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ERYTHROCYTE FRAGILITY IN AGING

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SUMMARY

Hemolysis experiments were run on normal human whole blood from young and old people. An equation was developed to fit the data and determine the mean erythrocyte fragility and breadth of the fragility distribution. Results indicate an increase in mean fragility and a broadening of the erythrocyte fragility distribution as a function of aging.

INTRODUCTION

Recent investigation by cone-plate viscometry has detected an age viscosity correlation for human whole blood. Blood from eleven old people (69–91 years old) was found to be approximately 12% more viscous than blood from eleven young people (12–29 years old) over a range of shear rates from 23 to 2.3 s^{-1} [1]. Increased viscosity suggests that red cell deformability decreases with age. Variation in erythrocyte membrane structure may introduce this deformability change.

The erythrocyte fragility test has previously been used as a measure of erythrocyte tensile strength, and has proven useful in the clinical detection of hypochromic and congenital hemolytic anemias [2]. The usual fragility testing involves hemolysis of whole-blood samples over a range of osmotic pressures introduced by a series of NaCl solutions. Hemolysis curves (percent hemolysis vs NaCl concentration) are constructed and represent the cumulative frequency distribution of individual erythrocyte fragilities present in the sample [3]. The NaCl concentration of 50% hemolysis represents the fragility of the greatest number of cells and may be taken as a measure of the mean erythrocyte fragility [4].

Presently, an aging study of erythrocyte fragility was undertaken to explore changes which may occur in red-cell membrane. Osmotically induced hemolysis experiments were performed on normal whole blood from young and old people by a modification of the method of Good [4]. The experiments indicate that the mean erythrocyte fragility and the distribution of fragilities (i.e. the distribution of types of erythrocytes) of a human blood sample vary as a function of age. A method for

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mathematical treatment of hemolysis data is presented by which parameters for breadth of the fragility distribution and the mean erythrocyte fragility are determined empirically.

METHODS AND PROCEDURE

Hemolysis experiments were conducted by modification of the method of Good [4]. Normochromic normocytic whole blood was classified by erythrocyte count, hemoglobin concentration, hematocrit corpuscular constants, and blood indices. All experiments were performed no later than four days after the fresh blood was drawn. Samples were oxygenated for 10 min by gentle swirling under a stream of purified oxygen immediately prior to hemolysis.

NaCl and NaOH were "Analyzed Reagent" grade from J. T. Baker. A series of forty stock hemolysis solutions ranging from 0.050 to 0.089 M NaCl were prepared by volumetric dilution of a 0.1000 M NaCl solution. NaOH solution (0.4%) was also prepared in advance.

Hemolysis was performed by adding 0.1 ml of oxygenated whole blood to each of forty tubes containing 3.0 ml of the stock hemolysis solutions thermostated at 20 °C. A fully hemolyzed standard was prepared by lysis with distilled, deionized water in an identical manner. After 5 min, the solutions were transferred to a centrifuge (International Centrifuge, Size 2, model 2, 12 inches head diameter, International Equipment Co., Boston, Mass.). Samples were centrifuged at $1207 \times g$ for 3 min. From each of the tubes, 1 ml of supernate was withdrawn and added to 2 ml of 0.4% NaOH. After 15 min, spectrophotometric absorbance of the alkali hematin was read at 540 nm. (Bausch and Lomb Spectronic-88, Rochester, N.Y.). The hemolysis fraction for each of the sample solutions was caculated as:

$$H = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \tag{1}$$

RESULTS AND DISCUSSION

Hemolysis curves (hemolysis fraction vs NaCl concentration) were constructed from the experimental hemolysis data in accordance with the equation:

$$H = \frac{1}{e^{\beta(X - X_{50})} + 1}, \tag{2}$$

where H is the fraction of cells hemolyzed, X is the molar sodium chloride concentration, X_{50} is the mean erythrocyte fragility (moles NaCl per l), and β is a measure of the breadth of the erythrocyte fragility distribution. In linear form,

$$\ln\left(\frac{1-H}{H}\right) = \beta X - \beta X_{50}, \tag{3}$$

the equation may be computer analyzed by a least squares formulation. Substituting $\ln(1-H/H)$ for Y in the following formulas,

$$\beta = \frac{N\Sigma XY - \Sigma X \Sigma Y}{N\Sigma X^2 - (\Sigma X)^2} \tag{4}$$

