Membrane Resistance of Human Red Cells

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ABSTRACT A method has been devised to measure the specific membrane resistance of single human red cells. The cells were sucked into a 3 to 5 micron diameter pore in the end of a glass tube. By passing a small current through the cells, the total cell resistance was measured. The dimensions of the cell were measured optically and the specific membrane resistance was then calculated. Leakage of current between the cell and the walls of the pore was minimized by filling this region with isotonic sucrose. The measured specific membrane resistance values of four human red cells were 6.3, 6.32, 10.0, and 19.7 ohm-cm^2.

INTRODUCTION

There is indirect evidence that the membrane resistance of the human red cell is much less than the membrane resistance of nerve and muscle cells.

Dirken and Mook (1931) showed that the half-time of Cl^- and HCO_3^- exchange across the membrane of the red cell is 0.2 sec., which is equivalent to a permeability constant of 2×10^{-4} cm/sec. (Glynn, 1957 *b*). Similar results have been obtained by Luckner (1939) and Tosteson (1956). These permeability constants are much greater than those measured for nerve and muscle membranes; therefore, the red cell membrane resistance should also be much less than that measured for nerve and muscle membranes (*i.e.*, for frog sartorius, $P_{Cl} = 4 \times 10^{-6}$ cm/sec. (Adrian, 1961) and $R_m = 4000$ ohm-cm^2 (Shanes, 1958)).

Another profitable method for studying the electrical properties of red cell membranes has been the use of a bridge circuit to measure the alternating current impedance of a suspension of red cells (Cole, 1932; Bothwell and Schwan, 1956). Estimates of red cell membrane resistance have varied from 0.1 ohm-cm^2 (McClendon, 1926) to infinite membrane resistance (Fricke, 1933). Due to the current leakage around the cells at low frequencies, this method cannot be employed to measure membrane resistance (Cole and Curtis, 1938).

This paper describes a method for measuring red cell membrane specific resistance and gives the results obtained on four human red cells.

MATERIALS AND METHODS

The cell-holding pipettes were made from 1.2 to 1.5 mm diameter pyrex tubing (Fig. 1). The pipettes were pulled very slowly with a standard micropipette puller with the heating element set at a relatively low temperature. When the glass was pulled to a diameter of 25 to 100 microns, it fractured at right angles to the axis of the pipette. The tip of the pipette was then flash-heated to produce a smooth tip with a cylindrical pore 3 to 5 microns in diameter by 5 to 20 microns in length (Strickholm, 1961).

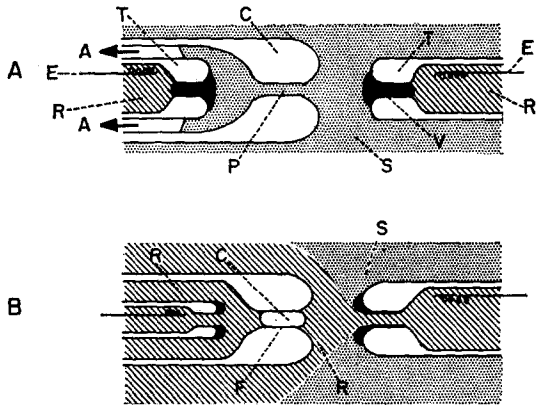

FIGURE 1 A. Schematic diagram of experimental set-up for measuring specific membrane resistance of human erythrocytes. *A*, a slight negative pressure applied to draw sucrose solution and cells into the cell-holding pipette. *C*, cell-holding pipette. *T*, perfusing tube. *V*, vaseline-paraffin plug. *S*, isotonic sucrose solution. *R*, mammalian Ringer's solution. *E*, tungsten electrode for resistance measurements. *P*, cell-holding pore. B. Schematic diagram showing configuration of apparatus less than 20 msec. after onset of perfusion. Sucrose solutions on both ends of cell have been replaced with mammalian Ringer's solution, but insulating film of sucrose between cell and pore wall is still intact. Resistance measurements are made during this period of time. *S*, isotonic sucrose solution. *R*, mammalian Ringer's solution. *C*, erythrocyte. *F*, film of sucrose between cell and pore wall.

