Deoxygenation-Induced Changes in Sickle Cell–Sickle Cell Adhesion

By Christopher L. Morris, Donald L. Rucknagel, and Clinton H. Joiner

The tendency for sickle cells to adhere to each other is increased in oxygenated sickle blood in parallel with cell density. The increased adherence of these cells occurred despite their reduced deformability and diminished ability to form rouleaux. Using a method developed in our laboratory, we measured the yield stress: a sensitive index of cell–cell adhesion of deoxygenated suspensions of sickle cells. Deoxygenation of whole sickle blood to 30 to 50 mm Hg caused a significant increase in yield stress of all sickle blood samples. Deoxygenation caused a significant increase in yield stress of both dense and light sickle cells. Deoxygenation-induced increases in yield stress occurred at higher oxygen tensions for dense (> 55 mm Hg) than for light sickle cells (< 45 mm Hg). The increase in yield stress on deoxygenation was correlated with hemoglobin polymerization as assessed morphologically by sickling or by changes in relative viscosity. Thus, deoxygenation-induced cell sticking must involve small areas of strong membrane adhesion because the changes in yield stress occurred despite a reduction in rouleaux formation and surface area of membrane contact. Sickle trait red blood cells also exhibited increased yield stress on deoxygenation but only under hypertonic conditions where sickling occurred. Thus, deoxygenation-induced cell adhesion did not require prior membrane damage because it occurred in sickle trait cells. No change in yield stress was seen when deoxygenated sickle cells were suspended in buffer, but the addition of physiologic amounts of fibrinogen to buffer restored the deoxygenation-induced increase in cell adhesion. We speculate that the increase in sticking among sickle cells on deoxygenation results from spicule formation and may involve interaction of fibrinogen and possibly other plasma proteins with the cell membrane.

© 1993 by The American Society of Hematology.

SICKLING CAUSES a sequence of biochemical and mechanical events that result in the formation of sickle cells with abnormal flow properties. Sickle cells are heterogeneous with respect to these abnormalities. It is recognized that the impairment in cellular rheology leads to clinical pain episodes, but how it does so remains controversial because causal relationships between these abnormalities and vasoocclusive events have been difficult to establish. Hebbel and coworkers found a strong correlation between clinical severity and the tendency for sickle cells to adhere to vascular endothelium. Recent studies using animal models have shown that the lightest fractions of sickle cells were most adherent to the vessel wall, probably because of the increased tendency for reticulocytes to adhere to endothelium. However, these models also showed that occlusion of microvessels did not occur in the absence of dense sickle cells even though the endothelial surfaces were covered with adherent light cells. The requirement for dense cells in causing vasoocclusion was confirmed in a separate animal model by Fabry and coworkers. These studies indicate that by itself the ability to adhere to vascular endothelium did not cause vessel obstruction in these models.

In contrast to the large amount of information available on sickle cell–endothelial cell adhesion, very little data exists on sickle cell–sickle cell adhesion. It would be expected that dense or rigid sickle cells should have a diminished ability to adhere to other cells because the membrane cannot form large contact areas. However, we showed that dense cells were responsible for increased sticking among oxygenated sickle cells. This phenomenon may explain why vasoocclusion in animal models required the presence of dense cells. Sticking among dense sickle cells within venular channels narrowed by adherent light cells could lead to obstruction, whereas the lighter sickle cells that have normal red blood cell–red blood cell (RBC–RBC) adherence and deformability were able to traverse these vessels.

Because increased cell rigidity did not prevent sticking between oxygenated dense cells, it is logical to ask whether deoxygenation affects sticking among sickle cells. This laboratory has developed a method that measures the strength of adhesion among RBCs in suspension. This quantity, termed the yield stress, is defined as the minimum stress required to initiate flow in a suspension of erythrocytes. The method measures the flow resistance of a sedimenting layer of RBCs under conditions of low shear force. The flow resistance is resolved into viscous and elastic components. The elastic resistance is the yield stress and reflects the strength of adhesive forces between cells. This study shows that deoxygenation is associated with increased sticking among sickle cells. We also show that sickle cells were heterogeneous with regard to the oxygen tension at which deoxygenation-induced increases in sticking were observed.

