Erythrocyte Deformation in Shear Flow: Influences of Internal Viscosity, Membrane Stiffness, and Hematocrit

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The effect of shear force (depending on shear rate and viscosity of extracellular medium) and hematocrit of RBC suspension on RBC deformation was studied quantitatively using a cone-plate rheoscope with various kinds of cells, i.e., partially hemolyzed (PH) cells, density-fractionated intact cells, and diamide-treated cells. The deformation index (DI) of ellipsoidally deformed cells was shown to be a function of \( \beta \gamma \eta_m / \eta_s \), where \( \gamma \) is applied shear stress, \( \eta_m \) and \( \eta_s \) are external and internal viscosities, respectively, and \( \alpha \) and \( \beta \) are adjustable parameters related to the membrane viscoelastic properties. The increase of suspension viscosity at higher hematocrits (Hts) generally enhanced the ellipsoidal deformation of cells, in the same manner as increasing the suspending medium viscosity of a diluted cell suspension. The suppressing effect on cell deformation appeared above a certain Ht. When intact cells were mixed with glutaraldehyde-treated, hardened cells, the ellipsoidal deformation of intact cells was disturbed. The suppression of deformation probably occurred through disturbance of laminar flow-lines around intact cells.

**MATERIALS AND METHODS**

**Preparation of PH Cells**

Pink ghosts were prepared by hypotonic hemolysis in a preliminary experiment, but ellipsoidal deformation under uniform shear flow was disturbed. Therefore, we prepared PH cells containing an appropriate amount of dextran by the following procedures.

Freshly drawn heparinized blood from healthy donors was centrifuged to remove plasma and buffy coat. The packed cells were then washed three times with an isotonic HEPES buffer (115 mmol/L of NaCl, 50 mmol/L of HEPES, 6 mmol/L of glucose, 5 mmol/L of KCl, pH 7.4). The washed cells were suspended in the isotonic HEPES buffer containing 38 mmol/L of L-α-lysophosphatidylcholine, palmitoyl (LPC, Sigma Chemical, St Louis) at a Ht of 45% (the RBCs changed from discocytes to spherocytic cells in the presence of LPC). After the suspension was allowed to stand for 5 minutes on ice, it was centrifuged to obtain packed LPC-treated cells. Two kinds of PH cells, having low internal viscosity (light PH cells) and high internal viscosity (heavy PH cells), were prepared by incubating LPC-treated cells in hypotonic dextran solutions as follows.

**Light PH cells.** In step 1, 1 mL of the LPC-treated packed cells was suspended in 9 mL of ice-cooled hypotonic HEPES buffer (10 mmol/L of HEPES, 1 mmol/L of KCl, 1 mmol/L of glucose, 2 mmol/L of EGTA, 5 mmol/L of MgSO4, 0.8 mmol/L of ATP; pH 7.4, 30 mOsm/kg) containing 7% Dextran T-40 (Pharmacia Fine Chemicals, Uppsala, Sweden) and was incubated for 5 minutes on ice with gentle stirring (designated as step-1 suspension). In step 2, 9 mL of the same hypotonic 7% dextran solution was added to the step-1 solution, and the suspension was stirred gently for 5 minutes.

In step 3, to return the isotonicity, 1.44 mL of a hypertonic HEPES buffer (1.150 mmol/L of NaCl, 500 mmol/L of HEPES, 60 mmol/L of glucose, 50 mmol/L of KCl; pH 7.4) was added to the...
step-2 suspension. In step 4, to remove LPC from cells, 18 mL of the isotonic HEPES buffer containing 1.5% bovine serum albumin (BSA, fraction-V, purchased from Armour Pharmaceutical, Kankakee, IL) was added to the step-3 suspension, followed by incubation of the suspension for 1 hour at 37 °C. During the incubation, rescaling of cells was accomplished. In step 5, the resulting PH cells were washed once with an isotonic 7% dextran solution (100 mmol/L of NaCl, 50 mmol/L of HEPES, 6 mmol/L of glucose, 5 mmol/L of KCl, pH 7.4) to remove a minute amount of membrane fragment and washed twice with the isotonic HEPES buffer.

