RED BLOOD CELL DEFORMATION IN SHEAR FLOW

Effects of Internal and External Phase Viscosity and of In Vivo Aging

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ABSTRACT Shear deformation of young and old human red blood cells was examined over a range of shear stresses and suspending phase viscosities (η_o) using a cone-plate Rheoscope. The internal viscosities (η_i) of these cell types differ, and further changes in internal viscosity were induced by alteration of suspension osmolality and hence cell volume. For low suspending viscosities (0.0555 or 0.111 P) old cells tended to tumble in shear flow, whereas young cells achieved stable orientation and deformed. Changes in osmolality, at these external viscosities, altered the percentage of cells deforming, and for each cell type threshold osmolalities (Osm-50) were determined where 50% of cells deformed. The threshold osmolalities were higher for younger cells than for older cells, but the internal viscosities of the two cell types were similar at their respective Osm-50. Threshold osmolalities were also higher for the higher external viscosity, but the ratio of internal to external viscosities (i.e., η_i/η_o) was nearly constant for both external viscosities. Deformation of stably oriented cells increased with increasing shear stress and approached a value limited by cell surface area and volume. For isotonic media, over a wide range of external viscosities and shear stresses, deformation was greater for younger cells than for older cells. However, deformation vs. shear stress data for the two cell types became nearly coincident if young cells were osmotically shrunk to have their internal viscosity close to that for old cells. Increases in external viscosity, at constant shear stress, caused greater deformation for all cells. This effect of external viscosity was not equal for young and old cells; the ratio of old/young cell deformation increased with increasing η_o. However, if deformation was plotted as a function of the ratio $\lambda = \eta_i/\eta_o$, at constant shear stress, young and old cell data followed similar paths. Thus the ratio λ is a major determinant of cell deformation as well as a critical factor affecting stable orientation in shear flow.

INTRODUCTION

The ability of the human red blood cell (RBC) to deform is critical for effective blood flow in the cardiovascular system, since an 8 μm in diameter RBC must deform in order to traverse the 3 to 4 μm in diameter capillaries of the microcirculation (Braasch, 1971). RBC deformability is also an important determinant of blood flow in larger vessels and of the rheologic behavior of RBC suspensions in viscometers (Chien et al., 1967; Chien, 1975; Meiselman, 1980); pathologic changes of the RBC (Mohandas et al., 1983) or experimental modification of RBC rigidity (Corry and Meiselman, 1978; Cokelet, 1972) can greatly influence their mechanical and viscometric behavior. Various experimental approaches to the study of RBC deformation have been developed (e.g., micropore filtration, high-speed centrifugal deformation, micropipette aspiration); of particular interest to this investigation is the Rheoscope system developed by Schmid-Schonbein and co-workers (Schmid-Schonbein, 1975; Fischer and Schmid-Schonbein, 1977; Fischer et al., 1978*a*). In this instrument, red cells in dilute suspension are subjected to a uniform shear field between a transparent, counter-rotating cone and plate, and are observed via light microscopy in the stationary plane midway between the two moving surfaces. Salient observations using the Rheoscope include: (*a*) if the viscosity of the external phase (i.e., suspending medium) is low, RBCs tend to tumble and behave unstably in the shear field; (*b*) above a critical level of external phase viscosity, the cells become stably oriented and elongate in the direction of shear; (*c*) when stable orientation is achieved, the cell membrane rotates about the cytoplasm in a fashion called "tank-treading;" (*d*) both the degree of cell deformation and the frequency of membrane rotation (tank-treading frequency, TTF) increase with increasing shear rate; (*e*) at constant levels of shear rate, the extent of RBC deformation increases as the external medium is made more viscous (Schmid-Schonbein, 1975; Fischer and Schmid-Schonbein, 1977; Fischer et al., 1978*b*).

The behavior of tank-treading erythrocytes in a uniform shear field has been the subject of recent theoretical analyses (Keller and Skalak, 1982; Tran-Son-Tay et al., 1984). For a specified model of the RBC membrane surface velocity, Keller and Skalak (1982) predicted that the cell and membrane motions will depend on the shape of

the cell, the ratio of internal/external viscosities (i.e., η_i/η_o), and the ratio of the energy dissipation in the membrane to that in the internal fluid (d). Although the extent of deformation of the cell was not predicted, the critical conditions necessary to enable stable orientation accompanied by tank-treading, as well as the dependence of TTF on the variables noted above, were derived. By extending this theory to explicitly consider the mechanism of energy dissipation in the membrane, Tran-Son-Tay et al. (1984) have shown that observations of cell elongation and TTF can be used to obtain values for the membrane viscosity and the dissipation ratio d. The magnitude of this dissipation ratio has also been determined by Fischer (1980) from calculations based on similar experimental observations, but with a different model for the membrane velocity during tank-treading; the two approaches yield similar values for d, i.e., larger than or of the order of unity.

The present study was designed to provide experimental information about the factors that affect stable orientation and the degree of deformation of RBCs in the shear field of a Rheoscope. Density-separated young and old cells were compared, over a wide range of fluid shear stresses, using external phase viscosity and suspending medium osmolality as the primary experimental variables. The selection of age-separated RBCs was predicated on the known changes in membrane and cytoplasmic viscosity and cell geometry, which normally occur during the in vivo aging process (for review see Nash and Meiselman, 1981); the cytoplasmic viscosity of these cells can also be varied as a function of osmotically induced cell shrinkage or swelling (Linderkamp and Meiselman, 1982).

