Antisickling Effects of 2,3-Diphosphoglycerate Depletion

By W.N. Poillon, B.C. Kim, R.J. Labotka, C.U. Hicks, and J.A. Kark

Elevation of 2,3-bisphosphoglycerate (2,3-DPG) in sickle erythrocytes (SS RBCs) and concomitant acidification of the cell interior promotes polymerization by decreasing the solubility (c_m) of deoxyhemoglobin S. The antisickling effect of 2,3-DPG depletion was evaluated after activation of the 2,3-DPG phosphatase activity of bisphosphoglycerate mutase by glycolate-2-phosphate, leading to rapid loss of intracellular 2,3-DPG. To ensure its maximal reduction in a physiologic medium, isomotic CO_2/bicarbonate-buffered saline, pH 7.0, was used. Substitution of K⁺ for Na⁺ as the major extracellular cation suppressed K⁺:Cl⁻ cotransport, prevented cell shrinkage, and allowed demonstration of the full antisickling effect of 2,3-DPG depletion. The modest effect on solubility per se of removing intraerythrocytic 2,3-DPG (Δc_m = 1.6 g/dL) was amplified into a much larger antisickling effect by interaction with three other cellular variables affecting solubility and polymer content (intracellular pH, O₂ saturation, and mean cell hemoglobin concentration). Acting in concert, these four antisickling effects (three solubilizing, one osmotic) reduced polymer fraction of glycolate-treated SS RBCs by 32% to 63%, with a concomitant decrease in sickling of 46% to 95% at the nominal pO₂ of the microcirculation (20 mm Hg). A decrement in sickling of this magnitude should significantly ameliorate the vasoocclusive severity of sickle cell disease.

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affinities of control and treated cells were measured in a Hemoxy Analyzer (TCS Medical Products Co, Huntingdon Valley, PA), which was also used to evaluate sickling as a function of Po2.

Cell preparation. Informed consent was obtained before blood collection from adult sickle cell anemia (SCA) patients in the steady state who were homozygous for Hb S (Hb SS). Blood specimens (approximately 30 mL) were drawn in tubes anticoagulated with heparin and used for metabolic depletion experiments within 24 hours. Hemoglobin phenotypes (SS) were verified by cellulose acetate electrophoresis at pH 8.6 (Helena Labs, Beaumont, TX). Blood was centrifuged at 670g for 10 minutes, followed by aspiration of plasma and buffy coat. Packed RBCs were washed twice in isotonic heparin and used for metabolic depletion experiments within 24 hours. Control medium consisted of 115 mmol/L KCl, 5 mmol/L NaCl, 2 mmol/L CaCl2, 1 mmol/L MgCl2, 2 mmol/L NaHPO4, 1 mmol/L inosine, 24 mmol/L NaHCO3, and 10 mmol/L glucose, with a final pH of 7.4 (37°C) obtained by bubbling with CO2 (5.6%) for 30 minutes to achieve a PCO2 of 40 mm Hg. Medium for treated cells contained 30 mmol/L glycolic acid and 5 mmol/L glucose at a final pH of 7.0 (37°C). Osmolality was maintained at 290 mOsm/kg by reducing KC1 to (approximately 30 mL) were drawn in tubes anticoagulated with L glycolic acid and 5 mmoL glucose at a final pH of 7.0 (37°C). Blood of 115 mmol/L KCI, 5 mmol/L NaCl, 2 mmol/L CaCl2, NaHCO3, and 10 mmol/L glucose, with a final pH of 7.4 (37°C) was prepared for morphologic sickling, as described above.

Osmolality was maintained at 290 mOsm/kg by reducing KC1 to (approximately 30 mL) were drawn in tubes anticoagulated with L glycolic acid and 5 mmoL glucose at a final pH of 7.0 (37°C). Blood of 115 mmol/L KCI, 5 mmol/L NaCl, 2 mmol/L CaCl2, NaHCO3, and 10 mmol/L glucose, with a final pH of 7.4 (37°C) was prepared for morphologic sickling, as described above.

