The Electrophoretic Mobility of Red Blood Cells of Normal Human Beings

By JOHN ANGERS AND ANTONIO ROTTINO

ALTHOUGH pioneer work in the electrophoretic study of particles[1,2] and of the blood cell[3-7] has been done (for reviews see references 8, 9, 10), the electrophoretic technic has not been extensively used for determining whether the surface charge of the red cell varies in health and disease. Present work in our laboratory indicates that such changes do occur in disease states and that this phenomenon should be further studied.

MATERIALS AND METHODS

Red cells were collected from 200 healthy persons. A medical history was taken and physical examination, urinalysis and red and white blood cell counts performed in each case. Both sexes were represented and the group was composed chiefly of blood donors, students of nursing and laboratory personnel, from 17 to 55 years of age. To control personal bias, two series were run—one by one of the authors° (121 samples) and one by a technician whom he had trained (79 samples). To test reproducibility many specimens were run from two to five times, at short intervals. The determinations were done on washed red blood cells, and on unwashed cells of 10 persons as well.

Preparation of Suspensions

Blood was collected by venepuncture into a tube containing ethylenediaminetetracetic acid. One cubic centimeter of blood was washed three times with 10 cc. of one per cent NaCl solution. Immediately before the experiment was started, one or two cubic millimeters of these washed cells were drawn into a white cell pipette and mixed with 25 cc. of Michaelis buffer of ionic strength 0.172. Successive tests were run at pH 9, 7, 5.5 and 4.

Apparatus

Of the various electrophoretic cells† available[9,11] we chose that of Ponder.[12] The electric circuit consisted of a "Heath Kit" variable-voltage, regulated power supply, Model PS-2. To adapt this to our purpose we introduced in series with it the following: a 25 milliampere meter, a 4250 ohms resistor, a 2500 ohms potentiometer and a doublepull-doublethrow switch to reverse the polarity when necessary. The power supplied was 155 volts and current 8 milliamperes.

A monocular microscope platform was modified to accommodate the vertical cell as described by Ponder.[12] With the cell in place the platform is in a vertical position and the microscope barrel horizontal. The optical system consists of a 0.45 mm. phase objective (Ph 20), a long-focus condenser and an eyepiece of 20x magnification with an inserted micrometer disc (Zeiss). With these optics the disc lines projected at the level of the cell

From the Hodgkin's Disease Research Laboratory, (Supported in part by U. S. Public Health grant CY4069, the Damon Runyon Memorial Fund for Cancer Research, and the Nias Foundation), St. Vincent's Hospital, New York, N. Y.

We gratefully acknowledge the assistance of Dr. Eric Ponder for instruction in the use of the electrophoretic apparatus.

Submitted July 15, 1960; accepted for publication Sept. 25, 1960.

°Dr. John Angers.

†Made by Mr. Otto Kessler, of Fisher Scientific Company, New York, N. Y.

appear 25 μ apart. The actual measurement was made by superimposing the image of the disc upon a hemocytometer placed at the level of the electrophoretic cell.

Any good light source was found suitable. A flask filled with water was placed between the light source and the stage. This prevented heating of the cell with resultant convection currents and bubble formation on the wall of the cell. If bubbles form, the cell must be cleaned out with acid and water.

A metronome set at one beat per second was used as the timing device.

The electrodes were made of pure copper wire, 3 mm. thick and 3 cm. long. They were cleaned daily, since a deposit forms on them after being used four or five times.

The inside of the apparatus was cleaned by removing the glass male electrode and applying oral suction to each internal section. The cleansing fluid was a 40 per cent solution of nitric acid, which must not be allowed to touch the metal base. The base and outside of the glass were cleaned with 1 per cent alcohol. Once-weekly cleaning was done with sulphuric acid chromate solution.