MATERIALS AND METHODS

Media and RBC Preparation

Media of various viscosities were prepared by dissolving either dextran T70 (Dx70, weight average molecular weight = 70,300, lot 11073) or dextran T500 (Dx500, weight average molecular weight = 527,000, lot 13748) at various concentrations in phosphate buffer (0.03M KH_2PO_4 + Na_2HPO_4); both dextran fractions were obtained from Pharmacia Fine Chemicals, Inc. (Piscataway, NJ). The viscosities of the solutions were measured at 25°C using a Wells-Brookfield cone-plate viscometer (½ RVT-200; Brookfield Engineering Laboratories, Inc., Stoughton, MA) and were adjusted to desired levels by dilution with dextran-free phosphate buffer. Osmolality was then measured and adjusted to 294 ± 5 mOsm/kg by addition of NaCl; such a procedure yields dextran solutions that are isotonic in that they do not significantly affect RBC volume (Nash and Meiselman, 1983*b*; see Discussion). In experiments where RBC shrinkage or swelling was desired, appropriate increases or decreases were made in the quantity of added NaCl. Finally, the pH of the buffered dextran solutions was adjusted to 7.40 ± 0.02 at 25°C.

Blood was obtained from healthy adult laboratory personnel via venipuncture into heparin (5 IU/ml). Populations of relatively young and old cells were obtained by centrifugal density fractionation (i.e., 12,000 *g*, 7 min) as previously described (Pfafferott et al., 1982). The 5% least dense (i.e., young) and 5% most dense (i.e., old) cells were separated and washed in pH = 7.40 phosphate buffer with NaCl. After washing, packed cells were suspended at ~2% hematocrit in dextran solutions of desired viscosity and osmolality. The osmolality of the buffer used for washing the cells was equal to that of the dextran solution used for their final suspension. All RBC studies were completed within 6 h of venipuncture. For the donors used in the present study, density fractionation yielded mean corpuscular hemoglobin concentration (MCHC) values averaging 32.4 and 41.3 g/dl for the young and old cell samples, respectively (means from three separate fractionations). Also, for another series of donors, the densities of fractionated cells were tested using the method of Danon and Marikovsky (1964). Density distributions were measured by centrifugation of cell samples layered onto cushions of phthalate ester oils having a range of known densities. For each oil density, the percent of cells finally resting above or below the oil was measured. In most cases, for either young or old cells, a change in oil density of 0.004 g/ml was sufficient to change the result from 100% above to 100% below the oil cushion. Averaged over six experiments, results for the density ranges of young and old cell fractions were 1.108–1.113 and 1.128–1.133 g/ml, respectively.

Rheoscope Measurements

RBC behavior was studied using a counter-rotating cone-plate Rheoscope (Fischer et al. 1978*a*, *b*; Pfafferott et al., 1982), which was mounted on an inverted microscope (Leitz Diavert, Leitz, Wetzlar, Federal Republic of Germany) equipped with a 40× (NA = 0.70) phase contrast objective. Cells suspended in dextran solution (see above) were placed in the gap between the plate and 1.5° cone, and were subjected to a uniform shear field at various shear stresses. RBC were observed at a radial distance of 1,200 μm from the center of rotation and were photographed using high contrast film (Kodak 2415; Eastman Kodak Co., Rochester, NY) and short duration flash illumination. To quantitate the deformation of stably oriented cells, the projected length (L) parallel to the direction of shear and the perpendicular width (W) were measured from the photographic negatives via a video camera, monitor, and video micrometer system (model 201; Bacon Instrument Co., Pasadena, CA). Cell deformation was then expressed either in terms of the ratio L/W (Tran-Son-Tay et al., 1984) or the degree of deformation, $D = (L - W)/(L + W)$ (Fischer et al., 1978*b*). Note that the value of D is zero in the absence of deformation and increases, with a maximum possible value of unity, as cellular deformation increases. In addition, video recordings were made in some experiments in order to evaluate the fraction of cells that achieved stable orientation (see below). All measurements were carried out at room temperature (23 ± 1°C). Three experimental protocols were employed.

(A) RBC Deformation in Isotonic Solutions of Differing Viscosity. For both young and old cells, deformation was measured for eight external phase viscosities, η_o, covering the range of 0.0673 to 2.27 P using Dx500 solutions of varying concentrations (i.e., 5 to 28 g %); the osmolality of these solutions was held constant at 294 ± 5 mOsmol/kg. For each viscosity, a fixed set of 10 shear stresses (τ, calculated as the product of η_o times the shear rate in the cone-plate gap) was applied in the range 2.5 to 500 dyn/cm^2. At each combination of η_o and τ, deformation was measured for 25 to 30 stably oriented cells.

(B) Percentage of Cells Deforming in Solutions of Differing Osmolality but Constant Viscosity. The percentage of either young or old cells achieving stable orientation and deformation in the direction of flow was quantitated as a function of medium osmolality. This was done for two Dx70 solutions with relatively low viscosities (η_o = 0.0555 P or 0.111 P, concentrations 10 or 15 g %), using a constant applied shear stress (τ = 500 dyn/cm^2). From video recordings of RBC motion in the Rheoscope, 100 cells were classified as either oriented and deformed or as not stably oriented (i.e., tumbling); in this particular experimental series, deformation was not determined. The measurements were repeated over a range of osmolalities (see Results) such that the percentage of cells deforming varied from 0 to 100%.

(C) Young vs. Old RBC Deformation at Equal MCHC. In one set of experiments designed to test the effect of MCHC on deformation in the Rheoscope, young cells were shrunk in hypertonic phosphate

buffer (455 mOsm/kg) so that their measured MCHC was comparable to that of old cells in isotonic buffer (i.e., 298 mOsm/kg). The deformation of young and old cells in isotonic Dx70 solutions and of young cells in the hypertonic Dx70 solution was evaluated over the shear stress range of 2.5 to 500 dyn/cm^2; 25 to 30 cells were measured at each stress level. For this set of experiments, the viscosity of all Dx70 solutions was held constant at 0.210 P (concentration 20 g %). Note that when measuring deformation in this experimental series and in *A* above, some cells were not stably oriented at the lowest τ and/or η_o. These undeformed cells were assigned the value of $L/W = 1$ (i.e., $D = 0$), and were included when calculating sample means and standard deviations.

