Increased adhesive forces between sickle erythrocytes and endothelial cells (EC) have been hypothesized to play a role in the initiation of vasoocclusion in sickle cell anemia. Erythrocyte/human umbilical vein EC interactions were studied under controlled flow conditions for normal (AA), homozygous sickle cell (SS), sickle cell trait (AS), mechanically injured normal, and "high-reticulocyte control" RBC by using video microscopy and digital image processing. The number of adherent RBC was determined at ten-minute intervals during a washout period. Results indicate that SS RBC were more adherent than AA RBC. Mechanically injured (sheared) AA RBC were also more adherent than control normal cells but less adherent than SS RBC. AS RBC did not differ significantly in their adhesive properties from normal RBC. Less-dense RBC were more adherent to EC than dense cells for normal, SS, and high-reticulocyte control RBC. The number of cells adherent at a given time during washout was a very strong function of wall shear rate. In addition, at all shear rates studied, the average velocity of individual SS RBC in the region near the EC surface was approximately half that of AA RBC at the same bulk volumetric flow rate through the flow chamber. These findings suggest that the increased adhesion of sickle RBC is at least partially related to the increased numbers of less-dense RBC present. Increased adherence of the less-dense cells to the EC lining vessel walls could contribute to microvascular occlusion by lengthening vascular transit times of other sickle cells.

RBC-ENDOTHELIAL CELL (EC) interactions may be important in the vasoocclusive phenomena observed in sickle cell disease. Evidence has been presented by several investigators that suggests that sickle cells adhere abnormally to EC under static conditions as well as under uncontrolled conditions of flow. The abnormal adherence of sickle cells has been correlated with the vasoocclusive severity of the disease. Interactions between blood cells and endothelial surfaces in blood vessels occur under dynamic conditions. Flow inhibition or promotion of blood cell adhesion to the endothelium may occur depending on the velocity and morphology of the moving cells. Presumably, cells in the vicinity of the endothelium that are moving slowly would have a greater chance of making contact and interacting with the endothelial surface.

Altered flow characteristics of sickle blood under both oxygenated and deoxygenated conditions have been reported in microvascular studies using intravital microscopy techniques. LaCelle reported that adherence of sickle cells to endothelium occurred at low flow rates. Klug and Lessin reported that sickle RBC adherence to the vessel wall did not occur at moderate and high flow rates. The mean transit time of sickle RBC was higher than that for normal RBC during their passage through the coronary circulation. In a rat mesococum microvascular preparation, it appeared that the cells actually trapped were mainly the dense sickle RBC.

The shear rate (flow rate) is an important dynamic variable present in vivo that will affect the propensity of erythrocytes to adhere to the vessel wall. In the studies described later, we consider the case of laminar flow between two parallel surfaces as a simplified model of a blood vessel. In this case, movement of fluid in the direction of the applied pressure gradient results in the development of a velocity gradient within the fluid. The velocity gradient, or shear rate, is defined as the difference in velocity between two adjacent layers of moving fluid divided by the distance between them and has the units (cm/s)/cm, or "inverse seconds" (s^{-1}). Time average wall shear rates vary throughout the circulation and range from 0 to 5,000 s^{-1}. The highest values occur in the arterioles and microcirculatory vessels and the lowest values in the small venules and veins. There is a rapid drop in the average vessel wall shear rate as a cell exits the capillaries and enters the venules. Using a jet shearing technique, Smith and LaCelle have recently shown that the number of sickle cells adherent to cultured endothelium decreased as the wall shear rate produced by the fluid flow was increased after a 30-minute static incubation.

In our studies, the effect of wall shear rate on the adhesion of RBC to EC was examined under controlled flow conditions. Velocity gradients utilized in these experiments approximate those found in venules, which are probably important sites for adhesive events. Leukocyte margination in vivo is usually seen to occur in this part of the circulation. Fluid drag forces on attached cells (related to the wall shear rate) are quite low in these vessels. Videotaped experiments were analyzed by using digital image analysis techniques to determine the velocities of individual sickle and normal RBC in the vicinity of a cultured human endothelial cell monolayer.