Heavy PH cells. The above procedures for the preparation of light PH cells were followed with a minor modification: In step 2, instead of the hypotonic 7% dextran solution, 9 mL of the hypotonic HEPES buffer containing 30% dextran was added to the step-1 suspension.

Preparation of Density-Fractionated Intact Cells

Intact cells were fractionated by density-gradient centrifugation. An isotonic suspension of Percoll (Pharmacia) in the HEPES buffer was used: 75.6% Percoll to obtain low-density cells and 82.8% Percoll to obtain high-density cells. One milliliter of packed cells was poured on 13.5 mL of Percoll medium and then spun at 7,000 g for 20 minutes at 20 °C. The low-density cells (10% to 15% of all cells) gathered in the upper half of the tube (in 75.6% Percoll) while the high-density cells (30% of all cells) sedimented in the lower half of the tube (in 82.8% Percoll). The low-density cells and high-density cells can be assigned to young and aged cells, respectively. The fractionated cells were washed three times with the isotonic HEPES buffer to remove Percoll. The fractional percentage was calculated on the basis of hemoglobin concentration.

Preparation of Diamide-Treated Hardened Cells
(Diamide-Treated Cells)

The fractionated low-density cells were treated in the isotonic HEPES buffer and incubated with various concentrations of diamide (diazene dicarboxylic acid bis(N,N-dimethylamide, Sigma) for 30 minutes at 37 °C (Ht 10%). After incubation, cells were washed three times with the isotonic HEPES buffer.

Preparation of Glutaraldehyde-Treated, Hardened PH Cells (GA-Treated PH Cells)

Light PH cells were suspended in the isotonic HEPES buffer containing 0.1% glutaraldehyde at a Ht of 10% and incubated for 30 minutes at 37 °C with gentle stirring. To remove unreacted glutaraldehyde, cells were washed three times with the isotonic HEPES buffer.

Determination of Mean Corpuscular Volume (MCV)

MCV was determined from the number of cells measured by an automatic counter (Model CC-110, Toa Electric, Tokyo), and the packed cell volume was determined by the method of England and Down, as follows. Cells were washed with the isotonic HEPES buffer containing 1.5% BSA and suspended in the same solution. Ten microliters of [125I]-labeled BSA solution (150 μCi/mL) were added to 5 mL of the cell suspension (Ht 50%). The radioactivities of supernatant and sedimented fraction were measured by an autowell γ counter (ARC-605, Aloka, Tokyo) after centrifugation for 10 minutes at 6,000 g with a microhematocrit tube using a microhematocrit centrifuge (Model KH-120, Kubota, Tokyo) and the packed cell volume was calculated after the volume of medium trapped among cells was corrected.

The Ht value of the cell suspension was determined after correcting the trapped medium volume. For the GA-treated PH cell suspension, the Ht was calculated from the number of cells in the suspension and the MCV of original cell before GA treatment.

Electrophoretic Analysis of Membrane Proteins

The polyacrylamide gel electrophoresis (PAGE) in 1% sodium dodecyl sulfate (SDS) was performed on a slab gel. The densitometric scanning of Coomassie brilliant blue R-250-stained gel was performed by a densitometer (Dual-wavelength TLC Scanner, CS-900, Shimadzu, Kyoto, Japan). After diamide treatment, the degree of cross-linking of membrane proteins was estimated by the method of Maeda and colleagues.

Determination of Amounts of Membrane Lipids

To estimate the loss of membrane lipids during the preparation of PH cells, the amounts of cholesterol and total phospholipids were determined by the method of Zlatkis and Zak and Bartlett, respectively.

