DIRECT MEASUREMENT OF POTENTIAL DIFFERENCE ACROSS THE HUMAN RED BLOOD CELL MEMBRANE

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ABSTRACT The electrical potential difference across the human red cell membrane has been measured directly. A biological amplifier with neutralized input capacity was used. Human red cells in modified Ringer solution were impaled individually with 3 M KCl-filled glass microelectrodes. Movements of the microelectrodes were effected by Leitz micromanipulators.[1] Results showed a potential difference of -8.0 ± 0.21 (SEM) mv, the inside being negative with respect to the outside. This value is approximately that calculated by using the Nernst equation considering the intracellular and extracellular chloride concentrations.

As a control, similar measurements were made on nylon microcapsules containing hemoglobin. The measured potential of -0.52 ± 0.02 (SEM) mv, which agreed very well with the value calculated on the basis of Donnan equilibrium, was much smaller in magnitude as compared to the results for the red cell, and there was evidence of fixed charges on the microcapsule membrane. There was no evidence of this in the case of the red cell.

INTRODUCTION

A difference of concentration of ions, protein molecules, and other charged particles on the opposite sides of a cell membrane will give rise to an electrical potential difference across the membrane. Such a concentration difference in the red cell is the result of the selective permeability of the membrane, the conditions for osmotic equilibrium, and the active transport mechanism (the sodium-potassium exchange pump.)

A direct measurement of electrical potential difference across the human red cell membrane has not been feasible until very recently, partly due to the inadequacy of microelectrodes and electronic equipment used on an object of such small size and capacitance. The human red cell is a biconcave disc with a thickness of about 2.3 μ and diameter of 8.1 μ (1). The capacitance is approximately estimated by the classical equation for the capacitance of a charged sphere (i.e. $C = 4\pi\epsilon r$) to be

[1] E. Leitz, Wetzlar, Germany.

of the order of 0.04 $\mu\mu$F. Lassen and Sten-Knudsen (2) have also reported success in such measurements in which a piezoelectric device was used to advance the pipette to penetrate the cell. Their paper has just been published and was not available to us when our work was done. Tables are not given of the individual voltages nor statistical evaluation, but calculation from the points on their graphs indicated that the mean voltage found was -5.1 mv with a very high standard deviation which we calculated as $\pm$ 2.7 mv. We were able to watch the penetration of the needle tip into and out of individual cells as relative to simultaneous jumps in voltage, and the standard deviation found in our work was much less ($\pm$ 0.91 mv). Another difference in our work from that of Lassen and Sten-Knudsen was that we used cells suspended in a modified Ringer's solution, while they used serum.

APPARATUS

Microscope A Nikon inverted microscope[2] made it possible to work conveniently from the top. The red cells on a slide would settle and remain on the bottom so that they could be individually impaled without having to be held by a second instrument. If the usual hanging drop method were used, we would not have this advantage.

Amplifier The biological amplifier (Bioelectric Instruments Inc.)[3] that we used featured a neutralized input capacity. The input impedence was larger than 10^{12} ohm, and this allowed the grid current to be less than 10^{-12} amp. The *neutralized input capacity* of the amplifier improved the response as shown in Fig. 1, *A* and *B*. 95% response of the amplifier with recorder was of the order of 1 msec. In the experiments on red cells, where the micropipette penetrated the membrane, the over-all response in voltage recorded had a time of less than 2 sec.

Microelectrodes Our microelectrodes were glass microelectrodes, filled with 3M KCl. The glass capillaries were special pyrex capillaries 520 119, supplied by Walter A. Carveth Ltd., Toronto, Canada in 6 inch long stock, 1 mm OD. A vertical micropipette puller (David Kopf Instruments)[4] could consistently produce micropipettes with 0.25 β tip diameter. However, only a few per cent of the microelectrodes produced were found to be satisfactory for our work. Only those which were significantly smaller than 0.25 μ in tip diameter were useful for our purpose since larger ones caused extensive damages to the cell membrane and usually resulted in the complete destruction of the cell during impalement. In these cases the membrane was evidently unable to seal around larger microelectrodes. Microelectrodes which were found to be satisfactory usually had resistances of about 50 MΩ, and had tip potentials measured to be about 5 mv, which did not change when the electrode was brought in contact with the glass slide. A silver–silver chloride wire provided the electrical contact between the KCl in the active electrode and the input to the amplifier. The second electrode was a similar microelectrode connected to the calibrator output of an oscilloscope, which was switched to ground during measurements. Such a connection made it very convenient for calibration purposes as well as for constant checking of the over-all gain of the system and the condition of the microelectrodes being used.