MATERIALS AND METHODS

Blood was drawn into heparinized tubes from patients with homozygous sickle cell disease (SS), volunteers with sickle cell trait (AS), other patients with high reticulocyte counts ("high-reticulocyte controls"), and normal subjects (AA). After centrifugation at 1,000 g for ten minutes, the plasma and Buffy coat were removed. The RBC were washed three times with medium 199 supplemented with 20% fetal calf serum (FCS, Hyclone Tissue Culture Products, Logan, UT), 100 U/mL penicillin, and 100 µg/mL streptomycin and then suspended at the desired hematocrit in the supplemented medium. For experiments involving density-fractionated RBC, cells...
in plasma were separated into density fractions by centrifugation at 30,000 × g for one hour at 30°C in an angle rotor. The column of RBC was separated into five equal fractions. Sheared RBC were obtained by subjecting suspensions of cells to a subhemolytic shear stress of 1,500 dynes/cm² for two minutes in a Rice University concentric cylinder viscometer described previously. 14,16

Endothelial cell cultures. Human umbilical vein EC were harvested from umbilical cords by using culture procedures adapted from those of Gimbrone et al 8 and grown to confluence on glass slides. To remove the EC, the veins were cannulated, rinsed with 100 mL phosphate-buffered saline (PBS) and then filled with 0.03% collagenase in PBS and incubated for 30 minutes at room temperature. After incubation, the enzyme solution was flushed through the cord with 100 mL PBS, and the effluent was collected and centrifuged at 100,000 × g for ten minutes. The cell pellet was resuspended in medium 199, supplemented with 20% FCS and antibiotics, and seeded onto 75 × 38-mm glass slides (Fisher, Medford, MA) that had been pretreated with 0.5 mol/L NaOH for two to three hours and rinsed. Cultures were incubated at 37°C and became confluent after three to four days. Experiments were conducted within four days after the cultures reached confluence. Identification of the monolayer as EC was obtained by positive immunofluorescent staining for factor VIII-related antigen and by morphological assessment. Viability was assessed by trypan blue exclusion.

RBC-EC adherence assay. EC monolayers on glass slides formed the base of a modified Richardson flow chamber that was mounted in a video microscopy system on the microscope stage (Fig 1). The chamber depth was controlled by the thickness of the gasket, and for the experiments presented here, was machined to either 170 μm or 104 μm. RBC suspensions at a 1% hematocrit were perfused into the chamber for five to ten minutes at a constant flow rate. After incubation, the enzyme solution was flushed through the cord with 100 mL PBS, and the effluent was collected and centrifuged at 100,000 × g for ten minutes. The cell pellet was resuspended in medium, supplemented with 20% FCS and antibiotics, and seeded onto 75 × 38-mm glass slides (Fisher, Medford, MA) that had been pretreated with 0.5 mol/L NaOH for two to three hours and rinsed. Cultures were incubated at 37°C and became confluent after three to four days. Experiments were conducted within four days after the cultures reached confluence. Identification of the monolayer as EC was obtained by positive immunofluorescent staining for factor VIII-related antigen and by morphological assessment. Viability was assessed by trypan blue exclusion.

RBC suspensions and culture medium enter and exit the chamber through two slits machined in the polycarbonate plate. Flow is controlled by using the Harvard syringe pump. The red cell suspensions were maintained in a 37°C water bath, and the chamber was maintained at 37°C by an air curtain incubator. The number of adherent RBC was determined at 1, 10, and 20 minutes of rinse for the same single microscope field. After 20 minutes of rinse, multiple microscope fields were examined. The initial count was made after static incubation and a one-minute period of rinsing to remove nonadherent settled cells. In the continuous-flow experiments where the incubation period was eliminated, the rinse immediately followed the RBC perfusion, and the number of adherent RBC was determined at ten and 20 minutes for the same microscope field. A video camera (RCA TC 1005; Lancaster, PA) mounted to a Nikon Diaphot microscope was used to record and display experiments. Experiments were recorded on video tape (Gyrr TLC 2001; Anaheim, CA) for later playback and analysis and displayed on a high-resolution TV monitor.

The wall shear stress in the flow chamber was calculated by using the momentum balance for a newtonian fluid and assuming a parallel-plate geometry and fully developed flow. In easily measurable experimental quantities, it is given by the formula τ = 3μQ/2ab dynes/cm² (wall shear stress), where Q is the flow rate, cm³/s; μ is the fluid viscosity, dynes · s/cm²; 2a is the gap width (chamber depth), cm; and b is the slit width, cm.

The wall shear rate (γw) is given by γw = Q/2ab(s⁻¹).