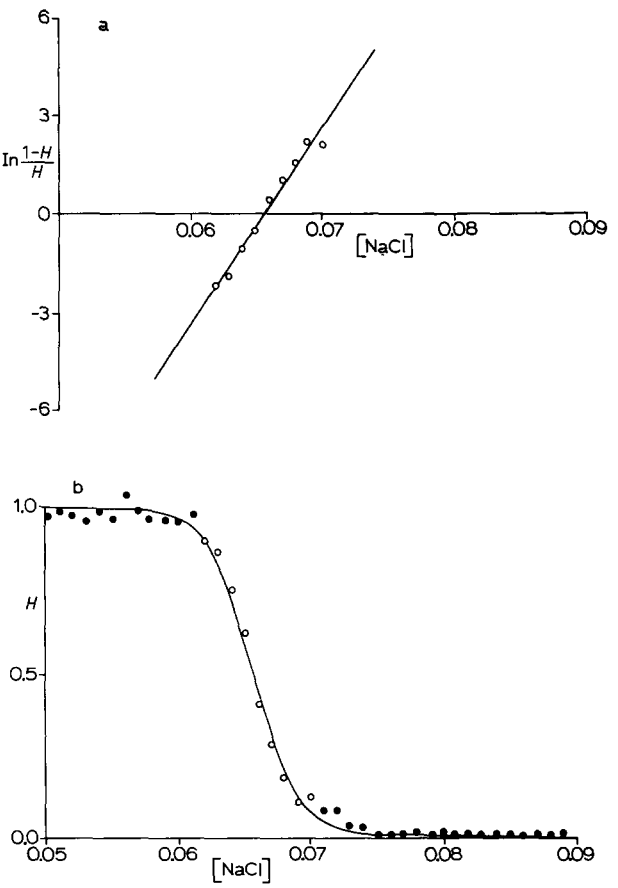
and

$$X_{50} = \left[\frac{(\Sigma X^2 \Sigma Y - \Sigma X \Sigma XY)}{N\Sigma X^2 - (\Sigma X)^2}\right] \cdot \left[-\frac{1}{\beta}\right] \tag{5}$$

where N is the number of experimental points (X, Y).

Curves of the sigmoid form (Eqn 2) constructed with the parameters from Eqns 4 and 5 were found to fit the experimental data with a maximum deviation of 3.2%. Typical hemolysis curves for a 36-year-old male and an 86-year-old female are shown in Fig. 1. The linear fits by Eqn 3 shown in Fig. 1a and 1c were used to compute the parameters for the sigmoid fit by Eqn 2 shown in Fig. 1b and 1d.

As stated previously, the hemolysis curves represent cumulative frequency distributions of individual erythrocyte fragilities present in the sample. The relative number of cells which hemolyze at any given NaCl concentration is found by differentiation of Eqn 2. The distribution function obtained is:



