Relative Viscosity and Specific Gravity of Human Blood During Cold Storage (34752)

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The viscosity of blood is influenced by several parameters. Recently, Stone and coworkers (1) have shown that concentration, shape, and size of erythrocytes from blood of different mammalian species have a significant effect on the flow rate of the blood through a capillary tube. Other parameters which must be considered are deformability and charge density of erythrocytes, and hydrodynamic effects. Dintenfass (2) has shown that at hematocrit concentrations of 98% or higher, considerable differences in the internal viscosity of red cells are observed. The size and shape of the erythrocyte may be influenced by hydrodynamic effects such as axial accumulation and orientation with flow (3). During storage of blood, alterations occurring in the metabolic activity and in the structural integrity of the red cell could influence various parameters which may affect the viscosity and the specific gravity of the blood. Previous studies (4, 5) on the preservation of human red cells have shown considerable alterations in the biochemical components and in the physical characteristics of the cell membrane during prolonged cold storage. Therefore it was considered of interest to study the relative viscosity and specific gravity of blood as well as the density distribution of red cell population during the preservation of human blood at 2° under blood banking conditions for a period up to 42 days.

Materials and Methods. Units of blood were obtained from eight healthy male donors, 18–25 years of age. The blood was drawn into plastic bags containing 67.5 ml of ACD (acid–citrate–dextrose solution, National Institute of Health, Formula A)/450 ml of blood. The bags were stored at 2° and at selected intervals (indicated in the figures and tables) aliquots of blood were removed for analysis under sterile conditions.

Hematocrit concentration was determined by dipping the hematocrit tube into the blood and rapidly removing it when a 50–60 cm length had been filled. The dry end was sealed with putty and the glass capillary tubes then were spun at 11,000 rpm for 15 min in an International microcapillary centrifuge. Maximum sedimentation was obtained in 10 min. Each determination was performed in triplicate; the variation obtained was less than ± 1.5.

Samples with various hematocrit concentrations were prepared by adding to, or removing plasma aliquots from tubes containing centrifuged blood. Resuspension of packed erythrocytes was achieved by gentle agitation. Plasma samples were also prepared.

Viscosity determinations were done using an Ostwald viscosimeter with a 0.6-mm capillary diameter, 9.5-cm capillary length, 3.2-ml efflux volume and an efflux time for water at 37° of about 60 sec. All determinations were made at 37.0° ± 0.1 at a flow rate that did not produce turbulence. Since we were interested in relative viscosity, the flow time for water was compared with the flow time of various samples of blood using the same volume of fluid, the same conditions and the same viscosimeter in all determinations. Thus, the Poiseville (6) equation for viscosity (η) can be simplified as follows: $\eta_B/\eta_w = f_B\rho_B/f_w\rho_w$ where the subscripts B and w refer to blood and water, respectively, ρ represents the density of the fluid (g/cm^3) and f the flow time (sec). Each viscosity determination was performed at least in triplicate and the average value was then calculated. The deviation obtained among the individual values for each sample was less than 5%.

Relative viscosities reported in the literature (1) are referred to the viscosity of water arbitrarily taken as unity (water = 1.00); for simplicity, we have assumed the same expression. The viscosity of various samples of blood is relative to the viscosity of water at 37° and a sample with an apparent specific viscosity 3.5 is therefore 3.5 times more viscous than water under identical experimental conditions.

The density measurements were made with a 5-ml calibrated pycnometer and using an analytical balance for weighing the pycnometer containing air, water, or blood samples after equilibration at 37°. The specific gravity for the various blood samples was then calculated. All determinations were performed in triplicate; only small variations to the third decimal place were observed.

The density distribution of red cell population was determined by the method of Danon and Marikovsky (7) using phthalate ester mixtures of known specific gravity as separating liquids in microhematocrit capillary tubes. Fractionation of red blood cells into low density (young) and high density (old) groups was achieved by the procedure described by Brok and co-workers (8) using phthalate esters in siliconized test tubes of 8-mm internal diameter. On the basis of the specific gravity of red cells and the two-phase centrifugation method, two extreme age groups were identified and designated young and old cells, representing 5 and 10% of the total cell population, respectively. Osmotic fragility of these two groups of cells and of fresh and 42-day-old cells was determined as previously described (4).