Velocity determination using image analysis. Taped experiments were analyzed by using a digital image processor (Model 327; Perceptive Systems Inc, Houston) for image quantification. To determine the velocity of individual cells, sequential images separated by a given delay time were digitized from tapes. The position of a particular red cell was noted in each digitized frame, and the velocity was determined from the number of pixels (picture elements into which a digitized frame is divided) a cell had moved between frames for a given delay time. All velocities were converted to micrometers per second by using a calibration on the microcomputer. Velocity measurements were made during the cell perfusion before the rinse cycle was initiated. At least 200 cells were tracked at each shear rate and results presented as a velocity histogram. For a given video frame, all nonadherent cells in focus near the EC surface were automatically tracked and entered into the histogram.

Statistical methods. The data of multiple microscope fields after 20 minutes of rinse were analyzed by using an f test that distinguishes between the treatment variance (different blood samples, eg. HbAA, HbSS, HbAA [high-reticulocyte], or HbAS sheared) and the variances due to the random or uncontrolled variables (different donors, different EC monolayers, field-to-field variances within a monolayer, etc). For comparisons of several single field measurements at different times (1, 10, and 20 minutes of washout) the Student’s t test was used.

RESULTS

When examined under flow conditions after a static ten-minute incubation period, sickle RBC were more adherent to EC than normal RBC (Table 1). A larger fraction of settled RBC remained adherent for SS RBC (1%) than for AA RBC (0.16%) after one minute of rinsing. These percentages are the ratio of adherent cells after one minute of rinse (Table 1), divided by the cell density of the initial monolayer of RBC in contact with the endothelium after static incubation, and multiplied by 100. RBC from individuals with AS did not differ significantly from normal RBC in their adhesion to EC. Mechanically traumatized (sheared) RBC, both normal and sickle trait, were also more adherent than normal RBC, but not as adherent as sickle RBC. Results from
experiments performed using RBC from a splenectomized patient with HbAA indicated that these RBC behaved like normal cells with respect to adhesion to EC (data not shown).

Experiments on density-separated sickle, normal, and high-reticulocyte control RBC revealed that the least-dense fraction (fraction with the highest percentage of reticulocytes) was more adherent than the denser fractions and the unfractionated sample (Table 2). The high-reticulocyte control RBC were more adherent than normal, which suggested that the degree of adhesion may be partially related to the percentage of young cells in the cell population.

The same differences in adhesion between the RBC populations were noted when the ten-minute incubation period was eliminated so that the entire experiment was conducted under flow conditions (Table 3). Under conditions of continuous flow a smaller chamber gap width was utilized, thereby reducing the total flow area and increasing the potential for RBC-EC interactions. In the data presented in Table 3, the flow rate was decreased so that the smaller gap width maintained the same wall shear stress of 1 dyne/cm² (wall shear rate of 100 s⁻¹).

To determine the dependence of sickle and normal RBC adherence to EC on the wall shear rate, experiments were performed at shear rates ranging from 25 s⁻¹ to 500 s⁻¹. The RBC adherence after the 20-minute rinse period as a function of wall shear rate is plotted in Fig 2. For both sickle and normal RBC, adhesion decreases as shear rate increases. Little or no adhesion is observed after 20 minutes of washout at shear rates exceeding 170 s⁻¹ for normal RBC and at shear rates exceeding 260 s⁻¹ for sickle RBC. The difference between normal and sickle cell behavior with respect to adhesion appears to be more pronounced at the lower shear rates.

Typical velocity distributions of sickle and normal RBC obtained from video tapes at two specific wall shear rates are plotted in Figs 3 and 4 in terms of the percentage of total nonadherent RBC in the frame traveling in a particular velocity range. At each shear rate (flow rate), it is apparent that a higher proportion of sickle RBC are traveling at slower velocities—whereas a higher proportion of normal RBC are traveling at the higher velocities. Sickle cells move more slowly across the endothelial surface than normal cells. The average velocity of all of the near-wall nonadherent cells in a given frame was usually at least twice as high for normal RBC as it was for sickle RBC at the same bulk volumetric flow rate.