Measurement of Cellular Deformation

Cellular deformation was observed at 25 °C with a cone-plate rheoscope constructed in our laboratory combining an inverted microscope (Model IMT, Olympus Optics Co., Tokyo), a transparent cone-plate viscometer (Model B, Tokyo Keiki) and a flashlight (Model MS-230, Sugawara, Tokyo). Two experimental protocols were used as follows. First, cells were suspended in isotonic dextran solutions to make a Ht of 0.4% to observe cell deformation in a diluted suspension. The shear rates were changed stepwise in the range of 75.2 to 752 s⁻¹. The dextran concentration was varied in the range of 7.5% (4.0 cP) to 20% (18.3 cP). Second, for observation of the cellular deformation at higher Hts, cells were suspended in either isotonic dextran solutions (6% or 10%) or plasma with varying Ht (4% to 55%), and a constant shear rate (752 s⁻¹) was applied.

The osmolarity of dextran solutions was adjusted with NaCl by using a Halbimikro osmometer (Type M, Knauer, FRG) after dissolving an appropriate amount of dextran in a hypotonic HEPES buffer (50 mmol/L of HEPES, 5 mmol/L of KCl, 6 mmol/L of glucose). The degree of deformation of stably oriented and deformed cells was expressed by the DI, defined by DI = (L - B)/(L + B), where L and B are the lengths of long and short axes of ellipsoidally deformed cells. For each sample, DIs of 60 to 80 cells were measured.

Measurement of Viscosity

To measure the viscosity of hemolysate and cell suspension, a cone-plate viscometer (Model E, Tokyo Keiki) was used at 25 °C. For measurement of intracellular viscosity, the hemolysates of both fractionated intact cells and PH cells were prepared. The packed cells were suspended in 3 vol of hypotonic HEPES buffer (30 mOsmlkg, 5 mmol/L of HEPES, 10 mmol/L of NaCl; pH 7.4). After membrane components were removed by centrifugation (10,000 g for 20 minutes), the hemolysate was concentrated by ultrafiltration (using a collodion bag; SM13200, Sartorius-Membranefilter GmbH, Göttingen, FRG) until the hemoglobin concentration of the hemolysate reached that of the intracellular hemoglobin concentration of original cells. The viscosity of hemolysate of intact cells was expressed by the DI, defined by DI = (L - B)/(L + B), where L and B are the lengths of long and short axes of ellipsoidally deformed cells. For each sample, DIs of 60 to 80 cells were measured.

RESULTS

Characterization of PH Cells

To facilitate the optical transparency of cell suspension and to avoid the decrease of internal viscosity, PH cells were prepared in the hypotonic dextran solution. Intact cells...
resisted hypotonic hemolysis in the presence of dextran. Thus, to enhance hemolysis, cells were pretreated with LPC. By suspending LPC-treated cells in the hypotonic solution of different concentrations of dextran, the internal viscosity of PH cells could be varied. After rescaling, LPC in the cell membrane was removed by washing with BSA solution and the cell shape could be easily reverted to biconcave disc. Although progressive loss of membrane materials caused by increasing LPC concentration has been reported, at a low concentration such as was used in the present study, no loss of cholesterol and phospholipids of either LPC-treated cells or PH cells was detected.

In this study, two kinds of PH cells, heavy and light, were prepared. The typical data about the properties of these cells are summarized in Table I, together with data about density-fractionated intact cells for comparison. PH cells deformed to the flat ellipsoid with unequal axial lengths in shear flow as well as intact cells did, as reported by Suda and co-workers. Thus, the degrees of deformation are expressed by DI in Table 1.

Deformation of Cells in Diluted Suspension

To investigate the influence of viscosity of suspending medium and shear rate on the degree of deformation, the DIs of cells suspended in the isotonic solution of various concentrations of dextran (hematocrit, 0.4%) were measured at various shear rates. Fischer and colleagues have shown that the DI of intact cells is a function of γ₁⁵⁺, where γ and η are shear rate and medium viscosity, respectively. In the present study, taking the intracellular viscosity into account, the variable γ₁⁵⁺ is modified to γηα/(ηm/ηa), where ηa is the viscosity of the suspending medium, ηm is the intracellular viscosity, and α is the adjustable parameter.