Miscellaneous Techniques

Solution osmolalities were determined by freezing-point depression (model 2007; Precision Systems, Inc., Sudbury, MA) and pH via a radiometer (model PHM 71; Radiometer Co., Copenhagen, Denmark) operating at 25°C. Hemoglobin concentrations were measured via the cyanmethemoglobin method and hematocrits by the microhematocrit technique. From these measurements, MCHC was calculated. Values for MCHC in the nonisotonic media used for experimental series *B* (see above) were calculated from the measured isotonic values, using previously determined volume vs. osmolality data for young and old cells (Linderkamp and Meiselman, 1982). Internal viscosities (η_i) for cells were calculated using the relation between hemoglobin solution concentration and viscosity at 25°C given by Ross and Minton (1977).

RESULTS

Two major differences between the behavior of young and old cells were immediately evident in the Rheoscope: (*a*) In isotonic suspending media with relatively low viscosity (η_o = 0.0555 or 0.111 P) most old cells tumbled, whereas young cells deformed and adopted a stable orientation (e.g., Fig. 1). (*b*) At equal τ, the degree of deformation, *D*, of young cells exceeded that of old cells for isotonic suspending media with $\eta_o \gtrsim 0.18$ P (e.g., Fig. 3 *b*). These differences were further investigated by systematically testing the effects of variations of external phase viscosity and osmolality on cellular alignment and deformation.

The observed percentages of cells that were stably oriented, and were deformed rather than tumbling, are plotted in Fig. 1 as functions of suspending medium osmolality. Young and old cells were compared at two different external viscosities; τ was 500 dyn/cm^2 in all cases. For each external viscosity, the percent deforming decreased as osmolality was increased (i.e., as cell volume fell and MCHC rose). The data were analyzed by linear regression of percent deforming against osmolality, with the straight lines shown in the figure drawn from these regression analyses. Clearly, the lines for young cells are shifted to the right (i.e., higher osmolality) relative to those for the old cells; to induce tumbling, young cells had to be hyperosmotically shrunk. In addition, for both cell types, the lines are shifted to the right for the higher compared to the lower external phase viscosity. Thus, to cause tumbling at a higher external phase viscosity, a higher medium osmolality is required. These effects of osmolality presumably occurred via its influence on cell volume, and hence on MCHC and internal viscosity. The linear regression analysis allowed prediction of the osmolality at which 50% of cells would deform (Osm-50), i.e., the median transition or threshold osmolality for stable orientation of the cells. From these Osm-50, young and old cell MCHC and internal viscosity were calculated (i.e., MCHC-50 and η_i-50, see Methods). The values obtained for Osm-50,

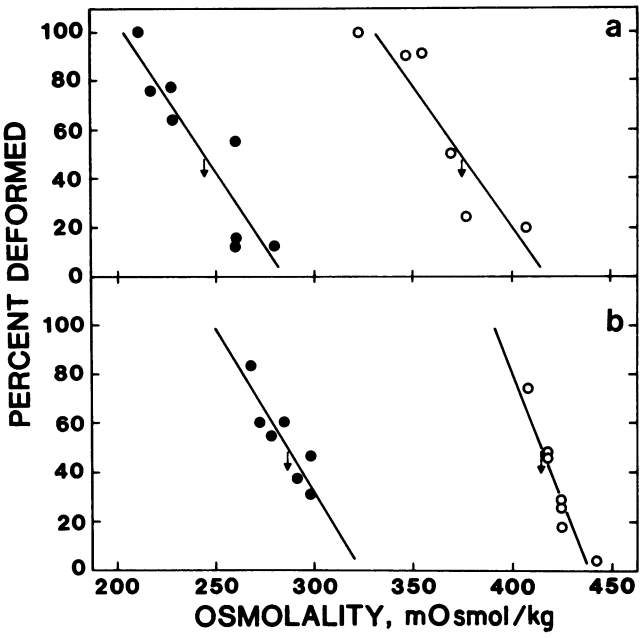

FIGURE 1 Percent of cells stably oriented and deformed plotted as a function of suspending phase osmolality; shear stress was held constant at 500 dyn/cm^2. Data for young (○) and old (●) RBCs are shown for two different suspending phase viscosities (η_o): (*a*) η_o = 0.0555 P and (*b*) η_o = 0.111 P. Linear regression lines are drawn for each set of data and down-pointing arrows indicate the threshold osmolality (Osm-50) where 50% of cells deformed. Correlation coefficients for the linear regressions were all >0.888 ($p < 0.01$).

TABLE I
THRESHOLD PARAMETERS FOR STABLE ORIENTATION OF CELLS IN THE RHEOSCOPE

	External viscosity			
	η_o = 0.0555 P		η_o = 0.111 P	
	Young cells	Old cells	Young cells	Old cells
Threshold osmolality (Osm-50, mOsm/kg)	374	244	415	286
Resultant MCHC-50 (g/dl)	36.3	36.9	38.3	40.2
Internal viscosity (η_i-50, Poise)	0.138	0.152	0.194	0.281
Viscosity ratio (η_i-50/η_o)	2.49	2.74	1.75	2.53

Values for MCHC-50 and η_i-50 were calculated from the measured isotonic MCHC values and the known effect of osmolality on young and old cell volume (Linderkamp and Meiselman, 1982), and from the relation between hemoglobin solution concentration and viscosity given by Ross and Minton (1977). Threshold osmolalities were determined for cells subjected to a fixed shear stress of 500 dyn/cm^2.