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Table 1. Effect of Loss of 2,3-DPG on pH of Human Red Cells as Measured by 31P-NMR Spectroscopy

<table>
<thead>
<tr>
<th>Type of Cells and System</th>
<th>pH</th>
<th>% Loss of 2,3-DPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.24</td>
<td>22</td>
</tr>
<tr>
<td>Glycolate</td>
<td>7.33</td>
<td>74</td>
</tr>
<tr>
<td>Sickle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.19</td>
<td>9</td>
</tr>
<tr>
<td>Glycolate</td>
<td>7.27</td>
<td>51</td>
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</table>

RESULTS

Effect of glycolate on intracellular pH. Using 31P-NMR spectroscopy, we measured the effect of incubation with glycolate in isosmotic HEPES-buffered saline, pH 7.4, on pH and the change in 2,3-DPG concentration for both AA and SS RBCs. Results are shown in Table 1. In both cases, loss of 2,3-DPG in glycolate-treated cells was substantially greater than in controls (respectively, 74% v 22% for AA and 51% v 9% for SS). Furthermore, for both cell types, pH was higher for 2,3-DPG-depleted cells than for controls (by 0.09 unit for AA and 0.08 unit for SS cells). These increments in pH agree well with those calculated using the Duhm factor (see Materials and Methods; 0.08 and 0.07, respectively). This result validates use of this factor to estimate pH in control and glycolate-treated SS RBCs.

Effect of glycolate on O2 affinity and sickling tendency. Results for a representative experiment on glycolate-induced depletion of 2,3-DPG in isosmotic high-K medium are shown in Table 2. ATP values were relatively unchanged for control and treated cells after 6-hour incubation, whereas 2,3-DPG concentrations increased by 6% for control cells and decreased by 83% for 2,3-DPG-depleted cells. MCHC, which reflects cellular hydration status, decreased for both control and treated cells after 6-hour incubation (by 2.1 and 4.3 g/dL, respectively), indicating that cell swelling was twice as large in the glycolate-treated cases.

The effect of 2,3-DPG depletion on %RSC as a function of pO2 at 5 to 51 mm Hg is shown in Fig 1, with the corresponding plot for O2 saturation shown in Fig 2. For control cells, %RSC increased gradually below 60% saturation, to a value of 62% near complete deoxygenation; for treated cells, %RSC was relatively flat throughout the range of O2 tension examined, ranging from 3% at 31 mm Hg to 21% at 5 mm Hg. Thus, near full desaturation, the sickling tendency of 2,3-DPG-depleted SS RBCs was lower than that of 2,3-DPG-replete cells by 66%.

The two sickling parameters that characterize SS RBCs in isosmotic CO2/bicarbonate-buffered saline (derived from Fig 1) are shown in Table 2. Sickling parameter 1 (%RSC at pO2 = 20 mm Hg, the nominal O2 tension of the microcirculation) was 29% lower for treated cells. A reduction of this magnitude reflects the significant decrease of polymer content in SS RBCs devoid of 2,3-DPG. Sickling parameter 2 (%RSC at pO2) was 11% lower for treated cells, indicative of inhibition of intracellular gelation independent of changes in O2 affinity. The percentage decrease in sickling (71% and 48%, respectively) shows that the sickling potential of these 2,3-DPG-depleted SS RBCs has been substantially diminished.

Table 2. Effect of 2,3-DPG Depletion by Incubation With Glycolate at Acid pH on O2 Affinity and Sickling Tendency of SS RBCs

<table>
<thead>
<tr>
<th>System</th>
<th>Incubation Time (h)</th>
<th>MCHC (g/dL)</th>
<th>ATP (µmol/mL RBC)</th>
<th>2,3-DPG (µmol/mL RBC)</th>
<th>P50 (mm Hg)*</th>
<th>Sickling Parameters†</th>
<th>1</th>
<th>2</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>36.4</td>
<td>1.10</td>
<td>7.45</td>
<td>27.5</td>
<td>12</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>Treated</td>
<td>0</td>
<td>34.3</td>
<td>1.16</td>
<td>7.92</td>
<td>21.5</td>
<td>12</td>
<td>23</td>
<td>12</td>
</tr>
</tbody>
</table>

*Derived from ODCs used to generate sickling curves shown in Fig 1.
† These parameters are derived from Fig 1 as (parameter 1) reversibly sickled cells (%RSC) at pO2 = 20 mm Hg, the nominal O2 tension of the microvasculature, and (parameter 2) %RSC for O2 tension at half-saturation (P50).
‡ The low 2,3-DPG concentration in treated cells is probably an overestimate, because its detectable limit by the coupled enzymatic oxidation of reduced nicotinamide-adenine dinucleotide (NADH) is being approached.
reflecting the sparing effect on polymerization of the ele-

ment between treated and control cells for six measured cells in whole blood was much less for patient 
to a

vated Hb F level for patient are translocated across the cell membrane.