PH cells. The DIs of light PH cells, at various shear rates and viscosities of suspending medium, are plotted against DI in Fig 1A. At certain DIs, the plots of log γηα/(ηm/ηa) shown in Fig 1B give parallel straight lines with the same slope (−0.6), where interpolated γ values were read at the crossing points on lines a through d of Fig 1. Thus, DI is expressed as a function of γηα/(ηm/ηa)⁰.⁶. In Fig 2A, all DIs of light PH cells and heavy PH cells are plotted against γηα/(ηm/ηa)⁰.⁶, and all points lie on the same curve in spite of the difference in ηa between light and heavy PH cells.

Table 1. Hematological Indices and Deformability of PH Cells and Fractionated Intact Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>MCV (μm²)</th>
<th>[hb]L⁺ (mmol/L Cell)</th>
<th>γ = 150 s⁻¹</th>
<th>γ = 376 s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>96.0</td>
<td>2.58</td>
<td>3.5 (2.8)</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>Heavy</td>
<td>75.2</td>
<td>3.73</td>
<td>9.8 (5.0)</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low density</td>
<td>89.0</td>
<td>5.05</td>
<td>10.3</td>
<td>0.31 ± 0.04</td>
</tr>
<tr>
<td>High density</td>
<td>81.5</td>
<td>6.20</td>
<td>20.4</td>
<td>0.22 ± 0.05</td>
</tr>
</tbody>
</table>

PH, partially hemolyzed; MCV, mean corpuscular volume.

*Intracellular hemoglobin concentration.
†Intracellular viscosity. The value in parentheses is the viscosity of hemolysate (prepared from intact cells) having the same hemoglobin concentration as that of PH cells.
‡Deformation index (DI) was measured at shear rates (γ) of 150 and 376 s⁻¹ when cells were suspended in 20% dextran solution (18.3 cP).
§Intact cells were fractionated by density gradient (described in Materials and Methods section).
Solution at Various Hts

Fractionated high-density cells (\(\alpha\)) were suspended in solutions of 10% (4.0 cP) to 20% (18.3 cP), and heavy PH cells (\(\beta\)) were suspended in solutions of 11% (6.1 cP) to 20%. DI was measured at shear rates of 75.2, 150, 376, 752 s\(^{-1}\). Data of light PH cells are the same as in Fig 1A. DI is represented by a mean of 60 to 80 cells.

Deformation of PH Cells Suspended in 6% Dextran

Deformation of PH cells is shown in Fig 4 (line B). The ellipsoidal deformation of PH cells in relatively low Ht range (4% to 25%) is shown in Fig 2A. DI is represented by a mean of 60 to 80 cells. With appropriate \(\alpha\) values for cells treated with 0. 0.55, 0.65, and 0.95 mmol/L of diamide are 0.8, 0.5, 0.35, and 0, respectively. DI is represented by a mean of 60 to 80 cells. (B) Both parameters \(\alpha\) and \(\beta\) are altered. Data and symbols are the same as in Fig 3A; \(\beta\) values for cells treated with 0, 0.55, 0.65, and 0.95 mmol/L of diamide are 1, 0.7, 0.5, and 0.4, respectively (\(\alpha\) values are the same as in Fig 3A). The dotted line is the regression curve of DI vs. \(\gamma_{\text{ex}}(\eta_{\text{ex}}/\eta_{\text{in}})^{0.6}\). Measurements were impossible due to cell crowding. At a Ht of 45%, however, cells were photographically observed to deform to flat ellipsoid but variously distorted shapes and orient with their long axes parallel to the flow direction (Fig 4, line B).

Heavy PH cells. The ellipsoidal deformation could not be observed for heavy PH cells. Presumably, the value of \(\gamma_{\text{in}}(\eta_{\text{in}}/\eta_{\text{ex}})^{0.6}\) was not high enough to induce such deformation (eg, \(\gamma_{\text{in}}(\eta_{\text{in}}/\eta_{\text{ex}})^{0.6} = 19\) dynes/cm\(^2\) at a Ht of 20%) due to the higher intracellular viscosity of heavy PH cells as compared with that of light PH cells.