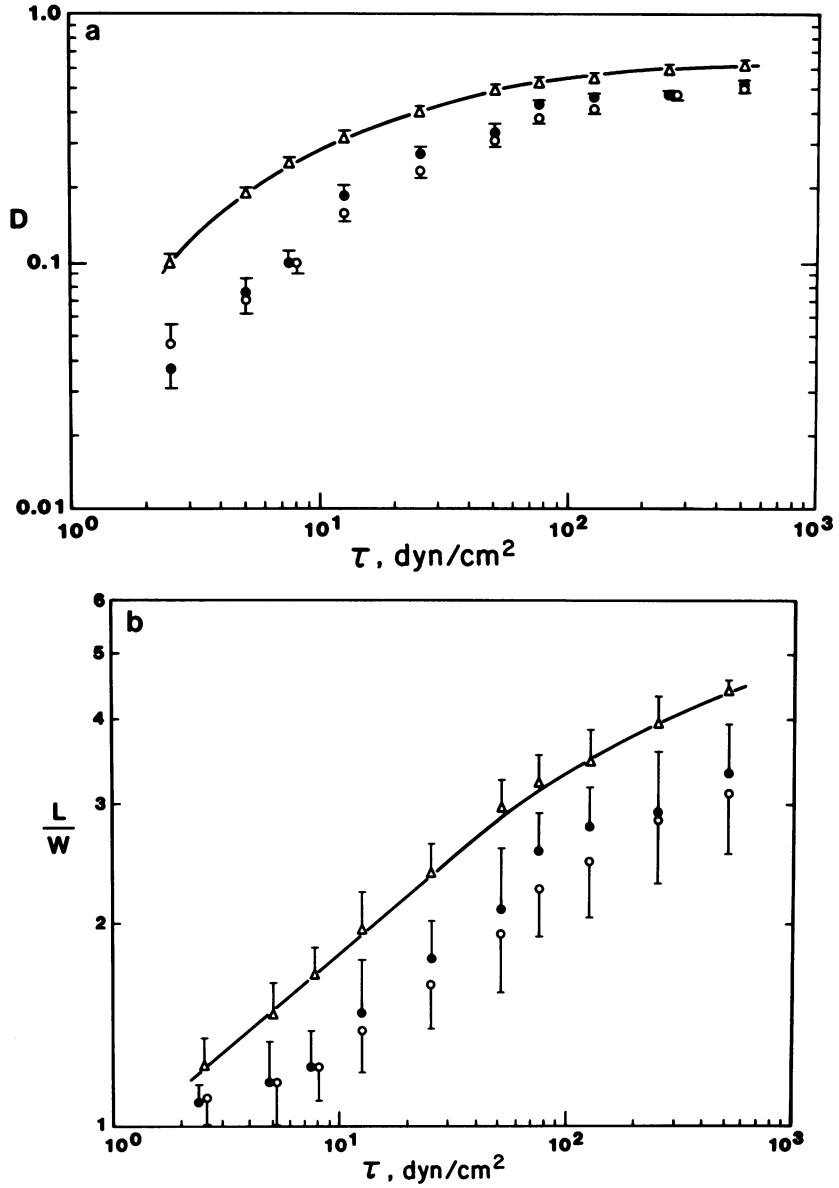

FIGURE 2 Cell deformation plotted as a function of shear stress (τ). Young cell data are shown for suspensions at 298 (Δ) and 455 (O) mOsm/kg; old cell data (●) are for 298 mOsm/kg. For all samples, the suspending phase viscosity was 0.21 P. (*a*) Degree of deformation (D) vs. τ; error bars represent one standard error of the mean. MCHC values for the different samples were: Δ, 33.3 g %; O, 42.0 g %; ●, 41.5 g %. (*b*) Deformation (L/W) vs. τ; error bars represent 1 SD of the mean. For both D and L/W data, differences between young and old cell deformation at 298 mOsm/kg were statistically significant for all τ ($p < 0.01$). For young cells at 455 mOsm/kg vs. old cells at 298 mOsm/kg, differences were only significant ($p < 0.01$) for τ = 25, 75 and 125 dyn/cm².

MCHC-50, η_i-50, and the ratio η_i-50/η_o are given in Table I for young and old cells at the two different η_o. Note that while young and old cells had nearly equal MCHC-50 for a given suspending medium, the MCHC-50 and η_i-50 were higher for the higher external viscosity. However, the ratios η_i-50/η_o were similar for all cases; differences in this ratio may arise from the extremely strong dependence of hemoglobin solution viscosity on concentration (e.g., for η_o = 0.111 P, the 5% difference in MCHC-50 yields a 45% change in η_i-50). Thus, a slight inaccuracy in values for MCHC-50 could give rise to large uncertainties in η_i-50 estimates.

Alteration of either the external phase viscosity or osmolality affected the degree of deformation of stably oriented cells. Typical deformation data are shown in Fig. 2 for young and old cells in isotonic medium (η_o = 0.210 P); using logarithmic scales, Fig. 2 *a* shows degree of deformation D vs. τ and Fig. 2 *b* shows the same data presented as L/W vs. τ. Also shown in Fig. 2 *a* and *b* are data for young cells tested with the same η_o but with the suspending phase osmolality adjusted to 455 mOsm/kg; at this osmolality the MCHC of the young cells was within ~1% of that of the old cells in the isotonic medium. As noted earlier, when suspended in identical media, young

cell deformation exceeded old cell deformation at equal τ. However, shrinkage of the young cells reduced their deformation; equalization of MCHC caused young and old cell deformation to become nearly coincident. Evidently, alteration of MCHC, and hence η_i, strongly influences cellular deformation in the Rheoscope. Note that the curves for D vs. τ (Fig. 2 *a*) tend to flatten at high τ, whereas this tendency toward a maximum deformation is not clearly evident in Fig. 2 *b*. On the other hand, if linear scales are used for the deformation data, then curves for either D or L/W vs. τ are flattened at high stresses and show evidence for the existence of maximum cell deformation levels; such linear scales were not used herein, however, since they compress the data for low τ and tend to obscure inter-sample differences in deformation in this region.

