. x does not enter critically into the evaluation of κ_c. Fricke's equation holds for particles of a conductivity κ_c in a medium κ_s and applies in the case of interest to us, provided that the erythrocyte membranes are short-circuited, i.e. at sufficiently high frequencies. A similar expression, replacing the κ's by ε's, was used to determine the internal dielectric constant ε_c. Since $\varepsilon_c/\varepsilon_s \sim 0.7$, the pertinent form factor is 1.80. In the case of tightly packed cells with 1 to 5% volume of trapped serum, the trapped serum was considered a suspension of "serum spheres" in an erythrocyte medium. The resulting values ε_c and κ_c deviate only a few per cent from ε and κ, since the effect of the trapped serum is small. In applying equation (1) also for

ε, the assumption was made that a Maxwell-Wagner dispersion, which is caused by the difference in internal and external impedances, is of negligible magnitude, as has been shown to be true by Schwan (4). The applicability of equation (1) is supported by the fact that it yields the same data for ε_e and for κ_e in the case of whole blood and packed cells (Table I).

TABLE I

INTERNAL CONDUCTANCE κ_e AND DIELECTRIC CONSTANT ϵ_e OF FRESH HEPARINIZED BEEF BLOOD, TIGHTLY PACKED CELLS AND CELLS RESUSPENDED IN SERUM. TEMPERATURE, 25°C

	Hematocrit	κ_e 70 to 100 Mc	ϵ_e 250 Mc
	Vol. %	*mmho/cm*	
Fresh blood	37.4	4.4	51
Tightly packed	98.2	4.3	52
Resuspended	49.1	4.6	52

The absolute accuracy of the bridge in the range used is stated by the manufacturer to be about 3% for both conductance and capacitance. Since our sample holder was calibrated with standard solutions, our experimental error in κ was smaller. It was determined largely by the resolution of the bridge and found to be near 1%.

RESULTS

Frequency Dependence. A suspension of erythrocytes is a heterogeneous medium from an electrical point of view. Consequently, it exhibits a Maxwell-Wagner type of frequency dependence of the dielectric constant ϵ and the conductivity κ. This dispersion, termed β-dispersion by Schwan (4), has been investigated by Fricke and Morse (2), Schaefer (see Rajewsky, reference 11), Daenzer (see Rajewsky, reference 11), and Fricke and Curtis (3) and is caused by polarization of the cell membranes. According to these investigators the characteristic frequency for the β-dispersion of blood is between 2 and 4 Mc and depends on the capacitance of the red cell membrane. The spectrum of electrical time constants involved is rather small. The erythrocyte membrane appears to be practically short-circuited at frequencies in excess of 50 to 100 Mc. It is therefore permissible to apply equation (1) above 50 to 100 Mc, as if membranes were not present.

Fig. 2 shows the high frequency end of the β-dispersion for both the conductivity κ and the dielectric constant ϵ of tightly packed erythrocytes of man and beef. It may be seen that κ levels off between 70 and 100 Mc. The small increase in κ of 3% between 70 and 100 Mc is mostly due to relaxation effects of hemoglobin, observed at very high frequencies (4). The increase in κ due to the microwave frequency dependence of water is ten times less pronounced and is therefore negligible. The contribution of the hemoglobin dispersion increases κ at frequencies in excess of

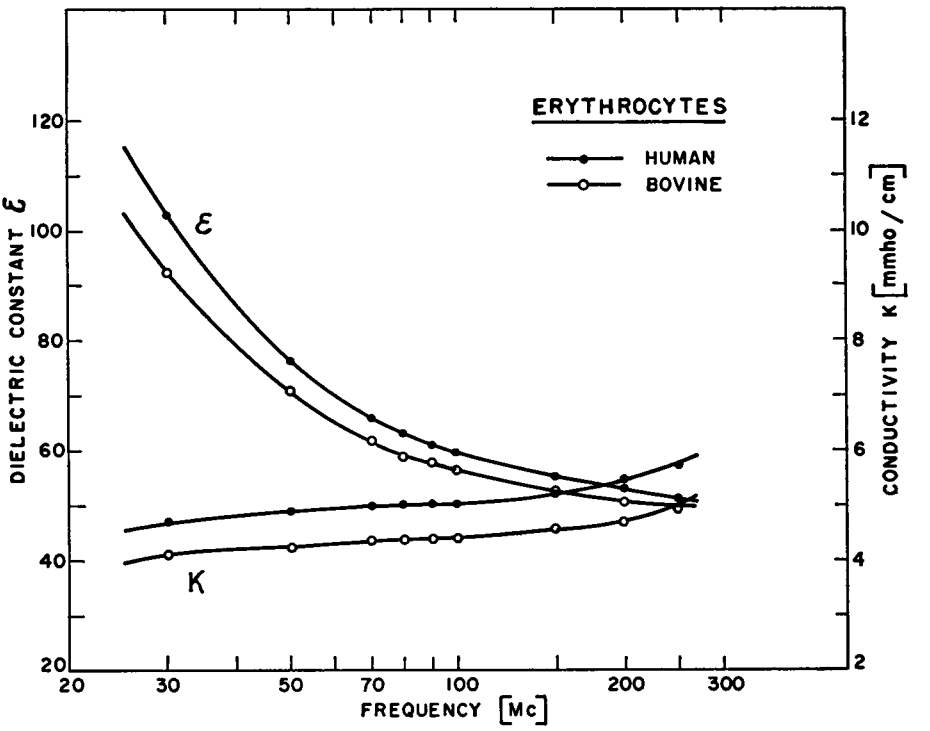

FIGURE 2 Dielectric constant ϵ and conductivity κ of packed human and bovine erythrocytes versus frequency.