$CuSO_4$ solution was prepared by dissolving cupric sulfate in deionized, distilled water until saturation occurred. The adsorbing agent used on the fritted glass was Kaolin powder, USP grade. Michaelis buffer was prepared according to the method of Bull.[13]

The filling of the electrophoresis cell was done as follows: If the plug of the horizontal arm of the glass receptacle for the copper electrode had been put in in such manner that it was flush with the end of the arm, the horizontal arm was filled with a saturated solution of CuSO4 containing 5 per cent agar and the vertical arm with a saturated solution of CuSO4; if, however, the plug had been slightly recessed into the arm, the resulting cavity at the end of the arm was packed with Kaolin and a pure saturated solution of CuSO4 used throughout the glass receptacle. The addition of agar increased the internal resistance of our unit to 15 x 10^3 ohms when filled with Michaelis buffer, the resistance being measured with a standard Wheatstone bridge..* The cell was filled with buffer of pH 7, the electrode receptacle was fitted into the female receptacle of the cell, and the cell was screwed into position on the microscope stage. A 30 cc. beaker containing the suspension of red cells was placed at the lower end of the electrophoresis cell and through a rubber tube at the top of the cell the suspension was drawn into the cell with gentle oral suction. If air bubbles formed during filling of the apparatus, it was emptied and filled again, as bubbles cause erroneous results. Immediately before turning on the current the three-way cocks were opened in a manner to allow the current to pass without interruption from one electrode to the other.

The microscope was focused upon the red cells. The depth at which this is done is critical and once determined remains constant thereafter. The critical point is at a locus 0.21 mm. beyond the inner surface of the anterior wall of the cell, at the junction of the midpoint of the length of the cell and the midpoint of the width of the cell.

After setting the metronome in motion, the distance that the red cell fell was measured per unit of time. First the velocity of a red cell falling against gravity alone was measured. We found that when the cells fell or rose at a rapid rate there were convection currents that led to erroneous results. Immediately after making the measurement against gravity alone for 5 seconds the current was put on for five seconds. The lesser velocity was subtracted from the greater, leaving the velocity due to the electrical force applied; this was divided by five, giving the velocity per second. The average of five readings on the same specimen was used in calculating mobility.

The mobility of the red cell in the suspended buffer is expressed as velocity per unit of electrical field strength imposed on the red cell by the power supply. The field strength, E, will depend, therefore, on the voltage supplied, the current generated, and the resistance offered by the buffer. The relationship is expressed by the formula:

$$\text{Mobility} = \frac{\text{Velocity}}{E} = \frac{\dfrac{\text{Distance travelled}}{\text{time}}}{E}$$

*Model RC 16, of the Industrial Instrument Company, Inc.

Distance was measured with the optical system by means of the micrometer disc which served as a background for the moving red cells. The time was five seconds, and the average velocity of five red cells was calculated. The field strength E in volts per centimeters acting on the red cell was calculated by the formula $E = \frac{I}{A\lambda}$, where I is the intensity of current expressed in amperes (in our case $= 8 \times 10^{-3}$ amperes), A is the measured cross-sectional area of the electrophoretic cell in centimeters, which for our cell is ($A = 1.1 \times 0.1 = 0.11$ cm.^{2} and λ is the specific conductivity cell with a known standard. For our particular Michaelis buffer it was $\frac{1}{60}$. Hence it follows that if I is kept constant, E will

$$\frac{E}{I} = \frac{A\lambda}{} = \frac{8 \times 10^{-3} \times 60}{11 \times 10^{-2}} = 4.36 \text{ volts/cm.}$$

Typical example: Mobility $= \frac{d/t}{E} = \frac{5.8}{E}$ μ per second

$$= \frac{5.8}{4.36} = 1.33 \ \mu\text{/sec./volt/cm.}$$

Results

Table 1 shows the mean mobilities and their standard deviations of single determinations performed by one person upon the washed red cells of 121 normal individuals, and by a second person upon those of 79 other normal individuals.

To determine the constancy of red cell mobility with time, determinations were made upon the cells of one individual at intervals for nine months. These results are shown in table 2. They show that the electrophoretic mobility of red cells of a person in health remains constant.

To determine the extent of the difference in mobility between washed and unwashed cells, the unwashed cells from 10 normal persons were studied (table 3). There is evidently something adsorbed to the unwashed red cell which lowers its electrophoretic mobility. This substance can be eluted by washing the red cells in one per cent NaCl solution. Performance of electrophoresis on washed red cells thus insures constancy and reproducibility of results.

To determine to what extent variations in the measurements were significant, specimens from five normal persons were subjected to three or four runs spaced an hour apart. The results of one such experiment are shown in table 4. The difference in mean value was .05 μ and the range was from 0 to .10 μ; the latter range occurred twice in 60 determinations. The same variation was found in the results obtained by both technicians.