Results. The relative viscosity and the specific gravity of fresh, 21- and 42-day-old blood, as a function of the hematocrit content, are shown in Fig. 1. All the individual experimental values for relative viscosity and specific gravity obtained on all the samples from the eight units of blood were first plotted as a function of percentage hematocrit. The best fitted curves were then determined for all the samples from fresh, 21- and 42-day-old blood; these curves are represented in Fig. 1. An indication of the variation in the individual sample values can be obtained from the data shown in Table I. The curves in Fig. 1 show that not only in fresh blood but also in blood stored for 21 or 42 days, the relative viscosity follows an exponential relationship whereas the specific gravity has a linear relationship with respect to the hematocrit content. It is also evident that at any hematocrit value the relative viscosity and the specific gravity of 42-day-old blood is greater than that of 21-day-old blood which is slightly higher than that of fresh

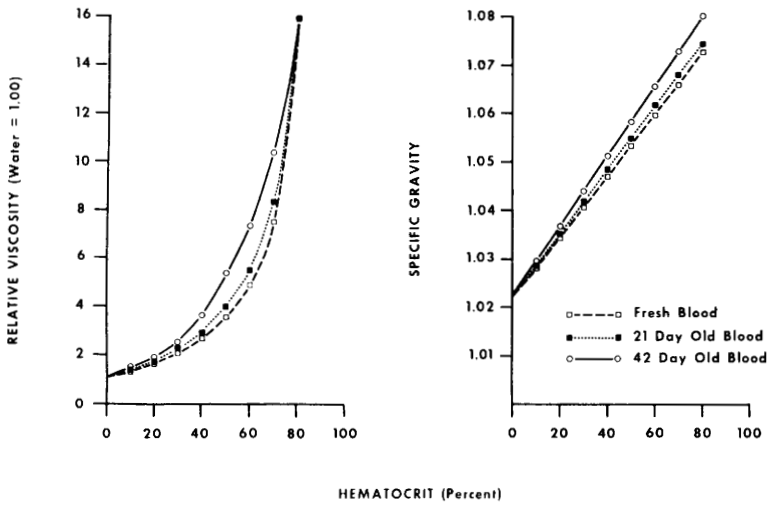

FIG. 1. Comparison of relative viscosity and specific gravity between fresh, 21- and 42-day-old blood as a function of hematocrit content. On the ordinates, plasma values are indicated.

TABLE I. Relative Viscosity and Specific Gravity of Fresh (T_0) and 42-day-old (T_{42}) Blood at Various Hematocrit Concentrations.[a]

Hematocrit (%)	Relative viscosity (av ± SD)		Sp gr (av ± SD)	
	T_0	T_{42}	T_0	T_{42}
40	2.69 ± 0.14	3.71 ± 0.13	1.0482 ± 0.0012	1.0513 ± 0.0010
50	3.59 ± 0.19	5.38 ± 0.21	1.0533 ± 0.0015	1.0584 ± 0.0010
60	4.88 ± 0.17	7.35 ± 0.15	1.0596 ± 0.0018	1.0657 ± 0.0013
70	7.50 ± 0.24	10.36 ± 0.31	1.0660 ± 0.0017	1.0728 ± 0.0020

[a] Values between T_0 and T_{42} at each percentage of hematocrit are significantly different ($p < 0.01$).

blood. Little or no difference is observed in the values obtained for plasma which are represented by the intersections on the ordinate.

Table I indicates average values with standard deviation of relative viscosity and specific gravity at 40, 50, 60, and 70% hematocrit for fresh and 42-day-old blood. Since the various determinations for the eight units of blood were made at different hematocrit levels, in order to obtain the data in Table I, the results for each unit of blood were plotted and the values at the above hematocrit were read from each individual curve. The averages and the standard deviation were then calculated for each hematocrit concentration. Statistical *t* test analysis of the differences between the values obtained for fresh and 42-day-old blood at each percentage hematocrit show that these differences are significant beyond 0.01 and 0.001 level of significance.