100 Mc. Thus, the measurement of κ between 70 and 100 Mc gives a "true" internal conductance which is neither affected by the dielectric losses of the proteins nor by the presence of the cell membranes.

The dielectric constant behavior reflects several mechanisms. A rather pronounced frequency dependence, caused by the aforementioned β-dispersion, terminates near 100 Mc. A change by about 10 to 20 dielectric units is due to the frequency dependent properties of hemoglobin and occurs, in the main, above 100 Mc (4).

Experiments are described below which are related to the lysis of the cell membrane. They indicate strongly that ϵ measured at 200 to 250 Mc permits evaluation of an internal dielectric constant which is unaffected by membrane polarization.

Membrane Lysis by Saponin and Toluene. The addition of a sufficient amount of saponin to a suspension of erythrocytes breaks down their membranes (3). Thus, it is possible to measure internal conductance also at low frequencies after complete destruction of the membranes. This conclusion is supported by the investigation of tightly packed erythrocytes at 1 kc. Packed erythrocytes were allowed to stand for 1 hr at 25°C so that they might react with saponin and reach final conductivity values. Results are given in Table II. The conductivity without

TABLE II

SPECIFIC CONDUCTIVITY κ OF TIGHTLY PACKED BEEF ERYTHROCYTES AFTER ADDITION OF DIFFERENT AMOUNTS OF SAPONIN. MEASUREMENTS AT 1 KC AND 25°C

mg Saponin/ml cells	κ
	mmho/cm
0	0.19
5.6	2.04
11.0	3.73
16.4	4.53
21.6	4.76
31.6	4.93
41.3	4.88
50.4	4.83
59.2	4.88

the addition of saponin is very low, reflecting the high electrical resistance of the membrane and the tight packing of the cells. After complete breakdown of the electrical resistance of the membrane, the conductivity reached a level of 4.8 mmho/cm, in agreement with the high frequency values shown in Fig. 4 and Table III.

Fig. 3 illustrates the breakdown of the membrane capacity. Saponin powder was added to tightly packed bovine erythrocytes, and the mixture incubated at room temperature for 4 hr. The graph demonstrates that the membrane is electrically nonapparent (short-circuited) at frequencies higher than 100 Mc, since saponin does not affect the dielectric constant above 100 Mc. The conductivity of the erythrocyte suspension changes accordingly (Fig. 4). The value at 0.5 Mc is not essentially different from the 100 Mc for the sample with a content of 20 mg of saponin per milliliter. It may be seen, too, that the conductivity of the erythrocyte suspension rises slightly with the amount of saponin added. This rise is caused by

TABLE III

FREQUENCY DEPENDENCE OF THE SPECIFIC CONDUCTIVITY OF THE WATER PHASE OF PACKED BEEF ERYTHROCYTES AFTER TOLUENE LYSIS. TEMPERATURE, 25°C

Frequency	κ
Mc	*mmho/cm*
0.5	4.72
10	4.71
30	4.72
50	4.77
70	4.87
80	4.87
90	4.95
100	4.97

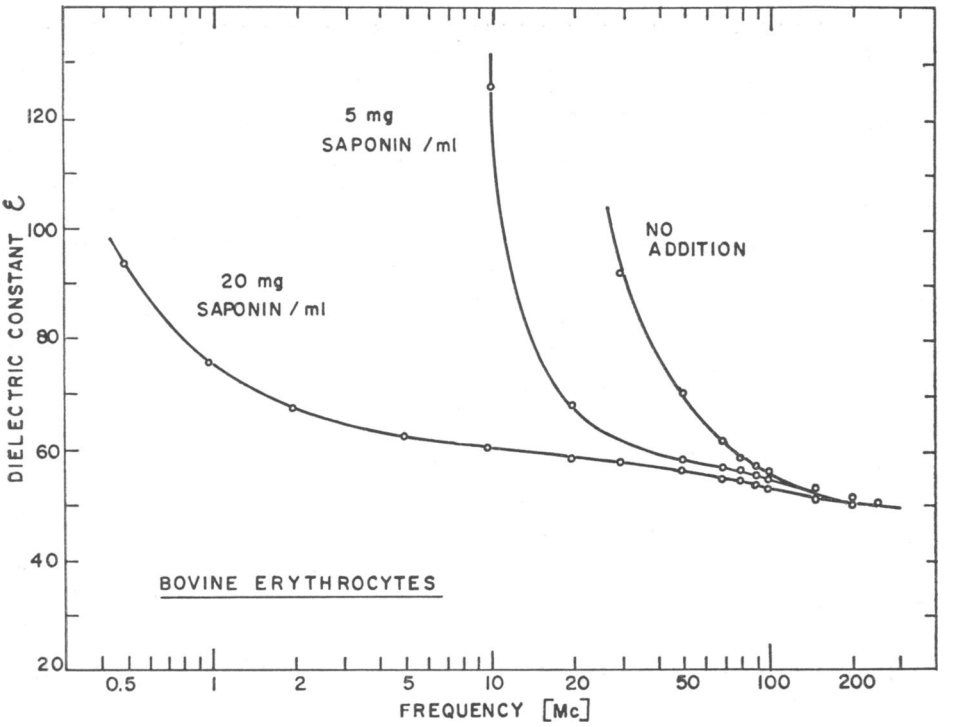

FIGURE 3 Effect of saponin on the frequency dependence of the dielectric constant ϵ of packed bovine erythrocytes. The increase of ϵ of the 20 mg saponin/ml curve below 5 Mc is due in part to electrode polarization.

the introduction of ions into the saponin powder. Toluene lysis causes a similar slight increase of the high frequency conductivity. The experiment supports the conclusion that the membrane is practically short-circuited above 70 Mc and that the measured conductivity yields internal conductivity values of the red blood cell if evaluated as discussed above.