We next wished to know whether different instruments made according to the same specifications would cause results to vary. We therefore filled cell #1 with a sample of blood and made a determination, then filled cell #2 with the same sample and made a determination. Ten different specimens were thus studied. This difference was from 0 to .011 μ, with a mean variation of .004, and is identical to that found when five determinations on the same sample are made in the same cell.

Reproducibility was further tested by performing the test on four specimens

Table 1.—*Electrophoretic Mobility of Human Red Blood Cells in Microns/sec./volt/cm.*

pH	9	7	5.5	4
Series 1	1.30 ± .03	1.32 ± .05	1.06 ± .11	.87 ± .13
Series 2	1.33 ± .05	1.27 ± .05	1.16 ± .04	1.08 ± .08
Abramson[3]	—	1.31 ± .02	—	—
Ponder & Furchgott[6]		1.03		

Table 2.—*Electrophoretic Mobility of Sample of Red Blood Cells from One Person, Taken at Approximately Monthly Intervals for Eleven Months*

Date	pH 9	pH 7	pH 5.5	pH 4
7-28-59	1.33	1.33	0.92	0.80
11-9-59	1.33	1.28	0.98	0.96
12-9-59	1.33	1.30	0.96	0.94
1-12-60	1.35	1.33	0.96	0.92
2-23-60	1.28	1.30	0.96	0.89
3-23-60	1.30	1.28	0.98	0.89
5-18-60	1.33	1.28	1.12	1.05
6-27-60	1.35	1.19	1.12	1.05

Table 3.—*Difference in Mobility Between Washed and Unwashed Red Blood Cells*

pH	9	7	5.5	4
Mean mobility of washed RBC	1.25	1.17	0.86	0.60
Mean mobility of unwashed RBC	1.07	1.01	0.84	0.53

Table 4.—*Variation When Multiple Determinations Were Done on the Same Sample Over a Period of 4 Hours*

pH	9	7	5.5	4
Mobility	1.38	1.33	0.98	0.89
	1.35	1.38	1.00	0.94
	1.35	1.28	0.98	0.89
	1.38	1.33	1.02	0.94

with our own equipment in our own environment and then repeating it on the same specimens in Dr. Ponder's laboratory with his apparatus but using our own micrometer disc. Identical results were obtained.

For practical reasons the red cells were sometimes refrigerated overnight. Table 5 shows that the mobility of red blood cells was not affected by storage at 4 C. overnight, while similar storage for 36 hours or more caused considerable change.

Discussion

It was our aim in this work to establish the range of the electrophoretic mobility of the erythrocytes of healthy persons. As might be expected, we found some variation—an expression of the individuality of the red cells—but

Table 5.—*Change of Electrophoretic Mobility Due to Storage of Red Cells for 24, 30 and 40 Hours*

pH	9	7	5.5	4
Mobility	1.33	1.28	0.98	0.96
+24 hrs.	1.30	1.33	1.05	0.94
+30 hrs.	1.25	1.28	1.00	0.92
+40 hrs.	1.40	1.19	1.17	1.05

the range was quite narrow, particularly at pH 9 and 7. Our values were close to those obtained by Abramson[3] for normal persons. Differences in our results and those obtained by Ponder and Furchgott[6] may be due to difference in the environmental temperature (22 vs. 26 C.) and to possible differences in the calibration of the grating used in the eyepiece for measuring distance.

Using the same equipment, material and environment, results obtained by two different persons in our laboratory varied slightly. There was a 2 per cent difference in mobility at pH 9 and 7, 9 per cent at pH 5.5, and 10 per cent at pH 4. In general this is good agreement.

The importance of meticulous technic should be emphasized. It is highly important that the electrical system should function properly, that solutions be prepared precisely and remain constant, that glassware be absolutely clean, and that the apparatus be set on a vibration-free base. Temperature control of the area of operation is important; a draft blowing upon the apparatus causes eddy currents. Coating of the electrodes is a source of trouble and they must be cleaned constantly. The light source must be cool. It is of the utmost importance that the red cell selected for measurement should come into focus at a constant point.[10] For our cell, this point is 0.21 mm. beyond the inner surface of the anterior wall of the cell.