MATERIALS AND METHODS

Background. RBC sedimentation is synonymous with the phase separation of blood at low shear rates and results in formation of a network of adherent cells. The yield stress is defined as the minimum force required to cause the collapse of this network into a liquid state. Yield stress depends on the hematocrit within the cell network and the concentration of proteins that support cell adhesion. At equal hematocrit, differences in yield stress reflect different concentrations of these proteins, differences in surface area of membrane contact, or differences in the adhesive properties of the RBC surface.

Yield stress method. The yield stress chamber method has been described in detail elsewhere. Briefly, a wedge-shaped chamber is
fi ed with blood and allowed to sediment under gravity. The chamber is mounted on a microscope stage and the extent of sedimentation is visually determined from the distance, between the focal point of the upper surface of the chamber and the sedimenting cell-layer surface, which is measured with the microscope micrometer. The cell-layer hematocrit is equal to the initial hematocrit of the blood put into the chamber multiplied by the width of the chamber and divided by the distance the cell layer has sedimented. After the desired cell layer hematocrit is achieved, a shear force is imposed on the layer by tilting the microscope. The velocity of the cell-layer surface is recorded at several specified points along the chamber. Each point corresponds to a different shear force that is proportional to the width of the chamber at that point.

The relationship between the applied shear force and cell-layer velocity is determined by the total flow resistance of the blood (viscous plus elastic resistance). The viscous resistance is obtained by measuring bulk viscosity over the same range of hematocrits (eg, cell-layer hematocrit) as the yield-stress measurements. The total flow resistance is then resolved into a viscous and elastic term using the Casson equation, an empirical relationship that describes the behavior of particle suspensions that form rodlike aggregates owing to surface forces between the particles. The yield stress is the elastic resistance term calculated from the Casson equation. The yield stress is nearly independent of the deformability properties of the RBC. Suspensions of deformable RBCs or glutaraldehyde-hardened RBCs in buffer have nearly identical yield-stress measurements that are only slightly greater than zero. This reflects the fact that although rigid cells have a much greater flow resistance than deformable cells, there is no significant adhesion between these cells in the absence of plasma. Thus, changes in yield stress are specific for adhesive interactions between cells and are independent of changes in cell rigidity per se.

**Blood samples.** Blood was obtained in accordance with the committee on human subjects from patients followed regularly at Children's Hospital Medical Center. All patients were afebrile and free of pain or objective signs that could be attributed to their disease. Blood samples were drawn from the antecubital vein into plastic syringes containing 1.4 to 9.0 (vol:vol) acid citrate dextrose anticoagulant to facilitate hematocrit as the yield-stress measurements. For some experiments, the degree of cell-layers had a clottable fibrinogen concentration of 2,15 mg/dL.

**Yield stress experiments.** One milliliter blood samples of the desired oxygen tension were transferred from tonometer to yield-stress chamber. Chambers were purged before being loaded with blood with the appropriate gas mixture. Yield stress measurements were performed at various hematocrits between 35% and 50%. To facilitate comparison of data, the yield stress for each whole-blood experiment was interpolated to 45% hematocrit and for density-fractionated experiments to 40% hematocrit. Relative viscosity (bulk viscosity divided by plasma viscosity) was determined at 225 °C in a water-jacketed Brookfield viscometer (model LVT cone plate with a 0.8° cone; Brookfield Engineering Laboratories Inc, Stoughton, MA) with gas inflow settings at 25°C. Hematocrits were adjusted in autologous plasma to obtain viscosity measurements at similar hematocrits as the yield-stress measurements. For some experiments, the degree of sickling was measured by removing 0.05 mL of blood from the yield-stress chamber and mixing with 1 mL of paraformaldehyde that had been equilibrated with the same gas mixture as the cell suspension. Sickled forms were defined as cells with a two to one or greater ratio of length to width or cells with two or more spicules. Five hundred cell counts were performed and the results expressed as a percentage.

**RESULTS**

The effect of oxygen tension on the yield stress of whole sickle blood, measured in samples from eight patients, is shown in Fig 1. These samples, suspended in autologous plasma, showed a twofold to threefold increase in yield stress as the oxygen tension was reduced below 50 mm Hg, which indicated that sticking among sickle cells increased dramatically on deoxygenation. The yield stress of normal blood
was not affected by deoxygenation. The oxygen tension at which yield stress of sickle blood began to increase varied somewhat among the samples tested but was generally between 30 and 50 mm Hg.