The density distribution of red blood cells in relation to the time of storage is shown in Table II. The results indicate that, as the time of storage of blood is prolonged, a greater percentage of cells will penetrate the clear layer of phthalate ester mixtures, demonstrating a greater increase in the number of cells with higher density during storage, in agreement with the results obtained in the determination of the specific gravity. The data in Table II also show that the magnitude of change in the density of the red cells is greater between 21 and 42 days than between 0 and 21 days of storage. This pattern is also observed in the changes of relative viscosity and specific gravity in Fig. 1.

When cells from fresh blood are separated into two extreme groups, young and old cells, the osmotic fragility of the erythrocytes in these two groups is significantly different as shown in Fig. 2A. At different NaCl concentrations, the young cells (low density) are more resistant to osmotic fragility than the old cells (high density). If osmotic fragility curves of fresh and 42-day-old cells are also plotted (Fig. 2B) and compared to those of the two cell populations (Fig. 2A), the osmotic resistances of fresh and low density cells are very similar, whereas the osmotic fragility of 42-day-old cells is greater than that of high density cells. This would indicate that, in regard to osmotic fragility, the deterioration of the cell by *in vitro* storage is greater than that caused by physiological aging of the cell *in vivo*.

Discussion. The relationship between blood viscosity and hematocrit content has been

TABLE II. Density Distribution of Red Blood Cells in Relation to Time of Storage.

Sp gr of separating liquid	% of packed cells at bottom		
	Fresh cells (av ± SD)	21-day-old cells (av)	42-day-old cells (av ± SD)
1.122	0 —	0	0 —
1.118	0 —	0	3.5 ± 0.3
1.114	3.1 ± 0.3	3.5	7.2 ± 0.5
1.110	10.2 ± 0.7	12.6	16.2 ± 1.0
1.106	28.7 ± 1.2	30.8	38.2 ± 1.8
1.102	57.8 ± 3.4	63.2	73.5 ± 2.3
1.098	87.5 ± 2.5	90.4	94.5 ± 3.8
1.094	97.0 ± 4.0	99.0	100.0 —
1.090	100.0 —	100.0	100.0 —

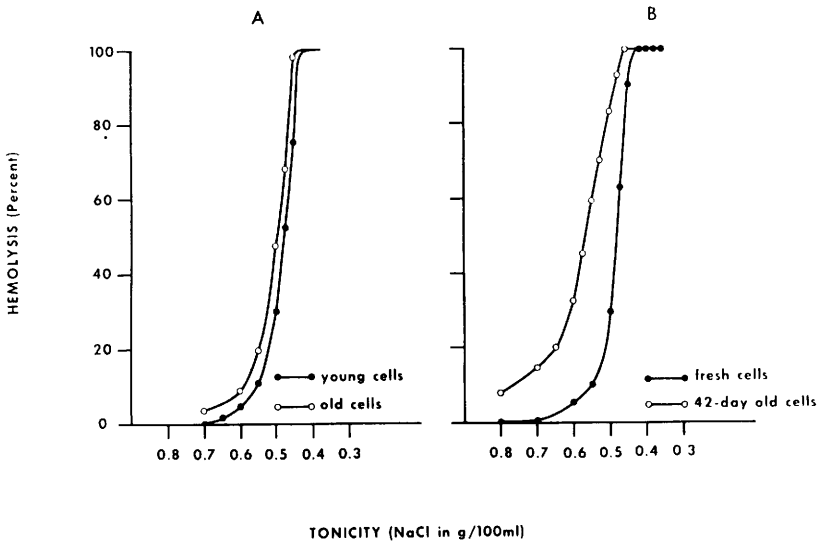

FIG. 2. Osmotic fragility of low density (young) and high density (old) red cells (A) and of red cells prepared from fresh and 42-day-old blood (B).