It is possible to remove the membrane of blood corpuscles by shaking packed erythrocytes in toluene. The lipids are dissolved in the toluene phase; the cell interiors with their electrolytes and proteins remain in the water phase. The following procedure was used: two parts of the beef erythrocytes and one part of toluene were shaken at room temperature for 3 hr and both phases separated by centrifuging for 15 min at 27,000*g*. The lower watery layer was then investigated. The results are shown in Table III.

Since the conductivity of untreated packed erythrocytes is roughly 0.2 mmho/cm at 0.5 Mc (Table II), the toluene treatment causes a marked change of conductivity. The value of 4.7 mmho/cm indicates that the membrane resistance breaks down completely. A remaining small frequency dependence of the sample

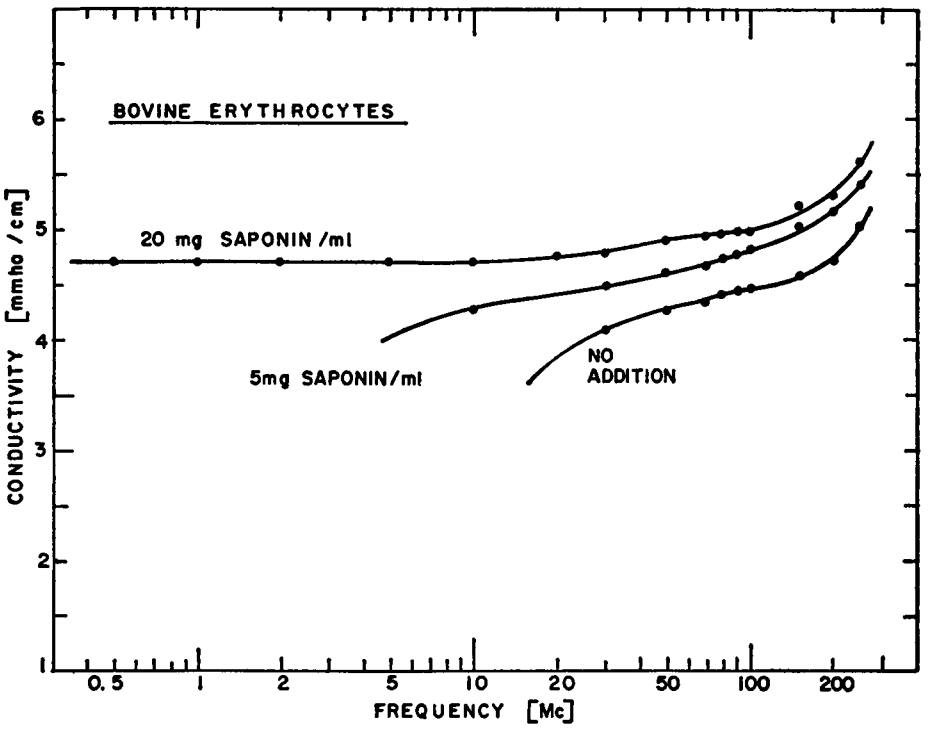

FIGURE 4 Effect of saponin on the frequency dependence of the conductivity of packed bovine erythrocytes.

conductivity is largely explained by the frequency-dependent dielectric properties of the highly concentrated proteins (4). The average conductivity between 70 and 100 Mc is 4.92 mmho/cm, which is only about 10% higher than the conductivity of the untreated red blood cells. A similar increase was found after saponin lysis. The lysis of the membrane and the transfer of the normal cell interior into the state of a highly concentrated solution of hemoglobin and electrolytes in water is common to both treatments. This change in organization may be responsible for the slight difference in conductivity values. At the moment we are only interested in the conclusion that after toluene lysis this conductivity is almost the same as that observed for normal red blood cells.

Influence of Storage Time. In order to investigate the influence of storage time on the internal conductance of human erythrocytes a few samples of human blood[1] were examined. No difference in the conductivity between freshly heparinized human red blood corpuscles and cells stored in the usual mixture of serum and ADC-Medium for 4 to 24 days could be detected.

[1] Supplied by the blood bank of the Hospital of the University of Pennsylvania. We are grateful to Dr. George F. Springer for his help and advice.

Internal Conductance and Dielectric Constant of Different Species. The internal dielectric properties of erythrocytes of 7 different species are listed in Table IV. All values are based on measurements of tightly packed erythrocytes from freshly heparinized blood and are not affected by the Co_2 partial pressure. The temperature of the sample, measured at each frequency, varied between 24° and 26°C. The conductivity values were corrected to 25°C by using the known

TABLE IV

INTERNAL SPECIFIC CONDUCTIVITY κ_c AND DIELECTRIC CONSTANT ϵ_c OF RED BLOOD CELLS. TEMPERATURE, 25°C

Species	κ_c 90 Mc	ϵ_c 250 Mc	Number of samples
	mmho/cm		
Man	5.18	50.1	5
Beef	4.35	51.3	8
Sheep	4.39	50.4	3
Dog	4.63	50.9	1
Cat	5.26	53.1	1
Rabbit	5.89	55.1	1
Chicken	4.90	52.1	1

temperature dependence of about +2% per degree centigrade (12). The dielectric constant was corrected by using a temperature dependence of 0.3 dielectric units per degree centigrade.