Whole-blood viscosity was measured on each of the samples depicted in Fig 1 and increased at oxygen tensions between 30 and 50 mm Hg. In Fig 2, yield stress was plotted as a function of whole-blood relative viscosity (whole blood divided by plasma viscosity). Points from individual blood samples measured at different oxygen tensions were connected by lines. There was considerable intersample variation in both measurements because of differences in plasma fibrinogen concentration that affected both parameters and differing levels of sickling at each oxygen tension. Nevertheless, within a given sample, changes in yield stress were tightly correlated with changes in viscosity. Because it is known that the increase in viscosity on deoxygenation of sickle blood is caused by hemoglobin polymerization, these data indicate that changes in yield stress are also correlated with polymerization.

We previously showed that the yield stress of oxygenated dense sickle cells were significantly greater than normal RBCs in both measurements because of differences in plasma fibrinogen concentration that affected both parameters and differing levels of sickling at each oxygen tension. Nevertheless, within a given sample, changes in yield stress were tightly correlated with changes in viscosity. Because it is known that the increase in viscosity on deoxygenation of sickle blood is caused by hemoglobin polymerization, these data indicate that changes in yield stress are also correlated with polymerization.
in contrast to light sickle cells, which had nearly normal yield stress. Therefore, we tested whether cell density influenced deoxygenation-induced changes in yield stress. A dramatic increase in yield stress was apparent on deoxygenation of both light (Fig 3A) and dense sickle cells (Fig 3B). The absolute magnitude of the deoxy yield stress was not significantly different for light and dense cells, \( P = 0.15 \). However, the magnitude of the increase in yield stress was significantly greater for light cells (0.094 ± 0.025 dyne/cm²) than for dense cells (0.049 ± 0.014 dyne/cm²) \( P = 0.025 \). Therefore, we tested whether cell density influenced the magnitude of the increase (deoxy minus oxy yield stress) for light cells versus 77% ± 38% for dense cells \( P = 0.002 \). This was mostly caused by the higher yield stress of oxygenated dense sickle cells, which we have previously described. These results indicate that both dense and light sickle cells contributed to increased cell sticking on deoxygenation.

Because polymer formation correlated with increased yield stress for whole blood, and dense cells form polymer at higher oxygen tensions than light cells, we tested whether the oxygen tension at which yield stress began to increase was higher for dense cells compared with light sickle cells. The relationship of yield stress with oxygen tension for light and dense cells is shown in Figs 4A and 4B, respectively. The yield stress of light sickle cells increased very rapidly at oxygen tensions...
below 35 and 43 mm Hg. In contrast, the yield stress of dense sickle cells increased at oxygen tensions between 56 and 64 mm Hg in 4 of the 6 experiments. Three experiments showed no significant change in yield stress at 65, 66, and 71 mm Hg. Taken together, these data indicate that yield stress increases at higher oxygen tensions for dense cells than light cells. These differences were also apparent when comparing the hemoglobin saturations at which increases in yield stress began: 70% to 80% for dense cells and 40% to 65% for light cells. The increase in yield stress on deoxygenation of dense cells was less than for light cells (55% v 300%). However, the changes were considerably greater than the variation related to reproducibility of the yield-stress measurement, which was 10% in our previous study.

The greater sensitivity of dense cells to reduced oxygen tension (compared with light cells) is consistent with a strong dependence of cell stickiness on polymer formation, as seen in whole blood. In Fig 5, the data in Figs 4A and 4B are

---

**Fig 5.** The yield stress of density-fractionated sickle cells was plotted as a function of relative viscosity as the oxygen tension was reduced. The data were from 10 experiments of fractionated cells suspended in identical aliquots of cross-matched plasma and includes the six studies shown in Fig 4. All yield-stress measurements were interpolated to 40% hematocrit. The normal range encompassed the range of yield stress and relative viscosities (at 40% hematocrit) obtained from 36 normal blood samples using the same yield-stress chamber apparatus and cone-plate viscometer as the present study. Sickle data overlapping the normal range were oxygenated light cells. The data were analyzed by standard linear regression technique and Pearson bivariate correlation test ($r = .74$).

