AGGLUTINATION BY POLYLYSINE OF YOUNG AND OLD RED BLOOD CELLS

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FOREWORD

This investigation was initiated by the Pathology Branch, Toxic Hazards Division, Biomedical Laboratory, Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The research was conducted by Weizmann Institute of Science, Rehovoth, Israel, under Air Force Contract AF 61(052)-879, through the European Office of Aerospace Research, United States Air Force, in support of Project No. 7164, "Biomedical Criteria for Aerospace Flight," Task No. 716404, "Physiological Criteria for Altered Environments." Captain H. P. Kaplan of the Pathology Branch was the technical monitor.

This technical report has been reviewed and is approved.

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ABSTRACT

The rate of agglutination by polylysine of young and old human erythrocytes was automatically recorded and correlated with measurements of the electrophoretic mobility of these cells. Old red cells have a reduced electrophoretic mobility as compared with young cells, as well as a higher rate of agglutination than young cells. Mild treatment of red cells with receptor destroying enzyme (RDE) which reduces their surface charge results in an increased rate of agglutination. The results indicate that there is correlation between the rate of agglutination and the surface charge of the red cell.

SECTION I

INTRODUCTION

It has been shown previously (ref 1, 2) that positively charged polyelectrolytes (polybases) induce at very low concentrations agglutination of erythrocytes. Polylysine induced agglutination was not due to the decrease in the overall negative charge of the erythrocyte. Polylysine molecules are capable of attachment to negatively charged components of the cell membrane, thus linking cells (i.e., causing agglutination) in spite of the mutual repulsion of the negatively charged surfaces. The negatively charged component of the surface of the cell membranes on which the polylysine molecule is attached has not been identified.

Furchgott and Ponder (ref 3) and Winkler and Bungenberg de Jong (ref 4) suggested that the charge of the red cell surface was due to a phospholipid. On the other hand, Hirst (ref 5) proposed, on the basis of studies on erythrocyte-virus adsorption and elution, that the sites of adsorption of the red cell may be mucoprotein in nature. Hanig (ref 6) showed that adsorption of myxovirus followed by elution resulted in a marked decrease in the electrophoretic mobility of red cells. Stone and Ada (ref 7) examined the changes in the electrophoretic mobility of red cells after their treatment with various myxoviruses. They found that treatment of the cells with receptor destroying enzyme (RDE) derived from vibrio cholerae cultures almost completely removed the negative charge. Gottschalk and Lind (ref 8) demonstrated that the substrate of the RDE on the red cell surface was neuraminic acid. Cook, Heard, and Seaman (ref 9) have shown that there is a reduction in electrophoretic mobility after treatment of red cells with neuraminidase. Glaeser and Mel (ref 10) have recently demonstrated by different methods that the negative charge on the red cell surface can be almost wholly ascribed to the carboxylic group of neuraminic acid.

It has been reported recently (ref 11) that polylysine interacts with solubilized membrane constituents, N-acetylneuraminic acid being the major component in the reaction of the negatively charged red cell surface with polylysine. The question arises whether neuraminic acid is also the site of interaction with polylysine on the surface of the intact red cell.

In the present study, an attempt has been made to determine whether any correlation existed between the rate of agglutination by polylysine and the surface electric charge. This was studied by correlating the rate of agglutination with the electrophoretic mobility and analysis of neuraminidase treated cells for the rate of agglutination. Similarly, it was of interest whether the naturally occuring reduction in surface charge of the old red cell, as compared with the young cell (ref 12, 13) can be correlated with the difference in rate of agglutination by polylysine of cells of the two age groups.

SECTION II

MATERIALS AND METHODS

Human citrated blood was taken by venipuncture (about 10 ml). Density distribution of the red blood cells (DDC) in each blood sample was determined using a set of phthalate-ester mixtures[1] of specific gravity decreasing in decrements of 0.004 (ref 14). After centrifugation at 10,000 g, the two tubes were selected in which the separating fluid isolated 10% of the total volume of the blood in the tube above and below respectively. That above represented young cells and that below represented old cells. The fluid having a specific gravity (sp gr) of 1.110 was generally used to separate old cells (bottom) and that having a sp gr of 1.098 for separating young cells (top). The fraction remaining on top of the fluid of higher specific gravity was resuspended in plasma, layered over a low density mixture (sp gr 1.062) and recentrifuged in order to expose the top fraction to the same treatment as that of the bottom fraction and remove the leucocytes and platelets. Microfuge (Beckman-Spinco) with polyethylene conical tubes at 10,000 g was used. The two separated fractions were resuspended in 0.9% NaCl solution buffered to pH 7.3 with veronal acetate buffer. The cells were washed twice in tenfold volumes of the same solution and finally resuspended in phosphate buffered saline at a concentration of 1 to 20 packed cells for the agglutination measurements and 1 to 500 for the measurements of the electrophoretic mobility.