**Fig 2.** (A) Plot of deformation index (DI) vs. \(\gamma_{\text{ex}}(\eta_{\text{ex}}/\eta_{\text{in}})^{0.6}\) of partially hemolyzed (PH) cells. Light PH cells (\(\alpha\)) were suspended in isotonic dextran solutions of 7.5% (4.0 cP) to 20% (18.3 cP), and heavy PH cells (\(\beta\)) were suspended in solutions of 11% (6.1 cP) to 20%. DI was measured at shear rates of 75.2, 150, 376, 752 s\(^{-1}\). Data of light PH cells are the same as in Fig 4 (line B). The ellipsoidal deformation of PH cells in relatively low Ht range (4% to 25%) is shown in Fig 2A. DI is represented by a mean of 60 to 80 cells. (B) Both \(\alpha\) and \(\beta\) are altered. Data and symbols are the same as in Fig 3A; \(\beta\) values for cells treated with 0, 0.55, 0.65, and 0.95 mmol/L of diamide are 1, 0.7, 0.5, and 0.4, respectively (\(\alpha\) values are the same as in Fig 3A). The dotted line is the regression curve of DI vs. \(\gamma_{\text{ex}}(\eta_{\text{ex}}/\eta_{\text{in}})^{0.6}\). Measurements were impossible due to cell crowding. At a Ht of 45%, however, cells were photographically observed to deform to flat ellipsoid but variously distorted shapes and orient with their long axes parallel to the flow direction (Fig 4, line B).

**Fig 3.** Plot of deformation index (DI) vs. \(\gamma_{\text{in}}(\eta_{\text{in}}/\eta_{\text{ex}})^{0.6}\) for diamide-treated cells. (A) The parameter \(\alpha\) is varied whereas \(\beta\) is fixed at unity. Fractionated low-density cells were treated with diamide. Diamide concentration: 0 mmol/L (\(\alpha\)), 0.55 mmol/L (\(\beta\)), 0.65 mmol/L (\(\gamma\)), 0.95 mmol/L (\(\delta\)). Cells were suspended in isotonic dextran solutions of 15.5% (11.0 cP) to 20% (18.3 cP), and DI was measured at shear rates of 75.2, 150, 376, 752 s\(^{-1}\); \(\alpha\) values for cells treated with 0, 0.55, 0.65, and 0.95 mmol/L of diamide are 0.6, 0.5, 0.35, and 0, respectively. DI is represented by a mean of 60 to 80 cells. (B) Both parameters \(\alpha\) and \(\beta\) are altered. Data and symbols are the same as in Fig 3A; \(\beta\) values for cells treated with 0, 0.55, 0.65, and 0.95 mmol/L of diamide are 1, 0.7, 0.5, and 0.4, respectively (\(\alpha\) values are the same as in Fig 3A). The dotted line is the regression curve of DI vs. \(\gamma_{\text{in}}(\eta_{\text{in}}/\eta_{\text{ex}})^{0.6}\) of intact cells shown in Fig 2B. The degrees of cross-linking of spectrin of cells treated with different concentrations of diamide are shown in Table 2.

This fact implies that PH cells are less deformable than intact cells; presumably, the PH cell membrane would be impaired during preparation of PH cells.

**Diamide-treated cells.** Diamide has been shown to induce cross-linking of membrane proteins to decrease cellular deformability.\(^{15,23}\) When a high concentration of diamide is applied, a buckling pattern (furrows parallel to the flow direction) appears\(^{24}\) and, in addition, hemoglobin is modified.\(^{15}\) Therefore, to study the influence of cross-linking of membrane proteins (mainly spectrin) on deformation, the concentration of diamide must be lowered to <1 mmol/L (no buckling pattern was detected).