---

**Fig 6.** Deoxygenation-induced changes in yield stress of sickle trait (AS) and normal (AA) RBCs. All yield-stress values were interpolated to 45% hematocrit. Cells were suspended in autologous plasma. Each experiment consisted of cells in isotonic (295 to 300 mOsm) or hypertonic (473 to 510 mOsm) plasma. Each sample was equilibrated with 20% oxygen 5% carbon dioxide and 95% nitrogen 5% carbon dioxide. The numbers in parentheses were the percentage of sickled forms in each sample. The results include three experiments with AS cells (○, ●) and two experiments with AA RBCs (□, ▲). Deoxygenation-induced increases in yield stress occurred only in the samples that sickled.
plotted as a function of the measured viscosity to illustrate the correlation between yield stress and polymer formation of density-fractionated sickle cells. These experiments were performed with the cells suspended in identical aliquots of cross-matched normal plasma, which eliminated any differences in yield stress or viscosity related to plasma factors. Compared to the results with whole blood, the correlation between polymer formation and cell stickiness was much stronger ($r = .74$).

Sickle trait blood (AS) also showed increased yield stress on deoxygenation (Fig 6). Normal (AA) and AS RBC were suspended in isotonic (280 to 300 mOsm) or hypertonic (450 to 500 mOsm) autologous plasma and incubated with and without oxygen. Sickling occurred only in the deoxygenated AS cells that were suspended in hypertonic plasma. The numbers next to each data point indicate the percentage of sickled cells. Although hypertonic plasma caused a slight increase in the oxy yield stress of both AA and AS samples, deoxygenation caused no further increase in AA yield stress or in the yield stress of isotonic AS cells that did not sickle. There was, however, a twofold to threefold increase in the yield stress of hypertonic AS cells that sickled. These results indicate that the presence of deoxygenated sickle hemoglobin in the absence of hemoglobin polymerization did not cause an increase in yield stress. In addition, prior membrane damage, as may be present in homozygous sickle cells regardless of cell density, was not necessary to cause the observed increases in adhesion among sickle cells.

Deoxygenation-induced increases in yield stress required the presence of one or more proteins present in plasma, as shown in Fig 7. Sickle cells suspended in buffer had minimal yield stress under both oxy and deoxy conditions (Fig 7C). The relative viscosity increased twofold to fourfold for these buffer suspensions, and visual inspection showed that 59% to 74% of the cells were sickled in the deoxygenated samples. This result indicates that the increased cell rigidity and blood viscosity that accompany sickle hemoglobin polymerization were not responsible for the deoxygenation-induced increase in yield stress. In contrast, sickle cells suspended in PBS containing 0.2 g/dL of purified human fibrinogen (Fig 7B) had similar deoxygenation-induced increases in yield stress as sickle cells suspended in plasma with the same fibrinogen content (Fig 7A). These results indicate that expression of deoxygenation-induced sticking of sickle cells requires a soluble cofactor present in plasma and that fibrinogen is one of these cofactors. We have not yet determined whether the ability of fibrinogen to support sticking among sickle cells is specific or if other plasma proteins are capable of supporting deoxygenation-induced sticking.

**DISCUSSION**

The yield stress of blood is a sensitive index of the strength of adhesion between cells. In this study we found a dramatic deoxygenation-induced increase in the yield stress of sickle blood. The increase in cell sticking occurred with both light and dense sickle cells and sickle-trait cells, was correlated closely with polymer formation (as measured by increasing viscosity) in these cells, and occurred over physiologically relevant oxygen tensions, especially for dense sickle cells. Deoxygenation of sickle cells in the absence of plasma proteins did not result in increased cell sticking; however, the

![Fig 7. Suspending phase requirements for expression of deoxygenation-induced changes in yield stress. All yield stress values were interpolated to 40% hematocrit. The normal range, mean ±2 SEM, was obtained from published results using 40% hematocrit and the fibrinogen concentration of the suspending media. Density-fractionated cells suspended in plasma or PBS-fibrinogen were equilibrated with three to four oxygen tensions. Suspensions in buffer were oxygenated in room air and deoxygenated with 100% nitrogen. The dashed lines (--) indicate the expected increase in yield stress for the suspensions of dense sickle cells (see Fig 4). (A) Dense (C) and light (B) sickle cells were suspended in plasma with fibrinogen concentration of 0.31 g/dL. (B) Dense (B) and light (D) sickle cells were suspended in PBS containing 0.20 g/dL clottable human fibrinogen. The deoxygenation-induced increase in yield stress was similar to that seen with plasma. The lower absolute value of the yield stress compared with the plasma samples was caused by the lesser fibrinogen concentration of the suspending media. In addition, to these results with homozygous sickle cells, suspensions of AS RBCs in hypertonic PBS-fibrinogen (0.19 g/dL) also showed deoxygenation-induced increases in yield stress on three separate experiments similar to the results in hypertonic plasma (data not shown). (C) Dense (C) light (D), and nonfractionated (A) sickle cells suspended in PBS. The yield stress of these suspensions did not change with deoxygenation. The magnitude of the yield stress was small and within the range of normal RBCs suspended in PBS obtained from earlier published results (0.006 to 0.015 dyne/cm²).
the fraction of cells sickled in the deoxygenated specimens