Young and old cell deformation was also compared for a series of different suspending phase viscosities; at each η_o, a fixed set of shear stresses was applied. In Fig. 3 *a* the degree of deformation is plotted as a function of τ on a linear-log scale; young and old cell data are shown for

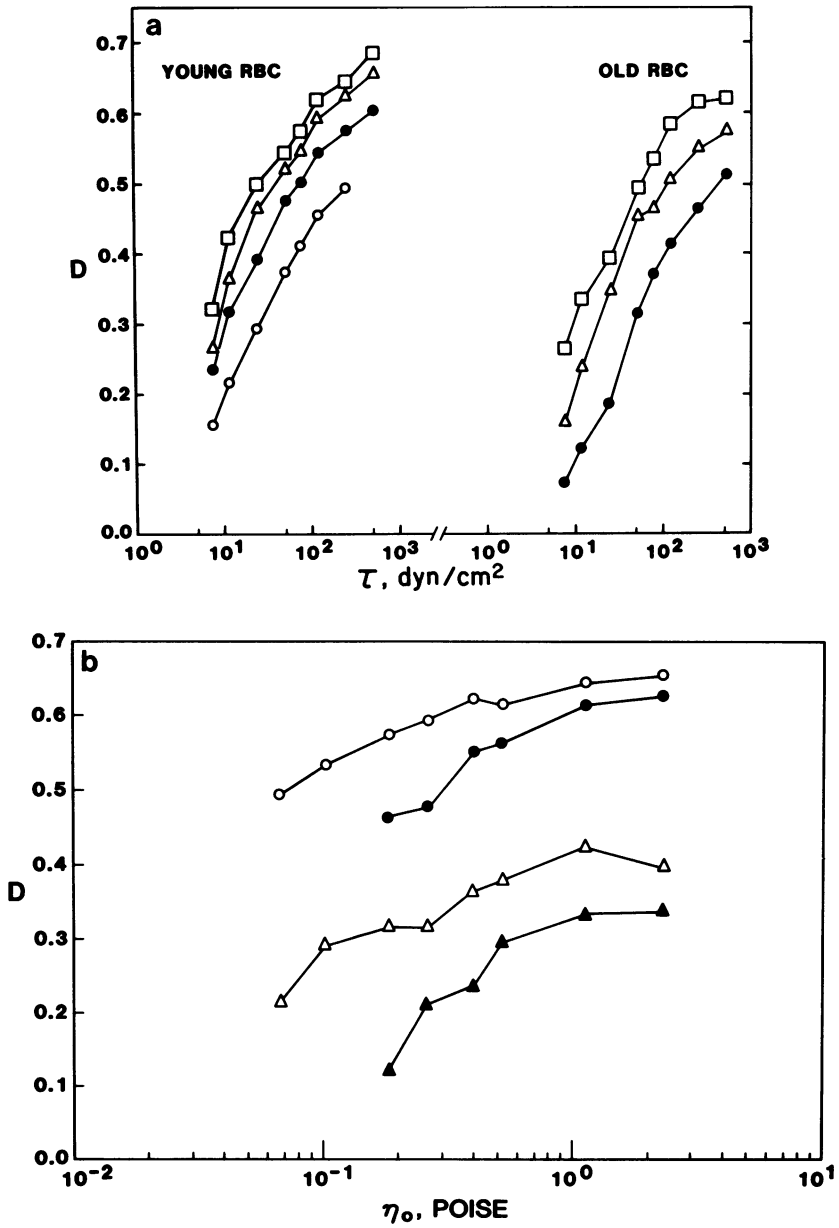

FIGURE 3 (*a*) Degree of deformation (D) plotted as a function of shear stress (τ). Young and old cell data are shown for various constant suspending phase viscosities (η_o): ○, 0.0673 P; ●, 0.183 P; △, 0.393 P; □, 1.12 P. Old cell data are not indicated for η_o = 0.0673 P as they tended to tumble in this medium. At each τ, differences between the D values obtained for each η_o were significant ($p < 0.05$ or better) (*b*) Degree of deformation (D) plotted as a function of suspending phase viscosity (η_o). Young (△ and ○) and old (▲ and ●) cell data are shown for two constant shear stresses (τ, dyn/cm^2): △ and ▲, τ = 12.5 dyn/cm^2; ○ and ●, τ = 250 dyn/cm^2. For every combination of η_o and τ, young cell D was significantly greater than that for old cells ($p < 0.01$). Also, D was significantly correlated with ln (η_o) for each of the curves shown ($p < 0.01$).

several η_o. In Fig. 3 *b* these and additional data for other η_o are replotted; deformation is shown as a function of η_o for two constant values of τ. Note that, for all cells, deformation increases as η_o is increased, even while τ is constant. However, changes in η_o have an unequal effect on the deformation of young and old cells, in that differences between young and old cell deformation decrease with increasing η_o. This unequal effect of η_o can be quantitated by calculating the ratio of old/young cell deformation, $R = (L/W)_o/(L/W)_y$, for the various η_o and τ. For a given η_o, R is nearly constant and there is no significant correlation of R with τ. Thus, average R values (R_a) over a wide range of τ can be calculated for each η_o. These average values are shown in Table II; R_a increases with increasing η_o, showing that old cell deformation approaches young cell deformation if the external viscosity is sufficiently high. However, at the highest τ (500 dyn/cm^2), the R values for all η_o tend to converge (Table II). This convergence probably arises from the fact that at high stress, the deformations of young and old cells will approach maximal levels determined by their different geometries, regardless of the external viscosity (see Discussion).

From the above, it is evident that changes in both η_i and η_o influence cell deformation in the Rheoscope. Greater insight into the effects of η_i and η_o is gained when data for D vs. η_o (e.g., Fig. 3 *b*) are replotted as D vs. η_i/η_o, i.e., explicitly considering the unequal MCHC, and thus unequal η_i, of young and old cells. In Fig. 4 young and old cell deformation data are plotted against the viscosity ratio, $\lambda = \eta_i/\eta_o$; separate curves are shown for four different, constant levels of τ. Note that deformation decreases as λ increases, but young and old cells follow a similar path for a given τ. Thus Fig. 4 predicts that if young and old cells are sheared at equal τ and λ, their deformation will be nearly equal. This prediction is supported by the results obtained when η_i was hypertonically increased for young cells (Fig. 2); for this condition of equal λ, the resulting deformation was similar for the shrunk young and the isotonic old cells over a wide range of τ. Consequently, the ratio λ appears to be a primary determinant of the degree of deformation of red cells in shear flow. Irregularities in the curves shown in Fig. 4, where old cell deformation may fall slightly below young cell deformation at low λ and/or low τ, could result from inequality of other cellular factors affecting deformation (see Discussion).