by 0.012 unit. Thus, 2,3-DPG-depleted sickle erythrocytes 

0, tension of the capillary bed (sickling parameter I  

was greatest for patient B (95%) and least for patient D  

for patients B through D (range, 8.7% to 21.8%), presumably  

adjustment of the osmotic equilibrium by which Cl- and H+ 

in 

show the following: (1) a reduction in 2,3-DPG concentra-

tion that varied from -3.7 to -6.6 mmol/L, corresponding  

2,3-DPG depletion on erythrocytes from four Hb SS patients;  

(4) an inhibition of sickling at pO2  

least for patient D (1  1%); (6) an increase in c,,, estimated  

(see Materials and Methods) to vary from 4.4 to 6.0 g/dL, and (7) a decrease in the calculated value of f, that ranged  

portion of the Bohr effect due to 2,3-DPG binding would lower pH, by 0.05 unit, while the compensa-

ory osmoregulatory chloride/proton shift necessary to main-

tain electroneutrality would raise pH, by 0.12 unit; the net  

ApH, of 0.07 unit corresponds to a Ac,,, of 0.8 g/dL, and  

the loss of 2,3-DPG per se produces a Δc,,, of 1.6 g/dL  

(see Materials and Methods). Thus, the overall increment in  

solubility (2.4 g/dL) and pHI produced by 2,3-DPG depletion would increase c,,, for unliganded Hb S from 18.0 to 20.4  

g/dL and pHI from 7.41 to 7.48. The corresponding decrease  

in f, is from 0.60 to 0.52. Cell swelling that accompanies  

adjustment of the Donnan equilibrium causes a decrease in  

MCHC from 32.3 to 30.0 g/dL and f,, from 0.52 to 0.45, a  

reduction of 25% in polymer content overall relative to the  

2,3-DPG–replete species. Sunshine et al have shown that the  

O2 affinity of polymer is threefold lower than that of monomer and provided an empirical equation by which to  

erthrocytic variables (2,3-DPG, pHI, MCHC, and O2 satu-

ration) that interact to affect the polymerization of partially liganded Hb S in a 2,3-DPG–dependent fashion. Non-S hemoglo-

ins (Hbs F and A,) also inhibit polymerization, as shown in our recent report, but this sparing effect is inde-

pendent of the cell’s 2,3-DPG status. [In any case, for the patient from Table 3 (B) chosen for this analysis, the Hb F  

concentration is so low (3.0%) that its solubilizing effect (<1.0 g/dL) can be ignored.] Overall inhibition of polymer-

ization has been dissected into the component contributions 

made by each of the four variables. Those due to DPG/pH,  

and MCHC (direct effects) have been calculated on the basis  

of solubilizing or osmotic effects with Hb S in the unliganded  

state (pO2 = 0). The effect of oxygenation on solubility  

(an indirect effect) was then calculated for the measured O2  

saturations of control and glycolate-treated cells. A reason-

able estimate for the values of pHI corresponding to 2,3-

DPG–replete and 2,3-DPG–depleted sickle erythrocytes at  

each stage can be made as follows: for unliganded Hb S,  

loss of that portion of the Bohr effect due to 2,3-DPG binding would lower pH, by 0.05 unit, while the compensa-

atory osmoregulatory chloride/proton shift necessary to main-

tain electroneutrality would raise pH, by 0.12 unit; the net  

ApH, of 0.07 unit corresponds to a Ac,,, of 0.8 g/dL, and  

the loss of 2,3-DPG per se produces a Δc,,, of 1.6 g/dL  

(see Materials and Methods). Thus, the overall increment in  

solubility (2.4 g/dL) and pHI produced by 2,3-DPG depletion would increase c,,, for unliganded Hb S from 18.0 to 20.4  

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O2 affinity of polymer is threefold lower than that of monomer and provided an empirical equation by which to 

Red cell density profiles shown in Fig 3 for control and treated cells indicate that at the inflection point (D), gly-

colate-treated SS RBCs have densities lower than controls by 0.012 unit. Thus, 2,3-DPG–depleted sickle erythrocytes 

are considerably more hydrated than controls because of adjustment to the osmotic equilibrium by which Cl- and H+ 

are translocated across the cell membrane.

Osmotic and antisickling effects of 2,3-DPG depletion.