Stearic interactions do not explain the deoxygenation-induced increase in yield-stress measurements. Sickle cells suspended in buffer and deoxygenated to the same extent as plasma suspensions showed almost no change in yield stress. The increase in relative viscosity of these suspensions and the fraction of cells sickled in the deoxygenated specimens (54% to 74%) were similar to results in plasma. Therefore, stearic interactions based on the irregularity of the cell surface were similar. Thus, the deoxygenation-induced increase in yield stress must reflect increased stickiness of the sickle membrane.

Cell aggregation and rouleaux formation facilitate erythrocyte sedimentation and are the major determinants of yield stress for normal blood. Our observations of cells in the yield-stress chamber showed that deoxygenated sickle cells exhibited reduced sedimentation velocity and rouleaux formation. Rouleaux formation was diminished at the same oxygen tension at which the increase in yield stress became apparent. Thus, the deoxygenation-induced increase in yield stress was opposite to the effect of deoxygenation on rouleaux formation and cannot be attributed to it. Furthermore, because formation of rouleaux is a function of the surface area of the cells available for contact, the reduction of rouleaux in deoxygenated sickle cells probably represents a reduced area of contact between cells brought about by stearic factors related to their rigidity and irregular shape. If so, it is likely that the deoxygenation-induced increase in yield stress significantly underestimates the strength of adhesion between sickled cells and represents a different form of RBC-RBC adhesion from that occurring in rouleaux.

The mechanism(s) responsible for increased sticking between deoxygenated sickle cells is unknown. Previously we noted a similar correlation between increased yield stress and reduced cell deformability for oxygenated sickle cells. We found that the increase in yield stress of oxygenated whole sickle blood correlated with the number of dense cells. The prior study and our current results implicate sickle hemoglobin polymer as the cause for increased cell sticking. However, the requirement for plasma proteins for expression of the phenomenon indicates that additional events other than polymer formation are required to cause deoxygenated cells to stick.

One possible mechanism is the reduction in charge repulsion that occurs when membrane protrusions with a small radius of curvature are formed, as occurs when cells sickle. The phenomenon was first described for normal RBCs by Salsbury and Clark, who showed that IgG anti-AB0 blood group antibodies that caused echinocytosis were able to agglutinate RBCs, whereas antibodies that did not cause echinocytosis did not agglutinate. Other blood cells, phagocytic leukocytes and platelets, also become sticky on development of pseudopods with a small radius of curvature. Application of this concept to deoxygenated sickle cells would argue that the sharp curvature of the spicules makes these areas sticky and results in a more adherent agglutinate than the rouleaux that are formed under oxy conditions.

The requirement for plasma proteins is not explained by this mechanism, but it could be related to interactions occurring between certain proteins and the sickle membrane. The usual mechanisms that explain rouleaux formation, cross bridging or depletion aggregation, cannot explain the deoxygenation-induced adhesion phenomenon. Cross bridging involves weak membrane interactions that must occur over a large portion of the erythrocyte membrane to be stable. Depletion aggregation requires much higher concentrations of proteins than were present in our buffer-fibrinogen suspensions (0.2 g/dL). The osmotic energy of a polymer solution is proportional to the polymer concentration and radius of gyration of the molecule. For red blood cells suspended in dextran 500, depletion aggregation did not occur below concentrations of 2 g/dL and the equivalent concentration of fibrinogen to achieve a similar effect would be 50% higher owing to the smaller radius of gyration of the fibrinogen molecule. In addition, neither mechanism can stabilize interactions over a small fraction of the surface area of the membrane. Many of the perturbations of the sickle membrane, such as loss of phospholipid asymmetry and uncoupling of the lipid bilayer from the spectrin-actin cytoskeleton, are localized to the spicules formed during deoxygenation. Fibrinogen and other proteins may stick to these regions, or proteins may facilitate adhesion by weakly stabilizing an initial membrane contact, followed by secondary adhesive events between spicules and other portions of the cell membrane. Regardless of the mechanisms involved in deoxygenation-induced cell sticking, the process must be completely reversible. On reoxygenation of deoxygenated sickle cells, the yield stress returned to the original oxy value in three of three experiments. This could reflect shedding of the adherent spicule upon reoxygenation or reduction in the stickiness of the membrane.