The internal conductivity varies from species to species within rather small limits. The value for human red blood cells corresponds to a specific resistance of 193 ohm-cm. The difference in specific resistance between beef and man is statistically significant. The standard deviation for all samples of human blood is ±2.7 ohm-cm or ±1.4%; that for beef is ±2.3 ohm-cm or ±1.0%.

The differences in dielectric constant among the listed species are smaller than those in the conductivity values. The differences are statistically insignificant, except for rabbit and perhaps cat. The standard deviation for man is $\epsilon_c = 50.1 \pm 0.4$ or 0.8% and for beef is $\epsilon_c = 51.3 \pm 0.3$ or 0.6%.

The variations in dielectric constant observed from one species to another may be accounted for by variations in hemoglobin content within physiologically reasonable limits, since an increase of 1% in weight in hemoglobin causes a decrease of ϵ_c by about 1 (4).

DISCUSSION AND ADDITIONAL PERTINENT EXPERIMENTAL DATA

Internal Dielectric Constant

The empirical value of the internal dielectric constant of human erythrocytes at

250 Mc is 50 (Table IV). The same values were measured after lysis of the membrane by saponin and after removal of the membrane by toluene. Therefore, the dielectric decrement of 27 units compared with water is not due to the specific structure of the red blood cell.

A theoretical value for the dielectric constant of the erythrocyte interior can be estimated from that of water with corrections being made for the presence of hemoglobin and electrolyte. The frequency dependence of the dielectric decrement of hemoglobin solutions was measured between 100 and 1000 Mc by Schwan and Li (12). The specific decrement $\Delta\epsilon$ for 1 g of hemoglobin in 100 ml of solution is 0.8 at 100 Mc and rises to 1.2 at 1000 Mc. However, $\Delta\epsilon$ displays a concentration dependence. From the data given by Schwan and Li (12), extrapolation to the hemoglobin concentration in the red blood cell of 35 g/100 ml cell gives a specific dielectric decrement of 0.6 to 0.7. Hence, the whole decrement due to hemoglobin and the small fraction of other proteins and lipids is 23 to 26 units. This value is calculated on the basis of a dry weight determination of 37.5 g/100 ml cells. The dielectric decrement of an aqueous electrolyte solution is small. Measurements of Hasted, Ritson, and Collie (13) indicated a dielectric decrement of 1 unit for a 0.1 M KCl solution. For other 1:1 electrolytes, practically the same value applies. For the present purpose the internal electrolyte may be compared with a 0.1 M KCl solution. Hence, the total calculated decrement amounts to 24 to 27 units.

The measured decrement of 27 units is in good agreement with the calculated decrement of 24 to 27 units, considering the uncertain extrapolation of the dielectric increment to values which pertain to the high internal hemoglobin concentration. Since the concentration of hemoglobin is nearly the same in all investigated red blood corpuscles and the effect of the electrolytes is small, it is to be expected that the internal dielectric constant would vary from species to species far less than the internal conductivity.

Ion Content of Red Blood Cells and Internal Conductivity

The internal conducitivity of red blood cells is largely due to its ion content composed primarily of K^+, Na^+, Mg^{++}, Cl^-, and large ions, i.e., predominantly dissociated hemoglobin molecules. In an ideal electrolytic solution, mobility and limiting ionic conductance values for K^+, Na^+, Mg^{++}, and HCO_3^- are known and tabulated (14). The first and second columns of Table V give the ionic balance in the red blood cell of man (15, 16). The ionic balances for the other species investigated in this paper are not equally well known. Therefore, the following discussions refer only to man. We will compare the above reported empirical internal conductivity data with data calculated from the red cells' ion content. Various relevant factors will be considered in turn.

1. The calculation necessary to find an upper limit of the internal conductivity of red blood corpuscles in man is carried out in Table V. The third column gives

TABLE V

CALCULATION OF THE "IDEAL INTERNAL CONDUCTIVITY" OF HUMAN ERYTHROCYTES USING KNOWN CONCENTRATIONS OF THE DIFFERENT IONIC SPECIES AND LIMITING IONIC CONDUCTANCE VALUES *l*. THE VOLUME EFFECT OF HEMOGLOBIN IS NOT YET CONSIDERED. TEMPERATURE, 25°C

Ion	Concentration, c	Limiting ionic conductances, l	Conductivity contribution, $10^{-3} \cdot c \cdot l$
	meg/liter cells	*mho-cm²*	*mmho/cm*
Na	18.6	50.1	0.93
K	95	73.5	6.97
Mg	5.1	53.1	0.27
Cl^-	55	76.3	4.20
HCO_3^-	15	44.5	0.67
X^-	10	35.8	0.36
"HbO_2^-"	42	27	1.10
			14.50

limiting ionic conductances ℓ for all ions of interest.[2] The fourth column shows the corresponding contribution of the several ions to the "ideal specific internal conductivity" of the cell interior in mmho/cm. The addition of the individual contributions results in an ideal specific internal conductivity of $\kappa_1 = 14.5$ mmho/cm. The contribution of the "HbO⁻" amounts to 7.6% due to the net charge of 6.4 electron charges per molecule.