To date the significance of the electrophoretic data consists in its usefulness in obtaining insight into the submicroscopic structure of the red cell surface. For example, results of mobility measurements at various ionic strengths led Furchgott and Ponder[6] to conclude that the contour of the red cell is "bumpy". From pH mobility data the same authors proposed that the red cell surface is composed largely of lipid and dominated by strong acid groups—possibly the phosphoric acid groups of the cephalin molecule. The electrophoretic mobility of erythrocytes from various animals show that they differ from each other. The erythrocyte mobility of the dog is 1.65 μ/sec., of the rat 1.45, of the mouse 1.40, Rhesus monkey 1.33, of man 1.31, and of the rabbit 0.55 (3).

Summary

Values obtained for the electrophoretic behavior of the red blood cells of healthy individuals is presented. The technic and instrument are described in detail and the necessary attention to meticulous care is emphasized. The data presented show that in an electric field the mobility of the red blood cells of healthy persons is constant and reproducible. It was concluded that the method is extremely sensitive and precise and that it may prove of value in the study of various disease states.

SUMMARIO IN INTERLINGUA

Es presentate le valores obtenite in le studio del comportamento electrophoretic de erythrocytos ab subjectos human in bon stato de sanitate. Le instrumentation e le technica usate es describite in detalio. Es sublineate le necessitate de grande meticulositate methodologic. Le observationes monstra que in un campo electric le mobilitate de erythrocytos ab subjectos normal es constante e reproducibile. Le conclusion es que le methodo es extrememente sensibile e precise e que illo va possibilemente provar se de valor in le studio de varie statos pathologic.

REFERENCES

1. Abramson, H. A. and Michaelis, L. M.: The influence of size, shape and conductivity of microscopically visible particles on cataphoresis mobility. J. Gen.Physiol. *12*:582–598, 1929.
2. Quinke, G.: Pogg.Ann. *113*: 513, 1861. Quoted by Abramson in citation 8.
3. Abramson, H. A.: The cataphoretic velocity of mammalian red blood cells. J.Gen.Physiol. *12*:711–725, 1925.
4. — and Moyer, L. S.: The electrical charge of mammalian red blood cells. J.Gen.Physiol. *19*:601–607, 1936.
5. —: The isoelectric point of normal and sensitized mammalian erythrocytes. J. Gen.Physiol. *14*:163–177, 1930.
6. Furchgott, R. H. and Ponder, Eric: Electrophoretic studies on human red cells. J.Gen.Physiol. *24*:447–457, 1941.
7. Abramson, H. A., Gorin, M. H. and Ponder, Eric: Electrophoresis and the chemistry of cell surfaces. Cold Springs Harbor Symposium 8:72–79, 1940.
8. Shedlovsky, T. S.: Symposium on electrophoresis. Ann.Acad.Sc. *39*:1–212, 1939.
9. Briggs, D. R.: Electrophoresis. *In* Biochemical Research Methods, by Uber, pp. 271–299. New York, Interscience Publishing Co., 1950.
10. Brinton, C. C. and Lauffer, M. A.: The electrophoresis of viruses, bacteria and cells, and the microscope method of electrophoresis. *In* Electrophoresis, edited by M. Bier., pp. 427–492. New York, Academic Press, 1959.
11. Abramson, H. A.: Modification of the Northrup Kunitz microelectrophoresis cell. J.Gen.Physiol. *12*:469–472, 1929.
12. Ponder, E. and Ponder, R. V.: Electrophoretic mobility of red cells and their ghosts as observed with improved apparatus. J.Exper.Biol. *32*: 175–182, 1955.
13. Bull, H. B.: Electrokinetics. *In* Physical Biochemistry, Ch. 9. New York, J. Wiley & Sons, 1951.

STUDIES IN SICKLE-CELL ANEMIA. XII. FURTHER STUDIES ON HEPATIC FUNCTION IN SICKLE-CELL ANEMIA. *A. D. Ferguson and R. B. Scott.* Howard University, Washington, D. C. A.M.A.J.Dis.Children 97:418–425, 1959.

A number of children with sickle cell anemia were studied for alterations in their liver function. In addition to traditional liver function tests, deteminations of blood ammonia, urinary amino acid patterns and serum protein electrophoresis were carried out. One liver biopsy was also performed. As in other studies of this type, changes were detected in many of these parameters, but these were not conclusively diagnostic. The authors conclude that although the evidence for hepatic dysfunction is unquestionable in persons with sickle cell anemia, quantitation of this damage in children is difficult.—*A. I. C.*