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[2] Nippon Kogaku (U.S.A.) Inc., Garden City, N.Y.
[3] Hastings-on-Hudson, N.Y.
[4] Tujunga, Calif.

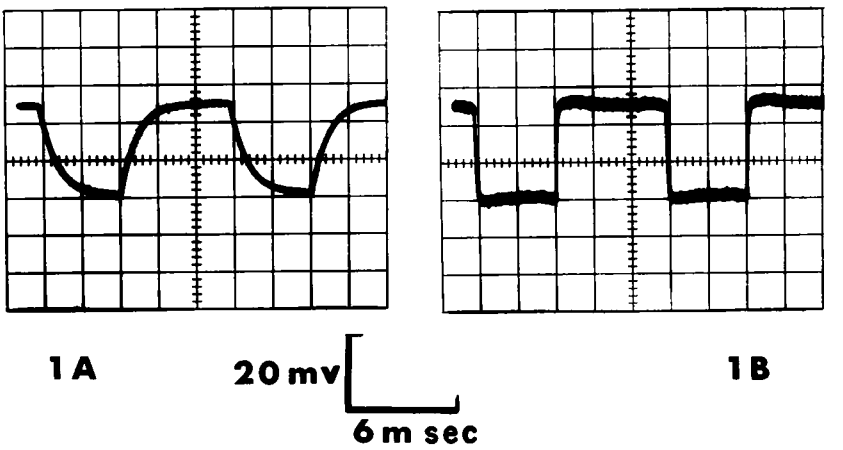

FIGURE 1 (*a*) Shape of calibrating square wave without neutralization. (*b*) Shape of calibrating square wave with neutralization.

Two Leitz micromanipulators were used to effect movements of the microelectrodes. A Beckman dynograph (Beckman Instruments, Inc., Fullerton, Calif.) recorded all the data.

PROCEDURE

Red cells from a finger prick were suspended in modified isotonic Tris-buffered Ringer solution.[5] A sample of very low hematocrit was placed in a cavity slide (with a coverslip forming the bottom) and the microelectrodes lowered into the solution. The amplifier was balanced, and a 10 mv square wave from the calibrator output of the oscilloscope was fed to the amplifier through the microelectrodes-liquid junctions system. The neutralization was adjusted for the best reproduction of the square-wave shape. The gain of the bioelectric amplifier with neutralization was set at 5. The cells were impaled individually at the rim. Since the cells adhered to the slide the final movement was conveniently effected by moving the stage so that the cell was moved against the tip of the microelectrode, thus eliminating the possible backlash of the microelectrode in the micromanipulator movement. The very tips of the microelectrodes that were small enough were not clearly visible due to the limitations of the optical resolving power. The actual penetration of the membrane by the microelectrode was indicated by a visible initial slight deformation of the membrane, followed immediately by a deflection of the beam of the oscilloscope. A measurement was considered satisfactory only if the deflection remained constant for at least 5 sec, and microscopic observation made it certain that the cell had been penetrated without contact of the needle with the glass coverslip. Fig. 2 shows a human red cell being successfully impaled by a microelectrode.

When a red cell had been impaled successfully, one of three things might happen. The cell might remain intact until the microelectrode was retracted. If the electrode was not retracted, usually after about 15 sec, the deflection would start to drift slowly towards the base line. A small vibration after entry might cause the red cell to suddenly become a "glassy"

[5] Modified isotonic Tris-buffered Ringer solution: NaCl 123.0 mM/liter; KCl 4.6 mM/liter; $CaCl_2$ 1.7 mM strokes/liter; $NaHCO_3$ 1.9 mM strokes/liter; Glucose 4.4 mM/liter; HCl 18.1 mM/liter; THAM 21.8 mM/liter.

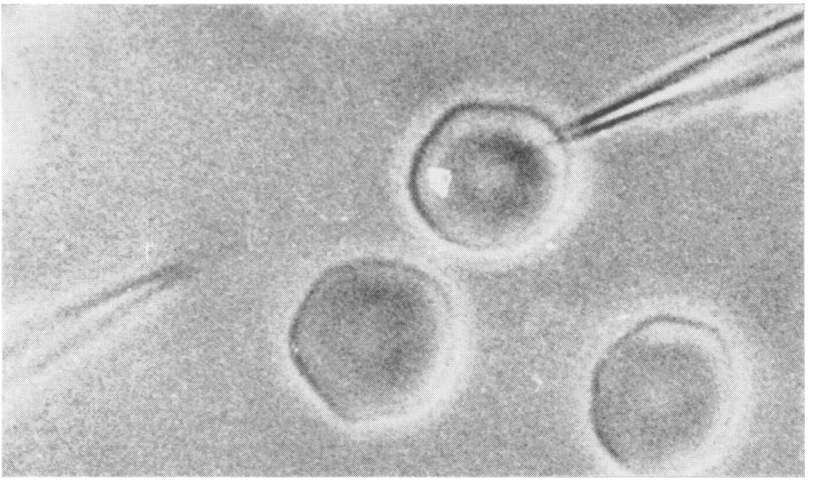

FIGURE 2 A red cell being successfully impaled by the active microelectrode. The ground electrode is shown on the left.