DIs of diamide-treated cells were measured in the dextran solution from 15.5% (11.0 cP) to 20% (18.3 cP). The parameter \(\alpha\) for diamide-treated cells was dependent on the degree of cross-linking, as estimated from the plot of log \(\gamma_{\text{ex}}(\eta_{\text{ex}}/\eta_{\text{in}})\) and log \(\eta_{\text{ex}}(\eta_{\text{in}}/\eta_{\text{in}})\) (not shown). In Fig 3A, DIs are plotted against \(\beta\) \(\gamma_{\text{in}}(\eta_{\text{in}}/\eta_{\text{ex}})^{0.6}\) with appropriate \(\alpha\) values for cells treated with diamide of various concentrations whereas \(\beta\) is fixed at unity. Furthermore, adopting an appropriate value of \(\beta\) for each curve shown in Table 2, all points can be brought on the same curve (Fig 3B), which curve agrees with the curve for intact cells (Fig 2B), ie, both \(\alpha\) and \(\beta\) decrease as the cross-linking proceeds.

**Table 2.** \(\alpha\) and \(\beta\) Values of Diamide-Treated Cells

<table>
<thead>
<tr>
<th>Diamide* (mmol/L)</th>
<th>Cross-linking† (%)</th>
<th>(\alpha)</th>
<th>(\beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>0.55</td>
<td>27.4</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>0.65</td>
<td>33.3</td>
<td>0.35</td>
<td>0.5</td>
</tr>
<tr>
<td>0.95</td>
<td>46.7</td>
<td>0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Diamide concentration for diamide treatment of intact cells.
† Percentage of spectrin polymerization (described in Materials and Methods section).
Deformation of PH Cells Suspended in 10% Dextran Solution at Various Hts

Light PH cells. In 10% dextran solution, light PH cells demonstrate advanced ellipsoidal deformation as compared with those in 6% dextran solution at lower Hts (<25%) as shown in Fig 4 (line A). When DIs are plotted against $\gamma \eta_p/\eta_0$ on a $\gamma^{0.6}$ graph, however, DI decreases from 0.28 to 0.23 despite the increase in $\gamma \eta_p/\eta_0$ from 60 dynes/cm$^2$ (at Ht = 4%, $\eta_p = 5.6$ cP) to 80 dynes/cm$^2$ (at Ht = 20%, $\eta_p = 7.1$ cP) as shown in Fig 5. In Fig 4 (line A), a photograph at Ht = 45% is also shown, in which distorted ellipsoidal deformation is observed.

Heavy PH cells. Although the intracellular viscosity was high, the ellipsoidal deformation was induced due to high viscosity of the suspending medium. In the Ht range of 4% to 20%, as shown in Fig 5, DIs lie on the DI $\gamma^{0.6}$ curve of light PH cells shown in Fig 2A. The suspension viscosity changes from 5.9 cP (Ht = 4%) to 7.6 cP (Ht = 20%).

Deformation of PH Cells Suspended in Plasma at Various Hts

Light PH cells. Cells deform to variously distorted discs at the lower Ht (<25%); however, distorted ellipsoidal deformation is conspicuous at Ht = 45%, as shown in Fig 4 (line C), at which cells deform to the same shape as that of light PH cells in 6% and 10% dextran solutions.

Heavy PH cells. Although the extracellular viscosity was low (1.8 cP), cells deformed but distorted in various shapes at the higher Ht range (35%, 45%, and 55%).

Deformation of Intact Cells in the Coexistence of Undeformable Cells

To investigate the influence of undeformable cells on the behavior of deformable cells in shear flow, a small amount of fractionated low-density intact cells (tracer cells) was mixed with either a larger amount of light PH cells (deformable cells) or GA-treated PH cells (light PH cells treated with GA, undeformable cells), and deformation of tracer cells was observed. GA-treated PH cells could not deform, and they oriented randomly in shear flow. Cells were suspended in...
16.7% dextran solution (12.7 cP) in which the Ht of tracer cells was kept constant (0.4%), and the Ht of light PH cell and GA-treated PH cell suspensions varied from 2.1% to 6.3%. On the flash-photographs, the intact tracer cells could be distinguished from the light PH or GA-treated PH cells by differences of cell shape and of darkness (due to higher hemoglobin concentration). The ellipsoidal deformation of intact cells was not affected in the deformable light PH cell suspension (Fig 6B,C,D), nor in the diluted GA-treated PH cell suspension (Ht = 2.1%) (Fig 6E). When the amount of the GA-treated PH cells was increased, however (to Ht = 6.3%), the ellipsoidal deformation was remarkably disturbed (Fig 6F,G).