DISCUSSION

Shear rate plays an important role in determining the rheological interactions of sickle cells in the circulation, particularly at the small vessel level (arteriole, capillary, venule). In cell filtration studies, the relative resistance to flow offered by both oxygenated and deoxygenated SS cells is much lower when the flow velocity is high. At low shear

Table 1. Adhesion of Normal, Sickle, Sickle Trait, and Sheared RBC to EC at a Wall Shear Stress of 1 dyne/cm²

<table>
<thead>
<tr>
<th>RBC</th>
<th>No. of Donors</th>
<th>1 min</th>
<th>20 min</th>
<th>Multiple Fields at 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (n = 19)</td>
<td>8</td>
<td>24.78 ± 16.46</td>
<td>8.11 ± 5.28</td>
<td>6.62 ± 6.10 (± 284)</td>
</tr>
<tr>
<td>SS (n = 13)</td>
<td>4</td>
<td>165.70 ± 142.62</td>
<td>52.56 ± 44.08</td>
<td>39.32 ± 29.72 (± 211)</td>
</tr>
<tr>
<td>AS (n = 2)</td>
<td>1</td>
<td>10.41</td>
<td>8.33</td>
<td>9.25 ± 8.25 (± 32)</td>
</tr>
<tr>
<td>AA sheared (n = 10)</td>
<td>6</td>
<td>40.83 ± 11.42</td>
<td>17.08 ± 8.66</td>
<td>22.25 ± 13.46 (± 165)</td>
</tr>
<tr>
<td>AS sheared (n = 2)</td>
<td>1</td>
<td>29.16</td>
<td>16.66</td>
<td>17.16 ± 9.04 (± 32)</td>
</tr>
</tbody>
</table>

NOTE: In these experiments, there was a ten-minute incubation period before the rinse was begun. The flow chamber gap width was 170 μm. The data at the one- and 20-minute rinses represent the means of single fields counted in n experiments. The data in the far right column reflect the sum of multiple fields counted in each experiment after a 20-minute rinse (± total fields in all n experiments). The differences between the AA controls and the SS, AA sheared, and AS sheared RBC had a level of significance of P < .01 (t test for multiple fields at 20 minutes and Student’s t test for single fields at 20 minutes) after 20 minutes of rinse. There was no significant difference between the adhesion of AA and AS RBC.

Table 2. Effect of Cell Density on Normal and Sickle Cell Adherence to EC After a 20-Minute Rinse at a Wall Shear Stress of 1 dyne/cm²

<table>
<thead>
<tr>
<th>SS</th>
<th>ISC (%)</th>
<th>Reticulocytes (%)</th>
<th>Adherent RBC* (%)</th>
<th>Total RBC (%</th>
<th>Reticulocytes (%)</th>
<th>Adherent RBC* (%)</th>
<th>Total RBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>16.9</td>
<td>16.1</td>
<td>87.75 ± 17.88</td>
<td>8.4</td>
<td>38.54 ± 19.54</td>
<td>0.8</td>
<td>6.25 ± 3.42</td>
</tr>
<tr>
<td>Top</td>
<td>9.6</td>
<td>32.2</td>
<td>92.42 ± 31.58</td>
<td>15.0</td>
<td>48.96 ± 13.83</td>
<td>3.3</td>
<td>11.17 ± 6.54</td>
</tr>
<tr>
<td>Middle</td>
<td>22.6</td>
<td>6.7</td>
<td>52.63 ± 11.67</td>
<td>6.0</td>
<td>22.13 ± 6.92</td>
<td>0.6</td>
<td>5.71 ± 2.58</td>
</tr>
<tr>
<td>Bottom</td>
<td>28.6</td>
<td>8.7</td>
<td>51.29 ± 13.29</td>
<td>1.0</td>
<td>22.92 ± 7.88</td>
<td>0.6</td>
<td>8.83 ± 4.00</td>
</tr>
</tbody>
</table>

NOTE: In these experiments, there was a ten-minute incubation period before the rinse was begun. The flow chamber gap width was 104 μm. The data are from a representative experiment for each RBC type. AA (high-reticulocyte) RBC were obtained from an iron-deficient patient responding to treatment. The differences between normal AA and the SS and AA (high-reticulocyte) RBC had a level of significance of P < .0005 (using the t test) for whole blood samples. For fractionated samples, there was no significant difference between the adhesion of middle and bottom cells, but the differences between the top and middle and the top and bottom cells had a level of significance of P < .0005 for SS and AA (high-reticulocyte) RBC.

Abbreviation: ISC, irreversible sickled cells.

*Average number of adherent RBC/mm² ± SD per microscope field (16 fields counted after a 20-minute rinse).

