Mobility of Human Red Blood Cells of Different Age Groups in an Electric Field

By A. Yaari

Small variations found in the electrical mobility of human red blood cells may be attributed to artifacts of the different techniques and instruments. Whether variations in the mobility are indeed due to technical artifacts or to the unequal surface charges of different cells in the same blood sample has not been clear. While Ruhenstroth-Bauer has stated that red cells of the same donor display a practically uniform charge, Danon and Marikowsky have reported that young red blood cells migrate in an electric field more rapidly than do old cells of the same blood sample.

In a preliminary study of the surface charge of the red blood cells of normal donors undertaken in order to establish the "normal range" we found a wide range of mobilities in each sample, which we were able to correlate with the age distribution of the cells. We assume that the density of red blood cells is correlated with the age of the cells as mentioned by others.

Materials and Methods

Siliconized (Silicalad, Clay Adams Corp.) glassware was employed using citrated blood (3.8 per cent sodium citrate) of eleven healthy donors. The density distribution of cells (D.D.C.) was determined using a battery of 20 phthalate ester mixtures with specific gravity decrements of 0.004 between each (Yeda Research and Development Co., Rehovot, Israel). Based on the specific gravity values obtained for the various age groups of cells, eight fractions from each sample were separated by a slight variation (Fig. 1) of the differential flotation method described by Brok et al.

The packed red cells below the separation fluids were resuspended in equal volume of plasma. This "reconstituted blood" was washed three times in NaCl solution buffered to pH 7.4. (NaCl 0.8 per cent, KCl 0.02 per cent, KH2PO4 0.02 per cent, Na2HPO4 0.115 per cent) and then resuspended in the same medium for electric mobility measurement. Whole blood, centrifuged and passed through separating fluid No. 18 (S.C.1.074) leaving only the buffy coat on top, served as a control to untreated washed cells.

The Ruhenstroth-Bauer Cytopherometer (C. Zeiss) was used at a current of 5 ma. at 24 C. A freshly prepared suspension of 0.1 per cent washed packed red blood cells in phosphate buffered NaCl solution was submitted for measurement. The mobility of 100 cells in each direction was measured. The formula \[ B = \frac{1}{T} \left( \text{cm}^2 \text{v}^{-1} \text{sec}^{-1} \right) \] was used to calculate the parameter. \( B \) is the mobility of a cell, \( T \) is the distance (cm.) traversed by the cell during the time \( T \) (sec.) and \( E \) is the field strength; where \( j \) is the current.

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First submitted March 5, 1968; accepted for publication June 5, 1968.

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Fig. 1.—Separation of red blood cells into age groups according to their specific gravity by the differential flotation method.

\[ p \text{ the specific resistance of the buffer solution, } h \text{ the height of the chamber (cm.)} \text{ and } t \text{ the depth of the chamber (cm.).} \]

**RESULTS**

The range of migration of red cells in all samples was \(0.855-1.47 \times 10^{-4}\) cm.\(^2\)v\(^{-1}\)sec.\(^{-1}\). The mobility of the youngest cell fraction was in the range \(1.47-1.25 \times 10^{-4}\) cm.\(^2\)v\(^{-1}\)sec.\(^{-1}\) and of the oldest \(1.00-0.855 \times 10^{-4}\) cm.\(^2\)v\(^{-1}\)sec.\(^{-1}\). A representative experiment is depicted in a histogram (Fig. 2). Average values obtained in the eleven experiments are presented in Table 1.

**DISCUSSION**

In 1940, Stephens\(^{15}\) found that young reticulocytes migrate more slowly under the influence of an electric field than do erythrocytes. Rottino et al.\(^{16}\) reported that the mobility of red cells obtained from the umbilical cord was higher than that of infants 48 hours after birth. Ponder and Ponder\(^{17}\) reported on the reduced mobility of cells "aged" in blood bank storage. However these changes were considered to be induced by deterioration rather than to aging in the physiologic sense.\(^{18,19}\) Danon and Marikowsky\(^{20}\) found a difference in the electrically induced mobility of old and young red cells when they tested minimal fractions taken from the top and bottom of a blood sample centrifuged in the presence of 30 per cent serum albumin.\(^{21}\) This difference in electric mobility of extremely young and old age groups of cells from the same blood samples was further confirmed by separating 10–15 per cent youngest and oldest cell fractions by differential flotation.\(^{22}\) Our results support the findings that young red cells have a higher negative charge than old cells. Furthermore, the results of measurements of the mobility of eight progressively denser fractions which were demonstrated to contain progressively older cells\(^{23,24}\) indicate that as red blood cells age their negative charge is progressively reduced. This phenomenon may be an important factor in
Table 1.—Average Per Cent Values Obtained in Eleven Experiments