We evaluated in detail the osmotic and antisickling effects of 2,3-DPG depletion on erythrocytes from four Hb SS patients; results are presented in Table 3. The percentage of dense cells in whole blood was much less for patient A (3.2%) than for patients B through D (range, 8.7% to 21.8%), presumably reflecting the sparing effect on polymerization of the elevated Hb F level for patient A. Composite data on the difference between treated and control cells for six measured (Δ2,3-DPG, ΔP50, ΔMCHC, ΔD50, and Δ sickling parameters 1 and 2) and two calculated (Δc,,, and Δf,) parameters show the following: (1) a reduction in 2,3-DPG concentration that varied from −37 to −6.6 mmol/L, corresponding to a loss of 2,3-DPG of 69% to 83%; (2) a significant increase in O2 affinity shown by a decrement in P50, ranging from −8.0 to −10.5 mm Hg; (3) a reduction in cell density with 
good correlation (r = .986) between ΔD50 and ΔMCHC, the two parameters that reflect the cell’s hydration status; (4) an inhibition of sickling at pO2 = 20 mm Hg, the nominal 
O2 tension of the capillary bed (sickling parameter 1), that was greatest for patient B (95%) and least for patient D (46%); (5) an inhibition of sickling at half-saturation (sickling parameter 2) that was greatest for patient B (68%) and least for patient D (11%); (6) an increase in c,,, estimated (see Materials and Methods) to vary from 4.4 to 6.0 g/dL, and (7) a decrease in the calculated value of f,, that ranged from −0.18 to −0.33, corresponding to decrements of 53%, 63%, 39%, and 32% for sickle erythrocytes from patients A, B, C, and D, respectively.

Direct and indirect effects of 2,3-DPG depletion on intra-
cellular polymerization. Table 4 quantitates all intra-

Fig 2. Extent of sickling (%RSC) as a function of O2 saturation (%HbO2) for control versus glycolate-treated SS RBCs (from patient A, Table 3), as described in the legend to Fig 1.

Fig 3. Red cell density profiles for control versus glycolate-treated SS RBCs (from patient A, Table 3), as described in the legend to Fig 1.
estimate $c_{sat}$ for partially oxygenated Hb S. Using this relationship (see Materials and Methods), we were able to calculate values of $c_{sat}$ for stripped Hb and 2,3-DPG-saturated Hb S (Hb-DPG) under conditions of $O_2$ saturation that correspond to a $pO_2$ of 20 mm Hg (34% and 59%, respectively). Values of $c_{sat}$ so obtained were 20.5 and 26.5 g/dL. (Incorporated into these numbers are decrements in $c_{sat}$ for Hb and Hb-DPG due to the loss of Bohr protons arising from partial saturation and depolymerization.) The corresponding values of $f_p$ were 0.52 and 0.19, an overall reduction in polymer content of 63% for 2,3-DPG-depleted cells.

### DISCUSSION

Interplay of cellular variables that inhibit polymerization and retard sickling of 2,3-DPG-depleted SS RBCs. Because incubation of treated cells at pH 7.4 led to incomplete depletion of their 2,3-DPG, it was necessary to reduce the pH to 7.0 and change the extracellular cation from Na$^+$ to K$^+$ to prevent potassium efflux. Under these conditions, 2,3-DPG was maximally depleted with minimal cell shrinkage.

In a preliminary experiment using the noninvasive technique of $^3$P-NMR spectroscopy to monitor pH$^2$ (Table 1), it was found that for both AA and SS RBCs, partial depletion

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**Table 3. Osmotic and Antisickling Effects of 2,3-DPG Depletion on Erythrocytes From Four SCA Patients With Varying Levels of Hb F**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dense Cells* (%)</th>
<th>% Hb F*</th>
<th>$\Delta$2,3-DPG (mmol/L)</th>
<th>$\Delta P_{50}$ (mm Hg)</th>
<th>$\Delta MCHC$ (g/dL)</th>
<th>$\Delta c_s$ (g/dL)</th>
<th>$\Delta c_p$</th>
<th>$\Delta f_p$</th>
<th>$\Delta c_{sat}$ (g/dL)</th>
<th>$\Delta f_{sat}$</th>
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<tbody>
<tr>
<td>A</td>
<td>3.2</td>
<td>17.2</td>
<td>-6.6</td>
<td>-8.0</td>
<td>-3.6</td>
<td>-0.0123</td>
<td>-29 (71)</td>
<td>-11 (48)</td>
<td>5.1</td>
<td>-3.31</td>
</tr>
<tr>
<td>B</td>
<td>12.1</td>
<td>3.0</td>
<td>-4.4</td>
<td>-10.5</td>
<td>-2.3</td>
<td>-0.0095</td>
<td>-42 (95)</td>
<td>-13 (68)</td>
<td>6.0</td>
<td>-0.33</td>
</tr>
<tr>
<td>C</td>
<td>8.7</td>
<td>0.8</td>
<td>-4.5</td>
<td>-8.0</td>
<td>-1.6</td>
<td>-0.0073</td>
<td>-22 (48)</td>
<td>-6 (21)</td>
<td>4.4</td>
<td>-0.21</td>
</tr>
<tr>
<td>D</td>
<td>21.8</td>
<td>1.9</td>
<td>-3.7</td>
<td>-10.0</td>
<td>-0.3</td>
<td>-0.0057</td>
<td>-27 (46)</td>
<td>-4 (11)</td>
<td>5.0</td>
<td>-0.18</td>
</tr>
</tbody>
</table>

