The critical importance of red cell hydration in sickle cell disease derives from the huge effects of hemoglobin concentration on the appearance of polymers of deoxygenated sickle cell hemoglobin. On deoxygenation of solutions of sickle hemoglobin, the delay time to polymer formation is inversely proportional to concentration (of deoxyhemoglobin S) with 30th to 50th order kinetics (1). Dilution of deoxyhemoglobin S whether by water or by nonsickling hemoglobin would increase the delay time (and polymer formation) sufficiently to allow the red cells to arrive in larger, less readily occluded veins, or even to return to the lung for oxygenation before polymerization has occurred.

The concentration of hemoglobin S may be decreased by increasing the proportion and concentration of fetal hemoglobin as in the successful clinical trials with hydroxyurea or perhaps by preventing cellular dehydration using pharmacological agents, one of which (clotrimazole) is under investigation (2).

While the hemoglobin concentration of normal red cells is tightly regulated over a narrow range, the hemoglobin concentration of circulating SS (sickle cell anemia) erythrocytes varies widely. The most dense SS cells fractionated on density gradients may have hemoglobin concentrations of 50 g/dl. The hemoglobin concentration of as many as 40% of SS cells may exceed 38 g/dl, a value found in $< 1\%$ of normal red cells. Deoxygenation-induced changes in shape and deformability accompany the changes in cell density that reflect dehydration of the sickle cells.

For over 20 yr, Bookchin and Lew have maintained a productive transatlantic collaboration on the pathophysiology of cellular dehydration in sicklers. Their central questions have been concerned with the nature of P_{sickle}, the unique increase in permeability to Ca^{2+} and other ions which allows the SS cell to become so dehydrated. They predicted and observed that the deoxy-dependent cellular dehydration associated with P_{sickle} was present in sickle reticulocytes (which become dehydrated on deoxygenation by both the Ca^{2+}-activated K channel and the K:Cl cotransporter), accounting for the presence of cells of all ages in the dense (dehydrated) fractions (3, 4). A main transporter responsible for sickle cell dehydration is the Ca^{2+}-activated K channel originally described by Gardos (5).

In a series of simple and carefully executed experiments, Lew and his colleagues report in this issue of the *Journal* new findings about the effects of repeat cycles of deoxygenation on the Ca^{2+}-activated K^+ channel in sickle red cells (6). Using the discocyte fraction of sickle cells of normal density (the most abundant fraction minimally contaminated with reticulocytes or with dense rigid cells), thiocyanate anions (SCN), and careful temperature control, they separated by density sickle cells having deoxygenation-induced active K_{Ca} channels. With each deoxy pulse, reversible activation of those channels in 10–45% of cells was observed. When the activated (dense) cells were removed, successive deoxy pulses generated activated cell fractions of similar magnitude. The proportion of dense cells did not increase after 20 min of deoxygenation, whereas reoxygenation and deoxygenation generated "new" dense populations with each cycle. The only cells resisting change in permeability had increased fetal hemoglobin. The proportion of SCN dense cells increased with increasing external calcium, leveling off at > 5 mM. The authors conclude that deoxygenation-induced calcium permeabilization in sickle cells is stochastic, that no defined subpopulation of sickle cells has increased susceptibility to activation of the K_{Ca} channel. With each deoxygenation cycle, some cells will have near maximal K_{Ca} channel activation, an activation that will be different with the next cycle. Repeated cycles and cellular dehydration lead to the dense cells circulating in sicklers. The molecular basis of these observations is not immediately evident. One is reminded of the relative instability of sickle hemoglobin which may generate reactive oxygen species that might damage transport channels. Yet the observed effects are seemingly completely reversible, although the authors have not yet examined transport by the reoxygenated dense cells removed after the first round of deoxygenation. It is hard to imagine oxidative damage that would have the patterns of plateau, reversibility, and reproducibility observed in these studies, and evidence of cell damage is lacking. The best explanation appears to be that of Lew and his colleagues (6), that the deoxyhemoglobin polymer binds to the cytoplasmic surface of the red cell membrane to distort and open/widen an existing channel or to create a new channel through which cations and water pass to the exterior. Oxyhemoglobin S exhibits increased (versus hemoglobin A) electrostatic binding to the membrane anion exchanger (band 3), and the NH_2-terminal segment of band 3 binds in the central cavity of the deoxyhemoglobin tetramer (7). In addition to these intimate associations of hemoglobin with band 3, Joiner observed inhibition of cation fluxes by stilbene disulfonates (8). While not entirely specific, such an inhibition also implicates band 3 as a participant in sickle permeability induced by deoxygenation. The plateau would then result from the sol-polymer equilibrium with some of the polymers resident at fixed sites in the deoxy SS cell where they increase P_{sickle}. While complete deoxygenation was used in these studies, sickle cell polymers have been demonstrated in partially deoxygenated sickle cells. On reoxygenation, polymers disappear and the cell is again susceptible to deoxygenation-induced polymer formation. These are important concepts in the cellular pathophysiology of sickling. Studies of the cellular localization of sickle polymers and of the properties of cells that have undergone a bout of deoxygenation-induced increase in density will provide further evidence about the structural and functional basis of P_{sickle}.

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