AGGLUTINATION MEASUREMENTS

Polylysine hydrobromide (0.1 ml), degree of polymerisation n = 100 according to viscosity measurements[2], dissolved in 0.9% NaCl (50 μg/ml) was added to 0.5 ml of the red cell suspension. The suspension was stirred with the help of a syringe and immediately introduced into the microcuvette of the Fragiligraph[3]. The external medium was the same as the suspending medium (0.9% NaCl) and was kept at a thermostatically controlled temperature of 25 C. The rate of agglutination of each cell suspension was determined according to the slope of the recorded curve obtained as previously described (ref 15). The level of agglutination reached after 4 minutes reaction time was taken as a standard.

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[1] Yeda Research and Development Co. Ltd., Rehovoth, Israel.

[2] Yeda Research and Development Co. Ltd., Rehovoth, Israel.

[3] Elron, Electronic Industries, P.O. Box 5390, Haifa, Israel.

TREATMENT WITH RECEPTOR DESTROYING ENZYME (RDE)

Packed cells (0.1 ml) from each separated fraction were added to 0.5 ml RDE solution diluted in the ratio 1:100, 1:200, 1:300, 1:400, 1:500, 1:600 in 0.9% NaCl solution buffered to pH 7.4 with veronal acetate buffer + 0.1% CaCl. The reaction mixture was incubated at 37 C for 1 hour. In one experiment, the dilution was constant (1:600) but incubation times of 20 minutes, 40 minutes, and 60 minutes were employed. After incubation the cells were washed twice with large volumes of 0.9% NaCl and resuspended at a concentration of 1 volume of packed cells per 20 volumes of 0.9% NaCl. The agglutination rate measurements on the RDE treated red cells were performed as described above.

ELECTROPHORETIC MOBILITY MEASUREMENTS

Electrophoretic mobility measurements were carried out as described by Nadell and Creger (ref 16). Packed cells of the two age groups were diluted 1 to 500 in phosphate buffer, pH 7.3. The electrophoretic mobility of 20 erythrocytes from each sample was determined. The polarity of the electrodes was reversed after each individual cell reading. The final electrophoretic mobility value was calculated on the basis of the mean of the 20 measured time values.

SECTION III

RESULTS

The rate of agglutination of the young cells and that of the old cells is given in Table I. It can be seen that in every blood sample, the rate of agglutination of the old cells is higher than that of the young ones. Two typical curves illustrating this difference are shown in fig. 1 (A and B).

Microelectrophoretic measurements were performed on six blood samples in order to find whether there is a correlation between the increased rate of agglutination of the old cells and their reduced electrophoretic mobility. The mean electrophoretic mobility of the old cells was 1.11 μ/V/sec/cm (ranging from 1.06 to 1.16), while the mean rate of agglutination of these cells appeared as 33 mm deviation from the base line in 4 min (range = 21.0 - 36.5 mm) (fig. 1D). As regards the top fraction (young cells), the mean electrophoretic mobility was 1.32 μ/V/sec/cm (ranging from 1.28 to 1.36), and the mean rate of agglutination was represented by a deviation of the curve of 18 mm in 4 min (range = 16.5 - 25.0 mm) (fig. 1A). In the measurement of the rate of agglutination as well as in electrophoretic mobility determinations, the passage of the cell through the phthalate mixture, as a factor in itself was found experimentally not to have any influence on the measurements.

TABLE I

RATE OF AGGLUTINATION OF YOUNG AND OLD RED CELLS

Blood Sample	Rate of agglutination* Top (young)	Bottom (old)	Blood Sample	Rate of agglutination* Top (young)	Bottom (old)
No. 1	22	33	No. 9	17	24
2	25	36.5	10	17	24
3	16.5	24	11	15.5	22
4	18	21	12	19	33
5	21	34	13	20	28
6	18	21	14	21	25
7	21	34	15	18	28
8	18	21	16	19	28

*Measured as deviation of the curve (as light transmission increases) in mm after standard time of 4 min.

The agglutination experiments with cells that were previously treated with RDE did not show consistent results. Different blood samples reacted differently with RDE. In some blood samples a dilution of 1 to 600 of the original enzyme solution completely inhibited agglutination. Other samples showed complete inhibition only when enzyme dilution 1 to 200 was used. However, a consistent difference in the rate of agglutination between young and old red cells from the same RDE treated blood samples was found. When a high titer (1 : 200) of enzyme was used, a slight agglutination in the young cell fraction could still be observed while no agglutination at all could be detected in the old cell fraction (fig. 1C). As the titer of RDE was reduced, the rate of agglutination increased. In some experiments treatment at low concentration of RDE modified the surface characteristics of young cells until their agglutination rate was the same as that of untreated old cells. In some experiments, when the RDE titer was kept constant (1 : 600) and cells from the top fraction were incubated for 20 minutes, these top fraction cells had the same rate of agglutination as the untreated bottom fraction cells (fig. 1D).