*Parameters in columns 4 through 9 refer to the measured difference between glycolate-treated and control cells after incubation in isosmotic $CO_2$ bicarbonate (high-K) medium for 6 hours at 37°C; those in columns 10 and 11 have been calculated by the treatment given in Materials and Methods by which the four variables that affect polymerization in a 2,3-DPG-dependent manner are quantitated.

† Values are for whole blood, before treatment.

† See notes to Table 2 for definition of sickling parameters 1 and 2; numbers in parentheses refer to percentage reduction in each parameter relative to controls.

‡ Calculated from the conservation of mass equation as the difference in $f_p$ for treated v control cells with the measured values of $c_p$ (MCHC) and estimated values of $c_{sat}$ corresponding to the partial saturation at $pO_2 = 20$ mm Hg; the value of 69.3 g/dL was used for $c_p$, the polymer concentration.

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**Table 4. Direct ($c_{sat}$, $c_p$) Versus Indirect (oxygation-dependent) Effects of 2,3-DPG Depletion on Intracellular Polymerization**

<table>
<thead>
<tr>
<th>Parameter Affected</th>
<th>Species</th>
<th>Estimated pH$^3$, 2,3-DPG (mmol/L)</th>
<th>% $HbO_2$</th>
<th>$c_p$ (g/dL)</th>
<th>$c_{sat}^*$ (g/dL)</th>
<th>$f_p$</th>
<th>Intracellular Variable</th>
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<tbody>
<tr>
<td>$c_{sat}$</td>
<td>Control</td>
<td>Hb-DPG</td>
<td>7.41</td>
<td>6.16</td>
<td>0</td>
<td>32.3</td>
<td>18.0</td>
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<tr>
<td></td>
<td>Treated</td>
<td>Hb</td>
<td>7.48</td>
<td>1.75</td>
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<td>32.3</td>
<td>20.4</td>
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<tr>
<td></td>
<td></td>
<td>$\Delta$</td>
<td>0.07</td>
<td>-4.41</td>
<td>0</td>
<td>2.4</td>
<td>-0.08</td>
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<tr>
<td>$c_{sat}$, $c_p$</td>
<td>Control</td>
<td>Hb-DPG</td>
<td>7.41</td>
<td>6.16</td>
<td>0</td>
<td>32.3</td>
<td>18.0</td>
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<tr>
<td></td>
<td>Treated</td>
<td>Hb</td>
<td>7.48</td>
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<td>30.0</td>
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<td>$\Delta$</td>
<td>0.07</td>
<td>-4.41</td>
<td>-2.3</td>
<td>2.4</td>
<td>-0.15</td>
</tr>
<tr>
<td>$c_{sat}$, $c_p$, $P_{50}$</td>
<td>Control (pH, 7.5 mm Hg)</td>
<td>Hb-DPG</td>
<td>7.314</td>
<td>6.16</td>
<td>34.5</td>
<td>32.3</td>
<td>20.5$^4$</td>
</tr>
<tr>
<td></td>
<td>Treated (pH, 7.0 mm Hg)</td>
<td>Hb</td>
<td>7.351</td>
<td>1.75</td>
<td>585</td>
<td>30.0</td>
<td>26.5$^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\Delta$</td>
<td>0.04</td>
<td>-4.41</td>
<td>-2.3</td>
<td>6.0</td>
<td>-0.33</td>
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</table>

*These data pertain to patient B in Table 3. Values of 2,3-DPG concentration, %$HbO_2$ (et $pO_2 = 20$ mm Hg), and $c_p$ (i.e., MCHC) were measured before and after incubation in isosmotic $CO_2$ bicarbonate (high-K) medium for 6 hours at 37°C. Because the 2,3-DPG assay tends to overestimate low concentrations, depletion was assumed to be complete for the glycolate-treated species (despite its measured value of 1.75 mmol/L). Values of pH$^3$, $c_{sat}$, and $f_p$ were calculated according to the treatment outlined in Materials and Methods.