sphere which would rapidly free itself from the microelectrode tip. A fairly strong vibration causing movement of the microelectrode might result in the cell membrane being torn apart completely. In each of the last two cases where the cell was destroyed, the potential profiles would show a rapid drift back to the zero line. On occasions, after a successful impalement the stage was rapidly moved further so that the tip of the active microelectrode went through the opposite membrane and emerged from the other side of the cell. An immediate return of the deflection to the base line was usually recorded in the potential profile in such a case. In these cases the cell ruptured.

Damage of the cells would inevitably contaminate the tip of the microelectrode. Experience showed that no more than a few measurements could be made with an individual microelectrode. A microelectrode was considered no longer useful when the response to the calibrating voltage showed a decrease, indicating an increase in impedence, or if the base line began to fluctuate.

As a control, similar measurements were made on nylon microcapsules containing hemoglobin. Nylon microcapsules were prepared according to the standard procedure as outlined by Chang (3). The final concentration of hemoglobin in the microcapsules was approximately 20% of that in normal red cells, and the microcapsules studied had diameters ranging from 10 to 500 μ.

There is, of course, no question of "active transport" in the case of these microcapsules, so the small difference in voltage reported between inside and out would be entirely due to the presence of the nonpermeating hemoglobin molecules inside, by the Donnan equilibrium. Correspondence of this with the experimentally obtained voltage difference might serve as a check on the magnitude of such aspects as liquid junction and tip potentials.

RESULTS AND DISCUSSION

Fig. 3 shows a typical trace of the potential profile obtained in a measurement of the red cell membrane potential. A deflection was observed when the active electrode penetrated the membrane and entered the cell interior. On the retraction of

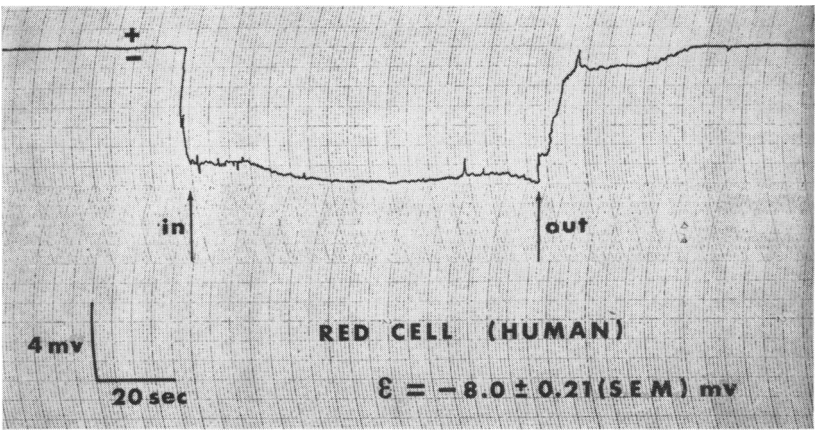

FIGURE 3 A typical potential profile obtained in a measurement of the electrical potential difference across the human red cell membrane.

the microelectrode the deflection rapidly returned to zero. In this particular case, the deflection was quite constant for as long as 1 min, the longest we observed. Drifting of the beam towards the base line was beginning to become evident immediately prior to the retraction of the microelectrode.

From our experiments, a mean value (in 20 measurements where our criteria were completely satisfied) of -8.0 ± 0.21 (SEM) mv was obtained for the electrical potential difference across the human red cell membrane, the interior being negative with respect to the exterior.