Fig. 1 illustrates the method employed to measure the specific membrane resistance. First the experimental chamber was filled with isotonic sucrose solution (308 mM). A slight negative pressure was then applied to draw the sucrose solution into the cell-holding pipette, so that the meniscus covered the tip of the perfusing tube (Fig. 1A). The immersion of the tip of the perfusing tube prevented trapping of air bubbles during the perfusion. Next, one drop of blood obtained by finger puncture from the experimenter, was added to the experimental chamber (Fig. 1B). A slight negative pressure was again applied to the cell-holding pipette and a single red cell was drawn up into the cell-holding pore. In order to conform to the geometry of the pore, the cell assumed a cylindrical shape with hemispherical ends. Next, a step of positive pressure was applied simultaneously to both perfusing tubes. This re-

placed the sucrose solution on both sides of the cell with mammalian Ringer's solution in less than 20 msec. Since a considerably longer period of time was required for the insulating layer of sucrose solution to diffuse away, the leakage currents were presumably very small in the first few milliseconds following the perfusion. Small square waves of current 60 msec. long were passed through the cell *via* electrodes not shown during this period of time and the voltage between the electrodes, E, (see Fig. 1A) was recorded on a cathode ray oscilloscope and on an ink-writing oscillograph. Resistance was calculated from the known current and the measured voltage drop.

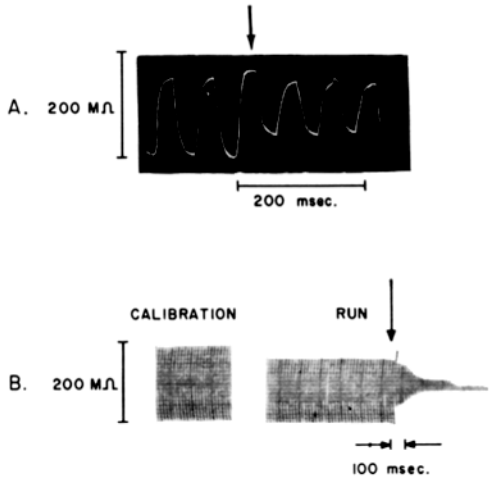

FIGURE 2. Resistance measurements on a single red cell. A. cathode ray oscilloscope recording of sudden change in resistance following perfusion. Since 60 msec. square waves of current were used to measure resistance, the height of the pulse is a measure of the resistance at that time. Note that there is a sudden drop in resistance following the onset of perfusion at arrow (probably within 20 msec.). B. same as A, except recorded on an ink-writing oscillograph and with a slower time base. Note the sudden drop in resistance following perfusion. Also note that it takes approximately 1 sec. for the resistance to reach the base line.

The perfusing tubes were manufactured in the same manner as the cell-holding pipettes, except that 0.6 to 1.0 mm pyrex tubing was used. The tubes were partially filled with mammalian Ringer's solution and the tips sealed with a vaseline-paraffin plug. The vaseline-paraffin ratio was adjusted so that a slight positive pressure within the perfusing tube would "blow out" the plug and permit perfusion. Both tubes were connected to a source of 30 pounds per square inch air pressure through a fast acting solenoid valve. When the solenoid opened, the air pressure forced open the vaseline-paraffin plugs and the solutions at the ends of the cell were replaced with mammalian Ringer's solution in less than 20 msec., probably in about 5 msec. (see Fig. 4).

The experimental chamber, containing the sucrose solution and the red cells, was mounted on the stage of a binocular microscope. The tip of the cell-holding pipette

and the tip of the outer perfusing tube were brought into the field of the microscope so that all manipulations could be done under direct visual control. A long working distance 40 × Cooke objective was used.

Since the perfusions and measurements were set in motion within several milliseconds after the onset of the experiment, these operations were triggered electronically.