2. The discrepancy between the ideal specific internal conductivity of 14.5 mmho/cm and the measured value of 5.18 mmho/cm is pronounced. A part of this discrepancy is explained by the volume fraction taken by the hemoglobin. The hemoglobin concentration of human erythrocytes is 35 g/100 ml of erythrocytes. If we assume a partial volume of 0.75 for hemoglobin and the generally accepted value of 0.3 g of bound water per gram of dry hemoglobin (4, 16, 17), the volume concentration of the hydrated hemoglobin in the cell amounts to 0.37. Furthermore, the assumption can be made that bound water does not dissolve salts. Work by Drabkin (16) with crystals of human hemoglobin supports this view. The same assumption was made by Sorensen (18) and Adair and Adair (19) in order to calculate hydration values of several proteins. Their hydration values fall in the range from 0.2 to 0.3 g of bound water per gram of dry protein. With this assumption the "ideal" conductivity κ_2 of the electrolyte surrounding the hydrated hemoglobin molecules will be larger than the figure of 14.5 mmho/cm by the ratio of the total volume to the volume not taken by the hydrated proteins:

[2] The conductance contribution per ion for the case of an infinite dilution decreases somewhat as the ion concentration increases. It is typically about 20% smaller for 0.1 M solutions of univalent ions.

$$\kappa_2 = \kappa_1 \frac{1}{1 - 0.37} = 23.0 \text{ mmho/cm}.$$

This calculation tacity assumes that the hemoglobin molecules are uncharged. The small contribution of the hemoglobin conductivity of 7.6% has already been considered as part of the surrounding medium.

3. The conductivity of the electrolyte surrounding the hydrated protein κ_2 and the total conductivity of the cell interior κ_c are different. A pertinent use of equation (1) permits calculation of the internal conductivity κ_c. If the very low conductivity of the hydrated hemoglobin is neglected,

$$\kappa_c = \kappa_2 \frac{1 - p}{1 + \frac{p}{x}}. \tag{2}$$

For spherical shape, $x = 2$. Shince $p = 0.37$, $\kappa_c = 0.53\ \kappa_2$, or $\kappa_c = 12.2$ mmho/cm, which is much higher than experimentally observed.

4. The result of our calculation shows that correction for hemoglobin with a hydration shell of 0.3 g/g of dry protein does not explain the discrepancy between calculated and experimental data. In an attempt to remove the discrepancy we shall allow now for a larger hydration shell. We can try to calculate the necessary volume fraction p of the hydrated hemoglobin by equating the calculated value κ_c with the experimental one and using the equation

$$\kappa_1/\kappa_c = 1 + \frac{p}{x} = 1 + p/2 = 14.5/5.2 = 2.8. \tag{3}$$

This cannot be satisfied since $p < 1$ by definition. Even if other than a spherical shape were considered and the lowest possible value of x, i.e. $x = 1$, were introduced, an impossible demand for a $p > 1$ would still result. Clearly, it is not possible to explain the large discrepancy between measurement and calculation by large hydration shells of nonsolvent water. Introduction of an activity coefficient which takes cognizance of the nonideal state of the electrolytic solution surrounding the hemoglobin molecules will not change this situation. The discrepancy between calculation and experiment is large, and the effect of ion concentration on individual ion conduction is small, as has been indicated before.

5. Next, we may consider the possibility that bound water is a solvent for ions. Thus, we gain a degree of freedom in fitting experimental and theoretical values. In essence, this introduces a new definition of "bound water." Bound water is now that part of the whole water in which the mobility of ions is zero, but ions are present. The pertinent calculation (8) gives the following result: The range of generally accepted hydration values between 0.2 and 0.5 g of bound water per gram of dry protein corresponds to having 3- to 8-fold more ions in the bound water than in free water. Such a high accumulation or "binding" of the electrolyte in the bound

water appears contradictory to experimental facts. Carr (20) did not find any binding of potassium or sodium by a large number of proteins. Drabkin (16) found a nearly 50% lower concentration of phosphate buffer in macrocrystals of human hemoglobin in equilibrium with the mother liquid. Values of Adair, tabulated by Bateman (17) and Alexander and Johnson (21), show the same results. We are forced to conclude that the discrepancy between measured and calculated internal conductivity cannot be explained in terms of the above considered model of hydrated uncharged protein molecules suspended in an electrolyte.

Conductance in Suspensions of Erythrocyte Ghosts

The discrepancy between measured and calculated internal conductivity values reflects a state of the ions inside the cell which is far from that characteristic of an ideal electrolyte solution. Obviously, the model underlying the calculations does not present an appropriate approximation of the red cell interior. But if red blood cells are hemolyzed in a large volume of distilled water, the interior should approach the behavior of an ideal electrolyte solution with increasing dilution. For "infinite" dilution we expect agreement between theoretical and experimental values. Experiments exhibit the expected behavior, as will now be shown.

From a tightly packed sediment of human erythrocytes a dilution series was made. Ten milliliters of cells were diluted and hemolyzed first with 10 ml of distilled water and then further in steps of two until the cells were diluted to 1/2048. The conductivity of this series of suspensions was measured at 1 kc. In Fig. 5 the abscissa gives the reciprocal volume fraction of the cells. The specific conductivity divided by the volume fraction of the cell is plotted on the ordinate. Corrections caused by the volume fraction taken by ghosts and Hb are necessary in order to arrive at figures characteristic of the electrolyte phase, excluding the macromolecular components. They have been applied using a pertinent simplification of equation (1) [equation (2)] since ghosts behave as particles of very low conductance at low frequencies. Equal Hb concentration in the intact cell and outside the cell after lysis has been assumed. The change in ionic mobility with concentration is not considered since it is negligible. The figure shows that the corrections are smaller at higher dilution ratios and negligible for dilutions in excess of 100. The corrected figures might be termed specific conductivity per cell and are obviously a measure of all the ions originally inside the cell and their mobility. Changes of the specific conductivity upon complete lysis reflect changes in either mobility or total number of ions available for conduction purposes. The specific conductivity per cell changes with the dilution factor. It levels off at 14 mmho/cm in agreement with the theoretical value of 14.5 mmho/cm discussed above.