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rates, significant problems may develop in the microcirculation due to obstructed flow caused by sickle cells becoming stiff and/or by SS cells interacting with EC. In a recent report, Rodgers et al.\textsuperscript{19} presented evidence that microcirculatory flow in patients with sickle cell disease is periodic. The increased adhesion of sickle cell RBC to microvascular or venule endothelium may be a rheological factor that contributes to local flow disturbance and induces oscillation. On the other hand, the high local shear stresses obtained in oscillatory flow could facilitate the passage of red cells through small vessels by helping them to deform and may dislodge sickle cells already adherent to the endothelium. Less-dense sickle RBC, which appear to be more adherent than heavier cells, may play a role in initiating vasooclusive by sufficiently retarding microvascular flow to extend the transit times of more-dense upstream cells beyond the delay time required for gel formation. This phenomenon may be particularly important in the venules where the chances for adhesive events are increased because of the low shear rates involved and where cell transit times may be approaching the pregelation times for heavier sickle cells. In this case, extending transit times even slightly could result in increased polymer content and sickling. Recent reports have shown that polymer fraction may be correlated with vasoocclusive severity,\textsuperscript{20} though not in all cases.\textsuperscript{21} Whether interactions between sickle erythrocytes and vascular EC are significant enough to play a role in the obstruction of flow would be influenced by the wall shear rate. Results from our studies suggest that adherence of sickle cells is enhanced at low shear rates but is not very different from that of normal RBC at high shear rates (where long-term adherence to endothelium for all RBC is very low).

Digital image analysis techniques were used to analyze videotaped flow experiments to gain insight into dynamic RBC-EC interactions by determining the velocities of individual sickle and normal RBC. The velocity of cells plays an important role in the promotion or inhibition of cell adhesion. Because of the inward radial migration and wall exclusion of RBC, which are important in flow channels whose height is many times that of the red cell, most of the RBC in a suspension will be flowing at relatively high speed in the core of the channel. Erythrocytes that are traveling in the cell-poor region near the wall are actually moving at different rates that, in some cases, may be slow enough to allow adhesive interactions to take place between the RBC and the endothelial monolayer at a wall shear rate of 126 s\textsuperscript{-1}. Cells moving at less than 1 \( \mu m/s \) were not included in the distribution because they were considered to be adherent. More than 200 individual cell velocities were obtained for both SS and AA samples. The average velocities were 60.2 \( \mu m/s \) for SS RBC (\textsuperscript{22}) and 121.7 \( \mu m/s \) for AA RBC (\textsuperscript{23}).

### Table 3. Adhesion of Normal, Sickle, and Sheared Red Cells to EC Under Conditions of Continuous Flow

<table>
<thead>
<tr>
<th>RBC</th>
<th>10 min (SD)</th>
<th>20 min (SD)</th>
<th>Multiple Fields (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS (n = 12)</td>
<td>14.77 ± 6.49</td>
<td>11.36 ± 8.16</td>
<td>11.48 ± 6.83 (s = 176)</td>
</tr>
<tr>
<td>AA (n = 12)</td>
<td>88.98 ± 22.56</td>
<td>59.09 ± 35.08</td>
<td>45.12 ± 26.76 (s = 164)</td>
</tr>
<tr>
<td>AA (sheared) (n = 6)</td>
<td>22.92 ± 9.85</td>
<td>20.14 ± 6.55</td>
<td>20.65 ± 7.80 (s = 95)</td>
</tr>
<tr>
<td>AA (high reticulocyte) (n = 5)</td>
<td>80.46 ± 32.64</td>
<td>49.81 ± 18.82</td>
<td>43.71 ± 23.59 (s = 76)</td>
</tr>
</tbody>
</table>

NOTE. In these experiments, there was no static incubation period before the rinse was begun. The flow chamber gap width was 104 \( mm \), and the wall shear stress was 1 dyne/cm\(^2\). The data at the ten- and 20-minute rinses represent the means of single fields counted in \( n \) experiments. The data in the far right column reflect the sum of multiple fields counted in each experiment after a 20-minute rinse (s, total fields in all \( n \) experiments). The differences between the AA control and the SS, AA sheared, and AA (high-reticulocyte) RBC all had a level of significance of \( P < .0005 \) (using the \( t \) test) for multiple fields at 20 minutes. For single fields at 20 minutes using the Student's \( t \) test, \( P < .0005 \) for AA v SS and AA v high-reticulocyte AA, whereas \( P < .025 \) for AA v sheared AA.

*AA (high-reticulocyte) RBC were obtained from two donors, a patient responding to treatment for iron deficiency and a patient with hereditary spherocytosis.

Fig 2. Effect of shear rate on adherence of sickle and normal RBC to EC after 20 minutes of washout at a fixed wall shear rate. The chamber used for these studies was the same as described in Table 3, and all experiments were done under continuous flow (no incubation). Data points are means with SD. (\( \Phi \)), SS; (\( \lambda \)), AA.