TABLE II
RELATIVE DEFORMATION OF YOUNG AND OLD CELLS AT DIFFERENT EXTERNAL VISCOSITIES

External viscosity (η_o, Poise)	Relative deformation $R_a = (L/W)_o/(L/W)_y$	R value at 500 dyn/cm^2
0.183	0.707 ± 0.049	0.778
0.260	0.773 ± 0.050	0.717
0.393	0.788 ± 0.030	0.772
0.521	0.850 ± 0.061	0.798
1.12	0.853 ± 0.059	0.791
2.29	0.931 ± 0.073	0.820

R_a values are the mean ± SD of the R values obtained at the seven shear stresses ($\tau \geq 12.5$ dyn/cm^2) that were applied for each η_o. For each constant η_o, there was no significant correlation of R with τ, but R_a did correlate with η_o ($p < 0.01$).

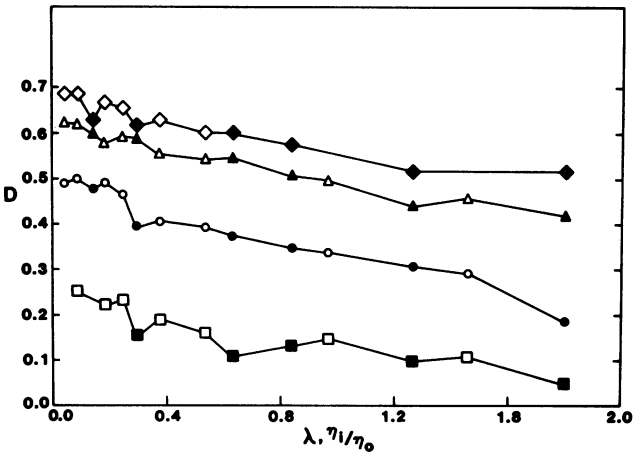

FIGURE 4 Degree of deformation (D) plotted as a function of the viscosity ratio $\lambda = \eta_i/\eta_o$, where η_i is the cytoplasmic viscosity and η_o is the suspending phase viscosity. Data for young (□, ○, △, ◇) and old (■, ●, ▲, ◆) cells are shown for various constant shear stresses (τ, dyn/cm^2). □ and ■, τ = 5 dyn/cm^2; ○ and ●, τ = 25 dyn/cm^2; △ and ▲, τ = 125 dyn/cm^2; ◇ and ◆, τ = 500 dyn/cm^2. For each τ, D was significantly correlated with λ ($p < 0.01$).

DISCUSSION

Both attainment of stable orientation and subsequent cell deformation in the Rheoscope were strongly influenced by cellular internal viscosity (η_i) and external suspending phase viscosity (η_o). In general, older cells showed a greater tendency to tumble and, when stably oriented, deformed less than younger cells. These differences were essentially eliminated if the MCHC, and hence the internal viscosities, of young and old cells were brought to similar levels. Increases in external viscosity caused the degree of deformation to be greater for all cells at a given shear stress, and also decreased differences between young and old cell deformation. The latter effect on old and young cells was clarified if deformation was plotted as a function of the ratio $\lambda = \eta_i/\eta_o$ for various fixed τ; young and old cell data followed similar curves, with D decreasing as λ increased. Thus, differences between young and old cell deformation appear to result mainly from unequal MCHC and/or λ.

The presence of dextran itself, at varying concentrations, is not expected to significantly affect the behavior of cells in the Rheoscope other than via its effect on the suspending medium viscosity. In previous studies, using a wide range of Dx70 and Dx500 concentrations, we have found minimal effects of these polymers on red cell geometry and membrane elasticity (Nash and Meiselman, 1983*b*). Although the microscopically measured cell volume and surface were increased slightly for Dx70 concen-

trations above 2.5 g %, we concluded that this apparent increase was due to an optical artifact induced by the increased refractive index of the suspending medium. Further, it is unlikely that dextran alters RBC membrane viscosity (η_m). Sutera et al. (1985) have shown that time constants for red cell shape recovery (t_c) can be measured using a Rheoscope. By this method, they obtained t_c for cells suspended in dextran (molecular weight = 2×10^6, $\eta_o = 0.35$ P); these t_c were similar to values obtained by other investigators (Hochmuth et al., 1979) using micropipettes for cells in dextran-free buffer. Since t_c is equal to the ratio of membrane viscosity to membrane elasticity (Hochmuth et al., 1979), dextran had not apparently altered the membrane viscosity. We have also carried out Rheoscope t_c measurements (Tran-Son-Tay, R., G. B. Nash, and H. J. Meiselman, unpublished observations), and have observed that for Dx70 solutions with η_o up to 0.8 P, t_c values obtained are very close to those measured for the same donors via micropipettes. At $\eta_o = 1.6$ P, a slight increase in t_c occurred in the Rheoscope, but this is probably due to increased viscous drag acting on the cell during shape recovery rather than to an actual change in membrane viscosity.

Variations of media osmolality (and hence of cytoplasmic viscosity) indicated that, for a given, low, external phase viscosity, an estimate could be made of the threshold osmolality where 50% of cells would deform and 50% would tumble in the shear flow. The threshold osmolalities were different for young and old cells, but their MCHC and η_i at these osmolalities were similar (Table I). If suspending phase viscosity was altered, different threshold internal viscosities were found. However, these η_i-50 changes were such that the ratios η_i-$50/\eta_o$ were nearly independent of cell age and η_o. The transition from unstable to stable orientation of RBC in shear flow has previously been observed (Bessis and Mohandas, 1975; Morris and Williams, 1979) and threshold external viscosities measured. Our data show that the ratio λ (i.e., η_i/η_o) primarily determines this transition, rather than absolute values for either η_i or η_o. This finding agrees well with the theory of Keller and Skalak (1982), which predicts that the transition to stable orientation for a tank-treading cell will occur for specific relations between cell shape and the ratios λ and d (d equals the ratio of energy dissipated in the membrane to that dissipated in the cytoplasm during tank-treading).