Similar measurements were made at 90 Mc, the frequency range used to determine the internal conductivity of the intact cells. Table VI and Fig. 6 give the results in the same type of presentation as used in Fig. 5. Since the membranes of

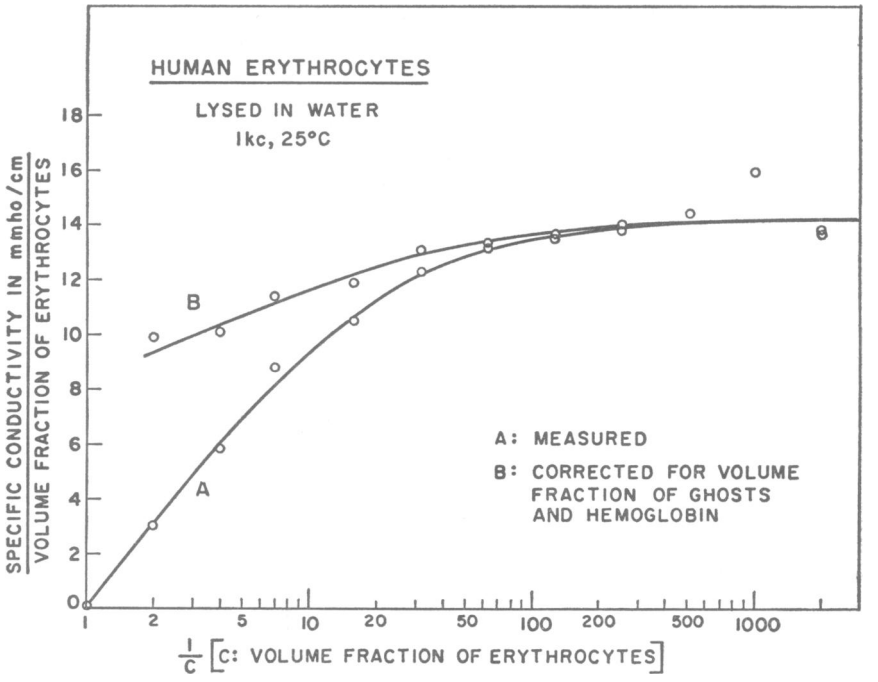

FIGURE 5 Conductivity of human erythrocytes, per volume fraction of erythrocytes versus dilution factor. Packed erythrocytes were diluted and hemolysed in H_2O. Measurements at 1 kc and 25°C.

the ghosts are short-circuited at 90 Mc, it is not necessary to correct for the ghost volume. The shape of the corrected and noncorrected curve is similar to that in the 1kc case. The internal conductance in the ideal state is 13.5 to 14 mmho/cm, which is practically identical with the 1kc measurement and with the theoretical value of the ion phase.

The *B* curves displayed in Figs. 5 and 6 are almost identical within the accuracy of the experiments. This is to be expected if the type of lysis which is achieved by the use of distilled water leads to an equilibrium of internal and external ions. It furthermore indicates that the application of the correction for Hb, which was based on the assumption of equal Hb concentration inside and outside the cells after H_2O lysis, was not grossly in error.

The deviation of the experimental data for low dilution ratios from the calculated value approached for high dilutions can be explained by either one of the following two mechanisms or any combination of them: (*a*) a certain percentage of the ions are bound by the proteins, and this percentage changes with the average separation of the protein molecules, i.e. the dilution factor; (*b*) the presence of the proteins causes an ionic mobility change as the total cell content is diluted. In any

TABLE VI

EVALUATION OF THE FRACTION OF IONS NOT CONTRIBUTING TO THE ELECTRICAL CONDUCTIVITY

1 Relative conc. of red blood cells c	2 Vol. fraction of hydrated Hb p	3 κ	4 $\frac{\Delta\kappa}{c} - f(p)$	5 Equiv. KCl conc. in cell water	6 Equiv. conductance Δ	7 $\frac{14}{1-p}\frac{\Delta}{\Delta_\infty}$	8 Fraction of immobile ions
		mmho/cm	*mmho/cm*	*mole/liter*	*mho-cm²*	*mmho/cm*	
1	0.35	4.97	8.9	0.13	126.3	18.2	0.51
1/2	0.17	4.05	10.6	0.065	131.7	14.9	0.29
1/4	0.088	2.54	11.3	0.033	135.8	13.9	0.19
1/8	0.044	1.47	12.2	0.016	139.3	13.7	0.11
1/16	0.022	0.81	12.5	0.008	142.0	13.5	0.074
1/32	0.011	0.45	12.9	0.004	144.2	13.6	0.052
1/64	0.0055	0.26	13.3	0.002	145.7	13.7	0.029
1/128	0.0028	0.16	13.7	0.001	146.8	13.8	0.007
$1/\infty$	0	0.04	(14.0)	0	149.9	14.0	0

Column 1: Volume fraction of red blood cells.

Column 2: The volume fraction of the hydrated hemoglobin molecules has been calculated under the assumption of 35 g of dry protein per 100 g of wet cells and 0.3 g of hydration water per gram of dry protein. The density of dry protein was taken as 1.3 g/cm³.

Column 3: Specific conductivity at 90 Mc and 25°C. The number corresponding to $c = 1/\infty$ refers to the distilled water used.