The composition of the mammalian Ringer's solution (in mM) was NaCl, 154; KCl, 5.6; $CaCl_2$, 2.16; Na_2HPO_4, 0.16; NaH_2PO_4, 0.44; $NaHCO_3$, 4.16; and dextrose, 5.5.

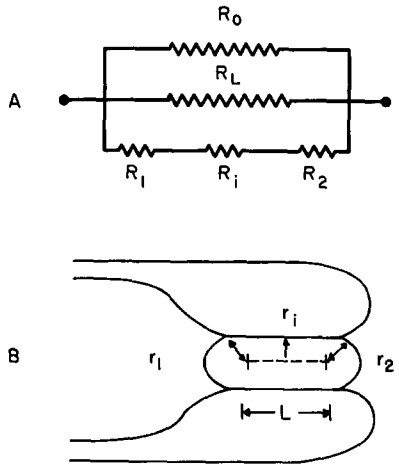

FIGURE 3. A. Equivalent circuit used in deriving the equation for specific membrane resistance in terms of experimentally derived data. R_L, leakage resistance between cell and pore wall, which is assumed to be infinite due to the use of an insulating sucrose solution between the cell and pore wall. R_1, resistance of inner hemispheric cell membrane. R_i, resistance of cell cytoplasm. R_2, resistance of outer hemispheric cell membrane. R_o, resistor shunted across input of resistance-measuring system. B. Cellular dimensions used to derive equation for specific membrane resistance.

Fig. 2 shows the resistance measurements from a successful experiment on a single red cell. The resistance between the electrodes shown in Fig. 1 can be calculated from resistance measurements like those shown in Fig. 2. A 180 megohm resistor was shunted across the input to the resistance-measuring preamplifier to improve frequency response. The resistance between the electrodes just prior to perfusion is approximately 1100 megohms in this example. At the onset of perfusion the resistance suddenly drops (less than 20 msec.) to 240 megohms. During the next second, the resistance slowly decays to 5 to 20 megohms. The resistance 10 to 40 msec. after the onset of perfusion was used in the calculation of the specific membrane resistance.

THEORY

The equivalent circuit (Fig. 3) was used to derive an equation for specific membrane resistance in terms of the various experimentally derived parameters. The equation is

$$R_m = \frac{\dfrac{R_t R_o}{(R_o - R_t)} - \dfrac{\rho_i L}{\pi r_i^2}}{\dfrac{1}{2\pi r_1^2} + \dfrac{1}{2\pi r_2^2}} \qquad (1)$$

where R_L = resistance of the insulating layer of sucrose between the cell and the pore wall. In this case it can be assumed to be infinite and is neglected.

R_m = specific membrane resistance.

R_o = 180 megohms = resistor shunted across the input circuit of the resistance-measuring system (used to improve the input circuit time constant).

R_t = total resistance of both the cell and the leakage pathway measured 5 to 25 msec. after the onset of the perfusion.

ρ_i = specific resistivity of the intracellular fluid. Assumed to be 193 ohm-cm (Pauly, 1959).

L = axial length of the cell (neglecting the hemispheric caps).

r_i = radius of the cylindrical portion of the cell.

r_1 = radius of the inner hemisphere of the cell.

r_2 = radius of the outer hemisphere of the cell. Usually $r_1 = r_2 = r_i$.

The resistance of the intracellular fluid within the hemispheric caps was neglected. This assumption introduces an error of less than 1 per cent, since R_i is only about 3 per cent of R_t.

RESULTS

Specific membrane resistance measurements were obtained from four single human red cells. The values of specific membrane resistance obtained in these four experiments were 6.3, 6.32, 10.0, and 19.7 ohm-cm^2. The average of these values was 10.6 ohm-cm^2.

The technical problems involved in performing these experiments were formidable. 2 to 3 days were required to set up the apparatus for an experiment. The vast majority of these experiments were not productive. The most frequent cause of experimental failure was blowing the cell out of the pore at the instant of perfusion. In 4 months of steady work, only four successful experiments were performed.