Here the threshold value of λ (i.e., η_i-$50/\eta_o$) was found to have an average value of 2.38 ± 0.44 (mean ± SD, Table I). If cell shape at the transition is taken to be an oblate ellipsoid (an approximation to the undeformed biconcave state) and cell sphericity is equal to 0.72 (Linderkamp and Meiselman, 1982), then for this λ, the dissipation ratio, d, is calculated to be ~0.5 using the theory of Keller and Skalak (1982). This is of the same order but less than the values obtained by previous workers (i.e., $d = 1$ to 2 according to Fischer [1980] and $d = 2$ to 4 according to Tran-Son-Tay et al. [1984]). Both directly calculated the energy dissipated by tank-treading, deformed cells using experimental data obtained over a range of shear rates, whereas the d value obtained in the present study refers to cells in the transition region between tumbling and stable orientation. The cells used in the present study generally had higher internal viscosities and were less elongated than those in the previous studies (Fischer, 1980; Tran-Son-Tay et al., 1984), and consequently d values might differ inasmuch as raising η_i is expected to cause d to fall.

Cell deformation increased when external phase viscosity was increased (e.g., Fig. 3). This is opposite to the response of a fluid drop, for which D will decrease if η_o increases at constant τ (Taylor, 1934). Fischer et al. (1978*b*) have previously observed increased D with increased η_o, at constant τ, for unfractionated RBC populations. To eliminate this η_o effect, they plotted D as a function of a modified shear stress equal to $\dot{\gamma}\eta_o^s$, and were able to collapse data for different η_o onto the same line. The necessary value for s varied somewhat between blood donors, with an average value of 1.5. The success of this procedure implies that the effect of η_o on D is solely a function of changes in the external medium. However, we noted that changes in η_o had unequal effects on young and old cell deformation (e.g., the ratio $[L/W]_o/[L/W]_y$ increases as η_o increases, Table II). Although the separate young and old cell data obtained in the present study could be treated in the manner of Fischer et al. (1978*b*), the value for s required to collapse the data differed between young and old cells (see Appendix). This difference in s values suggests that the effect of η_o on cell deformation is mediated by cellular factors that are unequal for young and old cells. On the other hand, if young and old cell deformation data were plotted as a function of the ratio λ, the curves followed similar paths for a wide range of η_o and τ (Fig. 4). Thus, increases in deformation as η_o is raised can be best described in terms of changes in the ratio of η_i/η_o, and this ratio appears to be a major determinant of cell deformation in shear flow. Its equalization for young and old cells, either by altering external phase viscosity (Fig. 4) or internal cytoplasmic viscosity (Fig. 2), caused their deformation behavior to become nearly equal.

The mechanism whereby changes in external phase viscosity affect cell deformation might be related to changes in membrane TTF. As shear rate increases, TTF also increases, approximately linearly; η_o has little effect on this relation (Fischer et al., 1978*b*). Thus, at constant shear stress ($\tau = \dot{\gamma}\ \eta_o$), $\dot{\gamma}$ and TTF will decrease as η_o increases. Therefore, the increased cell deformation, which occurs when η_o is increased (at constant τ), is associated with a decreased TTF. This implied dependence of D on TTF may be a consequence of the viscoelastic nature of the red cell membrane, which requires a finite time to deform fully in response to an applied force (Evans and Hochmuth, 1976). When the membrane experiences a cyclically varying shear force, the extent of its deformation will

depend on the frequency of the variation. In the Rheoscope, if the tank-treading period is of the same order as the time constant of the membrane (defined by the membrane elasticity and viscosity), then a decrease in TTF could increase the membrane deformation and thus the cell elongation. Values for TTF given in previous studies (Fischer et al., 1978*b;* Tran-Son-Tay et al., 1984), for comparable shear and viscosities, do indeed range from below to above $1/t_c$, where t_c is the viscoelastic time constant (~0.1 s at 25°C; Hochmuth et al., 1979).

Although presently available theory cannot explicitly predict D or L/W vs. τ data in terms of cell mechanical properties, factors such as membrane elasticity, membrane viscosity, cytoplasmic viscosity, and cell geometry are expected to influence deformation. The influence of the membrane shear elastic modulus was not evaluated in the present study, as this property does not vary significantly during in vivo aging (Nash and Wyard, 1981; Linderkamp and Meiselman, 1982). However, both membrane and cytoplasmic viscosities (η_m and η_i, respectively) do increase during aging (Linderkamp and Meiselman, 1982; Nash and Meiselman, 1983*a*). Since energy dissipated in the membrane and cytoplasm of a tank-treading cell is proportional to these viscosities, and since the ratio of dissipation in the membrane to that in the cytoplasm is of the order of unity (Fischer, 1980; Tran-Son-Tay et al., 1984), both η_m and η_i are likely to affect RBC deformation in shear flow. In the present study, differences in the deformation response of young and old cells were largely abolished when their MCHC and hence internal viscosities were made nearly equal (Fig. 2). This result appears to suggest that membrane viscosity differences have little effect on the relative deformation of young and old cells. Note that alteration of MCHC may also affect η_m, particularly if MCHC is osmotically raised above 40 g/dl (Nash and Meiselman, 1983). Thus, the decrease in deformation associated with shrinkage of young cells (Fig. 2) may also partly result from an increase in their η_m. However, we have previously found that if MCHC levels of young and old cells are equalized, the η_m of old cells still remains greater than that of young cells (Nash and Meiselman, 1983), and thus lower deformation of the old RBC would be expected. RBC membrane viscosity may also be shear rate dependent (Chien et al., 1978; Tran-Son-Tay et al., 1984), with η_m decreasing as shear rate increases. However, since the shear rate dependence of η_m appears similar for young and old cells (Tran-Son-Tay et al., 1984), it should have little effect on the relative deformation of these cell fractions at different shear rates. Note that at constant τ, increasing η_o (and hence decreasing shear rate) would result in increasing η_m. Such an effect would tend to cause deformation at a given τ to be smaller for greater η_o; this is opposite to the experimentally observed influence of η_o on deformation (Fig. 3). Thus, any shear rate dependence of η_m has a lesser effect in determining the dependence of deformation on η_o than other factors (e.g., the ratio λ, or variation in TTF).