Deoxygenation-induced changes in yield stress were correlated with sickle hemoglobin polymerization, measured as an increase in viscosity, and with morphologic sickling. The oxygen tension at which yield stress increased was higher for dense than for light sickle cells. This reflected the fact that hemoglobin polymerization occurred at higher oxygen tension in dense cells as a consequence of their higher hemoglobin concentration and lower oxygen affinity. The correlation between polymerization and yield-stress changes was reinforced by experiments with sickle-trait cells, which showed increased adhesion only when sickling occurred; thus, the presence of deoxygenated sickle hemoglobin was not sufficient to trigger adhesive changes but polymerization was required. In addition, these data show that prior membrane damage characteristic of sickle RBCs was not necessary for the deoxygenation-induced increase in adhesion because it was induced in the undamaged sickle trait membrane.

The fact that both the absolute magnitude and percent increment of yield stress in deoxygenated sickle cells was higher in light cells compared to dense cells is consistent with the greater degree of sickling known to occur in less dense cells. This result suggests, but does not prove, that deoxygenation-induced cell adhesion was caused by mechanical changes in the membrane related to sickling, as opposed to polymer formation per se. Additional experiments will be
necessary to differentiate the effects of sickling from hemoglobin polymerization on cell adhesion.

Our studies were performed at very low shear rates, but may be physiologically relevant. Animal studies showed that normal spleen and bone have areas of extremely low shear rates with periods of stopped flow up to 3 minutes in bone marrow (BM) sinusoids.\(^9\) RBC velocity in BM sinusoids of the rabbit were shown to vary between 0 and 0.2 mm/s, with sinusoid diameters varying between 15 and 60 \(\mu\)m. A simple Poiseuille model (wall-shear stress = 8 \(U \mu/d\)), with \(U = 0.2\) mm/s, \(d = 15\) to 60 \(\mu\)m, and \(\mu = 0.03\) (bulk viscosity of 3 cp) gives a peak wall-shear stress in the sinusoid between 0.8 and 3.0 dyne/cm\(^2\). Deoxy sickle blood yield-stress values were 0.2 to 0.3 dyne/cm\(^2\) at 40% to 45% hematocrit and increased to over 0.5 dyne/cm\(^2\) at hematocrits between 45% and 50% or at fibrinogen concentrations \(\geq 0.4\) g/dL. Thus, the shearing forces available within some marrow sinusoids are often less than the adhesive forces within a sedimented layer of sickle cells. In addition, sedimentation of cells in these sinusoids during periods of stopped flow would allow preferential flow of the low-viscosity cell-depleted layer to occur on resumption of flow causing accumulation of sedimented clumps of sickle cells within the sinusoid. Thus, in some sinusoids, sickle cell aggregates could, under the right conditions, remain undispersed and result in progressive obstruction of marrow sinusoids. It should also be noted that the cells most likely to be retained under oxygenated conditions (dense cells) developed increased sticking most quickly under deoxygenated conditions.

Kaul et al\(^2\) have suggested that adhesion of light deformable sickle cells to endothelial surfaces may initiate vasoocclusion through secondary entrapment of dense sickle cells. Our data suggest that another consequence of adherence of light sickle cells to endothelium is increased opportunity for deoxygenation-induced cell adhesion, as the movement of sickle cells is slowed through these narrowed vessels. Our speculation highlights the complex interplay between sickle cell--sickle cell adhesion, sickle cell--endothelial cell adhesion, and the influence that deoxygenation-induced changes in the stickiness of the sickle cell have on this relationship.

ACKNOWLEDGMENT

The authors thank Elaine Gillette and Lana Hackworth for their assistance in screening patients and obtaining blood for these studies.

REFERENCES


Deoxygenation-induced changes in sickle cell-sickle cell adhesion

CL Morris, DL Rucknagel and CH Joiner