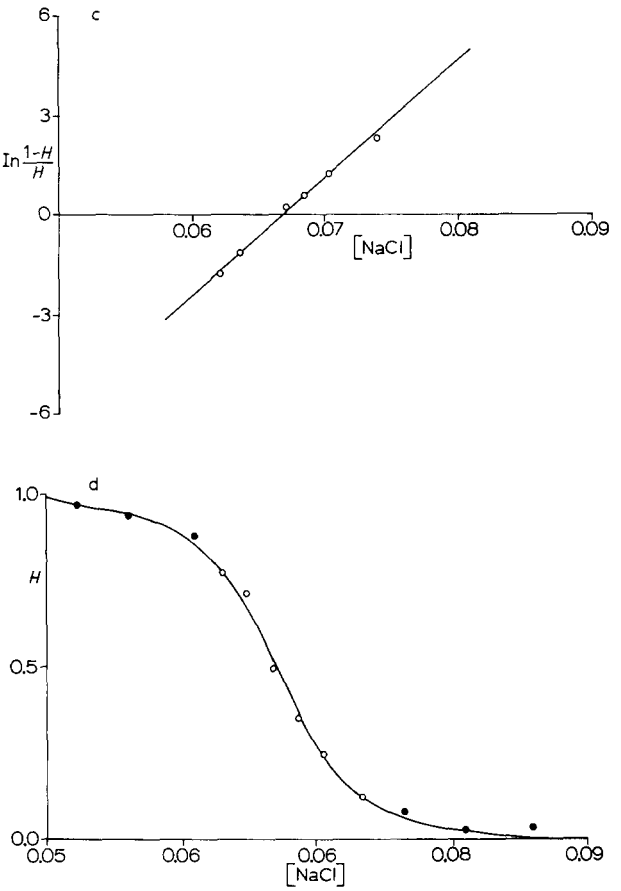


Fig. 1. a. Least squares fit of hemolysis data from a 36-year-old male by Eqn 3. b. Use of parameters from the fit in Fig. 1a to construct a hemolysis curve by Eqn 2. Maximum deviation from experimental points is 3 %. c. Least squares fit of hemolysis data from an 86-year-old female by Eqn 3. d. Use of parameters from the fit in Fig. 1c to construct a hemolysis curve by Eqn 2.

$$\frac{dH}{dx} = \frac{-\beta e^{\beta(X-X_{50})}}{[e^{\beta(X-X_{50})}+1]^2} \tag{6}$$

The value of X_{50} controls the position of the distribution maximum, and, as β decreases, the distribution broadens.

Results of hemolysis experiments on nomorchromic normocytic blood from twelve young (19–36 years old) and fifteen old people (64–90 years old) are given in Table I (lines 1 and 2) along with averaged clinical parameters. The results indicate that both the mean fragility and the breadth of the fragility distribution vary with age.

The values of X_{50} are 0.0665 ± 0.0022 and 0.0712 ± 0.0039 moles of NaCl per l for young and old respectively. Expressing the mean fragility as a measure of the average cell's ability to resist deviations from isotonicity (0.154 M NaCl), the young

TABLE I

Blood group and number of samples	Age range (years)	Red blood cell (10^6 count/mm^3)	Hemoglobin (g/100 ml)	Hematocrit* (%)	Mean corpuscular volume (μm^3)	X_{50}** (M NaCl)	β
Young normochromic normocytic blood (12)	19–36	4.7±0.4	14.5±1.0	42±3	88±2	0.0665±0.0022	509±66
Old normochromic normocytic blood (15)	64–90	4.7±0.3	13.8±1.1	43±3	91±2	0.0712±0.0039	353±20
Young normochromic normocytic blood sickle trait (3)	7–30	4.3±0.1	13.2±0.1	39±0	91±1	0.0695±0.0047	319±45

* Wintrobe method.

** Underscored figures are mathematically derived and experimentally insignificant except in roundoff.

samples could resist a change of 0.0875 molar whereas the old could resist only a change of 0.0828 molar. This reflects an approximate increase in mean fragility of 5.4% within the experimental age range.

The β parameter of the distribution breadth decreased markedly within the age range studied. The decrease in β, which reflects a broadening of the distribution, was from 509 ± 66.6 for young to 353 ± 20.4 for old.

Sigmoid hemolysis curves constructed from Eqn 2 using the average X_{50} and β parameters for young and old are overlayed in Fig. 2. The mean erythrocyte fragility (0.5 hemolysis fraction) is denoted by the dotted line to the X-axis. The relative positions of the two curves show that the majority of old cells are less resistant to osmotic pressure then young cells. The majority of the cells in the old samples exhibit a decrease in deformability consistent with previous findings by viscosity measurement [1].