Sources of Error

DIMENSIONAL MEASUREMENTS An important source of error in these experiments is inaccuracy in the measurements of cell dimensions. Small errors in the measurement of cell radii cause large errors in the calculated resistance. For example, assume a cell with R_m = 10 ohm-cm^2 and dimensions of $r = r_1 = r_2 = r_i$ = 2 microns and L = 15 microns. If r were misread as 1.5 microns, then equation (1) would have given R_m = 5.65 ohm-cm^2. If r were misread as r = 2.5 microns, equation (1) would have given R_m = 15.7 ohm-cm^2. Dimensional errors of this magnitude could easily have been made.

CELL MEMBRANE DEFORMATION. Another potential source of error is the deformation of the membrane which may have occurred when the cell assumed a cylindrical shape within the cell-holding pipette.

LEAKAGE AROUND CELL The initial experiments, without the sucrose insulating film, gave values of specific membrane resistance in the range of 0.5 to 2.0 ohm-cm^2. When the question of current leakage around the red cell arose (Cole and Curtis, 1938), bubbles of air the same size as red cells were drawn into the pore and the resistance measured. These resistance values were of the same order of magnitude as with a red cell in the pore. These results are explained on the basis that a thin film of saline between the cell and the pore wall provides a leakage pathway around the cell.

The thickness of the proposed saline film can be approximated by using the equation

$$\Delta x = \frac{\rho_o L r[R_m - (R_e + \rho_i L)]}{2R_m(R_e + \rho_i L)} \tag{2}$$

where the following assumptions and terms are used

Δx = thickness of saline film

ρ_o = 60 ohm-cm = specific resistivity of saline film.

r = $r_1 = r_2 = r_i$ = 2 microns = pore radius

R_m = 10 ohm-cm^2 = specific membrane resistance, calculated from experiments using sucrose insulating film.

L = 15 microns.

ρ_i = 193 ohm-cm.

R_e = 1 ohm-cm^2 = specific membrane resistance calculated from experiments in which the sucrose insulating film was not used.

Using the above assumptions, $\Delta\chi$ is 600 A in thickness. A film of this thickness could not be visualized with the equipment employed, since it is below the resolving power of the light microscope.

If the saline in the leakage pathway is replaced with an isotonic solution of sucrose in distilled water, the specific resistance increases by approximately 20,000 times (Stämpfli, 1954). In practice, an increase in specific resistance of 100 times would be adequate. Using the above example and assuming $\rho_0 = 6 \times 10^3$ ohm-cm for sucrose, this would give a leakage resistance of 900 megohms. Therefore, since the leakage is in parallel with the total cell resistance of 80 megohms ($R_c = R_m/\pi r^2 = 80$ megohms, where R_c is total cell membrane resistance), the leakage resistance has been neglected in calculating R_m from equation (1).

DIFFUSION TIME A perfusion system was used to replace the sucrose solution (bathing the ends of the cell) with Ringer's solution, without replacing the insulating film of sucrose solution located between the cell and the pore wall. The time required for the sucrose solution to diffuse out from between the cell and the pore wall can be calculated using the diffusion equations for a plane sheet (Crank, 1956). These equations may not be valid for

very thin films of fluid. They were used to provide an order of magnitude calculation of the maximum diffusion time. Assuming an initially uniform concentration of sucrose within the film and a zero concentration at the boundaries, the time required for the sucrose at the center of the sheet to drop to 0.9 of the initial value was taken as the diffusion time. The diffusion time was 13.5 msec. This relatively short diffusion time required that the perfusion of the ends of the cell be completed within a few milliseconds, if the insulating film of sucrose was to be effective.