Fig 3. Velocity distribution for sickle and normal RBC near the EC monolayer at a wall shear rate of 126 s\textsuperscript{-1}. Cells moving at less than 1 \( \mu m/s \) were not included in the distribution because they were considered to be adherent. More than 200 individual cell velocities were obtained for both SS and AA samples. The average velocities were 60.2 \( \mu m/s \) for SS RBC (\textsuperscript{22}) and 121.7 \( \mu m/s \) for AA RBC (\textsuperscript{23}).
EC lining the vessel wall. From qualitative observations of videotaped experiments, sickle RBC traveling near the EC surface appeared to be moving more slowly than normal RBC near the EC surface for a given bulk volumetric flow rate. Quantitative velocity measurements presented earlier utilizing digital image processing confirmed these observations. Some red cells (particularly in the case of sickle RBC) appeared to be sliding slowly across the endothelial surface as if temporary contacts between the cell surfaces were being made but were not strong enough for the red cell to adhere. Differences in the adherence properties of sickle and normal RBC (sickle RBC may be more "sticky") could account for the slower velocities of sickle RBC near the EC monolayer.

The increased adhesion of less-dense normal and high-reticulocyte control RBC suggests that the increased adhesive properties of SS RBC may be, at least in part, a reflection of their young mean age. However, other factors may be involved. Mechanical damage of the red cell can also lead to increased RBC/EC adhesion as shown by our results with sheared normal RBC (Tables 1 and 3). Several investigators have shown that sickle RBC are much more susceptible to shear damage than normal cells and that hemolysis can occur at stress levels that are obtained physiologically. Sublytic mechanical damage to sickle cells in the circulation may also predispose them to increased EC adhesion.

Exposure of normal RBC to subhemolytic shear stresses is known to induce a deformability defect and lead to calcium accumulation without alterations in morphology. After sublytic shear stress exposure, both AA and AS RBC were more adherent to EC than normal RBC. Further study of these stressed cells may aid in determining the characteristics of the SS RBC that make them more adherent.

Plasma factors have been shown to influence sickle RBC adhesion. Results obtained in this study indicate that adhesion differences between erythrocytes can be demonstrated in the absence of specific human plasma factors peculiar to SS plasma because these experiments utilized washed red cells and a tissue culture suspending medium. Specific SS plasma factors may play a modulating role in the adhesion process but were not necessary to demonstrate altered adhesion in sickle RBC in our system.

Previous experimental evidence has suggested at least two phenomena that may predispose to vasoocclusion in sickle cell disease: (a) intracellular gel formation, which leads to shape change and decreased deformability of SS RBC, and (b) increased adhesion of SS RBC to EC. In this study, the adhesion process was examined under controlled fluid mechanical conditions to gain insight into events that may lead to vasoocclusion but do not directly involve the deformability of the SS RBC. Cells with maximum intracellular gel formation and shape change are the most-dense cells, whereas our studies indicate that the most-adhesive cells are the least-dense RBC. Although the least-dense cells are hemodynamically the most competent in deoxygenated blood, their tendency to adhere could sufficiently retard flow in the small vessels to extend transit times for other cells beyond the critical delay time required for gel formation. The delay time for HbSS gelation is strongly dependent on the intracellular hemoglobin concentration. Thus, dense cells with high mean corpuscular hemoglobin concentrations may have a profound effect on the microcirculatory flow if their capillary transit times are increased. The adhesion of light (young) cells may lengthen transit times for other cells in the microvasculature, thereby leading to gel formation in dense cells and resulting in vasoocclusion. Recently, increased adhesion of reticulocytes in SS disease to fibronectin-coated Petri dishes has been demonstrated in a static system. The abnormal adherence of young cells may be of little or no consequence in other diseases that are characterized by increased numbers of reticulocytes but do not involve vascular occlusive events. Increasing capillary transit times would not result in changes in the rheological properties of RBC for these diseases. Increased adherence to endothelial cells, the only factor besides delay time thought to be correlated with disease severity, may be an important feature of young RBC in sickle cell disease.

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SICKLE CELL-ENDOTHELIAL CELL INTERACTIONS


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Endothelial cell interactions with sickle cell, sickle trait, mechanically injured, and normal erythrocytes under controlled flow

GA Barabino, LV McIntire, SG Eskin, DA Sears and M Udden