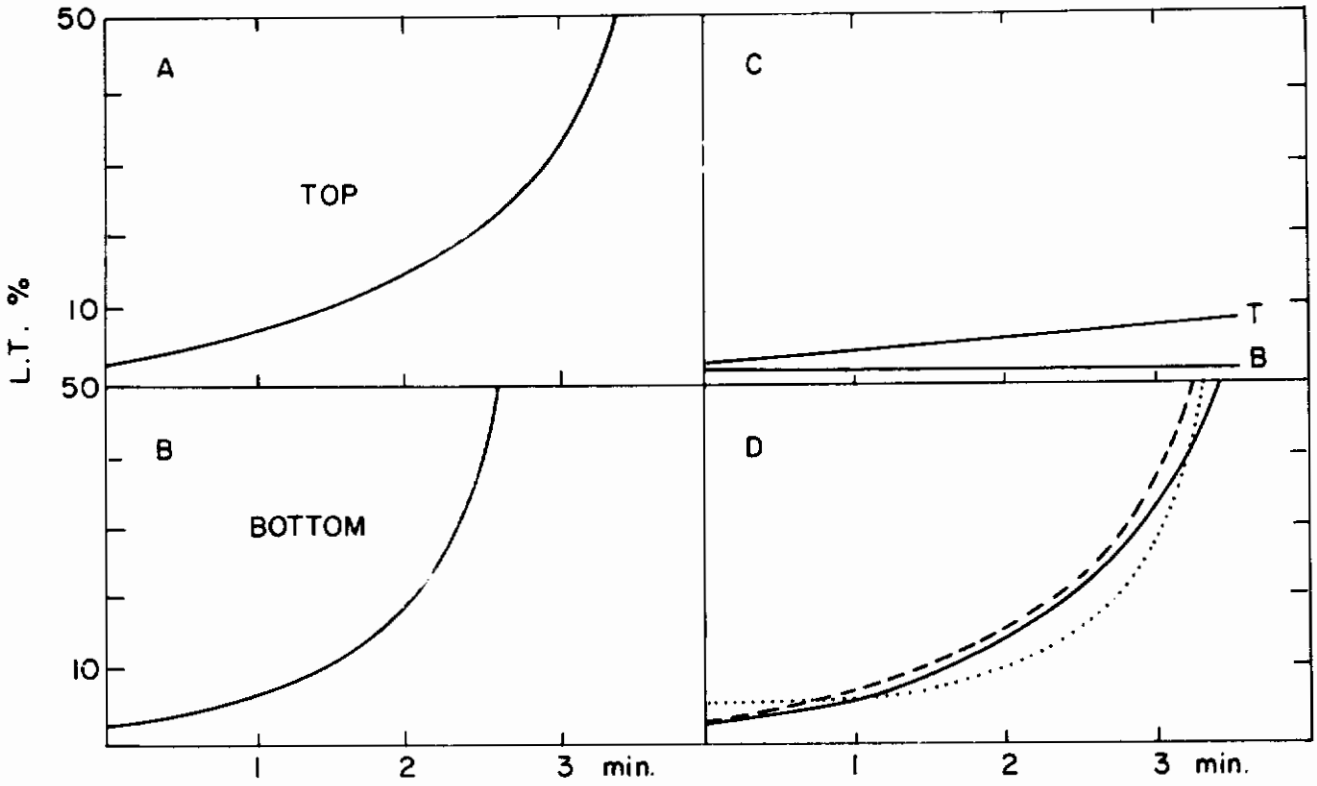

Figure 1. The rate of agglutination according to the slope of the recorded curve in a Fragiligraph reached after four minutes reaction time.

(A) rate of agglutination of the young cell fraction (TOP)
(B) rate of agglutination of the old cell fraction (BOTTOM)
(C) high titer RDE treated (T) young cell fraction
(B) old cell fraction
(D) 20 minutes incubation with RDE (1 : 600)
_____ young cell fraction - treated
------- old cell fraction - untreated
..... young cell fraction - untreated

SECTION IV

DISCUSSION

N-acetylneuraminic acid (NANA) may be considered as the main carrier of the negative charge of the red cell surface membrane (ref 17). As a soluble component of the red cell membrane, NANA has been found to interact with polylysine (ref 11), since neuraminidase-treated red cells have a reduced electrophoretic mobility (ref 7, 9), and old red blood cells have also been found to have a reduced surface charge (ref 12, 13). It is reasonable to assume that a positively charged polyelectrolyte will agglutinate old and young red cells at different rates. The results show that old red cells which have a reduced negative charge have a higher rate of agglutination than the young cells. The smaller repulsion forces of the less negatively charged old red cells apparently increase the probability of mutual approach of the cells, till they are close enough to be linked by the polylysine molecule. The mean electrophoretic mobility and the mean rate of agglutination of separated young and old red cells indicate that there is a correlation between the rate of agglutination and the electric charge of the red cell surface.

Complete inhibition of agglutination or a reduced rate of agglutination was obtained by treating red cells with RDE. The rate of agglutination of young red cells could be reduced to that of untreated old red cells. When old and young red cells were treated with high concentration of RDE, the old cells did not agglutinate, while some agglutination still took place in the young cells (fig. 1C).

It may be concluded that the different rates of agglutination between young and old cells may be ascribed to the different negative surface charge. Moreover, the rate of agglutination by polylysine of young and old red cells, as well as of RDE treated cells, indicates that NANA is the site of interaction with polylysine in the intact red blood cells.

Further work, including mathematical analysis of the phenomenon associated with the measurement of the rate of agglutination by the method used in this study is in progress.

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