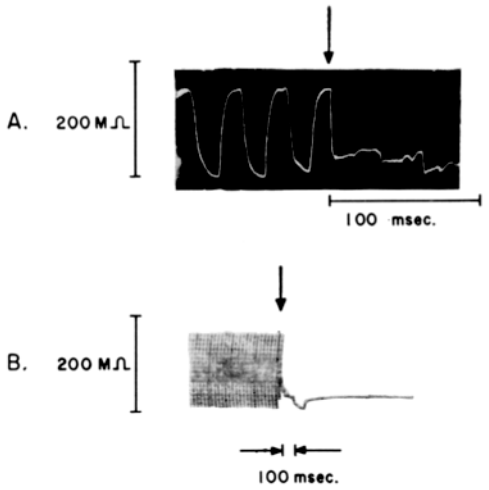

FIGURE 4. Resistance measurements with no red cell in the cell-holding pipette showing the rapid drop in resistance following the onset of perfusion. Otherwise this record is the same as that shown in Fig. 2. The arrow indicates the onset of perfusion. These measurements show that at the onset of perfusion the resistance drops to base line in less than 20 msec.

It should be noted from the resistance measurements in Fig. 2 that the decay of resistance to base line, due to the diffusion of sucrose away from the space between the cell wall and the pore wall, took longer than predicted from the diffusion calculations. This simplified the resistance measurements and increased their reliability.

The evidence that the sucrose solutions bathing the ends of the red cell are replaced within 20 msec. is as follows:—

1. The resistance measurements shown in Fig. 2 illustrate a sudden drop in resistance at the onset of perfusion.

2. Fig. 4 shows the resistance measurements obtained in an experiment in which there was no red cell in the cell-holding pipette. The resistance drops to base line within 20 msec.

3. In another experiment, the cell-holding pipette was plugged with vaseline and a dye was placed in the inner perfusing pipette. At the onset of perfusion, the sucrose was instantaneously replaced with saline colored with dye. The replacement occurred so fast that it could not be visualized.

DISCUSSION

Membrane Resistance in Other Tissues

These measurements show that the specific membrane resistance of human red cells is much lower than that of most nerve and muscle membranes. Examples of R_m in ohm-cm^2 in various tissues (Shanes, 1958) are as follows: Squid giant axon (*Loligo*), 1500; Cuttlefish giant axon (*Sepia*), 9200; frog node, 8 to 20; frog internode, 100,000; frog sartorius muscle, 4000; cat motoneuron, 500; and goat Purkinje fibers, 1900. An example of a membrane with a lower specific membrane resistance than the human red cell membrane is the intercalated disk of cardiac cells. The specific membrane resistance of the intercalated disk is of the order of 1 ohm-cm^2 (Weidmann, 1960; Woodbury and Crill, 1961).

The Basis for the Low Membrane Resistance in the Human Red Cell

The basis for the low specific membrane resistance of human red cells lies in the ready permeability of the red cell membrane to anions (Cl^- and HCO_3^-). Dirken and Mook (1931), using a dialysis flow method, calculated the time course of the exchange of Cl^- for HCO_3^- when a suspension of red cells was suddenly mixed with CO_2-saturated plasma. The half-time of the exchange of Cl^- and HCO_3^- across the red cell membrane was 0.2 sec., which is equivalent to a permeability constant of 2×10^{-4} cm/sec. (Glynn, 1957 *b*).

Luckner (1939) obtained similar results by suddenly increasing the CO_2 concentration and measuring the subsequent change in Cl^- concentration with an Ag/AgCl electrode. He found the half-time of the exchange to be 0.1 to 0.12 sec., which is equivalent to a permeability constant of 4×10^{-4} cm/sec. (Glynn, 1957 *b*).

Tosteson (1959) measured the time course of the efflux of Cl^{38} from human red cells by means of the dialysis flow method. For human red cells, the half-time of the exchange was 0.21 sec. and the chloride efflux was 1.31×10^4 pmoles/cm^2-sec. He also estimated the total specific membrane resistance by calculating that part of the total specific membrane resistance due to chloride ions. The following equation was used

$$R_{Cl} = \frac{RT}{F^2 M_{Cl}} \tag{3}$$

where R is the gas constant, T is the absolute temperature, F is the Faraday, M_{Cl} is the chloride efflux, and R_{Cl} is the chloride specific membrane resistance. Assuming that M_{Cl} is 1.3×10^4 pmoles/cm^2-sec., the chloride resistance is 19 ohm-cm^2.