Column 4: Specific conductivity of the cell electrolyte divided by the volume fraction of the red blood cells. $\Delta\kappa$: specific conductivity κ (Column 3) reduced by the conductivity of the distilled water used for dilution purposes (0.04). The function $f(p) = (1 + p/2)/(1 - p)$ transforms the conductivity of the solution into the conductivity of the cell electrolyte.

Column 5: Equivalent KCl concentration in the cell water. Defined as one-half of the water concentration of the ions K^+, Na^+, Mg^{++}, Cl^-, HCO_3^-, and X^- (Table V).

Column 6: Equivalent conductance of equivalent KCl solution (Column 5) at 25°C (Extrapolated data from B. E. Conway, Electrochemical Data, Amsterdam, Elsevier Publishing Co., 1952).

Column 7: Theoretical conductivity of the cell water. The factor $(1 - p)$ takes into account the volume fraction of the solution available for the small ions. It has been assumed that the hydration water of the proteins does not dissolve small ions. The factor Δ/Δ_∞ accounts for the lower conductivity of the equivalent KCl solution in the cell water. Δ_∞ is the equivalent conductance of an infinitely diluted KCl solution (last figure of Column 6).

Column 8: Fraction of ions not contributing to the electrical conductivity of the cell water. The value has been obtained by subtracting the values of Column 4 from those of Column 7 and dividing by the value of Column 7.

case, we can calculate a number which may either be interpreted as the fraction of immobilized ions or the reduction factor in average ionic mobility. This number has been calcuated and is given in Table VI as a function of the relative cell concentration (the experimental data of Fig. 6 have been used). In Fig. 7 the "effective" fraction of immobile ions is plotted as a function of the volume fraction of the hydrated protein. The relative conductance deficiency can be described by a power function of the protein concentration. The power factor is 0.68. This value

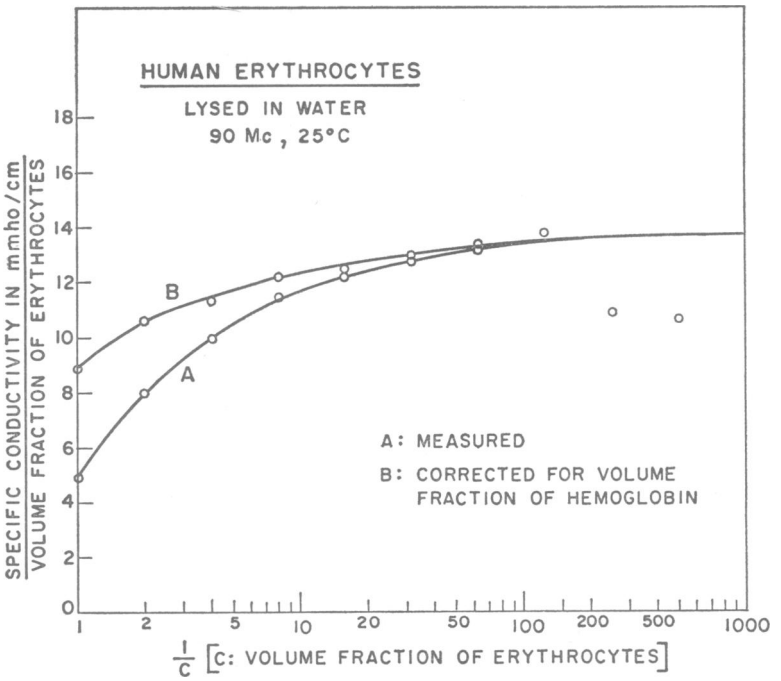

FIGURE 6 As in Fig. 5, measurement at 90 Mc, where the ghost membranes are electrically transparent. The deviation of the two points at the highest dilution ratios is due to the fast-increasing experimental error at the higher dilution ratios.

is close to the square root relationship of Kohlrausch's limiting law for electrolytes.

Discrepancy Between "Ideal" and the Actual Conductance. The ideal internal conductance of human red cells was reported above to be 2.7 times larger than the measured one. Appropriate consideration of the volume fraction occupied by hemoglobin combined with a reasonable assumption of 0.3 g of hydration water per gram of hemoglobin reduces this factor from 2.7 to 2.4. Hence, the volume concentration of the hemoglobin alone cannot account for the large discrepancy between ideal and actual conductivity. If we take into account the effect of the concentration on the equivalent conductance, the value is lowered from 2.4 to 2.0. Hence, a factor 2 still remains unexplained. Since nearly the same discrepancy was found in liver cells (12), eye lenses (22), and nerve fibers (24), and can be deduced from data for frog muscle (23), we must conclude that the lower actual conductance is a feature of the cell plasma and of protein solutions of high concentration. In Ehrlich ascites tumor cells with a protein content of less than 15%, the internal conductance is much closer to the theoretical value (25), as is to be expected. The reduced conductance can be caused by ions bound to hemoglobin or by a decreased ionic mobility.