<table>
<thead>
<tr>
<th>S.G. of Separating Fluid</th>
<th>Mobility *</th>
<th>1.4–1.35</th>
<th>1.35–1.30</th>
<th>1.30–1.25</th>
<th>1.25–1.20</th>
<th>1.20–1.15</th>
<th>1.15–1.10</th>
<th>1.10–1.05</th>
<th>1.05–1.00</th>
<th>1.00–0.95</th>
<th>0.95–0.90</th>
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<tr>
<td>1.074</td>
<td>1.8 (2–1)</td>
<td>3.5 (4–3)</td>
<td>11 (12–10)</td>
<td>19.3 (20–18)</td>
<td>24 (26–22)</td>
<td>18.1 (19–17)</td>
<td>10.8 (11.5–10)</td>
<td>6.8 (7.5–6.2)</td>
<td>2.7 (3–2.3)</td>
<td>2 (2.5–1.5)</td>
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<tr>
<td>1.118</td>
<td>6.3 (7–6)</td>
<td>29.8 (30–39)</td>
<td>41.9 (43–41)</td>
<td>22 (23–21.5)</td>
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<tr>
<td>1.114</td>
<td>3.2 (4–3)</td>
<td>26.6 (27–28)</td>
<td>52.8 (54–52)</td>
<td>13.9 (14–13)</td>
<td>3.5 (4–3)</td>
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<tr>
<td>1.110</td>
<td>2.6 (3–2)</td>
<td>22 (23–21)</td>
<td>57.5 (58–57)</td>
<td>15.5 (16–15)</td>
<td>2.4 (3–2)</td>
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<tr>
<td>1.106</td>
<td>3.5 (4–3)</td>
<td>30 (31–29)</td>
<td>57.5 (58–57)</td>
<td>7.4 (8–7)</td>
<td>1.6 (2–1)</td>
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<tr>
<td>1.102</td>
<td>3.6 (5–3)</td>
<td>22.8 (24–21)</td>
<td>61.5 (62–61)</td>
<td>9.9 (10–9)</td>
<td>2.2 (3–2)</td>
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<tr>
<td>1.098</td>
<td>3.3 (5–3)</td>
<td>31.6 (33–30)</td>
<td>56 (53–57)</td>
<td>77 (79–75)</td>
<td>1.4 (2–1)</td>
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<tr>
<td>1.094</td>
<td>3.7 (4–2)</td>
<td>26.2 (28–26)</td>
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<td>1.090</td>
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<td>56.5 (58–55)</td>
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* The Values x 10^-4 cm^2 sec^-1 V^-1.
Per cent range of Mobilities in brackets.
Fig. 2.—Bar graph illustrating the mobility of cells in an electric field in the eight fractions separated according to their density. On top of each bar which constituted the peak of a fraction, its fraction number, as well as the percentage it constituted of the whole population (see Fig. 1), is given. Each bar indicates the o/oo of cells that migrated at the speed noted in the abscissa. Since the various fractions had overlapping migration speeds, the additive effect from other fractions at each migration speed is shown on top of the column indicating the appropriate values in dotted lines. Fractions No. 1, 5 and 8 are shadowed to illustrate the distribution of migration speeds within these three representative fractions.

determining the sequestration of old cells in the reticulo-endothelial system as has already been suggested.20,21

SUMMARY

Using citrated blood of eleven healthy donors the density distribution of red blood cells (D.D.C.) was determined and eight fractions from each sample separated. Separated red cells were resuspended in their own plasma and employed for the electric mobility measurements.

The Ruhenstroth-Bauer Cytopherometer was used at a current of 5 ma. at 24 C. The range of migration of red cells was found to be 0.855 to 1.47 × 10^{-4} cm^2v^{-1}sec^{-1}. In separated fractions the older the cells the slower they migrated in the electric field.
MOBILITY OF HUMAN RED BLOOD CELLS

SUMMARIO IN INTERLINGUA

A base de sanguine citrate ab dece-un donatores normal, le distribution de densitate de erythrocytos esseva determinate, e in cata specimen octo fractiones esseva separate. Separate erythrocytos esseva resuspendite in lor proprie plasma e usate in le mesurationes de mobilitate electric.

Le cytopherometro Ruhenstroth-Bauer esseva usate con un currente de 5 ma a 24 C. Le area de migration del erythrocytos esseva identificate como limitate per 0.855 e 1.48 \times 10^{-4} \, \text{cm}^2 \cdot \text{v}^{-1} \cdot \text{sec}^{-1}. In fractiones separate il esseva trovate que le rapiditate del migration del cellulas in le campo electric declinava con le avantiamento del etate del cellulas.

ACKNOWLEDGMENTS

This investigation was supported by a grant of the B. de-Rothschild Foundation for the advancement of Science in Israel. The author wishes to thank Professor D. Danon and Dr. F. Doljanski for their helpful remarks and criticism.

REFERENCES

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