$^3$Based on the physiologic solubility of deoxy-Hb S presented in an earlier report. Measurements were made for stripped Hb and Hb-DPG in 9.05 mol/L Bistris, 0.1 mol/L KCl, 0.02 mol/L $NaCl$, pH 7.2 (290 mOsm/kg $H_2O$) at 37°C (see Materials and Methods for derivation of individual values) and have been corrected for the solubilizing effect of Bistris (46 g/dL per mol/L).

$^4$Calculated from conservation of mass relationship: $f_p = c_{sat} - c_{sat}^* = c_p - c_{sat}^*$, where $f_p$ is the polymer fraction and $c_p$, $c_{sat}$, and $c_{sat}^*$ are the polymer, total hemoglobin, and saturation concentrations, respectively (see Table 3, footnote $^4$ for details).

$^4$Release of Bohr protons on ligation caused by shift of allosteric equilibrium and depolymerization lowers pH by 0.10 and 0.13 units for control and treated cells, respectively.

$^5$Oxygen saturation at the nominal $pO_2$ of the tissue capillary bed (20 mm Hg).

$^{11}$Estimated from the empirical relationship between $c_{sat}$ and fractional $O_2$ saturation presented in Materials and Methods (based on Fig 4 in Sunshine et al$^{29}$).
of 2,3-DPG resulted in alkalinization of the cell, the magnitude of which agreed well with that estimated using the Duhm factor for AA RBCs enriched in 2,3-DPG (−0.023 pH unit/mmol/L, 2,3-DPG). This finding allowed us to calculate pH, for our experiments, based on 2,3-DPG concentrations and the dependence of pH, on 2,3-DPG concentration.

The principal observation of this study was the finding that depletion of 2,3-DPG to levels below 0.8 to 2.0 mmol/L (probably overestimates; see notes to Table 2) resulted in a considerable reduction in the sickling tendency of erythrocytes from four different patients. Results indicate that the antisickling effect at the p02 of the microcirculation (20 mm Hg) resulted in a considerable reduction in the sickling tendency of erythrocytes. Overall, then, four intraerythrocytic variables affecting sickling: (1) direct inhibition by elevation of c

Value for the antisickling effect at half-saturation (column 9 in Table 3) are similarly affected. Although there is no obvious explanation for this variation in effects on reversible sickling for the four patients, Hb F levels do not appear to play a role, because the reduction in sickling for patient A is less than for patient B (71% v 95%, respectively). This disparity somehow involves departure from an assumption necessary to this analysis—that the four variables affecting polymerization in a 2,3-DPG–dependent manner (2,3-DPG/pH, O2 saturation, and MCHC) are distributed homogeneously throughout the red blood cell population. The actual heterogeneous distribution of these variables may account for the individual variations in antisickling observed. Nevertheless, the order of the three measures of antisickling (%RSC at pO2 of 20 mm Hg and at P50, and calculated ∆P50) are identical for the four patients, showing that both measured and calculated indices of sickling and polymer content are internally consistent.

The data in Table 3 show that the depletion of 2,3-DPG from SS RBCs by manipulation of glycolysis significantly retards their propensity to sickle after deoxygenation. Furthermore, the data in Table 4 show that the relatively modest solubilizing effect of 2,3-DPG depletion per se is amplified into a much larger one by the concomitant alkalinization and increase in O2 affinity of the cell. These effects on c

Three strategies would produce a therapeutic inhibition of the intracellular polymerization that underlies sickling: (1) direct inhibition by elevation of c

The degree to which the potential antisickling effects accompanied by increased O2 affinity, was not rejected for clinical trials based on this fact or the minor increase in red blood cell mass it consistently produced. The degree to which the potential antisickling
effect of 2,3-DPG depletion would offset the negative features of increased O₂ affinity to have to be tested empirically by an in vivo study.

The findings of this in vitro study provide compelling evidence that 2,3-DPG is not a benign component of the sickle erythrocyte, but rather plays an essential role in the pathogenesis of polymer formation. Sustained reduction of this glycolytic intermediate could, therefore, substantially decrease Hb S polymerization in vivo, providing a therapeutically useful modality for ameliorating the vasoocclusive complications of sickle cell disease.

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Antisickling effects of 2,3-diphosphoglycerate depletion

WN Poillon, BC Kim, RJ Labotka, CU Hicks and JA Kark