**DISCUSSION**

**Contribution of Intracellular Viscosity and Membrane Stiffness to Cellular Deformation**

Increased viscosity of suspending medium is well known to enhance the deformation of RBCs, although the degree of deformation is influenced by intracellular viscosity. Pfafferott and co-workers showed that the differences between young and old cell deformation were eliminated if the DI was plotted as a function of the ratio of intracellular/extracellular viscosities for various shear stresses.

In the present study, we demonstrated the effect of extracellular/intracellular deformations and membrane stiffness on the cellular deformation and revealed that DI is a function of $\beta \gamma \eta_a(\eta_a/\eta_m)^n$. For various kinds of cells, $\alpha = 0.6$ is unanimously applicable (Fig 2A,B). Taking $\beta = 1$ for the intact cells, $\beta$ should be reduced to 0.7 for PH cells to fit the data of DI of PH cells on the same curve, DI $= \gamma \eta_a(\eta_a/\eta_m)^n$, of intact cells, indicating that PH cells are less deformed than intact cells. A remarkable difference of cell volume between light and heavy PH cells is detected (Table 1), whereas the surface area of these cells remains constant (because no loss of membrane lipids is detected). This may affect the cellular deformation through the change of surface/volume (S/V) ratio which is one of the determinants of deformability. No difference, however, in the relation DI $= \gamma \eta_a(\eta_a/\eta_m)^n$ between light and heavy PH cells (Fig 2A) suggests that such change of S/V ratio does not shift the curves DI $= \gamma \eta_a(\eta_a/\eta_m)^n$ throughout. Therefore, the decrease in $\beta$ of PH cells is presumably due to the increased membrane stiffness caused by changes in membrane integrity.

Although the intracellular viscosity of light PH cells is about half that of fractionated low-density cells, DI of light PH cells are close to that of fractionated low-density cells at given shear stresses (Table 1), because the effect of lowered intracellular viscosity of PH cells on DI is canceled by the increased membrane stiffness.

Age-related changes in membrane viscoelastic properties were reported. In this study, no difference in the DI $= \gamma \eta_a(\eta_a/\eta_m)^n$ curve between low-density cells and high-density cells was detected (Fig 2B). This result may imply that the age-dependent changes in membrane properties have no significant effect on the ellipsoidal deformation in shear flow.

The influence of cross-linking of membrane proteins is remarkable. When the intact cells (of low density) are treated with diamide, two parameters, $\alpha$ and $\beta$, must be reduced (Table 2) to bring all data to the same curve in the plot of DI $= \gamma \eta_a(\eta_a/\eta_m)^n$ (as shown in Fig 3).

The mechanical property of the membrane is physically expressed by the three intrinsic material constants of membrane, ie, elastic modulus, area compressibility, and membrane viscosity. The alteration of area compressibility would not be detectable in the present method, because discocytes deform easily at constant area but strongly resist change in area. Nash and Meiselman demonstrated that the ghost has a greater shear modulus but a similar membrane viscosity. Their result may correspond to the present result, ie, the partial hemolysis causes the change in $\beta$ but not in $\alpha$. The parameter $\beta$ may be related to the shear modulus. According to dimensional analysis, $\beta$ should have a dimension of (dynes/cm²)$^{-1}$. Therefore, a reciprocal of $\beta$ would express the relative shear modulus, and the shear modulus of PH cells may be $\sim 1.4$ times higher than that of intact cells. The diamide treatment is also known to increase the shear modulus and may cause increased membrane...
viscosity by restricting the flexibility of cytoskeleton. This may lead to the decrease of two parameters, $a$ and $b$.