Assuming equal chloride and bicarbonate resistances and a chloride resist-

ance of 19 ohm-cm², the membrane resistance is 9.5 ohm-cm². This is comparable to the four values of membrane resistance (6.3, 6.32, 10.0, and 19.7 ohm-cm²) obtained by the direct resistance measurements described in this paper.

Tosteson (1959) points out that the rate constant for Cl^- efflux is over one million times greater than that for K^+ efflux, but that the half-time of the Cl^- efflux is only one-fiftieth the half-time of the tritiated water influx.

Water-Filled Pores in the Red Cell Membrane

There is evidence that water-filled pores exist in the human red cell membrane (Solomon, 1960). Pappenheimer, Renkin, and Borrero (1951) and Koefoed-Johnsen and Ussing (1953) first demonstrated that the difference between the permeability constants for diffusion and filtration of water could be interpreted as indicating the existence of water-filled pores in the membrane. Using this water flux approach, Paganelli and Solomon (1957) measured the rate of diffusion of water across the membrane under isotonic conditions, using tritiated water as a tracer. This value was compared with the rate of flow of water under an osmotic pressure gradient, and the difference between the two rates was used to calculate an effective pore radius of 3.5 A.

Another approach which can be interpreted as demonstrating the existence of water-filled pores is the restricted filtration method which uses small lipid-insoluble, non-electrolyte molecules as probes to measure the size of the pores (Goldstein and Solomon, 1960). This method involves the measurement of the osmolarity of solutions of non-lipid–soluble, non-electrolyte molecules, which when exposed to red cells will cause zero initial change in net water flux across the membrane. This experimental value plus several mathematical manipulations was used to obtain the effective pore radius of 4.2 A.

It is of interest to derive the number of pores per red cell using the experimentally determined specific membrane resistance of 10.6 ohm-cm². The equation giving N = pores/red cell is

$$N = \frac{\rho_2 \Delta x A}{\pi r_p^2 R_m} = 4700 \text{ pores/red cell} \qquad (4)$$

where it is assumed that

Cl^- and HCO_3^- carry all the current across the membrane through cylindrical water-filled pores.

r_p = pore radius = 3.5 A (Solomon, 1960).

ρ_2 = (Cl^- + HCO_3^-) resistivity = 2 × 60 = 120 ohm-cm.

Δx = membrane thickness = 100 A (Glynn, 1957 *b*).

A = surface area of red cell = 160 microns² (Ponder, 1948).

R_m = specific membrane resistivity = 10.6 ohm-cm².

Solomon (1960) obtained 0.9 × 10^5 pores/cell in a calculation assuming

$A_w/\Delta x = 3.3 \times 10^{-4}$ cm (A_w is the total cross-sectional area of all the pores), a membrane thickness of 100 A, and a pore radius of 3.5 A.

A possible explanation for the discrepancy in these two independent calculations is that the plasma resistivity within the pores is much greater than the 120 ohm-cm assumed. Another possibility is that only a fraction of the pores used for water transport are also used for the transport of Cl^- and HCO_3^-.

Glynn (1957 *a*) has calculated that there are approximately 1200 sites for potassium influx on the red cell membrane, using data on the inhibition of potassium influx by scillaren. Solomon, Gill, and Gold (1956) estimated that there are less than 1.2×10^4 sites for potassium influx from data on the inhibition of potassium influx by ouabain. The agreement between these values of the number of sites of potassium influx per red cell and the number of pores per cell derived from the above resistance calculations raises the possibility of a connection between the sites of potassium influx and the pores or sites of Cl^- or HCO_3^- flux.

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