known for several years. Denning and Watson (9) reported an increase in blood viscosity with an increase in the number of erythrocytes. Trevan (10) formulated a quantitative relationship between viscosity of blood and hematocrit value. He reported a linear function with hematocrit below 40% and a logarithmic function when the percentage hematocrit was above 40. In a recent investigation, Strumia and Phillips (11) in their studies on the effect of mean corpuscular volume of human erythrocytes, varying from 58 to 116.8 μ^3, concluded that the relative viscosity of whole blood depends upon the relative volume of the red cell mass, regardless of the size and number of individual red cells constituting the mass. In our study, the cell mass is measured by hematocrit content and an exponential curve reflects the relationship between relative viscosity and percentage hematocrit even at hematocrit values below 40%; this relationship is similar to that observed by Stone and co-workers (1) with blood from several mammalian species. When the time of storage of blood is increased, the relative viscosity and the specific gravity of the blood also increase. After 21 days of storage, the increases observed are very moderate when compared to the values for fresh blood; however, at 42 days of storage these increases are significantly higher, indicating that greater *in vitro* deterioration of erythrocytes occurs after a storage period of 21 days.

It could be objected that by using a capillary viscosimeter such as the Ostwald viscosimeter no consideration was given to the anomalous effects reported by Fahraeus and Lindqvist (12) and to the shear rate effect. We were interested in the apparent viscosity of blood at different storage periods and measured under exactly identical conditions. Furthermore, a viscosimeter with a capillary tube 0.6 mm in diameter has been reported (1) not to show that Fahraeus and Lindquvist effect observed with smaller bore tubes; in addition, when a large diameter viscosimeter is employed the shear rate is sufficiently high so that any reduction in shear rate which might occur in the flow of more viscous blood samples has no appreciable effect on the apparent viscosity. Stone and co-workers (1) have demonstrated no appreciable difference in results obtained in the capillary viscosimeters and data obtained on the same blood samples using a cone-plate viscosimeter at high shear rate. Coulter and Pappenheimer (13), investigating the transistion from laminar to turbulent flow in blood, determined that this transition occurs at a Reynolds number of 970 $\pm$ 80. Since it was calculated (1) that in the capillary used in these studies

the Reynolds number for water is 120, these investigations were conducted at flow rates at which turbulence would not be expected to occur. Furthermore, in the determination of relative or apparent viscosity of blood (Fig. 1 and Table I), measurements were made at high shear rates; under such conditions, red blood cell aggregation is insignificant (14) and red blood cell deformation is the predominant rheological determinant (15).

The increase in specific gravity of red cells with physiological aging in the circulation has been known for several years. This feature has been used as a basis for separating the cell population in various groups by centrifugation of the cells in their own plasma or in a medium of higher specific gravity (7, 16–18). When red cells are stored for a prolonged period of time, their specific gravity increases (Fig. 1). The percentage of cells found in the high density group, following centrifugation in the presence of phthalate ester mixtures of varying density, is also higher than that obtained with fresh cells, in agreement with the results obtained in the determination of the specific gravity.

The red cell during storage undergoes several chemical and physical alterations. Some of these alterations have been reported previously (4, 5). Different physical parameters have been investigated in these studies, which may help in the elucidation of the complex changes occurring during storage of blood.

Summary. Eight units of blood were preserved under blood banking conditions for a period of 42 days. Aliquots were assayed for relative viscosity, specific gravity, and density distribution of red cell population at the time of collection of blood, at 21 and 42 days of storage. Relative viscosity demonstrated an exponential, and specific gravity a linear, relationship with red blood cell mass, measured as hematocrit content. The three parameters investigated showed significant changes during the preservation of blood. The magnitude of these changes is greater between 21 and 42 days than between 0 and 21 days of storage. Red cells from fresh blood were separated into two extreme age groups, low density (young) and high density (old cells); the osmotic fragility of the erythrocytes in these two groups was different and the high density cells demonstrated greater osmotic resistance than cells from 42-day-old blood.

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