**Ht Dependency of Cellular Deformation**

The shear rate applied in this study ($\dot{\gamma} = 752 \text{ s}^{-1}$) corresponds to the calculated wall shear rates in artery and capillary ($\sim 1,000 \text{ s}^{-1}$), assuming that Poiseuille flow and the behavior of PH cells in flow is similar to that of intact cells. Therefore, the present study gives information on RBC behavior in blood flow.

The quantitative measurement of PH cell deformation is possible up to the Ht value of ~20%. When the light and heavy PH cells are suspended in 6% and 10% dextran solutions, respectively, progressive deformation due to increase of suspension viscosity is observed. The increase in suspension viscosity owing to increasing Ht or dextran concentration induces the same degree of ellipsoidal deformation (Fig 5). However, the value of $\gamma_{\eta_0} (\eta_0/\eta_0)^{1/2}$ is below ~20 dynes/cm², both light and heavy PH cells suspended in any medium cannot deform, because at low value of $\gamma_{\eta_0} (\eta_0/\eta_0)^{1/2}$, the shear force is not enough to induce the deformation. On the other hand, DI of light PH cells suspended in 10% dextran at $\gamma_{\eta_0} (\eta_0/\eta_0)^{1/2} = 60$ dynes/cm² is smaller than that at $\gamma_{\eta_0} (\eta_0/\eta_0)^{1/2} = 60$ dynes/cm² as shown in Fig 5. In this decline phase, the ellipsoidal deformation is rather suppressed, probably because the cell crowding disturbs the uniform stream lines. Concerning the effect of Ht, Kon and Kon have shown that the degree of deformation and orientation is suppressed as the Ht is increased above a certain value depending on extracellular viscosity; ie, the critical Ht value, at which the suppressing effect begins, shifts toward a lower Ht as the extracellular medium increases in viscosity. At higher Hts (~25%), quantitative measurement of DI is not possible, even with PH cells. The degree of deformation of PH cells in plasma is qualitatively the same as that in 6% and 10% dextran solutions at a Ht of 45%, however. The ellipsoidal deformation of intact cells at a higher Ht is shown by Fischer and Schmid-Schönbein. The present result is explained as follows: The cellular deformation of PH cells in plasma is progressively induced with increasing Ht, but in 6% and 10% dextran solutions the suppressive mechanism becomes operative on cells at the higher Ht.

The influence of cell concentration was described by Goldsmith and Marlow; ie, the progressive deformation of tracer amount of intact cells in the ghost cell suspension (intact cells did not deform to ellipsoid but to irregularly distorted ellipsoid) with increasing ghost concentration; thus, they have proposed that the collision between cells induces such irregular deformation. In the present study, the fractionated low-density cells deformed to ellipsoid in light PH cell suspension but not in GA-treated PH cell suspension. This phenomenon may be due to the disturbance of laminar flow caused by tumbling of the hardened cells, which consequently reduces the effective shear stress acting on intact cells. Tumbling of rigid discs in Poiseuille flow is known, and a similar disturbing effect of hardened cells on intact cell deformation was reported by Kon and coworkers. Therefore, the ellipsoidal deformation of intact cells is maintained by ellipsoidal deformation of surrounding cells.

In short, the present study reveals that: (a) The degree of deformation of cells is determined by the applied shear stress ($\gamma_{\eta_0}$), the viscoelastic membrane properties (reflected on the parameters $a$ and $b$), and the ratio of suspending medium viscosity to internal viscosity ($\eta_0/\eta_0$); and (b) increasing suspension viscosity ($\eta_0$) by raising Ht generally enhances cellular deformation, but above a certain Ht deformation is suppressed. At physiological Ht, cellular deformation is mainly influenced by Ht, and the contribution of viscosity of extracellular medium is not significant.

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