There is general agreement in the literature that the voltage to be expected inside the red cell will be that given by the Goldman's constant field equation (4) as developed by Hodgkin and Katz (5).

i.e.
$$E = \frac{RT}{ZF} \ln \frac{P_K(K^+)_{in} + P_{Na}(Na^+)_{in} + P_{Cl}(Cl^-)_{out}}{P_K(K^+)_{out} + P_{Na}(Na^+)_{out} + P_{Cl}(Cl^-)_{in}}$$

where P_{Cl}, P_K, etc., are the permeabilities of the membrane to the various ions present. It is to be noted that this is an approximate equation, based not on thermodynamic equilibrium but on postulated mechanism of a "steady state" or flux equilibrium, as where "active transport" of certain ions (metabolic "pumps") is responsible for large steady-state difference in concentrations inside and out. Since the red cell membrane is several thousand times more permeable to chloride ions than it is to sodium and potassium ions, the steady-state potential may be approximated by considering the chloride concentrations alone. Thus the potential is given approximately by the Nernst equation:

$$E = \frac{RT}{ZF} \ln \frac{(Cl^-)_{out}}{(Cl^-)_{in}}$$

where E = potential in volts; R = 8.31 J/mole °K; T = 296°K; Z = −1 for chloride ions; F = 96,500 coul/mole; $(Cl^-)_{in}$ = 73 meq/liter H_2O; $(Cl^-)_{out}$ = 110 meq/liter H_2O.

Such a calculation predicts a value of −10 mv. The values of the chloride concentration used in the calculation are taken from the literature (6) and are experimentally measured values for cells in plasma, while our measurements had been done with red cells in modified Ringer's solution. If the ratio of the extracellular chloride concentration to the intracellular concentration is lowered by 10%, the calculated value would have coincided with our experimental result of −8.0 mv.

Fig. 4 shows a typical trace of the potential profile obtained in one of the measurements in our control experiment with the microcapsules. A very sharp spike was evident as the nylon membrane was penetrated and the microelectrode entered in the interior. This initial deflection was immediately followed by a comparatively gradual deflection of the beam towards a maximum negative value (usually −2 to −4 mv), before it decayed to a much smaller value which remained constant until the microelectrode was retracted, at which time the deflection showed an immediate sharp return to the base line. The final constant value of the transmembrane potential in the microcapsules was found to be -0.52 ± 0.02 (SEM) mv (35 measurements). This compared very well to the expected value from considering the ionic distributions caused by the presence of the nondiffusible hemoglobin in the intramicrocapsular volume (Donnan equilibrium.) The nylon membrane of course did not possess any active transport mechanism and the ions therefore must be passively distributed, with hemoglobin being limited to the interior.

It is known that surface charges do develop from the particular way the nylon microcapsules are made (3). The appearance of the spike prior to the attainment of the steady intracapsular potential of −0.52 mv is likely to arise from the presence of the fixed charges on the nylon membrane. Such "artifacts" due to surface charges are not, however, seen in the red cell records.

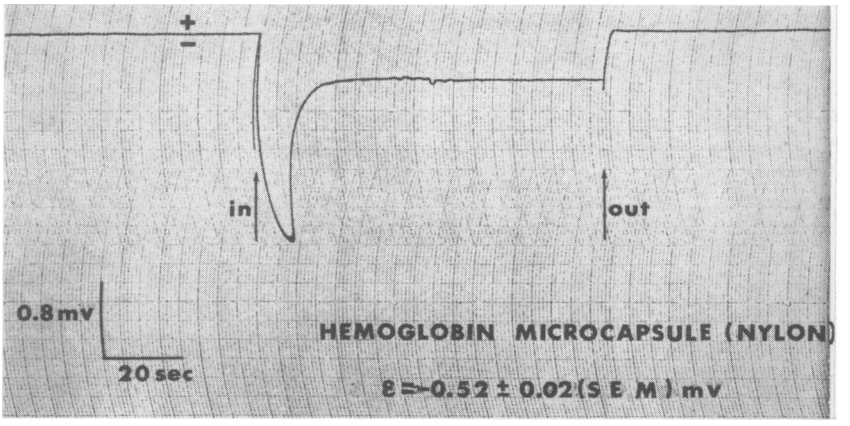

FIGURE 4 A typical potential profile obtained in a measurement of microcapsule potential.

The discrepancy between our result and the prediction would be partly accounted for if a small shift of chloride concentration occurred in cells when suspended in Ringer solution, or if the values of concentrations quoted are in slight error. An obvious uncertainty in our result is the error due to liquid junction potentials differing when the microelectrode is inside from when it is outside the cell. A liquid junction potential can be calculated by the Henderson equation (7) if the mobilities and concentrations of the ions on both sides of the liquid interface are known. Cole and Moore (8) in their study of the squid axon in sea water estimated the liquid junction potentials between 3 M KCl and sea water to be about 1 mv and that between 3 M KCl and axoplasm to be about 3 mv so that the total correction would be 4 mv. In the case of the red cells, unfortunately, the conductances of the anions and cations in the red cell cytoplasm cannot be measured and, therefore, the KCl red cell cytoplasm liquid junction potential cannot be estimated.

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