With respect to cytoplasmic viscosity, it can be seen in the series of experiments summarized by Fig. 4 that differences in deformation between young and old cells at equal τ were greatly reduced if the ratio $\lambda = \eta_i/\eta_o$ was equalized. Thus, the effect of internal viscosity on cell deformation appears to be mediated by this viscosity ratio, i.e., rather than η_i or η_o independently influencing deformation in shear flow, their ratio is the critical parameter. Of course, such cell fractions have different membrane viscosities (i.e., old greater than young), which may be the reason why old cell deformation tends to be less than that for young cells at the lower values for λ and τ in Fig. 4.

The maximum deformation achievable, at high τ, is limited by constraints of cell geometry, i.e., surface area (SA) and volume (V); for constant SA and V, the maximum L/W ratio and D occur for a unique prolate ellipsoidal shape. Using previous data for cell SA and V (Linderkamp and Meiselman, 1982), maximum D and L/W values can be calculated; for D the maximum values are 0.64 and 0.71 for old and young cells, respectively. These values are close to those experimentally measured at the highest η_o and τ (e.g., in Figs. 3 *A* or 4). Also, the limiting value for the ratio $(L/W)_o/(L/W)_y$ is predicted to be 0.77; this computed value is similar to the R value obtained at 500 dyn/cm^2 (Table II). Thus, at high shear stresses, red cells approach a predictable limiting ellipsoidal form, which is reflected in flattening of deformation vs. τ curves.

APPENDIX

In an analysis of the effects of suspending phase viscosity η_o on the deformation of unfractionated human erythrocytes, Fischer et al. (1978*b*) determined that the use of a modified shear stress term allowed the deformation data for different η_o to collapse onto a single line. The form of the modified stress term was $\eta_o^s\dot{\gamma}$, where $\dot{\gamma}$ is the shear rate in the gap of the Rheoscope; for the range of η_o from 0.117 to 0.510 P, the exponent s was empirically found to be 1.48 ± 0.21 (mean ± SD for 12 samples). The same approach (i.e., use of a modified shear stress term) was applied to the D vs. τ data obtained in the present study (e.g., Fig. 3 *a*), to evaluate its applicability to the deformation behavior of age-separated red cell fractions in various viscosity media. Note that the use of shear stress rather than shear rate as the independent variable does not vitiate the analysis, since for the Newtonian media employed, $\eta_o\dot{\gamma} = \tau$ and thus $\eta_o^s\dot{\gamma} = \eta_o^r\tau$, where $r = s - 1$. Thus the appropriate value of r was found to collapse either the young or old cell data, and were then used to obtain s values for comparison with the results of Fischer et al. (1978*b*).

The experimental results of Fischer et al. (1978*b*), and those obtained in the present study (Fig. 3 *A*), indicate a linear relation between cell deformation (D) and either $\ln(\tau)$ or $\ln(\eta_o\tau)$. Further, the effect of changes in η_o is to vertically displace the deformation data rather than to alter the slope of the D vs. $\ln(\eta_o\tau)$ relationship (i.e., only the intercept varies). Thus, for young or old cells in a suspending medium of viscosity η_{ON}:

$$D_N = B_N + M\ln(\eta_{ON}\tau) \quad \text{(A1)}$$

and

$$D_N = B + M\ln(\eta_{ON}^r\tau)\,, \quad \text{(A2)}$$

TABLE III
PARAMETERS DERIVED FOR MODIFIED SHEAR STRESS TECHNIQUE

Cell type	Mean M	$-(1-r)M$	r	s
Young RBC	0.0868	−0.0182	0.790	1.79
Old RBC	0.0935	0.0317	1.34	2.34

where M is the slope (which remains constant) and B represents the new intercept resulting from adding the exponent r in the ln term. Subtracting Eq. A2 from Eq. A1 yields

$$B_N = B - (1 - r)\, M \ln(\eta_{ON}) . \qquad (A3)$$

Values for B and M for the various experimental η_{ON} are calculated via Eq. A1 by linear regression of D_N against $\ln(\eta_{ON}\tau)$. Then, according to Eq. A3, regression of the resulting B_N vs. $\ln \eta_{ON}$ will yield a line whose slope is $-(1 - r)M$. The mean value of M (which is essentially independent of η_o) is obtained from the N applications of Eq. A1, thus allowing r to be calculated.

Young and old cell deformation data obtained in isotonic Dx500 media were separately analyzed using the above procedure, with the following constraints. (*a*) Deformation data obtained at 2.5 and 5.0 dyn/cm^2 were excluded from the regression calculated via Eq. A1, as these low shear stresses exhibited some deviation from linearity. For the data obtained at the remaining eight shear stresses, linear correlation coefficients exceeded 0.95. (*b*) Since old cells failed to exhibit stable orientation at the two lowest levels of media viscosity (i.e., 0.0673 and 0.101 P), old cell deformation data for six media were included (0.183, 0.260, 0.393, 0.521, 1.12, and 2.29 P), whereas young cell data from the complete span of eight η_o values were used in the analysis.

The computed values of r and s are shown in Table III. Note that: (*a*) the s values are higher (40% on average), but of the same order as the value of 1.48 ± 0.21 obtained by Fischer et al. (1978*b*) for unfractionated human cells; (*b*) use of the appropriate r values for a given cell type resulted in D vs. $\ln(\eta_o'\tau)$ curves that were essentially independent of η_o (data not shown); (*c*) the s and r values for young and old cells differ considerably, indicating that the dependence of cell deformation on external phase viscosity is mediated by cellular factors that differ between these cell types.

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