Protein-Ion Interaction and Binding of Small Ions. An extensive review

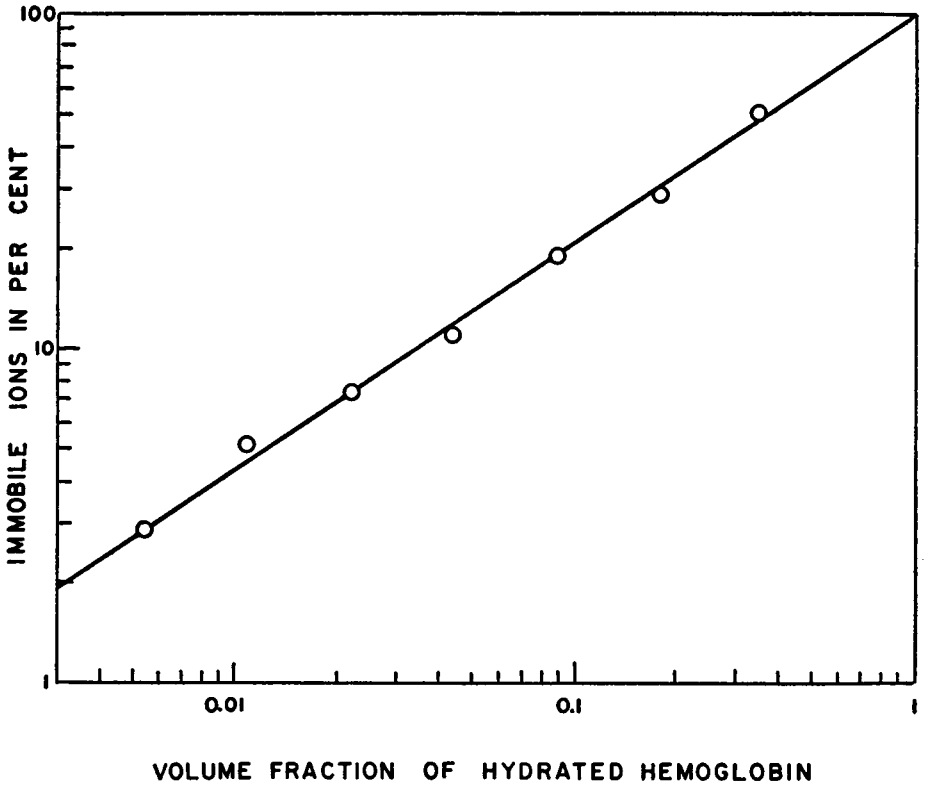

FIGURE 7 Immobile ions in per cent of all small ions in the hemoglobin solution. The points have been obtained by the procedure outlined in Table VI.

of the present knowledge of protein-ion interaction was given by Klotz (26). Data for the binding of cations by serum albumin and other proteins were obtained by equilibrium dialysis, titration, electrophoresis, and partition analysis experiments. Serum albumin exhibits an unusually high affinity for cations as well as anions in comparison with other proteins (27–31).

Unfortunately, the binding capacity of hemoglobin is poor. Near the isoelectric point there is no binding of K^+ and Na^+ (30, 31), even in 12% solutions (Klotz, personal communication). Ca^{++} and Mg^{++} will be bound to a larger extent. Even a complete binding of these ions could not explain the actual conductance in view of their small concentration. In order to explain the conductance deficiency each hemoglobin molecule would have to bind nearly 20 small ions. On the other hand, the osmotic pressure which is calculated on the assumption that all ions are free is well supported by experimental data. Hence, it is more likely that the lower conductance is due to a decreased mobility of the ions.

Decreased Mobility. The ions are subjected to electrostatic fields caused

by the nearly 200 charged groups of the hemoglobin molecule. This may well result in a lowered mobility in a manner similar to that stated in the theoretical treatment of the conductivity of concentrated electrolyte solutions.

Another mechanism, which may well affect the movement of the ions, is due to hydrodynamic interaction between the moving small ion and the macromolecules. The movement of the ions near the macromolecular surface should be retarded by the viscous drag resulting from the proximity of this surface. This effect on the over-all conductivity of a suspension of macroscopic spheres is usually very small; however, in the case of a suspension of macromolecules of high concentration this effect should show up because of the large surface area of all the macromolecules. From the Hb content and the radius of Hb molecules of 27 A, a total Hb surface 2.8×10^6 cm^2/g of red blood cell plasma is derived. The cell water, if spread equally over this surface, would have a thickness of 23 A. Hence, the radii of the hydrated potassium, sodium, and chloride ions of about 1 to 2 A are no longer very small compared with the average distance between ions and proteins.

A thorough mathematical treatment of the friction of small spheres moving through a suspension of large spheres is not available, but two simple geometrical arrangements have been calculated by Ladenburg (32). In the case of the movement of a sphere in the center of an infinitely long cylinder filled with a viscous liquid, the frictional resistance of the sphere is increased by the factor $(1 + 2.4\ R/d)$, where R is the radius of the sphere, and d the radius of the cylinder. In the case of moving a sphere between and perpendicular to two parallel plates of infinite extent, the frictional resistance of the sphere is increased by a factor $(1 + 3.3\ R/h)$, where h is the distance between the plates. Hence, the influence of the surfaces increases the frictional resistance of a sphere about 30% for R/d or R/h values near 0.1.

In the movement of small ions between the protein molecules, both cases are involved to a certain degree. Since R/d or R/h is of the order 0.1, the electrical mobility of the ions in the protein solution should be some 30% smaller. Compared with the simple geometrical arrangement of Ladenburg, the affect in the Hb solution could be even larger since many of the small ions are probably located as counter ions close to the dissociated groups at the surface of the protein.

At the present time, it is not possible to calculate accurately the degree of the conductivity deficiency. The considerations above indicate that probably the major part of the conductivity deficiency is caused by hydrodyamic and possibly by electrostatic effects also. Since the electric mobility of a particle and its diffusion constant are directly related to each other, one can expect a lower diffusion constant of the electrolytes and nonelectrolytes within the cell. This diminution should effect the rate of all diffusion-limited biological processes.

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