The composite hemolysis distribution curves are shown graphically in Fig. 3.

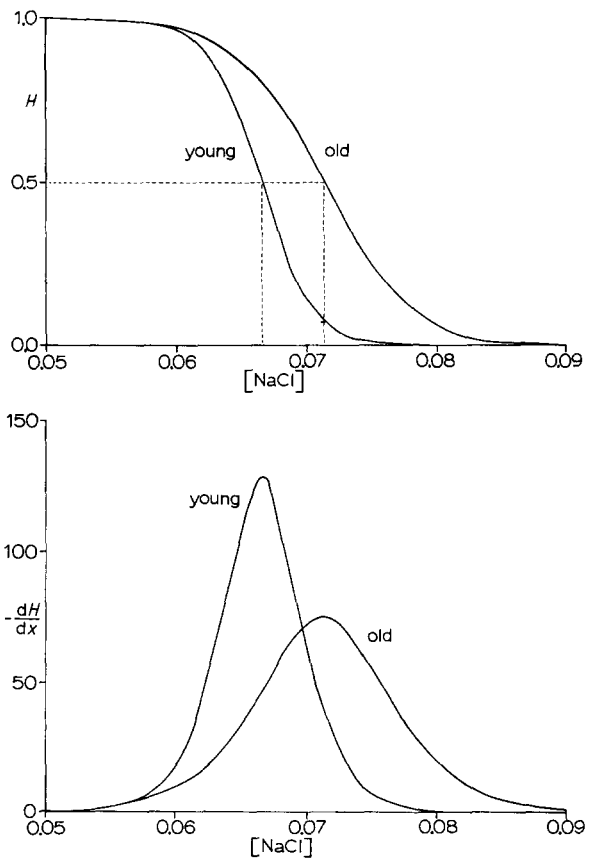


Fig. 2. Average hemolysis curves by Eqn 2 for 12 young (19–36 years old) and 15 old (64–90 years old) people. The more aged group exhibits a higher mean fragility (dotted lines) and a wider range of hemolysis.

Fig. 3. Distribution analysis by Eqn 3 of the average curves shown in Fig. 2. A broader and shifted distribution for the old population is evident.

Notice that the distribution shifts toward higher NaCl concentration and broadens for the old samples as opposed to the young. Thus the number of distinct types of erythrocytes recognizable by fragility testing increases as a function of age. This may be interpreted as a natural response to the increase in mean fragility, in that a given population of red cells from an aging person has a greater variety of types of cells, making a small fraction of cells susceptible to hemolysis with any given osmotic pressure change.

Hemolysis experiments run on three young (7–30 years old) sickle trait whole-blood samples (Table I, line 3) showed an average X_{50} of 0.0695 ± 0.0048 moles of NaCl per l and an average β of 319 ± 45.1. Although based on only three subjects, these results indicate that sickle-trait blood from young people may exhibit some characteristics similar to normal blood from older people. This is also consistent with previous findings by cone-plate viscometry [5].

Processes which may lead to the decrease in erythrocyte deformability with aging are under investigation.

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REFERENCES

1 Cerny, L. C., Cook, F. B. and Valone, F. (1972) Exp. Gerontol. 7, 137–142
2 U.S. Dept. of the Army, TM 8 - 227 - 4, Laboratory Procedures in Clinical Hematology, (March 1963) U.S. Gov't. Printing Office, Washington, D. C., 4–27 – 4–30
3 Marks, A. A. and Johnson, A. B. (1958) J. Clin. Invest. 37, 1542–1548
4 Good, W. (1971) Exp. Physiol. Biochem. 4, 163–181
5 Cerny, L. C., Cook, F. B. and Stasiw, D. M. (1974) A Model for the Sickle Cell: Theory and Experiment, Biorheology, in the press